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The skin barrier function gene *SPINK5* is associated with challenge proven IgE-mediated food allergy in infants

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Abbreviations

SNP: Single nucleotide polymorphism

OFC: Oral food challenge

SPT: Skin prick test

SPINK5: *Serine peptidase inhibitor Kazal type 5*

LEKTI: lymphoepithelial Kazal-type-related inhibitor

AIMs: Ancestry informative markers

TEWL: Trans-epidermal water loss

Word count: 3,640

Background: A defective skin barrier is hypothesised to be an important route of sensitisation to dietary antigens, and may lead to food allergy in some children. Missense mutations in the *Serine peptidase inhibitor kazal type 5 (SPINK5)* skin barrier gene have previously been associated with allergic conditions.

Objective: To determine whether genetic variants in and around *SPINK5* are associated with IgE mediated food allergy.

Method: We genotyped 71 'tag' single nucleotide polymorphisms (tag-SNPs) within a region spanning ~263 kilobases (kb) including *SPINK5* (~61kb) in n=722 (n=367 food allergic, n=199 food sensitised, tolerant and n=156 non-food allergic controls) 12-month infants (discovery sample) phenotyped for food allergy with the gold standard oral food challenge (OFC). Transepidermal water loss (TEWL) measures were collected at 12-months from a subset (n=150) of these individuals. SNPs were tested for association with food allergy using the Cochran-Mantel-Haenszel test adjusting for ancestry strata. Associations analyses were replicated in an independent sample group derived from four paediatric cohorts, total n=533 (n=203 food allergic, n=330 non-food allergic), mean age 2.5 years, with food allergy defined by either clinical history of reactivity, 95% positive predictive value (PPV) or challenge, corrected for ancestry by principal components.

Results: *SPINK5* variant rs9325071 (A⟶G) was associated with challenge proven food allergy in the discovery sample (P=0.001 | OR=2.95 | CI=1.49-5.83). This association was further supported by replication (P=0.007 | OR=1.58 | CI=1.13-2.20) and by meta-analysis (P=0.0004 | OR=1.65). Variant rs9325071 is associated with decreased *SPINK5* gene expression in the skin in publicly available genotype-tissue expression data, and we generated preliminary evidence for association of this SNP with elevated TEWL also.

Conclusions: We report, for the first time, association between *SPINK5* variant rs9325071 and challenge-proven IgE-mediated food allergy.

Key words: *Food allergy, LEKTI, skin barrier, skin barrier function, SPINK5*

Introduction

Cutaneous exposure to foods via a defective skin barrier is hypothesised as a route of sensitisation to foods. Infants with early onset eczema are significantly more likely to develop food allergies (Martin *et al.*, 2015) and in this context it has been proposed that disrupted skin barrier function in early life may facilitate sensitisation to foods due to passage of food antigens across the impaired epicutaneous barrier. According to the "Dual Allergen Hypothesis" the timing and balance of cutaneous exposure relative to oral exposure can result in food allergy (Allen & Koplin, 2016; Lack, 2008). Mutations in skin barrier integrity genes

are therefore potential risk factors for the development childhood food allergy. In support of this, loss of function mutations in the epidermal gene encoding *filaggrin* (*FLG*) have been associated with risk of peanut sensitisation (Tan *et al.*, 2012) and peanut allergy (Brough *et al*., 2014; Venkataraman *et al*., 2014; Brown *et al*., 2011). Yet the role of other skin barrier genes in the development of food allergy is underexplored.

Another gene of relevance to skin barrier integrity is *SPINK5*. *SPINK5* encodes the protein product lympho-epithelial Kazal-type-related inhibitor (LEKTI), a serine protease inhibitor. LEKTI is involved in the regulation of the desquamation process, i.e. the shedding of the outermost layer of the epidermis, by inhibiting serine proteases kallikrein (KLK) 5, KLK7 & KLK14 (Deraison *et al*., 2007). Mutations in *SPINK5* are associated with the rare recessive skin condition Netherton Syndrome (NS) (Chavanas *et al*., 2000), a condition frequently co-associated with atopic manifestations, including food allergy (Walley *et al.*, 2001). Single nucleotide polymorphisms (SNPs) at *SPINK5* have now been identified as associated with risk of asthma (Walley *et al.*, 2001; Kabesch *et al.*, 2004) and eczema (Walley *et al.*, 2001; Nishio *et al*., 2003; Kato *et al.*, 2003; Kusunoki *et al.*, 2005; Weidinger *et al.*, 2008; Zhao *et al*., 2012). However, the results have not always been consistent (Hubiche *et al.*, 2007; Kabesch *et al.*, 2004; Jongepier *et al.*, 2005; Fölster-Holst *et al*., 2005). To date no studies have directly examined the association of *SPINK5* variants with challenge-proven food allergy, despite clear association of this gene with skin barrier integrity and atopic manifestations. A study of 115 Japanese children with atopic dermatitis (AD) reported that a *SPINK5* SNP, rs2303067 [1258: G⟶A(Glu420Lys)], associated with severity of AD was also a risk factor for food allergy (Kusunoki *et al.*, 2005), although this study was not able to assess the role of *SPINK5* in food allergy risk independent of AD status. Here we sought to specifically investigate the relationship between *SPINK5* SNPs and food allergy in a cohort of one-year-old infants with challenge-proven clinical outcomes. With replication in an independent sample, our data seeks to investigate whether *SPINK5* variants are genetic risk factors for food allergy.

Methods

Study populations

For the discovery analysis, DNA samples were obtained from the HealthNuts (HN) cohort, a longitudinal, population-based study of food allergy based in Melbourne, Australia (Osborne *et al*., 2011). Briefly, recruitment took place between 2007 and 2011 of 5,276 12 month-old

infants presenting for scheduled immunisations at council run clinics. All infants underwent skin prick testing (SPT) to egg white, peanut, sesame, and 1 or 2 other foods (cow's milk or shrimp). Those with detectable SPT (1 mm or greater than negative controls) were invited to the Royal Children's Hospital within 1-2 months to repeat the SPT. On the same day they underwent an open oral food challenge irrespective of wheal size using pre-determined objective diagnostic criteria (Koplin *et al*., 2015), which has been widely published as the gold standard for infants (Sampson *et al*., 2012). Approximately 200 individuals SPT negative to the panel tested were also invited as negative controls and underwent food challenges. Ten millilitres of blood was collected after food challenges from 836 individuals and DNA was extracted for genetic studies. Ethical approval for this study was obtained from the Office for Children Human Research Ethics Committee (HREC) (CDF/07/492), the Department of Human Services HREC (10/07) and the Royal Children's Hospital (RCH) HREC (27047).

Replication was sought in an additional panel of 203 food allergic children and 330 non-atopic controls drawn from the Peanut Allergen Threshold study (PATs) of peanut allergy (Zurzolo *et al*., 2013), the Probiotic and Peanut Oral ImmunoTherapy (PPOIT) study (Tang *et al.*, 2015), Barwon Infant Study (BIS) (Vuillermin *et al*., 2015) and the Melbourne Atopy Cohort study (MACs) (Lowe *et al.*, 2016) (see **supplementary figure 1**). Replication sample ethics approvals for each study were as follows: PATS: HRECApp32166A and 2012P002475; PPOIT: Approved by RCH Human Research and Ethics Committee HREC 27086Q; MACS: initial approval by the Mercy Maternity Hospital Ethics Committee (R88/06), 18 year follow-up, including collection of DNA, was approved by the Royal Children's Hospital (HREC 28035); BIS: Barwon Health Human Research Ethics Committee HREC (10/24).

For the discovery analysis, phenotypes of HN cases and controls were defined by food challenge outcomes as follows: **Food allergy:** detectable SPT wheal ≥(1 mm) wheal to peanut, egg white, cow's milk or sesame AND evidence of clinical reactivity by OFC. **Food sensitised-tolerant:** detectable SPT wheal ≥(1 mm) to peanut, egg white, cow's milk or sesame but negative OFC. **Non-allergic:** No detectable SPT wheal to peanut, egg white, cow's milk or sesame and negative OFC.

For the replication analysis, case phenotypes were defined through a combination of challenge proven outcomes where available (BIS cohort), or by evidence of sensitisation (SPT or sIgE) with clear history of immediate-type clinical reactions within 1-2 hours of

exposure (PPOIT and PATs cohorts), or using accepted 95% positive predictive values for SPT wheal sizes (MACs cohort) (Peters *et al.*, 2013) (**Supplementary table 1**). For all studies eczema status was ascertained by history of doctor-diagnosed eczema, or nurse observed eczema at presentation to clinic.

Genotyping and Quality Control (QC)

Tag SNPs across the *SPINK5* region were chosen on the basis of linkage disequilibrium (LD), calculated using HapMap data in Haploview (Barrett *et al.*, 2005). 77 tag SNPs capturing 387 alleles with LD of r^2>=0.8 (mean=0.97) in and around *SPINK5* within a region of ~263 kilobases (kb) were selected for genotyping (HapMap Genome Browser Phase 1, 2 & 3, chr5:147,267,000 to 147,530,000) (**Supplementary figure 2**). SNPs were genotyped using Agena Bioscience iPLEX Gold chemistry and the MassARRAY mass spectrometer system. Primers were designed using Agena Bioscience online tools with MassArray Design 3.1 software to perform multiplexing of primers. In addition, a panel of 49 Ancestry Informative Markers (AIMs) were genotyped from the panel described in (Bousman *et al.*, 2013). Non-conservative genotype calls were visually inspected in TYPER 4.0 (Agena Bioscience) and samples re-genotyped where a call could not be made. Genotypes were analysed using PLINK (Purcell *et al.*, 2007). Individuals and SNPs with a genotyping success rate of less than 95% were removed. Tag SNPs with minor allele frequency (MAF) of <0.02, or deviation from the Hardy–Weinberg equilibrium (HWE) (p<0.01) were removed. These quality control measures resulted in a final cohort of n=722 (n=367 food allergy cases, n=199 food sensitised-tolerant and n=156 non-allergic controls) genotyped for 71 tag SNPs. A power calculation was conducted in Quanto to assess the power of the study, it predicted sufficient power (80%) to detect effects sizes over 1.5 at an alpha<0.05 for common SNPs. For detailed QC breakdown see **supplementary figure 1**. For the replication study the top four SNPs associated with food allergy from the discovery analysis (rs9325072, rs3815741, rs4705054, rs9325071, **supplementary figure 3A**) above the nominal p-value (<0.05) were selected for replication. After QC in the replication study there were 906 individuals genotyped for three SNPs (rs9325072, rs4705054, rs9325071, **supplementary figure 3B**). All individuals in the replication sample were genotyped using the AIM panel and ancestry was genetically inferred (see **Supplementary figure 4)**, described in the supplementary methods.

Trans-epidermal water loss measures

Measures of trans-epidermal water loss (TEWL) were available for a subset of HN participants with *SPINK5* genotype data (n=150). Participants were given time to acclimatise to the clinic room (20 minutes), during this time clothing was removed from the testing site. Atmospheric temperature and humidity were recorded, as well as skin temperature. Atmospheric temperature was on average 24°C (min: 20.8°C; max: 27°C). TEWL measures were recorded using the Tewameter® TM300 at the mid-lower back until five consecutive measures within standard deviation of ≤ 0.2 were achieved. Raw TEWL (g/m^2 h) readings were positively skewed, and therefore log transformed prior to analysis.

Data analysis

Ancestry strata were ascertained indirectly by parental country of birth in the HealthNuts study and samples were classified as Caucasian (both parents born in Australia, Europe, UK, Northern America or New Zealand, n=503), Asian (both parents born in South East Asia, n=74) or mixed Asian-Caucasian (n=145). We used genome-wide SNP data, available on a subset (n=344) of the discovery sample, and identity-by-state clustering analysis against the human reference populations to establish the accuracy of our predicted ancestry strata to assess the accuracy of our parent country of birth as proxy measure of ancestry (**Supplementary figure 5**). The strata we defined based on parental country of birth were highly correlated with genetically determined ancestry (93.7% correlation for "Caucasians" and 93.0% correlation for "Asians") (**Supplementary figure 5**). All analyses of the discovery study were modelled using Cochran-Mantel-Haenszel tests adjusting for sex and ancestry strata in PLINK, and the genomic inflation factor was 1.10, indicating only modest inflation (Yang *et al.*, 2011).

The primary analysis of the discovery cohort tested the association between *SPINK5* SNPs and food allergic cases and non-food allergic controls. A secondary analysis was conducted in the discovery cohort to tease apart the relationship between the variants and clinically reactive (challenge proven food allergy) or asymptomatic (sensitised-tolerant) food allergy using the same analysis model. An additional secondary analysis was conducted to measure allele frequencies between food allergic cases and food sensitised-tolerant cases, to test whether there was evidence for a stronger association amongst the clinically distinct outcomes. Further, to test whether the observed genetic associations for food allergy were driven by co-morbid eczema, an additional analysis for an association with challenge proven food allergy was conducted excluding infants with eczema.

In the replication phase principal components (PC) from the AIMs data were used to adjust for heterogeneity arising from population structure. Genetically inferred ancestry determined 657 individuals to be of European descent, 217 of mixed European-Asian descent and 32 of Asian descent. Logistic regression, adjusted for PCs, sex and study in PLINK was used to test for differences in minor allele frequencies between food allergic and non-food allergic individuals of the replication sample. Finally, due to differences in phenotype criteria used amongst these studies, a sensitivity analysis was conducted restricting to phenotype and age matched cases and controls (the Barwon Infant Study). BIS utilised oral food challenge to define case-control status at 12-months of age, consistent with measures used in the discovery analysis. SimpleM (Gao *et al.,* 2008), a method of correction for multiple-testing of correlated SNPs was used to define multiple-testing adjusted p-value thresholds for the discovery and replication. SimpleM is a PC analysis approach which captures the correlation of SNPs in the dataset and calculates the number of independent tests M_{eff}. Inferred M_{eff} in the discovery was 47, the derived significance threshold was therefore calculated with the formula 0.05/ M_{eff}. This formula gave the corrected significance threshold of 0.001. For replication the calculated M_{eff} was 7, giving a derived significance threshold of 0.007.

Meta-analysis of the discovery and replication panels was performed using the PLINK meta-analysis function for food allergy (n=570) vs non-allergic controls (n=486) using the association files from the discovery Cochran-Mantel-Haenszel test and the replication logistic regression adjusted for ancestry principal components.

For the TEWL analysis associations between log(TEWL g/m^2 h) and each variant were analysed using linear regression to assess whether there was a difference in measurable skin barrier functionality in those with the risk variant (n=150). TEWL recordings were analysed by genotype of SNPs associated with food allergy.

Results

Characteristics of the study participants

Overall 722 infants, 367 food-allergic, 199 food sensitised-tolerant and 156 non-allergic controls were included in the discovery analysis (**Table 1**). The proportion of clinically allergic children in this population was 50% whilst the rate of food sensitisation was 78%. Egg allergy was the most common type of food allergy at 89.3%, followed by peanut 32.2% and sesame 6.7%. Amongst the food allergic group, 56.4% had concurrent atopic eczema,

which was higher when compared with the food sensitised group (37.7%) or non-allergic controls (25.0%).

SPINK5 variants associated with food allergy

When comparing challenge-proven food allergy cases with non-allergic controls in the individuals of the discovery study we identified a haplotype block of correlated SNPs (**Supplementary figure 3**) associated with food allergy (nominal P<0.05) (rs9325072 C⟶T: P=0.001 | OR=2.95 | CI=1.49-5.83; rs3815741 A⟶G: P=0.002 | OR=2.76 | CI=1.44-5.31; rs4705054 A⟶T: P=0.01 | OR=1.94 | CI=1.17-3.24; rs9325071 A⟶G: P=0.02 | OR=1.83 | CI=1.11-3.03) (**Table 2a**). One variant rs9325072 reached the derived multiple-testing adjusted significance threshold of 0.001. To test whether these genetic associations were being driven by co-morbid eczema the analysis was then repeated in infants without eczema (leaving n=174 food allergic cases and n=90 non-food allergic controls). The top associations remained consistent, illustrating that the association is with food allergy and not eczema and that eczema is not involved in the mechanism for this association (**Supplementary table 2**).

A secondary analysis of food sensitised-tolerant (i.e asymptomatic food allergy) compared to non-food allergic controls was conducted. One SNP associated with challenge-proven food allergy showed a significant (nominal P<0.05) association with the food sensitised, tolerant phenotype (rs9325072 P=0.04 | OR=2.11 | CI=1.01-4.41) (**Table 2b)**. A comparison of the allele frequencies between symptomatic food allergic individuals and asymptomatic food sensitised individuals for the top four SNPs from the primary analysis did not support a significant difference between these two groups (**Table 2c)**.

Replication of candidate risk variants

Comparing food allergic cases to non-food allergic controls in the replication sample, only one association (rs9325071, fourth most significant association in the discovery sample) remained consistent with the discovery (P=0.007 | OR=1.58 | CI=1.13-2.20) (**Table 3a**) and reached the derived multiple-testing corrected significance threshold. Again this association remained after infants with eczema were removed to ascertain if the association was driven by comorbid eczema. In the sensitivity analysis restricted to only infants with oral food challenge outcomes, the direction of effect was similar but 95% Cis included 1 (rs9325071: OR=1.40, CI=0.66-2.93).

In a meta-analysis of data from discovery and replication (n=1056), 570 food allergy cases and 486 non-allergic controls, an association with variant rs9325071 (P=0.0004 | OR=1.65 | Q=0.62 | I=0.00) was further supported (**see Table 3b**). There was strong evidence against a null hypothesis of homogeneity across studies (the discovery and the replication samples) of the magnitude of the association (effect size), as detected by Cochrane's Q statistic, between variant rs9325072 and rs4705054 and the risk of food allergy (**see Table 3b**).

Evidence of skin barrier impairment via trans-epidermal water loss measurement

The analysis of transepidermal water loss (TEWL) provided some preliminary evidence that there may be a recessive effect on skin barrier permeability by genotype for food allergy associated SNPs (**Supplementary table 3**). However, the number of individuals with both minor allele genotypes and TEWL data in this analysis was underpowered to conclude anything more than a suggestive result that warrants further exploration.

Expression Qualitative Trait Loci (eQTL)

To further explore function of the replicated food allergy associated variant, publicly available gene expression data by tissue type was accessed from the GTEx Project database. Replicated variant rs9325071 was significantly associated with decreased *SPINK5* expression in the skin ($P=8.9x10^{6}$) in the GTEx database, suggesting the variant is a functional eQTL.

Discussion

In this study we report a novel genetic association between *SPINK5* variants and IgE-mediated food allergy. This is the first time these variants have been examined in the context of challenge-proven food allergy, the genetic mechanisms of which are still largely unknown. This study adds to a growing list of candidate gene associations and provides the foundation for extensive testing in other populations.

We focused on *SPINK5* with a prior expectation that this gene may harbour variants associated with food allergy due its critical role in skin barrier integrity and its co-association with atopic food allergy in patients with Netherton syndrome. The integrity of the skin barrier is increasingly recognised as a critical protective factor against IgE-mediated food allergy.

Using a tag-SNP approach provided broad genotype coverage of common variants in *SPINK5* and revealed SNPs in a haplotype block to be associated with clinical food allergy. The high

level of correlation between these variants suggests they are frequently co-inherited and future fine mapping studies are now needed to resolve the specific causal variants.

Within this haplotype block, SNP rs9325071 was shown to be associated with food allergy in both the discovery and replication sample. Analysis of publicly available gene expression data revealed rs9325071 G allele carriage to be associated with decreased *SPINK5* expression in the skin, suggesting a plausible functional role for this variant in skin barrier integrity. Additionally, individuals in this study who were homozygous for the rs9325071 minor allele exhibited higher levels of TEWL. While these data lend further support for a functional impact of rs9325071 on skin barrier integrity, our sample size was small and thus the findings need to be considered cautiously. We can reasonably conclude that the overall trend may suggest a potential functional role for this SNP by way of reduced LEKTI expression (the product of *SPINK5*) and increased skin permeability. Skin barrier impairment may allow for increased allergen absorption across the skin and when this precedes oral allergen ingestion, which is largely tolerogenic (Du Toit *et al.,* 2015), food sensitisation may develop (Brough *et al., 2015*). A similar pathway has been proposed for household peanut exposure in infants with *FLG* LOF mutations (Brough *et al., 2014*). This is further supported by observations in a murine model of skin barrier impairment induced by tape stripping, stimulating thymic stromal lymphopoietin (TSLP) production (Leyva-Castillo *et al.*, 2013). These mice subsequently became sensitised to the locally applied allergen, with detectable systemic allergen specific immune responses (Leyva-Castillo *et al.*, 2013). An alternative mechanism of action might occur via impaired LEKTI signalling and TSLP expression. LEKTI-deficient keratinocytes produce uninhibited KLK5, perpetuating pro-allergic Th2 signalling, including elevated TSLP expression (Briot *et al.*, 2010). LEKTI expression in the thymus has also been hypothesised to influence T cell differentiation and result in bias towards pro-allergic immune responses (Chavanas *et al*., 2000). LEKTI may also have an additional role in the mucosal epithelia where it has been implicated in immunological activity and inflammatory responses in the mucosal epithelia. Skin barrier impairment, induced Th2 signalling as a consequence of LEKTI deficiency, and/or disruption of LEKTI in the mucosal epithelia represent biologically plausible pathways for future functional studies to investigate the role of *SPINK5* polymorphisms in food allergy. Knowledge of food allergy associated skin barrier SNPs, in this case rs9325071, may be useful for identifying patients in whom repairing the skin barrier, or providing protection through the use of emollients, would constitute a treatment priority to either prevent or help manage food allergy.

A strength of this study was our ability to explore whether *SPINK5* variants were associated with the risk for developing food sensitisation, or clinical food allergy, due to the availability of challenge-proven phenotypic groups in the discovery sample. The replicated food allergy associated SNP, rs9325071, was associated with clinical food allergy (P=0.02 | OR=1.83 | CI=1.11-3.03) in the discovery analysis but was not associated with asymptomatic food sensitisation (P=0.26 | OR=1.38 | CI=0.79-2.41), suggesting it may predispose to a more severe clinical phenotype. However, the sample size available for the sensitised group was smaller than that for the allergic group, and this may have limited our power to detect association with sensitisation. Larger studies would be required to confirm this finding.

An important finding from this study was that *SPINK5* variation increases the risk of clinical food allergy independently of eczema. This was an important mechanism to consider given food allergy and eczema are frequent co-morbidities, and previous studies have reported *SPINK5* variants associated with maternal transmission of atopic dermatitis (Walley *et al.*, 2001). It is likely that *SPINK5* polymorphisms are a risk factor for both food allergy and atopic eczema.

A limitation of the study is the sample size of the discovery cohort which did not allow for robust associations after multiple testing correction. Thus we relied heavily on a replication sample, which showed the strongest associations in the discovery cohort to be false positives. Further strengths of this study include the highly clinically relevant case definitions, the replication in an independent population, and the inclusion of functional data. A limitation of this study was the potential heterogeneity between the discovery and the replication samples in terms of age and phenotype definitions. To address this we provided a supportive sensitivity analysis in infants with outcomes defined only by oral food challenge, to remain consistent with the discovery study. The effect size estimates resulting from this replication sub-analysis were consistent with the effect size estimates for the overall replication sample, providing evidence that heterogeneity arising from differences in outcome definitions was not introducing confounding. Another limitation was the use of parental country of birth as a proxy measure of ancestry rather than genetically determined ancestry in the discovery population. To address this parental country of birth was compared to available genome-wide SNP data on a subset of the discovery sample, providing evidence that parental country of birth clustered well with genetically inferred ancestry. A final potential limitation is that oral food challenges were not double-blinded placebo controlled. However, as published in the

PRACTALL guidelines, at 12-months of age infants are unlikely to have false-positive results due to subjective symptoms (Sampson *et al.*, 2012).

In conclusion, with replication in two populations we present cogent evidence that *SPINK5* variants are associated with clinical food allergy in children.

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Figures & tables

Table 1. Clinical characteristics of 722 infants included in the discovery cohort.

	Non-allergic controls (NA) N=156	Food sensitised, tolerant cases (FS) N=100	Food Allergy cases (FA) N=367
Infant demographics			
Age in months at recruitment (mean, SD)	12.6 (0.65)	12.6 (0.68)	12.7(0.75)
Gender (% male)	47.4%	54.3%	58.9%
Reported ethnicity			
Asian	3.3%	17%	14%
European	86.8%	66%	61.5%
Mixed European/Asian	9.9%	24.1%	24.4%
Infant clinical characteristics			
History of eczema	25.0%	37.7%	56.4%
Nurse observed	10.9%	19.1%	29.2%
History of doctor diagnosis	14.1%	18.6%	27.2%
TEWL	n=38	n=38	n=66
Average TEWL (g/m^2 h)	16.9 (16.1)	16.7 (11.5)	16.8 (16.3)
Food sensitisation			
Egg sensitisation	0%	29.6%	93.7%
Sesame sensitisation	0%	5.5%	9.0%
Peanut sensitisation	0%	30.2%	49.9%
Food allergy			
Egg allergy	0%	0%	89.3%
Sesame allergy	0%	0%	6.7%
Peanut allergy	0%	0%	32.2%
Family characteristics			
Any siblings	54.5%	48.7%	44.3%
Asthma			
Maternal asthma	19.9%	21.1%	22.9%
Paternal asthma	18.6%	15.6%	19.1%
Sibling asthma	10.9%	9.6%	10.1%
Hay fever			
Maternal hay fever	22.4%	15.1%	24.0%
Paternal hay fever	35.3%	28.1%	37.9%
Sibling hay fever	5.8%	3.5%	6.5%
Eczema			
Maternal eczema	22.4%	14.1%	22.1%
Paternal eczema	14.7%	10.1%	12.3%

Sibling eczema	22.4%	16.6%	17.2%
Food allergy			
Maternal food allergy	12.2%	6.0%	6.3%
Paternal food allergy	11.5%	4.5%	4.4%
Sibling food allergy	10.9%	5.5%	6.8%

Table 2. **A.** Associations between SPINK5 SNPs and food allergy corrected for ancestry strata with Cochran-Mantel-Haenszel 2x2xK test and adjusted for gender. **B**. Associations between SPINK5 SNPs and food sensitised-tolerant phenotype. **C**. Analysis of food allergic cases vs food sensitisation

			A. Food allergy vs non-food allergic				*B. Food sensitised, tolerant vs non-food allergic*				*C. Food allergic vs food sensitised, tolerant*			
SNP	A1	A2	P	OR	L95	U95	P	OR	L95	U95	P	OR	L95	U95
rs9325072	T	C	0.001	2.95	1.49	5.83	0.04	2.11	1.01	4.41	0.14	1.38	0.90	2.10
rs3815741	G	A	0.002	2.76	1.44	5.31	0.07	1.92	0.94	3.92	0.09	1.43	0.94	2.18
rs4705054	T	A	0.01	1.94	1.17	3.24	0.34	1.33	0.75	2.35	0.06	1.43	0.98	2.09
rs9325071	G	A	0.02	1.83	1.11	3.03	0.26	1.38	0.79	2.41	0.15	1.31	0.90	1.90
rs4371740	T	A	0.06	1.39	0.99	1.97	0.32	1.22	0.83	1.78	0.36	1.14	0.86	1.51
rs17704764	G	A	0.07	0.69	0.47	1.03	0.51	0.87	0.57	1.33	0.30	0.83	0.58	1.18
rs17641748	A	G	0.05	1.76	0.99	3.12	0.78	1.10	0.57	2.12	0.10	1.46	0.93	2.27
rs6580526	T	C	0.05	0.72	0.52	1.00	0.39	0.85	0.59	1.23	0.23	0.84	0.64	1.11
rs1120680	G	A	0.05	2.07	0.97	4.43	0.18	1.73	0.76	3.93	0.55	1.17	0.71	1.93
rs6580514	G	A	0.11	0.77	0.55	1.07	0.03	0.66	0.46	0.96	0.39	1.14	0.85	1.52
rs7700964	T	C	0.12	0.76	0.54	1.07	0.23	0.79	0.54	1.16	0.81	0.96	0.72	1.29
rs9325057	A	G	0.09	0.73	0.50	1.05	0.26	0.79	0.52	1.19	0.62	0.92	0.67	1.28
rs1860933	A	T	0.12	1.31	0.94	1.83	0.48	1.14	0.79	1.66	0.32	1.15	0.87	1.52

rs10068913	C	T	0.10	1.31	0.95	1.82	0.32	1.20	0.84	1.73	0.44	1.11	0.85	1.46
rs10463393	C	T	0.13	0.78	0.56	1.08	0.29	0.82	0.57	1.19	0.68	0.94	0.71	1.25
rs4705047	T	G	0.20	0.76	0.50	1.16	0.51	0.86	0.54	1.35	0.50	0.88	0.61	1.27
rs12658421	A	G	0.17	0.79	0.56	1.11	0.25	0.80	0.55	1.17	0.92	0.99	0.74	1.32
rs7701442	T	G	0.20	0.65	0.34	1.26	0.49	0.78	0.38	1.60	0.65	0.87	0.47	1.61
rs13160662	A	G	0.19	0.80	0.58	1.12	0.51	1.13	0.79	1.63	0.03	0.74	0.56	0.98
rs7719581	C	T	0.31	1.24	0.82	1.87	0.91	0.97	0.61	1.55	0.29	1.21	0.86	1.70
rs2303067	A	G	0.22	1.23	0.88	1.70	0.73	0.94	0.65	1.35	0.11	1.25	0.95	1.65
rs6884703	C	T	0.26	1.26	0.84	1.90	0.79	0.94	0.59	1.49	0.16	1.27	0.91	1.78
rs7704889	A	G	0.30	1.21	0.85	1.73	0.17	1.32	0.89	1.95	0.50	0.91	0.68	1.21
rs11745768	A	G	0.24	0.71	0.40	1.26	0.18	0.65	0.34	1.24	0.83	1.06	0.61	1.85
rs1153090	A	G	0.28	1.20	0.86	1.68	0.86	1.04	0.71	1.50	0.23	1.19	0.90	1.57
rs10491343	T	A	0.32	1.38	0.73	2.62	0.42	1.34	0.66	2.70	0.99	1.00	0.61	1.64
rs1345689	G	A	0.40	0.85	0.59	1.23	0.29	0.80	0.53	1.21	0.72	1.06	0.77	1.46
rs1432975	T	A	0.41	1.18	0.79	1.76	0.43	1.19	0.77	1.85	0.89	0.98	0.71	1.34
rs7715716	G	A	0.61	1.14	0.70	1.86	0.91	0.97	0.56	1.69	0.71	1.08	0.72	1.62
rs17599675	C	T	0.48	0.87	0.60	1.27	0.73	0.93	0.61	1.41	0.90	0.98	0.71	1.35
rs6894548	A	C	0.49	1.13	0.80	1.59	0.98	1.00	0.68	1.45	0.37	1.14	0.86	1.51

rs17538716	A	G	0.55	1.15	0.73	1.83	0.09	1.53	0.93	2.50	0.11	0.75	0.52	1.07
rs1422589	G	A	0.57	0.91	0.66	1.26	0.26	0.81	0.57	1.17	0.37	1.13	0.86	1.50
rs1862330	T	C	0.53	1.12	0.79	1.60	0.29	1.24	0.84	1.83	0.47	0.90	0.67	1.20
rs9325061	G	A	0.49	0.89	0.63	1.25	0.12	0.74	0.51	1.08	0.33	1.16	0.86	1.55
rs1154729	T	C	0.48	1.15	0.79	1.67	0.05	1.51	1.00	2.27	0.07	0.76	0.56	1.02
rs2287773	T	C	0.37	1.61	0.55	4.69	0.95	0.96	0.29	3.25	0.24	1.67	0.70	3.95
rs17704205	T	G	0.88	1.04	0.60	1.82	0.82	0.93	0.50	1.74	0.64	1.12	0.69	1.82
rs17775739	T	C	0.45	0.75	0.36	1.57	0.41	0.70	0.30	1.62	0.82	1.08	0.56	2.07
rs7443321	T	C	0.45	0.75	0.36	1.57	0.41	0.70	0.30	1.62	0.82	1.08	0.56	2.07
rs4362936	T	C	0.64	1.12	0.69	1.84	0.76	0.92	0.52	1.60	0.25	1.27	0.84	1.93
rs7709676	T	G	0.50	1.16	0.76	1.79	0.06	1.57	0.99	2.48	0.07	0.74	0.53	1.03
rs17107473	A	T	0.80	1.09	0.57	2.07	0.20	0.60	0.27	1.33	0.15	1.58	0.85	2.94
rs7721995	T	C	0.71	1.07	0.75	1.52	0.28	1.24	0.84	1.82	0.28	0.85	0.64	1.14
rs1422587	A	G	0.62	0.92	0.65	1.29	0.56	0.89	0.61	1.31	0.81	1.04	0.77	1.40
rs17718041	T	C	0.81	0.92	0.45	1.88	0.16	0.52	0.21	1.30	0.10	1.81	0.88	3.73
rs6867877	A	G	0.75	0.93	0.59	1.47	0.17	1.40	0.86	2.27	0.02	0.65	0.45	0.93
rs4421091	G	A	0.49	0.89	0.63	1.25	0.56	0.89	0.61	1.31	0.94	1.01	0.75	1.36
rs4487480	A	T	0.84	1.07	0.54	2.13	0.56	1.23	0.59	2.56	0.64	0.88	0.51	1.52

rs6896204	G	A	0.77	1.12	0.52	2.42	0.31	0.62	0.24	1.58	0.16	1.64	0.82	3.29
rs6892970	C	T	0.76	1.13	0.52	2.42	0.33	0.62	0.24	1.60	0.17	1.62	0.81	3.26
rs7714069	G	T	0.77	1.12	0.52	2.42	0.44	0.70	0.28	1.74	0.24	1.50	0.76	2.95
rs6874765	A	G	NA	NA	NA	NA	0.59	NA	NA	NA	0.15	0.00	NA	NA
rs1864997	G	A	0.81	1.05	0.70	1.59	0.32	1.25	0.80	1.96	0.19	0.80	0.58	1.12
rs7732713	T	A	0.79	1.10	0.57	2.11	0.34	1.40	0.70	2.81	0.50	0.84	0.50	1.41
rs10515597	A	G	0.89	0.95	0.43	2.07	0.09	0.42	0.15	1.18	0.06	2.22	0.96	5.12
rs10515601	T	G	0.91	1.03	0.64	1.65	0.34	0.77	0.45	1.32	0.20	1.31	0.87	1.99
rs13436856	T	A	0.91	1.02	0.70	1.50	0.25	1.28	0.84	1.93	0.12	0.78	0.58	1.07
rs13188824	T	C	0.85	1.07	0.53	2.17	0.72	0.86	0.38	1.95	0.42	1.30	0.69	2.47
rs7725292	A	G	0.86	1.04	0.65	1.67	0.27	1.33	0.80	2.21	0.19	0.78	0.54	1.13
rs10491340	G	A	0.96	0.98	0.43	2.22	0.11	0.42	0.14	1.26	0.07	2.23	0.91	5.50
rs17774892	G	A	0.85	1.08	0.50	2.33	0.16	0.50	0.18	1.34	0.08	1.96	0.92	4.16
rs9325073	C	G	0.88	1.03	0.70	1.53	0.66	1.10	0.72	1.70	0.62	0.92	0.67	1.27
rs17637711	T	C	0.90	1.06	0.44	2.58	0.70	0.81	0.29	2.32	0.43	1.39	0.61	3.19
rs13185274	A	G	0.81	0.96	0.66	1.38	0.98	1.00	0.66	1.49	0.77	0.96	0.70	1.30

†A1 is the minor allele, OR the odds ratio, L95 & U95 are the lower and upper limits to the 95% confidence interval and P column indicates the P value for the association. A. 367 food allergic cases vs 156 non-food allergic controls. B. 199 food sensitised-tolerant cases vs 156 non-food allergic controls. C. 367 food allergic cases vs 199 food sensitised-tolerant cases.

Table 3. A. Associations between SPINK5 SNPs and food allergy in replication sample adjusted for ancestry using principal components. **B.** Meta-analysis of discovery and replication associations between SPINK5 SNPs and food allergy

	A		*B. Replication results*					*C. Meta-analysis results*					
SNP	A1	A2	OR	SE	L95	U95	P	P	P(R)	OR	OR(R)	Q	I
rs9325072	T	C	0.72	0.22	0.47	1.10	0.13	0.71	0.62	1.07	1.42	0.0006	91.59
rs4705054	T	A	0.79	0.19	0.54	1.15	0.22	0.59	0.66	1.09	1.22	0.005	87.06
rs9325071	G	A	1.58	0.17	1.13	2.20	0.007	0.0004	0.0004	1.65	1.65	0.62	0

†A. Replication analysis adjusted for gender, ancestry (by principal components) and study. B. Meta-analysis P(R) is the P-value estimate deriving from the random-effects meta-analysis model, OR(R) is likewise the odds ratio estimate derived from the random-effects model. Q is the P-value from Cochrane's Q statistic of effect size heterogeneity between studies and I is the I2 heterogeneity index (0-100)