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Factors influencing the development and prognosis of eczema

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Faculty of Medicine, Dentistry & Health Sciences

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Allergy and Lung Health Unit

Submitted in total fulfilment for the degree of *Doctor of Philosophy (Medicine, Dentistry and Health Sciences)*

Dedication

This PhD work is dedicated to my parents, who have always supported and motivated me to continue moving forward. To my wife, Ludmila, whose companionship provided me with emotional support. Also, to my brother, sisters and friends who provided me much-needed support during my PhD.

Thesis Abstract

Eczema (or atopic dermatitis) is a skin condition characterised by impaired skin barrier function and its chronic and inflammatory nature; it affects children and adults worldwide and considerably impacts their quality of life. Throughout this thesis, I used the term eczema as interchangeable with atopic dermatitis. Eczema is the most frequent skin inflammatory disease in children and adults. Eczema has a wide variation in global prevalence, but it is common in developed and developing countries. Australia is among the countries with the highest prevalence of eczema. The prevalence from 6 to 7 years varied from 0.9% to 22.5%, and from 13 to 14 years, the prevalence ranged from 0.2% to 24.6%. Moreover, it is widely accepted that infants have the highest prevalence rates, which are between 6 and 31%. There are no worldwide estimates of adult prevalence; however, the World Allergy Organization reported a range of 2 to 10%. Eczema is the leading contributor to skin-related disability-adjusted life years or DALYs. The high DALY rate is due to years spent living with a disability. The peak eczema prevalence is during early life, which increases the potential years spent living with this condition. The exact pathogenesis remains unknown; the origin of eczema is most probably due to multiple pathogenic pathways in which there is an interplay between skin inflammation due to skin barrier dysfunction, innate and adaptive immunology, the effect of scratching and environmental factors. There needs to be more information on the environmental factors determining eczema development and its phenotypes, especially in adults. Moreover, eczema has a heterogeneous presentation, which varies in severity, age of onset, and response to treatment. For these reasons, the aims of my doctoral research are to assess(1) the eczema latent classes and their association with risk factors and allergic comorbidities in adulthood, (2) whether the exposure to air pollution is associated with eczema and food allergies in the first ten years of life, (3) whether the association between exposure to air pollution is associated with the incidence and prevalence of atopic and non-atopic eczema in middle-aged adults, (4) the associations between water hardness and eczema symptoms development and (5) the potential associations between occupational exposures and eczema prevalence in middle-aged adults.

For the first aim, I used latent class analysis to determine eczema subclasses (Chapter 4). For this research project, I used data from 619 participants from the Melbourne Atopy Cohort Study baseline and all data collection points. There were data collection points from birth to 2 years (every four weeks), from age 3-7 years (yearly surveys), one study at 12 years, another at age 18, and a final one at 25 years. I determined five different eczema subclasses, which had different risk factors and allergic comorbidities. “early-onset persistent”, “early-onset resolving”, “mid-onset persistent”, “mid-onset resolving” and “minimal eczema”. Filaggrin null mutations were associated with the early-onset persistent (OR=2.58 [1.09-6.08]) and mid-onset persistent class (OR=2.58 [1.32-5.06]).

Compared with “minimal eczema”, participants from early-onset persistent class had higher odds of eczema (OR=11.8 [5.20-26.6]) and allergic rhinitis (OR=3.13 [1.43-6.85]) at 18 and at 25 years eczema (OR=9.37 [3.17-27.65]), allergic rhinitis (OR=3.26 [1.07-9.93]) and asthma (OR=2.91 [1.14-7.43]). Likewise, mid-onset persistent class had higher odds of eczema (OR=2.59 [1.31-5.14]), allergic rhinitis (OR=1.70 [1.00-2.89]) and asthma (OR=2.00 [1.10-3.63]) at 18 and at 25 years eczema (OR=6.75 [3.11-14-65]), allergic rhinitis (OR=2.74 [1.28-5.88]) and asthma (OR=2.50 [1.25-5.00]). Allergic and food sensitisation in early life was more common in those in the persistent eczema subclasses.

To achieve the second aim, I assessed the association between air pollution associated with eczema and food allergies in the first ten years of life (Chapter 5). The HealthNuts cohort collected data when the participants were 1, 4, 6 and 10 years respectively. The air pollution data was assigned using a satellite-based land-use regression model. Eczema was defined at 1 year by the parent report and at 4, 6 and 10 years using the International Study of Asthma and Allergies in Childhood definition. Food sensitisation was defined using skin prick tests and food allergy using oral food challenges in each wave. A total of 4,668 infants were included in the analyses. NO2 exposure at 4 years was associated with food allergy prevalence at 6 years (aOR:1.63[95%CI:1.00-2.64]). A higher concentration of PM2.5 at 1 year was associated with having food allergy at 4 years (aOR:1.39[95%CI:1.07-1.81]) per 1.6 µg/m3 PM2.5 increase) and at 6 years (aOR:1.32[95%CI:1.02-1.71]) per 1.6 µg/m3 PM2.5 increase). Air pollution exposure at 1 year was associated with persistent food allergy and peanut allergy. In contrast, there was no evidence of associations between air pollution markers and eczema during the same period.

To accomplish the third aim, I analysed the effect of ambient air pollution markers on eczema in a cohort of adults (Chapter 6). The study population came from the Tasmanian Longitudinal Health study, and the specific data for this project came from participants of the 2002 and 2012 proband studies. The air pollution data came from satellite-based land-use regression models based on the participants’ home locations. Participants completed a self-administered postal survey and attended a clinic visit. Of 3153 participants in both follow-ups, 2369 had valid skin prick tests. For males, a 2.3 ppb increase in baseline NO2 was associated with increased odds of prevalent eczema (OR=1.15 [95%CI 0.98-1.36]) and prevalent atopic eczema (OR=1.26 [1.00-1.59]). These associations were not seen in females (P for interaction=0.08, <0.01). For both sexes, a 1.6 µg/m3 increase in PM2.5 exposure at follow-up was associated with increased odds of aeroallergen sensitisation (OR=1.15 [1.03-1.30]).

To achieve the fourth aim, I investigated how water hardness is related to eczema in adults (Chapter 7). I used the data from the UK Biobank study; the initial assessment visits were done between 2006

and 2010. Detailed data were collected through a touchscreen questionnaire, a nurse-led interview and the participant's medical history. In 2014, a subset of participants repeated the baseline assessments and assisted with the imaging visits where similar data was collected. Data on domestic water supply was obtained from local water supply companies in England, Wales, and Scotland. This data was linked to the participants based on their approximate home location. 306,531 participants with a mean age of 57 years nested across 7642 postcodes were included in the baseline analysis, and 31,036 participants nested across 3695 postcodes were included in the follow-up analysis. We observed an increase in the odds of eczema at baseline (OR: 1.02 95%CI 1.01-1.04) per 50 mg/L of CaCO3 increase. Furthermore, exposure to domestic hard water (>200 mg/L of CaCO3) was associated with increased odds of prevalent eczema at baseline (OR: 1.12 95%CI 1.04-1.22). Moreover, there was a significant linear trend (p-value: <.001) in which increasing levels of hard water increased eczema prevalence risk. No association was observed with the incident eczema or eczema at follow-up. The intraclass correlation coefficient for postcode was 1.6% (95%CI:0.7-3.4) and remained unexplained by area-level socioeconomic measures.

To realise the fifth aim, I assessed the association between several occupational exposures and eczema using a job exposure matrix (Chapter 8). The data for this study came from 3,609 participants from the 2012 follow-up from The Tasmanian Longitudinal Health study when participants had a mean age of 53. Participants completed a self-administered postal survey that collected sociodemographic characteristics and medical diagnoses and completed the lifetime work history calendar. Cumulative exposure to mould was associated with increased odds of current eczema (adjusted odds ratio (aOR)=1.08 [95%CI:1.00-1.18] per 5 years exposure-equivalent). Similarly, current occupational exposure to animals (aOR=2.88 [1.34-6.22]), storage mites (aOR=2.78 [1.29-5.99]) and endotoxin (aOR=1.96 [1.05-3.66]) were associated with increased risk of current eczema. Furthermore, increased odds of NAE was associated with current exposure to animals (aOR=5.33[1.37-20.7]), storage mites (aOR=5.39[1.39-20.9]), and metalworking fluids (aOR=1.11[1.01-1.23]). Current exposures to isocyanates (aOR=4.65[1.04-21.4]) and acrylates (aOR=7.44[1.38-24.8]) was associated with AE.

Altogether, the findings of my doctorate work have important public health and research impacts and have reduced the knowledge gaps in this area of research (Chapter 9). I showed that early detection of eczema subclasses might be important as persistence classes were associated with more allergic comorbidities. The identification of eczema subclasses may aid in class-specific preventive or therapeutic strategies. I provided evidence of environmental risk factors for eczema in adults, an area that is lacking research. Increased exposure to air pollution was associated with eczema outcomes in adults. Increasing levels of hard water were related to an increased risk of

eczema. Occupational exposures to animals, storage mites, endotoxin, metalworking fluids, isocyanates and acrylates were associated with eczema outcomes in adults. Raising awareness and limiting exposures to these risk factors is likely to aid the reduction of eczema prevalence. Air pollution exposure may be a risk factor for food allergy in infants. Avoidance of air pollution in early life could potentially be a food allergy preventive measure. The strengths of this body of research are (1) the data used for this work came from a large prospective cohort study, (2) the use of well-characterised definitions of allergies and (3) the use of contemporary statistical methods, including multilevel modelling and latent class analysis.

Declaration

This is to certify that

I. The thesis comprises only my original work towards the PhD, except where indicated in the Acknowledgements.

II. Due acknowledgment has been made in the text to all other material used.

III. The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

……..

Diego J Lopez Peralta

7 September 2023

Preface

The data that supports this thesis came from the Melbourne Atopy Cohort Study, the Tasmanian Longitudinal Health Study, the UK Biobank study and the HealthNuts cohort study. The data from each cohort study have been collected in multiple follow-ups and involved numerous researchers. This thesis includes three first-author publications and one manuscript that is under consideration for publication and one manuscript draft. All these research manuscripts have several co-authors duly acknowledged in the authoring section of each manuscript. In addition to my PhD publications, I co-authored five research articles and was the first author of two research articles. I conducted the analyses and interpretation of results and wrote up all the thesis research manuscripts. The co-authors have contributed towards the conception and design of the study, acquisition of data, interpretation of data, the revision of important intellectual content and approved the final submission. The editorial assistance involved minimal revisions for each publication that did not change the main results in meaningful ways. The editorial changes involved grammar improvements, adding table legends, running logistic regression diagnostics and editorial formatting of the manuscript. All the co-authors have signed and returned the authorisation forms and agreed to include the published manuscript as a part of this thesis. Also, my principal supervisor has provided declarations of collaborative work. To the best of my knowledge and belief, I certify that this work does not contain any information that has been used in my name to be awarded any other degree or diploma from a university or other tertiary institution. My thesis does not have content or material previously written or published by another person except for texts with appropriate citations.

……..

Diego J Lopez Peralta

7 September 2023

Acknowledgements

I want to acknowledge Professor Adrian Lowe, my principal supervisor, his commitment and good predisposition to our meetings, his patient guidance to topics that were new to me, and his caring mentoring were fundamental to the success of my PhD. I also thank Associate Professor Caroline Lodge, a co-supervisor; her knowledge of the cohort studies included in this thesis helped me navigate the datasets. I sincerely thank Dr Dinh Bui, a co-supervisor, who provided support and code examples that helped me learn new statistical methods. I also thank Dr Nilakshi Waidyatillake, a co-supervisor, who provided encouragement and relevant suggestions throughout my candidature. Finally, Associate Professor John Su, who is a co-supervisor, his vast content expertise and suggestions helped guide my research.

I was fortunate to complete my doctorate in the Allergy and Lung Health unit from the Centre of Epidemiology and Biostatistics. I want to acknowledge the support and very friendly predisposition of all its members, especially Professor Shyamali Dharmage, who dedicated a lot of time and effort to providing significant feedback to my research work. Also, I want to thank Sabrina Idrose, a PhD student during my candidature, who provided me with advice based on her experience. I also value the assistance and input the other co-authors have offered to my analyses and manuscripts.

The data collection process took a lot of work, years of preparation, challenging field and lab work and data management by numerous researchers working at different levels. I want to thank the cohort studies' researchers, participants and funding bodies, which provided data for my analysis during my doctorate.

Also, I want to acknowledge my PhD funding bodies which are the Becas Don Carlos Antonio López scholarship from Paraguay, which provided a stipend for the duration of my PhD and the Melbourne Research scholarship, which provided a fee offset for this degree. Also, the Population Health Investing in Research Student's Training (PHRIST) and the Melbourne Conference Scholarship (MCS) funded conference and travel grants during my PhD.

And last but not least, my wife, Ludmila, my parents, brother, sister and friends whose support was always there when I needed it.

Publications, presentations, research supervision, research initiatives, and other research-related outcomes during the PhD candidature

First author publications arising from this thesis

1. **Lopez DJ**, Lodge CJ, Bui DS, Waidyatillake NT, Abramson MJ, Perret JL, Su JC, Erbas B, Svanes C, Dharmage SC, Lowe AJ. Establishing subclasses of childhood eczema, their risk factors and prognosis. Clinical & Experimental Allergy. 2022 Sep;52(9):1079-90.
2. **Lopez DJ**, Lodge CJ, Bui DS, Waidyatillake NT, Su JC, Perret JL, Knibbs LD, Erbas B, Thomas PS, Hamilton GS, Thompson BR, Abramson MJ, Walters EH, Dharmage S, Bowatte G, Lowe AJ. Association between ambient air pollution and development and persistence of atopic and non-atopic eczema in a cohort of adults. Allergy. 2021 Aug;76(8):2524-34.
3. **Lopez DJ**, Singh A, Waidyatillake NT, Su JC, Bui DS, Dharmage SC, Lodge CJ, Lowe AJ. The association between domestic hard water and eczema in adults from the UK Biobank cohort study. British Journal of Dermatology. 2022 Nov 1.

First author publications arising from this thesis (under consideration or draft)

1. **Lopez DJ**, Alif SM, Dharmage SC, Lodge CJ, Bui DS, Moual NL, Waidyatillake NT, Su JC, Abramson MJ, Walters EH, Hamilton GS, Bowatte G, Erbas B, Benke GP, Perret JL, Lowe AJ. Assessing associations between occupational exposures and eczema in middle aged adults
2. **Lopez DJ**, Lodge CJ, Bui DS, Waidyatillake NT, Su JC, Lowe AJ, Peters RL. Associations between air pollution and development and persistence of eczema and food allergy across the first ten years of life: a population-based cohort study

International conference abstracts and presentations arising from this thesis

1. **Lopez DJ**, Lodge CJ, Bui DS, Waidyatillake NT, Abramson MJ, Perret JL, Su JC, Erbas B, Svanes C, Dharmage SC, Lowe AJ. Establishing subclasses of childhood eczema, their risk factors and prognosis. World Congress of Epidemiology 2021
2. **Lopez DJ**, Singh A, Waidyatillake NT, Su JC, Bui DS, Dharmage SC, Lodge CJ, Lowe AJ. The association between domestic hard water and eczema in adults from the UK Biobank cohort study. World Allergy Conference 2022. Available at: https://aai.org.tr/content.php?id=111
3. **Lopez DJ**, Alif SM, Dharmage SC Lodge CJ, Bui DS, Moual NL, Waidyatillake NT, Su JC, Abramson MJ, Walters EH, Hamilton GS, Bowatte G, Erbas B, Benke GP, Perret JL, Lowe AJ. Exploration of associations between occupational exposures and current eczema in middle aged adults. World Allergy Conference 2022. Available at: https://aai.org.tr/content.php?id=111

Other first-author publications during the PhD candidature

1. **Lopez D**, Waidyatillake N, Zaror C, Mariño R. Impact of uncomplicated traumatic dental injuries on the quality of life of children and adolescents: a systematic review and meta-analysis. BMC oral health. 2019 Dec;19(1):1-2.
2. **Lopez DJ**, Hegde S, Whelan M, Dashper S, Tsakos G, Singh A. Trends in social inequalities in early childhood caries using population-based clinical data. Community Dentistry and Oral Epidemiology. 2022 Nov 24.

Other co-author publications during the PhD candidature

1. Mariño R, Priede A, King M, Adams GG, **Lopez D**. Attitudes and opinions of Oral healthcare professionals on screening for Type-2 diabetes. BMC Health Services Research. 2021 Dec;21(1):1-8.
2. Mariño R, Delany C, Manton D, Reid K, Satur J, Crombie F, Wong R, McNally C, **Lopez D**, Celentano A, Lim M. Preparedness for practice of newly qualified dental professionals in Australia-educator, employer, and consumer perspectives. BMC Medical Education. 2022 Dec;22(1):1-2.
3. Turton B, Chher T, Hak S, Sokal-Gutierrez K, **Peralta DL**, Laillou A, Singh A. Associations between dental caries and ponderal growth in children: A Cambodian study. Journal of global health. 2022;12.
4. Mariño R, Delany C, Manton DJ, Reid K, Satur J, Crombie F, Wong R, McNally C, Adams GG, **Lopez D**, Celentano A. Preparedness for practice of newly qualified dental practitioners in the Australian context: an exploratory study. BMC medical education. 2022 Dec;22(1):1-2.
5. Zeleke, BM, Lowe, AJ, Dharmage, SC, **Lopez, DJ**, Koplin, JJ, Peters, RL, et al. Epidemiology of eczema in South-Eastern Australia. Australas J Dermatol. 2022; 00: 1– 10. https://doi.org/10.1111/ajd.13966

Other conference abstracts and presentations during the PhD candidature

1. **Lopez DJ**, Hegde S, Whelan M, Dashper S, Tsakos G, Singh A. Trends in social inequalities in early childhood caries using population-based clinical data. International Association for Dental Research 2022

Student supervisions

I have supervised one Master of Public Health students for final year research project at The University of Melbourne. The project title was “Assessing indoor air pollution profiles as risk factors for eczema”.

Review of manuscripts

I made 14 Peer reviews in the following journals:

1. Dental traumatology,
2. Pediatric Allergy and Immunology
3. Scientific Reports journal

Media engagement

2 Plain language statements

1 radio interview

Grants, awards and scholarships

1. Becas Don Carlos Antonio López scholarship (US$90.000 Stipend)
2. The Melbourne Research scholarship (PhD fee offset)
3. PHIRST 2020 (AU$550.70)
4. PHIRST 2021 (AU$250)
5. MCS 2022 (AU$1500)
6. PHIRST 2022 (AU$1350)
7. Australian Dental Research Foundation (Completed and submitted a grant application, unsuccessful)
8. Centre for Air pollution, energy and health Research SEED funding (Completed and submitted a grant application, unsuccessful)

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List of Abbreviations

AD	Atopic dermatitis
ADQ	Atopic dermatitis quick scores
AE	Atopic eczema
AEDS	Atopic eczema/dermatitis syndrome
AHR	aryl hydrocarbon receptor
AIC	Akaike information criteria
AIHW	Australian Institute of Health and Welfare
ANU	Australian National University
aOR	Adjusted odds ratio
BIC	Bayesian information criteria
$CaCO_3$	Calcite
$CaMg(CO_3)_2$	Dolomite
CO	carbon monoxide
DAG	Directed acyclic graph
DEPs	diesel exhaust particles
DHT	dihydrotestosterone
DMR	Distance to major roads
DNA	deoxyribonucleic acid
ECRHS	European Community Respiratory Health Survey
EDC	epidermal differentiation complex
EOP	Early onset persistent
EOR	early onset resolving
FLG	filaggrin
GWAS	genome-wide analysis
H&R	Hanifin & Rajka criteria
ICC	Intraclass correlation coefficient
ICD	International Statistical Classification of Diseases
IgE	Immunoglobulin E
IL	Interleukin
IMD	Index of Multiple deprivation
IQR	Interquartile range
ISAAC	International Study of Asthma and Allergies in Childhood
JEM	Job-exposure matrix
LCA	Latent class analysis

LEKTI	lymphoepithelial kazal type-related inhibitor type 5
LLCA	Longitudinal latent class analysis
LUR	land-use regression
MACS	Melbourne Atopy Cohort Study
MACS	Melbourne Atopy Cohort Study
mg/L	milligrams per litre
MIMS	Monthly Index of Medical Specialties
MOP	mid onset persistent
MOR	mid onset resolving
MOR	Median odds ratio
NAE	Non-atopic eczema
NO_2	Nitrogen dioxide
NOx	Nitrogen oxides
O_3	Ozone
OAs-JEM	Asthma specific-job exposure matrix
OFC	Oral food challenge
ORs	Odds ratios
OS	Ordnance Survey
OSD	occupational skin diseases
PCA	pyrrolidine carboxylic acid
PIAG	Patient Information Advisory Group
PM	particulate matter
PM_{10}	Particles matter of less than 10 μm in diameter
$PM_{2.5}$	Fine particulate matter with an aerodynamic diameter of 2.5 μm or less mass
QoL	Quality of life
RCT	Randomised controlled trial
ROS	Reactive oxygen species
SALIA	Study on the influence of air pollution and lung function, inflammation and Aging
SB	stratum basale
SC	stratum corneum
SEIFA	Socio-Economic Indexes for Areas
SG	stratum granulosum
SOx	Sulfur oxides
SPINK5	Serine peptidase inhibitor, Kazal type 5

SPRRs	short proline-rich proteins
SPT	Skin prick testing
SS	stratum spinosum
TAHS	Tasmanian Longitudinal Health Study
TEWL	Transepidermal water loss
Th_2	T helper cell type 2
TJs	tight junctions
TSLP	Thymic stromal lymphopoietin
UK	United Kingdom
UKWP	United Kingdom Working Party
VOCs	volatile organic compounds

Notes

Author accepted version of published articles.

Chapters 4, 6 and 7 from this thesis include published articles. The "author accepted" version of articles have been included in each chapter. This is the final draft as accepted by the publishers, including any changes based on referees' suggestions before it has undergone copy-editing, typesetting and proofing. For copyright reasons, the published version cannot be included in this thesis. However, the relevant citation is provided at the beginning of each chapter.

Referencing

Chapters 4 to 8 of this thesis include research papers that have been published as an article, are currently under review for publication or in draft stage. In each of this chapters, references were provided separately, and they are located at the end of each respective article. References from other chapters appear at the end of this thesis under the heading "References". Throughout this thesis the American Medical Association 11th edition referencing style (https://endnote.com/style_download/ama-11th-american-medical-association-11th-edition/) was used.

Supplementary material and Appendices

Supplementary material related to the chapters 4 to 8 are included at the end of those chapters under the heading "Supplementary Materials". Appendices from the overall thesis are attached at the end of the thesis.

Chapter 1 - Introduction

1.1. Overall aim and rationale

The rationale for my doctoral work was that eczema is a significant public health concern, with a considerable effect on the quality of life of the patient associated with substantial morbidity. However, there is limited information on the risk factors determining eczema development, especially in adults. Firstly, eczema has a heterogeneous presentation, which varies in terms of severity, age of onset, and response to treatment, suggesting underlying phenotypes that can incorporate the variability of eczema development and progression. These phenotypes may be differentially associated with risk factors and progression to other allergic comorbidities. Secondly, there are conflicting results regarding the effect of outdoor air pollution on eczema prevalence in middle-aged adults, making any public health action difficult. Thirdly, while there is evidence for associations between hard water and eczema, this evidence is limited to children, with no previous studies have considered this association in adults. Fourthly, occupational exposure as a risk factor for eczema has not been evaluated. Defining these associations is essential to understand better the development, persistence and pathophysiology of eczema in adults.

For these reasons, my doctoral research overall aims were twofold: to investigate the effects of air pollution, hard water and occupational exposures in eczema prevalence, identify eczema subclasses, and assess their associations with risk factors and progression to other allergic comorbidities. A better understanding of the potential risk factors and subclasses of eczema may lead to reduced adverse outcomes, improved treatment and prevention interventions and an improved long-term prognosis. In the sections below, I have expanded on the rationale for the specific projects within my thesis.

1.1.1 Eczema background

Eczema, also known as atopic dermatitis, is a chronic inflammatory skin condition characterised by defective skin barrier function affecting around 5% to 10% of adults and up to 20% of children. [1] The prevalence of eczema varies worldwide, as reported by phase three of the International Study of Asthma and Allergies in Childhood (ISAAC) study, a global multi-centre study that uses uniform methods that allow direct comparison of their results. [2] The prevalence in the age group 6 to 7 years varied from 0.9% (Jodhpur, India) to 22.5% (Quito, Ecuador) and for the age group 13 to 14 years, the prevalence varied from 0.2% (Tibet, China) to 24.6% (Barranquilla, Colombia). [2]

Eczema has a heterogeneous presentation, which varies in severity, age of onset, and response to treatment. [3] This variation is believed to be determined by an interplay of genetics and environmental factors. [3,4] While a genetic skin barrier flaw is associated with an increased risk of eczema, this risk factor is not a requirement for eczema, which can develop by other pathological

pathways. [5] As such, eczema onset is probably determined by a complicated pathogenesis mechanism. [6]

Eczema can negatively affect the patient's and their family's quality of life. The disease can manifest with different levels of severity of itching and scratching, sleep deprivation, pain, bleeding and sometimes skin infections. [7] Moreover, eczema can lead to dietary limitations and food allergy, as suggested by the dual-allergen-exposure hypothesis. [8] Eczema has the highest effect on DALY (disability-associated life years) for patients with skin diseases worldwide. [9] Furthermore, eczema often places a financial burden on patients and their families through direct medical costs and decreased productivity. [10]

The World Allergy Organization has recognised two types of eczema. [11] Atopic eczema (AE) is associated with high levels of immunoglobulin E (IgE) mediated sensitisation and allergen-specific IgE. [11] Normal levels of total IgE and a lack of sensitisation to environmental allergens characterise non-atopic eczema (NAE). [11] In older patients, particularly those with chronic eczema, other non-atopic inflammatory mechanisms might be more commonly involved.

1.1.2. Phenotypes of eczema identified by latent class analysis

There may be different pathophysiological pathways for the development of eczema, which have not yet been fully characterised. Complex interactions between the environment and genetic factors determine the age of onset, severity and persistence of eczema. [6] For that reason, the concept of “eczema phenotypes” has been suggested, rather than a simple binary classification of eczema, as such phenotypes can incorporate the variability of eczema development and progression. [12] The classification of eczema phenotypes could identify risk factors for specific sub-types of eczema and enhance understanding of patterns associated with eczema onset and progression.

There were five previous analyses that have examined this heterogeneity in eczema onset and persistence during infancy and early childhood and the potential associations to other allergic comorbidities. [13-17] However, these previous studies only used a data points to determine eczema subclasses and a limited number of risk factors and allergic comorbidities at 6[15], 8[16], 10[14], 12, 16[13] and 26[17] years. These findings need to be replicated by assessing eczema subclasses targeting high-risk children with multiple early eczema assessments to draw firmer conclusions. Furthermore, assessing the longitudinal associations of a broad range of risk factors encompassing individual, environmental and genetic factors with eczema subclasses may provide a better understanding of which factors contribute to the development and persistence of eczema patterns.

Subsequent better characterization of eczema subtypes, including identification of associated risk factors and clinical trajectories, may lead to more nuanced and personalised prevention and therapeutic strategies.

1.1.3. Ambient air pollution as a risk factor for eczema

It has been proposed that ambient air pollution may lead to eczema and sensitisation via inflammatory oxidative stress leading to skin barrier dysfunction. [4] Air pollution can produce reactive oxygen species (ROS) and nitrogen species that lead to damage cell structures. [18] Air pollutants can also act as irritants and immunomodulators, leading to elevated levels of serum IgE. [19] Specifically, particulate matter with a diameter less than 2.5 micrometres ($PM_{2.5}$) may activate aryl hydrocarbon receptors to promote cell metabolism and inflammation. [19] Other proposed mechanisms are altering trans-epidermal water loss, increasing inflammatory signals and modifying the skin pH and microbiome. [20]

A reduced number of research articles have considered the association between air pollution exposure and eczema prevalence or incidence, and the findings have been inconsistent. Two paediatric studies showed no association between ambient air pollution and increased risk of eczema. One of these studies used data from ISAAC phase one, in which annual concentrations of PM_{10} at the city level were obtained for 105 ISAAC centres in 51 countries in 13 to 14 age groups. The authors concluded that PM_{10} was not associated with eczema. [21] The other study evaluated short-term exposure to $PM_{2.5}$ and concluded that it did not impact skin symptoms in schoolchildren. [22] here are other studies in which ambient air pollution was associated with increased eczema risk in children [23] and adults. [24] The study in 5338 schoolchildren (10.4±0.7 years) in France, the authors concluded that flexural dermatitis was significantly increased in residential settings with $PM_{2.5}$ concentrations exceeding 10 µg/m_3. [23] The study to examine these associations in adults investigated the influence of traffic related air pollution on eczema, finding that baseline concentrations of traffic-related air pollution (TRAP) markers (NO_2, NO_x, $PM_{2.5}$ and PM_{10}) were significantly associated with increased odds of incident eczema over a 19-year follow-up period, and these associations were stronger for non-atopic eczema.[24] Therefore, environmental factors, including air pollution, might be important for developing eczema in middle age, particularly NAE. A better understanding of the potential effects of ambient air pollution on adult eczema may lead to targeted interventions to prevent eczema.

1.1.3.1. Ambient air pollution, eczema and food allergy

Eczema and food allergies have both shown parallel increases in prevalence in the recent years, suggesting that shared environmental factors have a role in the development of these early-stage allergic diseases. [25] Food allergy is defined as an negative and immunological response to certain foods. [26] Nearly 10% of 1 year old infants have a food allergy. [27] Food allergies are determined by active immune responses to typically harmless food antigens with a loss of or failure to develop oral tolerance. [8] Hence, it is determined by disproportional immune responses to food antigens that involves mainly the T helper cell type 2 (Th_2) pathway related to the production of IgE

against food antigens.[28,29] It was observed that allergic disease frequently start in early life with eczema often accompanied by food allergy symptoms. [30] Recent literature reported that many countries worldwide have a high prevalence of eczema and food allergy, these disease are amongst the most frequent chronic non-communicable diseases in children. [27,31]

It was postulated that changes caused on by urbanisation and industrialisation can compromise the skin's epithelial barrier and the gastrointestinal tract, making people more susceptible to allergies.[32] As described above, reactive oxygen species generated by air pollutants can damage the epithelial cells (dermal and gastrointestinal) through oxidative stress.[33] The damage to epithelial cells can determine an inflammatory response, this process may drive further pruritus (itching of the skin) related with eczema.[34] As suggested by the "dual allergen exposure hypothesis",[35] it is plausible that the increased exposures to allergens through a disrupted skin barrier can lead to food allergy.[8] Ambient air pollution may influence eczema and eczema with concurrent food allergies (oral food challenge-proven) in infancy. There is a lack of studies in this area and a better understanding of these topics can lead to preventive and treatment strategies for these allergic diseases.

1.1.4. Domestic hard water as a risk factor for eczema

Evidence shows that domestic use of hard water may cause eczema symptoms in children. [36] Hard water contains high mineral content, usually calcium and magnesium. [37] Hard water sources usually are underground, and it is formed when minerals are dissolved in the water during the filtration through sedimentary rocks. In general terms, groundwater used is commonly characterised to present “hardness” at different levels, but the same issue can happen with larger surface water sources if the conditions are appropriate. The key minerals that contribute to water hardness are calcium in the form of calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)^2$), which is mostly associated with calcareous rocks and limestone. [38] Countries that used hard water in their water supply include Canada, England and Wales, Ireland, The United States and Australia. [21] According to the World Health Organization, domestic water is hard when the concentration of $CaCO_3$ is more than 200 milligrams per litre (mg/L). [38,39]

The proposed biological mechanisms by which hard water may lead to increased eczema severity and development are a combination of direct and indirect effects which damage the skin barrier function. [36] The direct effects include a rise in pH on the skin surface (healthy skin is usually mildly acidic), which can affect the barrier function. [36] Indirect effects include the increase in usage of detergents such as sodium lauryl sulphate to counteract the high mineral content to achieve a “lather”, resulting in increased deposition of these detergents on the skin, which reacts with the calcium in the hard water to form small chalk particles which can irritate the skin. [36] Moreover, there

is an increased allergen penetration due to a disruption in the skin barrier,[36] and increased bacterial colonisation of the skin. [40]

To date, all the literature focused on the effects of domestic water hardness on eczema has studied children. In contrast, previously, this association has yet to be examined in an adult population. Particularly in older patients and those with chronic eczema, the pathophysiology of eczema is not well understood, [11] and other potential inflammatory mechanisms might be involved. Therefore, a better understanding of the effects of domestic water hardness on adult eczema may lead to targeted intervention (i.e. use of water softeners) to decrease eczema recurrence and severity.

1.1.5. Occupational exposures

Occupational exposures to an irritant or allergenic substances, including chemicals and substances such as oils, detergents, shampoos, and cleaning agents on the skin, are responsible for developing occupational skin diseases (OSD), most commonly contact dermatitis, which predominantly affects the hands. [41] However, much less is known about the effect of occupational exposures on eczema prevalence.

Occupational exposures may influence the development of new eczema cases (incidence) and ongoing eczema symptoms (persistence) in adults. Occupational epidemiology of skin reactions has conventionally classified eczema as being due to a genetic predisposition rather than caused by contact with external agents. [42] However, more recent thinking considers eczema development and progression to be multifactorial and related to an interplay between genetic and environmental factors. [5,43] Moreover, occupational exposures have been associated with the development and progression of allergic asthma. [44,45] As such, it is plausible that occupational exposures could have a similar effect on eczema, particularly in some occupations exposed to animal dust, fish/shellfish, latex, solvents and wood dust. [46]

Earlier occupational studies regarding dermatitis have been mostly limited to contact dermatitis. [47] Exposures in the workplace may play a role in the adult eczema pathological pathway as a series of environmental risk factors. It has been suggested that occupational substances can act as risk factors, such as allergens on contact with the skin resulting in an allergic reaction. [48] Alternatively, irritants could disrupt the barrier function of the skin, which may make it more permeable to other allergens. [48] Most occupational irritants are chemicals, including metalworking fluids, cleaning agents and organic solvents, that can damage the epidermal barrier after continuous exposure to the irritant. [49] Also, there are allergens like latex, rubber additives in gloves, metals (nickel, chromium and cobalt), epoxies, preservatives, resins and acrylates, which can lead to allergic dermatitis. [49] To date, only two studies investigated associations between occupational exposures to dust gases and fumes with current eczema. [47,50] However, the occupational exposure definition in

both studies was non-specific, defined as self-reported exposure to gases, dust and fumes. Assessing the associations of specific occupational exposures may provide a better understanding of the likely environmental contributors to the development and persistence of eczema in adults.

1.1.6. Summary of the gaps in knowledge

There is a need for more conclusive evidence to understand better the risk factors of eczema development and progression. As a result, preventative and therapeutic interventions are constrained by insufficient knowledge of the various pathophysiological aspects of eczema and its prognosis. More details on these gaps in knowledge are provided in Chapter 2. Compared with eczema studies done in children, there have been fewer studies done in adults, and some risk factors were not evaluated in adult samples. There is a lack of studies evaluating the effect of air pollution on eczema and food allergies, understanding this topic can lead to preventive and treatment strategies for these allergic diseases. Furthermore, due to eczema's heterogeneous clinical presentation and progression, there is a need to characterise eczema phenotypes rather than a simple binary classification. The available evidence on latent eczema classes was lacking. It is essential to produce robust evidence to address these knowledge gaps.

1.2. My doctoral work

1.2.1. Knowledge-gaps addressed.

During my PhD, I addressed some of these knowledge gaps. Firstly, I performed a latent class analysis using data from 0 to 12 years from the Melbourne Atopy Cohort Study (MACS) and examined associations between the identified eczema subclasses and risk factors and allergic comorbidities. Secondly, I assessed the exposure to ambient air pollution associated with eczema and food allergies in children from the HealthNuts cohort study. Thirdly, I analysed quantitative data on the association between ambient air pollution and the development and persistence of atopic and non-atopic eczema in adults using data from a large cohort study, the Tasmanian Longitudinal Health Study (TAHS). Fourthly, to determine the association between domestic hard water and eczema, I analysed quantitative data using hard water GIS data and data from the UK biobank study, which is a very large prospective cohort from the UK. Finally, I assessed the association between occupational exposures and eczema using a job exposure matrix; the allergy and job calendar data came from the TAHS.

1.2.2. Aims

The overall aim of my PhD research was to assess the factors that influence eczema development in different settings and age groups, with a focus on adult-onset eczema, childhood onset persistent eczema, and risk factors in early life and adulthood.

The core component of my PhD included five research projects, the specific aims and research of each project are below:

1. To determine the longitudinal eczema subclasses according to eczema prevalence in early life and childhood.

1.1. To assess the associations between risk factors and these eczema subclasses

1.2. To determine which longitudinal eczema subclasses are most strongly associated with allergic outcomes in adulthood.

2. To assess whether the exposure to air pollution is associated with eczema and food allergies in the first ten years of life.

2.1. To examine if air pollution is associated with eczema and food allergies persistence in the first ten years of life.

3. To examine whether exposure to air pollution is associated with the incidence and prevalence of eczema in middle-aged adults.

3.1. To assess whether these association vary according to atopic or non-atopic status.

4. To determine the associations of domestic water hardness and eczema prevalence and incidence in a cohort of adults.

5. To evaluate potential associations between occupational exposures and eczema prevalence in middle aged adults.

5.1. To assess whether these association vary according to atopic or non-atopic status.

1.3. Thesis statement

This thesis is based on my PhD research work conducted for three years and a half. I used data from TAHS, MACS, UK biobank and HealthNuts cohorts to assess (1) eczema latent classes from infancy to later childhood and its association to risk factors and allergic comorbidities in early adulthood, (2) whether the exposure to air pollution is associated with eczema and food allergies, (3) whether the exposure to air pollution is associated with the incidence and prevalence of atopic and non-atopic eczema in middle-aged adults, (4) whether water hardness types and eczema symptoms development are associated, and (5) potential associations between occupational exposures and eczema prevalence in middle-aged adults. The strengths of this body of research are (1) the data used for this work came from a large population-based prospective cohort study, (2) the use of well-characterised definitions of allergies and (3) the use of contemporary statistical methods, including multilevel modelling and latent class analysis.

1.3.1. Thesis overview

Chapter 1 provides the summary for this doctorate work. The background information on eczema is introduced and its clinical and public health importance explained. The existing evidence and knowledge gaps are described and the research objectives justified.

Chapter 2 a literature review on the clinical presentation, epidemiology, pathophysiology, risk factors and treatment of eczema.

Chapter 3 presents a description of the general methods used in this PhD work.

Chapter 4 is a publication arising from my doctorate work that describes eczema subclasses during childhood, their risk factors and prognosis in adulthood.

Chapter 5 is a manuscript draft in which I assessed the associations air pollution and eczema and food allergies in the first ten years of life.

Chapter 6 is a publication arising from my doctorate work where I assessed the effect of ambient air pollution as a risk factor in the development and persistence of atopic and non-atopic eczema in a cohort of adults.

Chapter 7 involves a publication arising from this PhD work in which the association between domestic hard water and eczema is assessed in a large cohort of middle-aged adults from the UK using multilevel analysis.

Chapter 8 includes a publication in which I assessed the associations between occupational exposures and eczema in adults using a job exposure matrix.

Chapter 9 I provide an overall discussion and conclusion for each objective including a thesis summary. I also provide a summary of the implications and recommendations related to this PhD work as well as directions for future research.

Chapter 2 - Literature review

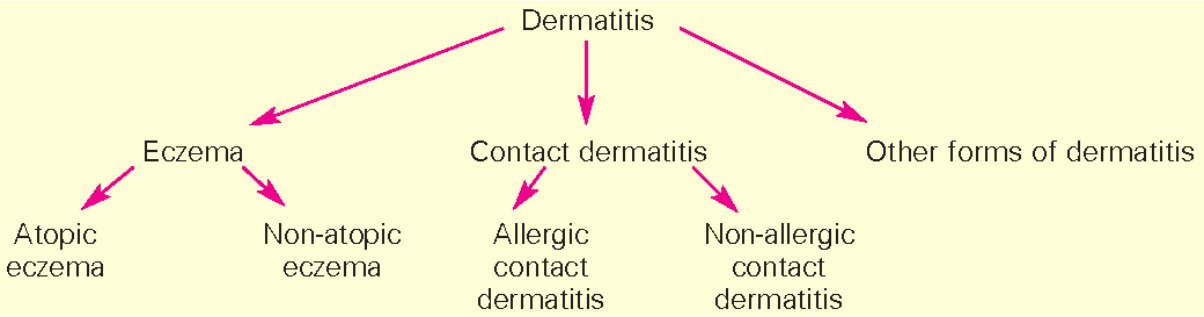
This chapter starts with a brief introduction on eczema. Then, I described the current eczema definitions, evaluate the evidence on eczema pathogenesis, pathophysiology, clinical presentation and diagnosis. Next, I reviewed the literature about the distribution of eczema in the general population and in specific sub-groups, eczema burden of disease and eczema classifications. After that, I assessed the evidence regarding eczema genetic and environmental risk factors. Finally, I examined the evidence-based treatment recommendations for eczema. At the end of each sub-section, a box containing a summary of each sub-section. Where knowledge gaps were highlighted, another box containing the research questions related to this thesis is presented.

2.1. An introduction to Eczema

Eczema, also known as "atopic dermatitis", is a frequent, chronic and recurring skin inflammatory condition characterised by an itchy red rash; [51] it is the most prevalent skin disorder in the developed world, with a lifetime prevalence of around 20%. [5] Its onset is usually before the age of 5 years. [52] It often improves or resolves during childhood, but may persist into adulthood. [51] Eczema is a heterogenous disease with wide variations in clinical features. Its morphological changes include vesicles (fluid-filled elevations), papules/nodules (dome-shaped elevations), plaques (flat-topped elevations), macular (impalpable) erythema, excoriations (scratch marks), and lichenification (skin thickening). [51] Changes are usually located in flexures (skin creases e.g. behind the knees and folds of elbows), in front of the ankles, under the buttocks, and around the neck, ears and eyes; the face is often involved in infant eczema. [51]

In 70% of individuals with eczema, the serum immunoglobulin E (IgE) and eosinophils are high. [51] Traditionally, two types of eczema have been recognised (Figure 2.1). The first form, non-atopic eczema (NAE), is characterised by normal levels of total IgE and lack of sensitisation to environmental allergens.[53] The second is an atopic type (AE) with skin inflammation driven by T-cell responses and T helper cell type 2 (Th_2) cytokines in the initial phase, which is usually associated with IgE-mediated sensitisation to environmental allergens and high levels of both total and allergen-specific IgE. [11] This form of eczema is strongly associated with increased tendency of developing other allergic conditions.[53]

Figure 2.1. The World Allergy Organization classification of eczema/dermatitis. (Source: E. Weinberg, et. al.; 2005) [54]



Eczema is a multifactorial disease that is determined by a complex interaction between genes and the environmental exposures. A key feature is an impaired skin barrier function usually associated with dry skin. [5] Eczema can have a substantial effect on the quality of life of both the patient and their family. Itching and scratching, soreness, bleeding, and sleep loss are all possible manifestations of the condition. [5] Eczema has also been linked to the development of allergic comorbidities such as allergic rhinitis and food sensitivities. [55] The economic costs associated with eczema are mostly indirect, such as lost workdays, travel expenditure for medical appointments, sleep loss, and productivity loss. [56] These aggravating factors, along with the potential to progress to other allergic diseases and high prevalence underpins the public health impact and importance of eczema.

Summary: An introduction to Eczema

- Eczema is a common, chronic and relapsing skin inflammatory condition characterised by an itchy red rash.
- Eczema is a heterogenous disease with wide variations in clinical presentations.
- Eczema development is multifactorial, determined by complex interactions between genes and environmental exposures.
- Eczema is commonly the first indication of allergic disease in children and is associated with increased risk of asthma, allergic rhinitis, and food allergy.
- Eczema is an important public health concern due to its high prevalence and effects on quality of life.

2.2. Eczema definitions

A variety of definitions for eczema have been proposed, and there remains a lack of consensus as to how to define eczema at this time. A disease definition must result in the best

possible differentiation from other diseases, be simple to recall and use, match the most recent clinical understanding of the condition, and be acceptable as a reliable research tool. [57] Eczema has a heterogenous presentation with different levels of severity, treatment response, age of onset and may progress to other atopic diseases. [30]

2.2.1. Eczema terminology

As knowledge about eczema has grown, new terminology has appeared, leading to the coexistence of several alternative denominations for eczema and a rise in definitional ambiguity (Table 2.1.). Previously, authors classified eczema as exogenous eczema (contact dermatitis) and endogenous eczema; which includes atopic dermatitis and other types dermatitis (nummular eczema, stasis dermatitis, xerotic eczema, nonspecific dermatitis). [58] Other authors used a wide range of synonyms for eczema (atopic dermatitis, atopic eczema, neurodermatitis), these denominations are frequently used interchangeably and listed as synonyms. [59] Despite several attempts to come to a consensus, two names—Eczema and Atopic Dermatitis (AD)—currently coexist and are often used as synonyms. [60]

There are debates over which definition is correct among clinicians and in the literature. If we consider the disease ontology, [61] atopic dermatitis is generally viewed as a more "specific" form of eczema (where eczema is considered as a synonym of dermatitis). However, the World Allergy Organization does not agree with this viewpoint,[11] while the International Statistical Classification of Diseases and Related Health Problems (ICD) [62] nomenclature does. Although the term atopic eczema was also suggested, it is used less commonly than eczema or atopic dermatitis. [60] Originally, the descriptor "atopic" implied a connection to type I hypersensitivity, [63] but atopic sensitisation in eczema has since been demonstrated to not always be present. [64] As such, it is nonsensical to refer to this condition as “non-atopic atopic dermatitis” as it makes more sense to use the term non atopic eczema. Moreover, eczema is the term most used by the lay population to refer to this disease, [65] so using the term eczema may make scientific research more accessible for the general public. For those reasons, I used the term “eczema” throughout this thesis.

Table 2.1. *Terms used to describe eczema in the scientific literature.* [66]

Terms used for eczema
Atopic dermatitis
Atopic eczema
Atopic neurodermatitis
Atopiform dermatitis
Besnier's prurigo
Childhood eczema
Dermatitis
Flexural eczema
Infantile eczema
Lichen agrius
Neurodermatitis
Porrigo larvalis

2.2.2. Genetic Definitions

Although genetic factors are involved in the aetiology of eczema and family history of atopic disease is the strongest known risk factor for eczema, there are no specific genetic markers for eczema that can predict the development of the disease. [67] Several susceptibility loci have been identified before, [67] more detailed description are provided in the Genetics section (2.8.1. Genetic risk factors). The most significant genetic factor in eczema is a mutation in the ATOD2, which is located on chromosome 1q21 in the "epidermal differentiation complex," contains the filaggrin gene, for which null mutations have been discovered. [67] When the cells of the granular layer break down into scales of the corneal layer, a process known as corneocyte formation, filaggrin plays a role in the aggregation of the keratin cytoskeleton. [68] However, not everyone that has a filaggrin gene mutation develops eczema and not everyone that has eczema has a filaggrin null mutation. [68]

2.2.3. Clinical definitions

There are no diagnostic laboratory markers or specific histopathologic diagnostic features in eczema, although the later can help to rule out differential diagnosis. [69] For that reason, eczema is diagnosed clinically and its golden standard is the diagnosis by an experienced clinician. [69] There is a

spectrum of dermatological manifestations which makes a precise clinical definition of eczema challenging.

Infantile eczema typically begins as rash or redness in the face, usually not affecting the centre of the face, and on the toddlers' extensor surfaces of their arms and legs as well as their trunk, occasionally exhibiting considerable oozing and crusting. [69] There are variations in localisations of eczema with age. [68] In juvenile eczema (until 13 to 15 years), there is eczematous involvement in flexural locations, most commonly in the neck and the hands, along with dry skin. [68] Moreover, adult eczema also features prominent involvement of wrists, ankles, facial and neck locations. [68] An increased transepidermal water loss (TEWL) can be a sign of skin barrier failure. [70] Flares (exacerbations) frequently begin as a worsening of the itching without any obvious skin lesions, followed by erythema, papules, and infiltration. Exacerbation can occasionally reveal important provoking factors, such as underlying infections or allergies. [69] The main symptom is pruritus (itch), which manifests as excoriations (skin lesions) and prurigo sores (itching papules). [68] Eczema is a chronic relapsing skin inflammatory disease in which constant scratching and rubbing can lead to lichenification (skin thickening). [69]

2.2.4. Epidemiological definitions and criteria

Several epidemiological criteria for eczema have been proposed, and there remains a lack of consensus mainly due to clinical, racial and age variations. Epidemiological research requires standardised definitions, and the majority of existing epidemiological criteria for eczema are based on skin signs of atopy. [71] One commonly used criteria is Hanifin and Rajka's criteria which involves having at least three of the following criteria: the presence of pruritus, the characteristic morphology and distribution, personal or family history of other allergic manifestations, chronic or persistently relapsing course, plus three minor criteria from a list of 21. [72] Another definition is from the UK working party and includes three of the following criteria in addition to itchy skin that must have been present within the past year: disease onset before the age of two, history of skin fold involvement, dry skin in general, other atopic diseases, and evident flexural eczema. [73] Similarly, the International Study of Asthma and Allergies in Childhood (ISAAC) definition of eczema includes itchy rash in the past 12 months, itchy rash coming and going for at least six months, and itchy rash in flexural locations (the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes). [74]

However, it has been suggested that the criteria of flexural pattern and early childhood onset may pose problems for diagnosis of eczema in adults as neither criteria may be present. [58] For those reasons, the Japanese diagnostic standards don't take the age of onset into account at all,

while the American Association of Dermatology diagnostic guidelines no longer consider early onset to be a necessary characteristic of eczema. [75,76] Additionally, both sets of recommendations distinguish between the age groups for the distribution of eczema, with the flexural form being more characteristic in children. [75] The Japanese diagnostic standards for a conclusive eczema diagnosis do not require personal or family history of atopy or IgE sensitisation. [75] Chinese researchers have developed a 3-item diagnostic criterion specific to adolescents and adults. [77] These include symmetrical eczema lasting more than six months (required), as well as a personal or family history of atopic disease (spanning up to three generations) and/or high serum levels of total IgE, allergen-specific IgE, and/or eosinophilia. [77] Due to its lack of specificity, pruritus is not considered of discerning value by these guidelines. [77] Additionally, due to limitations in clinical objectivity, xerosis (dry skin) is not included by them. [77] Several sets of diagnostic criteria have been developed; however, greater efforts need to be implemented to standardise criteria. Updates will also be required to account for new understandings of disease variations. For example, eczema is often more extensively extensor (rather than of flexural limbs), papular, follicular, lichenified, and pigmented in skin of colour.

Summary: Eczema definitions

- Eczema has a heterogenous presentation with different levels of severity, treatment response, age of onset and tendency to precede other atopic diseases.
- According to the WAO and others, eczema is synonymous with atopic dermatitis and atopic eczema.
- Although genetic factors are involved in the aetiology of eczema, there are no absolute genetic markers for eczema.
- Likewise, there are no diagnostic serological or histopathological markers for eczema. Eczema is diagnosed clinically by its symptoms and signs.
- There are variations in localisations of eczema with age.
- In chronic stage eczema, lichenification (skin thickening) is more prominent.
- The majority of epidemiological criteria are based on skin signs of atopy.

2.3. Eczema initiation and exacerbation

Eczema initiation and exacerbation is believed to be determined by a complex interplay of genetics and environmental factors.[3,4] More details are provided in the sections below.

2.3.1. Pathogenesis

Eczema is hypothesised to be caused by a variety of molecular and cellular pathways. The skin is the largest organ in the human body and one of its primary functions is to form a barrier to protect the body from outside harm, for example, toxic substances and microbes. [78] The epidermis, which is the outermost part of the skin, is divided into 4 main layers or stratums (Figure 2.2.): the stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS) and stratum basale (SB). The SC is the most external layer that is in contact with the outside world, and it is primarily composed of denucleated corneocytes that are bound to intercellular lipids analogous to brick and mortar. [79] Also, there are intercellular junctions that regulate the paracellular movement of water and solutes called tight junctions (TJs). [80] An abnormality of SC and TJs causes the skin barrier disruption in eczema.

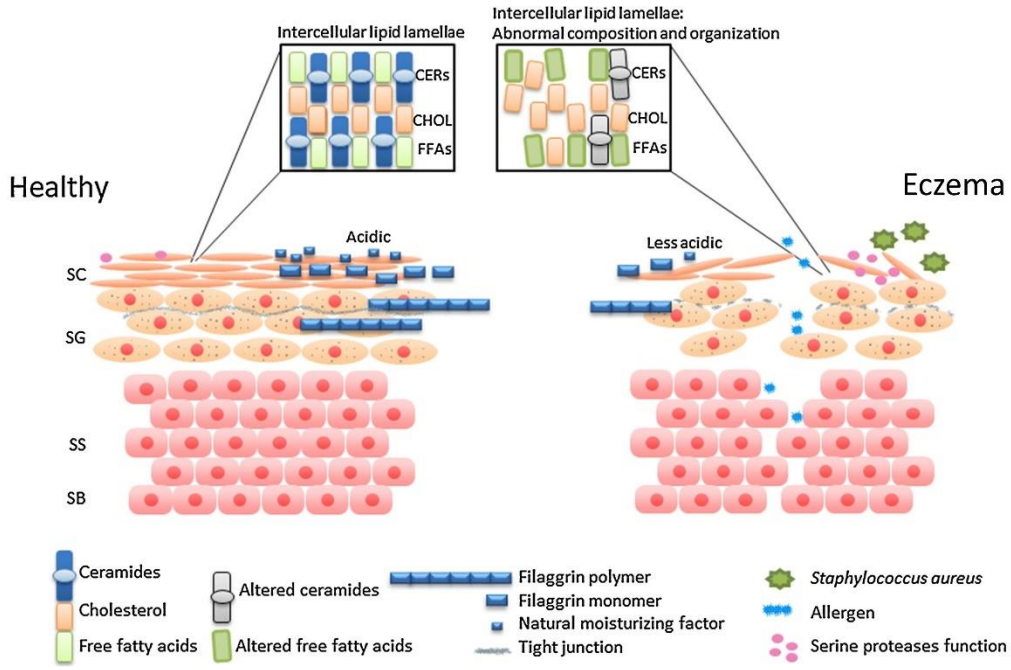
The mechanism of action of filaggrin *(FLG)* is critical for SC homoeostasis. *FLG* is an acronym for "filament-aggregating protein," which refers to the protein that binds to keratin intermediate filaments and is an important component of the skin's structural integrity. [81] *FLG* is produced and stored in keratohyalin granules as profilaggrin polymers, these are broken into *FLG* monomers where the SG-SC contact. [82] To strengthen the SC, these monomers join with keratin intermediate filaments. Finally, *FLG* is broken down into amino acids, urocanic acid, and pyrrolidine carboxylic acid (PCA) in the upper SC (Figure 2.2.). [83] PCA supplies natural moisturising components to the skin, while UCA is responsible for the skin's acid mantle. [83] *FLG* is needed for epidermis cornification and organisation as well as limiting transepidermal water loss and preserving SC hydration. [84] *FLG* levels are known to be lower in the epidermis of many eczema patients, and a null mutation in *FLG* is considered as one of the most important risk factor for the disease although this is not uniform across ethnicities and ages. [85] However, there is evidence that filaggrin mRNA and protein levels can be decreased in eczema affected skin, even in those with *FLG* wild type genotypes. [86] Hence, other elements like the cutaneous cytokine milieu or environmental impacts like low humidity or mechanical trauma have the ability to indirectly modify the expression of filaggrin. [87]

There are other SC components that are related to eczema development. [88] A variety of barrier-insufficient skin illnesses are caused by abnormalities in the enzymes that process intercellular lipids. [88] Furthermore, corneocyte shedding is strictly controlled by serine proteases and inhibitors of serine proteases. [88] The lymphoepithelial Kazal-type 5 serine protease inhibitor (*LEKTI*), which is encoded by the serine protease inhibitor Kazal-type 5 (*SPINK5*), is a serine protease inhibitor. [88] Mutations and genetic variations in these genes cause disorders with eczema-like symptoms. [89] Moreover, due to barrier disfunctions there are interactions between innate lymphoid cells (ILC)2, basophils, eosinophils, and mast cells. Epithelial-derived cytokines are produced and released after the skin barrier is disrupted, these cytokines activate ILC2. [88] Interleukin (IL)-4

produced by basophils can be detected near ILC2 in eczema skin lesions and these can directly activate ILC2 to produce T helper type 2 (Th_2) cytokines. [88] Prostaglandin D2, which is derived from mast cell degranulation, aids in the recruitment of ILC2 to the skin and the stimulation of Th2 cytokine production. [88] Cell adhesion molecules on keratinocytes (such as E-cadherin) are known to inhibit ILC2, however, in *FLG*-deficient individuals there is a loss of E-cadherin. [88]

There is evidence of a reduction in the length of ceramides and lipids in eczema and as a result a decrease in the skin barrier function. [90] Increase in inflammation and a decrease in its inhibition further fuels skin inflammation and barrier dysfunction. [88] During flares, atopic skin is characterised by loss of microbial diversity and increased S. aureus colonisation. [91]

Figure 2.2. Diagram of the epidermis in healthy and eczema individuals. (Source: P. Rerknimitr, et. al.; 2017) [88]



SC: Stratum corneum, SG: Stratum granulosum, SS: Stratum spinosum, SB: Stratum basale

Scratching in response to the itch of eczema further aggravates skin barrier dysfunction. [92] Immune responses can cause itching by secreting a variety of cytokines including IL-4, IL-13, and IL-5, which act as pruritogens. Scratching encourages the production of Th2 chemokines, eosinophil-recruiting chemokines, and thymic stromal lymphopoietin and may lead to a vicious itch-scratch cycle. [93] Furthermore, dry skin causes irritation that increases the density of epidermal nerve fibres and increases pruritus. [94]

Although a null mutation in the *FLG* gene is the strongest genetic risk factor for eczema, 60% of people who possess this null mutation do not have symptoms. [85] It is clear that other elements

are required for the disease to manifest, and the exact pathogenesis remains unknown. The origin of eczema is most probably due to an interplay between skin inflammation due to the skin barrier dysfunction, the innate and adaptive immunology, and the effect of pruritus.

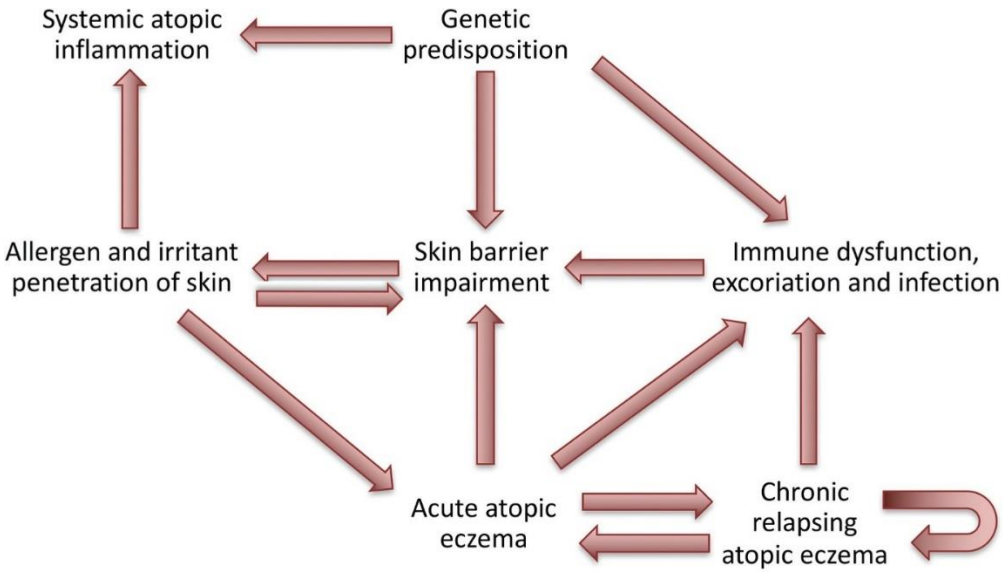
Summary: Pathogenesis

- Eczema is hypothesised to be caused by a variety of molecular and cellular pathways.
- An abnormality of the stratum corneum and tight junctions causes the skin barrier disruption in eczema.
- The metabolic process of *FLG* is critical for stratum corneum homoeostasis. A null mutation in *FLG* is considered as one of the most important risk factors for eczema in western societies.
- Due to barrier dysfunction, there are interactions between innate lymphoid cell (ILC)2, basophils, eosinophils, and mast cells.
- Scratching to relieve the itch associated with eczema can further aggravate the skin and impair the skin barrier function
- There is interplay between skin inflammation due to the skin barrier dysfunction, the innate and adaptive immunology, secondary bacterial infection, and the effect of pruritus in the development of eczema.

2.3.2. Pathophysiology

The pathophysiology of eczema is not fully understood. Eczema is a predominantly systemic T-helper (Th) 2-driven illness associated with increased atopic comorbidities, especially in individuals with moderate-to-severe disease. [95] Multiple studies have shown that similar factors that are responsible for its aetiology, epidermal barrier disruption, and immunological dysregulation play a role in the disease's progression (Figure 2.3.) . [96] Epidermal gene mutations, skin barrier dysfunction, immune dysregulation, neuroinflammation, altered lipid composition, and microbial imbalance, may all contribute to the progression of eczema. [96] Skin barrier defects are the most important pathologic findings in eczematous skin. [97]

Figure 2.3. Simplified representation of associated mechanisms in eczema pathophysiology. (Source: S. Brown, et. al.; 2017) [98]



The risks of severe eczema, prolonged disease, and skin infections are associated with *FLG* mutations, particularly homozygous mutations. [99] Moreover, alterations in the Th type 2 signalling pathway can be generated by polymorphisms of several immune pathway genes and lead to eczema progression. [100] Interleukin (IL) 4 and IL-13 upregulation decreases *FLG* expression and so compromises the skin barrier. [101] Furthermore, IL-31, IL-33, STAT-6, TSLP and its receptors (IL-7R and TSLPR), interferon regulatory factor 2, Toll-like receptor 2, and high-affinity IgE receptor (FcRI) gene are other immune-related genes that influence the development of eczema in some populations. [96] Additionally, the vitamin D receptor may be linked to eczema. [102] Of note, a growing body of research shows that environmental exposures cause eczema by altering DNA in keratinocytes and regulating posttranscription through the action of microRNAs. [100]

Histamine activates a subgroup of sensory neurones that have histamine H1 and H4 receptors, the activation of which may contribute to itching and allergic inflammation. [88] As mentioned in the previous section, scratching can lead to a vicious itch-scratch cycle. [93] In patients with eczema, IL-31 promotes elongation and branching of sensory nerves and likely to play a significant role in itch and skin sensitivity of eczema. [103] Additionally, it has been suggested that the production of lipocalin-2 by the activation of STAT3 in the astrocytes of the spinal dorsal horn contributes to chronic pruritus. [104]

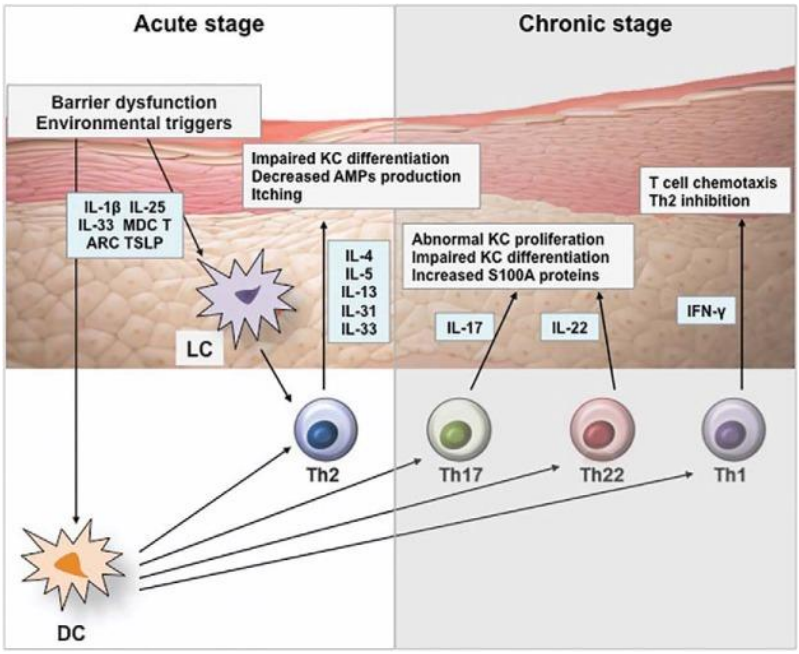
The lipid matrix, which is arranged in double bilayers situated between corneocytes, is made up of lipids including ceramides, long-chain FFAs, and cholesterol. [105] Enzymic processing of precursors produce the major lipid classes found in the skin, necessary to maintain the integrity of the epidermal barrier. [106] There is an altered lipid composition in skin affected by eczema. [106]

Regarding skin microbiome, there is decreased skin bacterial diversity associated with increased Staphylococcus, Corynebacterium, and with reduced Streptococcus, Acinetobacter, Corynebacterium, and Propionibacterium species during eczema flares. [107] On the other hand, there is greater skin bacterial diversity with increased abundance of Staphylococcus epidermidis and Streptococcus, Corynebacterium, and Propionibacterium species observed after eczema treatment and reduced eczema. [107] According to species-level research on eczema, patients with more severe disease had a higher preponderance of S. aureus, while those with milder disease had a greater abundance of S. epidermidis. [108] An unbalance in the gut microbiome with higher numbers of Escherichia coli, Clostridium difficile, Bifidobacterium and Staphylococcus has also been associated with eczema. [109,110] These findings are consistent with the theory that a particular microbial composition in the gut may inhibit Th2-shifted immunity and boosted regulatory immunity, regulatory dendritic cells and Treg cells, and this may be altered in eczema pathogenesis. [111]

Previous research has demonstrated the critical functions that type 2 immune cytokines, such as IL-4 and IL-13, play in the generation of chemokines, the breakdown of the epidermal barrier, the suppression of antimicrobial peptides, and allergic inflammation. [112] However, the pathophysiology of eczema is not solely attributed to Th2 immunity; even if type 2-driven inflammation is blocked, IL-17 can decrease *FLG* and involucrin expression. [113] Moreover, the beginning of Th1-cell activation as well as the continuous activation of Th2 and Th22 cells signal the transition to the chronic phase. [114] Keratinocytes are stimulated to release IL-1, IL-25, IL-33, MDC, TARC, and TSLP as a result of disrupted epidermal barrier and environmental triggers which activate dendritic cells and Langerhans cells. [96] Consequently, Th2 cells are stimulated by activated dendritic cells to release IL-4, IL-5, IL-13, IL-31, and IL-33, which impair keratinocyte differentiation, decrease AMP synthesis, and cause itch (Acute stage, Figure 2.4.). [96] Th1, Th22, and Th17 subsets are recruited during chronic eczema to cause aberrant keratinocyte proliferation and epidermal thickness (Chronic stage, Figure 2.4.). [96]

Eczema progression is determined by dysregulated immune responses to external antigens, which are made possible by defects in the epidermal barrier proteins like *FLG*, transglutaminases, keratins, and intercellular proteins. [115] This results in increased skin permeability and systemic inflammatory reactions. [115] Moreover, a compromised epidermal barrier increases allergen sensitivity which, besides leading to eczema, can increase the risk of food allergy, sensitisation of the airways and progression to other allergic diseases. [55]

Figure 2.4. Effects of cytokines on the epidermis of patients with eczema. (Source: K. Jihyun, et. al.; 2019) [96]



AD = atopic dermatitis (eczema); AMP = antimicrobial peptide; DC = dendric cell; IFN = interferon; IL = interleukin; KC = keratinocyte; LC = Langerhans cell; MDC = macrophage-derived chemokine; S100A = S100 calcium-binding protein A; Th = T-helper type; TARC = thymus and activation-regulated chemokine; TSLP = thymic stromal lymphopoietin.

Summary: Pathophysiology

- Eczema is a systemic T-helper (Th) 2-driven illness in which atopic comorbidities are more prevalent.
- Multiple factors contribute to eczema flares, with similarities to those responsible for initial eczema pathogenesis.
- Skin barrier defects are the most important early pathologic findings in eczematous skin.
- The risk of severe eczema, longer duration, and skin infections is associated with *FLG* mutations.
- Type 2 immune cytokines play a crucial role in the generation of chemokines, the breakdown of the epidermal barrier, the suppression of antimicrobial peptides, and allergic inflammation.
- Eczema progression is determined by dysregulated immune responses to external antigens due to barrier dysfunction, alterations in cell mediated immune responses, genetics, lipids, and microbiome.

2.4. Eczema clinical presentation

2.4.1. Eczema sign and symptoms

The most common acute symptoms/signs of eczema are skin redness, swelling, scaling, itch/burning and excoriations; vesicles (tiny blisters), exudation, and crusts indicate greater severity. [116] Chronic eczema changes include xerosis (dry skin), dyspigmentation, and lichenification. [117] Eczema is usually cyclical, it comes (flares-up) and goes (remission). Flare-ups may begin due to exposure to allergens or irritants, characterised by intense itching and signs of acute eczema. [116] The basic clinical presentation of eczema is similar to other types of dermatitis like contact dermatitis. [118] Typically, eczematous lesions can be classified in three types: acute, sub-acute and chronic: [119]

- Acute lesions typically include an itchy, erythematous (red) background with variable vesiculation, papulation, exudation, and crusting. Itchy results in scratching, excoriations (scratch marks) and exudation (serous secretions). Secondary infection (impetiginization is not uncommon).
- On anatomical locations where acute exacerbations are common, sub-acute lesions usually develop, usually as thicker, paler, dry, scaly, erythematous, and excoriated plaques. This is the next stage in the disease evolution.
- Chronicity results in lichenified (thickened) skin of chronic lesions following extensive tissue remodelling. The lichenified skin has thickened plaques, prominent skin lines, and dry, fibrotic papules. Fine scaling of the afflicted skin is a sign of xerosis (dry skin). [120] Patients with eczema have dry, scaly skin because the skin has undergone tissue remodelling with reduced keratinocyte water binding, on top of constitutional barrier defects. [120]

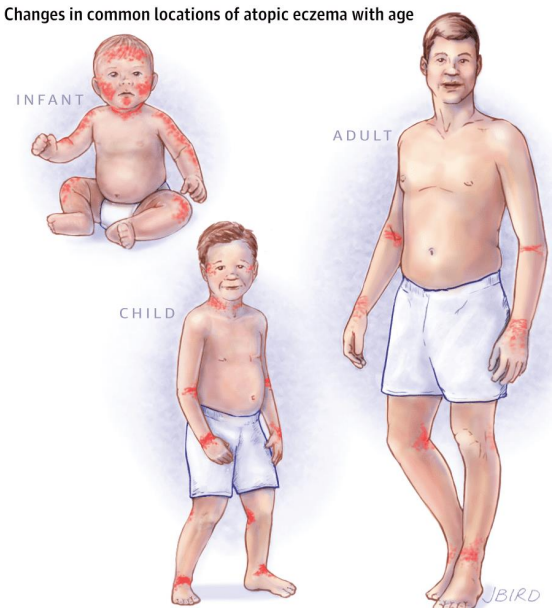
At any given time, patients may have all three types of lesions. Other clinical characteristics that may be present in eczema are those related to the “atopic syndrome”. [118] This includes facial distortions, a long, pale, atopic face, peri-orbital darkening beneath the eyes, a line or secondary crease that is accentuated below the margin of the lower eyelid, also known as the Denny Morgan fold, and other secondary mannerisms like horizontal nose rubbing (also known as the "side swipe" or the "allergic salute") and repeated sneezing. [118]

2.4.2. Variation with age

Depending on the patient's age, the clinical appearance of eczema varies significantly (Figure 2.5.). [121] Regarding age of eczema, at least 4 different types of clinical presentations have been described as infantile, childhood, adolescent/adulthood and elderly. [122] Except for some eczema in the very early stages of life, pruritus is usually the most characteristic symptom in all stages. [122]

- In infantile eczema (3 months to 2 years of age), the initial lesions usually appear on the cheeks from the second month of life and are characterised by oedematous (swollen) papules and papulovesicles. There is a potential for large plaques with leaking and crusting to form. Scalp eczema can appear as severely itchy scaly rough erythema on the scalp and mistaken for simple cradle cap or seborrheic dermatitis, both of which present earlier and lack itch. The diaper region is usually spared, whereas the scalp, neck, and extensor portions of the extremities, as well as the trunk, might all be affected. Eczema may be difficult to diagnose in its earliest stages before the more classic distribution and morphologies become apparent. [122] Other rashes of infancy including seborrheic dermatitis, irritant contact dermatitis (which may coexist), psoriasis, scabies, infective exanthems, and nutritional deficiencies may be confused with eczema. [122]
- In childhood eczema (2 to 12 years), acute lesions are often visible although chronic lesions with lichenification may start to appear. The popliteal and antecubital fossa sites (flexural eczema) and periorificial (near orifices) regions of the head become the predilection sites for eczema. The hands and wrists often display nummular plaques with oozing and crusting. Xerosis, or dry skin, becomes increasingly prevalent. [122] Secondary infection and airborne contact dermatitis are not uncommon.
- In adolescent/adult eczema (12 to 60 years), during this stage of life, lesions are more firmly established in traditional locations including the head, neck, and flexural areas. In addition, the condition can also affect the hands in adulthood (chronic hand dermatitis). The periorbital (near the eyes) regions are frequently affected by the disease in female patients. Eczema is more likely to be widespread and occasionally erythrodermic (erythema exceeding 90% body surface area) in patients who have a long-standing natural history of the disease. [122] Infection and secondry aeroallergic contact dermatitis may occur.
- Eczema in the elderly (more than 60 years) appears to be clinically underappreciated. Large eczematous itchy patches and plaques are common often with widespread xerosis. Sometimes the flexural areas are spared by the lesions. To establish precise clinical standards for its conclusive identification, this unique phenotypic unquestionably requires further study. A number of differential illnesses that could mimic eczema in the elderly should be ruled out, including allergic contact dermatitis and cutaneous T-cell lymphoma. [122]

Figure 2.5. Changes in anatomical location of Eczema by age groups. (Source: S. Sugerman, et. al.; 2014) [116]



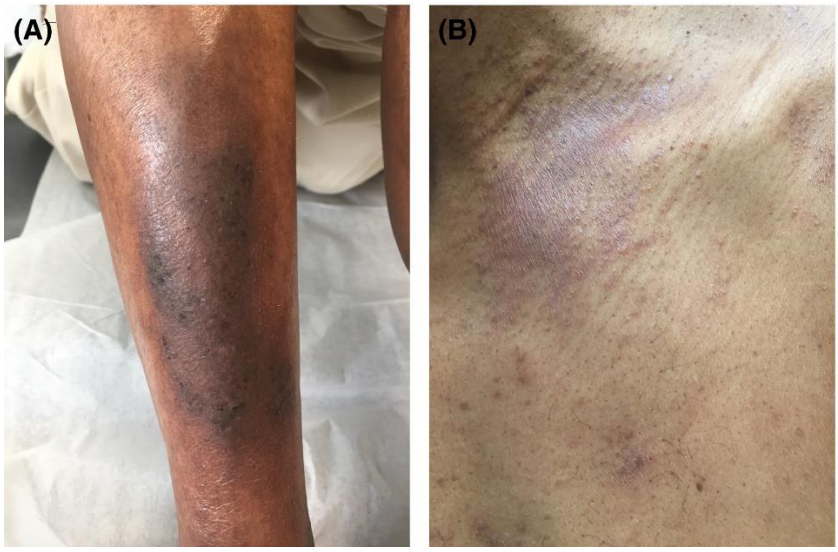
2.4.3. Variation by race

The presentation of eczema is believed to be consistent across race groupings. However, there are important distinctions in eczema visual appearance and distribution, which are underreported in the literature, associated with variations in skin pigmentation (Figure 2.6.). [123] When compared to Caucasians, Far East and South Asian people tend to have increased lesional demarcation, scaling, and lichenification. [124] Moreover, African heritage patients are less likely to develop flexural dermatitis and are more likely to present with extensor involvement. [125] Skin of colour is more likely to have perifollicular accentuation and scattered distinct papules on the extensor limbs and trunk, which can affect up to 54.1% of patients (Figures 2.6.). [125] Only those with dark skin have been observed to present with eczema in a lichen planus-like manner, which typically manifests on extensor surfaces, doesn't have lichenification, and heals more quickly. [126]

The presentation of eczema varies depending on the skin tone, even in patients who have lesions with a typical distribution and form. Darker skin makes erythema more likely to seem a darker tone of red or violet colour, it could also go unnoticed (Figure 2.7.). [123] Patients and healthcare professionals may be better able to detect underlying erythema if there is oedema, skin warmth, or scaling. [123] However, the severity of eczema in people with darker skin types is significantly underestimated when typical grading methods like SCORAD, SASSAD, NESS, and EASI are used. [125] Black children are 6 times more likely than White children to have severe eczema when erythema score has been taken into account. [123]

Figure 2.6. Clinical presentation by race. (Source: G. Giampiero, et. al.; 2021) [117]

Figure 2.7. Variation in eczema hue due to patient skin pigmentation. (Source: K. Bridget, et. al.; 2018) [123]



Diffuse xerosis, Dennie-Morgan lines, and palmar hyperlinearity are additional classic findings that are more frequently observed in skin types with darker pigmentation. [127] Additionally, Black individuals had higher rates of lichenification, prurigo nodularis, and periorbital dark circles than White patients. [125] This might be because Black people with eczema experience more pruritus, which causes them to rub and itch more frequently. [128]

Additionally, patients with darker skin types are more likely to experience post-inflammatory dyspigmentation. [129] Due to the contrast with normal skin, hypopigmented skin is frequently more obvious in those with darker complexion. [129] The ensuing dyspigmentation is frequently more upsetting than the skin symptoms of eczema. [123] Thankfully, changes to skin pigmentation may go back to normal after weeks to months. [123] However, in patients with darker skin, chronic excoriation linked to severe eczema may lead to irreversible depigmentation. [123]

Summary: Eczema clinical presentation

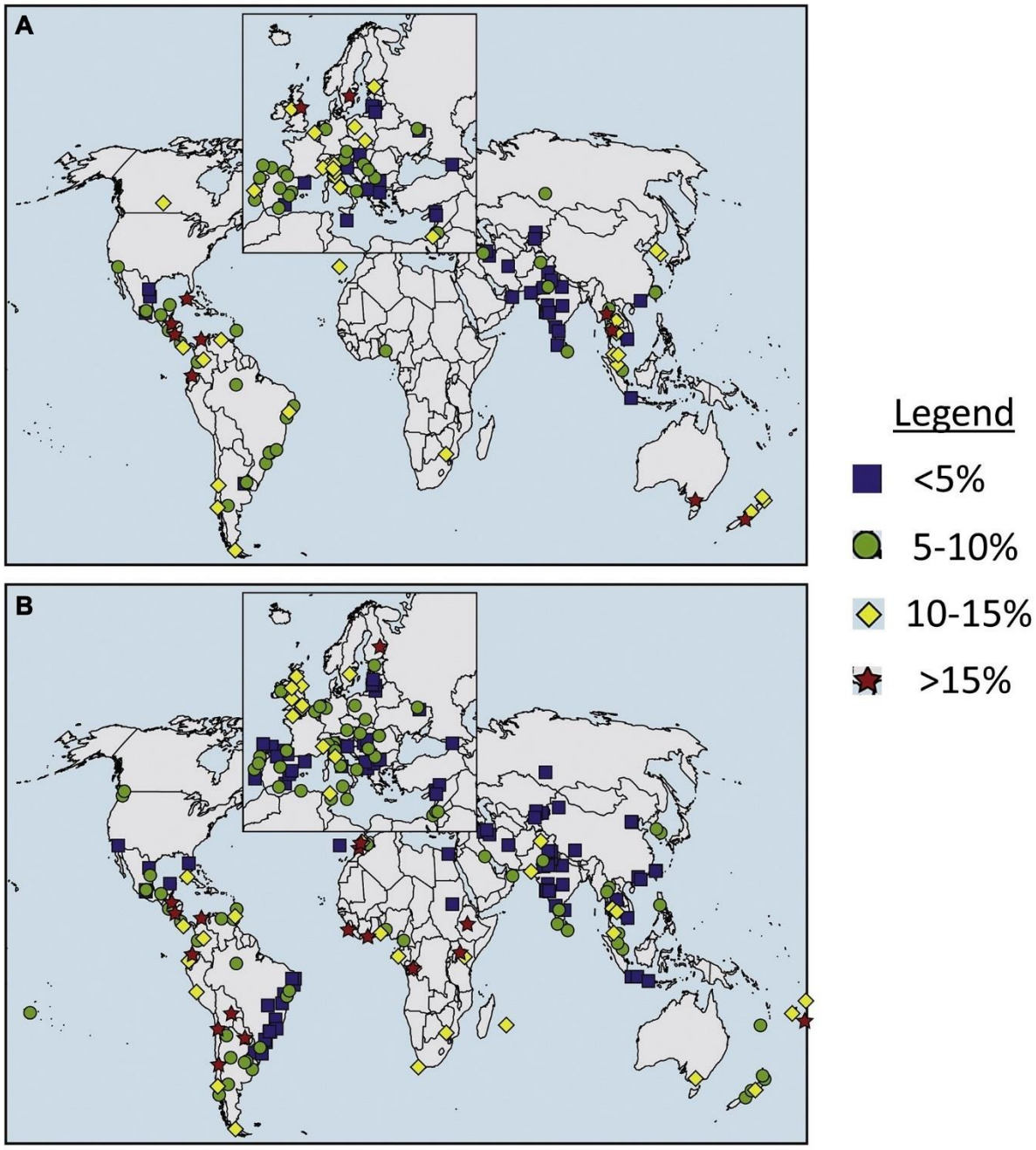
- The most common acute symptom is a very itchy rash with skin redness, oedema, and roughness.
- Eczema is usually cyclical; it comes and goes (flare-ups and remissions).
- Typically, eczema lesions can be classified in three types: acute, sub-acute and chronic.
- The clinical appearance of eczema varies significantly with age.
- The presentation of eczema may vary with ethnicity and constitutional skin colour.

2.5. Eczema epidemiology

2.5.1. Global epidemiology

In recent years, reports on the incidence and prevalence of eczema have primarily included general population surveys, children, or adult-specific investigations. [130] In the literature, there is one study that has taken a truly global approach, the International Study of Asthma and Allergies in Childhood (ISAAC) which involved close to a 100 develop and developing countries.[74] ISAAC Phase One (1992-1996, 56 countries) determined that the global population's 12-month period prevalence of eczema symptoms ranged from 0.6 percent to 20.5 percent, [131] and in Phase Three (2000-2003, 98 countries) it ranged from 0.9% to 22.5% for 6 to 7 years and 0.2% to 24.6% for 13 to 14 years (Figure 2.8.). [2] ISAAC suggested that the prevalence of eczema was increasing worldwide. Other reports suggest it affects 5 to 20% of children [132] and 2 to 10% of adults [1,133] internationally. However, ISAAC did not include estimates of younger children and toddlers where eczema prevalence is higher. [5] A systematic review reported that the prevalence of eczema in infants was between 6 to 31%. [134] Regarding incidence, a systematic review of epidemiological studies in children reported that the 1-year incidence varied from 10.2 in Italy to 95.6 in Scotland per 1,000 person-years in children. [135] Notably, I found no incidence studies on adults in the 21st century. [135] The epidemiological estimates of eczema have a considerable variation according to sociodemographic factors such as age, sex, ethnicity and geographical region.

Figure 2.8. World map showing the prevalence of eczema in ISAAC Phase Three. (Source: J. Odhiambo, et. al.; 2009) [2]



World maps for the age groups of 6 to 7 years (A) and 13 to 14 years (B) which demonstrate the prevalence of current eczema symptoms. A centre is represented by each symbol. Blue squares represent a prevalence of less than 5%, green circles represent a prevalence of 5% to less than 10%, yellow diamonds represent a prevalence of 10% to less than 15%, and red stars represent a prevalence of 15% or more, Europe is depicted in more detail.

2.5.2. Epidemiological variations by age

Eczema is extremely common in children and is frequently thought of as primarily a paediatric disease by clinicians. [136] A common trend in the prevalence of eczema by age is that

children have increased prevalence when comparing to adolescents and adults. As a general rule, the prevalence is lower among older adult age-groups. [1] In the vast majority of cases, eczema manifests early in life, with 50% of cases starting in the first year and 85% starting by age 5 years. [137]

Previously, it was believed that almost all eczema resolved before reaching adulthood and that eczema was primarily a paediatric disease. However, it has been demonstrated that the prevalence of eczema does not decline as drastically as was once thought. [136] Around 3 out of 4 patients with eczema that started in childhood experience a spontaneous remission before adolescence. [138] As a result, 25% of children who are diagnosed with eczema still have symptoms as adults, either continuously or as a result of a re-occurence of symptoms following a period of symptom-free years. [138] Severe or longstanding childhood eczema is more likely to persist into adulthood. [139] Furthermore, it has been reported that adult-onset eczema can occur, with approximately 1 in 4 adults with eczema reporting adult-onset disease. [140] However, it is difficult to determine the percentage of adult-onset eczema as patients with adult recurrence of eczema may have forgotten their childhood eczema.

2.5.3. Epidemiological variations by Race

Recent literature has made clear that the patient's racial background may account for differences in the prevalence of eczema and its clinical presentation. [141] It is commonly known that black children are more likely than white children to receive an eczema diagnosis in the United States and the United Kingdom. [142] Furthermore, compared to patients from European American backgrounds, African Americans are more likely to experience extensor involvement and less frequently experience flexural involvement. [123] Moreover, darker skin types are also more likely to have hyperlinearity of the palms, diffuse xerosis, and Dennie-Morgan lines. [125] Lichenification and prurigo nodularis are more frequently observed in black individuals than in white people, possibly as a result of more intense itching and rubbing. [123]

The severity of eczema in people with darker skin types might be significantly underestimated when using typical measures that depend on erythema (redness of the skin), such as the EASI, SCORAD, and others. [123] Clinically, eczema has been reported as being different in East Asian populations (greater skin discoloration and frequent parakeratosis (immature keratinocytes) than in European American ones. [124] This clinical distinction is mirrored by biomarker findings which show higher levels of cytokines and chemokines associated with psoriasis in East Asian populations [124] Additionally, compared to white children living in comparable environmental conditions, Chinese infants in Australia have a considerably greater incidence of eczema. [143] Likewise, there may be difference in eczema clinical presentation by race for example between African, Chinese or Japanese

people. [142] These phenotypical variations are not usually taken into account by epidemiological criteria and other diagnostic parameters, which could lead to patients of different racial heritage being misclassified. [142] For that reason, the use of particular standards has been suggested for Japan and China. [142]

2.5.4. Epidemiological variations by Country

Wide variation of eczema epidemiological estimates was reported by geographical region as in the ISAAC Phase three study (2000-2003, 98 countries) (Figure 2.8.). According to a comparison of prevalence estimates from Phases one and three of the ISAAC project, eczema prevalence is rising among children aged 6 to 7 worldwide, both in developing and developed countries, as well as among children aged 13 to 14 in developing countries. [144] It was reported that the average eczema prevalence in African schoolchildren (4 to 20 years) (Ghana, Gabon, Rwanda, Tunisia, Namibia and Senegal) was around 3.85%. [130] In the USA, the prevalence in 652 infants (0 to 5 years) was 24% [145], in 4,898 children was 14.8% (5 to 9 years) [146] and in 1,278 adults (more than 18 years) was 7.3% [147]. A study from Canada in 8,334 schoolchildren (13 to 14 year-olds) reported a 10.4% eczema prevalence. [148] In Latin America, 93,851 children aged 6-7 years had an eczema prevalence of 11.3%, and 165,917 adolescents aged 13–14 years had a lower prevalence of 10.6%. [149] In Asia, eczema prevalence in children was reported in South Korea (15%) [150], Malaysia (13.4%) [151], Taiwan (10.7%) [152], China (12.94%) [153] and Japan (15.3%) [154]. In South Korea, the prevalence of eczema in 47,351 adult prevalence was estimated to be 3.1%. [150]

In Europe, UK children aged 17 and younger had a reported prevalence of 12.3%, adults aged 18 to 74 had a reported prevalence of 5.1%, and people over 75 had a reported prevalence of 8.7%. [155] In Denmark the incidence rate of eczema in infants was 15% and in Sweden was 10% in 2006. [156] In Poland, the eczema prevalence in children was 6.5%, in adolescents was 9% and in adults was 3.62%. [157] In Hungary, the eczema prevalence for people under 19 years was 17.5%. [158] Regarding Australia, a cross-sectional cohort study involving 4972 infants (1 year old) that was conducted from 2008-2011 in Melbourne determined that the eczema prevalence was 20.3%. [159] A recent study evaluated the eczema 12-month prevalence in four Australian cohort studies were analysed: Melbourne Atopic Cohort Study (MACS), HealthNuts, Tasmanian Longitudinal Health Study (TAHS), and the Australian arm of the European Community Respiratory Health Survey (ECRHS). [160] It determined that in 6 years old children, the eczema prevalence was 16.7% to 26.6%; in 6 to 12 years old children, it was 14.6% to 24.7%; at 18 years old, it was 15.1%; and at 53 years, it was 8.8%. [160] Finally, in New Zealand the eczema prevalence in children was 15% and adolescents was 8.8%. [161] As

such, there is a wide variation of eczema prevalence by geographical area and country which may be due to different eczema assessment methods and different ethnicities.

2.5.5. Epidemiological variations by Sex

A cohort study from the UK that evaluated 55,525 infants in 2018 showed that boys younger than one year of age had a higher incidence rate in comparison to girls of the same age. [162] However, boys of 5 years or older had a lower incidence rate when compared to girls of the same age group. [162] Moreover, boys are more likely than girls to develop eczema throughout childhood. [163] However, from adolescence, females become more likely to develop eczema than males. [142] After puberty, the prevalence of eczema in females increases and becomes equivalent or superior to that of males, it has been suggested that the menstrual cycle and sexual hormones influence IgE levels. [164] Similar observations were reported in a recent study that assessed four Australian cohort studies. [160] Furthermore, other allergic conditions like asthma show an increase in prevalence in girls and women starting around puberty. [165]

The literature suggest that estrogen and progesterone (female sex hormones) suppress Th1 and Th17 activities while mostly increasing the activities of regulatory T cells (Tregs) and Th2 cells, which is beneficial for the acceptance of allogeneic foetuses during pregnancy. [166] The increase of female sex hormones during menstrual cycles may make Th2 inflammation worse in eczema sufferers. [167] It has been observed that eczema worsens when estrogen and progesterone are both released during the luteal phase, which occurs just before menstruation. [168] Most androgens, including testosterone and dihydrotestosterone (DHT), decrease Th1, Th2, and Th17 activities while inducing Treg activity. [169] Moreover, dehydroepiandrosterone, a male-predominant steroid hormone, seems to have an inhibitory influence on the emergence of atopy and allergies. [142] In general, Th1 activities are more prevalent in males than in women in adolescents and adults, while Th2 activities are significantly more prevalent in women than in men. [169] In the same manner, males and females may have different behaviours that influence exposure to allergens, access to healthcare, or health-seeking activities that may affect the course of the eczema. [170]

2.5.6. Challenges and limitations in the study of eczema epidemiology

Studying the epidemiology of eczema presents a number of important difficulties. [142] First, as mentioned above, there are no objective diagnostic tests or acknowledged biomarkers for eczema which makes the objective diagnosis of eczema impossible. Moreover, defining eczema based on a community doctor diagnosis relies on patients with active symptoms to visit a doctor, which cause underestimation of eczema burden due to the often remitting/relapsing nature of eczema. Additionally, it is challenging to create consistent and reliable questionnaires for epidemiologic

research due to the absence of defined nomenclature for eczema worldwide, as there are synonyms such as atopic dermatitis, atopic eczema, and Atopic eczema/dermatitis syndrome (AEDS). In addition, there is a great deal of variation in eczema clinical presentation and distribution (e.g., flexural, extensor, head and neck areas, and generalised), morphology (e.g., oozing, scaling, lichenification, prurigo nodules, ill-demarcated, and psoriasiform), intensity, and time course (e.g., intermittent, chronic persistent disease, seasonal variation, and episodic flares). A variety of methods have been employed to evaluate the epidemiology of eczema, but none of them are uniformly reliable and as such variation in the report of eczema epidemiological estimates are to be expected.

Summary: Eczema epidemiology

- There are several challenges in the study of eczema epidemiology: lack of objective measures, problems with doctor diagnosed definitions, absence of defined nomenclature for eczema, heterogeneity of symptoms and severity
- Eczema affects around 5 to 20% of children and 2 to 10% of adults wordlwide.
- The epidemiological estimates of eczema prevalence and incidence have considerable variation according to sociodemographic factors such as age, sex, ethnicity and geographical region.
- Children have a higher prevalence of eczema than adolescents and adults and prevalence is frequently lower among older adult age-groups.
- Black children are more likely than white children to receive an eczema diagnosis in the United States and the United Kingdom.
- There may be differences in eczema clinical presentation by race for example African, Chinese or Japanese.
- There is a wide variation in eczema epidemiological estimates reported by geographical region.
- Boys are more likely than girls to develop eczema throughout childhood.
- From adolescence, women become more likely to develop eczema than men.

2.6. Eczema burden of disease

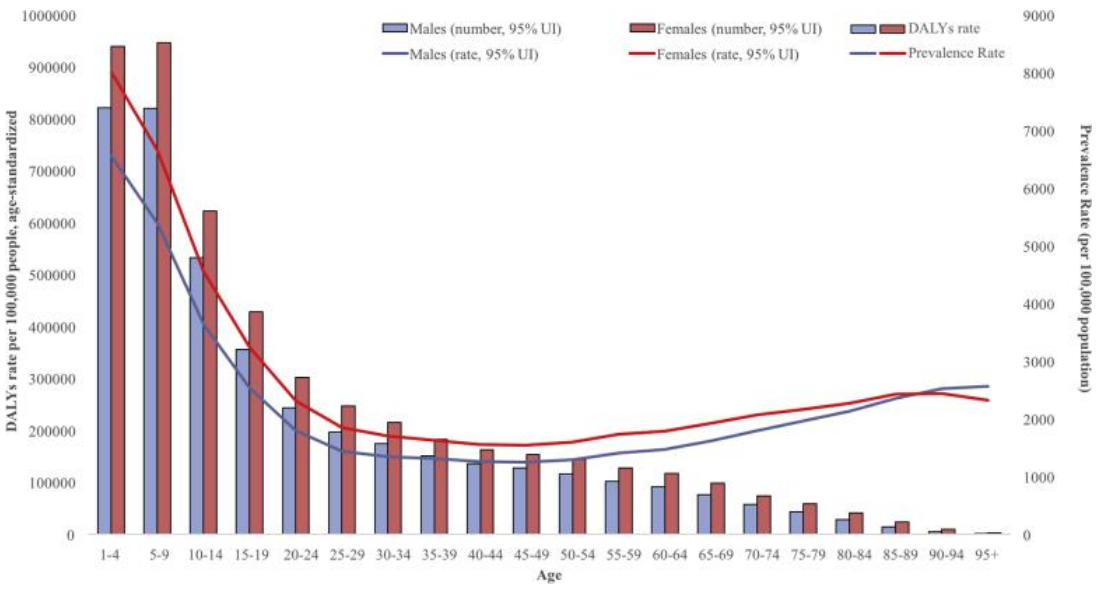
Eczema is recognised as a common disease that frequently manifests in infancy and can persist into adulthood. [171] As such, it can have a significant and prolonged burden of disease in people living with eczema.

2.6.1. Eczema disability-adjusted life years

For that reason, eczema is the leading contributor to skin-related disability-adjusted life years. [52]

Examining the methodology used to generate DALYs may help to explain this. DALY is the total number of years lost to premature death and years spent living with a disability (product of prevalent cases and disability weights). [172] Eczema has a high DALY rate because the peak eczema prevalence is during early life which increases the potential years spent living with a disability which is multiplied by increasing disability weights for mild, moderate, and severe eczema (Figure 2.9.). [52] As a result, eczema can negatively impact patients' and their families' lives at all stages of life. [10] This encompasses effects on quality of life (QoL) social, economic, and vocational. [10] Because of the high direct medical costs and lost productivity caused by eczema, patients, their families, and society as a whole are severely financially burdened. [10] The burden of eczema is comprised of all of these factors—QoL, social, economic, and vocational impact. [10]

Figure 2.9. Global 2017 eczema age standardised DALYs per 100,000 people in males and females. (Source: K. Urban, et. al.; 2021) [52]



The lines indicate prevalence rate, and the bars indicate DALYs rate.

2.6.2. Quality of Life and social impact

Qualitative data suggested that the impact in quality of life in children involved issues with physical effects (scratching and itching, sleeping, hurting, bleeding, and food restrictions), emotional effects (behaviour), social (both adults and kids may avoid socialising with children with eczema), clothing limitations, obstacles to bathing, playing (especially outside), swimming, and needing to hold kids' hands to prevent scratches. All of these factors reduced physical functioning. [173] Moreover, the high prevalence of eczema in younger age groups can place significant stress on a child during

crucial stages of psychosocial development. Eczema may cause embarrassment and lead tobullying, and there is evidence that people with skin conditions may become socially isolated, which can result in depression. [174] However, most studies have reported that the most significant impact came from itching and scratching which impacted sleep and socialisation. [175] [176] [177] As the symptoms of eczema worsened so did the impact on QoL. [178] These studies provide evidence of the substantial impact of eczema on the QoL of people living with eczema.

In adults the impacts on QoL are similar to those suffered by children with itching and scratching having the highest impact on QoL but there is also a large impact in their social interactions, clothing, shaving and wearing makeup choices. [179] Moreover, the anatomical location of eczema may also important. [180] Eczema located on visible areas of the body, the face, and genitals was related to the highest impact on QoL. [180] Furthermore, eczema with itching was linked to mental health issues like depression and suicidal ideation in adults. [181] Adults with eczema and fatigue were found to be substantially more likely than individuals without eczema or fatigue to rank their overall health as poor or fair. [182] This evidence shows that although eczema is not generally a life shortening condition it can have a significant impact on the patient's life.

Finally, the QoL impact of eczema is not restricted to those with eczema. In one study, 60% of the parents of children with eczema also reported emotional strain and sleep disturbance. [183] Similarly, a study that evaluated the mental health of mothers reported that mothers of children with eczema experienced significant impacts on their mental health. [184] Furthermore, the time required to care for a child with eczema can be onerous. On average, families with children that have severe eczema spent an additional 63 minutes per day caring for their child. [185]

2.6.3. Economic and vocational impact

Eczema has a wide range of severity, so the economic impact can differ from person to person. Calculating the economic costs is not straight forward and there are indirect, direct, and intangible costs to consider. Indirect costs include lower productivity at work, absenteeism (missing work), and harm to quality of life. [10] Direct costs include prescription drug costs, doctors visits, emergency room and hospital expenses, and over-the-counter pharmaceutical expenditures. [10] The unquantifiable detrimental effects of the illness, such as the neuropsychiatric effects on learning ability and the ramifications for quality of life, are examples of intangible costs. [186]

Several attempts to calculate eczema economic impact have been made. To comprehend the economic burden of eczema, one study followed a cohort of Korean juvenile and adult eczema patients, and estimated that on average, US$2281 was spent annually (per family). [187] The out-of-pocket costs (after insurance) of relapsing moderate to severe eczema in the paediatric population

were calculated in another study, and it was discovered that direct costs were US$75 for the month prior to the office visit, which can be extrapolated to be US$800 for the entire year out-of-pocket costs. [188] In an analysis of healthcare claims data that compared adult patients with eczema with matched patients without eczema, it was estimated that the direct costs averaged more than US$3000 more annually for patients with eczema across all insurance plans examined (Commercial: US$10,461 vs. US$7187, Medicare: US$16,914 vs. US$13,714, Medi-Cal: US$19,462 vs. US$10,408) and more than $5000 per patient with more severe eczema compared to less severe eczema. [189]

According to information from a study in the US in 2010, 75% of those who have eczema visited a doctor at least once in the previous year solely for their eczema. [190] It was reported that due to their eczema, 12.2% lost 1-2 days of work, and 2.3% missed 3 or more days. [190] In 2004, it was calculated that the financial costs of eczema in the United States were US$4.3 billion, these costs included indirect costs such as lost productivity of patients and carers and days of restricted activity and direct costs included things like doctors' visits and medication use. [186] That is the equivalent of $5.3 billion in 2015 according to the Consumer Price Index supplied by the US Bureau of Labor Statistics. [191] Furthermore, treating eczema and its concomitant diseases, such as allergic rhinitis and asthma, may account for more than one-fourth of medical expenses for patients with eczema. [192] In 2002, it was calculated that eczema in Canada costed C$1.4 billion overall. [193]

There is conflicting evidence that eczema may impact occupational choices and opportunities. Some studies suggest eczema does not impact job choice. One study of eczema patients prior to employment found that they did not avoid high-exposure occupations for their first job. [194] Similarly, another study reported that occupational exposures to irritants was comparable between employees with and without eczema, and that similar proportions of the 2 groups were employed in high-risk jobs. [195] On the other hand, the International Study of Life with Atopic Eczema (ISOLATE) involving 2002 participants reported that 32% of participants thought that eczema had an impact on their academic or professional lives, and 14% of the adults said that eczema had impeded their ability to advance in their careers. [196] Furthermore, it was suggested that having eczema may be a risk factor for other occupational skin disease like contact dermatitis. [197] As a result, eczema patients have reported avoiding particular occupations for example in the medical field, food preparation, housekeeping, hairdressing, and vehicle repair. [195]

2.6.4. Role of Eczema in progression of allergic disease: Atopic march

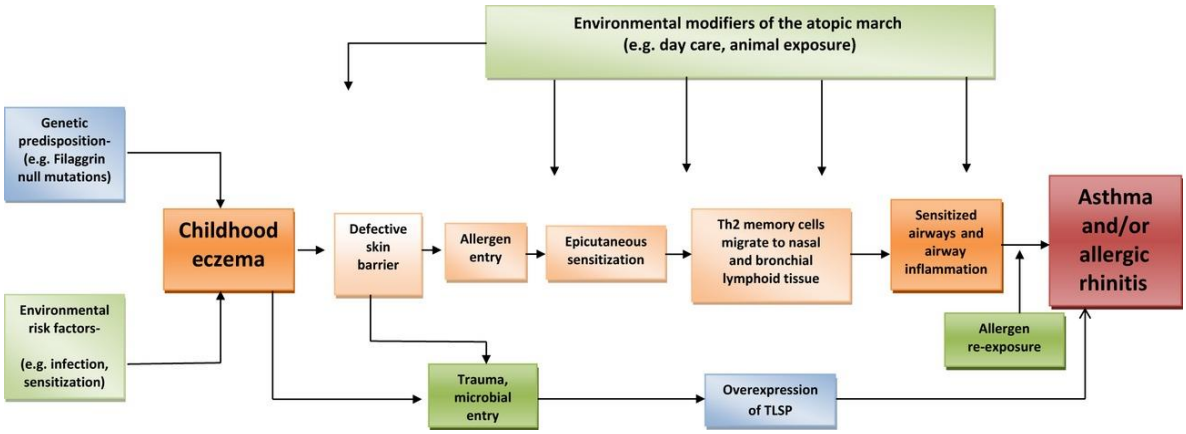
Eczema is often the first condition to be noted as indicating that a child has an allergic tendency and is associated with an increased risk of other forms of allergic disease. The natural course of atopic symptoms, known as the atopic march, is characterised by a regular progression of

allergic disease in children, with eczema typically developing in infancy or early childhood before the emergence of other allergy disorders later in childhood. [30] However, it was indicated that the atopic march does not always occur in this order, as some people with asthma go on to acquire eczema. [198] Furthermore, the atopic march has also been demonstrated to occur in adults at any age. [199] Recent thinking argues that eczema, wheeze, and rhinitis often coexist and it is better to understand their relationship as a multimorbidity framework, rather than a sequence or progression. [200] Long-term research has offered evidence of the occurrence of allergic comorbidities. [199,201,202] According to estimates, asthma and allergic rhinitis affect roughly one-third and two-thirds of eczema patients, respectively. [203] The observation of eczema, wheeze and allergic rhinitis co-occurrence does not imply that there is any particular link between them, and particularly not one that is progressive. [200] Nevertheless, the literature is supportive of a link between eczema and other allergic diseases like food allergies, allergic rhinitis and asthma. [30] Moreover, infants with eczema are almost five times more likely than infants without eczema to develop an IgE-mediated food allergy. [204] As such, eczema may be of importance in allergic disease co-occurrence.

The biological pathways that may underpin the development of the atopic march have not yet been clarified, despite the atopic march hypothesis's widespread acceptance. The involvement of a defective skin barrier function, a characteristic of eczema, is one of the most accepted theories on presumed biological factors underlying the atopic march (Figure 2.10.). [205] It is believed that this skin barrier disruption causes high molecular weight entities like germs, allergens and viruses to penetrate the skin and epidermal water loss. [205] The skin serves as a crucial barrier to the outside environment; while initially more permeable (during infancy), it becomes more impermeable with more age. [206] Infants' reduced barrier function is caused by the stratum corneum's low ceramide concentrations at least in part, these concentrations rise in the weeks following birth. [207] It was reported that ceramide levels are lower in eczematous skin as well, which may explain the skin barrier impairment in eczema. [208] Impaired skin barrier is linked to innate immune activation, which causes dysregulated immune responses to environmental antigens that are harmless, like microorganisms and allergens, and then causes skin inflammation, which eventually may result in the emergence of allergic sensitisation. [115] In turn, these dysregulated immune responses damage the barrier function further, creating a feedback loop of persistent inflammation and injury. [209] This mechanism is also called the barrier hypothesis and is a component of the dual allergen exposure hypothesis, the increased exposures to allergens through a disrupted skin barrier can lead to food allergy and further allergic diseases. [35] The is evidence of a link between eczema severity and the onset of other allergic disorders. [210] Up to the age of 18, early-onset (6 months) and chronic eczema is most significantly linked to an elevated risk of asthma and rhinitis. [210] However, there is ongoing

debate concerning the nature of these associations, with some arguing that eczema and its characteristic decreased skin-barrier function as possible starting points for causing the atopic march towards allergen sensitisation and allergic disease while others have suggested that the co-occurrence of allergic disease does not imply a progressive causal relation.

Figure 2.10. Potential biological mechanism from eczema to other allergic diseases. (Source: S. Dharmage, et. al.; 2014) [30]



2.6.4.1 Eczema and Food allergies

Food allergy occurs when the immune system mistakenly identifies a food as harmful and creates a defensive response against it [26]. It has a high prevalence and disease burden in many countries worldwide.[27,31] Eczema and food allergy symptoms typically manifest in infancy.[30] A systematic review of 66 studies reported a strong association between eczema, food sensitisation and food allergy with some evidence of eczema preceding the development of food allergy. [211] Another study reported a high risk of food allergy at 12 months for infants with eczema in the first year of life (OR 6.2, 95%CI 4.9-7.9). [204] These relationships were stronger in children who needed topical steroids and whose eczema started within the first three months; around half these infants exhibited food allergies. [204] Children with eczema are at an increased risk of developing food allergies, particularly to cow's milk, eggs, peanuts, and tree nuts. [204] It has been proposed that early-onset eczema may be on the pathological pathway to food allergy. [211] Given the strength of the association between food sensitisation and both early-onset and moderate-to-severe eczema, as well as the absence of sIgE in cord blood, it is most likely that food sensitisation largely occurs across the inflamed skin barrier in eczematous skin. [211] The prevalence increase of both eczema and food allergies suggest that both conditions are likely to share similar risk factors. [8]

Summary: Eczema burden of disease

- Eczema is the leading contributor to skin-related disability-adjusted life years.

- The burden of eczema is comprised of many factors—QoL, social, economic, and vocational impact.
- In adults, the impacts on QoL are similar to those in children but also there is a bigger impact in their social interactions, clothing, shaving and cosmetic product choices.
- Eczema economic burden was estimated to be equivalent of $5.3 billion in the US in 2015 including direct, indirect and intangible.
- The term "atopic march" refers to the clinical progression from eczema and food allergy, which is followed by the emergence of respiratory allergies (asthma and/or allergic rhinitis).
- This is ongoing debate concerning the role of eczema, and its characteristic decreased skin-barrier function, as possible causing the atopic march towards allergen sensitisation and allergic disease, with some arguing that co-occurrence of allergic disease does not imply a progressive or causal relation.
- There are strong associations between eczema, food sensitisation and food allergy with some evidence that eczema precedes the development of food allergy.

2.7. Classification of eczema

The heterogeneous presentation of eczema is the source of several eczema classifications in the literature. One of the most accepted classifications is the one proposed by the World Allergy Organization which classifies eczema into two types [11] as described before in the Eczema background section. Other authors have considered classifying eczema as morphologic eczema phenotypes, an eczema spectrum, which includes many types of morphologically different eczema, the conditions included in this classification are seborrheic eczema, prurigo nodularis, nummular eczema, stasis eczema, erythrodermic eczema, dyshidrotic eczema, nipple eczema, asteatotic eczema, lichenified dermatitis and Follicular/papular dermatitis. [117,212] Although there are conflicting views, most of the authors agree that eczema is viewed as an “umbrella” term that includes different conditions with differing pathogenesis and natural history. As long as the immunological mechanisms of these conditions remain unclear the collective of these conditions is referred to as eczema. [11,212]

2.7.1. International Classification of Diseases

To encourage cross-national comparability in the gathering, processing, classification, and presentation of mortality statistics, the International Classification of Diseases (ICD) was created. [213] However, the coding for eczema is not uniformly defined or applied. Adding to the varying views, there are separate ICD codes for "eczema" and "atopic dermatitis" (ICD-9: 691.8 vs 692.9; ICD-10: L20.x vs L30.9, respectively). The ICD-10 code L30.9 is intended to be used for unspecified dermatitis,

whereas the ICD-9 code 692.9 is intended to be used for unspecified contact dermatitis. As a result, the software may apply inaccurate ICD codes when healthcare practitioners enter the term "eczema" into an electronic health record. [66] Moreover, the ICD codes can create confusion and have a significant impact on epidemiology and health services research related to eczema. [66] To assess these possibilities, the validity of the ICD-9 codes 691.8 (AD) and 692.9 (eczema) was investigated using the Hanifin & Rajka criteria (H&R) and United Kingdom Working Party (UKWP) criteria as gold standard. [214] ICD-9 codes have a poor positive predictive value and it was suggested that ICD-10 codes may have the same systematic errors. [214] Similar misclassification can be introduced by the imprecision in the ICD-10 coding (Table 2.2). [215] Given the issues that arise to the inconsistent use of eczema classifications there is an urgent need for a unified classification of eczema.

Table 2.2. ICD-10 codes for eczema and similar conditions.

L20 Atopic dermatitis L20.0 Besnier's prurigo L20.8 Other atopic dermatitis L20.81 Atopic neurodermatitis L20.82 Flexural eczema L20.83 Infantile (acute) (chronic) eczema L20.84 Intrinsic (allergic) eczema L20.89 Other atopic dermatitis L20.9 Atopic dermatitis, unspecified
L26 Exfoliative dermatitis L30.1 Dyshidrosis [pompholyx]
L28 Lichen simplex chronicus and prurigo L30.2 Cutaneous autosensitization L28.0 Lichen simplex chronicus L30.8 Other specified dermatitis L28.1 Prurigo nodularis L30.9 Dermatitis, unspecified L28.2 Other prurigo L53 Other erythematous conditions
L29 Pruritus L29.0 Pruritus ani L29.1 Pruritus scroti

L29.2 Pruritus vulvae L29.3 Anogenital pruritus, unspecified L29.8 Other pruritus L29.9 Pruritus, unspecified
L30 Other and unspecified dermatitis L30.0 Nummular dermatitis
L53.8 Other specified erythematous conditions L53.9 Erythematous condition, unspecified

2.7.2. Eczema phenotypes

The difficulties of assigning a case definition to the disease which is popularly referred to as eczema is an indication that it is actually a collection of heterogeneous conditions with a range of overlapping clinical and histological characteristics. [212] Even the most skilled dermatologists frequently base their eczema diagnosis in accordance with clinical characteristics and histology. [212] Currently, clinicians have to continue treating this constellation of disorders under this general name until we can establish the pathophysiology, create diagnostic tools, and protocols to separate the individual conditions that are collectively referred to as eczema. [212]

There are different pathophysiological pathways for the development of eczema, which have not yet been fully characterised. The age of onset, severity and persistence of eczema are most likely determined by complex interactions between environmental and genetic factors. [6] For that reason, the concept of "eczema phenotypes" has been suggested, rather than a simple binary classification of eczema, phenotypes can incorporate the variability of eczema development and progression. [12] Understanding the traits of these patient clusters and their unique disease trajectories could aid clinicians in early decision-making regarding whether a paediatric patient with eczema should be closely monitored for their potential to develop comorbidities and/or be referred to a specialist for evaluation and follow-up. Also, it would allow the assessment of risk factors of specific phenotypes. [216] The classification of eczema phenotypes could allow for the identification of risk factors and enhance understanding of patterns associated with eczema onset and progression. I have examined eczema subclasses using a latent class analysis and assessed its associations with risk factors and allergic outcomes as a part of my doctoral work and made a publication presented in Chapter 4.

2.7.3. Eczema biomarkers

Another potential avenue to disentangle eczema heterogeneity is the identification of relevant biomarkers. A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic response to a therapeutic intervention." [217] As we have previously seen in other fields of medicine (i.e., oncology), where therapeutic decisions are made more frequently based on biomarkers, it is expected that a combination or a panel of multiple biomarkers will be employed for the stratification of complicated conditions by phenotypes like for eczema. [122] However, research in this space is at initial stages and a number of potential biomarkers for eczema have been identified (Table 2.3.). [122] Biomarkers could be used in early life eczema screening to identify those at high risk of developing eczema. An early intervention soon after birth can be beneficial, eczema is a disease that frequently develops in early life. [218] Other potential uses for biomarkers could be as diagnostic tools, to measure severity, to determine sensitization profiles, predict therapeutic response or as prognostic tools (Table 2.3.). [122]

Table 2.3. Potential candidates for biomarkers in eczema. [122]

Biomarker	Screening	Diagnostic	Severity	Sensitization	Predictive therapeutic response	Prognostic fate of eczema/comorbidities
Total/specific IgE				+++	Potential for prevention	?
TARC/CCL17	+		+++			
MDC/CCL22			+			
CTACK/CCL27			+			
*FLG*1/2	++				Potential for prevention	?
SPINK5/LEKTI	+					?
TSLP	+			?		Risk for viral complication
IL-31			+			
IL-33			+			Risk for viral complication
IL-22			+			
FcεRI/FcγRII		+				
IDO						Risk for EH
LL-37			+			
IL-18			+			
IL-16			+			
Soluble IL-2 receptor			+			
PARC/CCL18			+			

Biomarker	Screening	Diagnostic	Severity	Sensitization	Predictive therapeutic response	Prognostic fate of eczema/comorbidities
TEWL	++			?	Potential for prevention	?
Periostin			+			
BDNF			+			
IgE against *Malassezia* species			+			+

BNDF, Brain-derived neurotrophic factor; *CTACK*, cutaneous T-cell-attracting chemokine; *EH*, eczema herpeticum; *FLG*, filaggrin; *IDO*, indolamine-2,3-dioxygenase; *MDC*, macrophage-derived chemokine; *PARC*, pulmonary and activation-regulated chemokine; *SPINK5/LEKTI*, serine protease inhibitor Kazal-type 5/lympho-epithelial Kazal-type-related inhibitor; TARC, thymus and activation-regulated chemokine; TEWL, transepidermal water loss; TSLP, Thymic stromal lymphopoietin.

+, ++, +++: more + means increasing level of suitability for the biomarker type

?: not know

Summary: Classification of eczema

- The heterogeneous presentation of eczema is the source of several eczema classifications in the literature.
- The World Allergy Organization which classifies eczema in atopic and non-atopic.
- Eczema is viewed as an “umbrella” term that includes different conditions with differing pathogenesis and natural history
- The ICD have separated codes for "eczema" and "atopic dermatitis"
- The concept of “eczema phenotypes” has been suggested, rather than a simple binary classification of eczema, as such phenotypes can incorporate the variability of eczema development and progression.
- The classification of eczema phenotypes could allow for the identification of risk factors and enhance understanding of patterns associated with eczema onset and progression.
- Biomarkers could be used as screening tools, diagnostic tools, to measure severity, to determine sensitization profiles or as prognostic tools.

Research questions (Chapter 4):

1. What are the longitudinal eczema phenotypes according to eczema prevalence in early life and childhood?

1.1. How are these longitudinal eczema phenotypes associated with eczema risk factors?

1.2. How are these longitudinal eczema phenotypes associated with allergic outcomes in adulthood?

2.8. Eczema Risk factors

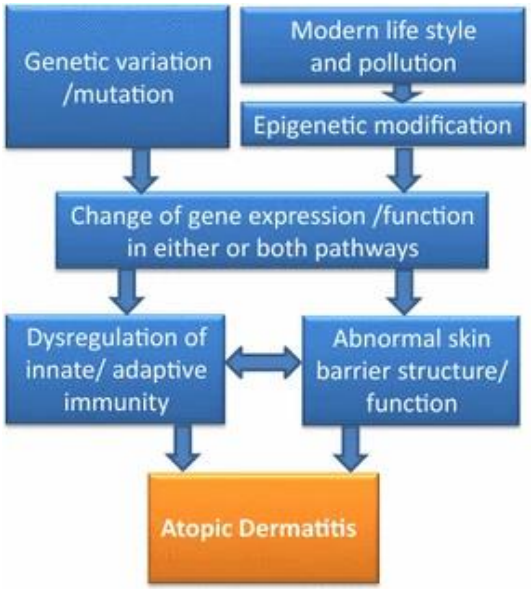
Despite constant research efforts and the modernization of molecular techniques, eczema is still a mysterious disease, and the cause (or causes) that mediate the development and progression of this skin illness are not well understood. [219] Recent literature suggests complex interactions between genetic and environmental variables are involved in eczema development. [6] The genetic and environmental factors related to eczema are described below.

2.8.1. Genetic risk factors

Understanding the genetic causes of eczema leads to identifying the biological pathways to eczema and identifying high-risk sub-populations that may benefit from specific interventions. There is strong evidence that the genetic predisposition contributes significantly to the development of eczema, [220,221] with ranges of concordance rates for identical twins 0.23 to 0.86 and dizygotic twins reaching 0.15 to 0.5. [222,223] Several studies on affected people and their families have looked more closely at eczema heritability. [223] The findings demonstrate that eczema is a disease influenced by several genes, their interactions, and environmental factors rather than following conventional Mendel's principles of inheritance. [223]

The methods for determining eczema genetic risk have been similar to those used for other complex conditions, such as genome-wide analysis (GWAS), the investigation of candidate genes and extreme phenotypes, transcriptome profiling analyses, epigenetic studies and exome or genome sequencing. [98] Based on the outcomes of these analyses, the genes involved in eczema pathogenesis primarily fall into two pathophysiologic categories: skin barrier genes and immune response genes (Figure 2.11.). [100] However, there are complex relationships between genes in both categories.

Figure 2.11. Potential genetic and epigenetic modifications in the pathophysiological pathway to eczema (atopic dermatitis). (Source: B. Lianghua, et. al.; 2016) [100]



2.8.1.1. Filaggrin and other skin barrier genes

Skin barrier disruption is widely acknowledged as a crucial component of eczema aetiology. [224] Chromosome 1q21 has a large group of genes involved in epidermal differentiation. [100] The loricrin, *FLG,* involucrin, short proline-rich proteins (*SPRRs*), S100A family, S100-fusion protein family, and late cornified envelope proteins make up this gene set, also known as the "epidermal differentiation complex" (EDC). [100]

The critical barrier protein (pro-)filaggrin seven encoded by the *FLG* gene has shown the highest genetic link with eczema. [99] The *FLG* null mutations *R501X* and *2282del4* were most often linked to eczema in European Caucasian patients. [99] Additionally, it has been demonstrated that eczema patients, particularly with *FLG* gene loss-of-function mutations, experience severe, chronic forms of eczema. [99] The *FLG* null mutation's relationship with eczema has revealed racial differences: The *FLG P478 S* and *C3321delA* mutations are linked to an elevated risk of eczema and recurrent skin infections in Asians. [225] *FLG* mutations are uncommon in African populations, and loss-of-function mutations in Filaggrin 2 (*FLG2*), but not *FLG*, are linked to elevated eczema risk in African American children. [84] There are reports of gene-environment interactions with the chemicals called phthalates found in a wide range of personal care items. [226] Children with *FLG P478S* mutations have higher skin phthalate absorption than other children, and levels of phthalate metabolites are strongly linked to an elevated risk of eczema in Chinese children. [226] Another study found that maternal *FLG* mutations increase the risk of eczema in children, even when children did not inherit the mutation, suggesting that maternal *FLG* mutations may be significant environmental risk factors for the kids. [227] As such, *FLG* is eczema's most important genetic risk factor.

The serine protease inhibitor of the Kazal type 5 (*SPINK5*) gene codifies the lymphoepithelial kazal type-related inhibitor type 5 (*LEKTI*), which is essential for maintaining the homoeostasis of the epidermis. [228] Netherton syndrome, a severe autosomal recessive skin condition characterised by eczema and sensitisation, was discovered to contain mutations in this gene that cause loss of function. [228] The literature showed that increased protease activity brought on by *SPINK5*'s malfunction boosted proteolytic activity in the epidermis, lowering DSG1 and *FLG* while increasing TSLP expression. [229] Additionally, it has been demonstrated that keratinocytes with less *FLG* express more IL-1 and TSLP. [229] Modifications of these genes contribute to eczema pathogenesis.

Other EDC genes have been associated with eczema development. An association between eczema and a *24-bp* deletion in small proline-rich proteins 3 (*SPRR3*) was suggested in a case-control study. [230] Claudin-1 (*CLDN1*) gene expression has been discovered to be downregulated in eczema skin; genetic variations and Th2 cytokines both contributed to the decline in *CLDN1* in eczema. [231] Null mutations in the gene *DSG1*, which codes for the crucial desmosome protein desmoglein 1, have been linked to severe dermatitis, many allergies, and metabolic wasting in humans. [232] It has been suggested that homozygous mutation of *TMEM79* has a purpose in the lamellar granule secretory system, which can lead to a skin barrier defect and result in eczema skin inflammation. [233] Moreover, a meta-analysis showed that a missense SNP (rs6694514) of TMEM79 was strongly associated with eczema. [234] Another gene linked to eczema development is *LAMA3* which encodes the alpha chain of *laminin 5*, *OVOL1* and *ACTL9*, essential proteins for skin integrity. [235] This information shows that the absence of structural and functional epidermal enzyme inhibitors compromises the skin barrier function and could mediate allergic inflammation-related immune reactions.

2.8.1.2. Adaptive and innate immune response genes

The research regarding candidate genes for eczema has also concentrated on the Th2 pathway because eczema is linked to allergen sensitisation, raised serum IgE, and increased expression of Type 2 cytokines (IL-4, IL-5, and IL-13) in both unaffected skin and skin lesions of eczema. [100] In fact, GWAS experiments have frequently found genetic risk loci for eczema in the vicinity of Th2 gene areas at *5q31*. [223] Downstream genes of the IL-4 receptor, like STAT6, have also been found to correlate positively with eczema. [236] Additionally, gene variations in the high-affinity IgE receptor's alpha and beta chains (FCER1A/B) have been linked to the pathophysiology of eczema. [237] In eczema skin lesions, Thymic stromal lymphopoietin (TSLP) expression is markedly elevated. [238] Since TSLP's main job is to stimulate the Th2 immune response, it has been assumed that it contributes significantly to the pathogenesis of eczema. [238] Genetic variations in TSLP have been linked to eczema. [239] Additionally, polymorphisms in the TSLP receptor IL-7R (*T244I* at exon six and *T46I* at exon 2) are linked to eczema. [240] The effect of impaired Th1 responses or reduced

inhibitory feedback was associated with eczema. [100] Th1 is promoted by IL12B, IL12 receptor beta 1 (IL12RB1), and IL-18. [241,242] Adaptive immune responses can be inhibited by both suppressors of cytokine signalling 3 (*SOCS3*) and IL-10. [243] As such, *SOCS3* polymorphism was associated with the higher development of eczema in European patients. [244] In addition, IL10 (-819 and -592 promoter polymorphisms) was found to be related to eczema in the Korean population. [245]

The increasing frequency of skin infections in eczema raises the possibility that the skin's inherent defence system is impaired. [100] The pathophysiology of eczema has been linked to genetic variations in several genes that operate as pattern recognition receptors. [246] These are *TLR2*, *TLR9*, *CD14*, *TOLLIP*, *MYD88, MAL, NOD1, NOD2,* and *NALP12*. [246] Moreover, human defensins, sphingosine, and antimicrobial peptides of the S100 protein all have polymorphisms linked to eczema development. [246,247] In addition to the direct effects of genetic changes in innate immune response genes, it is relevant to the pathogenesis of eczema, the attenuation of the antimicrobial response brought on by the overexpression of Th2 cytokines in the skin. [100] For instance, it has been shown that Th2 cytokines can inhibit human-defensin 3 and LL-37 gene expression in epidermal keratinocytes. [248] Immune responses and a malfunctioning epidermal barrier reciprocally interact to cause eczema.

2.8.2. Environmental factors

Environmental factors can increase the risk of eczema through different pathological mechanisms. The epidermal barrier is primarily formed by the stratum corneum, the epidermis' topmost layer. In this layer, corneocytes are linked together by corneodesmosomes and immersed in a lipid-rich intercellular matrix. [249] In particular, the envelope of corneocytes, made up of different structural proteins like loricrin, involucrin, filaggrin, and tiny proline-rich proteins, is crucial for preserving the physical integrity of the epidermis barrier. [249] The "outside-inside" concept states that the weakening of the skin barrier and subsequent entry of allergens and irritants lead to the development of eczema. [250] For instance, barrier damage brought on by air pollution oxidative stress on the skin. [250] The impact of external exposures (such as water hardness, pollution, and allergens) on the onset and course of eczema has been the subject of several studies. [6] These exposures probably work together to trigger the immunological responses leading to eczema. [6] These factors contribute to barrier disruption and reduced skin barrier function, leading to increased epidermal permeability and susceptibility to environmental risk factors that may set the itch and scratch cycle. [6] The environmental risk factors I examined in my doctoral work were air pollution, hard water and occupational exposures.

2.8.2.1. Air pollution

The term "air pollutants" refers to various compounds in the atmosphere that pose a risk to human health. [250] They can be categorised as primary or secondary pollutants and can take the form of gas or particle matter. [250] Sulfur oxides (SOx), nitrogen dioxides (NO_2), ammonia, carbon monoxide (CO), radioactive pollutants like radon, hazardous metals like lead and mercury, volatile organic compounds (VOCs), and particulate matter (PM) are some of the most significant main pollutants. [250] Through chemical and photochemical processes, secondary pollutants are created in the atmosphere from primary pollutants. For instance, smog, NO_2, sulfuric acid, and ground-level ozone. There are air pollutants in all places, and they can come from both indoor and outdoor sources. [250] Ambient air contains various outdoor air pollutants mixed in different proportions. They originate from a variety of places, both natural (i.e. Wildfires, volcanoes, biological degradation, etc.) and artificial (i.e., motor vehicles, biomass burning, power plants, etc.). [250] Typical outdoor air pollutants from fuel burning or motor vehicle emissions include sulphur dioxide, CO, and NO_2. [250] Ozone (O_3) is produced when VOCs and NO_2 react chemically in the presence of sunshine; O3 can cause health issues. [250] Another traffic-related outdoor air pollution recognised as detrimental to health conditions is diesel exhaust particles (DEPs). [251] PM is a combination of particles with different physical and chemical characteristics that may have different health impacts based on their chemical characteristics. [250] Depending on the particulate diameter is divided into three categories: particulate matter with a diameter of 10 m or less (PM_{10}), particulate matter with a diameter of 2.5 m or less ($PM_{2.5}$), and particulate matter with a diameter of 0.1 m or less. [250] The particles' size determines where they can deposit in the human body. [250] Various air pollutants can be found in indoor spaces from various sources, such as stoves, construction materials, furniture, and electronic equipment. [250] Mould spores, bacteria, house dust mites, and animal dander are the source of biological pollutants indoors. [250] Due to the fact that people spend a lot of their time indoors, indoor air pollution is a raising health concerns. [250] VOCs, PM, and pollutants from combustion, such as sulphur dioxide, carbon monoxide, and nitrogen dioxide, are also present in indoor air. [250] VOCs are frequently discovered at indoor and in homes. Depending on how well the building is ventilated, some interior PM comes from outside, but there are also various indoor PM sources. [250] These air pollutants may adversely affect the overall health of people exposed to them.

The effect of air pollutants on a person's health is related to the pollutant's hazard and exposure time. The three primary ways people are exposed to air pollution are through ingestion, inhalation, and skin contact. [250] There is another possible way the foetus's development may be impacted by prenatal exposure via the transplacental pathway. [252] Numerous environmental contaminants in contact with skin can get through the epidermal barrier. [252] These substances either

adhere to the stratum corneum, enter the epidermis, or are broken down by the body. [252] However, there are still significant challenges in determining how air pollution affects eczema patients. [252] The prevalence of eczema has been rising in recent years, especially in urban areas and recurrent eczema symptoms in adolescents can be explained by exposure to environmental triggers.

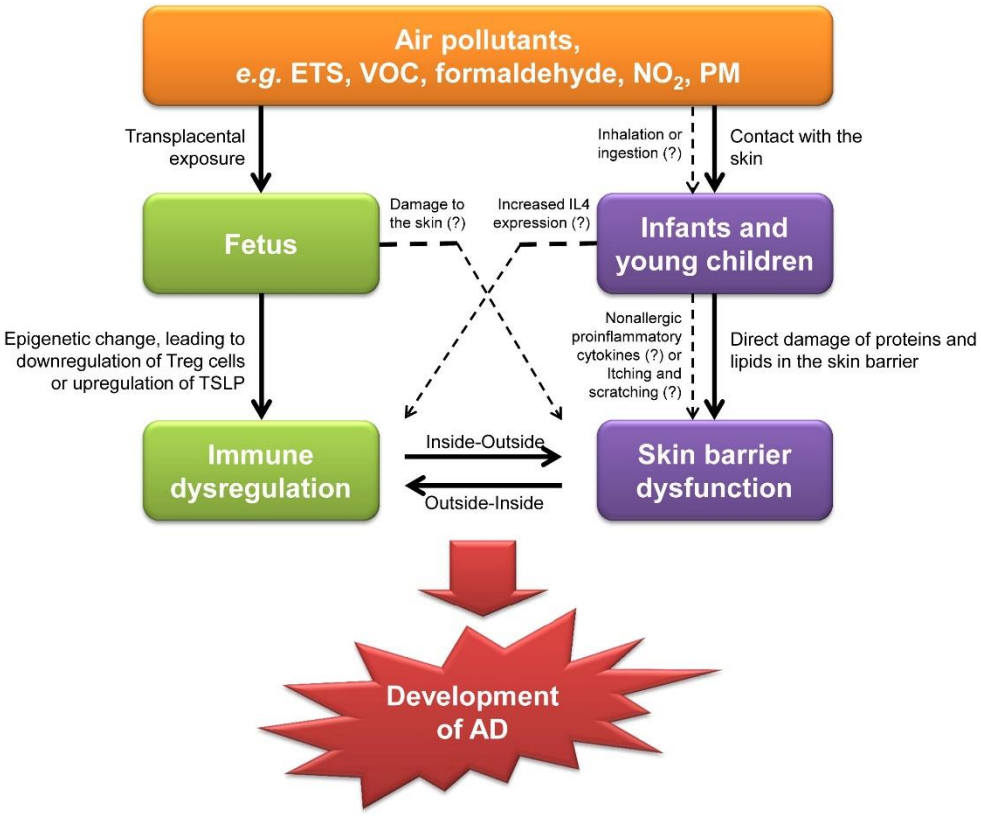
Several cross-sectional studies have suggested a correlation between more significant concentrations of air pollution and eczema in small towns and urbanised areas. [23,253,254] The associations between air pollution and eczema have been explored in the literature, mainly involving studies in children. In a French study, lifetime eczema was correlated with exposure to higher concentrations of PM_{10}, NO_2, NO_x, and CO in schoolchildren. [255] Another study found strong positive associations between the distance to the nearest main road and eczema in two birth cohort studies in the Munich metropolitan region. [254] It was also reported that NO_2 exposure was positively related to eczema (OR, 1.18). [256] Furthermore, $PM_{2.5}$ has also been linked to increased eczema prevalence in children. [23] Two recent systematic reviews and meta-analyses found positive associations between ambient air pollution and eczema development. [257,258] In an extensive nationwide survey of middle school students, eczema was associated with exposure to air pollutants, such as NO_x and CO. [259] One study from Germany reported that $PM_{2.5}$ (1.45; 95% CI, 1.06-1.99) and PM_{10} (1.36; 95% CI, 1.00-1.83) were associated with increased odds of incident eczema in middle aged women. [24] Another study showed that living close to heavy traffic was associated with eczema prevalence in adults (OR = 1.45, 95% CI 1.28–1.66). [260] A third study suggested that $PM_{2.5}$ (OR = 1.05, 95% CI 1.02–1.08) and the pollutant Standards Index (OR = 1.02, 95% CI 1.01–1.03) were associated with increased eczema prevalence in adults. [261] On the other hand, some studies found that exposure to air pollution was not significantly associated with developing eczema in children. [21,22,262] Hence, uncertainty exists as to the role of air pollution on eczema development. The main limitations of the literature in this topic are the lack of standardisation in the selection and methods to measure air pollutants, the understanding and selection of confounders and limited number of studies were done with adults samples. [263] A better understanding of the potential effects of ambient air pollution on adult eczema may lead to targeted interventions to prevent eczema. For that reason, and as part of my doctoral work, I assessed the longitudinal association of air pollution and eczema development and incidence in a cohort of adults. I published a paper that is available in Chapter 6.

The literature describes a few biological mechanisms by which air pollutants may lead to eczema and sensitisation. The most accepted pathway is via inflammatory reactive oxygen species (ROS) and nitrogen species leading to cell damage that promotes skin barrier dysfunction (Figure 2.12.); however, the potential mechanisms remain unknown. [4] It has been suggested that oxidative stress, which is an imbalance between oxidants and antioxidants in the skin, plays various roles in

the progression of eczema. [250] Air pollutants may affect the skin through direct percutaneous absorption or indirectly through inhalation and subsequent systemic inflammation by enhancing TH2 polarisation or triggering pruritus. [4] Another mechanism seems to be the COX-2 pathway; exposure to cobalt chloride resulted in the generation of ROS, the elevation of COX-2 expression, and nuclear factor B phosphorylation, all of which led to an increase in the secretion of IL-6 and IL-8. [264] Moreover, air pollution can also act as an irritant and immunomodulator, leading to elevated levels of serum IgE. [19] In particular, $PM_{2.5}$ may activate the aryl hydrocarbon receptor to promote cell metabolism and inflammation. [19] It was also suggested that exposure to VOCs during pregnancy may interfere with the development of the immune system of the unborn child, with the potential for epigenetic changes. [250] Other potential mechanisms include increased trans-epidermal water loss, interfering with the inflammatory signals and altering the microbiome and skin PH. [20] Also, it was suggested that chronic exposure to air pollution might introduce changes in the composition of the skin microbiome. [265] Most eczema patients have epidermal barrier disrupted without known genetic alterations, suggesting that in some people, eczema may result from exposure to environmental factors like air pollution alone.

In recent years there was a parallel increase in the prevalence of eczema and food allergy which may be due to environmental changes brought by urbanisation and industrialisation. [25] There has been growing interest in understanding the potential associations between air pollution and eczema and food allergies. [32] Infants are particularly vulnerable to the effects of air pollution, as epithelial barrier function and immune systems are still developing. [266] It is plausible that, as described by the "dual allergen exposure hypothesis",[35] the increased exposures to allergens through a disrupted skin barrier can increase the risk of food allergy.[8] Due to eczema flares and resolved periods and the difficulty of testing food allergies clinically, conducting any study aiming to assess both eczema and food allergies is challenging. [211] Performing oral food challenge (OFC) throughout infancy and prospectively screening for food sensitisation at regular intervals are the only ways to robustly study the onset of food allergy. [211] As such, there is limited evidence on this topic and more research needs to be done to better understand these relationships. As part of my doctoral work, I assessed the longitudinal association of air pollution and eczema and food allergies in the first 10 years of life. The manuscript of this research is available in Chapter 5.

Figure 2.12. Air pollution potential biological pathway to eczema. (Source: A. Kangmo, et. al.; 2014) [250]



Pregnancy-related epigenetic alterations brought on by transplacental exposure to air pollutants include overexpression of microRNA-223 and hypomethylation of the TSLP gene CGI. These modifications result in TH2 polarisation by decreasing the proportion of regulatory T cells or increasing the expression of TSLP protein. Several air pollutants that are in direct contact with the skin after birth cause ROS and reactive nitrogen species. Skin barrier failure is the outcome of the proteins and lipids in the epidermis being damaged by this oxidative stress. Skin barrier failure is brought on by prior immunologic dysregulation or TH2 polarisation, and vice versa. A healthy pregnancy, an infant, a young child in the developmental stage, or a person with a genetic susceptibility could suffer harm from air pollution.

Research questions:

2. Is the exposure to ambient air pollution associated with eczema and food allergies in the first ten years of life? (Chapter 5)

2.1. Is the exposure to air pollution associated with eczema and food allergies persistence in the first ten years of life?

3. Is the exposure to air pollution associated with the incidence and prevalence eczema in a cohort of middle-aged adults? (Chapter 6)

3.1. Do these associations vary according to atopic or non-atopic status?

2.8.2.2. Hard water

Hard water is characterised by high mineral concentrations (mainly calcium and magnesium ions), such as calcite, gypsum, and dolomite. [267] Divalent cations combine with anions in the water to

generate more stable salts. [268] Total hardness is measured by the concentration of calcium carbonate ($CaCO_3$) in the water, which determines whether water is soft (0-60 mg/L), moderate (61-120 mg/L), hard (121-180 mg/L), or very hard (>180 mg/L), includes hardness brought on by calcium and magnesium. [268] Hardness typically enters groundwater due to the water percolating through calcium- or magnesium-containing minerals. [268] Dolomite and limestone are the most prevalent sources of hardness because they add calcium and magnesium to the water. [268] Due to how hardness enters the water, groundwater often has higher hardness than surface water. [269] Domestic water hardness in the US, UK, and Australia range from soft to very hard the mineral content of drinking water in Asia is typically modest (between 2 and 80 mg/L). [270] Domestic water hardness varies based on the topography of the region.

There are various potential ways that hard water could harm the skin barrier and lead to eczema. The most accepted mechanism relates to the interaction between hard water and the surfactants (detergents) used in laundry products. [271] High calcium levels are believed to make surfactants less soluble, which could increase their deposition on the skin after washing. [272] Common wash products are surfactants, like the harsh anionic surfactant sodium lauryl sulphate (SLS), which are well-known skin irritants and significant environmental stressors that exacerbate eczema. [272] SLS residues left behind on the skin change the secondary structure of proteins and solubilise stratum corneum lipids. [271] Similarly, domestic use of hard water requires more soap than necessary to get a lather and build a soap film (calcium stearate) on the skin. [38,39] Another potential biological mechanism is related to the alteration of the skin's pH levels (normally mildly acidic); hard water is an alkaline solution that can increase the skin's pH. [271] Finally, the signalling in the epidermis may be altered by the high concentration of Calcium ions in hard water and lead to an impaired skin barrier function. [271] It is evident that persons who live in places with softer water also experience eczema, indicating that hard water is not a necessary cause but rather a component cause. [271] All of these potential mechanisms can lead to reduced barrier function, increased bacterial colonisation [271] and, allergen penetration, risk factors for eczema.

Most literature suggests that hard water is associated with increased eczema development. Several studies suggested that children living in areas with hard water have a higher prevalence of eczema than children living in softer water areas. [273-276] A cross-sectional study found that children in primary school who lived in hard-water areas had a higher chance of developing eczema than those who did not. [273] This association was supported by two more cross-sectional studies conducted among schoolchildren in Spain and Japan. [274,275] Likewise, a study that assessed a birth cohort from Denmark reported that for every five-unit rise in residential water hardness, the prevalence of eczema rose by 5% within the first 18 months of life. [276] Furthermore, a recent systematic review and

meta-analysis study assessed the pooled odds ratio estimate. It showed increased odds of eczema in infants and children exposed to hard water (OR 1.28, 95%CI 1.09-1.50) in comparison to those exposed to softer waters. [277] On the other hand, a few studies reported no statistically significant associations between hard water and eczema in infants or children. [278,279] In areas of the UK with hard water, a RCT found no significant impact of the use ion-exchange water softeners as therapeutic options to reduce the severity of eczema in children. [280] There is a distinct lack of studies assessing this potential association in adults. As such, I have evaluated the effect of domestic water hardness on eczema development in a large cohort of adults as part of my doctoral work; the resulting publication is available in Chapter 7.

Research question (Chapter 7):
3. Is domestic water hardness associated with eczema prevalence and incidence in a cohort of middle-aged adults?

2.8.2.3. Occupational exposures

Occupational exposures to irritant or allergenic substances, including chemicals and substances such as oils, detergents, shampoos, and cleaning agents on the skin, are responsible for developing occupational skin diseases (OSD). Most of the literature on OSDs is focused on contact dermatitis, a localised skin reaction that predominantly affects the hands, as opposed to generalised eczema. [41] As such, there needs to be more information about the potential effects of occupational exposure and eczema. Occupational epidemiology of skin reactions has conventionally classified eczema as an "endogenous" OSD, that is, occurring from "within" (i.e. immunological reaction due to a genetic predisposition to develop eczema) rather than caused by contact with defined external agents. [42] However, current understanding suggests that eczema initiation and exacerbation appear to be multifactorial. [5] Occupational exposures may be important in the pathological eczema pathway. Hence, as a part of my doctoral work, I evaluated the potential associations between a wide range of occupational exposures and eczema prevalence in middle-aged adults using a job exposure matrix, the resulting publication is available in Chapter 8.

As a sizable part of adult asthma and respiratory symptoms are caused by occupational exposures, [281] it is possible that occupational exposures may influence the prevalence of other allergic diseases such as eczema. Most occupational irritants are chemicals, including metalworking fluids, cleaning agents, and organic solvents; these can damage the epidermal barrier, particularly after repeated exposure. [49] In occupations involving exposure to animal dust, fish/shellfish, latex, solvents and wood dust. [46] Other allergens include latex, rubber additives in gloves, metals (nickel, chromium and cobalt), epoxies, preservatives, resins and acrylates, which can lead to allergies and

dermatitis. [49] The mechanism by which occupational exposures may result in eczema has yet to be fully understood. Occupational exposures can act as allergens on contact with the skin resulting in an allergic reaction. [48] While others can act as irritants to disrupt skin barrier function, increasing permeability to other allergens. [48]

Three studies have found positive associations between occupational exposures and current eczema. [47,50,282] An increased incidence of current eczema was linked to those occupationally exposed to gas dust or fumes (OR:2.08, 95% CI 1.40–3.08). [47] Similarly, Kurt et al. reported that work exposure to chemical fumes was linked to doctor-diagnosed eczema (OR: 3.7, 95%CI: 1.3–10.6). [50] In a study evaluating OSDs (both contact dermatitis and eczema), it was found that increased risk of OSDs was associated with jobs that exposed workers to acrylates, metalworking fluids, aliphatic amines, and high levels of isocyanates and organic solvents. [282] However, two of these studies [47,50] evaluated limited exposure definitions that were not time-specific and assessed only a few occupational exposures. The third study assessed all dermatitis as the outcome, which included eczema and contact dermatitis. [282] None of these studies considered the eczema atopy status and its association with occupational exposures. Further research is needed to understand the effects of every occupational agent. A better understanding of the associations between occupational exposures and eczema informs about the likely environmental contributors to the development and persistence of eczema in adults.

Research question (Chapter 8):

4. What are the potential associations between a wide range of occupational exposures and eczema prevalence in middle aged adults?

    4.1. Are these associations different according to atopic or non-atopic status?

Summary: Eczema risk factors

- Eczema pathogenesis involves complex interactions between genetic and environmental factors.
- There is strong evidence that the genetic predisposition contributes significantly to the development of eczema.
- The key barrier protein (pro-)filaggrin 7, which is encoded by the *FLG* gene, has been repeatedly shown to be a strong genetic risk factor for eczema.

- The "outside-inside" concept states that the weakening of the skin barrier and subsequent entry of allergens and irritants lead to the development of eczema.
- Environmental risk factors contribute to the barrier disruption and reduced skin barrier function leading to increased epidermal permeability and susceptibility to environmental risk factors that may set the itch and scratch cycle.
- The term "air pollutants" refers to a variety of compounds in the atmosphere that pose a risk to human health.
- Air pollution may be a risk factor for eczema and food allergy in children.
- Hard water is characterised by high mineral concentrations (mostly calcium and magnesium ions), such as those found in calcite, gypsum, and dolomite.
- Occupational exposures include irritant or allergenic substances, including chemicals and substances such as oils, detergents, shampoos, and cleaning agents.
- Air pollution, domestic hard water and occupational exposures may increase risk of eczema in adults.

2.9. Current treatment and management of eczema

Eczema treatment goals are to reduce symptoms (pruritus and dermatitis) and signs (redness), prevent and treat exacerbations (flare-ups), and minimise therapeutic risks prevent. Treatment involves preventing exposure to risk factors, maintaining appropriate skin care with emollients, and using medications, mainly steroid ointments. [283] Cutaneous hydration during eczema is essential as it improves the barrier function relieving itchiness and may reduce the use of topical steroids; as such, it should be used as a basic treatment. If basic treatment fails to control symptoms, standard medical treatment with topical steroids may be necessary. [284] Since eczema is a chronic condition, long-term therapy techniques are required. Clinical studies have employed three techniques to evaluate long-term control: regular evaluation of eczema outcomes, the medication used, and documentation of eczema flares/remissions. [285] Objective and subjective symptoms should be included when evaluating overall disease activity because they influence clinical severity. [286] n example of objective measures include the SCORAD index, [287] mild eczema is associated with SCORAD levels under 25, whereas severe eczema is associated with SCORAD levels over 50. [288]

Another important consideration is the occurrence of eczema flares. It can be challenging to define flare as a clinically meaningful phrase because of its diverse presentation. [289] A flare can be defined as "an acute, clinically significant worsening of eczema signs and symptoms necessitating

treatment intervention". [69] The most common methods in the past have been repeated assessments and subjective cut-offs of an pre-existing severity scale like SCORAD. [289] However, because the amount of tolerance to pruritus can differ significantly, it can be challenging to categorise flares solely on the severity of the itching. [286] Another way to identify flares is to measure the patient's medication intake. [69] Long-term remission without treatment is typically synonymous with cure. However, the amount of time without treatment needed to determine if a participant is cured or not has not yet been established. Remission obtained by avoiding allergens and irritants and using only emollients is not similar to remission brought on by immunosuppressive medications or biologics. [69] In chronic conditions, maintaining long-term remission is a common goal.

2.9.1. Basic therapy and skincare for eczema

2.9.1.1. Skin washing

The skin must be thoroughly and gently washed to remove crusts and mechanically eradicate bacterial pollutants to avoid complications like bacterial superinfection. [69] Skin cleansers come in various forms, such as syndets or aqueous solutions and may be used with or without antiseptics. Mechanical washing is more significant because some antiseptics have a relatively short duration of action. [290] Lower pH products are generally better for the skin barrier and should be used whenever possible. [69] The pH should be around 5-6 which is the physiological cutaneous range. [69] Avoiding harsh detergents and alkaline soap is the most crucial component of cleansing eczema skin. As substitutes, use emollient wash products (bath oils, shower emollient wash products) and leave-on emollient creams. Regarding bathing frequency, it was shown that no difference in eczema severity between bathing twice weekly or daily was found as long as skin moisture was controlled after the bath. [291] However, a prolonged bath time is linked to exacerbation of the symptoms. [292] As such, bathing or showering with lukewarm (less than 37 C, not hot) water 2 to 7 times per week using the “soak and seal” method with emollients and very mild surfactant wash products, bleach baths are also recommended. [69]

2.9.1.2. Emollient therapy

Emollients are moisturising products used topically to soothe and hydrate the skin. They apply a shielding coating to the skin to keep moisture from escaping. [69] Emollients can be a cost-effective way of avoiding flare-ups and needing Topical Corticosteroids and Topical calcineurin inhibitors. [69]

Most emollients are comprised of an occludent (such as petroleum) and a humectant (such as urea or glycerol). [293] It is well established that urea greatly lowers the chance of an eczema relapse. [294] Numerous non-medicated eczema topical therapy treatments contain known active

components, but they do not meet the criteria for being classified as topical drugs and do not require a licence. [69] These products, which the European standard refers to as "emollients plus," may include ingredients like bacterial lysates from Aquaphilus dolomiae or Vitreoscilla filiformis, flavonoids like licochalcone A, or a synthetic menthol derivative like menthoxypropanediol. [293] Despite this wide range of products and preparations, the evidence of differential efficacy between these treatments in eczema still needs to be provided. [69] It is suggested to use emollients freely every day, preferably with the "soak-and-seal" method, at least 30 grammes per day or one kilogramme per month for an adult. [69]

2.9.1.3. Allergen avoidance strategy

Acute as well as delayed-type hypersensitivity to environmental allergens is linked to eczema. Hen's egg, cow's milk, soy, wheat, tree nuts, and peanuts are the food allergens most commonly associated to eczema flares in newborns and young children. [69] Pollen-related allergies should be considered in older kids, teenagers, and adults. [295] The progression of the disease may be worsened by irritant causes, which include chemical and physical substances [69] Furthermore, contact sensitisation to chemicals, medications, plants, or metals can exacerbate the clinical severity. [296] It was suggested that an allergy workup for patients with moderate to severe eczema could be performed. Depending on the individual history, it may include serum IgE, skin prick tests, and patch testing to identify potential allergy sensitisation. However, elimination diets should only be prescribed in cases of confirmed food allergies, and dietary suggestions should be tailored to the individual patient to avoid complications related to dietary eliminations without an adequate substitution for the child's growth and general health. [69]

2.9.2. Topical anti-inflammatory treatment

Three key elements are necessary for effective topical anti-inflammatory therapy: adequate strength, adequate dosage, and proper application. [297] If possible, topical medication should be applied to moist skin, especially when using ointments.

2.9.2.1. Topical Corticosteroids

Topical corticosteroids remain the primary anti-inflammatory medication for eczema. Topical corticosteroid is available in various formulations (creams, ointments, lotions, or foams) and intensities (mild, moderate, strong, or super-potent). [298] Age, disease severity, and the location of eczema lesions all influence the patient's choice of the topical corticosteroid. In general, super-potent topical corticosteroids are not advised to treat eczema. [297] A once-daily application of a powerful Topical corticosteroid preparation is adequate to cure flares regularly, often for 3-5 days. [297] As soon as the flare's initial warning indications arise, a Topical corticosteroid should be initiated.

Itching is vital for determining how well a treatment works; therefore, weaning off should not start before the itch has subsided. [297] In low-severity cases, good SCORAD values around 15-20 are typically achievable with a monthly dosage of mild or moderate topical corticosteroids in the average range of 15g in infants, 30g in children, and up to 60 to 90g in adolescents and adults, along with extensive emollient usage. [297]

The belief of intolerance or resistance to topical therapies for eczema is often confused with insufficient topical corticosteroid application. It is difficult to use an upper limit to identify resistance because quantitative guidelines for topical prescription are rarely used. [286] Patients frequently use too few topical steroids, giving the impression that it has no effect. [69] The most beneficial strategy to use topical corticosteroids is during eczema flares; early intervention paired with subsequent emollient skincare to stabilise the flare can help to prevent steroid-related adverse effects of prolonged use of topical corticosteroids. [299] The negative effects of inadequate dosage could be worse than those of topical corticosteroid therapy. [300] To reduce the likelihood of causing systemic side effects topical steroids with short half-lives, such as prednicarbate or mometasone, reduces have been used. [69] Use of high-potency TCS for an extended period should be avoided, and instead, well-conducted systemic treatment may be safer and more beneficial for eczema. [69] Nevertheless, it is recommended to use topical corticosteroids alongside other treatment modalities (i.e., emollients).

2.9.2.2. Topical calcineurin inhibitors

Topical calcineurin inhibitors exhibit good anti-pruritic and anti-inflammatory properties while not having the side effects of corticosteroids, such as skin atrophy. [69] As such, they benefit patients who require long-term treatment in occlusive and sensitive skin areas. [298] While their anti-pruritic benefits are partially related to a particular impact on TRPV1 neurones in the skin; the anti-inflammatory effects are the consequence of a decrease in the generation of proinflammatory cytokines by mast cells and T cells. [301] A moderate potency topical corticosteroid has the same anti-inflammatory potency as 0.1% tacrolimus ointment. [302] However, a 1.0% pimecrolimus cream has clearly less potency. [303] Topical calcineurin inhibitors do not induce Skin atrophy. [303] Hence, It is recommended to use it as the first-line in sensitive body parts (like the eyelids), with tacrolimus preferred for long-term topical treatment and pimecrolimus preferred for mild eczema and severe eczema. [69]

2.9.2.3. Other topical treatments

One of the first treatments for eczema is the topical application of coal tar, which has a long clinical history but a steadily deteriorating clinical efficacy. [286] y activating the aryl hydrocarbon

receptor (AHR) and dephosphorylating STAT6, coal tar may prevent the Th2-mediated downregulation proteins in the skin barrier, reducing spongiosis, apoptosis, and CCL26 expression in eczema lesions. [304] Another topical treatment is related to the topical application of phosphodiesterase four inhibitors. Through the breakdown of cyclic adenosine monophosphate, phosphodiesterase 4 (PDE4) plays a crucial role in controlling the generation of inflammatory cytokines in eczema. [305]

2.9.3. Phototherapy

Phototherapy is a topical therapeutic option for eczema patients' skin lesions, itching, and insomnia with up to six-month remission times and no known harmful short-term adverse effects. [306] Several issues related to eczema are improved by phototherapy, including the suppression of Langerhans cells' ability to present antigens, the induction of apoptosis in infiltrating T cells and the induction of antimicrobial peptides. [307] A reduction in the colonisation of Malassezia species and S. aureus,[308] and a decreased ability to present antigens as a result of thicker stratum corneum. [309] Natural and artificial lights can be used, and the concurrent use of nb-UVB or medium-dose UVA with topical corticosteroids or a systemic drug is recommended. [69]

2.9.5. Systemic therapy

If eczema cannot be effectively managed with suitable topical and other therapies, a systemic therapy is required. In comparison to high doses for wide body areas over extended periods to control their eczema, it can be beneficial to reduce the overall dose of corticosteroids by using systemic corticosteroids. [310] Systemic corticosteroids' risk/benefit ratio is mostly unfavourable despite their quick effectiveness. [286] Due to the numerous long-term adverse effects, systemic corticosteroids 1-2 weeks is the maximum period of usage for severe acute exacerbations. [69] Another systemic therapy for both children and adults is cyclosporine A. [311] Its downside is that it necessitates regular blood pressure monitoring and renal impairment symptoms due to its narrow therapeutic index. [69] Similar systematic immunosuppressive treatments are methotrexate, azathioprine and Mycophenolate mofetil, but their action begins slowly (8 to 12 weeks). [286] Fast-acting medications called Janus kinase inhibitors to block various eczema pathways are very promising for treating adolescent and adult eczema. [69]

A different type of systemic therapy is known as biologics which targets type 2 immune responses. In the United States and Europe, dupilumab was authorised as a first-line treatment for moderate eczema in individuals 12 years of age and older. [69] It is an entirely human monoclonal antibody that inhibits IL-13 and IL-4 signalling by binding to the IL-4R. The phase 2 and phase 3

testing of numerous more biologics are underway, such as fezakinumab (anti-IL-22), tralokinumab (anti-IL-13), nemolizumab (anti-IL-31R), Tezepelumab (anti-TSLP), and etokimab (anti-IL-33). [5]

2.9.3.1. Systemic Antihistamines

In acute eczema flares, systemic antihistamines that inhibit the histamine 1 receptor (H1R) are routinely used to reduce itching. However, evidence suggested that they only had a marginal or non-existent impact on reducing pruritus in eczema. [69] For that reason, its use is not recommended.

2.9.5. Antimicrobial therapy

Eczema patients are more vulnerable to secondary skin infections, especially those caused by S. aureus, Malassezia furfur, and the herpes simplex virus. [5] Most antibacterial interventions aim to reduce the burden of S. aureus. If clinical symptoms of severe colonisation or bacterial superinfection are present, topical antiseptic medications and antiseptic baths should be considered in addition to sufficient anti-inflammatory treatment. [69] Topical antibiotic use for an extended period is not advised due to the possibility of heightened resistance and sensitisation. [69] In eczema patients with extensive and severe S. aureus infection, a brief course of systemic antibiotics, like first generation cephalosporin, could be prescribed. [69]

More commonly than in healthy people, eczema patients get viral infections with the herpes varicella zoster virus, simplex virus, smallpox virus, coxsackie virus, and molluscum contagiosum virus, which have the propensity to cause disseminated extensive disease. [312] It is recommended that all regional immunisation schedules be followed. Encouraging parents of children with eczema to immunise them thoroughly is vital. When eczema herpeticum is suspected, it must be treated without delay and with systemic antiviral medication, such as acyclovir. [69]

Malassezia spp., despite being a commensal on healthy human skin, is thought to play a pathogenic role in eczema because it may interact with barrier function and the skin's local immune system. Superoxide dismutase and thioredoxin proteins from humans and fungi have been shown to have molecular similarities, which may play a role in eczema pathogenesis. [313] Experts encourage that patients with eczema at head and neck locations, especially those with known IgE-sensitization to Malassezia spp., should consider antifungal treatment with either topical ketoconazole or ciclopirox olamine. [69]

2.9.6. Final considerations

One of the most typical reasons for treatment failure is poor adherence to the prescribed regimen. Therefore, the key to successfully treating eczema patients is actively using adherence-improving methods. [314] Patient features and attitudes, therapy effectiveness and duration, delivery methods, and disease chronicity are all factors that affect treatment adherence. Additionally, the

strength of the doctor-patient relationship, particularly the time the doctor has available for the patient and "shared decision-making," is crucial. [314]

In children, there are special considerations to be made. It is recommended that we pay closer attention to the tolerability, safety and hypoallergenic qualities of topical ingredients for treating and managing eczema in children, especially those under the age of two. Only skilled professionals should start a systemic treatment in children. [69]

Summary: Current treatment and management of eczema

- The main objective of eczema treatment is long-term control.
- The use of topical emollient therapy, avoid allergens based on a complete medical history and allergy testing, and choose topical anti-inflammatory medication is all part of standard eczema treatment.
- There are several new eczema medications that have recently become available in older children and adults, and further treatments are currently being developed
- Eczema patients are more prone to skin infections.
- Therapeutic patient education for kids, their parents, and adult eczema patients ensures and supports medical treatment since it is secure and cost-effective

2.10. Summary of the current knowledge and gaps

Eczema was reported to affect 5 to 20% [132] of children and 2 to 10% [1,133] of adults internationally. As such, it is a common disease that frequently manifests in infancy and can persist into adulthood. [171] For that reason, eczema is the leading contributor to skin-related disability-adjusted life years. [52] It impacts the quality of life and economic burden on the sufferer and family. Hence, identifying eczema risk factors and phenotypes can lead to prevention strategies that are likely to reduce its significant clinical and public health impact. Despite the mounting evidence of potential risk factors and eczema subtypes, the literature assessment of the available material revealed that there are still many places where understanding is lacking and eczema during adulthood is understudied and poorly understood. The concept of “eczema phenotypes” has been suggested, rather than a simple binary classification of eczema, as such phenotypes can incorporate the variability of eczema development and progression. [12] The classification of eczema phenotypes could identify risk factors and enhance understanding of patterns associated with eczema onset and progression. Moreover, the literature suggests a potential association between ambient air pollution and eczema; however, there are conflicting results. Also, the association between air pollution and eczema and challenge proven food allergy has not been assessed before. A better understanding of the potential effects of ambient air pollution on eczema and food allergy development may lead to

targeted interventions to prevent eczema and food allergy. Furthermore, there is a lack of evidence related to hard water association with eczema and a distinct lack of studies assessing this potential association in adults. Finally, occupational exposures may be important in the eczema pathological pathway. My doctoral work focused on addressing the highlighted knowledge gaps.

Chapter 3 - General Methods

I used data from two sizable Australian cohort studies and from one large cohort study from the UK. The Melbourne Atopy Cohort Study (MACS) provided data for one of my doctoral papers [315], while the Tasmanian Longitudinal Health Study (TAHS) provided the information for two research papers [316] and the UK (United Kingdom) Biobank provided data for one research paper. [317] In the following sections, I initially give a brief overview of the MACS, TAHS and UK Biobank followed by details on specific follow-ups and methodologies that are pertinent to my doctorate work.

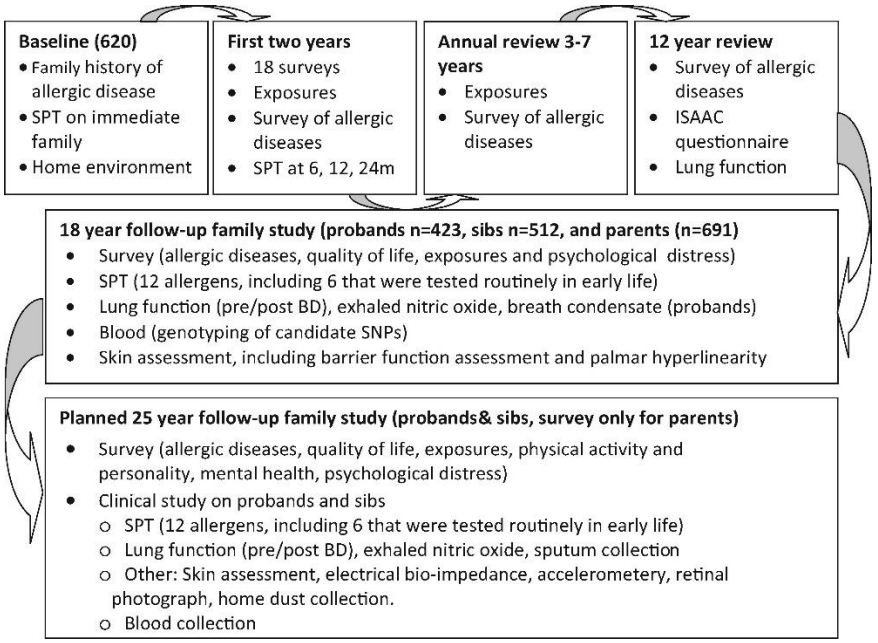
3.1. The Melbourne Atopy Cohort Study (MACS)

3.1.1. MACS summary

The Melbourne Atopy Cohort Study (MACS) began as a randomised controlled trial (RCT) of infant milk formulas but has since transitioned into a study of a birth cohort and their families. Eligible infants were required to have a first-degree relative with a history of asthma, eczema, allergic rhinitis, or a severe food allergy. Both the results chapters and the methods section refer to this group of infants as "probands". [315] MACS investigators recruited pregnant women who lived in Melbourne, Australia and a total of 620 probands born between 24 March 1990 and 1 November 1994 were enrolled in the study. [315] Following birth, data were collected at 27 distinct times, up to age 25 years (Figure 3.1.).

For my analyses, MACS was treated as an observational birth cohort study. Participants th recruited originally for a randomised controlled trial can be included in a cohort and its data used to test additional hypotheses concerning the relationship between baseline exposures and outcomes that were observed and measured during prospective follow-up. This practice is predicated on the verifiable hypothesis that the randomised intervention won't have an impact on the newly discovered associations of interest. [318] Although this cohort started as a RCT, prior research from this cohort demonstrated that the randomisation status (infant formula allocation) does not affect the association estimates if the randomisation is not related to the outcome of interest. [319]

Figure 3.1. MACS recruitment and follow-ups, the 25 year follow-up family study has now been completed. (Source: A. Lowe, et al.;2016) [315]



3.1.2. Study sample and data collection

After the initial RCT at baseline the study became a cohort study with several follow-ups (Figure 3.1.). [315] The aim of MACS was to supply data for the evaluation of the risk factors for asthma and allergies in childhood. [315] The baseline and all the follow-ups of MACS were relevant to this doctoral work.

At the baseline study, 620 probands and 1851 family members were involved. Expectant parents were asked to answer questions about their occupation, level of education, place of birth, place of residence, age, marital status, and smoking habits. The questionnaire also included details about allergy and illnesses in parents and siblings (Figure 3.1.). Additionally, details on social security benefits and exposures in the home environment (i.e. the type of flooring, heating system, and cooking methods) were collected.

After the baseline assessment and following the birth of the child, an allergy-trained nurse contacted the proband's parents by telephone and conducted a survey, from when the infant was aged 4 weeks, every four weeks until 64 weeks, then another survey at 18 months and at 2 years for a total of 18 follow-ups during the course of their first two years of life (Figure 3.1.). Two primary questionnaires were used throughout . The first questionnaire asked about when probands started eating various foods and Information about food reactions was documented. The second questionnaire gathered data on the emergence of allergic and atopic diseases, other newborn illnesses or conditions, contacts with medical personnel, and medication use. Then, when the infant was 6, 12, and 24 months old, clinical visits for skin prick testing (SPT) were conducted.

From 3 to 7 years of age, a yearly telephone survey was conducted for a total of 5 follow-ups. A thorough questionnaire was used to gather data on the socioeconomic situation of the probands' parents. A different questionnaire gathered data on food responses, immunizations, upper respiratory tract infections, other forms of infections, and respiratory/allergic illnesses. Probands were then invited to participate in a clinical evaluation and a survey at the age of 12 years. SPT was performed on the majority of the probands. Questionnaires given out during the 3 to 7 year follow-ups were again used at 12 years. In addition, data on allergies and asthma were gathered using a questionnaire based on the International Survey of Asthma and Allergy in Childhood (ISAAC). [74]

When probands were aged 18 years, they, along with siblings and parents were invited to participate in a thorough clinical evaluation. Regarding allergy and respiratory disorders, the same questionnaires that were used in the 12-year follow-up were also used in the 18-year follow-up. More specific data about the home environment, domestic exposures, smoking history, quality of life, education level, career activities, and general health and activity of the probands, siblings, and parents were gathered in addition to general questionnaires about allergies and respiratory diseases.

Finally, the MACS 25-year follow-up was recently completed. A similar survey to the 18-year-old follow-up where data on quality of life, exposures, allergic diseases, physical activity and mental health was collected. Also, there was a clinical visit where SPT was performed.

3.1.3. Data from MACS used in the analysis

3.1.3.1. Survey data from all time points

The specific survey data from MACS that was used in this doctorate work is described in Chapter 4 which involves a published research article. Overall, the survey data included sociodemographic characteristics, occupation, residence, health service use, medical diagnoses, smoking, reproductive histories, and allergic disease symptoms.

3.1.3.2. Distance to major roads

Distance to major roads was defined as straight-line distances from each parent-reported residential address during the first year of life of the probands to the nearest major road. The Public Sector Mapping Agencies Australia identified major highways as those with transport hierarchy codes 301 or 302 for the states of Victoria, Tasmania, Queensland, and New South Wales. Major roads included those that carried "huge traffic" and were classified as freeways, highways, arterial roads, and sub-arterial roads. [320] The busiest types of roads in Australia fall under this category. The participants were categorised into two groups: (1) living less than or at 150 m and (2) living further than 150 m. [321]

3.1.3.3. Nitrogen dioxide (NO_2)

A technique called land-use regression (LUR) can increase the precision with which air pollution exposure is estimated in epidemiological investigations. [322] The majority of LUR models are created for specific cities, which restricts their applicability to other sites. [322] A group of researchers using satellite measurements of tropospheric nitrogen dioxide (NO_2) columns in conjunction with other predictor factors created a model to predict NO_2 concentrations with countrywide coverage of Australia. [322] They projected the average ambient NO_2 concentrations measured by a national monitoring network from 2006 to 2011 using a generalised estimating equation model. [322] The best monthly model explained 76% of the spatial variation in NO_2 while the best annual model explained 81% of the variation. [322] They used this algorithms to forecast NO_2 concentrations at the approximately 350,000 census mesh blocks (each contains 30 to 60 dwellings) nationwide (a mesh block is the smallest spatial unit in the Australian census). [322] Average national concentrations, population-weighted, ranged from 7.3 ppb (2006) to 6.3 ppb (2011). [322] This data was linked to the participants from the MACS based on participants' geocoded addresses at baseline.

3.1.3.4. Skin prick tests (SPTs)

At 6, 12, 24 months and 12 years, standardised extracts (Bayer, Spokane, WA, USA) were used to test for aero allergen sensitization (rye grass, house dust mite and cat dander); and food allergen sensitization (egg white, cow's milk and peanut). [323] At 18 and 25 years, the skin prick test (SPT) panel was expanded by two food allergens: shrimp and cashew (ALK Abelló, Horsholm, Denmark) and three aero allergens: mixed grass, *Alternaria* and *Penicillium* (Hollister-Stier, Spokane, WA). [324] At both 18 and 25 years, histamine was employed as a positive control and normal saline was used as the negative control. For the test to be performed, the participant was in a comfortable position with their forearms or back at a convenient height. [325] A patient was given an explanation of the procedure, given reassurance if appropriate, and asked about any medications they were currently taking. Prior to the treatment, patients had to refrain from using skin moisturisers, antihistamines, and other medications that might interfere with the test. [325] The most common application site in older children and adults was the volar surface of the forearm, outer upper arm, and in infants, the back. [325] To identify the allergen, the locations for skin pricks were marked with numbers. Pricks were made right next to the numbers to prevent confusion between allergens. [325] To prevent overlapping reactions and false-positive results, the allergen pricks were at least 2 cm apart. The SPTs were all carried out by skilled research professionals. Perpendicular wheal diameters were measured in millimetres after 10 to 15 minutes of the prick, and an average was calculated. A positive control or allergen wheal of at least 3 mm in size was required for a valid SPT. A wheal size

of at least 3 mm bigger than the negative control (at 18 and 25 years) was regarded to be a positive SPT and to be a sign of allergic sensitisation. [325]

3.1.3.5. Atopic dermatitis quickscore (ADQ)

During the 18 year follow-up ADQ scores [326] were collected. The participant was asked to score the severity of the skin on various body areas in lay terms in the first section of the ADQ. [326] These evaluations follow an ordinal scale, with 0 denoting clear, 1 dry, 2 scaly, 3 redness, 4 fissures or apertures, and 5 denoting gushing. [326] Each body part, including the head, neck, trunk, arms, hands, legs, and feet, has its highest reported number recorded. [326] An ADQ skin severity score ranging from 0 to 35 is possible by adding the highest ratings for each body part. [326] The second part of the ADQ requests an assessment of the pruritus intensity for each of the same body locations (0 = not at all, 1 = a little, 2 = moderately bothered, 3 = rather bothered, 4 = very bothered, and 5 = highly affected/losing sleep). [326] The ADQ pruritus score, which has a possible range of 0 to 35, is obtained by adding the highest ratings for each body part. [326] The ADQ total score, which has a maximum achievable score of 70, is the sum of the skin severity and pruritus scores. [326]

3.1.4. Statistical analysis

The specific statistical methods related to MACS are described in Chapter 4 which involves a published research article. Overall, the statistical analyses involved longitudinal latent class analysis, logistic regression, and multinomial logistic regression.

First, longitudinal Latent Class Analysis (LLCA) aims to fit a model to longitudinal data including measured data such as eczema prevalence which are considered to be incomplete indicators of a set of unobserved latent classes. [327] The primary assumption of LLCA refers to the fact that any relationships between the observed variables are caused by the unobserved latent classes. [328] LLCA is a parametric mixture method because the likelihood of the observed or measured variables is a weighted sum, or mixture, of the probability of the variables for each latent class. [327] The prior probabilities of each latent class, or the likelihood of observing that class in the entire population, make up the mixture's weights. [327] LLCA accounts for correlations between repeated measures of eczema prevalence for each child when defining the classes. [329] The participants assigned to each subclass are alike to each other in regards to the indicator variables used (eczema prevalence), and the subclasses ought to correspond to groups of similar subjects. [328] Hence, latent classes are identified without exploiting the information on the time order of the observations, but also without forcing any parametric relationship between the repeated measures of eczema prevalence and time. [327] Latent classification starts with a single latent class and more classes are determined until the model fit measures, Akaike information criteria (AIC) and Bayesian information criteria (BIC), were optimised. [327] LLCA groups participants into classes with others who have comparable longitudinal

response patterns over a range of discrete time periods. [330] LLCA requires that the data is gathered at the same specific times (i.e. age). [330] Each participant should ideally be the exact same age at the time of each measurement. [327] In latent class analysis, proper class assignment is not guaranteed because class assignment is based on probabilities, the exact number or percentage of sample members within each class cannot be determined. [331] The model calculates two crucial measures: (1) the posterior probability of latent class membership for each participant and (2) the conditional probability of each variable's response within each class in order to construct the latent classes. [327] Details on how the participants were classified are provided in Chapter 4.

Second, logistic regression models involve a binary (0,1) outcome by maximum likelihood; it fits the possibility (odds ratio) of a positive response (1) given a set of covariates or regressors. [332] The maximum likelihood estimation is an iterative process. The "null" or "empty" model, or a model with no predictors, is represented by the log likelihood in the first iteration, known as iteration 0. The predictor(s) are incorporated into the model at the subsequent iteration. Since the objective is to maximise the log likelihood, the log likelihood grows with each iteration. The model is said to have "converged" when there is very little variation between iterations, at which point iteration ceases and the results are shown. [332] The natural logarithm of the odds is used by the logistic regression model as a regression function of the predictors. [332] When there is only one predictor, X, this has the formula ln[odds(Y=1)]=0+1X, where ln denotes the natural logarithm, Y denotes the outcome, and Y=1 when the event occurs (as opposed to Y=0 when it does not), 0 denotes the intercept term. [332] As such, 1 denotes the change in the logarithm of the probability of the event with a one unit change in the exposure or predictor or the regression coefficient. [332] By taking the exponential of 1, we can derive the ratio of the odds (the odds ratio) corresponding to a 1-unit change in X since the difference in the logarithms of two values is equal to the logarithm of the ratio of the two values. [332] Third, multinomial regression model is used when the outcome has more than two levels or categories with not evident order. [333] Similar to logistic regression, multinomial regression uses maximum likelihood estimation. [333] The multinomial logit model estimates k-1 models, where k is the number of levels in the outcome variable, and this is a key feature. [333] The standard interpretation of the multinomial logit states that because the parameter estimates are relative to the referent group, it is expected that for a unit change in the exposure variable, the logit of outcome m relative to the reference group will change by its respective coeficient estimate (which is in log-odds units), assuming the variables in the model are held constant. [333]

3.1.5. Ethics

The Mercy Maternity Hospital Ethics Committee approved the initial phases of the study up to the 12-year follow-up, and the 18 and 25-year follow-ups were approved by the University of

Melbourne and the Royal Children's Hospital Ethics Committees; all participants provided written informed consent. The secondary data analyses involving my PhD work is included within the ethics approval.

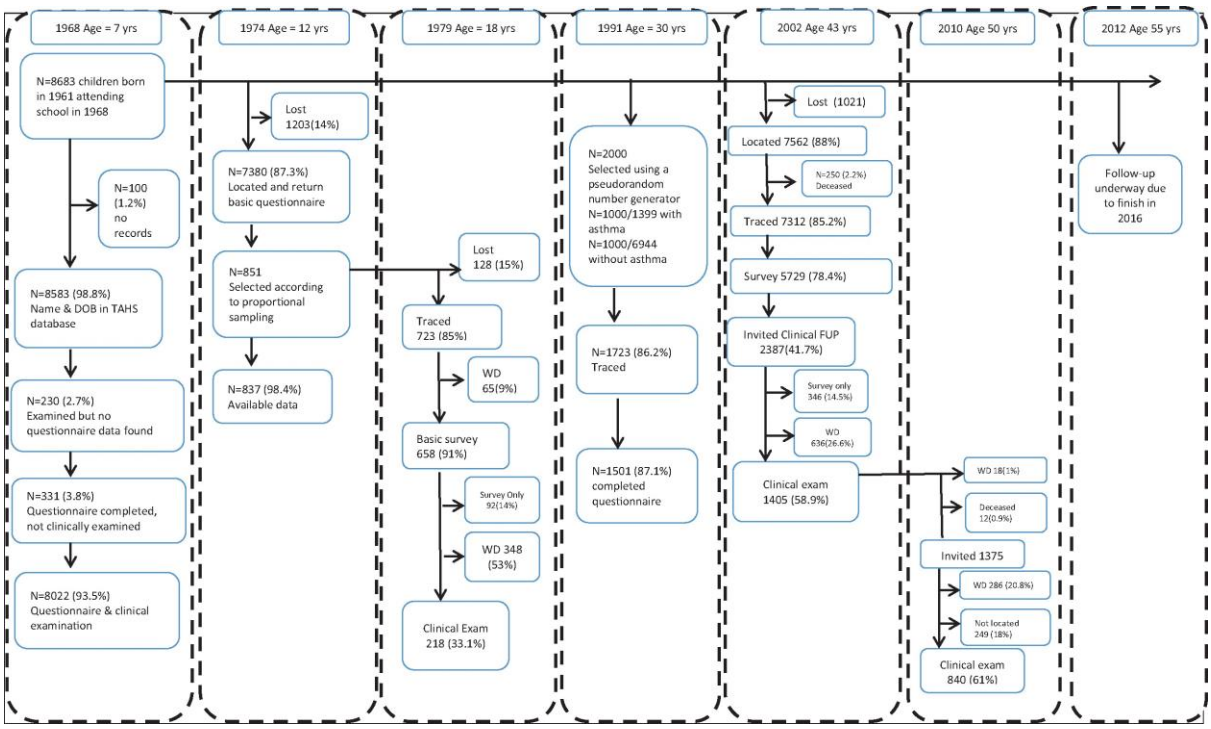
3.2. Tasmanian Longitudinal Health Study (TAHS)

I give a summary of the TAHS in this section and explain the relevant follow-ups and methods in length. Most of the data from the TAHS relevant to my doctoral work came from the TAHS fifth- and sixth-decade follow-ups. I used data from the fifth- and sixth-decade follow-ups when I assessed the association between air pollution and eczema prevalence and incidence in middle aged adults in Chapter 6. Likewise, I used data from the sixth decade follow-up to assess occupational exposures as risk factors for eczema prevalence in middle aged in Chapter 8.

3.2.1. TAHS summary

The aim of the TAHS was to assess the development and progression of asthma in children. [316] All Tasmanian schoolchildren born in 1961 and were 7 years old at the time of the baseline survey in 1968, were invited to participate in the study. At the baseline survey, questionnaires on both parents and all siblings were also conducted. As such, the TAHS among the longest running and largest family studies of respiratory disease in the world. [316] Since its inception, there were several rounds of follow-ups in 1974, 1981, 1992, 2002 and 2012 (Figure 3.2.).

Figure 3.2. TAHS recruitment and follow-ups. (Source: M. Matheson, et al.;2016) [316]



3.2.2. Study sample and data collection

The baseline and the follow-ups relevant to this doctoral work are described below. Likewise, I describe the methods of collection of relevant data: air pollution land use regression models, allergic outcomes and lifetime work calendar.

3.2.2.1. The baseline study in 1968

Study sample

Children that were 7 years old and from Tasmanian schools were recruited in 1968, their name was collected from the School Health Service files and private schools in Tasmania. The probands' age was chosen to ensure that the kids could perform spirometry well. All Tasmanian students who were enrolled in Tasmanian schools were send a survey with a letter to the child's parents outlining the survey and requesting their consent for their child to participate. To encourage the parents to cooperate and to swiftly return the completed surveys, there was publicity through television and press. These participants who were initially enrolled on the baseline were referred to as "probands".

Data collection

The schools gave surveys to a total of 8,683 children, this survey included questions on their medical history and that of their family (including parents and siblings). The parents of 8,583 (90.8%) of the children gave their consent and responded the survey. [316] Afterwards, the children were encouraged to take part in a medical examination with a focus on spirometry and symptoms of the upper and lower respiratory airways were collected with a questionnaire. The generated spirograms were read by two independent observers. The children's weights and heights were recorded using bathroom scales that underwent three-daily calibration. To ensure dependability, the measurements of height and weight were both collected by two observers. Parallel to the spirometry, school medical officials conducted a standard medical examination of the kids, placing particular emphasis on any clinical abnormalities in the upper airways, chest, and lungs. The results of the completed surveys were not known prior to the completion of these medical examinations.

3.2.2.2. Proband 2002 study

Since baseline, a number of follow-ups have been performed in TAHS but these were not relevant to my doctorate work. The proband 2002 study provided the baseline for the analyses performed in Chapter 6.

Study sample

The initial goal of this follow-up was to locate the residential addresses of the 8,583 probands who were recruited at the baseline and mail survey questionnaires to everyone who could be reached in the 2002–2008 period. [334] Since the baseline survey, a survey of the entire cohort has

not been done. Several national databases were employed for the aim of this linkage in order to determine the probands' present addresses. [334] First, the alive status of the probands was updated by the Australian Institute of Health and Welfare (AIHW) through data linkage with the National Death Index. Then, the Commonwealth electoral roll database was used to match the probands' data throughout the course of three rounds. In order to identify a change in surname, the female participants who were untraceable during the initial round of electoral record data checks were subsequently linked to the Tasmanian marriage records. The electoral roll was then used to link the identified female participants once more. [334] Then, the probands were sent mailouts by the Health Insurance Commission on behalf of TAHS, and, if the probands replied to the mailouts, their contact information was provided to TAHS investigators. Similarly, the Australian twin registry, which is a volunteer registry of more than 30,000 pair of twins, contacted identified twins on behalf of TAHS. [334] If probands could not be located using the methods explained before, the address information gathered during the 1991 survey was compared against the national electronic white pages telephone directory to see if it was current. [334]

Data collection

A postal survey was sent to 7,562 (88.1%) of the original proband cohort, and 5,729 (78.4%) of them provided responses to the survey (participants had an average aged of 42.8 years). The self-administered postal survey included information on sociodemographic traits, occupation, place of residence, use of health services, medical diagnoses, smoking, and past histories of pregnancy. [316] Of those that participated, 2,373 probands took part in a laboratory investigation. [334] From those, 59% (n=1,405) of individuals who were invited took part in a laboratory visit. In this visit, blood samples were taken for the purpose of genetic testing, skin prick testing was performed, and genetic analyses were done. Moreover, 1,372 (97.7%) of the 1,405 probands who underwent clinical evaluation and attendance had pulmonary function tests.

3.2.2.3. Proband 2012 study

Study sample

The sixth decade follow-up of TAHS was carried out from 2012 to 2016, and all live probands that could be traced were contacted and offered to participate. A total of 6,128 (71%) of the original probands who had valid contact information and were invited to participate in this comprehensive self-administer survey and clinical investigation. The study's participants had a mean age of 53 years old.

Data collection

There were 3,609 participants who responded a self-administered survey which included information on sociodemographic traits, occupation, place of residence, use of health services,

medical diagnoses, smoking and past histories of pregnancy. A self-selected subset of 2,659 (74%) had a laboratory visit which included skin prick testing.

3.2.3. Data from TAHS used in the analysis

3.2.3.1. Survey data from 1968, 2002 and 2012 proband study

The specific survey data from TAHS that was used in this doctorate work is described in Chapter 6 and Chapter 8 which involves two published research articles. Overall, the survey data included sociodemographic characteristics, occupation, residence, health service use, medical diagnoses, smoking, reproductive histories, and allergic disease symptoms.

3.2.3.2. Skin prick tests (SPTs) in the 2012 proband study

SPTs were carried out using a standard protocol. Eight aeroallergens were tested: cat, *Cladisporium species, Alternaria tenuis, Penicillium mix, Aspergillus fumigatus, perennial ryegrass*, and a mixture of grasses (including Kentucky bluegrass, Orchard, Redtop, Timothy, Sweet vernal grass, and Meadow fescue) (Hollister-Stier, Spokane, WA, USA). Also, four food allergens: milk, egg, peanut and shrimp. The SPT methods have been explained in detailed on section 3.1.3.4..

3.2.3.3. Distance to a major road (DMR)

Using ArcGIS 10.1, the distance in metres to the closest main road was calculated for each proband's residential address (ArcGIS 10.1, Redlands, CA: Environmental Systems Research Institute). Major roads were defined the same as in section 3.1.3.2..

3.2.3.4. Nitrogen dioxide (NO_2)

The land use regression method was described in section 3.1.3.3.. The NO_2 data from the LUR models[322] was linked to the participants from the 2002 proband study and 2012 proband study based on participants' geocoded addresses at each follow-up.

3.2.3.5. Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$)

Australia has low fine particulate matter ($PM_{2.5}$) concentrations and a somewhat varied range of potential sources of this air pollutant. Knibbs, et al. determined continental-scale LUR models with global geophysical estimates of $PM_{2.5}$ (SAT-$PM_{2.5}$). [335] These estimates were obtained by connecting satellite-observed aerosol optical depth to ground-level $PM_{2.5}$. [335] A group of investigators created four LUR models for the year 2015 using local land-use predictors and global SAT-PM2.5 data (10 km grid) (two satellite-based, two nonsatellite-based). [335] The researchers assessed model performance at 51 separate monitoring sites that weren't used for model development. [335] The most spatial variability in $PM_{2.5}$ was best explained by a LUR model that incorporated the SAT-$PM_{2.5}$ predictor variable (along with six other variables). [335] While local land-use predictors boosted the utility of global SAT-PM2.5 estimates, including improved characterisation of within-city gradients, SAT-$PM_{2.5}$ estimates also improved LUR model

performance. [335] As such, the reliability of LUR modelling at the continental scale for estimating $PM_{2.5}$ exposure in Australia was confirmed. [335] As with the NO_2 data, this data was linked to the participants from the 2002 proband study and 2012 proband study based on participants' geocoded addresses at each follow-up.

3.2.3.6. Lifetime work history calendar

The lifetime work history calendar data was gathered in the questionnaire during the 2012 proband studies. It includes information on thorough job histories, such as a list of all positions held during the course of a person's career, the nature of each position, the name and description of the industry, and the beginning and ending dates or years. Using only the job title allows for large-scale analysis, is relatively inexpensive, and is a simple way to get the occupational data. [336] However, data quality is not guaranteed by job title-based occupational information only. [337] As a result, it's important to gather data on subjects' specific tasks/activities and employment history in order to assign the most suitable job classification and related occupational exposures. Determining the coding's quality is crucial to ensuring accurate job classification and minimising bias. [336] In a previous study, [338] the 2012 lifetime work history calendar was quality checked and assigned an International Standard Classification of Occupations-88 (ISCO-88) four-digit classification for each participant. [339] Using a job exposure matrix, these data were used to assign cumulative and current occupational exposures to the participants during the 2012 proband study.

3.2.4 Statistical analysis

The specifics statistical methods related to TAHS are described in Chapter 6 and Chapter 8 which involve published research articles. Overall, the statistical analyses involved logistic regressions, multinomial logistic regressions and likelihood ratio tests. Logistic and multinomial regression methods have been described in section 3.1.4..

Likelihood ratio tests were performed to test for interactions in the assessed associations in Chapter 6. Likelihood ratio tests assess the distinction between nested models. [340] The first model may be formed by putting constraints on the second model's parameters, the second model is said to be nested in the first. [340] As such, one model includes a set of parameters (variables), and the second model has all of the first model's parameters plus one or more additional variables (interaction). [340] Hence, the null hypothesis that the parameter vector of a statistical model fulfils a smooth restriction can be tested. [340]

3.2.5. Ethics

TAHS has been approved by the Human Research Ethics Committee of the University of Melbourne; all participants provided written informed consent. The secondary data analyses performed for my PhD work is included within the ethics approval.

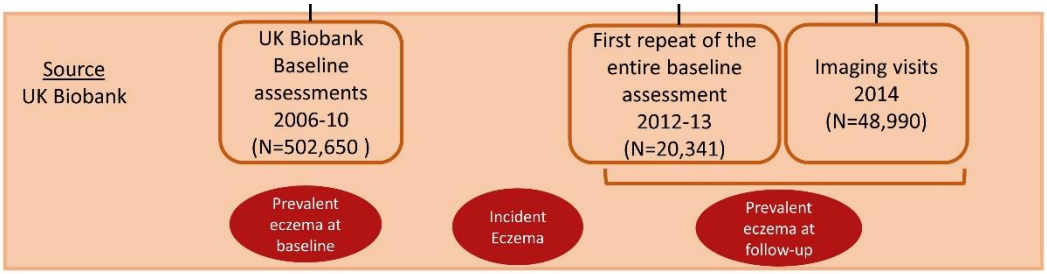
3.3. UK (United Kingdom) biobank

I provide an overview of the UK biobank and explain the relevant follow-ups and methods in detail. All data from the UK biobank relevant to my doctoral work came from the baseline assessments, the first repeat of the entire baseline assessments, and the Imaging visits. These data were used to assess whether domestic hard water was associated with eczema prevalence in adults. The results are described in Chapter 7.

3.3.1. UK biobank summary

The UK biobank is a very large population-based prospective cohort examining chronic disease risk factors, prevention, diagnosis, and treatment on a national scale. [341] The UK biobank aimed to be as representative as possible of the general population in the UK aged 40 to 69 years. After the baseline assessment (~500,000 participants) there were two follow-ups carried out by much smaller subsamples that were relevant to my research. These were: the first repeat of the entire baseline assessment (~20,000), and the Imaging visits (~50,000) (figure 3.3.).

Figure 3.3. UK biobank data sources relevant to this doctorate work. (Source: D. Lopez, et al.;2022) [342]



3.3.2. Study sample and data collection

The baseline and the follow-ups relevant to this doctoral work are described below. Similarly, I explained the methods of data collection: survey and hard water concentration data.

3.3.2.1. The baseline assessment

Study sample

All people who were registered with the National Health Service, aged 40 to 69 years and lived not more than 25 miles from one of the 22 research evaluation centres in Scotland, England, or Wales were invited to join the study. [317] A total of 502,650 participants (with a roughly even number of men and women) were recruited at baseline between 2006 and 2010. [317]

Data collection

The participants provided extensive baseline questionnaire data, physical measures, and biological samples. [317] Through a touchscreen questionnaire, participants submitted comprehensive demographic, socioeconomic, and health-related information, including their prescription histories. [317] Then, a nurse-led interview was conducted to cover general medical history. All positive disease

reports from the touchscreen questionnaire were asked again during the nurse led interview to increase accuracy.

3.3.2.2. First repeat of the entire baseline assessments

Study sample

The first repeat of the entire baseline assessments was carried out from August 2012 to June 2013 at Stockport, UK. Participants were invited by email or letter if they resided within 25 kilometres of the assessment centre, and 21% of those participants accepted the invitation. This follow-up involved a small subset of the baseline assessment sample; approximately 20,000 participants. [343]

Data collection

Participants attended a repetition of the baseline assessment visit, which involved collecting data on each participant's health and lifestyle, and other factors through a touchscreen questionnaire, a quick verbal interview, and a variety of physical assessments. [343]

3.3.2.3. Imaging visits

Study sample

The imaging visits were based on the rationale that to obtain the strongest evidence in certain diseases it was estimated that around 100,000 of the 500,000 UK Biobank individuals should complete an imaging visit. [344] The imaging sub-study started in 2014 and it is still ongoing but data on 48,999 participants have been already collected. [345] These data were included for the analyses in Chapter 7.

Data collection

Participants attended an imaging visit which included undergoing different imaging techniques for the carotid arteries, the brain, the heart, the entire body, the bones, and the joints. [344] By 2022, it is intended to scan 100,000 people, with another 10,000 participants undergoing repeat scans two to three years later. [346] Furthermore, repeated measures of the data collected at baseline are being collected. [345]

3.3.3. Data from UK biobank used in the analysis

3.3.3.1. Survey data

The specific survey data from UK biobank that was used in my PhD is described in Chapter 7 which involves a published research article. Overall, the survey data included information on the participant's socioeconomic status, health status (including eczema prevalence), and demographics. [317]

3.3.3.2. Note on international Classification of disease version 10 data (ICD-10)

I elected not to include ICD-10 codes L20 (eczema or atopic dermatitis) as a part of the definition of eczema for two reasons as stated by UK biobank documentation (available at: https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/first_occurrences_outcomes.pdf). First, the code lists have not been reviewed by clinicians or externally validated and should thus be thought of as a first pass to identify cases with any given condition. The diagnostic ICD and Read codes mapped to each 3- character ICD10 code have not been individually curated to pick out individual conditions sensitively or specifically. Second, as with all administrative data (which are subject to a range of potential biases and risk of being incomplete), the presence of a code should not be assumed to infer a confirmed diagnosis. Similarly, its absence does not guarantee that the participant was not affected by the health outcome, especially where the data source is missing (i.e. primary care for some participants) or the code was present but there was an invalid date, e.g. an event date before date of birth.

3.3.3.3. Hard water concentrations

Local water companies in the UK regularly assess the quality of domestic water and store the data which the public can access. The freedom of information act involves the disclosure of information held by governmental authorities or by persons providing services for them, [347] like water companies. A request for information disclosure may be made to any number of public organisations in accordance with the Act. [347] I made freedom of information [347] requests to 16 water companies in the UK, these were: Affinity water, Anglian Water, Bournemouth water, Bristol Water, Dŵr Cymru Welsh Water, Essex & Suffolk Water, Northumbrian Water Limited, Portsmouth water company, SES Water, South East Water, South West Region UK, Thames water, United Utilities, Wessex water, Yorkshire water and Scottish water. I requested from each company the data related to hard water annual concentrations, from 2005 and 2013, as Calcium carbonate concentrations from tap water at a postcode level. This data was then linked to the participants based on participants' geocoded approximate location at baseline and at follow-up using QGIS Geographic Information System (version 3.16.16. QGIS Association, 2021).

3.3.4. Statistical analysis

The specific statistical methods related to UK biobank are described in Chapter 7 which involves a published research article. The statistical analyses involved multilevel logistic regression analysis and logistic regression as a sensitivity test. Logistic regression methods have been described in section 3.1.4..

Multilevel logistic regression is suitable to study hierarchical data, where individual observations are nested within higher levels of classification. [348] In my study, the UK Biobank data was arranged in a

natural hierarchy, with observations at the individual level nested at a postcode level. As hierarchical data is composed by dependent observations, it is not correct to analyse with standard logistic regression approaches as the units of analysis are treated as independent. [349] The standard errors of regression coefficients will be underestimated as a result of failing to recognise hierarchical structures, which will cause an overestimation of statistical significance. [349] Multilevel logistic models can divide the outcome variation (random intercept) and predictor variance (random slope) into within- and between-unit components. [349] Furthermore, with multilevel logistic regression a researcher can assess the general contextual effects (i.e., the variation of the outcome by postcodes). I included measures of Intra cluster correlation and median odds ratio to measure the variance. The median odds ratio (MOR) describes the postcode-level variance on the odds ratio scale, as the median value of the distribution of odds ratios estimated when two participants with the same characteristics are selected from two different postcodes, comparing the one from the higher prevalence postcode to the one from the lower prevalence postcode. [350]

Finally, the estimated odds ratios from a multilevel logistic regression model are conditional on the random effect on postcodes being held constant. [348] Therefore, they are postcode-adjusted associations between water hardness and eczema outcomes. [348] An alternative way to interpret these estimates is using the population-average odds ratio, which is the average odds ratio comparing two participants from two different neighbourhoods who are the same in other covariates (apart from the covariate of interest). [348] It is possible to obtain estimated odds ratios that is not conditional to the random effect by using shrinkage factors to assess the estimated odds ratios. [351] If the intra cluster correlation is low the Shrinkage factors will be close to 1 which means that both the estimated odds ratios conditional on the random effects and the estimated population-average odds ratio are almost identical.

3.3.5. Ethics

The Northwest Multi-centre Research Ethics Committee has given UK Biobank its ethics approval. It has also requested permission from the Patient Information Advisory Group (PIAG) in England and Wales to obtain data that would allow UK biobank to invite potential participants. The data linking and analysis parts of this project were approved by the University of Melbourne Ethics Committees (ref: 2020-20674-13212-3).

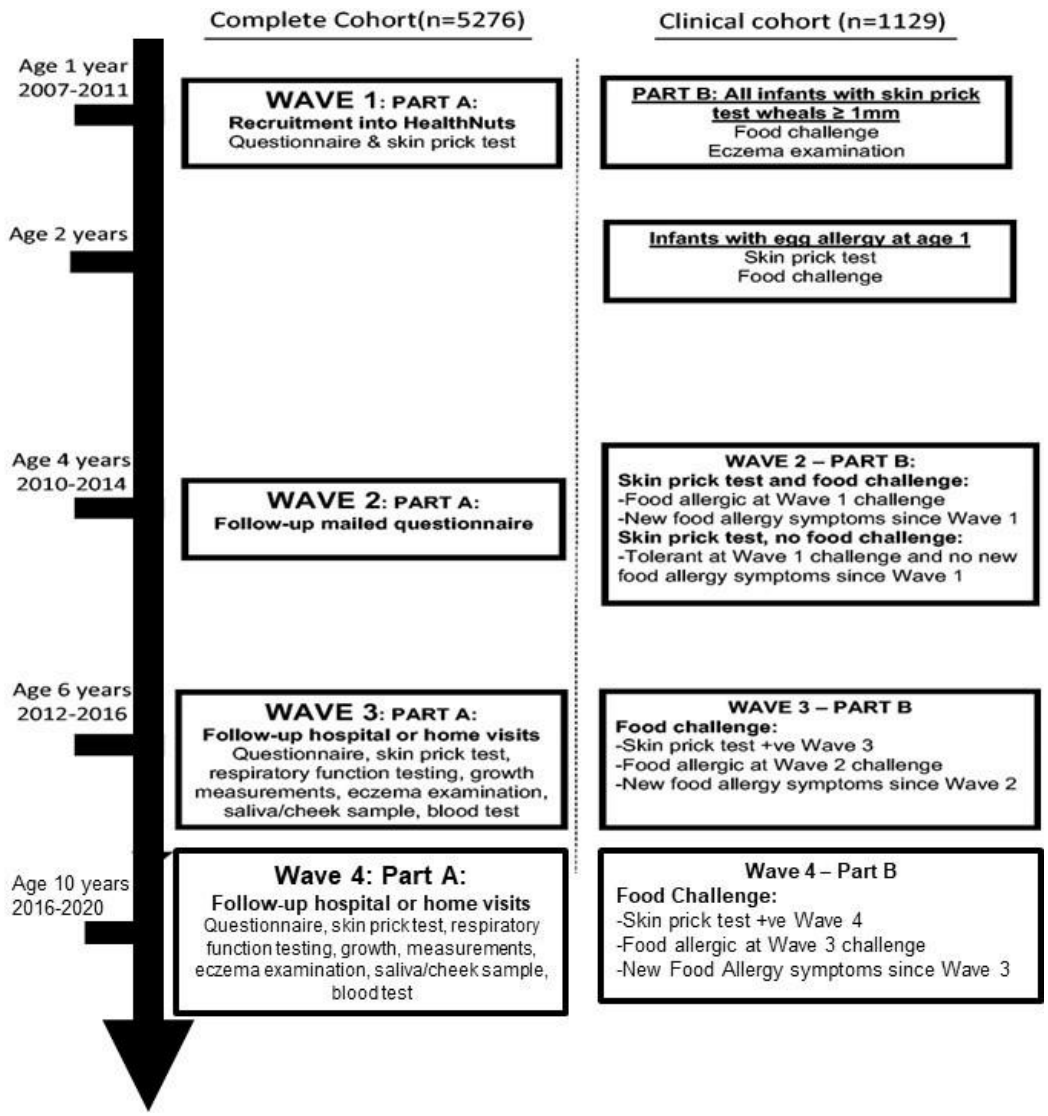
3.4. The HealthNuts study

I give an overview of the HealthNuts study and clarify the relevant methods and waves in detail. All the waves are relevant to my thesis, and these are Wave 1 to 4. I used this data to determine whether air pollution is associated to the development and persistence of eczema and food allergy across the first ten years of life. The results are described in Chapter 5.

3.4.1. The HealthNuts study summary

The HealthNuts study is a population-based, multi-wave, single-centre, prospective cohort examining the prevalence, causes and burden of allergic diseases (particularly food allergy) in early life. [352] The Wave 1 (Age 1 year) consisted of 5,276 infants recruited at 11 to 15 month-old from 138 council-run immunization sessions where they underwent skin prick testing screening. Children with a >=1mm skin prick test (section 3.1.3.4.) to a food allergen (peanut, egg white, sesame, and either cow's milk or shrimp) were invited to attend a food challenge at Melbourne's royal Children's Hospital. [352] A total of four waves were carried out (Figure 3.4.), parental questionnaires captured information on demographic factors, disease status and environmental exposures, including objective measures of food allergies (challenge-proven food allergy).

Figure 3.4. The HealthNuts study waves. (Adapted from Source: J. Koplin, et al.;2015)[352]



3.4.2. Study sample and data collection

The HealthNuts waves that were relevant to this doctoral work are described below. I explain the details related to the study sample and data collection.

3.4.2.1. Age 1 year (Wave 1)

Study sample

The study is established in Melbourne, Australia. Infants were enrolled between September 2007 and August 2011 when they arrived at immunisation clinics in greater metropolitan Melbourne and its immediate surroundings (within 70 km of the central business district) for their drop-in vaccination session. [352] In Victoria, vaccination coverage at age 1 is high (92% in 2007), and around half (46%) of newborns are inoculated at sessions conducted by local governments. [352] The HealthNuts study recruited participants from 138 locations after screening over 170 immunisation session sites. [352] All infants receiving their regular 12-month vaccines and between the ages of 11 and 15 months (inclusive) were eligible for recruitment (mean age 12.7 months), 5276 infants were recruited (74% participation). [352]

Data collection

A SPT was performed to four common food allergens at the time of recruitment (peanut, egg white, sesame, and either cow's milk or shrimp). After 15 minutes, the wheal size was measured, and the average wheal determined. Prior to hearing the SPT results and during the 15 minutes wait, the parents filled out a questionnaire. The questionnaire included information on the diet of the mother and the child, home address, family's history of allergic disease, exposure to pets and tobacco smoke, history of migration, and parents' country of birth. [352] Any infant with a detectable wheal (>=SPT 1 mm) to peanut, egg, or sesame to was invited to undergo an oral food challenge (OFC) to confirm food allergy.

3.4.2.2. Age 4 years (Wave 2)

Study sample

All enrolled children were asked to complete a survey at the age of 4 years, 4291 children participated. A brief telephone survey was given to parents if they did not complete the full questionnaire In addition, those participants with a history of food allergy or those reporting new food reaction suggestive of food allergy were invited to attend a clinical visit. [353]

Data collection

A similar questionnaire to the baseline assessment was completed by the participants. This questionnaire involved collecting the participant's health, socioeconomic and lifestyle data. In addition, 592 participants who had begun to have new symptoms of food allergy since Wave 1 and

those who had a history of food allergy at age 1 year were invited to participate in a clinical visit for evaluation, which involved a skin prick test and possibly an oral food challenge. [353]

3.4.2.3. Age 6 years (Wave 3)

Study sample

All the participants from the cohort were asked to carry out a health assessment and a questionnaire. [354] The questionnaire was completed by the parents and a health assessment was done. [352] A brief telephone survey was given to parents if they did not complete the full questionnaire. Moreover, permission from the participants' parents or legal guardians was sought for linking their study records with health registries.

Data collection

The questionnaire collected information on demographic characteristics, allergen exposure history, allergic disease and food allergy information and socioeconomic data. Physical measures, spirometry, and skin prick tests for food and aeroallergens were all a part of the comprehensive health evaluation. [353] Children with food allergies from prior waves, those reporting recent food reactions that might indicate an IgE-mediated food allergy, and those who had recently developed new food sensitivities but weren't eating or tolerating the index food were all offered oral food challenge. If the participant consented, its records were linked to Medicare/Pharmaceutical Benefits Scheme (PBS), Australian Childhood Immunisation Register (ACIR), School Entrant Health Questionnaire data and the Victorian perinatal database. [353]

3.4.2.4. Age 10 years (Wave 4)

Study sample

All participants from the original cohort who were contactable were invited to participate in the wave 4. This wave involved a questionnaire and health assessments.

Data collection

Participants completed a questionnaire and health assessments involving skin prick test and oral food challenge. The same data that was collected in wave 3 was collected again in this wave.

3.4.3. Data from the HealthNuts used in the analysis.

3.4.3.1. Questionnaire data

The specific survey data from the Healthnuts study that was used in my PhD is described in Chapter 5 which involves a research manuscript draft. Overall, the questionnaire data included information on the participant's demographics, home postcode, allergic status and parents' characteristics. [317]

The participants' postcode was used to assign them an Australian Bureau of Statistics' Statistical Areas Level 2 (SA2) code. The SA2 is a geographical region used for the collection and

dissemination of census data in Australia. SA2 are generally based on officially gazetted suburbs and localities. They are smaller than the ABS's Statistical Areas Level 1 and larger than the ABS's Mesh Blocks. The ABS's SA2s are used in the production of a range of ABS statistics, including population estimates and projections, and labor force data. The SA2 represent an economic and social society and have a population average of 10,000 people. [355]

3.4.3.2. Skin prick tests and oral food challenge

In waves 1 and 2, skin prick tests (SPT) were carried out on the infant's back using single-tine metal lancet. The tested food allergens included: egg, peanut, sesame, and either shrimp or cow's milk (after the first 2000 participants shrimp was replaced by cow's milk). Participants with detectable SPT (>=1mm) and/or a specific IgE level of 0.35 kU/L or greater were offered an oral food challenge (OFC) confirmation (egg, peanut, sesame, and either shrimp or cow's milk) at age 1 unless there was a known history of acute food allergy. [356] At wave 2, SPT was initially performed on children who had a food allergy but had a negative OFC at age 1 year and those who had reported symptoms of a new food allergy. If SPT showed signs of sensitisation and there was either no history of ingesting and developing tolerance or a history of ingesting and experiencing a reaction (if this reaction occurred recently, then the child was deemed allergic without OFC), participants were offered OFC to these foods (egg, peanut, sesame, and either shrimp or cow's milk). OFC required gradually increasing doses on day one in the hospital and continued eating of the maximum day one dose (2 teaspoon of peanut butter or tahini paste or 1 whole raw egg white) and continuing to do so on days two through seven. [357] The cessation criteria for a positive OFC result was determined by having at least one of this conditions within 2 hours of ingestion: (1) three or more noncontact hives occuring simultaneously that continue for at least five minutes, (2) perioral or periorbital angioedema, (3) vomiting, or (4) anaphylaxis.[357] If the participant had a positive OFC at age one, they had another OFC at age four to determine whether their food allergy had persisted or resolved. [352]

In waves 3 and 4, SPTs for aero and food allergens were performed using SPT. [353] Food sensitization was determined by a specific IgE level of 0.35 kU/L or greater and/or a SPT wheal size of 3 mm or larger. [353] Children with food allergies from prior waves, those reporting recent food reactions that might indicate an IgE-mediated food allergy, and those who had recently developed food sensitivities but weren't eating or tolerating the index food were all offered OFC. [353] In waves 3 and 4, the OFC protocol, although with larger dosages of food allergens, and stopping criteria was the same as in Wave 1.

3.4.3.3. Food allergy

At age 1 and age 4 assessments, food allergy was determined by a positive test of sensitization (specific IgE level (sIgE) of 0.35 kU/L or greater and/or SPT wheal of 2 mm or larger) and a positive OFC or recent reaction concordant with OFC positive result. [352]

Food allergy at age 6 and 10 years was characterised as per Table 3.1. and informed by sensitisation history, OFC and clinical signs and symptoms. [353]

Table 3.1. Food tolerance and food allergy definitions from 6- and 10-year-old children. [353]

Group	Food-tolerant	Food allergy		
		Definite food allergy	**Probable food allergy**	**Possible food allergy**
Assessment group*	•SPT result < 3 mm •Negative OFC result at age 6 y or 10 y •SPT result of 3-7 mm and ingestion history indicative of tolerance (had eaten the index food >3 times, no recent reactions, and not avoiding the index food; alternatively, negative OFC result at age 4 y)	•Positive OFC result and sensitised •Reaction consistent with IgE-mediated food allergy within the previous 12 mo and sensitised (no OFC) •SPT result ≥ 8 mm at age 6 y or 10 y and positive OFC result at age 4 y (no OFC at age 6 y or 10 y)	•SPT result ≥ 8 mm (no OFC at age 4 y or age 6 y or 10 y, no known tolerance) • SPT result of 3-7 mm and either a positive OFC result at age 4 y or a reaction consistent with IgE-mediated food allergy since age 4 y	N/A
Questionnaire only group†	Full questionnaire: child is eating the index food, and no reported allergic reactions Short questionnaire: child is not avoiding the index food because of allergy and no recent reactions to the index food	N/A	N/A	Parent-reported reaction consistent with IgE-mediated food allergy since age 4 y or parent report that the child is avoiding the index food because of an allergy

Children were classified into each group on the basis of meeting any of the listed criteria.
N/A, Not available.
∗Completed full assessment at age 6 or 10 years.
†Completed only questionnaires at age 6 or 10 years (no SPT data available).

3.4.3.4. Nitrogen dioxide (NO_2)

The land use regression method was described in section 3.1.3.3.. The NO_2 data from the LUR models [322] was linked to the participants based on participants' geocoded addresses at each wave.

3.4.3.5. Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$)

The land use regression method was described in section 3.2.3.5.. The $PM_{2.5}$ data from the LUR models [335] was linked to the participants based on participants' geocoded addresses at each wave.

3.4.4. Statistical analysis

The specific statistical methods related to the HealthNuts are described in Chapter 5 which includes a manuscript draft. The statistical analyses involved multilevel logistic regression analysis (section 3.3.4) and logistic regression as a sensitivity test (section 3.1.4.).

3.4.5. Ethics

The Office for Children (reference number CDF/07/492), the Victorian Department of Human Services (reference number 10/07), and the Royal Children's Hospital Human Research Ethics Committee all granted their clearance for the study (reference nos. 27047 and 32294). Parents or guardians provided their informed consent on behalf of the participants.

Chapter 4 - Establishing subclasses of childhood eczema, their risk factors and prognosis.

4.1. Section introduction

In this published paper, I aimed to investigate eczema heterogeneity by performing a longitudinal latent class analysis using 24-time points in the first 12 years of life. Subsequently, I performed regression analyses to assess the relevant early life risk factors and allergic outcomes at 18 and 25 years old. It was essential to determine eczema subclasses as the disease has a wide range of clinical and epidemiological variations, suggesting underlying phenotypes. Also, the subclassification provides a better understanding of eczema development and progression as there is uncertainty on the potential risk factors associated with eczema development and how eczema progresses to other allergic diseases. This paper is based on data from the MACS baseline and all its follow-ups up to 25 years of age. It contains participants' sociodemographic factors, economic variables and disease status.

4.1.1. Research question

- What are the longitudinal eczema subclasses according to eczema prevalence in early life and childhood?
    - How are these longitudinal eczema subclasses associated with eczema risk factors?
    - How are these longitudinal eczema subclasses associated with allergic outcomes in adulthood?

4.1.2. Research aim

- To determine the longitudinal eczema subclasses according to eczema prevalence in early life and childhood.
    - To assess the associations between risk factors and these eczema subclasses
    - To determine which longitudinal eczema subclasses are most strongly associated with allergic outcomes in adulthood.

4.1.3. Critical reflection on the work

For this project, I learned several new statistical analyses in theory and practice, which took me several weeks of web searching and many meetings with my supervisors. Once the analyses and manuscript were complete, my supervisors and I selected a journal, and I started the submission process, which was another learning process on its own. Looking at this project in hindsight, I realise that I could have done things faster if I had been more proactive at the time. However, it took me a

long time because I was developing my self-learning skills and trying to be more independent than when I was a master's student.

4.1.4. Publication citation

Lopez DJ, Lodge CJ, Bui DS, Waidyatillake NT, Abramson MJ, Perret JL, Su JC, Erbas B, Svanes C, Dharmage SC, Lowe AJ. Establishing subclasses of childhood eczema, their risk factors and prognosis. Clinical & Experimental Allergy. 2022 Sep;52(9):1079-90.

***Please note that the specific references from this accepted or submitted manuscript are at the end of this chapter and the in-text citation number restarts at 1.**

4.2. Author accepted version of the manuscript.

Title: Establishing subclasses of childhood eczema, their risk factors and prognosis.

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Conflicts of interest

D.J. Lopez has nothing to disclose. Dr N.T. Waidyatillake has nothing to disclose. Dr J.C. Su has nothing to disclose. Prof C. Svanes has nothing to disclose. Dr B. Erbas has nothing to disclose. Prof S. C Dharmage, A/Prof A.J. Lowe, Dr J. Perret, A/Prof C.J. Lodge, Dr D.S. Bui and Prof M.J. Abramson have received an investigator-initiated grant from GlaxoSmithKline for unrelated research. Prof M.J. Abramson holds investigator-initiated grants for unrelated research from Pfizer, Boehringer-Ingelheim and Sanofi. He has undertaken an unrelated consultancy for and received assistance with conference attendance from Sanofi. He has also received a speaker's fee from GSK. A/Prof A.J. Lowe has received in-kind contributions of emollients from PuraCap and Primus for use in eczema prevention trials that he leads.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Main text word count: 3484

Abstract

Background: The heterogeneity of development and progression of eczema suggests multiple underlying subclasses for which aetiology and prognosis may vary. A better understanding may provide a comprehensive overview of eczema development and progression in childhood. Thus, we aimed to determine longitudinal eczema subclasses based on assessments and identify their associations with risk factors and allergic outcomes.

Methods: A total of 619 participants with a family history of allergic disease were assessed at 24 timepoints from birth to 12 years. At each time, eczema was defined as the report of current rash treated with topical steroid–based preparations. Longitudinal latent class analysis was used to determine eczema subclasses. Subsequent analyses using regression models assessed the associations between eczema subclasses and potential risk factors and allergic outcomes at 18- and 25-years follow-ups (eczema, allergic rhinitis, asthma and allergic sensitisation).

Results: We identified five eczema subclasses “early-onset persistent”, “early-onset resolving”, “mid-onset persistent”, “mid-onset resolving” and “minimal eczema”. Filaggrin null mutations were associated with the early-onset persistent (OR=2.58 [1.09-6.08]) and mid-onset persistent class (OR=2.58 [1.32-5.06]). Compared with “minimal eczema”, participants from early-onset persistent class had higher odds of eczema (OR=11.8 [5.20-26.6]) and allergic rhinitis (OR=3.13 [1.43-6.85]) at 18 and at 25 years eczema (OR=9.37 [3.17-27.65]), allergic rhinitis (OR=3.26 [1.07-9.93]) and asthma (OR=2.91 [1.14-7.43]). Likewise, mid-onset persistent class had higher odds of eczema (OR=2.59 [1.31-5.14]), allergic rhinitis (OR=1.70 [1.00-2.89]) and asthma (OR=2.00 [1.10-3.63]) at 18 and at 25 years eczema (OR=6.75 [3.11-14-65]), allergic rhinitis (OR=2.74 [1.28-5.88]) and asthma (OR=2.50 [1.25-5.00]). Allergic and food sensitisation in early life was more common in those in the persistent eczema subclasses.

Conclusion: We identified five distinct eczema subclasses. These classes were differentially associated with risk factors, suggesting differences in aetiology, and also with the development of allergic outcomes, highlighting their potential to identify high risk groups for close monitoring and intervention.

Word count: 298

Keywords: Eczema, latent class analysis, genetic, food allergy

Key messages

- Five distinct eczema subclasses were identified, differentially associated with risk factors and allergic comorbidities
- Filaggrin null mutation and early allergic sensitisation were associated with the persistent eczema subclasses.
- Persistent eczema subclasses were associated with high allergic comorbidities at age 18 and 25 years.

Abbreviations used:

ADQ (Atopic dermatitis quick scores)

AIC (Akaike information criteria)

ANU (Australian National University)

BIC (Bayesian information criteria)

EOP (early onset persistent)

EOR (early onset resolving)

LCA (Latent class analysis)

LLCA (Longitudinal latent class analysis)

MACS (Melbourne Atopy Cohort Study)

MOP (mid onset persistent)

MOR (mid onset resolving)

NO_2 (Nitrogen dioxide)

RCT (Randomised controlled trial)

SPINK5 (Serine peptidase inhibitor, Kazal type 5)

SPT (Skin prick test)

Introduction

Eczema, also known as atopic dermatitis, is a chronic relapsing skin inflammatory disease characterised by defective skin barrier function. [1] The prevalence of eczema is around 20% in children and ranges from 5 to 10% in adults, [2] although reported prevalence varies widely between regions. *Filaggrin (FLG)* null mutations cause a skin barrier flaw which is associated with eczema. [3] However, this genetic factor is not an essential cause for eczema, which can develop through other biological pathways. [4] Eczema has a heterogenous presentation with different levels of severity, treatment response, age of onset and tendency to evolve to other atopic diseases. [5]

There may be different pathophysiological pathways for the development of eczema, which have not yet been fully characterised. The age of onset, severity and persistence of eczema are most likely determined by complex interactions between environment and genetic factors. [2] For that reason, the concept of "eczema phenotypes" has been suggested, rather than a simple binary classification of eczema, as such phenotypes can incorporate the variability of eczema development and progression. [6] The classification of eczema phenotypes could allow for the identification of risk factors and enhance understanding of patterns associated with eczema onset and progression.

Latent class analysis (LCA) is a parametric mixture method which can identify distinct underlying groups based on patterns over time. [7] Five studies have explored this heterogeneity in eczema onset and persistence during early childhood using LCA and its association to other allergic outcomes. [7-11] However, these studies assessed cross-sectional associations of eczema subclasses and a limited number of risk factors and comorbidities at 6[9], 8[10], 10[8], 12, 16[7] and 26[11] years. These findings need to be replicated by assessing eczema subclasses targeting high-risk children with multiple early eczema assessments to draw firmer conclusions and obtain a comprehensive overview of eczema development and progression in childhood. Furthermore, assessing the longitudinal associations of a broad range of risk factors encompassing individual, environmental and genetic factors with eczema subclasses may provide a better understanding of which factors contribute to the development and persistence of eczema patterns

Thus, we aimed to determine longitudinal eczema subclasses based on eczema prevalence at 24 time points from birth to 12 years of age and identify their associations with risk factors and allergic outcomes up to 25-years of age using the Melbourne Atopy Cohort Study (MACS).

Methods

Study design and population

Eczema data collected from the MACS from birth to 12 years of age were used to develop subclasses. MACS is a longitudinal study of a high-risk birth cohort that originally began as a randomised controlled trial (RCT). [12] Eligible infants (n=620) had at least one first degree family member with a history of eczema, asthma, allergic rhinitis, and/or severe food allergy. The recruitment process has been described previously. [13] Eighteen telephone surveys were conducted in the first two years where the primary carer reported information on skin conditions every 4 weeks until age 15 months and one at each age 18 and 24 months. An annual survey from three to seven years was collected, and then again at 12, 18 and 25 years respectively. The Mercy Maternity Hospital Ethics Committee approved the initial phases of the study up to the 12-year follow-up, and the 18 and 25-year follow-ups were approved by the University of Melbourne and the Royal Children's Hospital Ethics Committees. All mothers provided written informed consent (up to 12 years) and participants provided consent at 18 and 25 years.

When the randomization factor in RCTs does not affect the outcome of interest, the study can be considered an observational birth cohort. [14] A previously published article from MACS using an intention-to-treat analysis showed no difference in allergic disease outcomes among the 3 randomised groups. [15] Even so, we tested infant formula allocation as a potential predictor and confounder.

Eczema data from birth to 12 years

Up to two years, eczema at each of the 18 time points was defined by parental report of persistent skin rash for more than a week in the last 4 weeks in the face, trunk, legs, arms and others (excluding rash that only affected the nappy region or scalp) and report of treatment with eczema medication for one or more days. From 3 to 12 years, eczema was defined as the parental report of one or more episodes of eczema in the last 12 months, located in the chest, back, stomach, legs or arms and medication used for eczema for one or more days.

Skin prick testing

At 6, 12, 24 months and 12 years standardised extracts (Bayer, Spokane, WA, USA) were used to test for food allergens (cow's milk, egg white and peanut); and aero allergens (house dust mite, rye grass, and cat dander). [16] At 18 and 25 years, the skin prick test (SPT) panel was expanded by two food allergens: cashew and shrimp (ALK Abelló, Horsholm, Denmark) and three aero allergens: mixed grass, *Alternaria* and *Penicillium* (Hollister-Stier, Spokane, WA). [17] A positive SPT was defined as at least one allergen wheal size of greater than 2mm average diameter for children under 2 years, and 3 mm for participants older than 2 years. [18] Atopy was defined by sensitization to at least one of the allergens tested. If the participant were sensitised to one or more aeroallergen, it was considered aero sensitisation; the same definition was used for food allergens and food sensitisation.

Early life risk factors

Early-life risk factors were collected during the baseline and the 4 weeks survey. These were chosen based on reported associations with early-life eczema in the literature. [2] We classified early life risk factors as parental, child or environmental. Parental factors examined were maternal and paternal history of eczema and allergy (i.e. history of eczema, allergic rhinitis or asthma), education (more than 15 years of education), occupation status defined using the Australian National University (ANU), [19] smoking history (ever or never). Child factors included sex, weight (kg) at 4 weeks, older siblings (number), firstborn status, breast feeding at 4 weeks, *FLG* null mutations [20] and *Serine peptidase inhibitor, Kazal type 5* (*SPINK5*) mutations. [21] Environmental factors at baseline were presence of a dog or a cat, type of fuel used in cooking and heating, distance to major roads [22] and annual concentration of nitrogen dioxide (NO_2) [23] at baseline (Supplementary text 1) Data after 4-weeks of age were not used as risk factors due to the difficulty determining the sequence between these exposures and the development of eczema.

Allergic outcomes at 18 and 25 years

Allergic outcomes at age 18 and 25 were determined from surveys and clinical assessments: Current eczema, allergic rhinitis and asthma were defined by reports of (a) one or more episode in the last 12 months or (b) a history of the condition and use of medication for the condition in the previous 12 months. Allergic sensitisation, aero sensitisation and food sensitisation were defined by the results of the SPT as defined above. Also, atopic dermatitis quick scores (ADQ) which measured eczema severity were collected on the 18 year follow-up. [24] We elected not to include eczema at 18 and 25 years in the LCA to enable us to specify the temporal sequence of childhood eczema subclasses and subsequent allergic outcomes in adulthood.

Statistical analysis

Longitudinal Latent Class Analysis (LLCA) was used to explore the heterogeneity and determine classes eczema from birth to 12 years of age, we modelled "age" as an indicator of times when eczema prevalence was observed, [25] a detailed description of the modelling approach is described in Supplementary text 1. [26-29] Starting with a single latent class, additional classes were generated to the eczema model until the Bayesian and Akaike information criteria were optimised. [30] We included participants for whom data were available for at least half (12) of the time points studied. In LLCA, each participant is not allocated to a single class, but rather, have a probability for belonging to each identified class. [31] For that reason, we classified participants in two ways: 1)

Participants were assigned to the trajectory class for which they had the highest posterior probability of membership and 2) Weights, equal to the probability of membership to each eczema subclass from the LLCA for each participant, were used in the probability-weighted regression [32] models to account for the uncertainty in class assignment.

After the eczema subclasses were developed, we first examined the associations between the eczema subclasses (eczema subclasses as outcome) and established risk factors (parental, genetic or child) using multinomial regression. Secondly, associations between eczema subclasses (eczema subclasses as exposures) and allergic outcomes at 18 and 25 years (eczema, allergic rhinitis, asthma and sensitisation) were estimated using logistic regression. Directed acyclic graphs (Supplementary text 2) were developed to assess hypothesised causal relationships and to determine which confounders to include in each model. All analyses were carried out using the statistical software Stata (release 16; Stata Corporation, College Station, TX).

Results

A total of 619 participants had eczema data available from at least 12 time points (Figure 4.1). There were high proportions of parental eczema and allergy history (Table 4.1). Most parents were tertiary educated. Most had an older sibling at 4 weeks of age and were exposed to heating and cooking by indoor combustion of solid or gas fuel.

Figure 4.1. Participant flow diagram

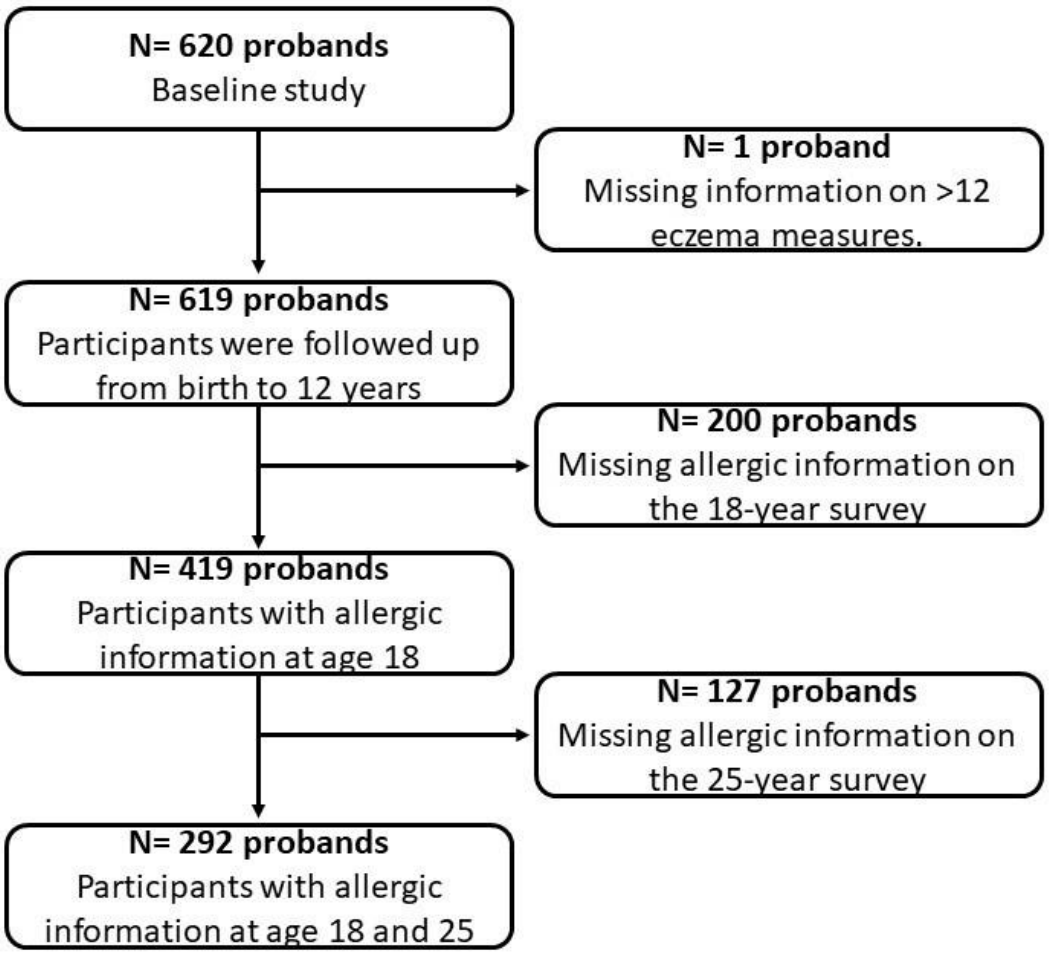


Table 4.1. Baseline characteristics of the MACS study cohort

Characteristic	Cohort (N=619)
Parental	%. (n/N)
Eczema history	54.5% (337/618)
Allergy history	89.0% (550/618)
Education (more than 15 years)	71.6% (443/619)
Occupation [ANU III score]† (Mean(SD))	46.0 (20.59)
Ever smoking	41.8% (258/617)
Child	
Female	49.1% (304/619)
Weight at 4 weeks [kg] (Mean(SD) Min-Max)	4.0 (0.6)
Older siblings (Median (IQR))	1 (1)
Firstborn status	40.1% (248/619)
Breastfeeding	89.4% (554/619)
FLG Null	12.1 % (52/429)
SPINK5‡	75.3% (323/429)
RCT allocation	
Standard cow's milk	33.1% (205/619)
Soy	33.6% (208/619)
Partially hydrolysed cow's milk	33.3% (206/619)
Environmental	
Presence of pets	49.3% (298/604)
Combustion heating §	75.1% (453/603)
Combustion cooking §	88.7% (535/603)
Distance to major roads <150m	23.3% (141/605)
NO_2 [ppb] (Median (IQR); 25-75%)	12.4 [3.39;10.9-14.3]

ANU: Australian National University SD: Standard deviation IQR: Interquartile range RCT: randomised controlled trial NO_2: Nitrogen dioxide ppb: parts per billion
†Continuous score 0-100
‡ Dominant G model
§ Gas, coal or wood

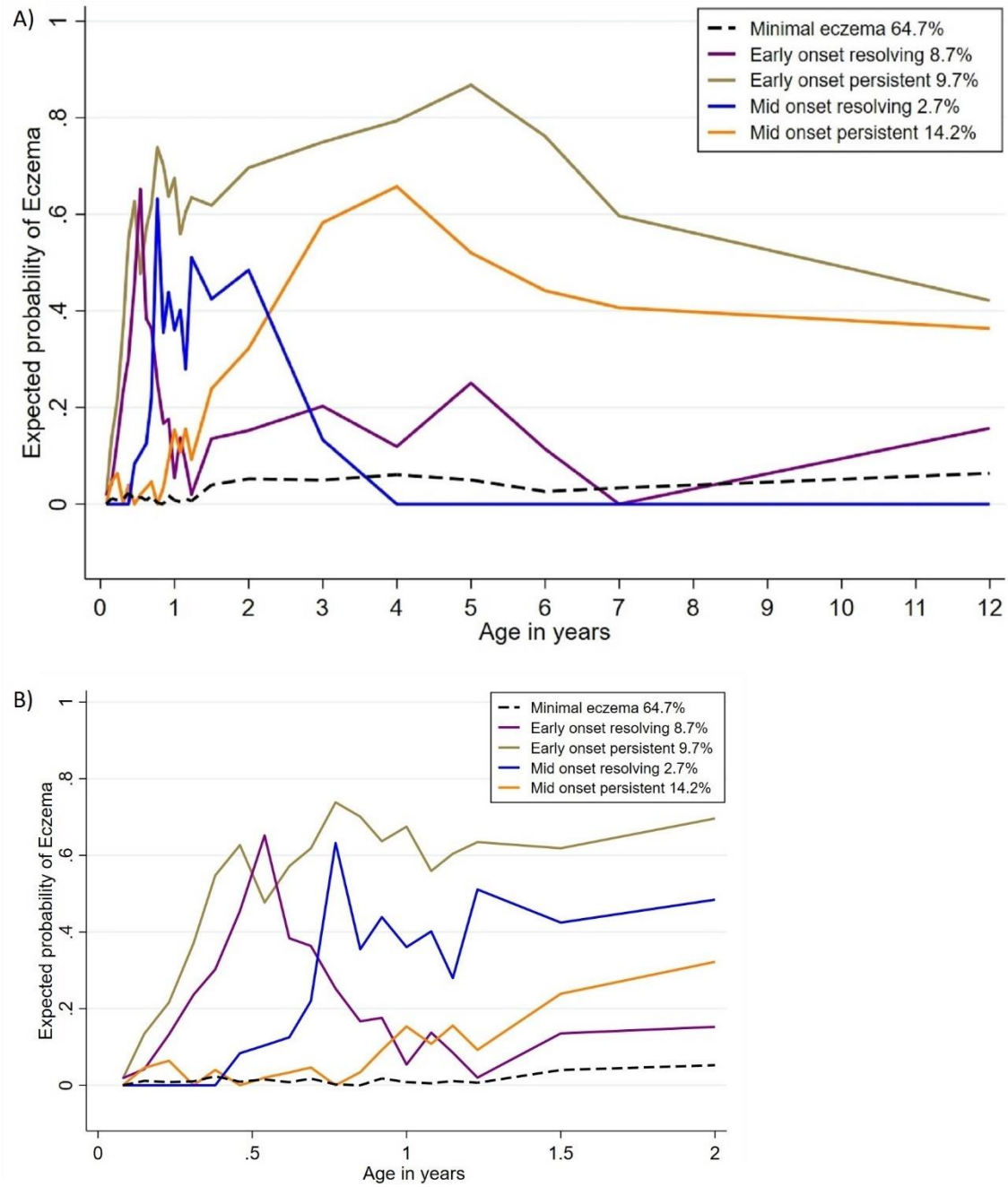
Information was missing for 200 probands (32.3%) in the 18-year survey and 327 (52.8%) in the 25-year survey. There were no important differences in the baseline characteristics between those followed and those lost to follow-up at 18 and 25 years, except that those lost were more likely to have parents who were not tertiary educated, who smoked, had a lower occupation score and were less likely to have been breast feed at 4 weeks (Table S4.1).

Eczema subclasses from birth to 12 years

A five-eczema class model was found to best fit the data (Table S4.2), a 6-class model was considered but did not reach convergence. These groups were labelled based on the pattern of prevalence of eczema (Figure 4.2) from birth to 12 years of age (A) and a zoom-in version of the same analysis is presented (B) because there were 18 time points from birth to 2 years. The "minimal eczema" subclass (64.7%, 419/619) was characterised by very low probability of eczema at all times, this group was used as the reference point for all analyses. The "early onset persistent" (EOP) eczema subclass (9.7%, 59/619) had a high probability of eczema early in life, that peaked at around 5 years of age, and continued to have a high eczema probability. The "early onset resolving" (EOR) eczema subclass (8.7%. 51/619) was characterised by a high probability of eczema in the first 6 months of life that then sharply declined. The "mid onset persistent" (MOP) eczema subclass (14.2% 74/619) had a low probability of eczema in the first year of life which increased thereafter. Finally,

the “mid onset resolving” (MOR) eczema subclass (2.7% 16/619) characterised by a sharp increase in cases at the one-year mark which started to decline at 2 years and completely resolved by 4 years. Furthermore, there were no important differences in the rates of loss to follow-up between the eczema subclasses.

Figure 4.2. Longitudinal trajectories of eczema subclasses from birth to 12 years of age.



A) Eczema subclasses from birth to 12 years B) Eczema subclasses in the first 18 time points from birth to 2 years

Early life risk factors

Early life risk factors distributions were different between the eczema classes (Table S4.3). Parental eczema history was associated with a two-fold increase in the odds of MOP (Table 4.2). Furthermore, higher occupation scores were associated with EOR. Overall, participants with *FLG* null

mutations were more likely to be associated with the persistent subclasses, both EOP and MOP. Participants with *SPINK5* G dominant mutation had a seven-fold increase in the odds of MOR. Female participants and those with parental allergy history had increased odds of MOP. Being firstborn was associated with decreased odds of EOP. Lastly, breast feeding at 4 weeks was associated with an approximately two-fold increase in the odds of MOP.

Table 4.2. Adjusted associations between eczema subclasses from 0 to12 years and early-life characteristics compared with the minimal eczema class.

Eczema subclasses†	Early onset persistent [EOP] (59/619)	Early onset resolving [EOR] (51/619)	Mid onset persistent [MOP] (74/619)	Mid onset resolving [MOR] (16/619)
Baseline risk factor	aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI) ‡
Parental factors‡				
Parental eczema history§	1.41 (0.79-2.51)	1.11 (0.64-1.91)	**1.98(1.28-3.08)***	0.89 (0.34-2.37)
Parental allergy history§**	1.35 (0.49-3.74)	1.70 (0.58-4.93)	0.90 (0.48-1.67)	2.39 (0.32-17.87)
Parental education (more than 15 years)	1.59 (0.80-3.16)	0.76 (0.39-1.46)	0.66 (0.42-1.05)	1.40 (0.47-4.20)
Occupation score per SD increase	1.04 (0.73-1.45)	**1.29 (1.00-1.69)**	1.01 (0.78-1.30)	0.89 (0.49-1.63)
Ever smoking	0.99 (0.90-1.70)	1.08 (0.99-1.17)	0.96 (0.89-1.04)	1.01 (0.84-1.21)
Child factors				
Female¶	0.79 (0.46-1.34)	1.15 (0.68-1.93)	**1.68 (1.12-2.54)**	0.59 (0.24-1.44)
Weight at 4 weeks (1 kg increase)	0.85 (0.52-1.39)	0.78 (0.50-1.23)	1.03 (0.69-1.52)	1.05 (0.48-2.34)
Older siblings	0.74 (0.49-1.11)	1.27 (0.90-1.78)	0.96 (0.69-1.33)	0.70 (0.34-1.43)
Firstborn status	**0.35 (0.15-0.82)**	1.46 (0.63-3.35)	0.64 (0.32-1.26)	0.32 (0.08-1.41)
Breast feeding	1.22 (0.47-3.14)	0.68 (0.30-1.53)	**2.13 (1.03-4.42)**	1.73 (0.62-4.82)
FLG Null¶	**2.58 (1.09-6.08)**	1.22 (0.44-3.38)	**2.58 (1.32-5.06)**	1.23 (0.17-9.07)
SPINK5¶	1.37 (0.62-3.02)	1.37 (0.66-2.84)	1.22 (0.70-2.14)	**7.02 (3.23-32.37)**
Environmental factors				
Presence of pets	1.00 (0.58-1.72)	1.12 (0.66-1.91)	0.98 (0.65-1.49)	1.21 (0.49-2.95)
Heating by combustion	2.22 (0.69-7.17)	0.53 (0.25-1.11)	1.05 (0.52-2.12)	0.61 (0.17-2.18)
Cooking by combustion	0.84 (0.44-1.61)	0.74 (0.41-1.34)	0.91 (0.55-1.50)	1.89 (0.54-6.61)
Distance to mayor roads	0.75 (0.39-1.47)	0.79 (0.41-1.52)	0.87 (0.53-1.44)	0.52 (0.13-2.14)
NO_2 (ppb) per unit increase	1.20 (0.87-1.66)	1.11 (0.87-1.42)	0.86 (0.64-1.15)	1.01 (0.65-1.58)

aMOR: adjusted multinomial odds ratio 95%CI: 95% confidence interval SD: standard deviation Kg: kilogram ppb: parts per billion

†Reference category: Minimal eczema

‡Adjusted multinomial odds ratio: sex, parental education, parental occupation, parental smoking.

§Further adjusted for: cooking by combustion of solid or gas fuel, heating by combustion of solid or gas fuel, NO_2, DMR, *FLG* and *SPINK5*, presence of dog or cat, older siblings, RCT: allocation, firstborn status and parental smoking history

¶Univariable association estimates

* P-values less than 0.05 are bolded

** all participants had at least one family member with a history of allergies

Allergic disease outcomes at 18 and 25 years

The prevalence of allergic diseases at 18 and 25 years varied widely between the identified eczema subclasses (Table S4.4). Particularly, members of the EOP subclass had higher odds of all subsequent allergic outcomes at ages 18 and 25 years (Table 4.3). Likewise, members of the MOP had high odds of eczema, allergic rhinitis and asthma at 18 and 25 years.

Table 4.3. Adjusted associations between allergic outcomes at 18 and 25 years and eczema subclasses from 0 to 12 years.

Eczema subclasses†	Early onset persistent [EOP] (38/419)	Early onset resolving [EOR] (37/419)	Mid onset persistent [MOP] (54/419)	Mid onset resolving [MOR] (10/419)
Allergic outcomes at 18 years	aOR (95%CI) ‡	aOR (95%CI) ‡	aOR (95%CI) ‡	aOR (95%CI) ‡
Current eczema [n:407]	**11.8 (5.20-26.6)***	1.73 (0.77-3.90)	**2.59 (1.31-5.14)**	0.72 (0.11-4.90)
Current allergic rhinitis [n:419]	**3.13 (1.43-6.85)**	1.39 (0.72-2.69)	**1.70 (1.00-2.89)**	1.67 (0.51-5.44)
Current asthma [n:413]	2.16 (0.94-5.00)	1.92 (0.91-4.06)	**2.00 (1.10-3.63)**	2.96 (0.84-10.49)
Allergic outcomes at 25 years				
Current eczema [n:241]	**9.37 (3.17-27.65)**	2.00 (0.81-4.97)	**6.75 (3.11-14-65)**	1.39 (0.23-8.34)
Current allergic rhinitis [n:240]	**3.26 (1.07-9.93)**	0.99 (0.42-2.32)	**2.74 (1.28-5.88)**	1.19 (0.33-4.32)
Current asthma [n:244]	**2.91 (1.14-7.43)**	1.53 (0.58-7.43)	**2.50 (1.25-5.00)**	2.23 (0.56-8.92)

n: number of observations in the regression model aOR: adjusted odds ratios 95%CI: 95% confidence interval.
†Reference category: Minimal eczema
‡Adjusted odds ratio: sex, parental history of eczema, parental history of allergies, parental education, parental occupation, parental smoking, cooking by combustion of solid or gas fuel, heating by combustion of solid or gas fuel, NO_2, DMR and Filaggrin null mutation.
*P-values less than 0.05 are bolded

Skin prick testing

The association of allergic sensitisation in early life (Table 4.4), adolescence and adulthood with eczema subclasses (Table 4.5) varied between subclasses. Participants in EOP had higher odds of developing allergic sensitisation through this period, particularly food sensitisation. Moreover, those with EOP were a) more likely to be food sensitised at six and 12 months than those from EOR (Table 4.6), b) have a higher proportion of very large (≥9 mm) SPT reactions to aero and food allergens in comparison with minimal eczema (Table S4.5) and c) have more severe eczema symptoms than other subclasses according to atopic dermatitis scores (Table S4.6). Likewise, participants from the MOP subclass had an increased risk of allergic sensitisation, but this occurred from 2 years following their increase in eczema symptoms and continued into adolescence. In comparison with EOP subclass, the participants of the EOR subclasses presented smaller increases on the risk of allergic sensitisation at 1 ,2 and at 18 years of age.

Table 4.4. Adjusted associations between eczema subclasses and early life allergic sensitisation and at 12 years with eczema subclasses as the outcomes.

Eczema subclasses†	Early onset persistent [EOP] (59/619)	Early onset resolving [EOR] (51/619)	Mid onset persistent [MOP] (74/619)	Mid onset resolving [MOR] (16/619)
Allergic sensitisation	aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI) ‡
Sensitisation at 6 months				
Allergic sensitisation [n:554]	**3.88 (2.10-7.15)***	1.14 (0.57-2.25)	1.41 (0.84-2.40)	1.48 (0.49-4.53)
Aero sensitisation [n:553]	**3.95 (1.87-8.36)**	1.31 (0.49-3.51)	1.91 (0.93-3.91)	3.03 (0.89-10.28)
Food sensitisation [n:554]	**4.55 (2.45-8.47)**	1.27 (0.63-2.59)	1.44 (0.81-2.54)	1.70 (0.55-5.24)
Sensitisation at 1 year				
Allergic sensitisation [n:543]	**6.34 (3.34-12.07)**	**2.09 (1.11-3.95)**	1.41 (0.84-2.37)	**5.06 (1.74-14.70)**
Aero sensitisation [n:543]	**4.18 (2.17-8.06)**	1.71 (0.82-3.60)	1.59 (0.86-2.91)	1.71 (0.45-6.40)
Food sensitisation [n:543]	**9.53 (4.91-18.50)**	**2.19 (1.12-4.30)**	1.41 (0.79-2.50)	**5.88 (2.21-15.67)**
Sensitisation at 2 years				
Allergic sensitisation [n:449]	**3.94 (1.93-8.02)**	**2.37 (1.26-4.43)**	**2.03 (1.18-3.50)**	1.49 (0.51-4.34)
Aero sensitisation [n:449]	**3.73 (1.89-7.37)**	**2.32 (1.22-4.39)**	**2.05 (1.17-3.59)**	0.77 (0.19-3.08)
Food sensitisation [n:449]	**5.95 (2.81-12.59)**	1.62 (0.75-3.53)	**2.06 (1.10-3.87)**	2.15 (0.68-6.75)
Sensitisation at 12 years				
Allergic sensitisation [n:344]	**4.58 (1.52-13.85)**	1.50 (0.69-3.28)	**1.90 (1.02-3.53)**	1.87 (0.40-8.77)
Aero sensitisation [n:344]	**4.66 (1.55-14.04)**	1.54 (0.71-3.37)	**1.86 (1.01-3.42)**	2.00 (0.43-9.19)

Food sensitisation [n:331]	**2.89 (1.11-7.54)**	1.99 (0.69-5.75)	**3.34 (1.65-6.76)**	1.25 (0.31-5.07)

n: number of observations in the regression model aMOR: adjusted multinomial odds ratio 95%CI: 95% confidence interval
†Reference category: Minimal eczema
‡Adjusted for: sex, parental eczema history, parental allergy history, parental education, parental occupation, Filaggrin null mutation, outdoor and indoor air pollution.
§Firth penalised logistic regression
*P-values less than 0.05 are bolded

Table 4.5. Adjusted associations between eczema subclasses as risk factor and allergic sensitisation at 18 and 25 years.

Eczema subclasses†	Early onset persistent [EOP] (59/619)	Early onset resolving [EOR] (51/619)	Mid onset persistent [MOP] (74/619)	Mid onset resolving [MOR] (16/619)
Allergic sensitisation	aOR (95%CI) ‡	aOR (95%CI) ‡	aOR (95%CI) ‡	aOR (95%CI) ‡
Sensitisation at 18 years				
Allergic sensitisation [n:389]	**4.63 (1.67-12.79)**	**2.40 (1.12-5.16)**	1.68 (0.94-3.00)	**9.58 (7.32-41.44)§**
Aero sensitisation [n:389]	**3.79 (1.47-9.76)**	**2.51 (1.17-5.40)**	1.75 (0.98-3.16)	**10.18(7.69-43.72)§**
Food sensitisation [n:382]	**4.35 (1.99-9.51)**	0.82 (0.26-2.60)	1.22 (0.58-2.58)	0.33 (0.10-1.09)§
Sensitisation at 25 years				
Allergic sensitisation [n:216]	2.50 (0.76-8.17)	1.04 (0.36-3.01)	1.43 (0.67-3.04)	5.53 (0.30-87)§
Aero sensitisation [n:215]	2.49 (0.76-8.14)	1.04 (0.36-3.01)	1.38 (0.65-2.94)	5.52 (0.32-87.6)§
Food sensitisation [n:195]	1.33 (0.28-6.23)	0.65 (0.08-4.92)	2.68 (0.81-8.85)	0.22 (0.01-5.69)§

n: number of observations in the regression model aOR: adjusted odds ratio 95%CI: 95% confidence interval
†Reference category: Minimal eczema
‡Adjusted for: sex, parental eczema history, parental allergy history, parental education, parental occupation, Filaggrin null mutation, outdoor and indoor air pollution.
§Firth penalised logistic regression
*P-values less than 0.05 are bolded

Table 4.6. Adjusted associations between early eczema subclasses and early life allergic sensitisation at 12 years as risk factors using Early onset resolving as the reference category.

	Early onset persistent [EOP] (59/619)
Allergic sensitisation	aOR (95%CI) ‡
Sensitisation at 6 months	
Allergic sensitisation [n:455]	**3.41 (1.48-7.87)***
Aero sensitisation [n:438]	**3.02 (1.00-9.10)**
Food sensitisation [n:455]	**3.57 (1.52-8.37)**
Sensitisation at 1 year	
Allergic sensitisation [n:478]	**2.44 (1.00-5.92)**
Aero sensitisation [n:459]	2.04 (0.70-5.98)
Food sensitisation [n:475]	**4.35 (1.85-10.23)**
Sensitisation at 2 years	
Allergic sensitisation [n:402]	1.66 (0.70-3.94)
Aero sensitisation [n:400]	1.61 (0.70-3.71)
Food sensitisation [n:391]	**3.66 (1.39-9.64)**
Sensitisation at 12 years	
Allergic sensitisation [n:344]	3.05 (0.84-11.05)
Aero sensitisation [n:344]	3.02 (0.84-10.92)
Food sensitisation [n:331]	1.45 (0.40-5.25)
Sensitisation at 18 years	
Allergic sensitisation [n:381]	2.11 (0.63-7.04)
Aero sensitisation [n:381]	1.68 (0.55-5.14)
Food sensitisation [n:374]	**5.10 (1.51-17.30)**

n: number of observations in the regression model aMOR: adjusted multinomial odds ratio 95%CI: 95% confidence interval
†Reference category: Early onset resolving

‡Adjusted for: sex, parental eczema history, parental allergy history, parental education, parental occupation, Filaggrin null mutation, outdoor and indoor air pollution.
*P-values less than 0.05 are bolded

Discussion

We identified 5 distinct eczema subclasses using LLCA from birth to 12 years of age. These subclasses were differentially associated with early life risk factors suggesting differences in their aetiology. Concerning genetic factors, both EOP and MOP were strongly associated with *FLG* Null, and MOP with *SPINK5* G dominant genotype. In terms of prognosis, both persistent classes were associated with a high allergy burden at 18 and 25. Furthermore, EOP had larger SPT reaction to food allergens and more severe symptoms of eczema than the other eczema subclasses. A positive skin prick in the first year of life was able to discriminate between those with early onset resolving versus early onset persistent eczema, which may help inform treatment strategy and prognosis.

Determining distinct patterns of eczema could provide a clearer picture of the disease pathways. In contrast to previously reported eczema LCAs, [7-10] we targeted high-risk children with 18 eczema assessments the first two years of life, permitting a more comprehensive overview of eczema in childhood as a basis for establishing subclasses. Our eczema subclasses have similar trajectories to those presented by Paternoster et al [7] in a similar time span (from birth to 11 and 16 years), except for the late onset resolving class which our analysis did not identify, possibly because we had a smaller sample size or a higher risk population. While Hu et al [8] also identified a 5-class model, the classes identified were somewhat different, however, the patterns for the "persistent" class were comparable to the persistent subclasses from our analysis. Moreover, Roduit et al [9] and Suaini et al [10] identified a 4-class model, they described similar trajectories to the similarly named subclasses in our model with minor differences. Ziyab et al [11] described five sex-specific trajectories, four trajectories applied to both sexes, these trajectories described similar eczema trends to our results . However, the late onset class, which was a female specific trajectory, was not identified in our results. Other studies examined patterns of severity of eczema [33] and the patterns of eczema into middle age [34] describing different patterns to our results. The reason for these discrepancies may be because of differences in the eczema definition, the number of time points assessed, different populations (multi-ethnic vs predominantly Caucasian), age of assessment, and study design. Nonetheless, studies that measured eczema during childhood described comparable eczema patterns to our results, providing robust evidence of an underlying classification of this disease.

Our findings suggest that certain eczema subclasses were associated with specific early-life risk factors. Although, the association between parental history of eczema with childhood eczema is well known [35], our results suggest that is more strongly associated with MOP subclass despite all members of the cohort having a family history of allergic disease. Likewise, associations between female sex and eczema have been reported [36] but the association was stronger with the MOP subclass. Breastfeeding at 4 weeks was associated with MOP, this may be due to breastfeeding delaying early manifestation of eczema [37] in children who are at high risk of developing this condition (genetic predisposition as noted by a high rate of *FLG* null mutations). The protein filaggrin facilitates the terminal differentiation of the epidermis and the formation of the skin barrier. Null mutations result in complete loss of processed functional filaggrin in the epidermis. [38] Accordingly, we have observed that *FLG* null mutations were strongly associated with both persistent eczema subclasses (EOP and MOP) which highlights a potential eczema development and progression pathway. Furthermore, *SPINK5* was linked to eczema in a previous study which suggested that the barrier function regulated by *SPINK5*, may also play an important role in the development of eczema. [39] While we observed that *SPINK5* dominant G model was associated with MOR, this analysis was not well powered and should be interpreted with caution.

Our results suggested that eczema subclasses from birth to 12 years were distinctly associated with allergic outcomes at 18 and 25 years. Allergic rhinitis is an inflammatory condition affecting nasal mucosal membranes. Defective skin and mucous membrane barriers may promote sensitization to environmental allergens and subsequent atopic diseases. [5] Even though, it has been shown that children with eczema have nearly 3-fold increased odds of developing allergic rhinitis at 5-year follow up compared to children without eczema [8,40], our results suggest that those with the persistent subclasses were at a higher risk. Furthermore, like our results suggested having severe, early-onset, or persistent eczema further increased the risks of developing allergic rhinitis and asthma. [40] Likewise, another study showed that children with infantile eczema have an increased risk of eczema, allergic rhinitis and asthma in preadolescence, compared to children without eczema. [41] Moreover, it has been estimated that approximately one-third of patients with eczema develop asthma and two-thirds develop allergic rhinitis. [42,43] Our results add to these findings by showing that EOP and MOP subclasses were at higher risk of further allergic disease. Additionally, children with eczema onset after 2 years have an increased risk of asthma, [44] in accordance with our observations that MOP (eczema onset around 1.5 years) was associated with increased odds of asthma at 18 and 25 years. Our finding of increased risk of allergic outcomes in in children with persistent eczema subclasses provides support to the atopic march theory, the progression from eczema to other allergic diseases. [5,42,43]

We observed a complex pattern of coexistence of allergic sensitisation with eczema during early life. Although it has been showed that early eczema is associated with an increased risk of food allergies, [45-47] our results suggest that participants from the EOP and MOP showed stronger associations with food allergens in the first 2 years of life. As such, a large proportion of children with eczema (around 30-40%) will have a coexisting food allergy [45] and our results suggest most of these belong to the persistent eczema subclasses.

There are several strengths in this study including its design with a prospective cohort having 26 data points with 25 years of follow-up and well-characterised definitions of eczema considering the change of distribution in skin involvement over childhood. Also, we objectively assessed associations with sensitisation at multiple time points by SPT using international standards. [18] However, our study also had some limitations, as the results were based on a high-risk cohort, the identified eczema subclasses and their associations might not be applicable to the general population. Nevertheless, the subclasses identified were similar to those found in unselected birth cohorts. [7] The definitions of eczema used in this study were selected to facilitate comparison with previous studies in this area [7] and modified from those used in previous MACS publications. [15] These measures had high agreement with the well-established eczema definition from the International Study of Asthma and Allergies in Childhood [48] (Supplementary text 3). Some of the data was self-reported and may be subject to measurement error. We elected not to use doctor diagnoses in the definition of eczema to avoid bias from health care seeking behaviour. Given the limited number of participants in the MOR class, its associations should be interpreted with caution. Loss to follow-up tended to be more pronounced among the less advantaged participants. However, there was no likely reason that the exposure-outcome associations would be different in the non-participants and thereby cause spurious results. [49] There were differences in the anatomical locations and time periods between the eczema definition up to 2 years and from 3 to 12 years, however, both included the report of eczema medication use. We did not include anatomical location of symptoms on the model, future research should look at the evolution of anatomical location of symptoms, and their severity, over time. Finally, as almost all the participants were Caucasian and Anglo-Celtic of European descent, the findings may not be generalizable to other ethnicities. Future research should focus on evaluating eczema subclassification in different ethnicities while using multiple data points. At this time, there are no birth cohorts available with such frequent collection of data to replicate these results.

Conclusion

We have identified distinct eczema subclasses from birth to 12 years, that were both differentially associated with early life risk factors and with the development of allergic outcomes at 18 and 25 years. Use of these eczema subclasses may aid in the identification of risk factors and causes of these specific forms of eczema. Food sensitisation in early life can potentially serve as a marker of persistent eczema subclasses. This can be clinically important as the persistent eczema classes were strongly associated subsequent allergic diseases in adulthood. It is possible that intensive treatment aimed at achieving eczema remission, might not only reduce the prevalence of eczema, but also the development of other allergic comorbidities. Future studies and interventions can be tailored to these eczema subclasses; early detection of eczema subclasses may be important for class-specific preventive or therapeutic strategies.

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4.3. Supplementary materials

Table S4.1. Summary statistics and predictors of loss to follow-up.

Characteristic	Summary statistics at baseline of lost to follow-up at 18 years (200/619)	Odds ratio of being loss to follow-up at 18 years	Summary statistics at baseline of lost to follow-up at 25 years (326/619)	Odds ratio of being loss to follow-up at 25 years
Parental factors		OR (95%CI)		OR (95%CI)
Parental eczema history	51.5% (103/200)	0.83 (0.60-1.17)	53.4% (174/326)	0.91 (0.66-1.24)
Parental allergy history	89.5% (179/200)	1.08 (0.63-1.86)	88.7% (289/326)	0.93 (0.56-1.54)
Parental education	63.5% (127/200)	**0.57 (0.39-0.82)**	65.4% (214/327)	**0.52 (0.36-0.75)**
Parental occupation‡ (Mean) (per 10 unit increase)	46.0 (20.59)	**0.89 (0.84-0.95)**	42.1 (28.1)	**0.91 (0.86-0.96)**
Ever smoking	53.54% (106/200)	**2.02 (1.43-2.85)**	49.39% (161/326)	**1.95 (1.41-2.70)**
Child factors				
Female Sex	48.5% (97/200)	0.96 (0.69-1.35)	45.57% (149/327)	0.74 (0.54-1.02)
Weight at 4 weeks [kg] (Mean)	3.9 (0.58)	**0.66 (0.49-0.89)** †	3.98 (0.57)	0.77 (0.58-1.02) †
Number of older siblings (SD)	1 (1)	1.07 (0.81-1.31) †	1 (1)	0.87 (0.75-1.01) †
Firstborn status	41.5% (83/200)	1.09 (0.78-1.54)	42.2% (138/327)	0.94 (0.78-1.08)
Breast feeding	85.57% (172/200)	**0.57 (0.34-0.96)**	88.11% (289/327)	**0.76 (0.45-1.27)**
Filaggrin Null	17.64 % (3/17)	1.59 (0.44-5.73)	11.8% (20/169)	0.96 (0.53-1.74)
SPINK5§	70.58% (12/17)	0.78 (0.27-2.27)	76.2% (130/169)	1.16 (0.85-1.82)
RCT allocation				
Standard cow's milk	34.0% (68/200)	Ref	33.3% (109/327)	Ref
Soy	35.0% (70/200)	1.02 (0.68-1.54)	28.75% (94/327)	0.73 (0.49-1.07)
Partially hydrolysed cow's milk	31.0% (62/200)	0.87 (0.57-1.32)	37.9% (124/327)	1.33 (0.90-1.97)
Environmental factors				
Presence of pets	50.75% (101/200)	1.09 (0.77-1.53)	49.7% (159/320)	1.21 (0.87-1.67)
Combustion heating ¶	90.45% (180/200)	1.31 (0.75-2.29)	88.1% (282/320)	0.88 (0.53-1.46)
Combustion cooking ¶	70.35% (140/200)	0.69 (0.47-1.01)	74.0% (237/320)	0.89 (0.61-1.28)
Distance to mayor roads	24.23% (47/200)	1.08 (0.72-1.61)	26.8% (85/317)	**1.51 (1.03-2.23)**
NO_2 [ppb] median (IQR)	3.7 (0.80)	0.98 (0.81-1.19) †	3.7 (0.87)	0.97 (0.81-1.16) †
Eczema classes				
Early onset persistent [EOP]	11.0% (22/200)	0.63 (0.28-1.43)	8.87% (29/327)	1.26 (0.59-2.67)
Early onset resolving [EOR]	7.0% (14/200)	0.62 (0.3-1.30)	8.56% (28/327)	0.98 (0.49-1.94)
Mid onset persistent [MOP]	10.0% (20/200)	1.01 (3.22-3.16)	11.01% (36/327)	1.03 (0.34-3.12)
Mid onset resolving [MOR]	3.0% (6/200)	0.83 (0.47-1.45)	2.45% (8/327)	1.21 (0.70-2.09)

Minimal eczema	69.00% (138/200)	0.59 (0.35-1.01)	69.11% (226/327)	0.97 (0.58-1.61)

SD: Standard deviation IQR: Interquartile range NO_2: Nitrogen dioxide ppb: parts per billion n: number of observations in the regression model aOR: adjusted odds ratios 95%CI: 95% confidence interval

†Per increase of 1 unit increase

‡Continuous score 0-100

§ Dominant G model

¶ Gas, coal or wood

Table S4.2. Latent class analysis model fit measures.

Model fit measures	**3 classes model**	**4 classes model**	**5 classes model**
Akaike information criterion	7099	6968	6939
Bayesian information criterion	7422	7374	7463
Entropy	0.872	0.862	0.876
Maximum Log Likelihood	-3476.57	-3388.26	-3364.91
LMR test nested 4-class solution to the 5-class solution*			p-value: 0.001
Classes	Class membership (95%CI)	Class membership (95%CI)	Class membership (95%CI)
1	69.59% (64.63%-74.13%)	65.19% (59.19%-70.74%)	64.70% (58.58%-70.38%)
2	8.84% (6.59%-11.75%)	10.78% (8.08%-14.23%)	8.70% (6.28%-11.94%)
3	21.57% (17.66%-26.07%)	9.84% (07.52%-12.77%)	9.70% (7.46%-12.52%)
4		14.2% (10.07%-19.64%)	2.66% (1.39%-5.01%)
5			14.24% (10.02%-19.83%)

* Both information criteria did not agree, so we considered the Lo-Mendell-Rubin likelihood ratio test comparing a nested 4-class solution to a 5-class solution, the test was significant suggesting that five classes had the better model fit.

Table S4.3. Baseline characteristics of the study cohort by eczema subclasses determined by highest probability.

Characteristic	Early onset persistent (59/619)	Early onset resolving (51/619)	Mid onset persistent (74/619)	Mid onset resolving (16/619)	Minimal eczema (419/619)
Parental factors					
Parental eczema history	57.6% (34/59)	56.9% (29/51)	74.3% (55/74)	56.3% (9/16)	50.1% (210/419)
Parental allergy history	89.8% (53/59)	94.1% (48/51)	87.8% (65/74)	93.8% (15/16)	88.1% (369/419)
Parental education	81.4% (48/59)	68.6% (35/51)	64.9% (48/74)	87.5% (14/16)	71.1% (298/419)
Occupation (ANU III score)† [Mean(SD) Min-Max]	46.1 (23.6) 5.9-91.9	50.5 (17.4) 21.0-79.3	46.2 (22.1) 7.8-99.2	48.5 (24.2) 5.9-77.7	45.3 (20.1) 0-94.4
Ever smoking	64.4% (38/59)	58.8% (30/51)	66.2% (49/74)	66.7% (10/15)	55.5% (232/418)
Child factors					
Female Sex	40.7% (24/59)	54.9% (28/51)	63.5% (47/74)	31.3% (5/16)	47.7% (200/419)
Weight at 4 weeks (kg) [Mean(SD) Min-Max]	4.0 (0.6) 2.2-5.6	3.9 (0.5) 3.0-5.6	4.0 (0.6) 2.4-5.8	4.2 (0.7) 3.2-5.8	4.0 (0.6) 2.6-5.3
Older siblings [Median (IQR) 25-75%]	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Firstborn status	30.5% (18/59)	43.1% (22/51)	29.7% (22/74)	37.5% (6/16)	43.0% (180/419)
Breast feeding at 4 weeks	91.5% (54/59)	82.4% (42/51)	95.9% (71/74)	100% (16/16)	88.54% (371/419)
Filaggrin Null	20.5% (8/39)	10.3% (4/39)	20.4% (11/54)	11.1% (1/9)	9.7% (28/289)
SPINK5‡	78.9% (30/38)	76.9% (30/39)	79.6% (43/54)	100.0% (9/9)	73.1% (211/289)
RCT allocation					
Standard cow's milk	20.3% (12/59)	43.1% (22/51)	37.8% (28/74)	31.3% (5/16)	32.9% (138/419)
Soy	44.1% (26/59)	25.5% (13/51)	27.0% (20/74)	50.0% (8/16)	33.7% (141/419)
Partially hydrolysed cow's milk	35.6% (21/59)	31.4% (16/51)	35.1% (26/74)	18.8% (3/16)	33.4% (140/419)
Environmental factors					
Presence of pets	88.1% (52/59)	88.2% (45/51)	89.2% (66/74)	81.3% (13/16)	90.9% (381/419)
Combustion heating §	91.5% (54/59)	78.4% (40/51)	87.8% (65/74)	81.3% (13/16)	86.6% (363/419)
Combustion cooking §	71.2% (42/59)	66.7% (34/51)	71.6% (53/74)	81.3% (13/16)	74.2% (311/419)
Distance to mayor roads	18.6% (11/59)	23.5% (12/51)	18.9% (14/74)	12.5% (2/16)	24.1% (101/419)
NO2 (ppb) [Median (IQR) 25-75%]	12.4 [2.95;11.3-14.3]	12.6 [3.55;11.3-14.9]	12 [4.3;9.9-14.2]	12.6 [3.43;10.9-14.3]	12.4 [3.3;10.9-14.2]

SD: Standard deviation IQR: Interquartile range NO_2: Nitrogen dioxide ppb: parts per billion

†Continuous score 0-100

‡ Dominant G model

§ Gas, coal or wood

Table S4.4. Prevalence of allergic disease at 18 years (N=419) and at 25 years (N=292) by eczema classes determined by highest probability.

Eczema phenotypes	**Minimal eczema**	**Early onset persistent**	**Early onset resolving**	**Mid onset persistent**	**Mid onset resolving**	**Cohort**
Allergic outcomes at 18 years						
Current eczema	13.9% (38/274)	58.3% (21/ 36)	22.9% (8/35)	40.4% (21/52)	10% (1/10)	21.9% (89/407)
Current allergic rhinitis	37.7% (106/281)	67.6% (25/37)	43.2% (16/37)	55.6% (30/54)	40% (4/10)	43.2% (181/419)
Current asthma	19.42% (54/278)	27.8% (10/36)	33.3% (12/36)	32.1% (17/53)	40% (4/10)	23.5% (97/413)
Any Allergic sensitisation	61.4% (162/264)	89.5% (34/38)	75.0% (27/36)	70.6% (36/51)	100.0% (8/8)	67.3% (267/397)
Aero sensitisation	60.2% (159/264)	86.8% (33/38)	75.0% (27/36)	70.6% (36/51)	100.0% (8/8)	66.2% (263/397)
Food sensitisation	13.6% (35/258)	39.5% (15/38)	8.3% (3/36)	16% (8/50)	0% (0/8)	15.6% (61/390)
Allergic outcomes at 25 years						
Current eczema	16.5% (29/176)	60.9% (14/23)	31.8% (7/22)	51.4% (19/37)	25% (2/8)	26.69% (71/266)
Current allergic rhinitis	54.8% (97/177)	79.2% (19/24)	56.5% (13/23)	56.5% (26/34)	62.5% (5/8)	60.2% (160/266)
Current asthma	20.9% (37/177)	41.7% (10/24)	31.8% (7/22)	37.8% (14/37)	37.5% (3/8)	26.5% (71/268)
Any Allergic sensitisation	61.7% (87/141)	83.33% (20/24)	64.7% (11/17)	67.9% (19/28)	100.0% (6/6)	66.2% (143/216)
Aero sensitisation	61.7% (87/141)	83.33% (20/24)	64.7% (11/17)	66.7% (18/27)	100.0% (6/6)	66.1% (142/215)
Food sensitisation	10.0% (13/130)	23.8% (5/21)	5.88% (1/17)	21.7% (5/23)	0% (0/6)	12.31% (24/195)

Table S4.5. Skin prick test wheal size per allergen type by eczema subclasses determined by highest probability.

Eczema phenotypes	Early onset persistent [EOP] (38/419)	Early onset resolving [EOR] (37/419)	Mid onset persistent [MOP] (51/419)	Mid onset resolving [MOR] (9/419)	Minimal eczema (269/419)
Aero allergens sensitisation†					
Wheal size					
0-2 mm	5 (13.2%)	10 (27%)	15 (29.4%)	2 (22.2%)	112 (41.64%)
3-5 mm	8 (21%)	7 (18.9%)	10 (19.6%)	0 (0%)	42 (15.61%)
6-9 mm	15 (39.5%)	15 (40.5%)	20 (39.2%)	6 (66.7%)	79 (29.37%)
More than 9 mm	10 (26.3%)	5 (13.5%)	6 (11.8%)	1 (11.1%)	36 (13.38%)
Fungi allergens sensitisation‡					
Wheal size					
0-2 mm	25 (65.8%)	36 (97.3%)	43 (84.3%)	7 (77.8%)	237 (88.10%)
3-5 mm	9 (23.7%)	1 (2.7%)	7 (13.7%)	1 (11.1%)	28 (10.41%)
6-9 mm	4 (10.53%)	0 (0%)	1 (1.96%)	0 (0%)	4 (1.49%)
More than 9 mm	0 (0%)	0 (0.00%)	0 (0%)	1 (11.1%)	0 (0%)
Food allergen sensitisation§					
Wheal size					
0-2 mm	24 (63.16%)	34 (91.89%)	46 (90.2%)	9 (100%)	236 (87.73%)
3-5 mm	6 (15.8%)	1 (2.70%)	3 (5.88%)	0 (0%)	24 (8.92%)
6-9 mm	5 (13.2%)	2 (5.4%)	1 (1.96%)	0 (0%)	3 (1.12%)
More than 9 mm	3 (7.89%)	0 (0%)	1 (1.96%)	0 (0%)	6 (2.23%)

†Aero allergens: house dust mite, cat dander, mixed grass and rye grass

‡ Fungi allergens: Alternaria and Penicillium

§ Food allergens: cow's milk, egg white, peanut, cashew and shrimp

Table S4.6. Descriptive statistics and rank-sum test of atopic dermatitis quick scores (N=91) at 18 years by eczema subclasses determined by highest probability.

Eczema phenotypes†	**Early onset persistent [EOP] (20/419)**	**Early onset resolving [EOR] (8/419)**	**Mid onset persistent [MOP] (0/419)**	**Mid onset resolving [MOR] (22/419)**	**Minimal eczema (41/419)**
Atopic dermatitis quick score (ADQ) ‡					
ADQ Total score (IQR; 25-75%)	**12.5; 6-18.5**	6; 6-12	9; 4-13	0	6; 4-10
ADQ Wilcoxon rank-sum test	**0.006**	0.118	0.150	-	ref

IQR: Interquartile range

†Reference category: Minimal eczema

‡ Eczema severity scores

Extended methods

Early life risk factors

Distance to major roads was defined as straight-line distances from each parent-reported residential address during the first year of life of the probands to the nearest major road. The participants were categorised into two groups: (1) living less than or at 150 m and (2) living further than 150 m. [1] Likewise, annual concentration of nitrogen dioxide (NO_2) was defined using a satellite-based land-use regression model. Briefly, the land-use regression model-predicted mean annual NO_2 levels were based on tropospheric NO_2 columns derived from satellite observations in combination with other predictors such as land use and roads, to estimate ground-level NO_2 across Australia.[2] Data after 4-weeks of age were not used as risk factors due to the difficulty determining the sequence between these exposures and the development of eczema.

Statistical analysis

This analysis assumed that all associations between the observed variables were due to unobserved classes. [3, 4] Longitudinal Latent class analysis (LLCA) accounted for correlations between repeated measures of eczema for each child when defining the classes. [3-5] The subjects assigned to each class were like each other according to the descriptor variables used, and the latent classes should correspond to clusters of similar subjects. Hence, latent classes are identified without exploiting the information on the time order of the observations, but also without forcing any parametric relationship between the outcomes and time. Starting with a single latent class, additional classes were added to the eczema model until measures that estimated model fit Bayesian (BIC), Akaike (AIC) information criteria were optimised. However, both information criteria did not agree, so we considered the Lo-Mendell-Rubin likelihood ratio test comparing a nested 4-class solution to a 5-class solution, the test suggested that five classes had the better model fit. Furthermore, entropy and content wise criteria were used to as indicators of better model fit, and they suggested that a 5-class model was more suitable. The subjects assigned to each class were like each other according to the descriptor variables used, and the latent classes should correspond to clusters of similar subjects. We included participants for whom data were available for at least half (12) of the time points studied. In LCA, proper class assignment is not guaranteed

because class assignment is based on probabilities, the exact number or percentage of sample members within each class cannot be determined. [6] Nevertheless, we classified participants in two ways: 1) Participants were assigned to the trajectory class for which they had the highest posterior probability of membership, were used as N in tables 2) Weights, equal to the probability of membership to each eczema subclasses from the LCA for each participant, were used in the probability-weighted regression[7] models to account for the uncertainty in class assignment (in Stata: [pweights]).

Firstly, associations between the eczema classes (eczema subclasses as outcome) and established risk factors (parental, genetic or child) were tested using multinomial regression. Second, associations between eczema classes (eczema subclasses as exposures) and allergic outcomes at 18 and 25 years (eczema, allergic rhinitis, asthma and sensitisation) were estimated using logistic regression. Directed acyclic graphs were developed to assess hypothesised causal relationships and to determine which confounders to include in each model. In both sets of analyses, weights, equal to the probability of membership of each eczema subclasses from the LCA for each child, were used. If there were 10 observations or less per cell [8] for some combination of risk factor and eczema subclasses or eczema subclasses and allergic outcomes, the Firth bias adjustment [9] (a generalisation of adding 0.5 to each cell) was used (using Stata's firthlogit command). [10] All analyses were carried out using the statistical software Stata (release 16; Stata Corporation, College Station, TX).

Extended results

Eczema Subclasses

In accordance with best practices multiple fit statistics were used to define the optimal class number. [6] The BIC score was lower for the 4-class model and the AIC score was lower for the 5-class model (Supplementary table 2). Furthermore, the Lo-Mendell-Rubin likelihood ratio test comparing both models suggested that the 5-class model had a better fit (p-value 0.001) and the 5-class model had better entropy scores. Statistical criteria should always be evaluated in conjunction with interpretability. [6] As such, past studies with participants of similar age range reported 5 and 6 class. [11, 12] For those reasons, the 5-class eczema model was considered the best fit to the data.

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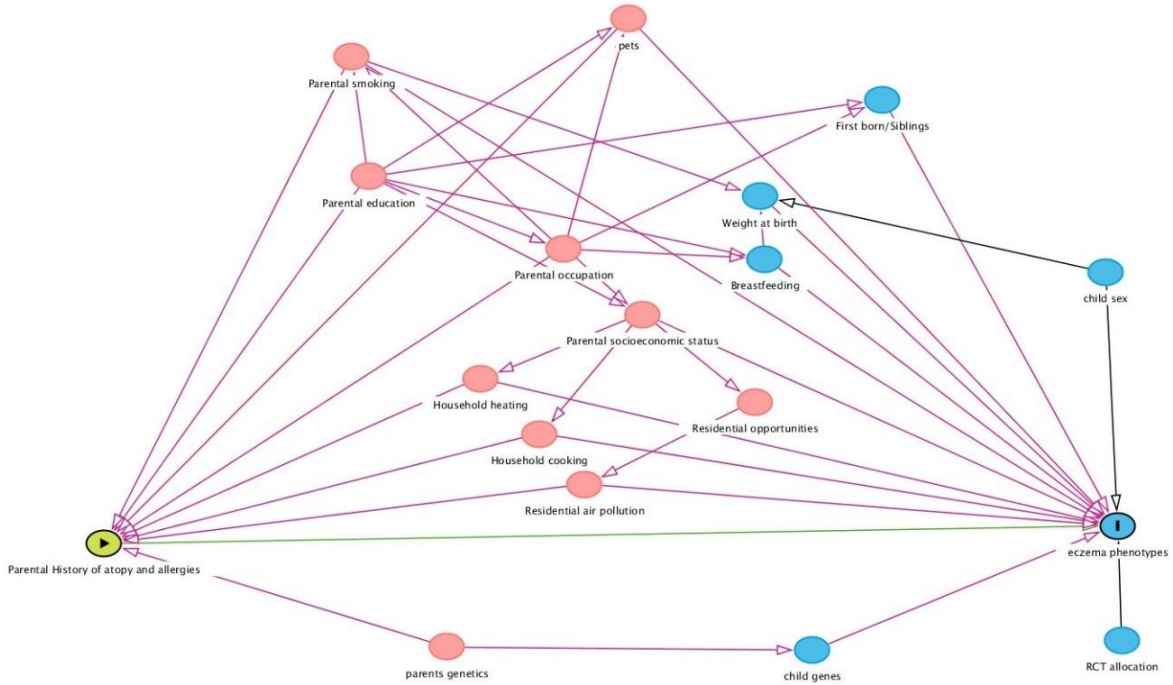
Early life risk factors directed acyclic graphs (DAG)

Figure S4.1. Minimal set of confounders for the association between early life risk factors and eczema phenotypes according to a DAG



Legend
- exposure
- outcome
- ancestor of exposure
- ancestor of outcome
- ancestor of exposure *and* outcome
- causal path

biasing path

Minimal set of confounders for multinomial regression model: sex, parental education, parental occupation, parental smoking.

Parental history of atopy and Parental history of allergies was further adjusted by cooking by combustion of solid or gas fuel, heating by combustion of solid or gas fuel, NO_2, DMR, filaggrin and SPINK5, presence of dog or cat, older siblings, RCT allocation, firstborn status and parental smoking history

Early life risk factors DAGitty code

```
dag {
bb="0,0,1,1"
"First born/Siblings" [pos="0.754,0.168"]
"Household cooking" [pos="0.474,0.626"]
"Household heating" [pos="0.426,0.550"]
"Parental History of atopy and allergies" [exposure,pos="0.119,0.776"]
"Parental education" [pos="0.335,0.273"]
"Parental occupation" [pos="0.494,0.372"]
"Parental smoking" [pos="0.321,0.109"]
"Parental socioeconomic status" [pos="0.558,0.462"]
```

"RCT allocation" [pos="0.952,0.916"]

"Residential air pollution" [pos="0.511,0.695"]

"Residential opportunities" [pos="0.650,0.582"]

"Weight at birth" [pos="0.654,0.300"]

"child genes" [pos="0.697,0.922"]

"child sex" [pos="0.936,0.404"]

"eczema phenotypes" [outcome,pos="0.946,0.752"]

"parents genetics" [pos="0.399,0.918"]

Breastfeeding [pos="0.658,0.386"]

pets [pos="0.547,0.057"]

"First born/Siblings" -> "eczema phenotypes"

"Household cooking" -> "Parental History of atopy and allergies"

"Household cooking" -> "eczema phenotypes"

"Household heating" -> "Parental History of atopy and allergies"

"Household heating" -> "eczema phenotypes"

"Parental History of atopy and allergies" -> "eczema phenotypes"

"Parental education" -> "First born/Siblings"

"Parental education" -> "Parental History of atopy and allergies"

"Parental education" -> "Parental occupation"

"Parental education" -> "Parental smoking"

"Parental education" -> "Parental socioeconomic status"

"Parental education" -> Breastfeeding

"Parental education" -> pets

"Parental occupation" -> "First born/Siblings"

"Parental occupation" -> "Parental History of atopy and allergies"

"Parental occupation" -> "Parental smoking"

"Parental occupation" -> "Parental socioeconomic status"

"Parental occupation" -> Breastfeeding

"Parental occupation" -> pets

"Parental smoking" -> "Parental History of atopy and allergies"

"Parental smoking" -> "Weight at birth"

"Parental smoking" -> "eczema phenotypes"

"Parental socioeconomic status" -> "Household cooking"

"Parental socioeconomic status" -> "Household heating"

"Parental socioeconomic status" -> "Residential opportunities"

"Parental socioeconomic status" -> "eczema phenotypes"

"RCT allocation" -> "eczema phenotypes"

"Residential air pollution" -> "Parental History of atopy and allergies"

"Residential air pollution" -> "eczema phenotypes"

"Residential opportunities" -> "Residential air pollution"

"Weight at birth" -> "eczema phenotypes"

"child genes" -> "eczema phenotypes"

"child sex" -> "Weight at birth"

"child sex" -> "eczema phenotypes"

"parents genetics" -> "Parental History of atopy and allergies"

"parents genetics" -> "child genes"

Breastfeeding -> "Weight at birth"

Breastfeeding -> "eczema phenotypes"

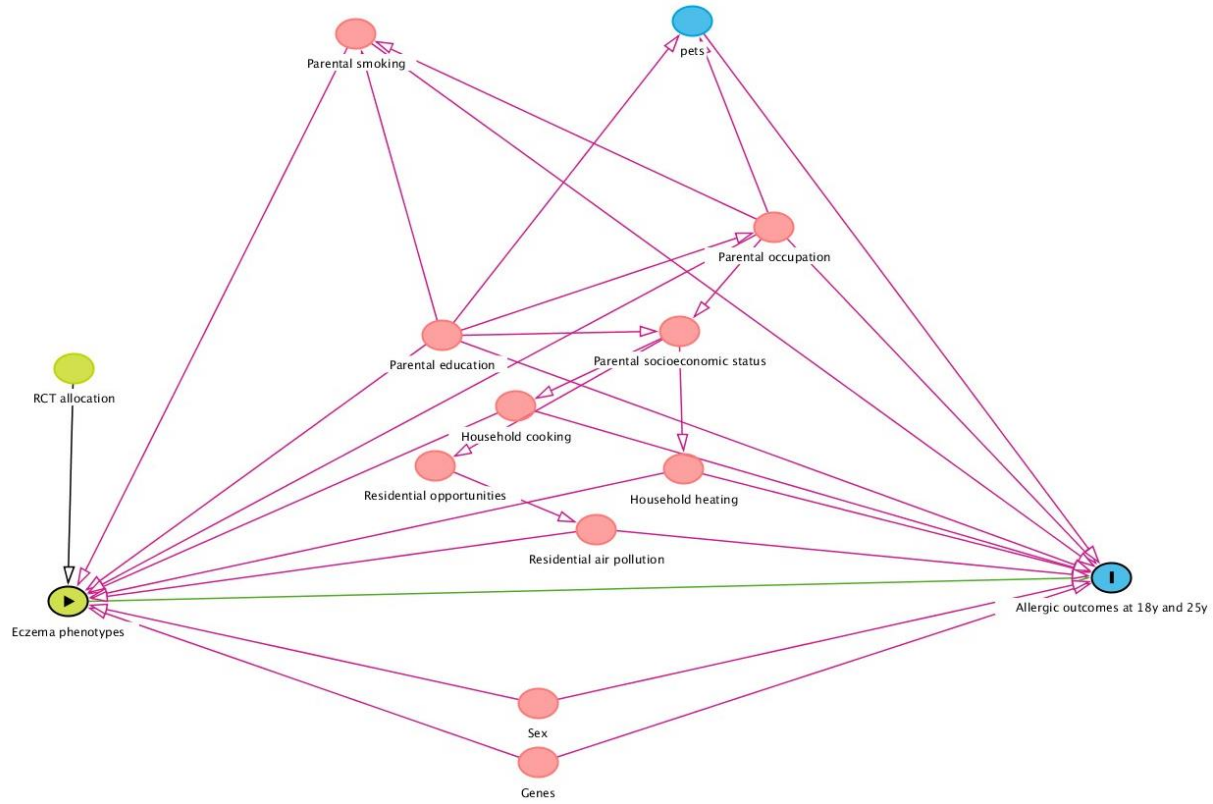
pets -> "Parental History of atopy and allergies"

pets -> "eczema phenotypes"

}

Allergic outcomes directed acyclic graphs (DAG)

Figure S4.2. Minimal set of confounders for the association between eczema phenotypes and allergic outcomes at 18 and 25 years according to a DAG.



Legend
exposure
outcome

ancestor of exposure
ancestor of outcome
ancestor of exposure *and* outcome
causal path
biasing path
Minimal set of confounders: sex, parental history of eczema, parental history of allergies, parental education, parental occupation, parental

smoking, cooking by combustion of solid or gas fuel, heating by combustion of solid or gas fuel, NO2, DMR and Filaggrin null mutation.

Allergic outcomes at 18y and 25y DAGitty code

```
dag {

bb="0,0,1,1"

"Allergic outcomes at 18y" [outcome,pos="0.889,0.721"]

"Eczema phenotypes" [exposure,pos="0.138,0.750"]

"Household cooking" [pos="0.460,0.514"]

"Household heating" [pos="0.581,0.590"]

"Parental education" [pos="0.407,0.428"]

"Parental occupation" [pos="0.646,0.297"]

"Parental smoking" [pos="0.345,0.063"]

"Parental socioeconomic status" [pos="0.578,0.423"]
```

"RCT allocation" [pos="0.142,0.468"]

"Residential air pollution" [pos="0.518,0.663"]

"Residential opportunities" [pos="0.402,0.586"]

Genes [pos="0.476,0.945"]

Sex [pos="0.476,0.873"]

pets [pos="0.587,0.048"]

"Eczema phenotypes" -> "Allergic outcomes at 18y"

"Household cooking" -> "Allergic outcomes at 18y"

"Household cooking" -> "Eczema phenotypes"

"Household heating" -> "Allergic outcomes at 18y"

"Household heating" -> "Eczema phenotypes"

"Parental education" -> "Allergic outcomes at 18y"

"Parental education" -> "Eczema phenotypes"

"Parental education" -> "Parental occupation"

"Parental education" -> "Parental smoking"

"Parental education" -> "Parental socioeconomic status"

"Parental education" -> pets

"Parental occupation" -> "Allergic outcomes at 18y"

"Parental occupation" -> "Eczema phenotypes"

"Parental occupation" -> "Parental smoking"

"Parental occupation" -> "Parental socioeconomic status"

"Parental occupation" -> pets

"Parental smoking" -> "Allergic outcomes at 18y"

"Parental smoking" -> "Eczema phenotypes"

"Parental socioeconomic status" -> "Household cooking"

"Parental socioeconomic status" -> "Household heating"

"Parental socioeconomic status" -> "Residential opportunities"

"RCT allocation" -> "Eczema phenotypes"

"Residential air pollution" -> "Allergic outcomes at 18y"

"Residential air pollution" -> "Eczema phenotypes"

"Residential opportunities" -> "Residential air pollution"

Genes -> "Allergic outcomes at 18y"

Genes -> "Eczema phenotypes"

Sex -> "Allergic outcomes at 18y"

Sex -> "Eczema phenotypes"

pets -> "Allergic outcomes at 18y"

}

Comparison between the International Study of Asthma and Allergies in Childhood (ISAAC) eczema definition and eczema, its location and medication report at 12, 18 and 25 years of age

ISAAC definition of eczema: [1] A positive response to all three questions: 'have you had an itchy rash in the past 12 months?', 'Have you ever had an itchy rash coming and going for at least six months?', and 'Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?'

There is a high agreement between the ISAAC definition and eczema defined by eczema report in the last 12 months, its location and eczema medication used (Table 1, 2 and 3)

	Eczema self-report		
	No	Yes	Total
ISAAC			
No	222	8	230
Yes	27	36	63
Total	249	44	293
Expected Agreement		88.05%	
Kappa statistic		0.6026	

Table 1. Eczema definition at 12-year follow-up

Table 2. Eczema definition at 18-year follow-up

	Eczema self-report		
	No	Yes	Total
ISAAC			
No	21	6	27
Yes	13	47	60
Total	34	53	87
Expected Agreement		78.16%	

Kappa statistic	0.5238

Table 3. Eczema definition at 25-year follow-up

	Eczema self-report		
	No	Yes	Total
ISAAC			
No	31	6	37
Yes	75	119	194
Total	106	125	231
Expected Agreement		64.94%	
Kappa statistic		0.4572	

Reference

1. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J.* 1995;8(3):483-491.

Chapter 5 - Associations between air pollution and development and persistence of eczema and food allergy across the first ten years of life: a population-based cohort study.

5.1. Section introduction

This manuscript draft evaluated the associations between air pollution and eczema and food allergies. There is a lack of evidence on the effect of air pollution on food allergies assessed with objective measures (oral food challenge-proven) and food allergy concurrent with eczema. A better understanding of these topics can lead to preventive and treatment strategies for these allergic diseases. To the best of my knowledge, this analyses was the first to assess air pollution as a risk factor for food allergy using an oral food challenge proven definition. [8] This research paper is based on data from the HealthNuts cohort study when the participants had 1, 4, 6 and 10 years old. The participant's parents completed a questionnaire which captured information on demographic factors and allergic disease status and a subset of the cohort underwent health assessments which included SPT screening and OFC in each wave.

5.1.1. Research question

- Is the exposure to air pollution associated with eczema and food allergies in the first ten years of life?
    - Is the exposure to air pollution associated with eczema and food allergies persistence in the first ten years of life?

5.1.2. Research aim

- To assess whether the exposure to air pollution is associated with eczema and food allergies in the first ten years of life.
    - To examine if air pollution is associated with eczema and food allergies persistence in the first ten years of life.

5.1.3. Critical reflection on the work

I worked with data from the HealthNuts cohort study, a world-leading cohort study on food allergies. For these analyses, I learned a lot of content knowledge regarding food allergies and their relationship with eczema in early life. I learned the data structure of the HealthNuts cohort and how the food allergy variables were coded. Thanks to my experience with previous research papers, I did the analyses much quicker than in my first few papers. I wrote up the manuscript draft, which my supervisors and the HealthNuts principal investigator revised. My plan is to include the food allergy

data at ten years in the analyses, which were collected recently and the data is being organised and cleaned, it will be available in late April. Including this data will add much more value to my analyses and results as I will have assessed the effect of air pollution in challenge proven food allergy in the first ten years of life. Once the analyses are completed, I intend to submit this work to a high-ranking allergy journal.

5.1.4. Manuscript Draft.

Lopez DJ, Lowe AJ, Peters RL. Associations between air pollution and development and persistence of eczema and food allergy across the first ten years of life: a population-based cohort study

***Please note that the specific references from this accepted or submitted manuscript are at the end of this chapter and the in-text citation number restarts at 1.**

5.2. Manuscript draft

Title: Associations between air pollution and development and persistence of eczema and food allergy across the first ten years of life: a population-based cohort study

Running Title: Air pollution exposures and eczema and food allergy

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Author Contributions: Conception and design: DJL, AJL, RLP Analysis and interpretation of data: DJL, AJL, RLP Drafting the article: DJL, AJL, RLP Revision for important intellectual content and approval of final submission: all authors.

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Conflict of Interest

A.J.L. declare they have received research funds from GSK's competitively awarded Investigator Sponsored Studies programme, for unrelated research. A.J.L have received grant funding from Sanofi Regeneron for unrelated research. A.J.L. has received donations of interventional product (EpiCeram) from Primus Pharmaceuticals for unrelated research. The other authors declare they have no conflicts of interest.

Availability of data and materials:

The data that support the findings of this study are available from the HealthNuts cohort study, but restrictions apply to the availability of these data, which were used under authorization from the HealthNuts investigators for the current study, and so are not publicly available. Data are however available from the HealthNuts investigators upon reasonable request.

Acknowledgments

We acknowledge the participants, investigators and founders of the HealthNuts study. DJL was supported by the University of Melbourne and Becas Carlos Antonio Lopez scholarship. The funding agencies had no direct role in the conduct of the study, the collection, management, statistical analysis and interpretation of the data, preparation or approval of the manuscript.

Abstract

Background: There is a lack of evidence on the effect of air pollution on oral food challenge-proven food allergy and concurrent eczema.

Objective: To assess whether the exposure to air pollution associated with eczema and food allergies in the first ten years of life.

Methods: The HealthNuts cohort collected data when the participants were 1, 4 ,6 and 10 years respectively. Nitrogen dioxide (NO_2) and fine particulate matter ($PM_{2.5}$) were assigned using a satellite-based land-use regression model. Eczema was defined at 1 year by the parent report and at 4, 6 and 10 years using the International Study of Asthma and Allergies in Childhood definition. Food sensitisation was defined using skin prick tests and food allergy using oral food challenges in each wave. We fitted multilevel logistic regression models with random intercepts for SA2.

Results: A total of 4,668 infants were included in the analyses. NO_2 exposure at 4 years was associate with food allergy prevalence at 6 years (aOR:1.63[95%CI:1.00-2.64] per 1). A higher concentration of $PM_{2.5}$ at 1 year was associated with having food allergy at 4 years (aOR:1.39[95%CI:1.07-1.81]) per 1.6 $\mu g/m^3$ $PM_{2.5}$ increase) and at 6 years (aOR:1.32[95%CI:1.02-1.71]) per 1.6 $\mu g/m^3$ $PM_{2.5}$ increase). Air pollution exposure at 1 year was associated with persistent food allergy and peanut allergy. In contrast, there was no evidence of associations between air pollution markers and eczema during the same period.

Conclusions: Air pollution exposure may be a risk factor for food allergy in infants. Avoidance of air pollution in early life could potentially be a food allergy preventive measure.

Abstract word count: 246

Key messages

- To the best of our knowledge our study is the first study to evaluate the association between air pollution and challenge-proven food allergy in infants during the first ten years of life.

- Air pollution exposure at 1 and 4 years was associated with increased odds of challenge-confirmed food allergy and peanut food allergy during early childhood.

-Exposure to air pollution at 1 year increased the risk of persistent food allergy and persistent peanut allergy.

Keywords: Eczema, Food Allergy, Air pollution, Early life

Abbreviation List

95% confidence intervals (95%CIs)

Australian Bureau of Statistics' Statistical Areas Level 2 (SA2)

culturally and linguistically diverse (CALD)

Diesel exhaust particles (DEP)

Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$).

immunoglobulin E antibodies (IgE)

International Study of Asthma and Allergies in Childhood (ISAAC)

kilounits per liter (kU/L)

micrograms per cubic meter air ($\mu g/m^3$)

Nitrogen dioxide (NO_2)

normalised difference vegetation index (NDVI)

odds ratios (ORs)

oral food challenge (OFC)

parts per billion (ppb)

reactive oxygen species (ROS)

skin prick testing (SPT)

socio-Economic Indexes for Areas (SEIFA),

specific IgE level (sIgE)

T helper cell type 2 (Th_2)

tumour necrosis factor (TNF)

Introduction

Food allergy and eczema are amongst the most frequent chronic non-communicable diseases in children, with an elevated prevalence and disease burden in many countries worldwide. [1,2] It has been observed that allergic disease typically manifests in infancy, beginning with eczema accompanied by food allergy symptoms. [3] Food allergy is an adverse, immune system-mediated reaction to foods. [4] It was observed that approximately 10% of infants (1-year-old) have a food allergy. [1] It is defined by a loss of or failure to develop oral tolerance, which is the active suppression of immune responses to typically harmless food antigens. [5] Food allergy is determined by imbalanced immune responses to dietary antigens, mainly involving the T helper cell type 2 (Th_2) phenotypes related to the production of immunoglobulin E antibodies (IgE) against dietary allergens. [6,7] Similarly, eczema is also characterised by TH_2 immunity dysregulation and increased serum IgE and eosinophilia. [8] Eczema (or atopic dermatitis) is a frequent, chronic and recurrent skin inflammatory condition characterised by an itchy red rash. [8] Eczema is the most prevalent skin disease in the industrialised world, with a lifetime prevalence of around 20%.[9] Eczema is strongly associated with an increased tendency to develop other allergic conditions. [10] Food allergies and eczema often coexist; both illnesses usually start in the first year of life and are both associated with genetic polymorphisms that can impair skin barrier function. [11] As such, they are likely to share similar risk factors. [5]

It has been hypothesised that environmental changes brought on by industrialisation and urbanisation can compromise the skin's epithelial barrier, the respiratory system, and the gastrointestinal tract, making people more susceptible to chronic diseases like allergies. [12] The term "air pollutants" refers to various compounds in the atmosphere that pose a risk to human health. [13] Air pollutants may generate reactive oxygen species, which damage the epithelial cells (dermal, gastrointestinal, and respiratory) through oxidative stress. [14] Cytokines are then created, leading to an inflammatory response; this process may drive further inflammation and pruritus (itching of the skin) associated with eczema compromising the structural integrity of the epidermal barrier. [15] Correspondingly, it has been reported that higher levels of air pollutants can be associated with an increased risk of eczema in children. [16,17] It is possible that, as described by the "dual allergen exposure hypothesis",[18] the increased exposures to allergens through a disrupted skin barrier can lead to food allergy. [5]

Limited studies have examined the potential association between ambient air pollution and food allergy. One recent study found positive associations between indoor and outdoor air pollution. [19] However, this study used self-reported measures of food allergies. Due to eczema's recurrent nature and the difficulty of testing food allergies clinically, conducting any study aiming to assess

both eczema and food allergies is challenging. [20] Performing oral food challenge (OFC) throughout infancy and prospectively screening for food sensitisation at regular intervals are the only ways to study the onset of food allergy robustly. [20] As such, there is a lack of evidence on the effect of air pollution on food allergies assessed with objective measures (oral food challenge-proven) and food allergy concurrent with eczema. A better understanding of these topics can lead to preventive and treatment strategies for these allergic diseases. Therefore, we aimed to assess whether exposure to air pollution is associated with eczema and food allergies in the first ten years of life.

Methods

Study design and population

The HealthNuts is a multi-wave population-based cohort of 5276 infants recruited at 12 months of age in 2007 from 138 council-run immunization sessions where they underwent skin prick testing (SPT) screening and OFC at Melbourne's Royal Children's Hospital in Victoria, Australia. [21] Data was collected in four waves when the participants were 1, 4, 6 and 10 years respectively; the methods have been described previously. [21-23] In brief, the participant's parents completed a questionnaire that captured information on demographic factors and allergic disease status. A subset of the cohort underwent health assessments, including SPT screening and OFC in each wave. [21]

Exposure definitions

The air pollution markers used in these analyses were Nitrogen dioxide (NO_2) and Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$). The annual average of each marker was defined in every wave.

Nitrogen dioxide (NO_2)

To assign mean annual Nitrogen dioxide (NO2) in parts per billion (ppb) from pregnancy through to 10 years of age, we used a satellite-based land-use regression model linked to the participants' geocoded residential address exposures. [24] Briefly, from satellite observations in combination with other predictors such as land use and roads, the land-use regression tropospheric NO2 columns, were derived, and then a model-predicted mean annual NO2 levels were estimated, to determine ground-level NO2 across Australia. [24]

Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$)

Similarly, using a satellite-based land-use regression model, $PM_{2.5}$ in micrograms per cubic meter air (µg/m^3) from pregnancy through to 10 years of age was linked to the participants' geocoded residential address exposures. [24] In brief, satellite-based estimates for Australia of ground-level $PM_{2.5}$ were used as a land-use regression predictor, with other spatial predictors of $PM_{2.5}$. This model explained 63% of spatial variability in measured annual $PM_{2.5}$. [25]

Outcome definitions

Eczema outcomes

At wave 1 (1 year), eczema was defined by the parent report of eczema or rash in the last 12 months and the use of eczema medication. [26] For the following waves (4, 6 and 10 years), the International Study of Asthma and Allergies in Childhood (ISAAC) definition of eczema was used. [27] Participants were classified as having prevalent eczema if they reported "yes" to all three questions: 'have you had an itchy rash in the past 12 months?', 'Have you ever had an itchy rash coming and going for at least six months?', and 'Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?'. Eczema persistence was defined as those participants that had eczema at 1 year and continued to have eczema in the following waves. Eczema remission was defined as those participants that had eczema at 1 year, but it remitted in the following waves.

Skin prick test and oral food challenge

At wave 1, SPT was carried out on the infant's back, and all infants with detectable (>=1mm) SPT to food allergens: peanut, egg, sesame, and either shrimp or cow's milk (after the first 2000 participants shrimp was replaced by cow's milk) were offered an OFC confirmation (sesame, peanut, egg, and either shrimp or cow's milk) unless there was a known history of acute food allergy. [28] For a positive OFC result it was needed to have at least 1 of the following: (1) three or more noncontact hives simultaneously that continue for at least five minutes, (2) perioral or periorbital angioedema, (3) vomiting, or (4) anaphylaxis. [29] Participants who reported symptoms of a new food allergy and those who were sensitised to a food allergen but had a negative OFC at age 1 year initially underwent SPT. If the participant had a positive OFC at age one, they had another OFC at age four to determine whether their food allergy had persisted or resolved. [21] If there was a history of ingestion and reaction or if SPT demonstrated evidence of sensitisation and there was no history of ingestion and tolerance, an OFC to these foods were recommended to the participants. [22] OFC and SPT at waves 1 and 2 followed the same protocol. [21]

At waves 3 and 4, SPT for aero and food allergens were performed (a combination of tests from Immunotek S.L.-Australasian Medical and Scientific Ltd. [Madrid, Spain] and Greer-Stallergens Greer Ltd. due to supply concerns during the trial). [30] A SPT wheal size of 3 mm or larger and/or sIgE of 0.35 kilounits per litre (kU/L) or greater to a food allergen was determined as food sensitisation. [30] Children with food allergies from prior waves, those reporting recent food reactions that might indicate an IgE-mediated food allergy, and those who had recently developed food sensitivities but were not eating or tolerating the index food were all given OFC. [30] Waves 3 and 4 followed the same protocol for SPT and OFC. [30] All clinical staff performing OFCs were blinded to previous history of reaction and SPT results.

Food allergy

At waves 1 and 2, food allergy was defined by: (1) A positive OFC result along with a positive test of sensitisation (SPT wheal of 2 mm or larger and/or specific IgE level [sIgE] of 0.35 kU/L or greater) and (2) if a participant experienced a reaction that complied with the OFC stopping criteria and had a positive test of sensitisation, they were also diagnosed with a food allergy without OFC. Food tolerance was defined as a negative OFC or test of sensitisation. Also, OFC, test of sensitisation, and clinical history informed the definition. [22]

At waves 3 and 4, food allergy was defined by: (1) Positive OFC result and sensitised (2) Reaction consistent with IgE-mediated food allergy within the previous 12 months and sensitised (no OFC) (3) SPT result ≥ 8 mm and positive OFC result at age 4 years (no OFC at age 6 years or 10 years). Food tolerance was defined as a negative OFC or SPT, a 3 to 7 mm SPT result, and an ingestion history indicative of tolerance. [31] Food allergy persistence was defined as those participants that had Food allergy at 1 year, and it continued in the following waves. Food allergy remission was defined as those participants that had Food allergy at 1 year, but it remitted in the following waves.

Neighbourhood unit

We used the Australian Bureau of Statistics Statistical Areas Level 2 (SA2) to represent a neighbourhood unit. The SA2 is a geographical region used to collect and disseminate census data in Australia. [32] The SA2 were used as a neighbourhood unit in the regression models because they represent an economic and social society in an area, are generally based on officially gazetted suburbs and localities and have an average population of 10,000 people. [32] The participant's approximate home address was used to assign an SA2 code in each wave.

Confounding variables

The parent (or guardian)-administered questionnaire at recruitment was used to gather data on various demographic and environmental characteristics. According to their parent's countries of origin and the language spoken at home (other than English), each infant's culturally and linguistically diverse (CALD) status was allocated and divided into ten general culturally diverse regions. Each participant's socioeconomic status was determined based on their local government region. Socio-Economic Indexes for Areas (SEIFA) [33] was used to assessed advantages and disadvantages (income, Assets, Expenditures). Environmental greenness was defined using a normalised difference vegetation index (NDVI) with values ranging from -1 to 1. A 1600 m radial buffer zone around the home address was assigned to each participant. [34,35] A directed acyclic graph (Figure 1) was developed to assess hypothesised causal relationships and determine the minimum confounders to include in the regression models to approximate a causal model. The minimum

confounders for the combined model are culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

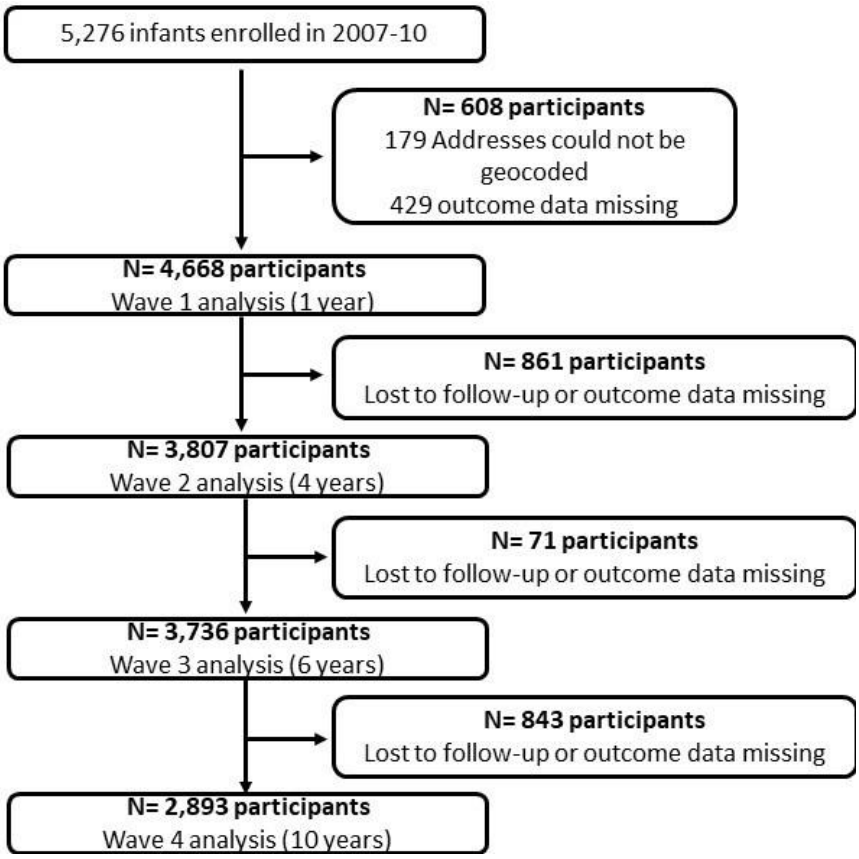
Statistical Analysis

The data were hierarchically structured, with participants (level 1) living within neighbourhoods (level 2). As such, the clustering effect of participants within higher-level units (neighbourhoods) needs to be accounted for when fitting regression models to avoid bias. [36,37] The assumption of independence required for standard regression modelling is likely to be violated by the correlation of outcomes between participants living in the same neighbourhoods. [36,38] If the environment in which a participant lives adds to the probability of children developing eczema or food allergy, then it is sensible to assume that there may be similar effects on children living in the same areas. Multilevel logistic regressions were fitted to examine the associations between air pollution exposure (annual average of each marker in each wave) and eczema and challenge-proven food allergy separately. The regression models incorporated the outcome (eczema or food allergy), exposure (i.e. NO2 or PM2.5), participant-level covariates (culturally diverse background, sex, parents' smoking status, immediate family allergic history and pet ownership), neighbourhood-level covariates (environmental greenness and SEIFA) and the neighbourhoods specific random effects (SA2). The potential presence of non-linearity of these associations was assessed by comparing two models using the likelihood ratio test with the exposure included as a continuous variable versus categorised quartiles. The coefficients represented the estimated effect per interquartile range (IQR) increase of air pollutant exposure and were expressed as odds ratios (ORs) with 95% confidence intervals (95%CIs). When the association was non-linear, the continuous air pollution exposure was replaced by a dichotomous variable (lower 25% vs upper 75% of the air pollution marker distribution). We performed a number of sensitivity analyses: 1) standard logistic regressions, explored the associations between air pollution markers and 2) peanut and egg food allergy and 3) food sensitisation regardless of food allergy. All statistical analyses were carried out using Stata (release 16; Stata Corporation, College Station, TX).

Results

A total of 5,276 infants were enrolled at baseline, of whom 608 participants were excluded from the 1-year analyses (N=4,668, Figure 5.1). Of these participants, 861 were excluded from the 4-year analyses (3,807), and further 71 participants were excluded from the 6-years analyses (3,736). Finally, 843 participants were excluded from the 10-year analyses (2,893). There were some differences between those followed and those lost to follow-up (Table S5.1); those lost were more likely to be smokers, have CALD status, be from lower socioeconomic status, and without a family history of allergies, eczema or food allergies.

Figure 5.1. Participants' flow diagram



The proportion of males and females was approximately equal in each wave (Table 5.1). Around a quarter of the participants have had CALD status. Most of the participants had a family history of allergies and had non-smoker parents. At wave 1, 1,236 participants had prevalent eczema (26.5%), and 494 participants had a prevalent food allergy (10.8%). In subsequent waves, the prevalence of eczema and food allergy decreased as the participants grew older. Air pollution markers decreased slightly, while greenness increased slightly over time.

Nitrogen Dioxide (NO_2)

There was no evidence of associations between NO2 annual average and prevalent eczema at each wave (Table 5.2). There was evidence of non-linearity of associations between NO2 and food allergy outcomes (Table S5.2). A dichotomous NO2 variable (lower 25% vs upper 75% of the air pollution marker distribution) was used in the regression models where food allergy was the outcome of interest. While the point estimate for the association between NO2 annual average and food allergy was greater than 1 at all times (Table 5.2) the only point where a significant association was observed was between higher concentration of NO2 at 4 years and food allergy at 6 years (aOR:1.63 [95%CI:1.00-2.64]). As a sensitivity analysis, the same variables were included in standard logistic regressions (Table S5.3). The association estimates were similar to those reported from the multilevel logistic models.

Table 5.1. Baseline characteristics of the included study sample per Wave.

Characteristic	Wave 1 (N= 4,668)	Wave 2 (N= 3,807)	Wave 3 (N=3,736)	Wave 4 (N=2,893)
	% (n/N)	% (n/N)	% (n/N)	% (n/N)
Individual level characteristics				
Mean Age (years)	1.05	4.08	6.3	10.38
Female	49.1% (2,294/4,668)	49.3% (1,877/3,807)	48.6% (1,815/3,736)	48.8% (1,412/2,893)
CALD status	18.1% (843/4,668)	16.5% (630/3,807)	17.1% (642/3,736)	15.4% (447/2,893)
Pet ownership	44.4% (2,071/4,668)	32.86% (1,213/3,807)	44.6% (1,667/3,736)	60.2% (1,743/2,893)
Family history of allergies	69.9% (3,262/4,668)	71.1% (2,719/3,807)	72.2% (2,670/3,736)	72.3% (2,100/2,893)
Smoking				
No	55.2% (2,578/4,668)	55.8% (2,126/3,807)	55.4% (2,071/3,736)	56.8% (1,644/2,893)
Former	23.0% (1,074/4,668)	35.8% (1,364/3,807)	33.9% (1,266/3,736)	32.7% (947/2,893)
Current	21.8% (1,016/	8.3% (317/3,807)	10.7% (399/3,736)	10.8% (296/2,893)
Eczema	26.5% (1,236/4,668)	11.2% (425/3,807)	13.4% (501/3,736)	13.4% (387/2,893)
Remission		21.9% (832/3,807)	20.2% (757/3,736)	24.2% (699/2,893)
Persistence		6.4% (245/3,807)	3.4% (125/3,736)	2.0% (59/2,893)
Food allergy	10.8% (494/4,592)	3.5% (132/3,775)	5.5% (165/3,016)	
Remission		10.5% (397/3,775)	7.8% (235/3,016)	
Persistence		3.5% (131/3,775)	3.4% (103/3,016)	
Peanut allergy	3.0% (139/4,592)	2.3% (87/3,775)	3.4% (103/3,016)	
Egg allergy	8.9% (407/4,592)	1.4% (54/3,775)	1.3% (39/3,016)	
Sesame allergy	0.6% (28/4,592)	0.5% (17/3,775)	0.6% (18/3,016)	
Food allergy & eczema	6.7% (308/4,668)	1.5% (58/3,807)	1.8% (68/3,736)	
Food sensitised	18.8% (876/4,668)	5.7% (217/3,807)	7.4% (276/3,736)	7.7% (224/2,893)
Area level characteristics				
SA2 (participants)	244 (19)	212 (17)	239 (14)	247 (11)
Greenness†*	.39 (.09; .01, .74)	.43 (.09; .01, .79)	.41 (.09; .01, .77)	.42 (.09; .01, 0.76)
SEIFA categories				
1	20.4% (952/4,668)	18.1% (688/3,807)	18.4% (688/3,736)	16.8% (484/2,893)
2	20.1% (937/4,668)	20.7% (785/3,807)	20.7% (775/3,736)	21.4% (618/2,893)
3	20.8% (969/4,668)	20.5% (789/3,807)	21.0% (785/3,736)	21.1% (616/2,893)
4	19.4% (905/4,668)	20.6% (783/3,807)	21.4% (800/3,736)	19.6% (566/ 2,893)
5 (Highest)	19.4% (905/4,668)	20.1% (762/3,807)	18.4% (688/3,736)	21.1% (609/ 2,893)
NO2‡ (ppb)‡	11.3 (3.2; 2.8, 22.5)	8.6 (2.6; .81, 17.2)	7.9 (2.5; .50, 16.4)	8.2 (2.5; .45, 16.4)
PM2.5‡ (µg/m^3)‡	8.4 (1.6; 2.5, 12.2)	8.1 (1.0; 1.01, 10.4)	7.7 (1.1; 1.14, 10.4)	7.3 (1.1; 1.0, 8.8)

CALD: Culturally and linguistically diverse SA2: Statistical area level 2 ppb: parts per billion µg/m3: micrograms per cubic meter air

† Mean (Standard deviation; min, max)

‡ Median (interquartile range; min, max)

*A greater greenness score (range: -1, 1) implies a greater proportion of vegetation.

Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$)

The associations between $PM_{2.5}$ annual average and prevalent eczema at each wave were non-significant (Table 5.3). In contrast, I observed significant associations between NO_2 annual average and food allergy (Table 5.3). An increasing level of $PM_{2.5}$ at 1 year was associated with higher odds of having food allergy at 4 years (aOR:1.39 [95%CI:1.07-1.81]) per 1.6 µg/m^3 $PM_{2.5}$ increase) and at 6 years (aOR:1.32 [95%CI:1.02-1.71]) per 1.6 µg/m^3 $PM_{2.5}$ increase). Likewise, higher concentrations of $PM_{2.5}$ at 4 years were associated with increased odds of having food allergy at 6 years (aOR:1.28 [95%CI:1.00-1.65]) per 1.0 µg/m^3 $PM_{2.5}$ increase). As a sensitivity test, the same

variables were included in standard logistic regressions (Table S5.4). The association estimates did not materially change in comparison to those from the multilevel logistic models. There was no evidence of non-linearity in these associations.

Table 5.2. Adjusted association estimates from the multilevel logistic regression models between NO_2 and allergic outcomes at each Wave.

Outcome	**NO_2 at 1 year** (IQR: 3.2 ppb)	**NO_2 at 4 years** (IQR: 2.6 ppb)	**NO_2 at 6 years** (IQR: 2.5 ppb)	**NO_2 at 10 years** (IQR: 2.5 ppb)
	aOR (95%CI) †	**aOR (95%CI)** †	**aOR (95%CI)** †	**aOR (95%CI)** †
Eczema				
1 year (N= 4,668)	0.95 (0.86-1.06) ‡			
4 years (N= 3,807)	1.07 (0.90-1.26) ‡	0.91 (0.78-1.07) ‡		
6 years (N=3,736)	0.85 (0.74-1.01) ‡	0.87 (0.75-1.02) ‡	0.89 (0.77-1.03) ‡	
10 years (N=2,893)	0.90 (0.76-1.07) ‡	0.97 (0.81-1.16) ‡	1.00 (0.85-1.18) ‡	1.03 (0.87-1.21) ‡
Food Allergy				
1 year (N= 4,668)	1.27 (0.98-1.64) §			
4 years (N= 3,807)	1.57 (0.96-2.58) §	1.33 (0.82-2.14) §		
6 years (N=3,736)	1.42 (0.88-2.29) §	**1.63 (1.00-2.64)** *§	1.39 (0.87-2.21) §	
10 years (N=2,893)				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.
†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.
‡ ORs (95% CIs) per an IQR increase in NO_2
§ Non-linear association, a dichotomous variable (lower 25% vs upper 75% of the air pollution marker distribution) was used.
*P-value less than 0.05 is bolded

Table 5.3. Adjusted association estimates from the multilevel logistic regression models between $PM_{2.5}$ and allergic outcomes at each Wave.

Outcome	**$PM_{2.5}$ at 1 year** (IQR: 1.6 µg/m^3)	**$PM_{2.5}$ at 4 years** (IQR: 1.0 µg/m^3)	**$PM_{2.5}$ at 6 years** (IQR: 1.1 µg/m^3)	**$PM_{2.5}$ at 10 years** (IQR: 1.1 µg/m^3)
	aOR (95%CI) †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡
Eczema				
1 year (N= 4,668)	0.99 (0.90-1.10)			
4 years (N= 3,807)	1.03 (0.89-1.20)	0.89 (0.77-1.03)		
6 years (N=3,736)	0.89 (0.77-1.02)	0.96 (0.83-1.10)	1.04 (0.91-1.18)	
10 years (N=2,893)	0.94 (0.81-1.10)	0.96 (0.21-1.12)	0.94 (0.81-1.08)	1.00 (0.85-1.17)
Food Allergy				
1 year (N= 4,668)	1.12 (0.97-1.29)			
4 years (N= 3,807)	**1.39 (1.07-1.81)**	1.21 (0.84-1.56)		
6 years (N=3,736)	**1.32 (1.02-1.71)**	**1.28 (1.00-1.65)**	1.23 (0.97-1.54)	
10 years (N=2,893)				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.
†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.
‡ ORs (95% CIs) per an IQR increase in $PM_{2.5}$
*P-value less than 0.05 is bolded

Remission and persistence of eczema and food allergy

Air pollution markers were not associated with eczema remission or persistence (Table S5.5). However, our results showed that increased concentrations of air pollution (both NO_2 and $PM_{2.5}$) at 1 year were associated with the persistence of food allergy mainly driven by peanut food allergy

(Table 5.4). Higher levels of NO_2 at 1 year were associated with increased odds of persistent food allergy at 4 (aOR:1.78 [95%CI:1.05-3.03]) and 6 years (aOR:1.94 [95%CI:1.03-3.68]). Similarly, increased concentrations of $PM_{2.5}$ were associated with higher odds of persistent food allergy at 4 (aOR:1.46 [95%CI:1.10-1.94] per 1.6 μg/m^3 $PM_{2.5}$ increase) and 6 years (aOR:1.54 [95%CI:1.12-2.14] per 1.6 μg/m^3 $PM_{2.5}$ increase). Notably, air pollution markers were associated with persistent peanut food allergy at 4 and 6 years, but there was no evidence of association with persistent egg allergy (Table 5.4).

Table 5.4. Adjusted association estimates from the multilevel logistic regression models between air pollution markers at 1 year and food allergy outcomes persistence and remission.

Outcome	**NO_2 at 1 year** (IQR: 3.2 ppb)	**$PM_{2.5}$ at 1 year** (IQR: 1.6 μg/m^3)
Any food allergy	**aOR (95%CI)** †	**aOR (95%CI)** †
0-4 year outcomes (N= 3,807)		
Never food allergy	Ref	Ref
Remitting food allergy	1.19 (0.89-1.59) §	1.04 (0.88-1.22) ‡
Persistent food allergy	**1.78 (1.05-3.03)** §	**1.46 (1.10-1.94)** ‡
0-6 year outcomes (N=3,736)		
Never food allergy	Ref	Ref
Remitting food allergy	0.95 (0.65-1.39) §	0.98 (0.80-1.21) ‡
Persistent food allergy	**1.94 (1.03-3.68)** §	**1.54 (1.12-2.14)** ‡
Peanut Allergy	**aOR (95%CI) †**	**aOR (95%CI) †**
0-4 year outcomes (N= 3,807)		
Never food allergy	Ref	Ref
Remitting food allergy	0.61 (0.23-1.65) §	0.73 (0.41-1.31) ‡
Persistent food allergy	**2.85 (1.30-6.23)** §	**1.52 (1.06-2.16)** ‡
0-6 year outcomes (N=3,736)		
Never food allergy	Ref	Ref
Remitting food allergy	0.75 (0.33-1.73) §	0.87 (0.53-1.42) ‡
Persistent food allergy	**3.26 (1.34-7.94)** §	**1.61 (1.09-2.38)** ‡
Egg Allergy	**aOR (95%CI) †**	**aOR (95%CI) †**
0-4 year outcomes (N= 3,807)		
Never food allergy	Ref	Ref
Remitting food allergy	1.33 (0.86-2.07) §	1.02 (0.81-1.29) ‡
Persistent food allergy	1.19 (0.56-2.53) §	1.29 (0.83-2.01) ‡
0-6 year outcomes (N=3,736)		
Never food allergy	Ref	Ref
Remitting food allergy	1.13 (0.78-1.63) §	1.02 (0.84-1.24) ‡
Persistent food allergy	1.59 (0.48-5.25) §	1.34 (0.71-2.51) ‡

aOR: adjusted odds ratio 95% CI: 95% confidence interval.
†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.
‡ ORs (95% CIs) per an IQR increase in $PM_{2.5}$
§ Non-linear association, a dichotomous variable (lower 25% vs upper 75% of the air pollution marker distribution) was used.
*P-value less than 0.05 is bolded

Egg and Peanut allergy outcomes

When exploring the associations between NO_2 and peanut or egg food allergy, there were significant associations with peanut allergy, while none with egg allergy (Table S5.6). An increase in the NO_2 concentration at 1 year was associated with having peanut food allergy at 1 year (aOR:2.08

[95%CI:1.25-3.47]), and exposure to NO_2 at 4 years was associated with having peanut allergy at 6 years (aOR:1.83 [95%CI:1.04-3.22]). When considering $PM_{2.5}$ as the exposure (Table S5.7) and increased in the concentration of $PM_{2.5}$ at 6 years was associated with having peanut food allergy at 6 years (aOR:1.34 [95%CI:1.02-1.76] per 1.1 μg/m^3 $PM_{2.5}$ increase).

We assessed the association between NO_2 and food sensitisation (Table S5.8). Increased levels of NO_2 at 1 year were associated with being food sensitised at 1 year (aOR:1.24 [95%CI:1.01-1.54]) and being peanut sensitised at 1 year (aOR:1.34 [95%CI:1.00-1.81]). While a higher concentration of NO_2 at 4 years was associated with being peanut sensitised at 6 years (aOR:1.64 [95%CI:1.07-2.51]). Likewise, we assessed the association between $PM_{2.5}$ and food sensitisation (Table S5.9). A higher concentration of $PM_{2.5}$ at 4 years was associated with food sensitisation at 4 years (aOR:1.44 [95%CI:1.11-1.88] per 1.0 μg/m^3 $PM_{2.5}$ increase) and sesame sensitisation at 4 years (aOR:1.81 [95%CI:1.09-3.01] per 1.0 μg/m^3 $PM_{2.5}$ increase). The results were different to the association estimates from the OFC food allergy analyses indicating that food sensitisation is a poor proxy measure.

Discussion

To the best of our knowledge, our study is the first study to evaluate the association between air pollution and challenge-proven food allergy in infants during the first ten years of life. In this population-based cohort of infants followed over the first ten years of life, based in Australia, where pollution levels are generally low, [24] we observed that air pollution exposure during the first year of life was associated with increased odds of challenge-confirmed food allergy during early childhood. Moreover, there was strong evidence that exposure to air pollution at 1 and 4 years increased the risk of food allergy and challenge-confirmed peanut allergy. Moreover, exposure to air pollution during the first year of life increases the risk of persistent food allergy and persistent peanut allergy. In contrast, there was an absence of associations between the air pollution markers and eczema prevalence or persistence during the same period. Additionally, there was evidence that increased levels of air pollution exposure were associated with increased odds of food and peanut sensitisation.

There is an increasing number of studies that have considered air pollution exposure as a risk factor for eczema in infants and children, but the results have been inconsistent so far. [39] On the one hand, studies found evidence of associations between air pollution and increased eczema prevalence in infants and children. [16,40-43] While other studies have reported null findings between air pollution and increased risk of eczema during early life. [44-49] Specifically, a study done in the Netherlands, where the mean NO_2 was 25.4 μg/m^3 and $PM_{2.5}$ was 16.9 μg/m^3, reported no evidence of an association between these air pollution markers and eczema in 8 years old children.

Concordant with our results, we found no evidence to support an association between air pollution (measured as NO_2 and $PM_{2.5}$) and eczema prevalence or persistence in the first ten years of life. The inconsistent results seen in the literature may be due to variations in the diagnosis criteria for eczema in various nations, temperature and humidity, and the method used to define air pollution (i.e. ground-level monitoring vs satellite-based estimations). [39] Furthermore, different types of air pollutants exist and are exposed to humans in different proportions and at varying levels throughout the world, [39] our results come from a low pollution setting.

Despite the abundance of data demonstrating the connection between air pollution and allergic respiratory diseases, the data on food allergy is scarce. [5] Moreover, previous studies on this topic have used self-reported food allergy or food sensitisation as a proxy for food allergy, which can be problematic because of recall bias or overestimation of true prevalence by around 50%, respectively. [5] A study from China reported that early life exposure to NO_2 was significantly associated with self-reported food allergy in young children (aOR:1.38 [95%CI:1.03–1.85] per IQR increase). [19] Another similar study from China reported that air pollution exposure in the postnatal period was associated with increased self-reported food allergy in pre-schoolers (aOR:1.30 [95%CI:1.01–1.66] per IQR increase). Food allergen sensitisation is a poor proxy measure of food allergy because some food-sensitised children can orally tolerate the food to which they are sensitised. [5] Nevertheless, a study from Canada reported that the NO_2 levels were significantly associated to peanut sensitisation (OR = 1.07, 95% CI:1.03–1.11) in infants. [50] Another study found that higher concentrations of $PM_{2.5}$ increased the risk of having food sensitisation in children aged 8 to 10 years. [51] Moreover, a meta-analysis determined that $PM_{2.5}$ was associated with having food sensitisation at 4 and 8 years of age and exposure to NO_2 was associated with the risk of food sensitisation in early life. [52] In the US, it was reported that $PM_{2.5}$ increased the risk of food sensitisation in infants. [53] A Swedish study showed that early life exposure to nitrogen oxides in the first year of life could increase food sensitisation at eight years, [54] however, the results could not be replicated when the whole cohort was analysed. [55] A study from the Netherlands also reported null results regarding the association between air pollution at birth and food sensitisation in eight year old children. [48] Our findings suggested associations between air pollution and food sensitisation, agreeing with most studies done in this area. [51-54] Furthermore, we found strong evidence of air pollution as a risk factor for food allergy and persistence.

The literature and our results suggested air pollution as a potential environmental factor in the prevalence and persistence of food allergy. [5] The underlying biological mechanisms still need to be fully clarified, but several possible explanations were proposed. [56,57] Air pollution may drive this effect through percutaneous absorption, inhalation, and systemic inflammation. [58] Air pollution

generates reactive oxygen species (ROS) and nitrogen species, which harm DNA, lipids, and proteins. [59] Air pollution can raise serum IgE levels because of their irritant and immunomodulatory effect. [60] It was proposed that Diesel exhaust particles (DEP) showed adjuvant activity for sensitisation against common allergens, and the allergic response could be more severe in a sensitised individual. [61,62] Furthermore, nitrogen dioxides have been linked to boosting the Th2 proallergic response and decreasing the Th1 response. [61] $PM_{2.5}$ may cause cell metabolism and inflammation by activating the aryl hydrocarbon receptor. [60] Other potential pathways are related to gene-environment interactions, variations in the tumour necrosis factor (TNF) and glutathione S-transferase P1 genes can affect allergic sensitisation in infants is developed when exposed to air pollution. [63] DNA methylation is also a possible mechanism, as early pregnancy $PM_{2.5}$ exposure was negatively correlated with placental global DNA methylation. [64] Another study reported that reduced inducible nitric oxide synthase methylation was substantially correlated with increased 7-day average $PM_{2.5}$ exposure. [65] The association between air pollution and food allergy may be influenced by cross-reactions between pollen, included as PM_{10} or $PM_{2.5}$, and specific food allergens. [54] Peanut allergens are known to cross-react with pollen in the air, [66] which may explain our finding of air pollution markers being highly associated with peanut food allergy and sensitisation. Moreover, our findings provide evidence to support the "dual allergen exposure hypothesis",[18] air pollution may be disrupting the epidermis barrier from the skin, gut or airways by the different pathways described before increasing trans-epidermal exposure to food allergens and promoting the development of food allergies.

Our study has many strengths, including access to data from a large multi-wave population-based prospective cohort study with extended follow-up for a 10-year assessment period. The use of objective measures, the criterion standard OFC to define food allergy to address the limitation of previous studies that used self-report or food sensitisation proxy definitions. Similarly, we used SPT to measure food sensitisation objectively. The extensive collection of covariates informed analyses and used land-use regression models to determine air pollution concentrations. Finally, we used multilevel analysis to account for the magnitude of the effect of clustering of the outcomes. Also, there were some limitations in our study. Eczema and some of the covariates definitions were based on self-reports which may lead to recall bias, but robust questions and definitions were used to account for this. There were participants lost to follow-up, which was more pronounced among the less advantaged participants (with high rates lost if parents were smokers and had lower SES), those with CALD status and those without a family history of allergies, eczema or food allergies. This differential loss to follow-up is frequent among prospective studies, and it generally does not cause selection bias when assessing exposure-outcome associations. [67] Another limitation relates to the

number of air pollutants we assessed; additional research is needed to evaluate the effect of other air pollution components on food allergy, although air pollutants are highly correlated. Lastly, external validity considerations must be made as the majority of the cohort were of Caucasian and Anglo-Celtic ethnicity.

Conclusion

Exposure to higher concentrations of air pollution was associated with the development and persistence of food allergy, particularly peanut allergy, in this low air pollution setting. In addition, air pollution may increase food sensitisation in early life. There was no evidence that air pollution exposure increased the risk of eczema. The main guidelines to prevent food allergy involve actively introducing allergenic foods into infants' diets from 6 months. [68] Avoidance of air pollution in early life could potentially be a preventive measure and is likely to improve several other child health outcomes. The use of air purifiers with HEPA filters may reduce air pollution exposure. Limiting outside activity on extremely poor air quality days may also be possible. At a societal level, it would help to take steps to improve air quality (such as avoiding driving, taking public transportation, and switching to non-combustion fuels may have a range of beneficial health effects.

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5.3. Supplementary material

Table S5.1. Baseline Characteristics of study participants lost to follow-up in Wave 4 (10 years).

Characteristic	Lost at 10 years (N= 2,384)	Not lost at 10 years (N=2,893)	Odds to be lost at 10 years
	% (n/N)	% (n/N)	OR (95%CI)
Female	49.2% (1,173/2,384)	48.6% (1,406/2,893)	1.03 (0.93-1.15)
CALD status	23.0% (549/2,384)	15.4% (445/2,893)	**1.68 (1.47-1.93)***
Pet ownership	40.9% (974/2,384)	45.2% (1,309/2,893)	**0.89 (0.79-1.00)**
Family allergies	65.94% (1,572/2,384)	72.2% (2,089/2,893)	**0.74 (0.66-0.84)**
Smoking			
No	51.8% (1,235/2,384)	58.2% (1,685/2,893)	Ref
Former	22.7% (540/2,384)	23.6% (682/2,893)	1.10 (0.96-1.26)
Current	25.5% (609/2,384)	18.2% (526/2,893)	**1.62 (1.41-1.86)**
Eczema	22.8% (544/2,384)	27.0% (781/2,893)	**0.80 (0.71-0.91)**
Food sensitised	15.3% (355/2,319)	18.5% (521/2,823)	**0.80 (0.69-0.93)**
Food allergy	8.1% (175/2,169)	13.1% (359/2,738)	**0.58 (0.48-0.70)**
Peanut allergy	2.1% (46/2,169)	4.0% (110/2,738)	**0.51 (0.36-0.72)**
Egg allergy	7.5% (163/2,169)	11.2% (308/2,738)	**0.64 (0.52-0.78)**
Sesame allergy	0.6% (12/2,169)	0.8% (21/2,738)	**0.70 (0.34-1.42)**
Food & eczema classification			
No eczema or food allergy	74.9% (1,533/2,046)	67.6% (1,750/2,588)	Ref
Food allergy only	2.8% (57/2,046)	5.2% (134/2,588)	**0.49 (0.35-0.67)**
Eczema only	17.3% (354/2,046)	19.2% (498/2,588)	**0.81 (0.70-0.95)**
Food allergy & eczema	5.0% (102/2,046)	8.0% (206/2,588)	**0.57 (0.44-0.72)**
Area level characteristics			
Greenness†§	.39 (.11; .01, .74)	.39 (.11; .01, .72)	1.15 (0.63-2.10)
SEIFA categories			
1	24.2% (576/2,384)	17.1% (494/2,893)	Ref
2	19.6% (467/2,384)	20.4% (591/2,893)	**0.67 (0.57-0.80)**
3	19.5% (466/2,384)	22.0% (636/2,893)	**0.63 (0.54-0.75)**
4	18.6% (443/2,384)	19.9% (576/2,893)	**0.67 (0.56-0.79)**
5 (Highest)	18.2% (432/2,384)	20.6% (596/2,893)	**0.63 (0.53-0.85)**
NO_2‡ (ppb)‡	11.2(9.5, 12.9)[2.9, 22.5]	11.2(9.8, 12.9)[2.82, 20.1]	**0.97 (0.95-1.00)**
$PM_{2.5}$‡ (µg/m^3)‡	8.4 (7.6, 9.3)[2.5, 12.2]	8.4 (7.7, 9.3)[3.2, 12.2]	0.98 (0.94-1.03)

CALD: Culturally and linguistically diverse Ref: Reference group ppb: parts per billion µg/m3: micrograms per cubic meter air

† Mean (Standard deviation; min, max)

‡ Median (25^{th} percentile, 75^{th} percentile)[min, max]

§ A greater greenness score (range: -1, 1) implies a greater proportion of vegetation .

*P-value less than 0.05 is bolded

Table S5.2. Linearity assessment. Adjusted association estimates between NO_2 categorized in 8 quantiles and food allergy at each Wave.

Outcome	Food allergy at 1 year	Food allergy at 4 years	Food allergy at 6 years
	aOR (95%CI) †	**aOR (95%CI)** †	**aOR (95%CI)** †
NO_2 at 1 year			
1st quantile	Ref	Ref	Ref
2nd quantile	1.33 (0.88-2.01)	1.14 (0.52-2.48)	0.88 (0.42-1.86)
3rd quantile	1.39 (0.92-2.11)	1.09 (0.50-2.42)	1.05 (0.51-2.17)
4th quantile	**1.71 (1.14-2.56)***	**2.13 (1.04-4.33)**	1.45 (0.74-2.84)
5th quantile	**1.65 (1.09-2.50)**	1.74 (0.83-3.66)	1.53 (0.76-3.06)
6th quantile	1.48 (0.96-2.27)	1.68 (0.78-3.62)	1.50 (0.74-3.04)
7th quantile	1.02 (0.64-1.64)	0.81 (0.33-2.03)	0.70 (0.30-1.67)
8th quantile	1.24 (0.77-2.00)	1.29 (0.53-3.13)	1.08 (0.46-2.51)
NO_2 at 4 years			
1st quantile		Ref	Ref
2nd quantile		1.07 (0.81-2.25)	0.86 (0.40-1.85)
3rd quantile		1.38 (0.68-2.80)	1.43 (0.72-2.85)
4th quantile		1.24 (0.60-2.58)	1.55 (0.78-3.08)
5th quantile		1.57 (0.77-3.21)	1.41 (0.70-2.85)
6th quantile		1.33 (0.62-2.82)	1.85 (0.91-3.75)
7th quantile		1.04 (0.45-2.38)	0.98 (0.43-2.26)
8th quantile		1.62 (0.69-3.82)	1.74 (0.75-4.05)
NO_2 at 6 years			
1st quantile			Ref
2nd quantile			2.09 (0.91-4.81)
3rd quantile			1.68 (0.70-4.02)
4th quantile			2.11 (0.91-4.90)
5th quantile			2.01 (0.86-4.71)
6th quantile			**2.67 (1.16-6.14)**
7th quantile			1.94 (0.80-4.71)
8th quantile			**2.51 (1.01-6.22)**

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

*P-value less than 0.05 is bolded

Table S5.3. Adjusted association estimates from the standard logistic regression models between NO_2 and allergic outcomes at each Wave.

Outcome	**NO_2 at 1 year** (IQR: 3.2 ppb)	**NO_2 at 4 years** (IQR: 2.6 ppb)	**NO_2 at 6 years** (IQR: 2.5 ppb)	**NO_2 at 10 years** (IQR: 2.5 ppb)
	aOR (95%CI) †	**aOR (95%CI)** †	**aOR (95%CI)** †	**aOR (95%CI)** †
Eczema				
1 year	0.95 (0.86-1.05) ‡			
4 years	1.07 (0.91-1.25) ‡	0.89 (0.68-1.17) ‡		
6 years	0.86 (0.74-1.01) ‡	0.87 (0.75-1.02) ‡	0.90 (0.78-1.03) ‡	
10 years	0.92 (0.78-1.08) ‡	0.97 (0.81-1.16) ‡	1.00 (0.85-1.17) ‡	1.03 (0.87-1.21) ‡
Food Allergy				
1 year	1.27 (0.99-1.64) §			
4 years	1.58 (0.97-2.57) §	1.33 (0.83-2.13) §		
6 years	1.43 (0.90-2.27) §	**1.63 (1.00-2.63)** *§	1.39 (0.87-2.21) §	
10 years				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ ORs (95% CIs) per an IQR increase in NO_2

§ Non-linear association, a dichotomous variable (lower 25% vs upper 75% of the air pollution marker distribution) was used.

*P-value less than 0.05 is bolded

Table S5.4. Adjusted association estimates from the standard logistic regression models between $PM_{2.5}$ and allergic outcomes at each Wave.

Outcome	**$PM_{2.5}$ at 1 year** (IQR: 1.6 μg/m^3)	**$PM_{2.5}$ at 4 years** (IQR: 1.0 μg/m^3)	**$PM_{2.5}$ at 6 years** (IQR: 1.1 μg/m^3)	**$PM_{2.5}$ at 10 years** (IQR: 1.1 μg/m^3)
	aOR (95%CI) †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡
Eczema				
1 year	0.99 (0.90-1.10)			
4 years	1.03 (0.89-1.20)	0.90 (0.78-1.03)		
6 years	0.89 (0.77-1.02)	0.96 (0.82-1.09)	1.04 (0.92-1.18)	
10 years	0.95 (0.81-1.11)	0.96 (0.82-1.12)	0.94 (0.81-1.08)	1.00 (0.85-1.17)
Food Allergy				
1 year	1.12 (0.97-1.29)			
4 years	**1.39 (1.07-1.80)**	1.21 (0.95-1.56)		
6 years	**1.31 (1.02-1.70)**	**1.28 (1.00-1.65)**	1.23 (0.97-1.54)	
10 years				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ ORs (95% CIs) per an IQR increase in $PM_{2.5}$

*P-value less than 0.05 is bolded

Table S5.5. Adjusted association estimates from the multilevel logistic regression models between air pollution markers at 1 year and eczema persistence and remission.

Outcome	**NO_2 at 1 year‡** (IQR: 3.2 ppb)	**$PM_{2.5}$ at 1 year‡** (IQR: 1.6 μg/m^3)
Eczema outcomes	**aOR (95%CI)** †	**aOR (95%CI)** †
0-4 year outcomes		
Never eczema	Ref	Ref
Remitting eczema	0.90 (0.79-1.02)	1.03 (0.91-1.16)
Persistent eczema	1.15 (0.90-1.47)	1.01 (0.82-1.25)
0-6 year outcomes		
Never eczema	Ref	Ref
Remitting eczema	0.94 (0.82-1.08)	0.98 (0.86-1.11)
Persistent eczema	1.04 (0.76-1.44)	0.92 (0.69-1.22)
0-10 year outcomes		
Never eczema	Ref	Ref
Remitting eczema	0.97 (0.84-1.12)	0.97 (0.85-1.11)
Persistent eczema	0.96 (0.64-1.46)	0.84 (0.57-1.25)

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ ORs (95% CIs) per an IQR increase.

*P-value less than 0.05 is bolded

Table S5.6. Adjusted association estimates from the multilevel logistic regression models between NO_2 and peanut food and egg allergy at each Wave.

Outcome	NO_2 at 1 year	NO_2 at 4 years	NO_2 at 6 years	NO_2 at 10 years
	aOR (95%CI) †‡	aOR (95%CI) †‡	aOR (95%CI) †‡	aOR (95%CI) †‡
Peanut allergy				
1 year	**2.08 (1.25-3.47)***			
4 years	1.80 (0.97-3.35)	1.70 (0.92-3.13)		
6 years	1.56 (0.90-2.69)	**1.83 (1.04-3.22)**	1.48 (0.86-2.55)	
10 years				
Egg Allergy				
1 year	1.12 (0.93-1.63)			
4 years	1.30 (0.62-2.73)	1.69 (0.81-3.54)		
6 years	1.07 (0.46-2.51)	1.74 (0.73-4.16)	2.08 (0.84-5.15)	
10 years				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ Non-linear association, a dichotomous variable (lower 25% vs upper 75% of the air pollution marker distribution) was used.

*P-value less than 0.05 is bolded

Table S5.7. Adjusted association estimates from the multilevel logistic regression models between $PM_{2.5}$ and peanut and egg allergy at each Wave.

Outcome	**$PM_{2.5}$ at 1 year** (IQR: 1.6 μg/m^3)	**$PM_{2.5}$ at 4 years** (IQR: 1.0 μg/m^3)	**$PM_{2.5}$ at 6 years** (IQR: 1.1 μg/m^3)	**$PM_{2.5}$ at 10 years** (IQR: 1.1 μg/m^3)
	aOR (95%CI) †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡
Peanut allergy				
1 year	1.11 (0.87-1.43)			
4 years	1.32 (0.96-1.81)	1.15 (0.85-1.55)		
6 years	1.26 (0.94-1.70)	1.28 (0.96-1.70)	**1.34 (1.02-1.76)**	
10 years				
Egg Allergy				
1 year	1.07 (0.92-1.25)			
4 years	1.34 (0.89-2.03)	1.02 (0.72-1.45)		
6 years	1.16 (0.72-1.88)	0.82 (0.55-1.22)	0.93 (0.63-1.38)	
10 years				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ ORs (95% CIs) per an IQR increase in $PM_{2.5}$

*P-value less than 0.05 is bolded

Table S5.8. Adjusted association estimates from the multilevel logistic regression models between NO_2 and food allergen sensitisation at each Wave.

Outcome	NO_2 at 1 year	NO_2 at 4 years	NO_2 at 6 years	NO_2 at 10 years
	aOR (95%CI) †§	aOR (95%CI) †§	aOR (95%CI) †§	aOR (95%CI) †
All food allergens				
1 year	**1.24 (1.01-1.54)**			
4 years	0.94 (0.58-1.54)	1.30 (0.81-2.13)		
6 years	1.27 (0.90-1.83)	1.31 (0.90-1.91)	1.18 (0.82-1.69)	
10 years				
Peanut sensitised				
1 year	**1.34 (1.00-1.81)**			
4 years	0.82 (0.49-1.37)	1.56 (0.90-2.71)		
6 years	1.32 (0.89-1.95)	**1.64 (1.07-2.51)**	1.31 (0.88-1.96)	
10 years				
Egg sensitised				
1 year	1.22 (0.98-1.54)			
4 years	1.16 (0.62-2.17)	1.82 (0.95-3.47)		
6 years	1.05 (0.56-1.99)	1.36 (0.69-2.68)	1.40 (0.71-2.74)	
10 years				
Sesame sensitised				
1 year	1.40 (0.76-2.60)			
4 years	0.76 (0.35-1.63)	1.10 (0.51-2.37)		
6 years	0.82 (0.35-1.94)	1.30 (0.52-3.25)	1.87 (0.75-4.67)	
10 years				

aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

§ Non-linear association, a categorical air pollution exposure was used

*P-value less than 0.05 is bolded

Table S5.9. Adjusted association estimates from the multilevel logistic regression models between $PM_{2.5}$ and food allergen sensitisation at each Wave.

Outcome	**$PM_{2.5}$ at 1 year** (IQR: 1.6 μg/m^3)	**$PM_{2.5}$ at 4 years** (IQR: 1.0 μg/m^3)	**$PM_{2.5}$ at 6 years** (IQR: 1.1 μg/m^3)	**$PM_{2.5}$ at 10 years** (IQR: 1.1 μg/m^3)
	aOR (95%CI) †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡	**aOR (95%CI)** †‡
All food allergens				
1 year	1.02 (0.91-1.15)			
4 years	1.03 (0.80-1.34)	**1.44 (1.11-1.88)**		
6 years	1.20 (0.98-1.46)	1.16 (0.95-1.42)	1.16 (0.96-1.39)	
10 years				
Peanut sensitised				
1 year	1.05 (0.89-1.24)			
4 years	0.88 (0.67-1.16)	1.29 (0.97-1.72)		
6 years	1.10 (0.89-1.37)	1.20 (0.96-1.50)	1.20 (0.98-1.46)	
10 years				
Egg sensitised				
1 year	1.03 (0.90-1.16)			
4 years	0.98 (0.71-1.35)	1.22 (0.90-1.68)		
6 years	1.20 (0.85-1.71)	1.02 (0.72-1.45)	0.98 (0.72-1.34)	
10 years				
Sesame sensitised				
1 year	1.00 (0.72-1.40)			
4 years	1.03 (0.67-1.58)	**1.81 (1.09-3.01)**		
6 years	1.31 (0.77-2.23)	1.01 (0.62-1.64)	1.20 (0.75-1.91)	
10 years				

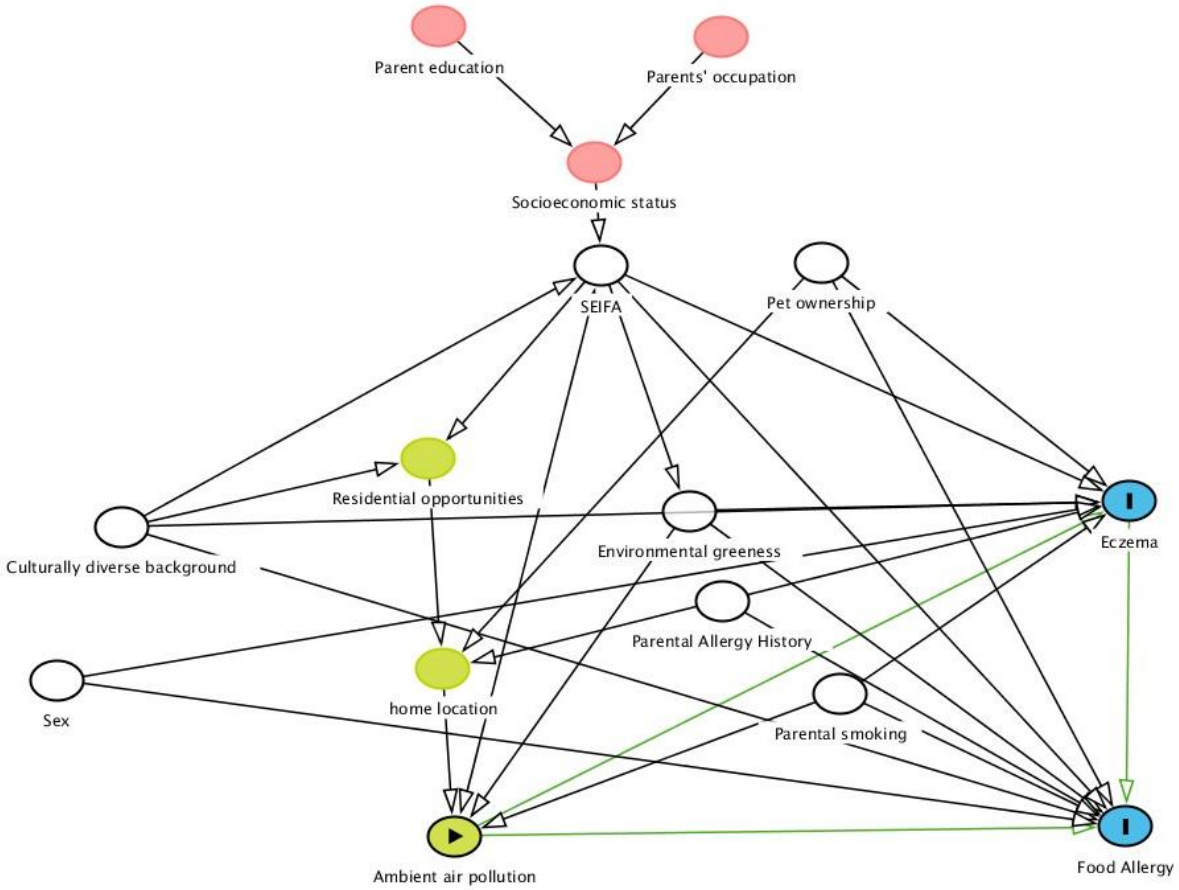
aOR: adjusted odds ratio 95% CI: 95% confidence interval.

†Adjusted for: Culturally diverse background, sex, parents' smoking status, immediate family allergic history, pet ownership, environmental greenness and SEIFA.

‡ ORs (95% CIs) per an IQR increase in $PM_{2.5}$

*P-value less than 0.05 is bolded

Figure S5.1. Minimal set of confounders for the association between air pollution and eczema and food allergy according to a DAG.



Chapter 6 - Association between ambient air pollution and development and persistence of atopic and non-atopic eczema in a cohort of adults.

6.1. Section introduction

My objective in this research project was to examine whether exposure to air pollution is associated with the incidence and prevalence of eczema in middle-aged adults and evaluate if this association was different depending on the atopic status of the participant. Evidence in this area conflicted with some studies reporting positive associations while others did not. Furthermore, most of the studies were on children, while few reported findings from adults. A better understanding of this topic can better inform prevention and treatment strategies. The data for this project came from the TAHS 2002 proband study and the TAHS 2012 proband study. The dataset includes the participants' sociodemographic factors, economic variables, disease status and air pollution exposures at both time points.

6.1.1. Research question

- Is the exposure to air pollution associated with the incidence and prevalence eczema in a cohort of middle-aged adults?
    - Do these associations vary according to atopic or non-atopic status?

6.1.2. Research aim

- To examine whether exposure to air pollution is associated with the incidence and prevalence of eczema in middle-aged adults.
    - To assess whether these association vary according to atopic or non-atopic status.

6.1.3. Critical reflection on the work

I had previous experience with the statistical methods (logistic and multinomial regressions) that I applied to this project, so the analyses were relatively easy initially. I spent considerable time improving my writing skills as a non-native English speaker with the feedback of my supervisors. I wrote the manuscript and submitted it to a journal, where the editor suggested a revision. One of the reviewers asked for logistic regression diagnostics (post-estimations), which I have never done before. Fortunately, my web search and self-learning skills improved, and I found a tutorial on how to do the diagnostics on Stata and included it with comments on the manuscript. With my supervisors' help, I resubmitted the manuscript, which was accepted for publication in the Allergy

journal which is the highest ranked journal in the Allergy field. Also, I developed a plain language statement about this research that was sent to the TAHS participants (Appendix A).

6.1.4. Publication citation

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***Please note that the specific references from this accepted or submitted manuscript are at the end of this chapter and the in-text citation number restarts at 1.**

6.2. Author accepted version of the manuscript.

Title: Association between ambient air pollution and development and persistence of atopic and non-atopic eczema in a cohort of adults

Running title: Ambient air pollution and atopic and non-atopic eczema in adults

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Lowe, C.J. Lodge, D.S. Bui, N.T. Waidyatillake, J.C. Su. Drafting the article : D.J. Lopez, A.J. Lowe, C.J. Lodge, D.S. Bui, N.T. Waidyatillake, J.C. Su. Revision for important intellectual content and approval of final submission : all authors.

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Dr. Lopez has nothing to disclose.

Dr. Lodge has nothing to disclose.

Dr. Bui has nothing to disclose.

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Dr. Thompson has nothing to disclose.

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Dr. Walters has nothing to disclose.

Dr. Dharmage has nothing to disclose.

Dr. Bowatte has nothing to disclose.

Dr. Lowe has nothing to disclose.

Main text word count: 3492

Abstract

Background: There is limited information on risk factors for eczema in adults. Recent evidence suggests that air pollution may be associated with increased incidence of eczema in adults. We aimed to assess this possible association.

Methods: Ambient air pollution exposures (distance from a major road, nitrogen dioxide [NO_2], fine particulate matter with an aerodynamic diameter ≤2.5 µm [$PM_{2.5}$]) were assessed for the residential address of Tasmanian Longitudinal Health Study participants at ages 43 and 53 years. Eczema incidence (onset after age 43 years), prevalence (at 53 years) and persistence were assessed from surveys, while IgE sensitisation was assessed using skin prick tests. The presence or absence of eczema and sensitisation was classified into four groups: no atopy or eczema, atopy alone, non-atopic eczema, and atopic eczema. Adjusted logistic and multinomial regression models were fitted to estimate associations between ambient air pollution and eczema, and interaction by sex was assessed.

Results: Of 3153 participants in both follow ups, 2369 had valid skin prick tests. For males, a 2.3 ppb increase in baseline NO_2 was associated with increased odds of prevalent eczema (OR=1.15 [95%CI 0.98-1.36]) and prevalent atopic eczema (OR=1.26 [1.00-1.59]). These associations were not seen in females (P for interaction=0.08, <0.01). For both sexes, a 1.6 µg/m^3 increase in $PM_{2.5}$ exposure at follow-up was associated with increased odds of aeroallergen sensitisation (OR=1.15 [1.03-1.30]).

Conclusion: Increased exposure to residential ambient air pollutants was associated with an increased odds of eczema, only in males, and aeroallergen sensitisation in both genders.

Keywords: adults, atopy, dermatitis, eczema, middle-age, ambient air pollution

Word count: 247

Introduction

Eczema, also known as atopic dermatitis, is a chronic inflammatory skin condition, characterised by defective skin barrier function and it affects around 5% to 10% of adults and up to 20% of children. [1] Eczema has a heterogeneous presentation, which varies in terms of severity, age of onset, and response to treatment. [2]

The World Allergy Organization has recognised at least two types of eczema. [3] An atopic type (AE) with skin inflammation driven by T-cell responses and Th_2 cytokines in the initial phase, which is usually associated with IgE-mediated sensitisation to environmental allergens and high levels of both total and allergen-specific IgE. This form of eczema is strongly associated with increased tendency of developing other allergic conditions. [4] The second form, non-atopic eczema (NAE), is characterised by normal levels of total IgE and lack of sensitisation to environmental allergens. [4] The pathophysiology of NAE is poorly understood[3], and, particularly in older patients and those with chronic eczema, other non-atopic inflammatory mechanisms might be involved. [4]

There have been relatively few studies of the risk factors for eczema in adults. [5] In paediatric studies, higher levels of ambient air pollutants have been associated with increased eczema prevalence. [6] It has been proposed that air pollutants may generate reactive oxygen species which damage the outer-most layer of the skin through oxidative stress. [7] This process may drive the inflammation and pruritus that are associated with eczema, and this may subsequently downregulate filaggrin expression, further compromising the structural integrity of the epidermal barrier. [8] The effect of ambient air pollution on the prevalence and incidence of eczema in adults has received less attention.

A recent longitudinal analysis of middle aged German women, which investigated the influence of traffic related air pollution on lung function, inflammation and Aging (SALIA) found that baseline concentrations of traffic-related air pollution (TRAP) markers (NO_2, NO_x, $PM_{2.5}$ and PM_{10}) were significantly associated with increased odds of incident eczema over a 19-year follow-up period, these associations being stronger for NAE. [5] Therefore, environmental factors, including air pollution, might be important for development of eczema in middle age, particularly NAE. These findings need to be replicated using similar longitudinal data to draw firmer conclusions. Better understanding of the potential effects of ambient air pollution on adult eczema may lead to targeted interventions to prevent eczema. Using data from a large established longitudinal health study, we investigated whether exposure to ambient air pollution was associated with the incidence and prevalence of AE or NAE in middle-aged adults of both sexes.

Methods

Study population

The Tasmanian Longitudinal Health study (TAHS) is a population-based prospective cohort study, that has followed participants since 1968 when 7-year-old children (98.7%, n=8583) attending schools in the Australian state of Tasmania were recruited. [9] Several follow-up surveys have subsequently been conducted and study methodology has been reported in detail elsewhere. [9] The data for this analysis came from participants of the 2002 and 2012 proband studies when participants had a mean age of 43 and 53 years old respectively. Participants completed a self-administered postal survey that collected the following baseline (2002) and follow-up (2012) data: sociodemographic characteristics, occupation, residence, health service use, medical diagnoses, smoking, reproductive histories, and symptoms. Only participants who completed both assessments and had valid skin prick tests (performed at the 2012 follow-up) were included in the analysis. The study was approved by the Human Research Ethics Committee of the University of Melbourne; all participants provided written informed consent.

Distance to a major road (DMR)

Straight-line distances from each participant's residence to the nearest major road in 2002 and 2012 proband studies were calculated using ArcGIS 10.1 software (Redlands, CA). Major roads were defined using public sector mapping agencies, and Australian transport hierarchy codes 301 and 302. [10] These included freeways, highways and arterial roads, which were likely to have high volumes of traffic. Participants were categorised into two groups: (i) living <200m; or (ii) living ≥200m from a major road. Major traffic pollutant concentrations tend to decay as the distance to major roads (DMR) increases, with most components of TRAP reaching near background concentrations at approximately 200m. [11]

Nitrogen dioxide (NO_2)

A satellite-based land-use regression model was used to assign mean annual NO_2 exposures for the 2002 and 2012 proband studies. [12] Briefly, the land-use regression model-predicted mean annual NO_2 levels were based on tropospheric NO_2 columns derived from satellite observations in combination with other predictors such as land use and roads, to estimate ground-level NO_2 across Australia. [12] As more than half of the ambient NO_2 is attributed to on-road sources, NO_2 is a reasonable proxy for TRAP. [13] The model's development and validation are described in detail elsewhere, and it explained 81% of spatial variability in measured annual NO_2 at all regulatory monitoring sites in Australia. [12] Mean annual residential exposures to outdoor NO_2 were estimated and assigned based on participants' geocoded addresses at baseline (2002 proband study) and follow-up (2012 proband study).

Fine particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$)

The methods are explained in more detail elsewhere. [14] In brief, satellite-based estimates for Australia of ground-level $PM_{2.5}$ were used as a land-use regression predictor, with other spatial predictors of $PM_{2.5}$. This model explained 63% of spatial variability in measured annual $PM_{2.5}$ (RMSE: 1 µg/m^3). [14] The mean annual residential exposures to outdoor $PM_{2.5}$ were estimated and assigned based on participants' geocoded addresses at baseline and follow-up. In Australia, traffic-related sources of $PM_{2.5}$ are estimated to account for only 17% of ambient $PM_{2.5}$ mass, while the majority of ambient $PM_{2.5}$ is from other anthropogenic sources (i.e. wood heaters, power stations, etc). [15]

Prevalent eczema at 53 years

Prevalent eczema at age 53 (2012 proband study) was determined using the International Study of Asthma and Allergies in Childhood (ISAAC) definition of eczema. [16] Participants were classified as having prevalent eczema if they reported "yes" to all three questions: 'have you had an itchy rash in the past 12 months?', 'Have you ever had an itchy rash coming and going for at least six months?', and 'Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?'

Incident eczema at 53 years

Incident eczema at 53 years was defined as eczema newly arising between the two proband studies i.e. between 43 and 53 years. The participants were classified as having Incident eczema if they answered "no" to ''Have you ever had eczema or any skin allergy?'' at baseline (2002 proband study), but reported eczema based on the ISAAC definition[16] and having eczema for the first time after baseline at ''How old were you when you first had this itchy rash?'' at the follow-up (2012 proband study).

Persistent current eczema

Persistent current eczema was defined as prevalent eczema at baseline that persisted to follow-up. Participants were classified as having persistent current eczema if they answered "yes" to ''Have you ever had eczema or any skin allergy?'' at baseline and reported eczema based on the ISAAC definition[16] at follow-up.

Atopic status

Subclassification as AE or NAE was based on skin prick testing (SPT) results at age 53 years. [9] In the 2012 proband study, SPTs were performed for eight aeroallergens: *Dermatophagoides pteronyssinus*, cat pelt, *Cladosporioides*, *Alternaria tenuis*, *Penicillium* mix, *Aspergillus fumigatus*, mixed grass pollen No. 7. Histamine was used as the positive control and normal saline as the negative control. After 10 to 15 mins, the wheal diameters were measured in two perpendicular directions in millimetres and an average was derived. A valid SPT was determined by a positive

control or allergen wheal equal to or greater than 3 mm in size and a negative control wheal equal to or less than 3 mm in size. A positive SPT was defined as a wheal size of at least 3 mm greater than the negative control and was considered to indicate sensitisation to that allergen. [17] Atopy was defined as sensitization to at least one of the allergens tested.

Statistical Analysis

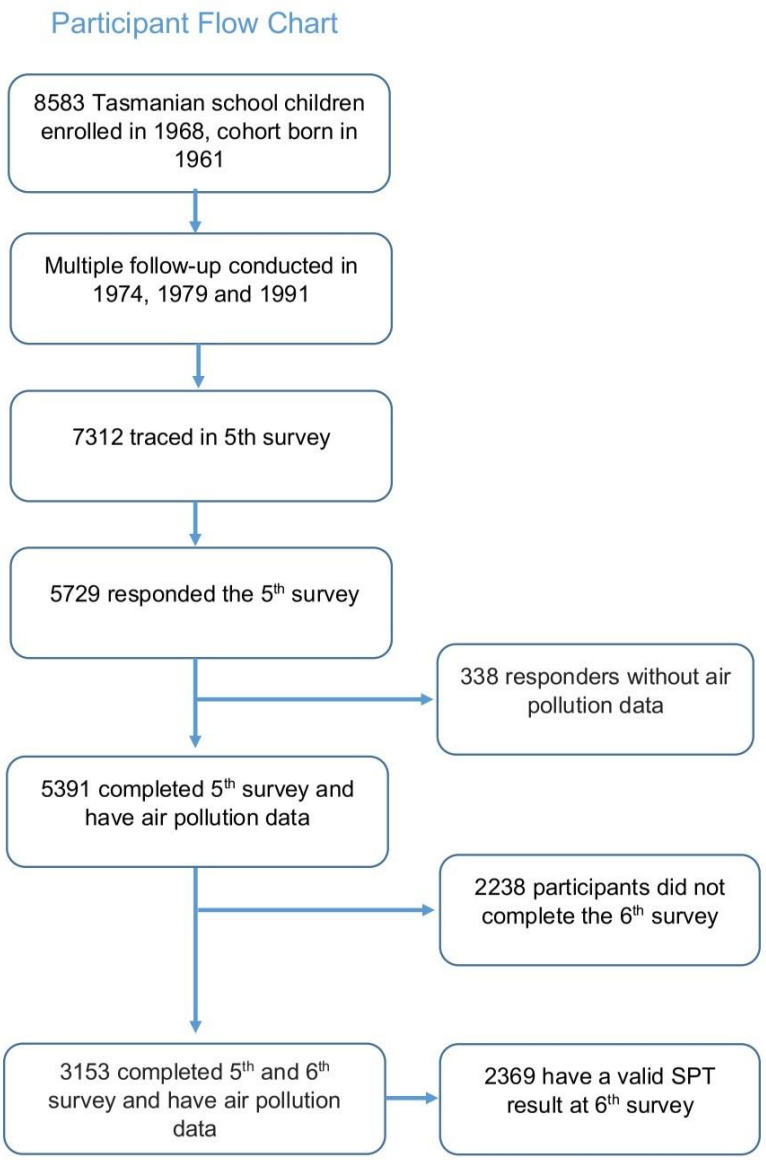
Associations between markers of ambient air pollution and the following outcome measures were assessed: 1) Prevalent eczema, 2) Incident eczema, 3) Persistent eczema, 4) Prevalent and incident eczema sub grouped by atopic status (neither, atopy alone, non-atopic and atopic eczema) and 5) Sensitisation (regardless of eczema status). Second, in accordance with the eczema and atopy classification used in the SALIA cohort study[5], we examined NAE incidence and prevalence using three increasingly restricted subgroups: 1) all participants, 2) participants without hay fever ever, 3) participants without hay fever ever and negative SPT.

Logistic regression and multinomial models were fitted to estimate the associations between baseline ambient air pollution and each outcome. The coefficients represented the estimated effect per interquartile range (IQR) increase of air pollutant exposure and were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A directed acyclic graph (DAG) (Figure S6.1) was developed to specify the hypothesised causal relationships and to determine which confounders to include in the model (Table S6.1). The potential presence of non-linearity of these associations was assessed using Stata's "fracpoly" command; no evidence of non-linearity of associations between ambient air pollution markers and eczema was identified. To detect outliers and influential data points, we separated the main multinomial regression models into sets of logistic regression models and plotted their residuals. Potential effect modification by sex was explored using likelihood ratio tests and a p value < 0.1 was considered as significant. A sensitivity analysis was performed, where associations were assessed only in the participants who did not change residential address during follow-up period. All analyses were carried out using the statistical software Stata (release 16; Stata Corporation, College Station, TX).

Results

Of 8583 Tasmanian school children enrolled in 1968 at age 7 years, 5729 were included in the 2002-08 proband study (Figure 6.1), of whom 338 were excluded because the participant did not provide a residential address allowing ambient air pollution exposure to be estimated. Of the remaining 5391 participants, 2238 were excluded because they did not participate in the 2012 proband study. Of the 3153 with data from both the 2002 and 2012 proband studies, 2369 had a valid SPT result. There were no important differences between those followed and those lost to follow-up, except that those lost were more likely to be smokers or exposed to smoke and being from lower socioeconomic status, less likely to report hay fever. (Table S6.2).

Figure 6.1. Participant flow chart.



The mean age at the 2012 follow-up was 53 years and 50.3% were males (Table 6.1). At 53 years 281 participants (8.96%) of the participants had prevalent eczema, 115 participants (3.67%) had incident eczema and 201 participants (6.38%) had persistent eczema (Table S6.3). When comparing baseline and follow-up concentrations, ambient air pollution markers decreased slightly over time (Table 6.1).

Table 6.1. Characteristics of participants included. (N=3153)

Co-variates	2002 Survey	2012 Survey
Age (years). Mean (SD); min-max	43 (0.82); 41-44	53 (0.95); 50.94-55.58
Male. %(n/N)	50.3 (1,586/3,153)	
Body mass index (kg/m^2). Mean (SD); min-max	26.27 (4.61); 16.31- 52.33	28.4 (5.48); 16.9-58.86
Heating by indoor combustion of solid† or gas fuel. %(n/N)	53.2 (1,679/3,153)	43.8 (1,383/3,153)
Cooking by indoor combustion of solid† or gas fuel. %(n/N)	24.1 (757/3,153)	28.7 (886/3,081)
Years of education. %(n/N)		
< Grade 12	34.7 (1,093/3,153)	32.7 (1,020/3,153)
Grade 12 or equivalent	9.5 (300/3,153)	7.8 (242/3,153)
> Grade 12	55.8 (1,755 /3,153)	59.6 (1,860/3,153)
Occupation. %(n/N)		
Managers and Professionals	30.9 (970/3,135)	35 (1,087/3,105)
Associate Professionals	11.4 (357/3,135)	14.1 (439/3,105)
Tradespersons and Advanced Clerical	20.7 (648/3,135)	18.8 (585/3,105)
Intermediate clerical and production	18 (563/3,135)	18 (558/3,105)
Elementary clerical, laborers and related	19 (597/3,135)	14 (436/3,105)
Smoking status. %(n/N)		
Never	44.5 (1,404 /3,143)	44.5 (1,402/3,120)
Former	31.6 (995/3,143)	38.4 (1,211/3,120)
Current	23.6 (744 /3,143)	16.1 (507 /3,120)
Current second-hand smoke. %(n/N)	11.6 (358 / 3,096)	7.8 (244/3,153)
Hay fever ever. %(n/N)	53 (1,660/3,153)	55.8 (1,757/3,153)
Living <200m from a major road	813 /3,153 (25.78%)	787 /3,048 (25.82%)
NO_2 (ppb) median [IQR; 25-75%le]	4.24 [2.27; 3.43-5.7]	2.72 [2.21; 1.93-4.14]
$PM_{2.5}$ (µg/m^3) median [IQR; 25-75%le]	6.48 [1.49; 5.76-7.26]	6.4 [1.56; 5.63-7.19]

† Solid fuels are coal and wood.

Association between ambient air pollution and prevalent eczema at age 53.

There was evidence that sex modified the association (P for interaction<0.1) between NO_2 and prevalent eczema (Table 6.2). Thus, in males, baseline exposure to NO_2 was associated with increased odds of having prevalent eczema at follow-up (adjusted odds ratio (aOR): 1.15 [95%CI 0.98-1.36] per IQR [2.27ppb] NO_2 increase), while higher exposure in females was associated, although non significantly, with reduced odds of prevalent eczema (aOR: 0.83 [95%CI 0.67-1.03 per 2.27 ppb NO_2 increase). Additional adjustment for family history of allergic disease did not materially alter the results.

Table 6.2. Adjusted† associations between ambient air pollution markers at baseline and prevalent eczema at 53 years, incident eczema‡ and persistent current eczema stratified by sex.

	Eczema prevalence (281/ 3,135) §	**Eczema incidence** (115/3,152) §	**Eczema persistence** (201/3150) §
Air pollution markers at baseline			
	aOR (95%CI) † p	aOR (95%CI) † p	aOR (95%CI) † p
DMR<200m	1.10 (0.83-1.45) 0.514	1.15 (0.76-1.75) 0.518	1.06 (0.76-1.48) 0.722
Males	1.17 (0.77-1.77)	0.88 (0.48-1.63)	1.26 (0.78-2.03)
Females	1.04 (0.71-1.53)	1.55 (0.86-2.79)	0.91 (0.57-1.44)
P for interaction	0.653	0.145	0.675
NO_2‡	1.01 (0.88-1.15) 0.890	1.10 (0.90-1.33) 0.781	0.96 (0.82-1.13) 0.604
Males	1.15 (0.98-1.36)	1.13 (0.88-1.43)	1.15 (0.94-1.40)
Females	0.83 (0.67-1.03)	1.02 (0.74-1.41)	0.75 (0.58-0.98)
P for interaction	**0.018***	0.530	**0.033**
$PM_{2.5}$‡	0.97 (0.84-1.13) 0.740	1.17 (0.92-1.47) 0.195	0.84 (0.71-1.00) 0.048
Males	0.96 (0.77-1.20)	1.21 (0.88-1.66)	0.91 (0.70-1.18)
Females	1.00 (0.82-1.23)	1.11 (0.79-1.56)	0.80 (0.63-1.01)
P for interaction	0.809	0.686	0.685

*P for interaction with p<0.1 are bolded

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and second-hand smoking.

‡ORs (95% CIs) are given per an IQR increase in air pollution marker

§Few missing observations

Likewise, associations also differed by sex when prevalent eczema was classified by atopy status (Table 6.3). In males, baseline NO_2 exposure was associated with increased odds of having AE (aOR 1.26 [1.00-1.59]) per 2.27 ppb NO_2 increase, while baseline $PM_{2.5}$ was associated with increased odds of having AE (aOR 1.47 [1.04-2.06]) per 1.56 µg/m^3 $PM_{2.5}$ increase. By contrast, in females, higher NO_2 exposure at baseline was associated with a reduced odds of having AE (aOR 0.65 [95% CI 0.43-0.99]) per 2.27 ppb NO_2 increase. A similar trend was seen at follow-up, NO_2 was associated with a reduced odds of AE (aOR 0.67 [0.44-1.01]) per 2.21 ppb increase (Table 6.3). When the same associations were assessed restricting only to those who did not change their address (non-movers), only negligible variation of the estimated effects were shown (Table S6.4).

Table 6.3. Adjusted associations between ambient air pollution exposure at the baseline and prevalent eczema at 53 years by atopy with stratified results by sex.

	No atopy or eczema (972/ 2,360)	**Atopy alone** (1,172/ 2,360)	**Non-atopic Eczema** (83 / 2,360)	**Atopic Eczema** (133 / 2,360)
Air pollution markers at baseline§				
	aOR (95%CI) †	aOR (95%CI) †	aOR (95%CI) †	aOR (95%CI) †
DMR	Ref group	1.15 (0.95-1.41) 0.150	1.46 (0.90-2.38) 0.128	0.93 (0.60-1.44) 0.837
Males	Ref group	1.09 (0.82-1.46)	1.41 (0.59-3.41)	1.05 (0.58-1.90)
Females	Ref group	1.22 (0.93-1.61)	1.54 (0.85-2.81)	0.81 (0.42-1.56)
P for interaction		0.541	0.908	0.613
NO_2‡	Ref group	1.06 (0.96-1.16) 0.246	1.11 (0.88-1.41) 0.375	1.02 (0.84-1.25) 0.810
Males	Ref group	1.09 (0.96-1.25)	1.17 (0.77-1.77)	1.26 (1.00-1.59)
Females	Ref group	1.03 (0.90-1.18)	0.89 (0.63-1.25)	0.65 (0.44-0.98)
P for interaction		0.954	**0.081**	**0.005**
$PM_{2.5}$‡	Ref group	1.05 (0.95-1.18) 0.320	0.93 (0.70-1.23) 0.611	1.07 (0.84-1.36) 0.577
Males	Ref group	1.17 (0.99-1.9)	1.11 (0.64-1.92)	1.47 (1.04-2.06)
Females	Ref group	0.99(0.85-1.15)	0.86 (0.61-1.20)	0.82 (0.59-1.14)
P for interaction	Ref group	0.317	0.800	**0.018**
Air pollution markers at follow-up				
DMR	Ref group	1.23 (1.01-1.51) 0.043	1.50 (0.91-2.46) 0.107	0.85 (0.54-1.34) 0.486
Males	Ref group	1.37 (1.03-1.83)	2.09 (0.9-4.83)	0.97 (0.53-1.79)
Females	Ref group	1.14 (0.86-1.51)	1.34 (0.71-2.51)	0.73 (0.37-1.47)
P for interaction	Ref group	0.435	0.479	0.567
NO_2‡	Ref group	1.05 (0.96-1.16) 0.293	1.10 (0.87-1.40) 0.428	0.86 (0.68-1.08) 0.176
Males	Ref group	1.06 (0.93-1.21)	1.17(0.78-1.75)	0.98 (0.74-1.29)
Females	Ref group	1.04 (0.91-1.20)	0.86 (0.60-1.24)	0.67 (0.44-1.01)
P for interaction	Ref group	0.551	**0.099**	**0.097**
$PM_{2.5}$‡	Ref group	1.18 (1.04-1.33) 0.010	1.03 (0.76-1.41) 0.833	1.00 (0.77-1.29) 0.986
Males	Ref group	1.26 (1.01-1.51)	0.97 (0.57-1.63)	1.22 (0.84-1.75)
Females	Ref group	1.13 (0.95-1.35)	1.09 (0.73-1.62)	0.83 (0.57-1.21)
P for interaction	Ref group	0.756	0.420	0.162

*P for interaction with p<0.1 are bolded

‡ORs (95% CIs) per an IQR increase in air pollution marker

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and second-hand smoking.

§An influential outlier was removed from this regression model

Association between ambient air pollution at baseline and incident eczema.

There was weak evidence of associations, nor effect modification by sex, between baseline ambient air pollution markers at baseline and incident eczema (Table 6.2). Similarly, when incident eczema was classified by atopy groups (Table S6.5), there was weak evidence of association.

Association between ambient air pollution at baseline and persistent current eczema.

There was evidence that sex modified the association (P for interaction<0.1) between NO_2 exposure and as a result, increased odds of persistent eczema was stronger in males than females (Table 6.2). Likewise, when persistent eczema was classified by atopy status (Table 6.4), there was evidence of interaction by sex (P for interaction<0.1). As such, baseline NO_2 exposure in males was found to increase persistent AE odds (aOR 1.25 [95% CI 0.95-1.65] per 2.27 ppb NO_2 increase, while in females, the association tended towards reduced odds of persistent AE (aOR 0.57 [95% CI 0.36-0.91] per 2.27 ppb NO_2 increase). Similarly, baseline $PM_{2.5}$ exposure in males increased the odds of persistent AE (aOR 1.53 [1.04-2.25] per 1.56 µg/m^3 $PM_{2.5}$ increase) and in females the odds of persistent AE was reduced (aOR 0.71 [0.49-1.02] per 1.56 µg/m^3 $PM_{2.5}$ increase).

Table 6.4. Adjusted associations between ambient air pollution exposure at the baseline and persistent eczema by atopy with stratified results by sex.

	No atopy or eczema (1,005/2,368)	Atopy alone (1,207/2,368)	Non-atopic Eczema (54/2,368)	Atopic Eczema (102/2,368)
Air pollution markers at baseline				
	aOR (95%CI) † p	aOR (95%CI) † p	aOR (95%CI) † p	aOR (95%CI) † p
DRM	Ref group	1.15 (0.96-1.42) 0.131	1.65 (0.92-2.96) 0.092	0.74 (0.44-1.25) 0.266
Males	Ref group	1.10 (0.82-1.46)	1.88 (0.66-5.31)	1.02 (0.51-2.03)
Females	Ref group	1.23 (0.84-1.61)	1.66 (0.81-3.40)	0.51 (0.22-1.17)
P for interaction		0.547	0.882	0.225
NO_2‡	Ref group	1.07 (0.97-1.17) 0.161	1.25 (0.96-1.63) 0.099	0.95 (0.75-1.20) 0.671
Males	Ref group	1.10 (0.97-1.26)	1.58 (1.12-2.32)	1.25 (0.95-1.65)
Females	Ref group	1.41 (0.91-1.19)	1.00 (0.68-1.47)	0.57 (0.36-0.91)
P for interaction		0.937	0.137	**0.011**
$PM_{2.5}$‡	Ref group	1.06 (0.95-1.18) 0.332	0.77 (0.55-1.08) 0.131	1.00 (0.77-1.31) 0.986
Males	Ref group	1.17 (0.99-1.38)	0.94 (0.48-1.87)	1.53 (1.04-2.25)
Females	Ref group	0.99 (0.85-1.15)	0780 (0.47-1.05)	0.71 (0.49-1.02)
P for interaction		0.296	0.645	**0.009**

*P for interaction with p<0.1 are bolded

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and second-hand smoking.

‡ORs (95% CIs) per an increase of 1 IQR in air pollution marker

Restricted definition of non-atopic eczema.

When using the same analytic approach as the SALIA cohort study[5] there were no significant associations between ambient air pollution markers at baseline and incident NAE with increasing the strictness (i.e. those participants without hay fever and SPT negative) of the definition (Table S6.6). However, a non-significant trend between DMR at follow-up and increased odds of prevalent NAE was seen, and this effect became stronger when the NAE definition became stricter (Table S6.6). Furthermore, when the analyses were restricted to women, there was an association between DMR at follow-up and increased odds of prevalent eczema (Table S6.7).

Association between ambient air pollution at baseline and aeroallergen sensitisation at age 53 years.

At baseline, increased exposure to $PM_{2.5}$ was associated with increased odds of being sensitised to aeroallergens (aOR 1.15 [95% CI 1.03-1.30]) per 1.56 µg/m^3 $PM_{2.5}$ increase) at age 53 (Table 6.5). Furthermore, there were associations between ambient air pollution markers and specific aeroallergen sensitisations that are described in more detail in Table S6.8.

Table 6.5. Adjusted associations between ambient air pollution markers and aeroallergen sensitisation with stratified results by sex.

	Aeroallergen sensitisation (1,218 / 2,207)	
	Air pollution markers at baseline	Air pollution markers at follow-up
	aOR (95%CI) † p	aOR (95%CI) † p
DRM	1.09 (0.91-1.32) 0.350	1.45 (0.95-1.39) 0.168
Males	1.07 (0.81-1.41)	1.27 (0.96-1.67)
Females	1.12 (0.86-1.45)	1.06 (0.81-1.39)
P for interaction	0.769	0.398
NO_2‡	1.04 (0.96-1.14) 0.322	1.02 (0.93-1.12) 0.630
Males	1.09 (0.96-1.23)	1.03 (0.90-1.16)
Females	1.01 (0.89-1.15)	1.03 (0.90-1.18)
P for interaction	0.765	0.573
$PM_{2.5}$‡	1.07 (0.96-1.19) 0.215	1.15 (1.03-1.30) **0.017**
Males	1.19 (1.01-1.40)	1.36 (1.06-1.50)
Females	0.99 (86-1.15)	1.09 (0.93-1.29)
P for interaction	0.178	0.456

*P-value less than 0.05 is bolded

†Adjusted for: age, sex, household cooking by indoor combustion of solid or gas fuels, household cooking by indoor combustion of solid or gas fuels, occupation, highest education level, smoking and second-hand smoking.

‡ORs (95% CIs) per an IQR increase in air pollution marker

Discussion

In this cohort of participants followed from 43 to 53 years of age, based in Australia where pollution levels are generally low, we observed that in males, higher exposure to ambient air

pollution was associated with increased odds of prevalent atopic eczema. In contrast, for females, higher exposure to NO_2 was associated with a paradoxical reduced odds of prevalent atopic eczema. Furthermore, the absence of associations between ambient air pollution and incidence of eczema might be due to the reduction in ambient air pollution concentrations over time. Additionally, there was evidence that increased levels of $PM_{2.5}$ exposure were associated with increased odds of allergic sensitisation, in both males and females.

There are a limited number of previous studies that have considered the association between ambient air pollution exposure and eczema prevalence or incidence, and the results were inconsistent. Two paediatric studies[18,19] showed no association between ambient air pollution and increased risk of eczema, while other studies suggested an association between ambient air pollution and increased eczema prevalence in children[6] and adults. [20,21] A study by Kim et al[22] found similar results in that NO_2 was associated with the prevalence of AE in male but not in female, however the study population were children. The reason for these sex specific effects is not clear, but may be partly be due to differences in skin morphology, occupational exposures in adults and behaviour. [23] As has been reported previously in this age group, [24] women tend to spent more time indoors compared to men, with men reported spending almost twice the amount of time outside compared to women (Table S6.9). Additionally, women are more likely to care about skin issues and avoid exposure to irritants compared to men. [25] Therefore, residual confounding may have introduced the sex-specific interaction in the association estimates. On the other hand, Gilmour et al. [26] observed that not all oxidative stress responses on the epithelial barrier are injurious and a lower level of oxidative stress might be paradoxically protective. To further elucidate the possible reasons for these sex specific effects, we recommend focused exploration of the physiological, barrier function and immune responses to ambient air pollution at low levels in men and women.

We were unable to replicate the results from the SALIA cohort of elderly women study. [5] However, we saw similar non-significant trends of associations between ambient air pollution markers at follow-up and NAE prevalence which may warrant further investigation. There are several reasons why we may not have observed the same associations. First, we used SPT results and hay fever rather than blood IgE levels and hay fever, to determine atopic sensitisation. However given the strong association between SPT and IgE[27] this would be unlikely to explain the differences in results between these studies. Second, our cohort was younger (baseline at 43 years followed-up to 53 years) than those in SALIA (baseline at 53 years followed-up to 73 years of age), making this a different time in women's reproductive lives, and changes in sex hormone levels may help explain the differences in results seen between these studies. Third, we included both sexes in our main analyses, whereas the SALIA study included only women. However, we did not observe increased

risk in women with higher ambient air pollution exposures. Finally, the sources of ambient $PM_{2.5}$[15] are different between Australia (mainly from wood heaters, power stations and off-road sources) and Germany (mainly on-road traffic) where the study was conducted. [5]

Our findings suggest an association between $PM_{2.5}$ and aeroallergen sensitisation, agreeing with other studies. [28,29] In a study with adult participants; living close to busy roads was associated with a higher risk of sensitisation to pollen. [28] Furthermore, a previous cross-sectional analysis using data from this cohort reported, in this low pollution setting, that increased levels of ambient air pollution conferred a higher development of atopy. [29] Of two studies that have not observed an association between ambient air pollution and allergic sensitisation, one study had a relatively small sample size and low power. [30] Another, in an adult population, reported a cross sectional association with DMR and NO_2 and aeroallergen sensitisation, but did not assess $PM_{2.5}$. [31]

It has been proposed that air pollutants may lead to eczema and sensitisation via inflammatory oxidative stress leading to skin barrier dysfunction. [32] Ambient air pollution may drive these effects either through direct percutaneous absorption or indirectly through inhalation and subsequent systemic inflammation. [32] These air pollutants produce reactive oxygen species (ROS) and nitrogen species that lead to damage of proteins, lipids, and DNA. [33] Air pollutants can also act as irritants and immunomodulators leading to elevated levels of serum IgE. [34] Specifically, $PM_{2.5}$ may activate the aryl hydrocarbon receptor to promote cell metabolism and inflammation. [34] Other proposed mechanisms are by altering trans-epidermal water loss, increasing inflammatory signals and modifying the skin pH and microbiome. [35] Our results support an effect of ambient air pollution on immune function, even in this low ambient air pollution setting.

Our study has both strengths and limitations. Strengths include access to a large population-based prospective cohort study with long follow-up allowing for a 10-year assessment period, well-characterised definitions of eczema with objective measures of SPT and land-use regression models. While the questions and definitions used were well validated, [36] a limitation is the reliance on self-reporting. Unfortunately, there were no data on the frequency or severity of symptoms and the aero-allergen SPT data were only available at follow-up, which may lead to some misclassification of atopy in the eczema subgroups. Although lost to follow-up tend to be more pronounced among the less advantaged participants, this differential loss to follow-up generally does not lead to selection bias in the measurement of exposure-outcome associations. [37] As such, while we cannot exclude this possibility, it seems unlikely. Also, it is possible that residual confounding could have been an issue. As such, further replication of these findings is required. Given the exploratory nature of these data with multiple associations being assessed, we have attempted to interpret the pattern of associations, rather than relying on any arbitrary p value threshold to draw conclusions. Finally, as

almost all the participants were Caucasian and Anglo-Celtic, the findings may not be generalizable to other ethnicities.

Conclusion

Our findings suggest that higher ambient air pollution exposure is associated with greater odds of AE in adult men and an increased odds of aeroallergen sensitisation in both sexes. Ongoing efforts to reduce ambient air pollution exposure are likely to have a range of health benefits. Nevertheless, further research is needed to identify the components of ambient air pollution that exert the major effects, the extent of exposure required to increase risk of eczema and the factors that determine individual susceptibility. Further efforts are required to harmonize measurement of ambient air pollution markers, case definitions, study designs, and assessment of confounding factors to aid in replication of findings in this area.

List of abbreviations

Atopic eczema (AE)

Directed acyclic graph (DAG)

Distance to major roads (DMR)

Fine particulate matter with an aerodynamic diameter of 2.5 μm or less mass ($PM_{2.5}$)

International Study of Asthma and Allergies in Childhood (ISAAC)

Interquartile range (IQR)

Nitrogen dioxide (NO2) exposure

Non-atopic eczema (NAE)

Odds ratios (ORs)

Skin prick testing (SPT)

Study on the influence of air pollution and lung function, inflammation and Aging (SALIA)

Tasmanian Longitudinal Health Study (TAHS)

Declarations section

Ethics approval and consent to participate

The 2002 and 2012 proband studies were approved by the Human Research Ethics Committee of the University of Melbourne, Melbourne. All participants provided written informed consent.

Availability of data and materials:

The data that support the findings of this study are available from the TAHS cohort 5th and 6th follow-up studies but restrictions apply to the availability of these data, which were used under authorization from the TAHS investigators for the current study, and so are not publicly available. Data are however available from the TAHS investigators upon reasonable request.

Competing interests:

MJA holds investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has also undertaken an unrelated consultancy for and received assistance with conference attendance from Sanofi. He has received a speaker's fee from GSK. The other authors declare that they have no competing interests.

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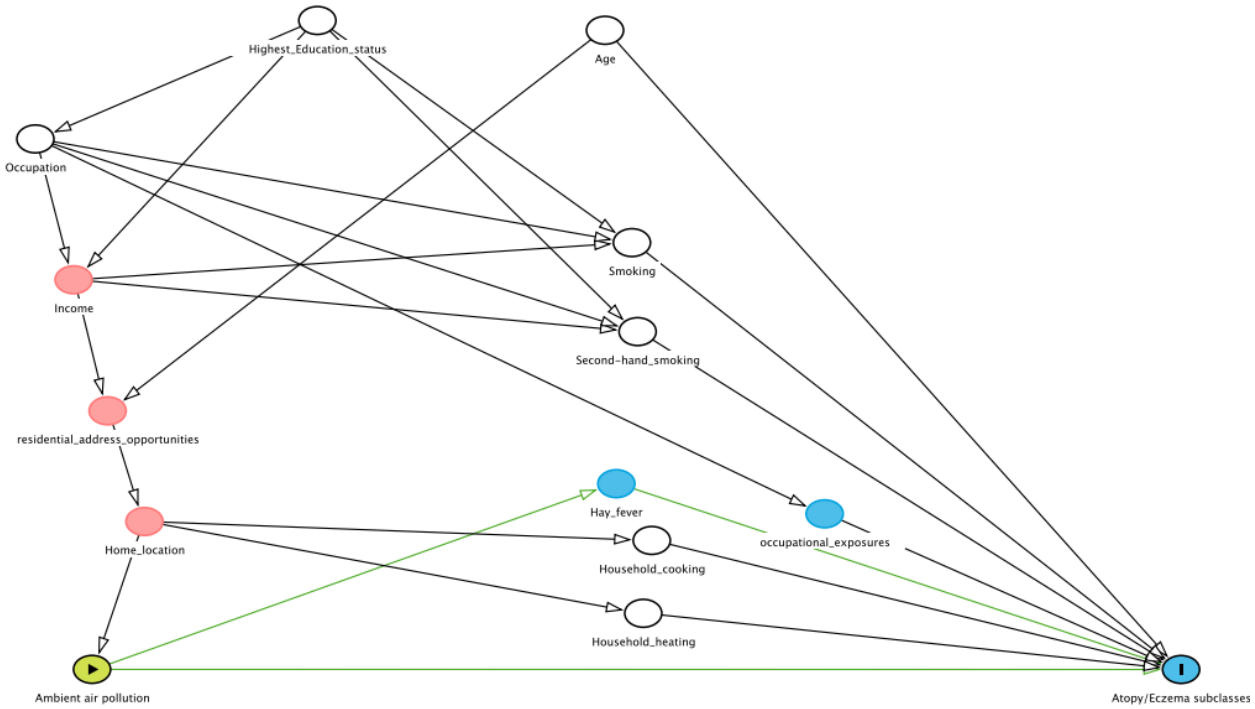
6.3. Supplementary material

Figure S6.1. Directed acyclic graph (DAG) of the association between ambient air pollution and eczema and atopy subclasses and its confounders.



Based on this DAG, we adjusted all associations for age, highest educational status of participants, household heating by indoor combustion of solid fuels (i.e. wood and coal) or gas, smoking (categorised as current, former, or never smoking), and exposure to second-hand smoke at home and work

Table S6.1. Confounder table of definitions

Confounder	
Age (per year)	Range: 41 -44, Continuous variable
Sex (dichotomous)	Male = 1, female=2
Heating fuel at 2002 (dichotomous)	No use of solid or gas fuel=0 use of solid or gas fuel=1
Heating fuel in 2012 (dichotomous)	No use of solid or gas fuel=0 use of solid or gas fuel=1
Education in 2002 (ordinal)	> grade 12 = 0, Grade 12 or equivalent = 1, < Grade 12 = 2
Smoking status at 2002 (ordinal)	Never=0 Former =1 Current= 2
Secondhand smoking status (dichotomous)	No=0 Yes= 1
Hay fever (dichotomous)	No=0 Yes= 1

Table S6.2. Summary statistics and predictors of loss to follow-up between baseline (2002 proband study) and follow-up (2012 proband study) stratified by sex.

Covariate	Summary statistics at baseline of participants lost to follow-up	Odds ratio of being loss to follow-up p	P for interaction by sex	Strata specific results by sex
Sex %(n/N)	Male. 1,231/2,238 (55.00%) [Ref.] Female. 1,007/2,238 (45.00%)	0.87 (0.79-0.97) 0.009	-	-
Age (years) Mean (SD);	43.04 (0.84)	0.99 (0.92-1.07) 0.830	0.307	
BMI (kg/m^2) Mean (SD)	27.08 (4.83)	1.04 (1.02-1.06) 0.001	0.062	Male 1.05 (1.02-1.08) Female 1.01 (0.97-1.04)
Solid fuel Heating† %(n/N)	No. 1,117/2,238(49.91%) [Ref.] Yes. 1,121/2,238 (50.09%)	0.88(0.79-0.76) 0.022	0.207	
Solid Fuel Cooking† %(n/N)	No. 1,763/2,231 (79.02%) [Ref.] Yes. 468/2,231 (20.98%)	0.84 (0.74-0.96) 0.008	0.216	
Highest education status. %(n/N)	less than grade 12. 1,110/2,233 (49.71%) [Ref.] grade 12. 206/2,233 (9.23%) more than grade 12. 917/2,233 (41.07%)	0.72 (0.68-0.76) 0.000	0.645	
Occupation %(n/N)	Managers. 465/2,208 (21.06%) [Ref.] Associate professionals. 218/2,208 (9.87%) Tradesperson. 408/2,208 (18.48%) Intermediary clerical. 488/2,208 (22.10%) Elementary clerical. 597/2,208 (19.04%)	1.21 (1.17-1.26) 0.000	0.317	
Smoking status %(n/N)	Never. 761/2,225 (34.20%) Former. 631/2,225 (28.36%) Current. 833/2,225 (37.44%)	1.43 (1.34-1.53) 0.000	0.600	Males 1.37 (1.25-1.50) Females 1.49 (1.36-1.65)
Secondhand smoking %(n/N)	No. 1,744/2,155 (80.93%) Yes. 411/2,155 (19.07%)	1.80 (1.55-2.10) 0.000	0.196	Male 1.78 (1.43-2.20) Female 1.84 (1.47-2.29)
Hay fever %(n/N)	No. 1,183/2,228 (53.10%) Yes. 1,045/2,228 (46.90%)	0.78 (0.74-0.88) 0.000	0.822	
Living <200m from a major road	587/2,238	1.02 (0.90-1.16) 0.714	0.067	Male 1.14 (0.96-1.35) Female 0.91 (0.76-1.08)
NO_2 (ppb) median [IQR; 25-75%le]	4.11 [2.27; 3.31-5.5]	0.97 (0.95-0.99) 0.013	0.223	
$PM_{2.5}$ ($\mu g/m^3$) median [IQR; 25-75%le]	6.41 [1.49; 5.57-7.16]	0.94 (0.90-0.98) 0.005	0.326	

† Solid fuel are coal and wood.

Table S6.3. Prevalence, incidence and persistence of eczema and their subclasses at 2012 survey.

Prevalence of Eczema	281/ 3,135 (8.96%)
Sub-classes	
Non-sensitised non-eczema	972/ 2,360 (41.19%)
Sensitised non-eczema	1,172/ 2,360 (49.66%)
Non-atopic Eczema	83 / 2,360 (3.52%)
Atopic Eczema	133 / 2,360 (5.64%)
Incidence of Eczema†	115/ 3,135 (3.67%)
Sub-classes	
Non-sensitised non-eczema	1,018/2,273 (42.97%)
Sensitised non-eczema	1,263/2,273 (53.31%)
Non-atopic Eczema	41/2,273 (1.73%)
Atopic Eczema	47/2,273 (1.98%)
Eczema persistence	201/3,135 (6.38%)
Sub-classes	
Non-sensitised non-eczema	1,005/2,368 (42.44%)
Sensitised non-eczema	1,207/2,368 (50.97%)
Non-atopic Eczema	54/2,368 (2.28%)
Atopic Eczema	102/2,368 (4.31%)

†Newly developed symptoms of eczema between 2002 and 2012 survey, 399 participants excluded who had symptoms before 2002 survey.

Table S6.4. Adjusted associations between TRAP exposure at the baseline and prevalent eczema at 53 years by atopy with stratified results by gender and for participants that did not change their address.

	No atopy or eczema (639/ 1,519)	**Atopy alone** (743/ 1,519)	**Non-atopic Eczema** (55/ 1,519)	**Atopic Eczema** (82/ 1,519)
Air pollution markers at baseline				
	aRR (95%CI) †	aRR (95%CI) †	aRR (95%CI) †	aRR (95%CI) †
DMR	Ref group			
Males	Ref group			
Females	Ref group			
P for interaction				
NO_2‡	Ref group	1.06 (0.96-1.16)	1.11 (0.88-1.41)	1.02 (0.84-1.25)
		0.246	0.375	0.810
Males	Ref group	1.05 (0.90-1.25)	1.36 (0.96-1.92)	1.13 (0.83-1.54)
Females	Ref group	0.99 (0.82-1.19)	0.88 (0.57-1.37)	0.61 (0.35-1.06)
P for interaction	Ref group	0.735	0.217	**0.092**
$PM_{2.5}$‡	Ref group	1.05 (0.95-1.18)	0.93 (0.70-1.23)	1.07 (0.84-1.36)
		0.320	0.611	0.577
Males	Ref group	1.18 (0.95-1.48)	1.46 (0.73-2.92)	1.34 (0.85-2.133)
Females	Ref group	0.96(0.72-1.17)	0.92 (0.57-1.47)	0.87 (0.55-1.38)
P for interaction	Ref group	0.317	0.800	0.340
Air pollution markers at follow-up				
DMR	Ref group			
Males	Ref group			
Females	Ref group			
P for interaction	Ref group			
NO_2‡	Ref group	1.05 (0.96-1.16)	1.10 (0.87-1.40)	0.86 (0.68-1.08)
		0.293	0.428	0.176
Males	Ref group	1.05 (0.89-1.24)	1.36 (0.96-1.93)	1.11 (0.81-1.53)
Females	Ref group	0.97 (0.81-1.17)	0.86 (0.55-1.34)	0.59 (0.33-1.03)
P for interaction	Ref group	0.782	0.182	**0.081**
$PM_{2.5}$‡	Ref group			
Males	Ref group			
Females	Ref group			
P for interaction	Ref group			

*P for interaction with p<0.1 are bolded

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and

‡ORs (95% CIs) per an increase of 1 IQR in air pollution marker

Table S6.5. Adjusted associations between ambient air pollution exposure at the baseline and incident of current eczema by atopy with stratified results by gender.

	No atopy or eczema (1,018/ 2,369)	Atopy alone (1,263/2,369)	Non-atopic Eczema (41/2,369)	Atopic Eczema (47/2,369)
Air pollution markers at baseline				
	aRR (95%CI) †	aRR (95%CI) †	aRR (95%CI) †	aRR (95%CI) †
DMR	Ref group	1.09 (0.89-1.32)	0.95 (0.46-1.97)	1.30 (0.67-2.47)
		0.407	0.884	0.441
Males	Ref group	1.06 (0.80-1.40)	0.63 (0.18-2.29)	0.88 (0.36-2.11)
Females	Ref group	1.10 (0.84-1.43)	1.20 (0.48-2.98)	2.68 (0.91-7.83)
P for interaction	Ref group	0.841	0.506	0.117
NO_2‡	Ref group	1.04 (0.95-1.14)	0.94 (0.63-1.40)	1.11 (0.83-1.48)
		0.380	0.763	0.474
Males	Ref group	1.08 (0.95-1.23)	0.99 (0.55-1.79)	1.18 (0.86-1.61)
Females	Ref group	1.01 (0.88-1.15)	0.89 (0.53-1.50)	0.75 (0.35-1.59)
P for interaction	Ref group	0.916	0.822	0.143
$PM_{2.5}$‡	Ref group	1.06 (0.95-1.18)	0.85 (0.57-1.27)	1.25 (0.85-1.83)
		0.321	0.426	0.253
Males	Ref group	1.19 (1.01-1.40)	1.15 (0.57-2.33)	1.43 (0.88-2.33)
Females	Ref group	0.98 (0.85-1.13)	0.74 (0.45-1.22)	1.10 (0.54-2.21)
P for interaction	Ref group	0.188	0.645	0.338

*P for interaction with p<0.1 are bolded

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and second-hand smoking.

‡ORs (95% CIs) per an increase of 1 IQR in air pollution marker

Table S6.6. Associations between ambient air pollution at baseline and incident symptoms of eczema after age 43 years following exclusion of atopic subjects, with increasing strictness from left to right (SALIA cohort analytical approach)

	All participants	Never hay fever	Never hay fever and negative SPT at follow-up
Air pollutants at baseline and incident symptoms of eczema after age 43 years*			
	aOR (95%CI) † p	aOR (95%CI) † p	aOR (95%CI) † p
N	2,475	1,241	583
DRM	1.15 (0.76-1.75) 0.518	0.65 (0.31-1.36) 0.253	0.43 (0.13-1.48) 0.182
NO_2‡	1.10 (0.90-1.34) 0.339	1.08 (0.78-1.49) 0.633	0.92 (0.53-1.61) 0.770
$PM_{2.5}$‡	1.16 (0.92-1.48) 0.193	1.11 (0.78-1.56) 0.563	0.64 (0.37-1.11) 0.113
Air pollutants at follow-up and prevalent symptoms of eczema up to 12 months before follow-up investigation			
N	2,906	1,372	603
DRM	0.96 (0.72-1.29) 0.779	1.36 (0.85-2.16) 0.199	1.89 (0.97-3.68) 0.062
NO_2‡	0.93 (0.80-1.07) 0.308	0.93 (0.72-1.21) 0.586	1.12 (0.81-1.56) 0.491
$PM_{2.5}$‡	0.93 (0.79-1.10) 0.410	1.03 (0.78-1.35) 0.835	1.05 (0.66-1.66) 0.836

†Adjusted for: age, sex, household cooking by indoor combustion of solid and gas fuels, household cooking by indoor combustion of solid and gas fuels, occupation, highest education level, smoking and

‡ORs (95% CIs) per an increase of 1 IQR in air pollution marker

Table S6.7. Associations between ambient air pollution at baseline and incident symptoms of eczema after age 43 years following exclusion of atopic subjects, with increasing strictness from left to right in females only (SALIA cohort analytical approach)

	All participants	Never hay fever	Never hay fever and negative SPT at follow-up
Air pollutants at baseline and incident symptoms of eczema after age 43 years*			
	aOR (95%CI) † p	aOR (95%CI) † p	aOR (95%CI) † p
N	2,475	1,241	583
DRM<200m	0.95 (0.59-1.52) 0.839	1.03 (0.48-2.22) 0.935	1.07 (0.36-3.12) 0.899
NO_2‡	0.98 (0.78-1.24) 0.887	0.94 (0.63-1.40) 0.759	0.81 (0.46-1.42) 0.455
$PM_{2.5}$‡	1.17 (0.91-1.5) 0.228	0.92 (0.60-1.41) 0.715	0.64 (0.36-1.16) 0.139
Air pollutants at follow-up and prevalent symptoms of eczema up to 12 months before follow-up investigation			
N	2,906	1,372	603
DRM<200m	0.88 (0.58-1.33) 0.542	1.39 (0.69-2.79) 0.355	2.40 (0.96-5.96) 0.060
NO_2‡	0.84 (0.68-1.05) 0.128	0.91 (0.61-1.36) 0.650	1.07 (0.66-1.73) 0.779
$PM_{2.5}$‡	0.98 (0.78-1.23) 0.869	1.44 (0.91-2.26) 0.119	1.52 (0.76-3.063) 0.235

†Adjusted for: age, sex, household cooking by indoor combustion of solid or gas fuels, household cooking by indoor combustion of solid or gas fuels, occupation, highest education level, smoking and second-hand smoking.

‡ORs (95% CIs) per an increase of 1 IQR in air pollution marker

Table S6.8. Adjusted† associations between air pollutants and specific aeroallergen.

Aeroallergen. % (n/N)	DMR	NO_2	$PM_{2.5}$
	aOR (95%CI) †	aOR (95%CI) †	aOR (95%CI) †
Air pollution markers at baseline			
House dust mite. (982/2,370)	1.03(0.85-1.21)	1.03 (0.95-1.13)	1.01 (0.91-1.13)
Cat pelt. (365 /2,363)	1.13 (0.87-1.47)	1.09 (0.98-1.23)	1.07 (0.93-1.25)
Cladosporioides. (149/2,361)	0.98 (0.69-1.52)	0.83 (0.69-1.01)	1.01 (0.82-1.26)*
Alternaria tenuis. (227/2,361)	0.97 (0.71-1.34)	**0.84 (0.71-0.99)***	0.99 (0.83-1.19)
Penicillium mix. (93/2,358)	1.19 (0.74-1.90)	0.98 (0.78-1.23)	0.83 (0.64-1.09)
Aspergillus fumigatus. (122/2,360)	**1.79 (1.20-2.66)***	1.07 (0.9-1.29)	1.07 (0.84-1.36)
Rye grass. (724/2,365)	1.19 (0.97-1.46)	1.05(0.96-1.16)	**1.22 (1.09-1.38)***
Mixed grasses. (727/2,367)	1.20 (0.98-1.47)	1.05 (0.96-1.16)	**1.19 (1.06-1.33)***
Air pollution markers at follow-up			
House dust mite. (982/2,370)	1.09 (0.90-1.33)	1.01 (0.92-1.10)	1.08 (0.96-1.22)
Cat pelt. (365 /2,363)	1.08 (0.83-1.41)	1.05 (0.93-1.19)	1.13 (0.96-1.33)
Cladosporioides. (149/2,361)	0.89 (0.62-1.39)	**0.79 (0.65-0.98)***	1.01 (0.79-1.29)
Alternaria tenuis. (227/2,361)	1.05 (0.76-1.45)	**0.79 (0.67-0.94)***	1.05 (0.86-1.29)
Penicillium mix. (93/2,358)	1.31 (0.83-2.09)	0.98 (0.78-1.24)	1.00 (0.74-1.37)
Aspergillus fumigatus. (122/2,360)	**1.86 (1.25-2.80)***	1.00 (0.82-1.22)	1.07 (0.82-1.42)
Rye grass. (724/2,365)	1.18 (0.96-1.45)	1.06 (0.96-1.17)	**1.33 (1.16-1.52)***
Mixed grasses. (727/2,367)	1.21 (0.99-1.49)	1.06 (0.96-1.17)	**1.29 (1.13-1.48)***

* P-value less than 0.05

†Adjusted for: age, sex, household cooking by indoor combustion of solid or gas fuels, household cooking by indoor combustion of solid or gas fuels, occupation, highest education level, smoking and second-hand smoking.

Table S6.9. Average outside time at 2012 follow-up by gender

Average outside time	Continuous variable (minutes)	Dichotomised at median
	Mean (SD); min-max	%(n/N)
Males	253 (152); 7.5-870	<50p. 34.47% (536/1,555) >50p. 65.53% (1,019/1,555)
Females	142 (94); 3-765	<50p. 69.75% (1,095/1,570) >50p. 30.25% (475/1,570)

Chapter 7 - The association between domestic hard water and eczema in adults from the UK biobank cohort study

7.1. Section introduction

In this published manuscript, I aimed to assess the effect of hard water (water with high mineral content) on eczema in adults in the UK. In the literature, there is evidence of hard water being a risk factor for eczema, but this evidence was solely focused on children populations. Improving the knowledge of the association between hard water and eczema in adults can lead to preventive strategies. The analyses from this research project used data from the UK Biobank baseline and two follow-ups. The UK biobank is a rich resource that contains a wide range of participants' data, including sociodemographic factors, economic variables and disease status. Also, I used the data from local water companies on the mineral concentrations of the water supply.

7.1.1. Research question

- Is domestic water hardness associated with eczema prevalence and incidence in a cohort of middle-aged adults?

7.1.2. Research aim

- To determine the associations of domestic water hardness and eczema prevalence and incidence in a cohort of adults.

7.1.3. Critical reflection on the work

The UK biobank is a massive database with data on around 500,000 participants that requires an application form and paying a fee. I applied to Population Health Investing in Research Students' Training and was awarded the fee. I think filling out a grant application is an important skill to have as a researcher. Once I got access to the data, it took me a few weeks to learn how to use this resource. The data dictionary was handy in this process. Also, I emailed all the water companies from the UK to request their water quality data that contained the mineral content data. I had to liaise with the representatives from each company. The time zone difference made the process a bit difficult, but overall, I was surprised by how well the companies replied to the requested data. Then, I applied a new statistical technique which involved more self-learning with the help of my supervisors. The publication process for this manuscript was speedy and straightforward. I think it was due to improvements in my writing skills and the appropriate selection of the journal.

7.1.4. Publication citation

Lopez, D.J., Singh, A., Waidyatillake, N.T., Su, J.C., Bui, D.S., Dharmage, S.C., Lodge, C.J. and Lowe, A.J., 2022. The association between domestic hard water and eczema in adults from the UK Biobank cohort study. British Journal of Dermatology.

***Please note that the specific references from this accepted or submitted manuscript are at the end of this chapter and the in-text citation number restarts at 1.**

7.2. Author accepted version of the manuscript.

Title: The association between domestic hard water and eczema in adults from the UK biobank cohort study

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Conflicts of interest

S.C.D., C.J.L. and A.J.L. declare they have received research funds from GSK's competitively awarded Investigator Sponsored Studies programme, for unrelated research. A.J.L. has also received donations of interventional product (EpiCeram) from Primus Pharmaceuticals for unrelated research. J.C.S. has been a consultant/speaker/investigator for AbbVie, Amgen, Bioderma, Bristol Myers Squibb, Ego Pharmaceuticals, Eli-Lilly, Janssen, LEO Pharma, L'Oreal, Mayne, Novartis, Pfizer, Pierre-Fabre, and Sanofi. The other authors declare they have no conflicts of interest.

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Bournemouth water; Bristol Water; Dŵr Cymru Welsh Water; Essex & Suffolk Water; Northumbrian Water Limited; Portsmouth water company; SES Water; South East Water; South West Region UK; Scottish water; Thames water; United Utilities; Wessex water and Yorkshire water for providing the water hardness data.

Bulleted statements

- Hard water is formed when minerals are dissolved in the water from filtration through sedimentary rocks. Several studies have reported a higher prevalence of eczema in areas with hard water. However, all studies on this topic have assessed this in infants and school-aged children, while this association has not been explored in adults.
- Our findings suggest that higher domestic hard water concentration exposure is associated with an increase in eczema prevalence in middle aged adults. Ongoing efforts to reduce hard water exposure may have a beneficial effect in reducing eczema prevalence in adults.

Main text word count:3046

Figure count: 1

Table count: 4

Abstract

Background: Eczema is a chronic inflammatory skin disease. Domestic water with high mineral content (hard water) is a risk factor for eczema in children, but this association has not been assessed in adults.

Objectives: To examine the association between domestic hard water supply and eczema prevalence and incidence in middle-aged adults and the contextual effect in eczema outcomes by postcodes in adults in the UK.

Methods: We used data from the UK Biobank study collected in 2006-2010 (baseline) and 2013-2014 (follow-up). Eczema prevalence at baseline (2006-2010), at follow-up (2013-2014) and incidence (new onset between baseline and follow-up) were determined from the touchscreen questionnaires and nurse-led interviews. Domestic hard water information was obtained in 2005 and 2013 from the local water supply companies in England, Wales, and Scotland as calcium carbonate ($CaCO_3$) concentrations. We fitted multilevel logistic regression models with random intercepts for postcode areas to examine the effect of domestic hard water on eczema outcomes and we measured components of variance.

Results: 306,531 participants with a mean age of 57 years nested across 7642 postcodes were included in the baseline analysis and 31,036 participants nested across 3695 postcodes were included in follow-up analysis. We observed an increase in the odds of eczema at baseline (OR: 1.02 95%CI 1.01-1.04) per 50 mg/L of $CaCO_3$ increase. Furthermore, exposure to domestic hard water (>200 mg/L of $CaCO_3$) was associated with increased odds of prevalent eczema at baseline (OR: 1.12 95%CI 1.04-1.22). Moreover, there was a significant linear trend (p-value: <.001) in which increasing levels of hard water increased eczema prevalence risk. No association was observed with the incident eczema or eczema at follow-up. The intraclass correlation coefficient for postcode was 1.6% (95%CI:0.7-3.4) and remained unexplained by area level socioeconomic measures.

Conclusion: Increasing levels of domestic hard water, as measured by $CaCO_3$ concentrations, were associated with an increased prevalence of eczema in adults but not increased incidence. Ongoing efforts to reduce hard water exposure may have a beneficial effect in reducing the burden of eczema in adults. Further research is needed to explore area level factors that may lead to eczema.

Keywords: adults, hard water, dermatitis, eczema, middle-age

Word count: 347

Introduction

Eczema (atopic dermatitis) is a chronic inflammatory skin disease, characterised by a defective skin barrier function. [1] Eczema has a heterogeneous presentation that varies by its severity, age of onset, response to treatment, and tendency to develop further atopic comorbidities. [2] Globally, it affects around 5% to 10% of adults and up to 20% of children. [3] Eczema is determined by an interplay of genetics, immunity, and environmental factors. [2,4,5]

One of the proposed environmental risk factors for eczema is domestic hard water, [6] which is water with a high mineral content. Hard water is formed when minerals are dissolved in the water from filtration through sedimentary rocks. [7] The key minerals that constitute hard water are calcium in the form of calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$), which are mostly associated with calcareous rocks and limestone. [8] According to the World Health Organization, domestic water is hard when the concentration of $CaCO_3$ is more than 200 milligrams per litre (mg/L) which leads to the need for excess use of soap to achieve lather and the formation of soap film (calcium stearate) on skin and clothes. [8,9]

A number of studies in children have observed a higher prevalence of eczema in areas with hard water. [10-12] A study of UK primary-school children aged 6-7 years suggested that those living with domestic hard water had an increased eczema prevalence. [10] Likewise, a study conducted in Spain showed that the lifetime prevalence of eczema in school children aged 6-7 years of age was higher in those that lived in areas with hard water. [11] A case control study of 80 young adults with a mean age of 26 years showed that skin sites washed with hard water significantly increased SLS deposits, which in turn increased transepidermal water loss and caused skin irritation. [12] All the studies in this area have assessed the impact of hard water on eczema in infants and school-age children, while this association has not been explored in adults. [13] Consequently, a better understanding of the association between domestic hard water and adult eczema may provide evidence to generate targeted interventions to decrease eczema flares and severity. We therefore examined the association between domestic hard water supply and eczema outcomes (prevalence and incidence) in middle-aged adults and the contextual associations by postcodes.

Methods

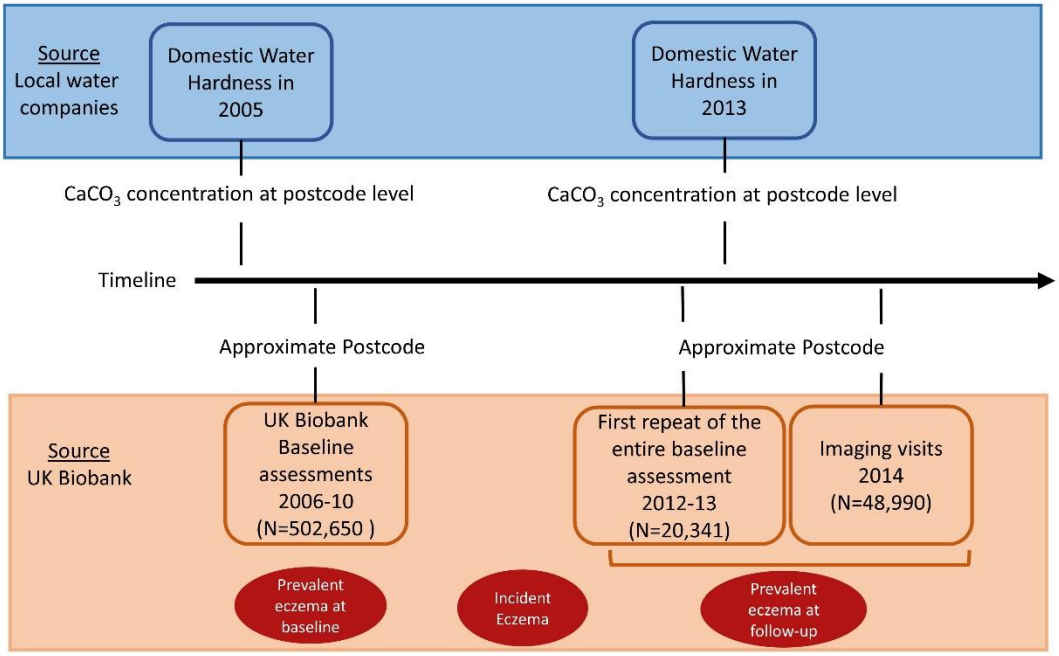
Design

The UK Biobank study is a population-based prospective cohort with the aim of improving the prevention, diagnosis and treatment of a varied range of serious chronic diseases. [14] The UK Biobank represents the general population in the UK. It aimed to be as inclusive as reasonably possible, with all people aged 40–69 years who were registered with the National Health Service and living up to about 25 miles from one of the 22 study assessment centres in Scotland, England and

Wales invited to participate. [14] The initial assessment visits were performed between 2006 and 2010, more details are provided elsewhere. [14] Detailed data on 502,650 people was obtained including the participant's demographic, socioeconomic and health-related information. [14] Medical and medication history was collected using a touchscreen questionnaire. A nurse-led interview was further performed, validating the medical history obtained from the touchscreen questionnaire by direct questioning. During the first repeat of the entire baseline assessment in 2012; data on around 20,000 participants was collected. [15] Furthermore, similar data was collected in the imaging visits in 2014 (Figure 7.1). [16]

UK Biobank has approval from the Northwest Multi-centre Research Ethics Committee. It has also sought approval in England and Wales from the Patient Information Advisory Group (PIAG) to access information that would allow it to invite potential participants. The University of Melbourne Ethics Committees approved the data linkage and analyses components of this project (ref: 2020-20674-13212-3).

Figure 7.1. Study data points diagram.



Postcode definition

The home location of each participant is based on the Ordnance Survey (OSGB) references, rounded to the nearest kilometre. We used the Ordnance Survey (OS) Code-Point®, a database of UK's 1.6 million postcodes available as part of the OS Open Data release in 2010. A set of British National Grid coordinates are attached to each postcode in the dataset, a postcode being an abbreviated form of address made up of combinations of between five and seven alphanumeric characters. A postcode may cover between 1 and 100 addresses, the average number per postcode

being 15 addresses. We assigned a postcode to each participant based on their approximate location and use the assigned postcodes as a link to the hard water locations.

Domestic hard water

Data on domestic water supply was obtained from local water supply companies in England, Wales, and Scotland. The hard water concentration range in the UK is wide and covers soft, hard and very hard water areas. Data included domestic water annual $CaCO_3$ concentrations in mg/l at area level measured 1 year before baseline (i.e. 2005) and at follow-up (i.e. 2013) assessments (Figure 7.1).

The concentration of $CaCO_3$ is directly related to the concentrations of calcium and magnesium in the water. [17] In this study domestic hard water has been classified in three ways: (1) as a continuous variable in increments of 50 mg/L, (2) as a dichotomous variable (World Health Organization criteria[8] considers a $CaCO_3$ concentration more than 200 mg/L as hard water and less than 200 mg/L as soft water), and (3) as a categorical variable, using the United States Geological Survey classification[18] of $CaCO_3$ concentration into 4 groups i.e. soft water (0 to 60 mg/L), moderately hard water (60 to 120 mg/L), hard water (120 to 180 mg/L), and very hard water (>180 mg/L).

Outcome definition

Prevalent eczema

Prevalent eczema was determined using the participants' answers to the touchscreen questionnaire and the nurse-led interview. We used the Monthly Index of Medical Specialties (MIMS) online from Australia and UK and professional advice to make a list of medication names which are indicated for treating eczema or atopic dermatitis. Then, we used this list to create a variable that represents current eczema medication at baseline and at follow-up (repeat assessment visit and imaging visit). Participants were classified as having prevalent eczema at baseline or at follow-up if they self-reported both eczema and eczema medication usage at each of the assessment times.

Incident eczema

Incident eczema was defined as new eczema arising after baseline (i.e. after 2010). Participants were classified as having incident eczema if they did not report ever eczema at baseline and reported ever having eczema at follow-up. Also, participants were considered as incident cases if they reported the year of doctor diagnosis after baseline on the follow-up (i.e. after 2010). Those with prevalent eczema at baseline were removed from analysis involving incidence.

Confounder variables

A directed acyclic graph (Figure S7.1.) was developed to assess hypothesised causal relationships and to determine the minimum set of confounders to include in the regression model to approximate a causal model. The confounder set included (1) individual level variables: age, ethnicity, sex, and income and (2) area level variables: Index of Multiple deprivation (IMD) and Townsend index at postcode level. Townsend deprivation index was calculated immediately prior to participant joining UK Biobank based on the preceding national census output areas. [19] The overall IMD can be used to rank areas according to the deprivation experienced by their residents and allow comparison of deprivation levels between areas. [20] To allow consistent score analysis across the countries we have used UK adjusted IMD scores as described elsewhere. [21]

Statistical Analysis

The data were hierarchically structured, with individuals (level 1) living within postcode areas (level 2) in the UK. A current recommendation when analysing multilevel data is to account for the effect of clustering of participants within higher-level units (i.e. postcodes) when fitting regression models to such data, as analysing variance gives added value. [22,23] We fitted multilevel logistic regressions to examine the association between domestic hard water and prevalent/incident eczema, since the assumption of independence required for standard regression modelling is likely to be violated by the correlation of outcomes between participants living in the same postcodes. [24] [22] First, we created an empty model which comprised random intercepts for postcodes to examine the general contextual effects, the extent in which the outcome varied between postcodes (Model 1). If the environment in which a participant lives contributes to the likelihood of an individual developing eczema, then it is reasonable to suppose that there may be similar effects upon participants living in the same areas and similar area level environmental exposures. Then, Model 2 was created which included the exposure (i.e. domestic hard water), individual-level covariates (age, ethnicity, sex, income), and the postcode-specific random effects (from Model 1). Finally, Model 3 incorporated adjustment by the individual level and postcode level covariates (IMD and Townsend Index) in addition to postcode-specific random effects. We reported the estimated odds ratios for multilevel logistic regression models. Furthermore, standard logistic regressions including individual level covariates were performed as a sensitivity analysis. To assess the general contextual effects (i.e., the variation of the outcome by postcodes), we included measures of intraclass correlation coefficient (ICC). ICC quantifies the proportion of observed variation in the outcome that is attributable to the effect of clustering. We also reported the median odds ratio (MOR) in supplementary text S7.1. To assess the potential public health impact of domestic hard water in eczema cases we calculated the population attributable fraction. The potential presence of non-linearity of these associations was assessed using Stata's "fracpoly" command. Hard water and participant data linkage was performed

using the QGIS Geographic Information System (version 3.16.16. QGIS Association, 2021). All other statistical analyses were carried out using Stata (release 16; Stata Corporation, College Station, TX).

Results

A total of 306,531 participants nested across 7642 postcodes were included in the baseline analysis (Figure S7.2.). At baseline, 55.6% of the participants were female. The 56 to 65-year age range was the most common. The vast majority of participants were of white ethnicity, and from England (Table 7.1). From the first repeat of the baseline in 2012 and the imaging visits, 31,036 participants were included in the follow-up analysis. Those included in the follow-up were more likely to have higher levels of income, to be white, and to live in England compared with those who were not followed. Moreover, participants lost to follow-up were more likely to live in hard water areas (Table S7.1).

Table 7.1. Baseline characteristics of the included study sample.

Characteristic	Baseline (N= 306,531)	Follow-ups (N=31,036)
	% (n/N)	% (n/N)
Individual level characteristics		
Female	55.6% (170,462/306,531)	51.1% (15,873/31,036)
Age		
Less than 45 years	9.1% (27,809/306,531)	8.1% (220/31,036)
46-55 years	24.9% (76,357/306,531)	26.4% (5,938/31,036)
56-65 years	47.4% (145,146/306,531)	52.2% (12,770/31,036)
More than 65 years	18.7% (57,219/306,531)	13.3% (12,108/31,036)
Ethnicity		
White	94.3%(288,970/306,531)	97.5% (30,154/30,942)
Mixed ethnic groups	0.6% (1,695/306,531)	0.4% (118/30,942)
Asian or Asian British		1.1% (354/30,942)
Black, African, Caribbean or	2.2% (6,879/306,531)	0.5% (170/30,942)
Black British	1.6% (4,908/306,531)	0.5% (146/30,942)
Other ethnic group		
	0.9% (2,645/306,531)	
Income		
Less than 18,000£	27.6% (70,580/256,055)	16.9% (4,741/28,038)
18,000 to 30,999£	27.0% (69,197/256,055)	30.5% (8,561/28,038)
31,000 to 51,999£	24.3% (62,311/256,055)	28.9% (8,099/28,038)
52,000 to 100,000£	16.9% (43,198/256,055)	18.9% (5,306/28,038)
Greater than 100,000£	4.2% (10,769/256,055)	4.8% (1,331/28,038)
Home location		
England	88.3% (263,899/298,909)	98.2% (29,859/30,398)
Wales	4.4% (13,294/298,909)	0.3% (96/30,398)
Scotland	7.3% (21,716/298,909)	1.5% (443/30,398)
Eczema outcomes		
Prevalence	1.11% (3,392/306,531)	1.03% (319/31,036)
Incidence		1.21% (382/31,036)
Area level characteristics		
Postcodes	7,642	3,695

Average participants per postcode	40	8
England IMD†*	18.3 (14.4; 0.61, 82)	16.5 (13.0;1.12, 81.6)
Townsend index score†*	-1.2 (3.1; -6.3, 10.9)	-2.0 (2.7; -6.3, 9.7)
Hard water variables		
Hard water (mg/L)†	140.0 (114.4; 5, 460)	85.1 (85.14; 1.5, 477)
Hard water (>200 mg/L)	36.3% (110,118/303,781)	12.4% (3,835/31,036)
Hard water category		
0 to 60 mg/L	37.2% (113,029/303,781)	57.2% (17,750/31,036)
60 to 120 mg/L	20.2% (61,436/303,781)	21.0% (6,523/31,036)
120 to 180 mg/L	5.8% (17,589/303,781)	6.7% (2,074/31,036)
More than 180 mg/L	36.8% (111,727/303,781)	15.11% (4,689/31,036)

IMD: Indices of multiple deprivation
mg/L: milligrams per litre
† Mean (standard deviation; min, max)
*A greater IMD or Townsend index score implies a greater degree of deprivation.

Table 7.2. Multilevel logistic regression models for prevalent eczema at baseline. (N=3,392/306,531)

	Model 1†	Model 2‡	Model 3§
Prevalent eczema at baseline	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Hard water (per 50 mg/L increase)			
OR	-	1.03 (1.01-1.05)	1.02 (1.01-1.04)
General contextual effects			
Postcode variance	.052 (.023-.112)	.054 (.026-.113)	.042 (.017-.101)
ICC	1.6% (0.7-3.4)	1.6% (0.7-3.4)	1.3% (0.4-2.9)
Hard water (>200 mg/L)			
OR		1.16 (1.07-1.25)	1.12 (1.04-1.22)
General contextual effects			
Postcode variance		.053 (.025-.111)	.043 (.018-.100)
ICC		1.6% (0.8-3.3)	1.3% (0.5-2.9)
Hard water groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		1.01 (0.91-1.12)	1.00 (0.91-1.11)
120 to 180 mg/L		1.08 (0.93-1.26)	1.05 (0.90-1.23)
More than 180 mg/L		1.17 (1.07-1.28)	1.13 (1.03-1.24)
General contextual effects			
Postcode variance		.053 (.020-.102)	.042 (.018-.100)
ICC		1.6% (0.8-3.3)	1.3% (0.4-2.8)

N: Proportion of prevalent eczema at baseline OR: cluster specific odds ratio of the association between domestic hard water and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

An increase in domestic hard water was associated with an increase in the odds of prevalent eczema at baseline (OR: 1.02 95%CI 1.01-1.04) per 50 mg/L of $CaCO_3$ increase (Table 7.2, Model 3). Overall, the estimates for the association between hard water and prevalent eczema at baseline were similar with and out adjustment for area level measures of socio-economic status (Table 7.2, Models 2 and 3). Participants exposed to domestic water with a concentration of 200 or more mg/L

of $CaCO_3$ had an association with an increase in the odds of prevalent eczema at baseline (OR: 1.12 95%CI 1.04-1.22) compared with participants exposed to water of less than 200 mg/L concentration. Furthermore, participants exposed to domestic water with a concentration of 180mg/L of $CaCO_3$ had increased risk of prevalent eczema at baseline (OR: 1.13 95%CI 1.03-1.24) compared with participants exposed to concentrations less than 60mg/L of $CaCO_3$. There was a significant linear trend (p-value: <.001) in which increasing levels of hard water exposure were associated with increased prevalent eczema risk at baseline. The total number of eczema cases in the UK biobank participants that could be attributable to domestic hard water was approximately 451 per 10,000 persons.

Table 7.3. Multilevel logistic regression models for incident eczema. (N=511/31,036)

	Model 1†	Model 2‡	Model 3§
Incident eczema	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Hard water (per 50 mg/L increase)			
OR	-	0.99 (0.93-1.05)	0.99 (0.93-1.05)
General contextual effects			
Postcode variance	.102 (.027-.364)	.104 (.030-.364)	.099 (.027-.365)
ICC	3.0% (0.8-9.9)	3.1% (0.9-9.9)	2.9% (0.8-10.0)
Hard water (>200 mg/L)			
OR		0.86 (0.64-1.17)	0.87 (0.64-1.17)
General contextual effects			
Postcode variance		.107 (.033-.353)	.103 (.031-.353)
ICC		3.2% (1.0-9.8)	3.0% (0.9-9.7)
Hard water groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		1.14 (0.93-1.39)	1.15 (0.94-1.41)
120 to 180 mg/L		1.04 (0.72-1.51)	1.06 (0.73-1.53)
More than 180 mg/L		0.92 (0.68-1.23)	0.93 (0.69-1.25)
General contextual effects			
Postcode variance		.102 (.030-.345)	.098 (.028-.344)
ICC		3.0% (0.9-.9.5)	2.9% (0.9-9.6)

N: Proportion of incident eczema OR: cluster specific odds ratio of the association between domestic hard water and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval

†Null models with no covariate adjustment

‡ Adjusted for: sex, age, ethnicity and income.

§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

The estimates for the association between hard water and incident incidence were relatively imprecise (Table 7.3, Models 2 and 3). Likewise, the estimates for the association between hard water and prevalent eczema at follow-up had wide confidence intervals in Model 2 and 3 (Table 7.4).

Table 7.4. Multilevel logistic regression models for prevalent eczema at follow-up. (N=319/31,036)

	Model 1†	Model 2‡	Model 3§
Prevalent eczema at follow-up	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Hard water (per 50 mg/L increase)			
OR	-	0.98 (0.91-1.05)	1.00 (0.94-1.07)
General contextual effects			
Postcode variance	.053 (.005-5.25)	.033 (.001-52.48)	.034 (.001-43.2)
ICC	1.6% (0.2-11.4)	4.1% (1.4-11.6)	1.0% (0.1-92.9)
Hard water (>200 mg/L)			
OR		0.92 (0.64-1.33)	1.00 (0.70-1.42)
General contextual effects			
Postcode variance		.046 (.001-9.65)	.043 (.001-13.6)
ICC		1.4% (0.1-74.6)	1.3% (0.1-80.5)
Hard water groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		0.88 (0.66-1.18)	0.95 (0.71-1.27)
120 to 180 mg/L		1.34 (0.90-2.00)	1.39 (0.93-2.07)
More than 180 mg/L		0.90 (0.63-1.28)	0.99 (0.70-1.39)
General contextual effects			
Postcode variance		.030 (.001-139.5)	.029 (.001-171.9)
ICC		0.9% (0.1-97.8)	0.9% (0.1-98.1)

N: Proportion of prevalent eczema at follow-up OR: cluster specific odds ratio of the association between domestic hard water and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

The estimated general contextual effects (variance components) were considered low in all models (Tables 7.2 & 7.3). For all outcomes, the variance estimates in model 1 (null model) were higher than those estimated on model 3. This means that the clustering effect seen on Model 1 was partly explained by the addition of individual level and area level covariates (IMD and Townsend index).

The estimated odds ratios are conditional on the random effect on postcodes being held constant. [22] We can estimate the population-average odds ratio based on the cluster-specific regression odds ratio using shrinkage factors. [25] Considering that the variance estimates for each eczema outcome were low, the corresponding shrinkage factors for prevalent eczema at baseline was 1.007, for incident eczema was 1.017 and for prevalent eczema at follow-up was 1.005. As the shrinkage factors are almost one, the population-average odds ratios are essentially equal to the cluster-specific odds ratios (to two decimal places).

As a sensitivity test, we used a model that only included individual-level covariates in a standard (i.e., single-level) logistic regression without considering postcode influence and postcode

level variables (Table S7.2). The association estimates between hard water and eczema outcomes were similar to those from the multilevel logistic models.

Discussion

In this large cohort of population-based adults from the UK we estimated the association between domestic hard water and eczema in adults. We showed that increasing levels of domestic hard water, as measured by $CaCO_3$ concentrations, were associated with an increase in prevalent eczema at baseline. The absence of a found association between domestic hard water and incident eczema may be due to a lack of statistical power. Furthermore, we observed minor variations in eczema outcomes across postcodes; postcode level measures of deprivation explained a minor proportion of this variation. The reduction of domestic hard water concentrations may therefore lead to a relatively small reduction in eczema prevalence in adults.

To the best of our knowledge this is the first study to evaluate the association between domestic hard water and eczema in a cohort of adults. The results from most studies assessing this association in infants and school-age children report higher risk of eczema prevalence associated with exposure to higher concentrations of hard water. [10,11,26-29] One observational study in children[30] suggested that water hardness was not associated with eczema, however, the comparison groups were not very different in terms of $CACO_3$ levels Likewise, a randomised controlled trial reported no good evidence that water softeners (a device that reduces tap hard water concentration) provided additional benefit for eczema patients in comparison to usual care. However, this trial only had a duration of 12 weeks and assessed eczema severity instead of prevalence. [31] A recent systematic review reported a pooled odds ratio of 1.28 (95%CI 1.09, 1.50; I^2 63%) based on 385,901 participants from different studies, showing increased odds of eczema prevalence in infants and children exposed to hard water compared with those exposed to soft water. [32] While the direction of association is concordant with what we have observed at baseline for eczema prevalence in adults when comparing participants living in areas of more than 200 mg/L of $CACO_3$ to participants in areas with less, the strength of association in this adult cohort is somewhat lower (OR:1.12 [1.04-1.22]). It is possible that the association wanes over time as eczema becomes less prevalent in middle aged adults. [33] Alternatively, individuals who have eczema that is exacerbated by hard water may employ other ways to protect their skin (i.e. lotions or creams), different shower duration, use of rainwater for washing or selectively move away from areas with hard water.

There are a number of biological mechanisms by which hard water may lead to increased risk of individuals developing and having more severe and persistent eczema by damaging the skin barrier function. [9] The most recognised mechanism involves the increase of use and deposition of detergents such as sodium lauryl sulphate (SLS) on the skin. SLS causes skin irritation and skin barrier

impairment, the extent of which is dependent on the hardness of the wash water. [12] SLS residues left on the skin alter protein secondary structure, solubilize stratum corneum lipids, and elevate skin surface pH in a dose-dependent manner. [12] Soap also reacts with calcium in hard water to form small chalk particles that can irritate the skin. [9] In addition, calcium and magnesium are alkaline metals that form a basic solution with water, which can increase skin pH (normally mildly acidic) and compromise barrier function. [12] Increased concentration of the metal ion (Ca^{2+}) in hard water may also alter calcium signalling in the epidermis to contribute to impaired skin barrier function. [12,34] These various potential mechanisms can then lead to increased allergen penetration and bacterial colonisation of the skin which are risk factors for eczema development and progression. [35]

We evaluated the general contextual effect (clustering effect) of individuals with eczema outcomes by postcodes. Previous studies provided evidence that the socioeconomic characteristics of the neighbourhood environment were related to individual risk of eczema. [36,37] The variance components estimated in our study showed that there is a minor contextual effect that influences eczema outcomes. The addition of individual level variables and the deprivation measures did not explain a major proportion of the variance. As such, there are other contextual characteristics that may lead to this unexplained variance in eczema. These may include air pollution, area effects (i.e. neighbourhood greenness, blueness etc.), or other neighbourhood level variables.

The strengths of our study include access to a large population based prospective cohort study of around half a million participants at baseline, extensive collection of covariates, and confirmation of responses from the touch screen questionnaire during the nurse-led interviews. Finally, we used multilevel analysis to formally examine the magnitude of the effect of clustering and account for it in our association estimates. An important study limitation was that we did not have access to data on the participant's usage of water softeners. Informal data suggested that in 2016 only 3% of households had a water softener fitted in the UK. Another limitation was that very mild cases of eczema that did not require any type of treatment will have been missed. Finally, there was a substantial and selective reduction in participation at the follow-up, participants lost to follow-up were more likely to come from hard water areas, which reduced our power to test associations with eczema incidence and eczema at follow-up.

Conclusions

Our findings suggest that higher domestic hard water concentration exposure is associated with an increase in odds of eczema prevalence in middle aged adults from the UK. Ongoing efforts to reduce hard water exposure in adults may have a relatively small beneficial effect in reducing eczema prevalence in adults. Furthermore, the estimates of clustering effect by postcodes in eczema

outcomes although small, remain mostly unexplained by area level socioeconomic measures, so further research is needed to explore which geographical factors may lead to eczema.

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7.3. Supplementary material

Supplementary text S7.1. Median odds ratio

To assess the general contextual effects (i.e., the variation of the outcome by postcodes), we included measures of variance (i.e., clustering). The median odds ratio (MOR) describes the postcode-level variance on the odds ratio scale, as the median value of the distribution of odds ratios estimated when two participants with the same characteristics are selected from two different postcodes, comparing the one from the higher prevalence postcode to the one from the lower prevalence postcode. [350]

Interpretation

The estimated general contextual effects (variance components) were generally considered low when considering the different water hardness definitions. The MOR suggested a 1.24 (95%CI 1.13-1.28) increase in the odds of prevalent eczema at baseline when comparing a participant from a high eczema prevalence postcode to a participant form a low prevalence postcode (Table 1).
The MOR suggest a 1.35 (95%CI 1.08-1.61) increase in the odds of incident eczema when comparing a participant from a high eczema incidence postcode with a participant from a low eczema incidence postcode (Table 2).
The MOR suggests a 1.25 (95%CI 0.62-1.88) increase in the odds of prevalent eczema at follow-up when comparing a participant from a high eczema prevalence at follow-up postcode with a participant from a low eczema prevalence postcode (Table 3).

Table 1. Multilevel logistic regression models for eczema at baseline. (N=3,392/306,531)

	Model 1†	Model 2‡	Model 3§
Prevalent eczema at baseline	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Water Hardness (per 50 mg/L increase)			
OR	-	1.03 (1.01-1.05)	1.02 (1.01-1.04)
General contextual effects			
MOR	1.24 (1.13-1.28)	1.25 (1.15-1.35)	1.22 (1.11-1.32)
Water Hardness (>200 mg/L)			
OR		1.16 (1.07-1.25)	1.12 (1.03-1.21)
General contextual effects			
MOR		1.25 (1.14-1.35)	1.22 (1.12-1.32)
Water Hardness groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		1.01 (0.91-1.12)	1.00 (0.91-1.11)
120 to 180 mg/L		1.08 (0.93-1.26)	1.05 (0.90-1.23)
More than 180 mg/L		1.17 (1.07-1.28)	1.13 (1.03-1.24)
General contextual effects			
MOR		1.25 (1.14-1.35)	1.22 (1.11-1.31)

N: Proportion of eczema at baseline OR: cluster specific odds ratio of the association between domestic water hardness and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval MOR: Median odds ratio.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

Table 2. Multilevel logistic regression models for eczema incidence. (N=511/31,036)

	Model 1†	Model 2‡	Model 3§
Incident eczema	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Water Hardness (per 50 mg/L increase)			
OR	-	0.99 (0.93-1.05)	0.99 (0.93-1.05)
General contextual effects			
MOR	1.35 (1.08-1.61)	1.36 (1.10-1.62)	1.35 (1.09-1.62)
Water Hardness (>200 mg/L)			
OR		0.86 (0.64-1.17)	0.87 (0.64-1.17)
General contextual effects			
MOR		1.37 (1.11-1.62)	1.36 (1.11-1.62)
Water Hardness groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		1.14 (0.93-1.39)	1.15 (0.94-1.41)
120 to 180 mg/L		1.04 (0.72-1.51)	1.06 (0.73-1.53)
More than 180 mg/L		0.92 (0.68-1.23)	0.93 (0.69-1.25)
General contextual effects			
MOR		1.36 (1.11-1.61)	1.35 (1.10-1.61)

N: Proportion of eczema incidence OR: cluster specific odds ratio of the association between domestic water hardness and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval MOR: Median odds ratio.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

Table 3. Multilevel logistic regression models for eczema at follow-up. (N=319/31,036)

	Model 1†	Model 2‡	Model 3§
Prevalent eczema at follow-up	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Water Hardness (per 50 mg/L increase)			
OR	-	0.98 (0.91-1.05)	1.00 (0.94-1.07)
General contextual effects			
MOR	1.25 (0.62-1.88)	1.20 (0.45-1.94)	1.19 (0.44-1.95)
Water Hardness (>200 mg/L)			
OR		0.92 (0.64-1.33)	1.00 (0.70-1.42)
General contextual effects			
MOR		1.22 (0.56-1.90)	1.22 (0.53-1.91)
Water Hardness groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		0.88 (0.66-1.18)	0.95 (0.71-1.27)
120 to 180 mg/L		1.34 (0.90-2.00)	1.39 (0.93-2.07)
More than 180 mg/L		0.90 (0.63-1.28)	0.99 (0.70-1.39)
General contextual effects			
MOR		1.18 (0.36-2.00)	1.18 (0.35-2.00)

N: Proportion of eczema at follow-up OR: cluster specific odds ratio of the association between domestic water hardness and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval MOR: Median odds ratio.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

Table S7.1. Lost to follow-up baseline characteristics.

Characteristic	Lost (N= 276,119)	Not lost (N=30,412)	Odds to be lost
	% (n/N)	% (n/N)	OR (95%CI)
Female	55.9% (154,469/276,119)	52.6% (15,993/30,412)	0.87 (0.85-0.89)
Age			
Less than 45 years	9.1% (25,124/276,119)	8.8% (2,685/30,412)	Ref
46-55 years	24.6% (67,824/276,119)	28.06% (8,533/30,412)	0.85 (0.81-0.89)
56-65 years	47.0% (129,790/276,119)	50.5% (15,356/30,412)	0.90 (0.87-0.94)
More than 65 years	19.33% (53,381/276,119)	12.6% (3,838/30,412)	1.49 (1.41-1.57)
Ethnicity			
White	94.3% (259,426/276,119)	97.2% (29,544/30,412)	Ref
Mixed or Multiple ethnic groups	0.6% (1,570/276,119)	0.4% (125/30,412)	1.43 (1.19-1.72)
Asian or Asian British	2.2% (6,536/276,119)	1.1% (343/30,412)	2.17 (2.61-3.54)
Black, African or Black British	1.6% (4,731/276,119)	0.6% (177/30,412)	3.04 (2.62-3.54)
Other ethnic group	0.9% (2,500/276,119)	0.5% (145/30,412)	1.96 (1.66-2.32)
Education			
College or University degree	35.7% (75,292/211,020)	45.6% (12,403/27,201)	Ref
A levels/AS levels or equivalent†	13.5% (28,393/211,020)	13.8% (3,760/27,201)	1.24 (1.20-1.29)
O levels/GCSEs or equivalent‡	27.7% (58,355/211,020)	23.0% (6,243/27,201)	1.54 (1.49-1.59)
CSEs or equivalent §	6.72% (14,175/211,020)	4.3% (1,163/27,201)	2.01 (1.89-2.14)
NVQ or HND or HNC or equivalent	9.1% (19,249/211,020)	6.9% (1,876/27,201)	1.70 (1.61-1.78)
Other professional qualifications	7.4% (15,556/211,020)	6.5% (1,756/27,201)	1.46 (1.38-1.54)
Income			
Less than 18,000£	28.9% (66,104/228,914)	16.5% (4,476/27,141)	Ref
18,000 to 30,999£	27.1% (62,035/228,914)	26.4% (7,162/27,141)	0.58 (0.56-0.61)
31,000 to 51,999£	23.8% (54,442/228,914)	29.0% (7,869/27,141)	0.47 (0.45-0.49)
52,000 to 100,000£	16.2% (37,038/228,914)	22.7% (6,160/27,141)	0.41 (0.39-0.42)
Greater than 100,000£	4.1% (9,295/228,914)	5.4% (1,474/27,141)	0.43 (0.40-0.45)
Home location			
England	87.5% (235,404/269,146)	95.7% (28,495/29,763)	Ref
Wales	4.9% (13,203/269,146)	0.3% (91/29,763)	17.56 (14.28-21.59)
Scotland	7.6% (20,539/269,146)	4.0% (1,177/29,763)	2.11 (2.00-2.24)
England IMD†	18.5 (14.6; 0.61, 82)	16.4 (12.8;0.61, 81.6)	1.01 (1.01-1.01)
Townsend index score†	-1.1 (3.2; -6.3, 10.9)	-1.9 (2.7; -6.3, 9.7)	1.10 (1.09-1.10)
Water Hardness (mg/L)†	147.0 (115.2; 5, 460)	93.4 (93.9; 5, 460)	1.00 (1.00-1.00)
Water Hardness (>200 mg/L)	38.4% (105,141/273,624)	16.5% (4,977/30,157)	3.16 (3.06-3.26)
Water Hardness			
0 to 60 mg/L	35.2% (96,318/273,624)	55.4% (16,711/30,157)	Ref
60 to 120 mg/L	19.9% (54,534/273,624)	22.9% (6,902/30,157)	1.37 (1.33-1.41)
120 to 180 mg/L	5.9% (16,229/273,624)	4.5% (1,360/30,157)	2.07 (1.95-2.19)
More than 180 mg/L	38.9% (106,543/273,624)	17.2% (5,184/30,157)	3.57 (3.45-3.68)
Prevalent eczema at baseline	1.10% (3,026/276,097)	1.20% (365/30,045)	0.91 (0.82-1.02)

A: Advanced As: Advanced Subsidiary level O: Ordinary GCSEs: General Certificate of Secondary Education CSEs: Certificate of Secondary Education NVQ: National Vocational Qualification HND: Higher National Diplomas HNC: Higher National Certificate IMD: Indices of multiple deprivation mg/L: milligrams per litre
† Mean (standard deviation; min, max)

Table S7.2. Odds ratios for logistic regression models for the adjusted association between water hardness definitions and eczema outcomes.

Domestic hard water	Prevalent eczema at baseline (3,392/306,531) aOR (95%CI) †	Incident eczema (511/31,036) aOR (95%CI) †	Prevalent eczema at follow-up (319/31,036) aOR (95%CI) †
Per 50 mg/L increase	**1.01 (1.00-1.03)**	0.99 (0.93-1.05)	0.98 (0.91-1.04)
More than 200 mg/L	**1.10 (1.02-1.18)**	0.83 (0.60-1.16)	0.90 (0.63-1.27)
Categorical variable			
0 to 60 mg/L [Ref]			
60 to 120 mg/L	0.99 (0.90-1.09)	1.07 (0.84-1.37)	0.86 (0.65-1.16)
120 to 180 mg/L	1.07 (0.92-1.24)	1.01 (0.64-1.58)	1.34 (0.90-1.99)
More than 180 mg/L	**1.11 (1.02-1.19)**	0.88 (0.64-1.23)	0.87 (0.62-1.21)

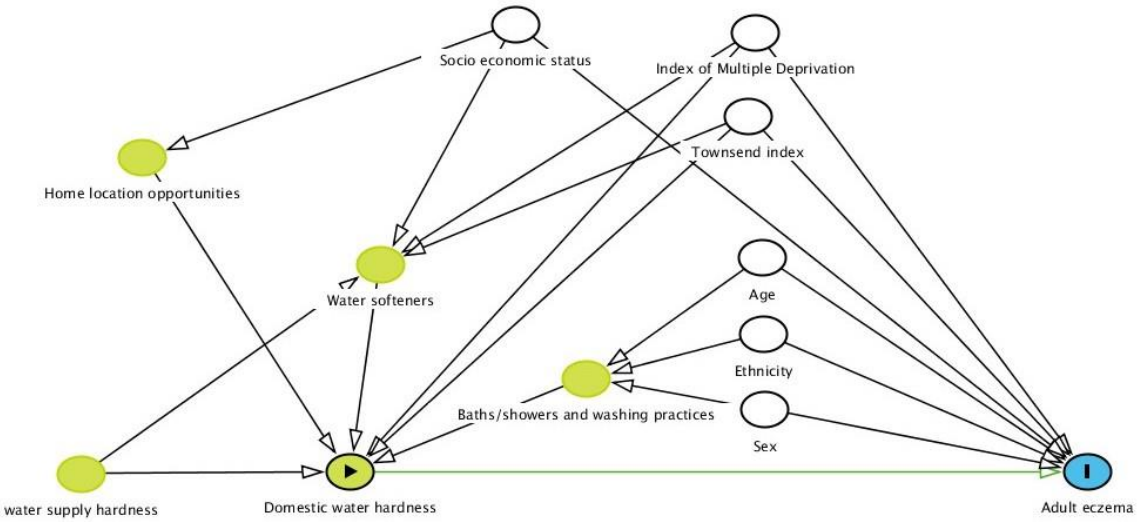
* P-values less than 0.05 are bolded
†Adjusted for: sex, age, ethnicity and income.

Table S7.3. Multilevel logistic regression models for ever eczema. (N=12,545/306,531)

	Model 1†	Model 2‡	Model 3§
Prevalent eczema at baseline	Coefficient (95%CI)	Coefficient (95%CI)	Coefficient (95%CI)
Hard water (per 50 mg/L increase)			
OR	-	1.01 (1.00-1.03)	1.01 (1.00-1.02)
General contextual effects			
Postcode variance	.110 (.092-.134)	.098 (.080-.120)	.091 (.074-.113)
ICC	3.3% (2.7-3.9)	2.9% (2.4-3.5)	2.7% (2.2-3.3)
Hard water (>200 mg/L)			
OR		1.09 (1.04-1.15)	1.08 (1.03-1.14)
General contextual effects			
Postcode variance		.097 (.079-.120)	.091 (.073-.113)
ICC		2.9% (2.4-3.5)	2.7% (2.2-3.3)
Hard water groups			
OR (Ref: 0 to 60 mg/L]		-	-
60 to 120 mg/L		1.00 (0.94-1.07)	1.00 (0.94-1.06)
120 to 180 mg/L		1.07 (0.98-1.17)	1.05 (0.96-1.15)
More than 180 mg/L		1.10 (1.04-1.16)	1.09 (1.03-1.15)
General contextual effects			
Postcode variance		.097 (.078-.119)	.091 (.073-.112)
ICC		2.9% (2.3-3.5)	2.7% (2.2-3.3)

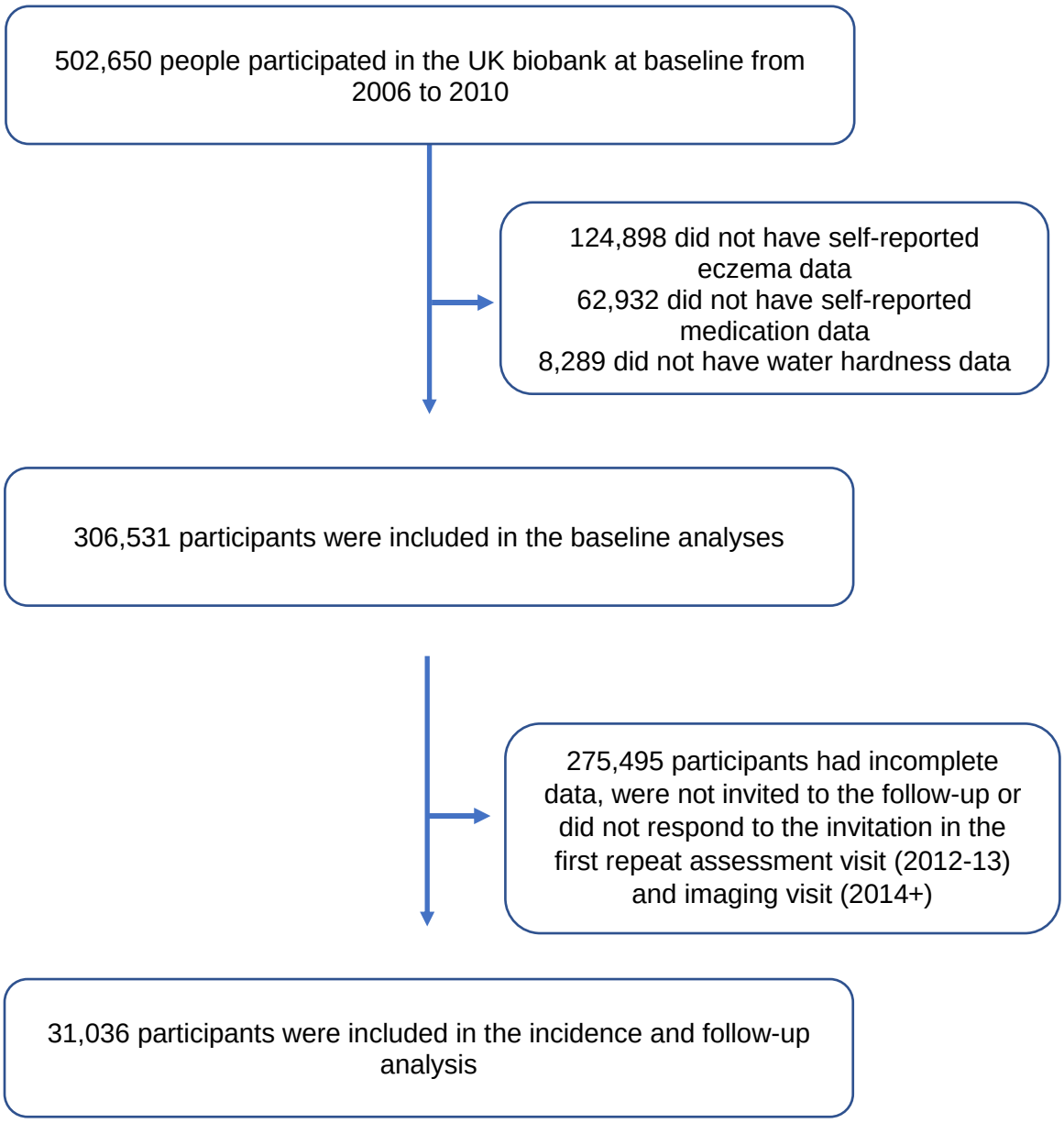
N: Proportion of prevalent eczema at baseline OR: cluster specific odds ratio of the association between domestic hard water and eczema outcomes ICC: intra cluster correlation 95% CI: 95% confidence interval.
†Null models with no covariate adjustment
‡ Adjusted for: sex, age, ethnicity and income.
§ Adjusted for: sex, age, ethnicity, income, IMD and Townsend Index

Figure S7.1. Direct acyclic graph of the association between domestic hard water and eczema in adults.



Legend
exposure
outcome
ancestor of exposure
ancestor of outcome
ancestor of exposure *and* outcome
causal path
biasing path

Figure S7.2. Participant Flowchart.



Chapter 8 - Assessing associations between occupational exposures and eczema in middle aged adults

8.1. Section introduction

This manuscript under review evaluated the associations between several occupational exposures and eczema. The literature suggested that occupational exposures may be significant as environmental risk factors of eczema. A better understanding of these potential associations can lead to preventive strategies. To my knowledge, this is the first manuscript evaluating this association using a job exposure matrix, as previous work used non-specific occupational exposure definition (every exposure to gas, dust and fumes). This research paper is based on data from the TAHS 2012 proband study when the participants had a mean age of 53 years. It contains participants' lifetime job history, sociodemographic factors, disease status and skin prick test data.

8.1.1. Research question

- What are the potential associations between a wide range of occupational exposures and eczema prevalence in middle aged adults?
    - Are these associations different according to atopic or non-atopic status?

8.1.2. Research aim

- To evaluate potential associations between occupational exposures and eczema prevalence in middle aged adults.
    - To assess whether these association vary according to atopic or non-atopic status.

8.1.3. Critical reflection on the work

This research project involved using a job exposure matrix which is a tool to assign occupational exposures according to the participant's job. We used a job exposure matrix for asthma as there were none for dermatitis or eczema exposures. I contacted the researcher responsible for developing this tool to request permission, and she agreed and offered to review the manuscript. Liaising with researchers from outside the university is an excellent experience to have much of the research collaborative. Once I had the job exposure matrix, I assigned the occupational exposures to each participant based on their job and generated both cumulative and current occupational exposures. I used STATA during this process, the coding was straightforward, and by this point, I feel comfortable using STATA. Once the analyses and manuscript were complete, I submitted the manuscript to a journal with the help of my supervisors and it is currently under review.

8.1.4. Manuscript under review.

Lopez DJ, Alif SM, Dharmage SC, Lodge CJ, Bui DS, Moual NL, Waidyatillake NT, Su JC, Abramson MJ, Walters EH, Hamilton GS, Bowatte G, Erbas B, Benke GP, Perret JL, Lowe AJ. Assessing associations between occupational exposures and eczema in middle aged adults

***Please note that the specific references from this accepted or submitted manuscript are at the end of this chapter and the in-text citation number restarts at 1.**

8.2. Submitted version of the manuscript.

Title: Assessing associations between occupational exposures and eczema in middle aged adults

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Abstract

Background: There is a scarcity of evidence on workplace exposures that may increase eczema in adults.

Objective: To investigate relationships between a wide range of occupational exposures and eczema in an adult population-based cohort.

Methods: A lifetime work history calendar was collected from participants of the Tasmanian Longitudinal Health Study when they were 53-years of age. We used the Occupational Asthma specific Job Exposure Matrix to determine current and cumulative (lifetime) exposures. Current eczema was determined using the report of flexural rash that was coming and going for at least six months in the last 12 months. Skin prick tests were used to further subgroup eczema into atopic eczema (AE) or non-atopic eczema (NAE). Logistic and multinomial regression models adjusted with for potential confounders were used adjusted with potential confounders.

Results: Of 2923 participants with complete work history, 2369 had a current job. Eczema prevalence was 9.1%. Cumulative exposure to mould was associated with increased odds of current eczema (adjusted odds ratio (aOR)=1.08 [95%CI:1.00-1.18] per 5 years exposure-equivalent). Similarly, current occupational exposure to animals (aOR=2.88 [1.34-6.22]), storage mites (aOR=2.78 [1.29-5.99]) and endotoxin (aOR=1.96 [1.05-3.66]) were associated with increased risk of current eczema. Furthermore, increased odds of NAE was associated with current exposure to animals (aOR=5.33[1.37-20.7]), storage mites (aOR=5.39[1.39-20.9]), and metal working fluids (aOR=1.11[1.01-1.23]). Current exposures to isocyanates (aOR=4.65[1.04-21.4]) and acrylates (aOR=7.44[1.38-24.8]) was associated with AE.

Conclusions: Several occupational exposures were associated with current eczema in middle aged adults. Further research with robust measures of occupational measures is needed to validate these results. Nevertheless, minimising harmful workplace exposures may reduce the eczema burden in adults.

Keywords: occupational, eczema, adults, workplace, exposures

Key points

Our results suggest that lifetime exposure to mould was associated with increased probability of eczema prevalence. Moreover, current exposure to animals, storage mites and the microbial group was associated with increased risk of non-atopic eczema. While current exposure to acrylates and isocyanates was associated with increased risk of having atopic eczema.

Author Contributions: Conception and design: D.J. Lopez, A.J. Lowe, S. Alif, S. Dharmage Acquisition of data: S. Dharmage, J. L. Perret, G.S. Hamilton, E.H. Walters, G. Bowatte, B. Erbas, G.P. Benke, S.M. Alif, A.J. Lowe Analysis and interpretation of data: D.J. Lopez, A.J. Lowe, C.J. Lodge, D.S. Bui, N.T. Waidyatillake, N. L. Moual, J.C. Su., M. J. Abramson, S Alif Drafting the article : D.J. Lopez, A.J. Lowe Revision for important intellectual content and approval of final submission : all authors.

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Conflict of Interest

S.C.D., C.J.L., M.J.A. and A.J.L. declare they have received research funds from GSK's competitively awarded Investigator Sponsored Studies programme, for unrelated research. AJL, SCD and MA have received grant funding from Sanofi Regeneron for unrelated research. A.J.L. has received donations of interventional product (EpiCeram) from Primus Pharmaceuticals for unrelated research. J.C.S. has been a consultant/speaker/investigator for AbbVie, Amgen, Bioderma, Bristol Myers Squibb, Ego Pharmaceuticals, Eli-Lilly, Janssen, LEO Pharma, L'Oreal, Mayne, Novartis, Pfizer, Pierre-Fabre, and Sanofi. M.J.A. holds investigator-initiated grants for unrelated research from Pfizer, Boehringer-Ingelheim and Sanofi. He has also undertaken an unrelated consultancy for Sanofi. He has also received a speaker's fee from GSK. The other authors declare they have no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the Tasmanian Longitudinal Health Study, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Tasmanian Longitudinal Health Study. Data request should be made at: https://tahs.com.au/data-request/

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Abbreviations

Adjusted odds ratio (aOR)

Asthma specific-job exposure matrix (OAs-JEM)

Atopic eczema (AE)

International Study of Asthma and Allergies in Childhood (ISAAC)

Job-exposure matrix (JEM)

Non-atopic eczema (NAE)

Reactive oxygen species (ROS)

Skin prick testing (SPT)

Socio-Economic Indexes for Areas (SEIFA)

Tasmanian Longitudinal Health Study (TAHS)

Introduction

Eczema, also known as atopic dermatitis, is a chronic relapsing inflammatory skin disease characterised by defective skin barrier function.[1] This condition differs from contact dermatitis which is a localised skin reaction, despite having similar characteristics including itchiness, erythema, irritation, and increased trans-epidermal water loss as a result of defective skin barrier.[2] Although, both skin disorders share clinical features, they are caused by different pathophysiological mechanisms.[2] Furthermore, their typical anatomical distribution and the extent of the eczematous lesions are also different.[2,3]

It is likely that specificity of workplace exposures affect how eczema develops in adults. In general, eczema development and progression appears to be multifactorial, related to an interplay between genetic and environmental factors including occupational exposures.[4,5] According to the World Allergy Organization, two types of eczema have been recognised, atopic (AE) and non-atopic eczema (NAE).[1] The pathophysiology of AE and NAE is not completely understood, and additional inflammatory pathways may be involved, especially in adult patients.[6] For this reason, it is important to determine occupational exposures that may act on the different pathophysiological pathways of eczema.

Occupational agents can act as allergens on contact with the skin resulting in an allergic reaction.[7] Other agents can act as irritants, leading to production of a cytokine cascade, skin barrier impairment and oxidative stress, all of which lead to an inflammatory response.[8] Three studies have found positive associations between occupational exposures and current eczema.[7,9,10] However, two of these studies[9,10] were limited by exposure definitions that were not time-specific (exposure ever) and evaluated only a few occupational exposures while the third study[7] did not evaluated eczema specifically. Assessing the associations between occupational exposures and eczema is needed to better understand the likely environmental contributors for onset and adult persistence. Given the few studies of this topic to date, we aimed to investigate relationships between a wide range of potential occupational exposures and eczema.

Materials and methods

Study design and population

The Tasmanian Longitudinal Health Study (TAHS) is a population-based prospective cohort.[11] The study methodology has been reported in detail elsewhere.[11] The data for this analysis came from participants of the 2012 follow-up study when participants completed a self-administered survey and clinic visit at a mean age of 53 years. The study was approved by the Human Research Ethics Committee of the University of Melbourne (HREC no. 040375); all participants provided written informed consent.

Exposure definitions

Occupational exposures

A lifetime work history calendar was completed by participants during the 2012 follow-up and used to define occupational exposures, participants were asked to list all jobs they had held in their lifetimes. [12] The job titles were coded according to the International Standard Classification of Occupations-88 four-digit classification. [13]

There was no existing job-exposure matrix (JEM) to assess eczema-specific exposures. [7] As asthma and eczema are likely to share similar occupational risk factors, [14] we used the occupational asthma specific-JEM (OAs-JEM) to assess occupational exposures. [15] Occupational exposures to 30 agents and seven exposure groups were determined using the updated version of OAs-JEM. [15] Details on the individual agents and exposure groups are provided elsewhere. [15,16]

Current occupational exposures

Each occupational exposure was assessed using discrete quantitative metrics i.e. 2:high (high probability of exposure and moderate-to-high intensity); 1:medium (low-to-moderate probability or low intensity of exposure, such as 'high probability and low intensity' or 'low probability and moderate to high intensity') and 0:no exposure (unlikely to be exposed, low probability and low intensity) to a potential occupational exposure. The current occupational exposures were dichotomised (i.e., high/low exposure versus not exposed) based on the exposures from the participant's current job or last job held in the last 12 months when they were assessed at the 2012 follow-up.

Cumulative occupational exposures

The cumulative occupational exposure was calculated by summing exposures over all jobs for each participant. Where participants had two different jobs at the same time, exposures from both jobs were averaged and rounded up to the largest integer. The weighted cumulative occupational exposure was calculated by multiplying the years of exposure by the exposure intensity (i.e. for low/medium exposure=1 and high exposure=4) for each job and then summed for each participant over the entire job history. [12] Then the cumulative occupational exposures were scaled to a continuous variable of cumulative exposure per 5 years exposure equivalent. Participants missing any years in the lifetime work history calendar were excluded from the cumulative exposure analyses.

Outcome definitions

Current prevalent eczema

Current prevalent eczema was determined using the International Study of Asthma and Allergies in Childhood (ISAAC) definition of eczema. [17] Participants were classified as having prevalent eczema if they reported "yes" to all three questions: 'Have you had an itchy rash in the past 12

months?', 'Have you ever had an itchy rash coming and going for at least six months?', and 'Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, around the neck, ears or eyes?'

Atopic status

Sub-classification as atopic eczema (AE) or non-atopic eczema (NAE) was based on skin prick testing (SPT) results at mean age 53 years. [11] SPTs were performed for eight aeroallergens: *Dermatophagoides pteronyssinus*, cat pelt, *Cladosporium cladosporioides*, *Alternaria tenuis*, *Penicillium* mix, *Aspergillus fumigatus*, rye grass, mixed grass pollen No. 7 and four food allergens: egg, milk, shrimp, peanut. Atopy was defined as at least one of the allergens tested having a wheal that was 3mm or larger than the saline control. [18]

Current prevalent asthma

Participants were classified as having current prevalent asthma, if they reported attacks of asthma or wheezy breathing (asthma and wheezy breathing was regarded as the same) in the last 12 months or use of asthma medication during the last 12 months.

Confounding variables

A directed acyclic graph was developed to specify the hypothesised causal relationships and to determine which confounders to include in the model (Figure S8.1). We adjusted for sex, smoking, pack-years, infantile eczema (report of both eczema and flexural eczema before 7 years of age), infantile allergies (report of eczema, hay fever or asthma at 7 years of age), parental allergic conditions (asthma or rhinitis), highest education at 53 years and Socio-Economic Indexes for Areas (SEIFA) score in deciles (10 categories) at 53 years. The baseline disease status (infantile eczema, infantile allergies, parental allergic conditions) was defined from the TAHS 1968 baseline survey when participants were 7 years of age. [11]

Statistical Analysis

Associations between the occupational exposures and the following outcome measures were assessed: 1) Current prevalent eczema and 2) Prevalent eczema sub-grouped by atopic status (No atopy nor eczema, atopy alone, eczema alone, and atopic eczema). Logistic and multinomial regression models were fitted to estimate the associations between occupational exposures and each eczema outcome. Associations between current and cumulative occupational exposures were assessed separately. Each occupational exposure agent and group was analysed separately, and the corresponding odds ratios and 95% confidence intervals presented in tables.

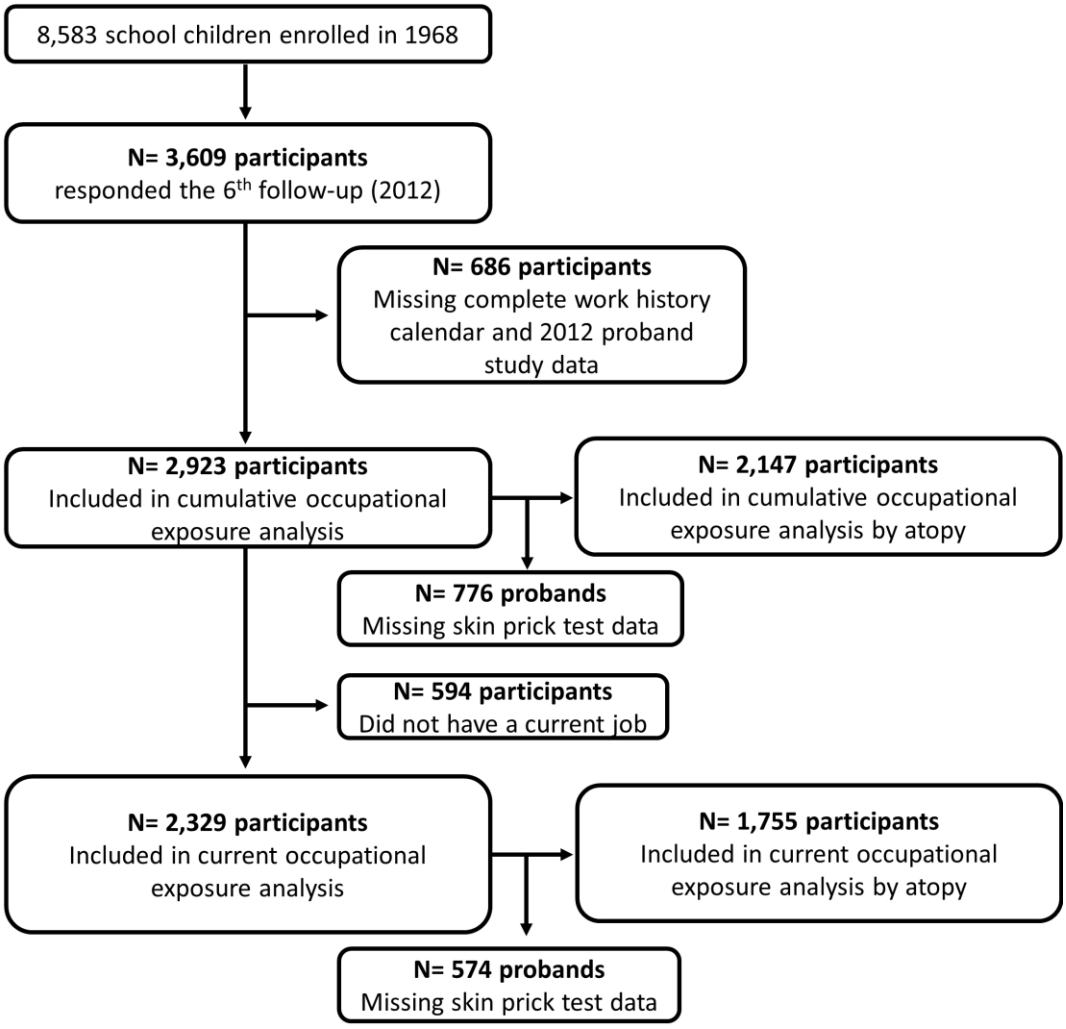
Potential effect modification by sex and by infantile eczema was explored by including an interaction term in the models. Linearity of the associations with cumulative exposures was confirmed by using Stata's "fracpoly" command. To evaluate the potential public health impact of

occupational exposures in eczema cases, we calculated the population attributable fraction. We performed a number of sensitivity analyses: 1) using a "universal un-exposed reference category" of participants with no exposure to any occupational exposure, and 2) we also explored the associations between occupational exposures and concurrent eczema and asthma outcomes (neither, eczema only, asthma only, or both) to determine if any associations with eczema could be due to concomitant asthma. All analyses were carried out using the statistical software Stata (release 16; StataCorp, College Station, TX).

Results

At mean age 53 years 3,609 participants completed the follow-up survey (Figure 8.1), of whom 686 were excluded from the cumulative occupational exposure analysis, because they did not have a complete work history calendar completed and/or 2012 follow-up data. An additional 594 participants were excluded from the current occupational exposure analyses because they did not have a current job.

Figure 8.1. Participant flow diagram.



The mean age was 53 years, 51% were females, most were educated beyond grade 12 and were never or former smokers (Table 8.1). The prevalence of current eczema was 9.1%. In those with a valid SPT, the prevalence of NAE was 3.3%, while 6% had AE. When comparing those lost to follow-up due to missing lifetime work history calendar data to those included, there were no important differences, except that those lost were more likely to be smokers or exposed to smoke, less educated, and came from lower socioeconomic strata (Table S8.1, S8.2). We have previously reported no important differences between those with or without a valid SPT. [19]

Table 8.1. Characteristics of participants included in the analyses.

Characteristic	Current exposure (N= 2,329)	Cumulative exposure (N=2,923)
Age (years). Mean (SD); range	53 (0.91) 51-55.5	53 (0.92); 51-55.5
Female. % (n/N)	50.75% (1,182/2,329)	51.11% (1,494/2,923)
Years of education. %(n/N)		
< Grade 12	31.52% (734/2,329)	32.98% (964/2,923)
Grade 12 or equivalent	7.13% (166/2,329)	7.73% (226/2,923)
> Grade 12	61.36% (1,429/2,329)	59.29% (1,733/2,923)
Childhood eczema. % (n/N)	13.83% (322/2,329)	13.55% (396/2,923)
Childhood allergies. % (n/N)	27.31% (636/2,329)	28.70% (839/2,923)
Parental allergy history. % (n/N)	27.48% (640/2,329)	27.68% (809/2,923)
Smoking status. %(n/N)		
Never	43.11% (1,004/2,329)	42.29% (1,236/2,923)
Former	41.61% (969/2,329)	41.12% (1,202/2,923)
Current	15.29% (356/2,329)	16.59% (485/2,923)
Pack years smoked. Median; 25-75%le	0.5; 0-14	0.6; 0-15
SEIFA deciles. % (n/N)		
1	8.50% (198/2,329)	9.89% (289/2,923)
2	17.48% (407/2,329)	17.79% (520/2,923)
3	8.84% (206/2,329)	8.93% (261/2,923)
4	8.80 % (205/2,329)	8.52% (249/2,923)
5	15.80% (368/2,329)	15.70% (459/2,923)
6	6.83% (159/2,329)	6.91% (202/2,923)
7	5.32% (124/2,329)	5.34% (156/2,923)
8	11.03% (257/2,329)	10.74% (314/2,923)
9	9.70% (226/2,329)	8.96% (262/2,923)
10	7.69% (179/2,329)	7.22% (211/2,923)
Current prevalent eczema	9.06 % (211/2,329)	9.13% (267/2923)
Eczema atopy groups†		
No atopy nor eczema	42.11% (739/1,755)	42.06% (903/2,147)
Atopy alone	48.95% (859/1,755)	48.67% (1,045/2,147)
Non-atopic eczema	3.08% (54/1,755)	3.26% (70/2,147)
Atopic eczema	5.87% (103/1,755)	6.0% (129/2,147)

SEIFA: Socio-Economic Indexes for Areas SD: standard deviation

†Only those participants with a valid SPT were included in the atopic groups

The most common occupational exposures for participants' current jobs were indoor cleaning products, exhaust fumes, latex and bleach, which ranged from 10.2% to 14.3% (Table S8.3).

Similarly, the highest cumulative exposures were to exhaust fumes, indoor cleaning products, latex and also organic solvents (Table S8.4).

Table 8.2. Adjusted associations between current and cumulative occupational exposure to individual agents and groups and prevalent eczema at 53 years, irrespective of atopic status.

Occupational exposure	Current exposure† aOR (95%CI) ‡	Cumulative exposure (5-years) aOR (95%CI) ‡§
	Prevalent eczema (211/2,329)	Prevalent eczema (267/2,923)
Individual Agents		
Animals	**2.88 (1.34-6.22)**	1.04 (0.98-1.11)
Fish/shellfish	1.55 (0.53-4.56)	1.03 (0.96-1.11)
Flour	1.17 (0.26-5.27)	0.94 (0.82-1.09)
Foods	1.03 (0.23-4.54)	0.97 (0.81-1.16)
Plants-related dusts	1.78 (0.81-3.92)	1.02 (0.97-1.08)
House dust mites	0.46 (0.06-3.44)	0.95 (0.78-1.16)
Storage mites	**2.78 (1.29-5.99)**	1.08 (0.96-1.24)
Plant mites	1.57 (0.68-3.62)	1.01 (0.96-1.07)
Enzymes	1.60 (0.46-5.58)	1.02 (0.84-1.23)
Latex	0.78 (0.45-1.34)	0.99 (0.97-1.02)
Textiles	2.52 (0.27-23.17)	1.03 (0.83-1.28)
Mould	1.72 (0.93-3.14)	**1.08 (1.00-1.18)**
Endotoxin	**1.96 (1.05-3.66)**	1.07 (0.90-1.28)
Drugs	0.97 (0.12-7.62)	0.88 (0.59-1.32)
High-level chemical disinfectants	1.11 (0.71-1.73)	1.00 (0.94-1.05)
Aliphatic amines	2.00 (0.64-6.24)	1.03 (0.95-1.11)
Isocyanates	1.98 (0.64-6.10)	1.04 (0.94-1.14)
Acrylates	1.87 (0.61-5.71)	1.12 (0.98-1.28)
Epoxy resins	1.80 (0.73-4.45)	1.02 (0.99-1.05)
Persulfates/henna	0.92 (0.11-7.52)	0.98 (0.92-1.05)
Wood dust	1.07 (0.40-2.82)	1.00 (0.93-1.08)
Metal and metal fumes	0.83 (0.41-1.64)	0.98 (0.92-1.04)
Metalworking fluids	1.14 (0.13-9.97)	0.99 (0.89-1.10)
Herbicides	1.24 (0.60-2.57)	1.02 (0.98-1.05)
Insecticides	1.71 (0.88-3.35)	1.02 (0.98-1.05)
Fungicides	1.67 (0.85-3.27)	1.02 (0.98-1.05)
Indoor cleaning	0.91 (0.59-1.40)	1.00 (0.97-1.03)
Bleach	0.90 (0.54-1.46)	0.99 (0.94-1.04)
Organic solvents	0.97 (0.58-1.61)	0.98 (0.94-1.02)
Exhaust fumes	1.35 (0.85-2.14)	1.00 (0.97-1.02)
Groups		
HMW sensitiser	1.08 (0.74-1.58)	1.00 (0.98-1.02)
Mites	1.52 (0.80-2.88)	1.01 (0.96-1.07)
Microbial exposure	**1.81 (1.04-3.16)**	1.03 (0.98-1.08)
LMW sensitiser	1.03 (0.72-1.47)	1.00 (0.98-1.02)
Irritants	1.20 (0.88-1.63)	1.00 (0.99-1.02)
Highly reactive chemicals	1.08 (0.76-1.55)	1.00 (0.98-1.02)
Biocides	1.15 (0.79-1.68)	1.00 (0.98-1.03)

Definitions of abbreviations: aOR: adjusted odds ratio CI: confidence interval HMW: high molecular weight LMW: low molecular weight

*P-value less than 0.05 is bolded

† Current occupational exposures: high/low exposure versus not exposed.

‡ Adjusted for: sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, pack-years and SEIFA deciles at 53 years.
§ Per 5 years exposure equivalent increase.

Current occupational exposures

Several current occupational exposures were associated with increased odds of current prevalent eczema, irrespective of atopic status (Table 8.2). The odds of prevalent eczema were increased with current exposure to animal antigens (adjusted odds ratio (aOR)=2.88[95%CI:1.34-6.22]), storage mites (aOR=2.78[1.29-5.99]) and endotoxin (aOR=1.96[1.05-3.66]). The current prevalence of eczema in those exposed to any animal, storage mite and endotoxin antigens was 14.6% (15/103) and in those unexposed 8.8% (196/2,226). The proportion of prevalent eczema cases that could be attributed to the combined animal, storage mite and endotoxin antigen exposures among the study population was 3.0% (95%CI:1-6%). When considering exposure groups, current microbial exposure (textiles, mould, endotoxin and metal working fluids) was associated with increased odds of prevalent eczema (aOR=1.81[1.04-3.16]).

Several current occupational exposures were differentially associated with AE or NAE (Table 8.3). Increased odds of having NAE were associated with current exposure to animal antigens (aOR=5.33[1.37-20.7]); current exposure to storage mites (aOR=5.39[1.39-20.9]); or current microbial exposure (aOR=2.68[1.06-7.50]). In the same manner, increased odds of AE were associated with current exposure to isocyanates (aOR=4.65[1.04-21.4]); or acrylates (aOR=7.44[1.38-24.8]). Reduced odds of having atopy alone was associated with either current occupational exposure to indoor cleaning products (aOR=0.76[0.61-0.95]) or irritants [15] (aOR=0.75[0.70-0.93]).

Cumulative occupational exposures

There was evidence of association between cumulative occupational exposure and eczema prevalence (table 8.2); mould (aOR=1.08[1.00-1.18]) per 5 years exposure equivalent). For sub-groups of eczema by atopy, cumulative occupational exposure (Table 8.4) to acrylates was associated with AE (aOR=1.21[1.02-1.44]), while cumulative exposure to metal working fluids was associated with NAE (aOR=1.11[1.01-1.23]).

Potential association-modification and sensitivity analyses

There was no evidence of any potential effect-modification by previous infantile eczema or sex (all p-values for interaction >0.1; data not shown). When we performed the same analyses using a universal not exposed reference category, similar results were found (Table S8.5), but two other associations were observed: between current mould exposure and prevalent eczema (OR=1.99[95%CI:1.07-3.73]) and current wood dust exposure and risk of NAE (OR=5.09[1.17-22.1]). From the analyses evaluating concurrent eczema and asthma (Table S8.6), similar associations to the main results were seen in the “eczema only” category.

Table 8.3. Adjusted associations between current occupational exposure and eczema by atopy groups at 53 years.

Eczema outcomes†	Atopy alone (859/1,755)	Non-atopic Eczema (54/1,755)	Atopic Eczema (103/1,755)
Occupational exposures ‡	aMOR (95%CI) §	aMOR (95%CI) §	aMOR (95%CI) §
Individual Agents			
Animals	0.88 (0.36-2.17)	**5.33 (1.37-20.7)**	1.68 (0.35-8.1)
Fish/shellfish	0.82 (0.35-1.89)	2.38 (0.49-11.6)	0.63 (0.08-4.99)
Flour	0.84 (0.28-2.56)	1.62 (0.18-14.4)	1.02 (0.12-8.67)
Foods	0.73 (0.25-2.14)	1.45 (0.17-12.7)	0.91 (0.11-7.62)
Plants-related dusts	0.91 (0.44-1.87)	2.06 (0.44-9.59)	1.41 (0.38-5.18)
House dust mites	0.54 (0.16-1.80)	-	-
Storage mites	1.06 (0.44-2.56)	**5.39 (1.39-20.9)**	1.81 (0.38-8.67)
Plant mites	0.84 (0.40-1.75)	2.08 (0.44-9.72)	0.88 (0.19-4.08)
Enzymes	0.83 (0.30-2.33)	1.43 (0.17-12.3)	1.88 (0.38-9.29)
Latex	0.72 (0.65-1.01)	0.63 (0.23-1.70)	0.55 (0.24-1.27)
Textiles	-	-	-
Mould	0.96 (0.56-1.66)	1.75 (0.50-6.19)	1.37 (0.50-3.78)
Endotoxin	0.93 (0.52-1.68)	1.26 (0.28-5.62)	1.59 (0.57-4.42)
Drugs	0.98 (0.29-3.30)	2.78 (0.31-25.1)	-
High-level chemical disinfectants	0.81 (0.58-1.12)	1.03 (0.45-2.32)	0.79 (0.38-1.61)
Aliphatic amines	1.13 (0.34-3.79)	4.76 (0.84-27.0)	2.87 (0.52-15.8)
Isocyanates	1.43 (0.46-4.46)	2.80 (0.29-26.8)	**4.65 (1.04-21.4)**
Acrylates	2.62 (0.70-9.85)	4.46 (0.42-47.6)	**7.44 (1.38-24.8)**
Epoxy resins	0.91 (0.42-1.98)	2.63 (0.54-12.9)	2.41 (0.73-7.95)
Persulfates/henna	0.47 (0.08-2.63)	-	2.05 (0.22-19.5)
Wood dust	0.95 (0.48-1.91)	3.42 (0.89-13.2)	0.38 (0.05-3.03)
Metal and metal fumes	1.09 (0.70-1.71)	0.82 (0.18-3.67)	1.10 (0.43-2.80)
Metalworking fluids	1.90 (0.36-10.2)	7.12 (0.56-27.2)	-
Herbicides	0.97 (0.54-1.73)	0.65 (0.08-5.01)	1.27 (0.41-3.88)
Insecticides	0.89 (0.49-1.64)	0.67 (0.87-5.17)	1.70 (0.61-4.75)
Fungicides	0.93 (0.51-1.69)	0.65 (0.09-5.03)	1.63 (0.58-4.57)
Indoor cleaning	**0.66 (0.49-0.89)**	0.60 (0.26-1.40)	0.81 (0.42-1.54)
Bleach	0.74 (0.5.-1.05)	0.61 (0.23-1.63)	0.67 (0.30-1.46)
Organic solvents	1.11 (0.77-1.61)	0.98 (0.33-2.87)	1.22 (0.59-2.53)
Exhaust fumes	0.72 (0.67-1.04)	1.42 (0.57-3.53)	0.95 (0.47-1.92)
Groups			
HMW sensitiser	0.81 (0.68-1.01)	1.18 (0.60-2.35)	0.71 (0.38-1.31)
Mites	0.79 (0.45-1.39)	2.20 (0.72-6.69)	0.81 (0.24-2.80)
Microbial exposure	1.02 (0.62-1.70)	**2.68 (1.06-7.50)**	1.47 (0.58-3.75)
LMW sensitiser	0.90 (0.70-1.16)	1.25 (0.65-2.41)	0.85 (0.49-1.46)
Irritants	**0.75 (0.70-0.93)**	1.23 (0.69-2.22)	1.00 (0.63-1.57)
Highly reactive chemicals	0.89 (0.69-1.16)	1.24 (0.64-2.41)	0.89 (0.51-1.54)
Biocides	0.82 (0.62-1.10)	1.04 (0.50-2.16)	0.85 (0.46-1.56)

Definitions of abbreviations: aMOR: adjusted multinomial odds ratio CI: confidence interval; HMW: high molecular weight; LMW: low molecular weight

* P-values less than 0.05 are bolded

†Reference category: No atopy nor eczema

‡ Current occupational exposures: high/low exposure versus not exposed.

§Adjusted multinomial odds ratio: sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, pack-years and SEIFA deciles.

Table 8.4. Adjusted associations between cumulative occupational exposure per 5 years exposure equivalent and eczema by atopy groups at 53 years.

Eczema outcomes†	Atopy alone (1,045/2,147)	Non-atopic Eczema (70/2,147)	Atopic Eczema (129/2,147)
	aMOR(95%CI)‡§	aMOR(95%CI)‡§	aMOR(95%CI)‡§
Individual Agents			
Animals	0.96 (0.90-1.01)	0.99 (0.86-1.14)	1.04 (0.96-1.13)
Fish/shellfish	0.98 (0.91-1.05)	1.03 (0.90-1.18)	0.75 (0.48-1.16)
Flour	1.01 (0.94-1.08)	0.98 (0.80-1.21)	0.86 (0.61-1.23)
Foods	1.04 (0.91-1.18)	1.07 (0.78-1.46)	0.99 (0.73-1.33)
Plants-related dusts	0.95 (0.93-1.02)	1.02 (0.94-1.11)	1.00 (0.93-1.09)
House dust mites	0.98 (0.88-1.08)	1.02 (0.82-1.27)	0.89 (0.59-1.35)
Storage mites	0.94 (0.84-1.04)	1.02 (0.80-1.32)	1.05 (0.86-1.28)
Plant mites	0.97 (0.93-1.02)	1.02 (0.94-1.11)	1.00 (0.92-1.08)
Enzymes	1.05 (0.89-1.22)	1.11 (0.80-1.55)	1.00 (0.73-1.37)
Latex	1.00 (0.98-1.01)	1.01 (0.97-1.05)	0.98 (0.93-1.02)
Textiles	1.17 (0.88-1.56)	1.31 (0.79-2.17)	1.34 (0.90-1.99)
Mould	0.96 (0.89-1.02)	1.01 (0.89-1.02)	1.02 (0.89-1.16)
Endotoxin	0.98 (0.93-1.03)	0.96 (0.83-1.12)	1.04 (0.96-1.12)
Drugs	0.98 (0.81-1.17)	0.74 (0.23-2.34)	0.79 (0.41-1.53)
High-level chemical disinfectants	0.98 (0.95-1.02)	1.02 (0.94-1.10)	0.97 (0.88-1.06)
Aliphatic amines	0.98 (0.91-1.05)	1.09 (0.98-1.20)	1.00 (0.86-1.16)
Isocyanates	0.99 (0.91-1.07)	0.97 (0.73-1.29)	1.10 (0.98-1.23)
Acrylates	0.94 (0.82-1.08)	1.02 (0.71-1.45)	**1.21 (1.02-1.44)**
Epoxy resins	0.99 (0.97-1.01)	1.01 (0.95-1.07)	1.02 (0.99-1.06)
Persulfates/henna	0.99 (0.95-1.03)	0.75 (0.08-2.12)	1.00 (0.93-1.08)
Wood dust	0.98 (0.93-1.03)	1.06 (0.97-1.16)	0.92 (0.78-1.07)
Metal and metal fumes	1.00 (0.96-1.04)	0.97 (0.85-1.10)	1.00 (0.83-1.08)
Metalworking fluids	1.00 (0.93-1.08)	**1.11 (1.01-1.23)**	0.82 (0.02-4.90)
Herbicides	0.98 (0.95-1.01)	1.00 (0.93-1.07)	1.00 (0.95-1.06)
Insecticides	0.98 (0.95-1.00)	1.00 (0.93-1.06)	1.01 (0.96-1.06)
Fungicides	0.98 (0.96-1.00)	1.00 (0.93-1.06)	1.01 (0.96-1.06)
Indoor cleaning	0.97 (0.95-1.00)	1.01 (0.96-1.07)	0.97 (0.92-1.03)
Bleach	0.97 (0.93-1.00)	1.00 (0.93-1.08)	0.94 (0.86-1.03)
Organic solvents	1.00 (0.97-1.03)	0.97 (0.89-1.06)	1.01 (0.96-1.06)
Exhaust fumes	0.98 (0.96-1.00)	1.01 (0.97-1.05)	0.96 (0.92-1.00)
Groups			
HMW sensitiser	0.99 (0.98-1.01)	1.01 (0.95-1.01)	0.98 (0.95-1.01)
Mites	0.97 (0.94-1.01)	1.01 (0.93-1.10)	0.99 (0.91-1.07)
Microbial exposure	0.99 (0.95-1.03)	1.05 (0.98-1.13)	1.01 (0.93-1.09)
LMW sensitiser	0.99 (0.97-1.00)	1.01 (0.97-1.05)	1.00 (0.97-1.03)
Irritants	0.98 (0.96-1.00)	1.01 (0.98-1.04)	1.00 (0.97-1.01)
Highly reactive chemicals	0.99 (0.97-1.00)	1.00 (0.96-1.05)	1.00 (0.97-1.03)
Biocides	0.98 (0.96-1.00)	0.99 (0.95-1.04)	0.99 (0.95-1.03)

Definitions of abbreviations: aMOR: adjusted multinomial odds ratio CI: confidence interval; HMW: high molecular weight; LMW: low molecular weight

* P-values less than 0.05 are bolded

†Reference category: No atopy nor eczema

‡Adjusted multinomial odds ratio: sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, pack-years and SEIFA deciles.

§ Per 5-year increase

Discussion

This paper explored the associations between a wide range of occupational exposures and eczema in isolation, as determined by the internationally accepted ISAAC definition. [17] In this cohort of middle-aged Australian adults, we found that current occupational exposure to animals, storage mites, endotoxin and the microbial group of exposures were associated with increased odds of prevalent eczema. Likewise, cumulative exposure to mould was associated with increased odds of eczema prevalence. When considering eczema by atopy groups, current exposure to animals, storage mites and the microbial group was associated with increased odds of NAE. In contrast current exposure to acrylates and isocyanates was associated with increased odds of having AE. Similarly, cumulative exposure to acrylates was found to be associated with increased odds of AE only and metal working fluids associated with increased odds of NAE only.

Previous studies suggested that in-utero and early life exposures to animal antigens were associated with reduced risk of eczema[20-23]. Few studies have reported on the association between animal exposure in later life and eczema. Working with animals leads to skin exposure to animal antigens and also endotoxin. [24] While exposure to endotoxins (i.e. farm living) may reduce the risk of allergies in early life, [25,26] this relationship may actually be reversed in later life as endotoxins also promote inflammation. [27] Endotoxins are a potent pro-inflammatory, neutrophilic agent[27] which has been associated with non-atopic allergies and asthma. Inhalable grain dust (with storage mites) is associated with increased odds of eczema. [28] Mite extracts (including storage mites) contain substances that modulate the production of proinflammatory cytokines secreted by human epidermal keratinocytes and dermal fibroblasts which can make the skin more permeable to allergens and irritants, and therefore may influence the pathophysiology of eczema. [29] Hence, it is plausible that occupational exposures to animals, endotoxin and storage mites could increase eczema prevalence.

We observed that cumulative occupational exposure to mould was associated with increased risk of eczema. Moulds produce volatile organic compounds, spores and mycotoxins. [30] These by-products can increase the risk of eczema in children through the generation of reactive oxygen species (ROS) [31] and nonspecific inflammatory mechanisms. [32] The prevalence of current eczema was higher adults living in homes with visible mould and damp. [33] Hence, occupational exposure to mould can be a risk factor for eczema.

Interestingly, in our study, cumulative exposure to metalworking fluids was associated with NAE, rather than AE. Metalworking fluids contain additives such as detergents, anti-foam agents, bactericidal agents and even trace metals and microorganisms, [34] which can irritate the skin and

some components can also be skin "sensitizers". [35] Dermatitis was seen in many parts of the body that are directly exposed to metalworking fluids and or indirectly from contaminated clothes. [36]

Exposures to isocyanates and acrylates are potentially associated with eczema. Isocyanates are highly reactive, low molecular-weight chemicals and direct skin contact can cause both irritant and allergic reactions[37] The loss of skin barrier function brought on by short-term high isocyanate exposure or long-term low isocyanate exposure might result in systemic allergen sensitivity and increase the risk of eczema. [37] Likewise, acrylates have been primarily linked to contact dermatitis. [38] However, exposure has been known to promote atopic eczema flares due to their high sensitising potency. [39] The lack of association with latex exposure may in part explained by the replacement of latex by other less allergenic alternatives in Australia. [40] Finally, our results extend those of Shakik et al, who assessed the associations between occupational exposures and dermatitis (eczema and contact dermatitis) and reported that occupations with exposure to isocyanates, metal working fluids and acrylates increased the risk of both types of dermatitis. [7]

The pattern of results seen in our analysis suggests that the associations between occupational exposures and eczema is not simply due to concomitant asthma. We observed only moderate overlap between the two conditions (~25% of those with eczema had asthma, compared with 12% for those without eczema). Moreover, all of the associations seen between occupational exposures and eczema were still present when those with concomitant asthma were excluded in a sensitivity analysis, and doing so identified additional occupational exposures of interest (plant-related dusts, mould, insecticides and fungicides).

This study has some important strengths. Strengths include access to a large population-based prospective cohort, an internationally accepted definition of eczema that attempted to exclude pure contact dermatitis, with objective measures of atopy (SPT) and the availability of work-history calendars for the participants' entire working lives. Furthermore, we adopted an asthma-specific JEM to standardise all exposure measurements given the absence of an eczema-specific JEM.

However, there were also some potential weaknesses. Some cases of contact dermatitis could have affected a flexural location and potentially be misclassified as eczema. However, this is unlikely given that contact dermatitis is much more frequently found on the hands, face or eyelids. [2] JEM may be subject to non-differential misclassification of exposures from heterogeneity of exposure in each job or occupation. While the questions and definitions used were well validated, [41] a limitation is the reliance on self-reporting mainly for the cumulative exposure measurement. There were participants missing years in the lifetime work history calendar who were excluded from the

cumulative analyses, but this proportion was low (1.1%), and those excluded had similar rates of prevalent eczema.

Although loss to follow-up tended to be more pronounced from the less advantaged participants, differential loss to follow-up generally does not lead to selection bias in the measurement of exposure-outcome associations.[42] We have attempted to address the "healthy hire" effect (participants with early life eczema avoiding occupations with specific exposures[43]) by adjusting for childhood eczema. We performed multiple comparisons, based on hypotheses that were biologically plausible. Our conclusions were based on the pattern rather than any specific association. However, as we did not adjustment for multiple comparison, there is some risk that some associations were spurious. It is possible that the association estimates are impacted by residual confounding factors such as household exposures. Few participants had experienced some occupational exposures, so the corresponding association estimates were imprecise and clear conclusions could not be drawn. Finally, given almost all the participants were Anglo-Celtic, findings cannot necessarily be generalizable to other ethnicities.

Conclusion

In this comprehensive explorative analysis that used a JEM to define occupational exposures, several agents were found to be associated with eczema in middle aged adults. Our study provided evidence that current exposures to animals, storage mites and endotoxin and exposure to metalworking fluids were related to prevalent eczema that was predominantly non-atopic in nature. Whereas chemical exposure to isocyanates and acrylates was associated with atopic eczema. Raising awareness and limiting occupational exposures during an individual's productive working life is likely to have a range of health benefits including the reduction of eczema prevalence. Further research involving the development of a JEM for eczema/dermatitis-specific exposures to advance research into occupational risk factors this disease, and potentially other skin conditions is needed.

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8.3. Supplementary material

Table S8.1. Lost to follow-up due to missing lifetime work history calendar data.

Characteristic	Lost (N= 686)	Not lost (N=2,923)	Odds to be lost
	% (n/N)	% (n/N)	OR (95%CI)
Age (years). Mean (SD); min-max	53 (1.09) 51-55	53 (0.92) 51-55	1.09 (0.99-1.19)
Female. % (n/N)	51.0% (340/667)	51.1% (1,494/2,923)	0.98 (0.83-1.16)
Years of education. %(n/N)			
< Grade 12	37.8% (239/632)	33.0% (964/2,923)	Ref
Grade 12 or equivalent	7.6% (48/632)	7.7% (226/2,923)	0.87 (0.62-1.21)
> Grade 12	54.6% (345/632)	59.3% (1,733/2,923)	**0.80 (0.67-0.96)***
Childhood eczema. % (n/N)	7.6% (45/589)	6.9% (201/2,923)	1.11 (0.80-1.55)
Childhood allergies. % (n/N)	26.6% (154/578)	28.7% (839/2,923)	0.90 (0.73-1.09)
Parental allergy history. % (n/N)	26.8% (141/526)	27.7% (809/2,923)	0.96 (0.78-1.18)
Smoking status. %(n/N)			
Never	35.2% (234/664)	43.3% (1,236/2,923)	Ref
Former	43.8% (291/664)	41.1% (1,202/2,923)	**1.27 (1.05-1.53)**
Current	20.9% (139/664)	16.6% (485/2,923)	**1.53 (1.22-1.93)**
Cigarette packs a year. Mean (SD); min-max	12.2 (16.5) 0-76	9.65 (15.3) 0-119	**1.01 (1.01-1.02)**
SEIFA deciles. % (n/N)			
1			
2	13.2% (80/606)	9.9% (289/2,923)	Ref
3	17.5% (106/606)	17.8% (520/2,923)	0.73 (0.53-0.99)
4	5.8% (35/606)	8.9% (261/2,923)	**0.47 (0.31-0.73)**
5	8.6% (52/606)	8.5% (249/2,923)	0.76 (0.52-1.12)
6	14.5% (88/606)	15.7% (459/2,923)	**0.70 (0.50-0.97)**
7	7.8% (47/606)	6.9% (202/2,923)	0.84 (0.56-1.25)
8	5.3% (32/606)	5.3% (156/2,923)	0.81 (0.53-1.26)
9	10.9% (66/606)	10.7% (314/2,923)	0.76 (0.53-1.09)
10	9.08% (55/606)	9.0% (262/2,923)	0.75 (0.52-1.10)
Prevalent eczema	7.43% (45/606)	7.2% (211/2,923)	0.79 (0.53-1.17)
	7.5% (50/667)	9.1% (267/2,923)	1.02 (0.78-1.33)

SEIFA: Socio-Economic Indexes for Areas SD: standard deviation

*P-value less than 0.05 is bolded

Table S8.2. Lost to follow-up due to missing skin prick test data in the cumulative analysis sample.

Characteristic	Lost (N= 893)	Not lost (N=2,327)	Odds to be lost
	% (n/N)	% (n/N)	OR (95%CI)
Age (years). Mean (SD); min-max	53 (0.95) 51-55	53 (0.91) 51-55	1.09 (0.99-1.19)
Female. % (n/N)	53.2% (468/880)	50.8% (1,182/2327)	1.09 (0.94-1.28)
Years of education. %(n/N)			
< Grade 12	38.5% (326/846)	31.5% (732/2,327)	Ref
Grade 12 or equivalent	9.2% (78/846)	7.1% (166/2,327)	1.06 (0.78-1.42)
> Grade 12	52.3% (442/846)	61.4% (1,429/2,327)	**0.70 (0.59-0.82)***
Childhood eczema. % (n/N)	6.4% (52/757)	7.1% (166/2,327)	0.90 (0.65-1.24)
Childhood allergies. % (n/N)	32.6% (261/800)	27.3% (636/2,327)	**1.28 (1.08-1.52)**
Parental allergy history. % (n/N)	28.1% (211/752)	27.5% (639/2,327)	1.03 (0.86-1.23)
Smoking status. %(n/N)			
Never	37.6% (330/877)	43.2% (1,004/2,327)	Ref
Former	41.9% (367/877)	41.6% (968/2,327)	1.15 (0.96-1.36)
Current	20.5% (180/877)	15.3% (355/2,327)	**1.55 (1.25-1.93)**
Cigarette packs a year. Mean (SD); min-max	11.6 (16.5) 0-92.5	9.02 (14.7) 0-119	**1.01 (1.01-1.02)**
SEIFA deciles. % (n/N)			
1	14.0 %	8.5% (197/2,327)	Ref
2	(115/823)	17.5% (406/2,327)	0.68 (0.51-0.91)
3	19.8% (163/823)	8.9% (206/2,327)	0.57 (0.40-0.82)
4	8.5 % (70/823)	8.8% (205/2,327)	0.53 (0.37-0.77)
5	7.7% (63/823)	15.8% (368/2,327)	0.60 (0.45-0.82)
6	15.7% (129/823)	6.8% (159/2,327)	0.64 (0.44-0.92)
7	7.3% (60/823)	5.3% (124/2,327)	0.58 (0.39-0.88)
8	5.1% (42/823)	11.04% (257/2,327)	0.56 (0.40-0.78)
9	10.2% (84/823)	9.7% (226/2,327)	0.39 (0.27-0.58)
10	6.3% (52/823)	7.7% (179/2,327)	0.43 (0.29-0.64)
Prevalent eczema	5.5% (45/823)		
	9.2% (81/799)	9.1% (211/2,328)	1.02 (0.78-1.33)

SEIFA: Socio-Economic Indexes for Areas SD: standard deviation

*P-value less than 0.05 is bolded

Table S8.3.1. Descriptive statistics of current occupational exposures by prevalent eczema status.

	No eczema (2,118/2,329)	Prevalent eczema (211/2,329)
Current occupational exposures	% (n/N)	% (n/N)
Individual agents		
Animals		
1: Low	1.37% (29/2,118)	2.37% (5/211)
2: High	0.28% (6/2,118)	1.90% (4/211)
Fish/shellfish		
1: Low	1.18% (25/2,118)	1.42% (3/211)
2: High	0.14% (3/2,118)	0.47% (1/211)
Flour		
1: Low	0.47% (10/2,118)	0.95% (2/211)
2: High	0.33% (7/2,118)	-
Food		
1: Low	0.85% (18/2,118)	0.95% (2/211)
2: High	0.09% (2/2,118)	-
Plants-related dusts		
1: Low	1.46% (31/2,118)	3.32% (7/211)
2: High	0.71% (15/2,118)	0.47% (1/211)
House dust mites		
1: Low	0.85% (18/2,118)	0.47% (1/211)
2: High	0.24% (5/2,118)	-
Storage mites		
1: Low	1.75% (37/2,118)	4.27% (9/211)
2: High	-	-
Plant mites		
1: Low	1.42% (30/2,118)	2.84% (3/211)
2: High	0.71% (15/2,118)	0.47% (1/211)
Enzymes		
1: Low	0.90% (19/2,118)	1.42% (3/211)
2: High	-	-
Latex		
1: Low	4.49% (95/2,118)	3.79% (8/211)
2: High	4.91% (104/2,118)	4.27% (9/211)
Textiles		
1: Low	0.19% (4/2,118)	0.47% (1/211)
2: High	-	-
Moulds		
1: Low	4.11% (87/2,118)	6.64% (14/211)
2: High	-	-
Endotoxin		
1: Low	3.21% (68/2,118)	5.21% (11/211)
2: High	0.24% (5/2,118)	0.95% (2/211)
Drugs		
1: Low	0.42% (9/2,118)	0.47% (1/211)
2: High	0.09% (2/2,118)	-
High-level chemical disinfectants		
1: Low	10.81% (229/2,118)	11.85% (25/211)
2: High	0.38% (8/2,118)	0.95% (2/211)
Aliphatic amines		
1: Low	0.76% (16/2,118)	1.42% (3/211)
2: High	0.05% (1/2,118)	0.47% (1/211)
Isocyanates		
1: Low	0.80% (17/2,118)	1.90% (4/211)
2: High	0.14% (3/2,118)	-
Acrylates		
1: Low	0.99% (21/2,118)	1.90% (4/211)
2: High	-	-
Epoxy resins		
1: Low	0.19% (4/2,118)	0.95% (2/211)
2: High	1.56% (33/2,118)	1.90% (4/211)
Persulfates/henna		
1: Low	-	-
2: High	0.42% (9/2,118)	0.47% (1/211)
Wood dust		
1: Low	1.65% (35/2,118)	2.37% (5/211)
2: High	0.42% (6/2,118)	-

Metal and metal fumes		
1: Low	5.15% (109/2,118)	4.27% (9/211)
2: High	0.61% (13/2,118)	0.47% (1/211)
Metal working fluids		
1: Low	0.28% (6/2,118)	0.47% (1/211)
2: High	0.05% (1/2,118)	-
Herbicides		
1: Low	2.22% (47/2,118)	2.37% (5/211)
2: High	1.27% (27/2,118)	1.90% (4/211)
Insecticides		
1: Low	1.46% (31/2,118)	2.84% (6/211)
2: High	1.89% (40/2,118)	2.37% (5/211)
Fungicides		
1: Low	1.46% (31/2,118)	2.84% (6/211)
2: High	1.89% (40/2,118)	2.37% (5/211)
Indoor cleaning		
1: Low	9.92% (210/2,118)	9.00% (19/211)
2: High	4.44% (94/2,118)	4.74% (10/211)
Bleach		
1: Low	7.70% (163/2,118)	7.11% (15/211)
2: High	2.50% (53/2,118)	2.84% (6/211)
Organic solvents		
1: Low	8.31% (176/2,118)	7.58% (16/211)
2: High	0.85% (18/2,118)	1.42% (3/211)
Exhaust fumes		
1: Low	6.94% (147/2,118)	8.53% (18/211)
2: High	3.68% (78/2,118)	4.27% (9/211)
Groups		
HMW sensitizer		
1: Low	9.30% (197/2,118)	10.90% (23/211)
2: High	7.18% (152/2,118)	7.11% (6/211)
Mites		
1: Low	2.97% (63/2,118)	5.21% (11/211)
2: High	0.94% (20/2,118)	0.47% (1/211)
Microbial exposure		
1: Low	4.39% (93/2,118)	6.64% (14/211)
2: High	0.28% (6/2,118)	1.42% (3/211)
LMW sensitizer		
1: Low	16.67% (353/2,118)	16.59% (35/211)
2: High	3.54% (75/2,118)	4.74% (10/211)
Irritants		
1: Low	21.15% (448/2,118)	22.27% (47/211)
2: High	13.13% (278/2,118)	15.64% (33/211)
Highly reactive chemicals		
1: Low	13.98% (296/2,118)	13.27% (28/211)
2: High	5.19% (110/2,118)	7.58% (16/211)
Biocides		
1: Low	10.81% (229/2,118)	11.85% (25/211)
2: High	4.86% (103/2,118)	6.16% (13/211)

Table S8.3.2. Current occupational exposures frequency Heatmap (Red=more frequent Green=Less frequent)

Individual agents
Indoor cleaning
Exhaust fumes
Latex
Bleach
High-level chemical disinfectants
Organic solvents
Metal and metal fumes
Insecticides
Fungicides
Herbicides
Moulds
Epoxy resins
Endotoxin
Plants-related dusts
Plant mites
Animals
Wood dust
Storage mites
Fish/shellfish
House dust mites
Isocyanates
Flour
Persulfates/henna
Acrylates
Foods
Aliphatic amines
Enzymes
Drugs
Metal working fluids
Textiles
Groups
Irritants
Highly reactive chemicals
LMW sensitiser
HMW sensitiser
Biocides
Microbial exposure
Mites
Reference
10%
5%
1%

Table S8.4.1. Descriptive statistics of cumulative occupational exposures per 5-year exposure equivalent by prevalent eczema status.

	No eczema (2,667/2,934)	Prevalent eczema (267/2,934)
Cumulative exposure (Per 5-year equivalent)	Mean (SD) Min-Max	Mean (SD) Min-Max
Individual agents		
Animals	0.30 (1.58) 0-30.4	0.40 (1.73) 0-16
Fish/shellfish	0.19 (1.30) 0-32	0.26 (2.11) 0-32
Flour	0.17 (1.37) 0-29.6	0.10 (0.60) 0-5.6
Foods	0.12 (0.80) 0-16.8	0.10 (0.56) 0-5.4
Plants-related dusts	0.43 (2.13) 0-32	0.52 (2.13) 0-23.2
House dust mites	0.11 (0.88) 0-25.6	0.08 (0.58) 0-8
Storage mites	0.22 (0.88) 0-8	0.27 (1.04) 0-8.4
Plant mites	0.43 (2.12) 0-32	0.50 (2.12) 0-23.2
Enzymes	0.10 (0.64) 0-9.2	0.11 (0.59) 0-5.4
Latex	1.51 (5.66) 0-56	1.41 (5.24) 0-29.6
Textiles	0.05 (0.52) 0-16.8	0.06 (0.51) 0-6.8
Moulds	0.42 (1.34) 0-14	0.53 (1.52) 0-8.4
Endotoxin	0.46 (1.85) 0-30.4	0.60 (2.06) 0-16
Drugs	0.04 (0.45) 0-8.8	0.02 (0.18) 0-2.2
High-level chemical disinfectants	0.88 (2.57) 0-32.8	0.88 (2.16) 0-16
Aliphatic amines	0.15 (1.27) 0-24.8	0.21 (1.97) 0-29.6
Isocyanates	0.15 (1.18) 0-23.2	0.19 (1.036) 0-9
Acrylates	0.10 (0.73) 0-8.8	0.18 (1.02) 0-9
Epoxy resins	0.75 (3.79) 0-35.2	1.00 (4.71) 0-36
Persulfates/henna	0.21 (2.20) 0-40	0.15 (2.14) 0-34.4
Wood dust	0.38 (1.85) 0-28.8	0.36 (1.79) 0-17.8
Metal and metal fumes	0.68 (2.31) 0-29.6	0.60 (2.39) 0-29.6
Metal working fluids	0.14 (1.26) 0-24.8	0.13 (1.83) 0-29.6
Herbicides	0.91 (3.33) 0-32	1.01 (3.85) 0-33.6
Insecticides	1.01 (3.70) 0-33	1.16 (4.32) 0-33.6
Fungicides	1.01 (3.70) 0-33	1.16 (4.32) 0-33.6
Indoor cleaning	1.84 (4.2) 0-42.4	1.87 (3.79) 0-23
Bleach	0.98 (2.89) 0-33.6	0.90 (2.50) 0-16.8
Organic solvents	1.30 (3.50) 0-40	1.13 (3.58) 0-35.2
Exhaust fumes	2.12 (5.57) 0-40	2.00 (5.54) 0-33.6
Groups		
HMW sensitiser	2.88 (6.56) 0-56	2.33 (6.01) 0-20
Mites	0.60 (2.35) 0-32	0.64 (2.25) 0-23.2
Microbial exposure	0.71 (2.35) 0-30.4	0.88 (2.83) 0-29.6
LMW sensitiser	2.81 (5.65) 0-40	2.86 (6.18) 0-36
Irritants	6.63 (8.69) 0-42.4	6.76 (8.98) 0-36.4
Highly reactive chemicals	2.94 (5.84) 0-40	3.01 (6.40) 0-36
Biocides	2.34 (5.12) 0-40.8	2.42 (5.16) 0-33.6

Table S8.4.2. Cumulative occupational exposures frequency Heatmap (Red=more frequent Green=Less frequent)

Individual agents	
Exhaust fumes	
Indoor cleaning	
Latex	
Organic solvents	
Insecticides	
Fungicides	
Bleach	
Herbicides	
High-level chemical disinfectants	
Epoxy resins	
Metal and metal fumes	
Endotoxin	
Plants-related dusts	
Plant mites	
Moulds	
Wood dust	
Animals	
Storage mites	
Persulfates/henna	
Fish/shellfish	
Flour	
Aliphatic amines	
Isocyanates	
Metal working fluids	
Foods	
Acrylates	
House dust mites	
Enzymes	
Textiles	
Drugs	
Groups	
Irritants	
Highly reactive chemicals	
HMW sensitiser	
LMW sensitiser	
Mites	
Biocides	
Microbial exposure	

Table S8.5.1. Adjusted associations between current occupational exposure to individual agents and groups and prevalent eczema at 53 years using a universal un-exposure reference category for exposures.

Occupational exposure	Current exposure aOR (95%CI) †
Eczema outcome	Prevalent eczema ‡ (211/2,327)
Individual agents	
Animals	**3.10 (1.42-6.77)**
Fish/shellfish	1.68 (0.67-5.00)
Flour	1.28 (0.28-5.77)
Foods	1.15 (0.26-5.11)
Plants-related dusts	2.11 (0.94-4.74)
House dust mites	0.48 (0.08-3.62)
Storage mites	**3.08 (1.41-6.72)**
Plant mites	1.86 (0.79-4.37)
Enzymes	1.77 (0.50-6.22)
Latex	0.84 (0.48-1.45)
Textiles	2.76 (0.30-25.7)
Moulds	**1.99 (1.07-3.73)**
Endotoxin	**2.23 (1.17-4.27)**
Drugs	0.94 (0.11-7.42)
High-level chemical disinfectants	1.16 (0.74-1.83)
Aliphatic amines	2.24 (0.71-7.02)
Isocyanates	2.18 (0.68-6.97)
Acrylates	2.04 (0.65-6.43)
Epoxy resins	2.03 (0.79-5.18)
Persulfates/henna	1.00 (0.12-8.19)
Wood dust	1.32 (0.49-3.56)
Metal and metal fumes	0.98 (0.48-2.00)
Metal working fluids	1.42 (0.15-12.7)
Herbicides	1.50 (0.71-3.16)
Insecticides	1.96 (0.99-3.91)
Fungicides	1.93 (0.97-3.85)
Indoor cleaning	1.00 (0.64-1.55)
Bleach	0.98 (0.59-1.63)
Organic solvents	1.09 (0.64-1.84)
Exhaust fumes	1.46 (0.89-2.37)
Groups	
HMW sensitiser	1.12 (0.76-1.65)
Mites	1.67 (0.87-3.21)
Microbial exposure	**2.05 (1.15-3.65)**
LMW sensitiser	1.08 (0.74-1.56)
Irritants	1.18 (0.87-1.60)
Highly reactive chemicals	1.13 (0.78-1.64)
Biocides	1.18 (0.80-1.75)

aOR: adjusted odds ratio 95%CI: 95% confidence interval HMW: high molecular weight LMW: low molecular weight

*P-value less than 0.05 is bolded

†Adjusted for: age, sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, packs a year and SEIFA deciles at 53 years.

‡ Universal reference category for exposures: participants with no occupational exposures

Table S8.5.2. Adjusted associations between current occupational exposure individual agents and groups and eczema by atopy groups at 53 years using a universal un-exposure reference category for exposures.

Eczema outcomes	No atopy nor eczema† (739/1,755)	Atopy alone (859/1,755)	Non-atopic Eczema (54/1,755)	Atopic Eczema (103/1,755)
		aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI)‡
Individual Agent				
Animals	Ref group	0.83 (0.33-2.04)	**6.25 (1.54-25.4)**	1.73 (0.31-8.45)
Fish/shellfish	Ref group	0.75 (0.32-1.74)	2.49 (0.48-12.8)	0.65 (0.09-5.23)
Flour	Ref group	0.72 (0.23-2.20)	1.58 (0.17-14.9)	1.03 (0.11-8.88)
Foods	Ref group	0.63 (0.21-1.88)	1.46 (0.16-13.6)	0.94 (0.11-7.92)
Plants-related dusts	Ref group	0.84 (0.40-1.71)	2.45 (0.50-11.9)	1.55 (0.41-5.87)
House dust mites	Ref group	0.48 (0.14-3.04)	-	-
Storage mites	Ref group	0.95 (0.39-2.31)	**6.41 (1.57-26.1)**	1.86 (0.38-9.08)
Plant mites	Ref group	0.76 (0.36-1.60)	2.47 (0.50-12.1)	0.96 (0.20-4.57)
Enzymes	Ref group	0.72 (0.26-2.04)	1.52 (0.17-13.9)	1.90 (0.38-9.56)
Latex	Ref group	0.80 (0.64-0.99)	0.72 (0.26-2.00)	0.60 (0.26-1.41)
Textiles	Ref group	-	-	-
Moulds	Ref group	0.86 (0.49-1.49)	2.39 (0.64-9.01)	1.49 (0.52-4.23)
Endotoxin	Ref group	0.82 (0.45-1.49)	1.66 (0.36-7.75)	1.65 (0.58-4.72)
Drugs	Ref group	0.92 (0.27-3.10)	2.87 (0.31-26.9)	-
High-level chemical disinfectants	Ref group	0.75 (0.54-1.05)	1.08 (0.46-2.52)	0.84 (0.40-1.75)
Aliphatic amines	Ref group	1.02 (0.30-3.42)	4.52 (0.72-28.4)	2.96 (0.53-16.6)
Isocyanates	Ref group	1.24 (0.39-3.91)	2.91 (0.24-35.9)	4.02 (0.83-19.8)
Acrylates	Ref group	2.34 (0.62-8.88)	4.62 (0.34-62.1)	**6.76 (1.19-38.5)**
Epoxy resins	Ref group	0.84 (0.38-1.85)	3.44 (0.59-20.0)	2.21 (0.64-7.65)
Persulfates/henna	Ref group	0.44 (0.08-2.45)	-	2.04 (0.21-20.3)
Wood dust	Ref group	0.91 (0.45-1.84)	**5.09 (1.17-22.1)**	0.45 (0.06-3.58)
Metal and metal fumes	Ref group	1.00 (0.63-1.59)	1.18 (0.24-5.72)	1.12 (0.43-2.92)
Metal working fluids	Ref group	1.86 (0.35-10.0)	11.7 (0.84-62.2)	-
Herbicides	Ref group	0.88 (0.49-1.59)	0.84 (0.12-6.78)	1.37 (0.43-4.33)
Insecticides	Ref group	0.81 (0.44-1.49)	0.89 (0.11-7.14)	1.75 (0.61-5.06)
Fungicides	Ref group	0.84 (0.45-1.53)	0.88 (0.11-7.08)	1.72 (0.59-4.95)
Indoor cleaning	Ref group	**0.77 (0.47-0.89)**	0.73 (0.31-1.76)	0.88 (0.45-1.70)
Bleach	Ref group	0.71 (0.50-1.01)	0.72 (0.26-1.98)	0.74 (0.33-1.66)
Organic solvents	Ref group	1.00 (0.69-1.46)	1.15 (0.37-3.55)	1.15 (0.37-3.55)
Exhaust fumes	Ref group	**0.73 (0.46-0.96)**	1.55 (0.59-4.10)	1.02 (0.49-2.13)
Group				
HMW sensitiser	Ref group	0.72 (0.54-1.01)	1.22 (0.60-2.50)	0.74 (0.40-1.38)
Mites	Ref group	0.71 (0.40-1.26)	2.45 (0.77-7.77)	0.85 (0.24-2.98)
Microbial exposure	Ref group	0.91 (0.62-1.53)	**3.49 (1.16-10.4)**	1.56 (0.59-4.09)
LMW sensitiser	Ref group	0.84 (0.65-1.09)	1.26 (0.64-2.50)	0.86 (0.49-1.51)
Irritants	Ref group	**0.75 (0.60-0.94)**	1.21 (0.68-2.19)	0.98 (0.62-1.54)
Highly reactive chemicals	Ref group	0.84 (0.64-1.09)	1.31(0.65-2.63)	0.89 (0.50-1.57)
Biocides	Ref group	0.77 (0.58-1.04)	1.09 (0.51-2.33)	0.88 (0.47-1.66)

aMOR: adjusted multinomial odds ratio 95%CI: 95% confidence interval

* P-values less than 0.05 are bolded

†Reference category: No atopy nor eczema
‡Adjusted multinomial odds ratio: age, sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, packs a year and SEIFA deciles.
§ Universal reference category for exposures: participants with no occupational exposures

Table S8.6.1. Adjusted associations between current occupational exposure individual agents and groups and asthma and eczema groups at 53 years.

Eczema outcomes	No eczema nor asthma† (2,002/2,555)	Asthma only (325/2,555)	Eczema only (171/2,555)	Eczema and Asthma (57/2,555)
		aMOR (95%CI) ‡	aMOR (95%CI) ‡	aMOR (95%CI)‡
Individual Agent				
Animals	Ref group	0.65 (0.20-2.18)	**3.22 (1.43-7.25)**	1.28 (0.17-10.0)
Fish/shellfish	Ref group	0.47 (0.11-2.04)	0.93 (0.22-3.98)	3.19 (0.65-15.6)
Flour	Ref group	1.77 (0.56-5.67)	0.92 (0.12-7.13)	2.30 (0.25-21.1)
Foods	Ref group	1.54 (0.50-4.79)	0.75 (0.10-5.72)	2.09 (0.23-18.9)
Plants-related dusts	Ref group	1.66 (0.77-3.55)	**2.36 (1.01-5.52)**	0.92 (0.12-7.07)
House dust mites	Ref group	-	-	1.75 (0.22-14.3)
Storage mites	Ref group	1.74 (0.74-4.07)	**3.64 (1.61-8.26)**	1.39 (0.18-10.8)
Plant mites	Ref group	1.68 (0.78-3.60)	**2.42 (1.03-5.67)**	-
Enzymes	Ref group	1.57 (0.50-4.91)	0.79 (0.10-6.03)	4.81 (0.94-24.7)
Latex	Ref group	**1.80 (1.20-2.69)**	0.76 (0.40-1.43)	1.27 (0.46-3.45)
Textiles	Ref group	-	2.61 (0.28-24.1)	-
Moulds	Ref group	1.38 (0.77-2.49)	**2.31 (1.23-4.37)**	0.47 (0.06-3.56)
Endotoxin	Ref group	1.13 (0.58-2.20)	**2.27 (1.15-4.49)**	1.24 (0.29-5.38)
Drugs	Ref group	3.52 (0.99-12.5)	-	8.00 (0.92-69.3)
High-level chemical disinfectants	Ref group	**1.54 (1.06-2.50)**	1.00 (0.59-1.69)	1.97 (0.89-4.37)
Aliphatic amines	Ref group	0.40 (0.05-3.10)	2.48 (0.80-7.73)	-
Isocyanates	Ref group	0.71 (0.16-3.15)	2.56 (0.83-7.90)	-
Acrylates	Ref group	0.69 (0.16-3.03)	2.37 (0.78-7.26)	-
Epoxy resins	Ref group	0.60 (0.18-2.00)	2.23 (0.89-5.55)	-
Persulfates/henna	Ref group	-	1.02 (0.12-8.31)	-
Wood dust	Ref group	1.32 (0.57-3.07)	0.92 (0.27-3.07)	1.70 (0.36-8.17)
Metal and metal fumes	Ref group	0.76 (0.40-1.43)	0.85 (0.40-1.82)	0.63 (0.14-2.84)
Metal working fluids	Ref group	-	1.24 (0.14-10.8)	-
Herbicides	Ref group	1.50 (0.81-2.77)	1.64 (0.76-3.56)	0.54 (0.07-4.11)
Insecticides	Ref group	1.18 (0.60-2.31)	**2.18 (1.07-4.42)**	0.63 (0.08-4.71)
Fungicides	Ref group	1.16 (0.59-2.27)	**2.13 (1.05-4.34)**	0.60 (0.08-4.52)
Indoor cleaning	Ref group	1.24 (0.86-1.78)	0.77 (0.46-1.29)	1.66 (0.76-3.62)
Bleach	Ref group	1.41 (0.95-2.11)	0.81 (0.45-1.46)	1.46 (0.61-3.52)
Organic solvents	Ref group	0.72 (0.44-1.17)	0.98 (0.55-1.72)	0.76 (0.26-2.21)
Exhaust fumes	Ref group	1.10 (0.72-1.70)	1.52 (0.91-2.53)	0.94 (0.34-2.59)
Group				
HMW sensitiser	Ref group	**1.46 (1.06-2.01)**	1.05 (0.67-1.62)	1.56 (0.76-3.17)
Mites	Ref group	1.26 (0.68-2.35)	1.78 (0.89-3.59)	1.05 (0.24-4.53)
Microbial exposure	Ref group	1.17 (0.65-2.09)	**2.22 (1.23-4.02)**	0.85 (0.20-3.69)
LMW sensitiser	Ref group	1.29 (0.94-1.75)	0.98 (0.65-1.48)	1.40 (0.71-2.76)
Irritants	Ref group	1.21 (0.92-1.59)	1.16 (0.82-1.64)	1.52 (0.83-2.79)
Highly reactive chemicals	Ref group	1.16 (0.84-1.59)	1.03(0.68-1.55)	1.40 (0.70-2.79)
Biocides	Ref group	**1.50 (1.08-2.08)**	1.12 (0.72-1.75)	1.63 (0.80-3.35)

aMOR: adjusted multinomial odds ratio 95%CI: 95% confidence interval

* P-values less than 0.05 are bolded

†Reference category: No eczema nor asthma

‡Adjusted multinomial odds ratio: age, sex, highest education status, childhood eczema, childhood allergies, parental history of allergies, smoking status, packs a year and SEIFA deciles.

Table S8.6.2. Percentage of participants by asthma eczema groups exposed to occupational exposures with fisher's exact test p-values.

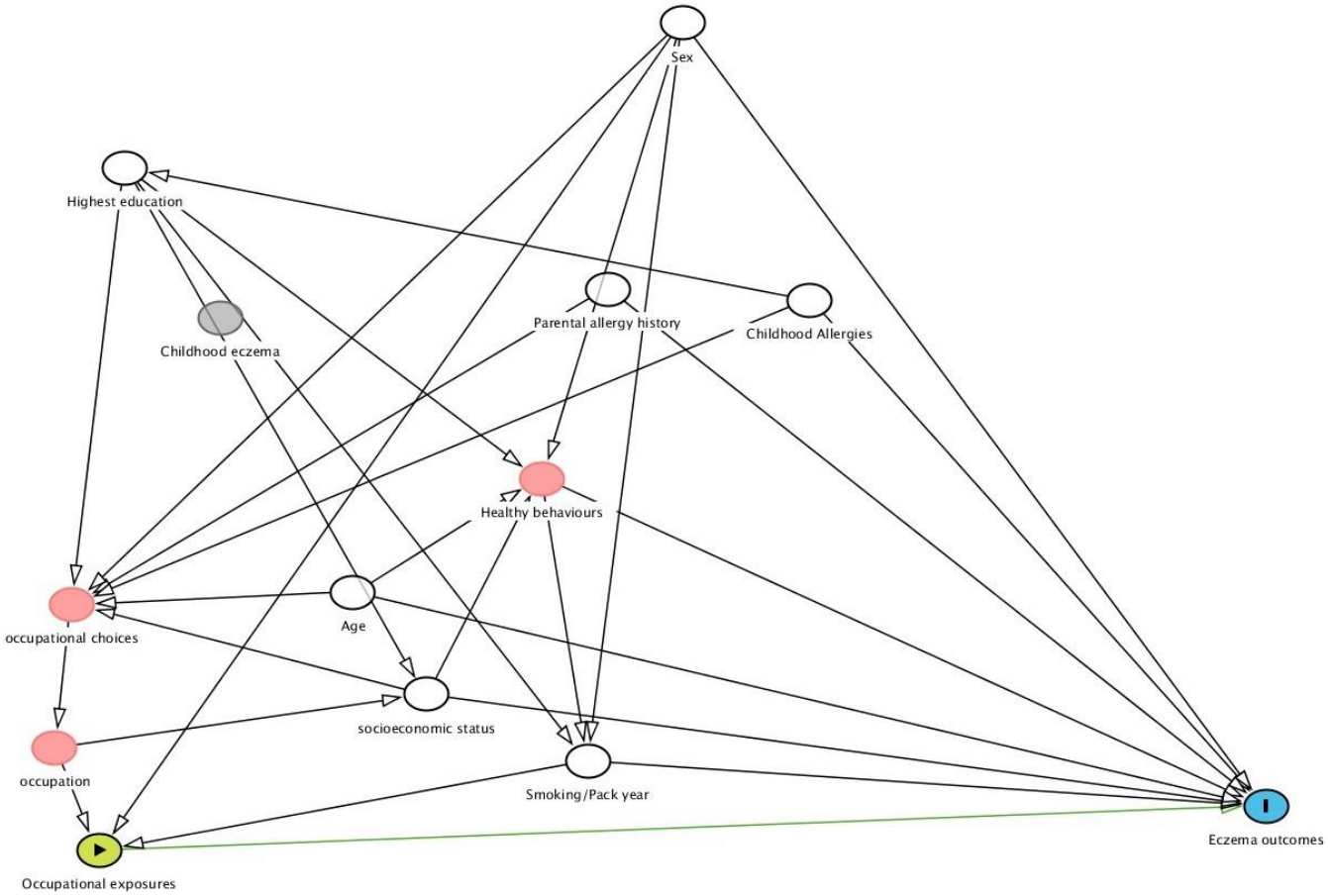
Eczema outcomes	No eczema nor asthma† (2,002/2,555)	Asthma only (325/2,555)	Eczema only (171/2,555)	Eczema and Asthma (57/2,555)
	% (n/N)	% (n/N) p-value‡	% (n/N) p-value‡	% (n/N) p-value‡
Individual Agent				
House dust mites	1.15% (23/2,002)	0% (0/325) 0.063	0% (0/171) 0.251	1.75% (1/57) 0.492
Plant mites	2.05% (41/2,002)	2.77% (9/325) 0.408	4.09% (7/171) 0.097	3.51% (2/57) 0.335
Textiles	0.20% (4/2,002)	0% (0/325) 1.00	0.58% (1/171) 0.336	0% (0/57) 1.00
Drugs	0.35% (7/2,002)	1.23% (4/325) 0.055	0% (0/171) 1.00	1.75% (1/57) 0.201
Aliphatic amines	0.95% (19/2,002)	0.92% (3/325) 1.00	2.34% (4/171) 0.101	0% (0/57) 1.00
Isocyanates	1.00% (20/2,002)	0.92% (3/325) 1.00	2.34% (4/171) 0.114	0% (0/57) 1.00
Acrylates	1.00% (20/2,002)	0.92% (3/325) 1.00	2.34% (4/171) 0.114	0% (0/57) 1.00
Epoxy resins	2.10% (42/2,002)	1.23% (4/325) 0.392	3.51% (6/171) 0.269	0% (0/57) 0.629
Persulfates/henna	0.55% (11/2,002)	0.31% (1/325) 1.00	0.58% (1/171) 1.00	0% (0/57) 1.00
Metal working fluids	0.40% (8/2,002)	0.31% (1/325) 1.00	1.17% (2/171) 0.183	0% (0/57) 1.00

* P-values less than 0.05 are bolded

†Reference category: No eczema nor asthma

‡Fisher's exact test

Figure S8.1. Direct Acyclic graph for the association between occupational exposures and eczema outcomes.



Chapter 9 - Implications, recommendations and conclusion

In this chapter, I first summarise the key findings of this doctorate thesis. I then outline my doctoral work's main methodological limitations and strengths and the generalizability of the results. Next, I describe the clinical and public health impact of my doctorate body of work and explain the potential future directions in this area of research. Finally, I conclude by summarising the findings drawn from my research.

9.1. Summary of key findings

In this section, I provide a summary of the key findings in relation to the aims and sub-aims of this thesis (Section 1.2.2.).

9.1.1. Aim 1: To determine the longitudinal eczema subclasses according to eczema prevalence in early life and childhood.

My longitudinal latent class analysis of eczema prevalence in the first 12 years of life used 24 data points. Unlike previous studies in this area, I analysed high allergic risk children with detailed data in the first two years of life. This number of data points is considerably more than in previous analyses. I determined five eczema subclasses, namely, “minimal eczema”, “early onset persistent”, “early onset resolving”, “mid onset persistent”, and “mid onset resolving” subclasses. Although my results were mostly consistent with the results from previous studies, [13-17] the eczema subclasses added to the literature by being the first analyses to provide detailed eczema trajectories of a high allergy-risk population that was measured 24 times.

Then, I assessed the associations between eczema subclasses and several early life risk factors using multinomial regression models with the “minimal eczema” subclass as the reference class. Participants with filaggrin null mutations were more likely to be a member of the persistent phenotypes (early onset persistent and mid onset persistent). The development of the skin barrier and the epidermis’ final differentiation is made easier by the protein filaggrin. Suppose there is a null mutation on the gene responsible for coding filaggrin. In that case, the epidermis loses processed functional filaggrin. [358] It is possible that breastfeeding delays the early presentation of eczema [359] in children who are at high risk of getting the condition since breastfeeding at four weeks was linked to mid onset persistent eczema (genetic predisposition as noted by a high rate of FLG null mutations) in my analyses. Furthermore, parental allergic history and being female were associated with increased odds of being a member of the mid onset persistent phenotype.

Similarly, I assessed the associations between the eczema phenotypes as predictors of allergic outcomes at 18 and 25 years using logistic regression models. The persistent classes were

associated with an increased risk of allergic comorbidities at 18 and 25 years. Conversely, members from the resolving phenotypes were not at a significantly higher risk of allergic outcomes than the minimal eczema phenotypes. In the literature, it is known that having infantile eczema can increase the risk of developing other allergies like asthma or allergic rhinitis. [203,360] My findings add to this area of research by showing that the persistent eczema subclasses were at higher risk of further allergic disease. I reported the findings related to Aim 1 in detail in the publication presented as Chapter 4.

9.1.2. Aim 2: To assess whether the exposure to air pollution associated with eczema and food allergies in the first ten years of life.

I examined whether air pollution was a risk factor for eczema and food allergy in the first ten years of life. My results suggested that air pollution exposure at 1 and 4 years was associated with increased odds of challenge-confirmed food allergy and peanut allergy during early childhood. Exposure to air pollution at one year of life increased the risk of persistent food and peanut allergies. To the best of my knowledge, this study was the first to assess air pollution as a risk factor for food allergy using an oral food challenge proven definition. [8] In contrast, there was no evidence of associations between air pollution and eczema during the same period. This finding adds to the existing literature, air pollution is unlikely to have a substantial impact on the development of eczema in children. Hence, my findings filled a knowledge gap in the literature and helped improve our understanding of potential environmental contributors to allergic disease. I reported the findings related to Aim 5 in depth in the manuscript draft presented as Chapter 5.

9.1.3. Aim 3: To examine whether exposure to air pollution is associated with the incidence and prevalence of eczema in middle-aged adults.

Using logistic and multinomial regression, I evaluated the potential associations of air pollution with eczema and allergic sensitisation in a group of middle-aged adults. I observed that higher levels of nitrogen dioxide at baseline (age 43) were associated with increased odds of prevalent eczema and atopic eczema at age 53, but only in males. Exposure to higher levels of particulate matter with a diameter of less than 2.5-micrometre was associated with increased odds of atopic eczema only in males and with aeroallergen sensitisation in both sexes. Only a few studies have previously examined this relationship, mostly in children, and the findings have not been consistent. Some studies reported no associations [21,22] while others reported positive associations between air pollution and eczema prevalence. [23,260,261] The results from my analyses helped to increase the understanding of this relationship in adults addressing one of the knowledge gaps in

this area of research. I reported the findings related to Aim 2 in detail in the publication presented as Chapter 6.

9.1.4 Aim 4: To determine the associations of domestic water hardness and eczema prevalence and incidence in a cohort of adults.

Using multilevel regression models, I assessed whether domestic water hardness was a risk factor for eczema prevalence in a cohort of adults. I found that the risk of eczema increased with increasing levels of calcium carbonate. Furthermore, those participants who lived in hard water areas with more than 200 mg per litre of calcium carbonate had an increased prevalence of eczema. When water hardness was assessed as a categorical variable, those living in locations with calcium carbonate concentrations higher than 180 mg had a higher risk of eczema prevalence. No association was observed with incident eczema or eczema at follow-up. These associations had not been previously assessed in adults. [361] As such, my findings are an essential knowledge contribution to the literature. Additionally, I found minimal variability in eczema outcomes between postcodes, and postcode-level deprivation indicators only partially explained this variance. I report the results related to Aim 3 in depth in the publication presented as Chapter 7.

9.1.5 Aim 5: To evaluate potential associations between occupational exposures and eczema prevalence in middle aged adults.

I investigated a wide range of occupational exposures, assessed using a job exposure matrix, and eczema outcomes in a population of middle-aged adults. This analysis was the first to assess this association using a job exposure matrix. I found that current occupational exposure to animals, storage mites, and endotoxin antigens were associated with increased prevalent eczema risk in adults. Similarly, I reported a positive association between cumulative or lifetime occupational exposure to mould antigens and the risk of eczema prevalence. Then, I tested whether the current or cumulative occupational exposure was associated with non-atopic or atopic eczema. My results suggested that current occupational exposure to animal and storage mites' antigens increased the odds of non-atopic eczema. In contrast, current exposure to isocyanate and acrylate antigens increased the odds of having atopic eczema. Moreover, cumulative occupational exposure to acrylate antigens increased the odds of atopic eczema, and metal working fluid exposure increased the odds of non-atopic eczema. The results from these analyses extend those reported before, which involved dermatitis as an outcome. [282] I stated the findings related to Aim 4 in depth in the publication presented as Chapter 8.

9.2. Methodological Strengths and Limitations

In this section, I consider the overall methodological strengths and weaknesses of my thesis. The specific strengths and limitations of each study were discussed in detail in the discussion section of each manuscript.

9.2.1. Methodological strengths

Overall, there were a number of methodological strengths in my thesis. First, I used data from multiple longitudinal cohort studies with strong epidemiological designs. These studies had multiple follow-ups, and a wide range of data including air pollution measured by land use regression models, work history calendars and the use of job exposure matrix categorizations, water hardness, health history, demographics and socioeconomic variables. Moreover, data on skin prick tests from multiple follow-ups were available in the TAHS, MACS and HealthNuts studies. These provided an objective measure of atopy within my analyses. Likewise, objective, gold standard, measures of food allergy measured by criterion standard oral food challenges were available in the HealthNuts study. Finally, I used various robust and relatively novel statistical methods for this area of research to address my research questions, including longitudinal latent class analysis, likelihood ratio tests, logistic and multinomial regression, geographic information system and multilevel logistic regression.

9.2.2. Methodological limitations

The following are methodological limitations within this body of work. One common issue with longitudinal cohort studies is the loss to follow-up over time. Ideally, long-term cohort studies should maximise follow-up to minimise attrition bias. In practice, this is challenging to achieve in long-term population-based studies. Attrition bias result from differences between participants at baseline and participants at follow-ups. Specifically, when data gathered in follow-ups are not missing at random and arise because the likelihood of loss to follow-up varies according to the outcome of interest or the level the exposure, then this may lead to biased estimates of longitudinal relationships. [362] If participants are randomly missing, the likelihood that a participant is lost to follow-up does not depend on the outcome or exposure of interest. In this situation, it is assumed that the remaining participants who are followed up are a randomly selected subsample of the initial study population. [363] The statistical power to identify specific relationships is, however, diminished when the loss to follow-up is wholly random, mainly when the cohort sample size is small. [363] In all the cohort studies included in this thesis, a significant number of participants were lost to follow-up (range: 32.3%-52.8%), and there were differences between those followed and those lost to follow-up. The participants lost to follow-up were from lower socioeconomic classes and had lower education levels, a common finding from other cohort studies. However, this differential loss to

follow-up does not usually lead to attrition bias as participants were missing at random. [364] Hypothetical scenarios have demonstrated that a loss-to-follow-up rate of up to 60% for missing at random is unlikely to produce considerable bias. [362] Therefore, it is not likely that the discrepancies between the lost and followed-up participants will introduce substantive bias.

Another limitation relates to the fact that the findings from this thesis are based on observational data. It is possible that some of the reported association estimates were confounded by factors that weren't measured in each of the included cohort studies. To reduce this possibility, I created direct acyclic graphs for each outcome exposure association assessed during this thesis to identify the relevant confounders. If there was no data about an identified confounder, I used a proxy measure (i.e. Socio-Economic Indexes for Areas instead of socioeconomic status). However, I acknowledge that my understanding of these associations was incomplete as there may be confounding pathways that I could not conceive, so residual confounding was possible.

The studies included in this thesis mostly relied on parental and participant reports of eczema and other allergic conditions. It is possible that some participants could not correctly recall the information regarding their allergic disease status, which is also known as recall error. Hence, there may have been some non-differential misclassification bias in outcome classification. To minimise this possibility, the surveys used to collect these data used detailed questionnaire methods. [365] The researchers from each cohort study used validated definitions (group of questions) that were related to the symptoms of eczema and another allergic disease rather than a single question about eczema or another allergic disease status. Also, the frequent evaluation of the allergic outcomes and the use of 12-month period prevalence measures rather than ever prevalence measures provided advantages in reducing recall bias in all the considered cohorts. In the UK biobank, each reported disease was confirmed in a nurse-led interview. [317] While I cannot exclude the potential for recall error, it would be minimised by the mentioned methods.

Finally, the external interpretation of my findings relies on the participants' representativeness based on demographic characteristics. The MACS is a high allergic disease risk cohort because only newborns with at least one immediate family member with a history of allergic disease were recruited. Moreover, the study samples included in my thesis primarily comprised participants from Caucasian and Anglo-Celtic backgrounds. Hence, the findings from this thesis should be generalised with caution to other populations.

9.3. Summary of the implications

I have previously stated the implications in detail regarding each study included in this thesis in Chapters 4, 5, 6, 7 and 8. Below I organised the summarised implications for public health research and clinicians.

9.3.1. Public health research

I advanced the research in this area, assessing eczema phenotypes and environmental risk factors with a focus on adults. First, I identified five distinct eczema subclasses differentially associated with risk factors and allergic comorbidities, which showed that eczema is a heterogeneous disease. As such, this subclassification may be helpful for researchers in this area. Second, I determined that increased air pollution exposure was associated with increased odds of eczema in males and aeroallergen sensitisation in both sexes in adults. As there were conflicting results in this area of research from previous papers, these findings provide a better understanding of the association between air pollution and eczema. Third, my findings showed that adults living in hard water areas had a higher risk of having eczema than those living in softer water areas. To my knowledge, this was the first time such results were reported which can inform future analyses related to eczema and other risk factors. Another important recommendation is to consider multilevel modelling when assessing exposures measured at the ecological level to account for the clustering of individual variables. Fourth, I determined that occupational exposures to animals, storage mites, endotoxin, metalworking fluids, isocyanates, and acrylates were associated with increased eczema prevalence in adults. Fifth, I found that no evidence of associations between air pollution markers and eczema during the first ten years of life. While exposure to higher concentrations of air pollution was associated with the development and persistence of food allergy, particularly peanut allergy in 4 and 6 years of age. This analysis was the first to evaluate the association between air pollution and challenge proven food allergy and the results can inform further research in this area. All the association estimates from the analyses included in my thesis can be used in a multistate life table to evaluate eczema morbidity in a population.

9.3.2. Clinical implications

My novel eczema subclassification may aid in class-specific preventive or therapeutic strategies. Clinical early detection of eczema subclasses may be important as persistence classes were associated with more allergic comorbidities at later ages. Air pollution has various adverse health effects, including increasing eczema prevalence; raising awareness and limiting exposures is likely to aid in the reduction of eczema prevalence. Using domestic hard water may increase the risk of eczema prevalence in at least some adults, and reducing the exposure may improve a patient

treatment prognosis. Occupational exposures to animals, storage mites, endotoxin, metalworking fluids, isocyanates, and acrylates may increase eczema risk; raising awareness and limiting occupational exposures during an individual's productive working life is likely to have a range of health benefits, including the reduction of eczema prevalence. Fifth, avoidance of air pollution in early life could potentially be a food allergy preventive measure. These recommendations may be included in prevention or treatment guidelines for eczema.

9.4. Summary of the recommendations and future research directions.

Below I summarised the overall recommendations for researchers, clinicians and public health policy and the future research directions according to the findings and objective of my doctoral thesis.

9.4.1 Recommendations for Researchers

I have found clear evidence for a latent eczema classification. It may be important to consider the eczema subclasses separately, as they have different risk profiles and prognoses. This result suggests that these five subclasses of eczema should not be clustered since these are different eczema presentations, each with a different prognosis and probable risk factors. It should be noted that to determine eczema subclasses using latent class analysis, repeated measures are needed. Even so, class-stratified research should clarify eczema's underlying causes and its natural course.

I have observed that exposure to air pollution in middle-aged adults confers a greater risk of allergic sensitisation and eczema prevalence in males. There have been a few studies in this area with adult populations with conflicting results. My findings provided evidence of this association even in a low air pollution setting. In this area of research, there is a need to standardise the method of measurement of air pollution markers, study designs, eczema definitions, and selection of confounding factors to aid in the replication of results. Also, researchers wanting to evaluate environmental risk factors for eczema should consider this positive association when specifying confounders to include in their models.

My findings provided evidence supporting that domestic hard water use is associated with increased eczema in middle-aged adults. The results presented in this thesis are the first to demonstrate this association in adults. My results indicate that hard water is a risk factor for adult eczema. Also, there was a small eczema clustering effect by postcodes in this cohort that remained unexplained. Other variables like air pollution, neighbourhood greenness or blueness could explain this clustering effect.

I reported associations between various occupational exposures and eczema in middle-aged adults. It was the first time such findings were reported, as previous literature in the area did not use a job exposure matrix to assign occupational exposures. As these analyses are of an exploratory nature, further studies can be done to evaluate each occupational risk exposure and corroborate this study's findings.

I have found that infants who are exposed to air pollution have a higher risk of developing food allergy and food sensitisation. No study in this field has ever used criterion-based oral food challenges to objectively measure food allergy in infants before. Understanding the various molecular mechanisms by which air pollution raises the likelihood of food allergies requires more study. Also, when deciding which confounders to include in their models, researchers looking to assess environmental risk factors for food allergies should take this positive connection into account.

I have found that higher exposure to air pollution in early life confers a greater risk of food allergy and food sensitisation in infants. No study in this area has examined the associations between air pollution and objectively measure food allergy with criterion based oral food challenges before. Further research is needed to understand the potential biological pathways by which air pollution increases the risk of food allergy. Also, researchers wanting to evaluate environmental risk factors for food allergy should consider this association when specifying confounders to include in their models.

9.4.2. Recommendations for Clinicians

The findings of this thesis could impact clinicians in a variety of aspects. Early detection of the persistent eczema subclasses could be necessary as these classes have the highest persistence of eczema symptoms and associations with allergic comorbidities. Developing a diagnostic method or marker to determine eczema subclasses is essential for class-specific preventive or therapeutic strategies.

My findings provided evidence for the effect of air pollution, hard water and various occupational exposures as environmental risk factors for eczema. These results should help clinicians consider these factors as potential triggers for patients and help promote allergen avoidance strategies.

Efforts to limit ambient air pollution exposure likely have several positive health effects, including decreasing eczema prevalence in adults and food allergy prevalence in infants and children. A decrease in the prevalence of eczema may result from limiting occupational exposures, possibly by wearing protective gear, to animals, storage mites, endotoxin, metalworking fluids, isocyanates and acrylates allergens during a person’s productive working life. People living with

eczema that does not improve with traditional treatment and live in hard water areas could take shorter showers, use rainwater or water softeners, or move away from places with hard water to protect their skin. Finally, the results from my thesis provide evidence that can be incorporated into eczema prevention and treatment guidelines.

9.4.3. For Public Health Policy

The early detection of eczema subclasses may be important to determine eczema prognosis. The results of supplementary analysis showed that the persistent classes had a higher rate of positive skin prick tests (Table S4.4). The routine use of skin prick tests in infants with eczema may not be warranted at this time, but there is prognostic value in doing so. The findings from this thesis regarding environmental risk factors for eczema provide evidence for further strengthening regulations to ambient air pollution, domestic hard water concentration and occupational exposures. Also, once further studies confirm the results which suggested that a range of occupational exposures increased eczema risk, there would be medico-legal considerations for occupational eczema development.

9.4. Future research or directions

The results from this doctorate work have raised several questions and indicated future direction for further research in this area of knowledge:

1. Future studies and interventions evaluating eczema outcomes can be customised to include the eczema subclasses that I have observed.
2. Future research can extend the latent class analysis to include the distribution of rash locations to determine if certain rash locations patterns have a higher probability of being classified in the persistent eczema subclasses.
3. Further research is needed to identify the components of ambient air pollution that are associated with eczema and food allergy, the extent of exposure required to increase risk of eczema and the factors that determine individual susceptibility.
4. To further elucidate the possible reasons for air pollution sex specific associations, I recommend a focused exploration of the behaviour and physiological responses to air pollution and how they differ between men and women.
5. Further research is required to determine the water hardness minimal safe threshold for eczema.
6. Further research is needed to explore geographical or ecological factors that may lead to eczema.

7. As the occupational exposure analyses were of explorative nature, future research should focus on evaluating the pathogenesis of specific occupational exposure identified as potential risk factors for eczema.
8. There is a need to develop a job exposure matrix that is specific to eczema and includes skin allergens or irritants identified in the literature like Portland cement or wet work exposures.
9. Further research is needed to determine the potential biological pathways by which air pollution increases risk to food allergy in children and eczema in adults.

9.5. Conclusion

In conclusion, my doctoral work: 1) determined five eczema subclasses from birth to twelve years with different prognosis and risk factors; 2) provided evidence that exposure to higher concentrations of air pollution was associated with the development and persistence of food allergy while air pollution did not increase risk of eczema in young children; 3) provided evidence suggesting increased exposure to air pollution was associated with eczema in males and with allergic sensitisation in both sexes; 4) suggested that domestic hard water usage was related to an increased risk of eczema in adults; and 5) proposed occupational exposures to animals, storage mites, endotoxin, metalworking fluids, isocyanates and acrylates were associated with eczema in middle-aged adults. My published papers are well accepted by the scientific community and are cited by new research in this area.

My findings highlight that using the eczema subclasses may help determine the risk factors and underlying causes of these types of eczema. Also, there is a need to increase public awareness and limit exposure to these environmental risk factors whenever possible, as it is probable to help in the reduction and treatment outcomes of eczema and food allergy. I expect that the findings from this thesis will add to the current understanding of allergic diseases and lead to new research on this topic.

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Appendix

Appendix A. TAHS Plain Language Statement Newsletter

TAHS
Tasmanian Longitudinal Health Study

Ambient air pollution and eczema in adults

Ambient air pollution is a health hazard as it increases the risk of respiratory problems, heart diseases and strokes. Recent evidence suggested that air pollution may also increase the risk of eczema in adults. In this article, we will discuss the recent findings from the Tasmanian Longitudinal Health study (TAHS).

What do we know about eczema in adulthood?

The majority of adults who have eczema had it as a child. It's possible for eczema to go away in childhood and return years later. For others, eczema in childhood never goes away, making it a lifelong condition. If you have never had eczema before, eczema that begins after 18 years of age is called "adult-onset eczema". A peak time for developing adult-onset eczema is around 50 years of age. There have been only a few studies on the causes of eczema in adulthood. It is believed that environmental factors, including air pollution, might play an important part in the development of eczema in middle age.

What is ambient air pollution?

The World Health Organization defines ambient air pollution as potentially harmful pollutants emitted by industries, households, and traffic (cars and trucks). Of these pollutants, fine particulate matter ($PM_{2.5}$) has the greatest effect on human health. In Australia, pollution from traffic accounts for only 17% $PM_{2.5}$ in our air, with most $PM_{2.5}$ coming from other combustion sources (i.e. wood heaters, power stations, etc). Likewise, nitrogen dioxide [NO_2] also has negative health effects. More than half of the ambient NO_2 is attributed to on-road sources in Australia. Other important air pollutants are carbon monoxide, particulate matter with a diameter less than 10 micrometre (PM_{10}), ozone and sulphur dioxide.

How does air pollution increase eczema risk?

It has been proposed that air pollutants may damage our skin by generating highly reactive molecules (reactive oxygen species) when it settles on the outer-most layer of our skin. This then leads to inflammation and itch that are associated with eczema. There are also genetic differences between people, such as filaggrin null mutations, that may make people particularly at risk of developing eczema when exposed to high levels of air pollution.

What was measured in the study?

The TAHS is a population-based study, that has followed participants since 1968. While TAHS started in Tasmania by Drs Heather Gibson, Brian Gandevia and Harrold Silverstone, 30% of the participants have now moved to other states making TAHS a national collaborative study. The study is now conducted with input from a network of clinical researchers from six states and lead by Allergy and Lung Health Unit, University of Melbourne. We followed up these participants when they were aged 43 years and again when they were 53 years old. We measured ambient air pollution exposures (distance from a major road, NO_2, $PM_{2.5}$) based on residential address and eczema prevalence (new and old cases of eczema), incidence (new cases of eczema) and persistence (lifelong disease). We also measured allergic sensitisation using skin prick tests.

What were the study findings?

We found that exposure to higher concentrations of air pollution is associated with a greater risk of atopic eczema (eczema with allergic sensitisation) in men and an increased risk of sensitisation to aeroallergens in both men and women. The reason why the increased risk was observed only in men is not clear but may partly be because of behavioural differences i.e. women tend to take better care of their skin than men, men spent more time outside than women, making them more exposed to air pollution.

So, what's next?

Ongoing efforts to reduce ambient air pollution exposure may help reduce the burden of eczema in adults, particularly men, and will have a range of other health benefits.

Further research is needed to identify which parts of air pollution increase the risk of eczema, the extent of exposure required to increase risk of eczema and the factors that make someone more susceptible to developing eczema if exposed to high levels of air pollution. We are now also exploring if indoor air pollution may have a role in adult eczema.