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Grandmaternal smoking increases asthma risk in grandchildren: a nationwide Swedish cohort

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ABSTRACT

Background: There is growing interest in exposures prior to conception as possible risk factors for offspring asthma. Although partially supported by evidence from limited human studies, current evidence is inconsistent, and based on recall of exposure status.

Objective: We aimed to investigate grandmaternal smoking during pregnancy and the risk of asthma in grandchildren using prospectively collected population-based data.

Methods: Information on grandmaternal and maternal smoking during pregnancy and grandchild use of asthma medications was collected from national Swedish registries. Associations between grandmaternal smoking during pregnancy (10-12 weeks), and asthma medication use in grandchildren were investigated using generalized estimating equations. Ages at which asthma medications were prescribed classified childhood asthma into never, early transient (0-3years), late onset (3-6 years) and early persistent (0-3 and 3-6 years) phenotypes.

Results: From 1982 to 1986, 44,583 grandmothers gave birth to 46,197 mothers, who gave birth to 66,271 grandchildren (born 1996-2010). Children aged 1-6 years had an increased asthma risk if their grandmothers had smoked during pregnancy, with a higher risk for more exposure (10+ cigs/day; adjusted OR 1·23; 1·17, 1·30). Maternal smoking did not modify this relationship.

Conclusions & clinical Relevance: Children had an increased risk of asthma in the first six years of life if their grandmothers smoked during early pregnancy, independent of maternal smoking.

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Importantly this exhibited a dose-response relationship and was associated with a persistent childhood asthma phenotype. These findings support possible epigenetic transmission of risk from environmental exposures in previous generations.

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Introduction

Asthma is a Health Priority Area in many regions worldwide, and remains the most common chronic disease in childhood. It affects 334 million people including 14% of children globally and the prevalence is continuing to rise.[1] There is emerging evidence that early childhood asthma may be linked with chronic obstructive pulmonary disease (COPD) in later life, an association which further increases the lifetime burden of asthma.[2]

As a heterogeneous condition, asthma is better understood in terms of phenotypes classified according to differences in age of onset, severity, and associated features. This concept was highlighted by Martinez et al [3] and has since been widely explored in many studies with differing methods.[4, 5] It is important to identify asthma phenotypes as they are likely to have differing underlying aetiologies and long term outcomes, but moreover, they are likely to require differing prevention and management approaches.

We have few answers concerning the aetiology of asthma. It is known to "run in families" with heritability estimates between 36% and 79%.[6] However, this high heritability has not been fully explained by genetic associations found in Genome Wide Association Studies.[7, 8] Environmental factors are believed to be responsible for the rapid rise in asthma prevalence in recent decades due to: the rapid time course; vastly different prevalences between and within countries[9], and; the inherited asthma risk of migrants.[10] Although there are established risks with some environmental exposures including; early childhood respiratory infection[11], atopy[12], traffic related air pollution[13], and tobacco smoke[14], most asthma remains unexplained.

Recently it has been suggested that environmental exposures may cause an inherited risk of asthma without changes in the genetic code. This is thought to occur through alteration of epigenetic programming that affects gene expression. Furthermore, it is believed that the "epigenetic marks" that occur from environmental exposures may be transmissible to future unexposed generations.[15] Establishment of this risk may contribute to our understanding of the reasons behind the rapid rise in asthma. In addition, an understanding of the possible inherited effect from exposures in previous generations may clarify the relationships between current exposures and risks.

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Tobacco smoke has been investigated for its potential role as a transgenerational risk factor for asthma. The previous literature in humans is scarce and inconsistent with only three published accounts.[16-18]. Two of these found an increased risk of asthma in the grandchildren of grandmothers who smoked whilst mothers were in utero. [16, 17] The third study, however, could not confirm this finding and suggested instead an increased risk for the daughters of fathers exposed in utero to smoking grandmothers. [18] . None of these studies had prospectively collected exposure measures. Although two of these studies are population based cohorts[17, 18], they require voluntary participation and are likely to differ from a whole population sample on important variables, including smoking prevalence and socio-economic status, along with many other factors which may influence the relationship between grandmaternal smoking and grandchild asthma[19].

We aimed to investigate tobacco smoke exposure as a transgenerational risk factor for asthma using prospectively collected data from the entire Swedish population.

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Methods

Anonymized data were obtained from the Swedish National Board of Health and Welfare and Statistics Sweden. These data were routinely collected as part of the medical record and have been approved and utilized for research of this kind without direct informed consent according to special register legislation. Permission for access to these data for this analysis was granted by the regional

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ethical committee of Umeå, Sweden. Cross-linkage between registries was performed using each individual's unique personal identification number.

Study Population

Eligible participants were Swedish families with information on three generations (grandmother, mother and child) within the pre-defined dates as explained in the following. Women giving birth to a female child in Sweden between 1982 and 1986 were designated grandmothers. Female children from the grandmothers who also gave birth to at least one child between 1996 and 2010 were designated mothers. The children (of either sex) from these mothers born in Sweden between 1996 and 2010 were designated children.

Exposure definition

Smoking in grandmothers was recorded at 10-12 weeks gestation as a three-level categorical variable.[20] Grandmothers were asked by specially trained midwives using a standardized questionnaire if they were currently smoking. Responses were categorized as: 1: No; 2; Smoking 1-9 cigarettes per day and; 3: Smoking 10 or more cigarettes per day. This information was used to create both a binary (smoking yes/no) and categorical variable for analysis.

Outcome definition

The Swedish Prescription Register started recording prescribed medications on 1/7/2005. All asthma medications purchased for the children between 1/7/2005 and 31/12/2013 were recorded.[21] Asthma was defined by the recorded purchase of steroid or leukotriene medications by the children in any given year between 1/7/2005 and 31/12/2013. For the purpose of this variable, children using only beta agonist medication were excluded. Childhood wheeze or asthma was defined by the purchase of any asthma medication (beta-agonists, inhaled steroids or leukotrienes) by the children in any given year between 1/7/2005 and 31/12/2013.

Other Variables

Smoking in mothers was recorded in registry data similarly to grandmothers (10-12 weeks gestation/ first trimester visit) with additional smoking questions in the third trimester (30-32 weeks).The smoking categories were the same as for grandmothers (not smoking, 1-9 cigarettes per day and 10 or more cigarettes per day). A further question concerning smoking was also asked of mothers: Were you smoking three months prior to this pregnancy? Mothers were also asked about exposure to oral moist tobacco snuff in the form of snus which has a 200-year history of use in Sweden. Use of snus in early pregnancy is reported by 2-3% of all women.[22] Snus exposure was recorded as a binary variable in the same time windows (ie: three months prior to pregnancy; in first trimester (10-12 weeks) and in third trimester (30-32 weeks). Nicotine exposure in mothers was defined as exposure to smoking or snus at any of these recorded time points.

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Grandmothers variables

Other recorded variables for grandmothers from the Medical Birth Register, the Educational Register, the Social allowance register, the Patient Register (Inpatient 1997-2012 and outpatient 2001-2012) and the Cause of Death Register included: age at mother's birth, BMI at 10-12 weeks gestation, marital status, asthma /hypertension/diabetes diagnosis at time of birth of mother, highest formal education, social allowance recipient, and county of residence at child's birth.

Mothers variables

Variables recorded for mothers from the Medical Birth Register, the Educational Register, the Social allowance register, the Patient Register (Inpatient 1997-2012 and outpatient 2001-2012) and the Prescription Register (2005-2013) included: prescribed asthma medication, asthma /hypertension/diabetes diagnosis at time of birth of children, highest formal education, social allowance, marital status, paid work, body mass index (10-12 weeks), mother's age at child's birth, gestational age, birth weight, Apgar score at 1 and 5 minutes, caesarian section, birth order. A variable of complicated childbirth was defined by any complications recorded during pregnancy and birth. (ICD8) We defined maternal asthma as any inpatient or outpatient admission for asthma and/or any use of asthma medication.

Childs variables

Variables recorded for children from the Medical Birth Register, the Patient Register (1997-2012 for inpatient care and 2001-2012 for outpatient care) and the Prescription Register (2005-2013) included: gestational age, birth weight, Apgar score at 1 and 5 minutes, mode of delivery (caesarian section vs vaginal), birth order, age, gender, any asthma medication and date purchased, specific asthma medication (steroids, Leukotriene inhibitors) and date purchased, inpatient and outpatient hospital visits for asthma

Grandfathers variables

A proxy variable for grandfathers smoking was defined using records of smoking related illnesses and deaths (Lung cancer, COPD) recorded from the Patient Register as hospital admissions (1997-2012) or outpatient attendances (2001-2012) or from the Cause of Death Register. Grandfather's smoking was defined as any inpatient or outpatient hospital visit for COPD or lung cancer, or COPD or lung cancer noted on the death register.

Statistical Methods

Logistic regression analyses with generalized estimating equations were used to account for inclusion of more than one child from each grandmother. Initial observations were performed on the entire sample, without allowance for year of age when medication was purchased. These analyses were then repeated stratified by age (year) of the child. A further analysis was performed for child asthma phenotypes for the subset of children with available information at all time points. Asthma phenotypes

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were defined as: 1. No asthma: No purchased asthma medication up to sixth year; 2. Early transient wheeze: only purchased at least one asthma medication before the third year; 3. Early persistent asthma: purchased at least one asthma medication before the third year and also between the third and sixth years; 4. Late onset asthma: no purchased asthma medication before the third year but purchased at least once between the third and sixth years.[3]

Variables tested as confounders and/or effect modifiers in the analysis included: child's gender, child's birth order, child's gestation, child's birthweight, mother's years of education, mother's family allowance, maternal nicotine exposure (smoking and snus), mother's birth order, mother's county of residence at child birth, mother's age (quartiles), mother's BMI, mother's asthma, mother's caesarian section, mother's pregnancy complications, mother's diabetes, grandmother's county of residence at child birth, grandmother's years of education, grandmother's social allowance, grandmother's asthma, grandmother's age (quartiles), grandmother's BMI, grandmother's diabetes, grandmother's hypertension, grandmother's pregnancy complications, proxy for grandfather's smoking. Variables were included in final models if effect estimates changed by >10% (confounders) or had a p-value <0·10 (effect modifiers). A model for confounders was also derived using directed acyclic graphs (DAGGITY software) (Supplement Fig 1)

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Results

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In the grandmother's generation, we identified 54,085 women who gave birth to 56,406 daughters in Sweden between 1982 and 1986, who in turn had given birth to 81,550 children in Sweden between 1996 and 2010. There were however 9,502 grandmothers who had no recorded information on smoking in at 10-12 weeks gestation. This lack of recorded information was related to the timing of implementation of data collection on smoking in the first trimester of pregnancy which began in January 1982. Data on smoking habits were, therefore, usually missing for those giving birth during the first part of 1982. We restricted the analysis therefore to 44,583 grandmothers with smoking information who had given birth to 46,197 mothers and who in turn had given birth to 65,956 children. Of these children: 13,442 had purchased asthma medication between 1/7/2005 and 31/12/2013; 10,119 had had an outpatient hospital visit for asthma since 2001 and; 3,303 children had been admitted for asthma or bronchitis at least once between 1997 and 2012. (Tables 1 and 2).

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Table 1 Grandmothers and Mothers demographics

		Grandmothers	Mothers
Mean age at mothers/child's birth years (range)		25·88 (13-52)	23·35 (13-28)
Mean body mass index (10-12 weeks gestation) (range)		22·20 (14·01-46·26)	24·66 (14·30 –54·97)
First child			39.31%
Caesarean section	No CS	91·44%	

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	Elective CS	4·26%	
	Emergency CS	4·30%	
Marital status	Married	93·05%	
	Single mother	3·44%	
	Other	3·51%	
Smoking 10-12 weeks	No smoking	59.12%	84.83%
	1-9 cigs/day	23.77%	12.19%
	10+ cigs/day	17.11%	2.99%
Diabetes (checkbox)		0·74%	
Hypertension (checkbox)		0·21%	
Asthma (Impatient or outpatient hospital contact)			3·37%
Purchased Asthma medication (any)			17·93%
Purchased medication (steroids/ Leukotriene inh.)			10·68%
Complicated Childbirth	No	64·79%	
	Yes	35·21%	
Highest formal education	<=11years	66·93%	24·48%
	12 years	12·26%	47·56%
	13+years	20·81%	27·96%
Received social allowance		3·01%	6·9%

Table 2 Child's demographics

Gender	male	51·58%
	female	48·42%
First child		68·79%
Purchased Asthma medication (any)		20·45%

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Purchased Asthma medication (steroids/Leukotriene inh.)		16·90%
Asthma (Inpatient or outpatient hospital contact)		8·85%
Any asthma medication by year of life	Year1	1·68%
	Year2	8·45%
	Year3	10·79%
	Year4	9·54%
	Year5	8·45%
	Year6	7·20%
	Year7	6·77%
	Year8	6·36%
Any Steroids/LT medication by year of life	Year1	1·19%
	Year2	6·74%
	Year3	9·19%
	Year4	8·23%
	Year5	7·15%
	Year6	6·02%
	Year7	5·30%
	Year 8	4·90%

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There was an increased risk of asthma or wheeze (purchase of any asthma medication between 1/7/2005 and 31/12/2013) in children whose grandmothers had smoked. This was found in the unadjusted model and models adjusting for: only maternal nicotine exposure and; for the 2 fully adjusted models (confounders chosen by: 1 evidence of change on odds ratios and by 2 Directed acyclic graph (supplement Fig 1)) (Table 3). The risk increased with increasing levels of grandmaternal smoking. There was a significant trend for increasing asthma risk in grandchildren with increasing cigarette consumption by grandmothers. (Table 3). No interactions were found for the variables outlined in methods for the association between grandmaternal smoking during pregnancy and asthma in grandchildren (all interaction p values > 0·1). The same pattern was seen when the outcome was inpatient or outpatient hospital visits for asthma or bronchitis (Supplement Table 1). The relative risk increase in asthma from grandmaternal smoking was 15% and the relative risk increase in hospital contact was 19%.

GMS at 10-12 weeks gestation

0 (ref)	1-9cigs/day	10+ cigs/day	P trend
Odds of asthma medication use in the child (unadjusted) Odds Ratio & 95% CI, [p] (n/N) N=65,956			
1 (7,416/38,992)	1·20 (1·14-1·26) [0·000] (3,456/15,680)	1.25 (1·19-1.32) [0·000] (2,571/11,284)	0.002
Odds of asthma medications in the child (adjusted for any maternal nicotine exposure) Odds Ratio & 95% CI, [p] (n/N) N=63,580			
1 (7,149/37,627)	1·17 (1·11– 1·23) [0·000] (3,319/15,070)	1·19 (1·12-1·25) [0·000] (2,451/10,883)	0.004
Odds of asthma medications in the child (adjusted*) Odds Ratio & 95% CI, [p] (n/N) N=48,971			
1 (5,496/29,165)	1·13 (1·07– 1·20) [0·000] (2,490/11,391)	1·15 (1·08– 1·23) [0·000] (1,911/8,415)	0.069
Odds of asthma medications in the child (adjusted for confounders identified by DAG) Odds Ratio & 95% CI, [p] (n/N) N= 62,899			
1 (7,154/37,678)	1.18 (1.12- 1.24) [0·000] (3,247/14,800)	1.23 (1.17 -1.30) [0·000] (2,382/10,421)	0.013

Table 3: Grandmaternal smoking (GMS) and the odds of any asthma medication use in the child

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* Adjusted for child's gender, child's birth order, mother's years of education, mother's family allowance, maternal nicotine exposure (smoking and snus), mothers birth order, mother's residence at child birth, mothers age (quartiles), grandmother's residence at child birth, grandmother's years of education, grandmother's social allowance, grandmother's asthma, grandmother's age(quartiles), grandmother's BMI, proxy for grandfather's smoking. Partially and Fully adjusted models have reduced numbers due to incomplete information on included covariates

DAG adjustments - grandmother's years of education, grandmother's social allowance, grandmother's asthma and proxy for grandfather's smoking

Stratification by Age

When stratified by age of purchased medications (Supplement Tables 2 & 3) there was a similar pattern for both any purchased asthma medications and any anti-inflammatory asthma medications. There was an increased risk of asthma or wheeze from ages one to six, but these associations reduced in strength at ages seven and eight.

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Asthma Phenotypes

Grandmaternal smoking was related to an increased risk of early persistent asthma whilst maternal smoking was related to an increased risk of the early transient asthma (Table 4). When a child had both grandmother and mother exposed, the risk of both early transient and early persistent asthma was increased. Neither grandmaternal nor maternal smoking was related to late onset asthma.

Table 4: Phenotypes of asthma (anti-inflammatory meds) in grandchildren by grandmaternal smoking (GM + or -) and maternal smoking (M + or -)

		GM- & M-	GM+ M-	GM- M+	GM+ M+
	N	Odds Ratio & 95% CI, [p] (n/N)			
Early Transient (0-3years)	10456	1 (121/3,395)	0·95 (0·71-1·29) [0·762] (70/2,033)	1·27 (0·97,1·66) [0·081](105/2,267)	1·38 (1·07,1·78) [0·014](141/2,761)
Early persistent (Both times)	10762	1 (197/3,471)	1·45 (1·17,1·79) [0·001](172/2,135)	1·10 (0·88,1·38) [0·392](145/2,307)	1·43 (1·17,1·75) [0·001](229/2,849)
Late Onset (3-6years)	10774	1 (257/3,531)	0·93 (0·75,1·16) [0·530](144/1,963)	0·92 (0·75,1·14) [0·445](155/2,317)	0·98 (0·80,1·19) [0·815](199/2,819)

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Discussion

Using prospectively collected data from national Swedish registers, we found that grandmaternal smoking whilst mothers were in utero was associated with an increased risk of asthma (use of prescribed asthma

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medications) in grandchildren aged one to six years. This risk was increased for the children of both non-smoking and smoking mothers. Our research is novel in three key respects. Firstly, it is the only study in this area to use prospectively collected data on smoking in pregnancy, thereby silencing concerns about recall bias. Secondly, we found a "dose-response" effect with higher levels of grandmaternal smoking associated with greater asthma risk, which adds to the evidence for a potentially causal link. And finally, we found a relationship between grandmaternal and maternal smoking and, distinct phenotypes of childhood asthma; maternal smoking was associated with the early transient asthma phenotype, whilst grandmaternal smoking was associated with the early persistent asthma phenotype.

Only three published studies have previously investigated this association. In 2005, Li et al[16] found that children were at an increased risk of asthma if their grandmothers had smoked whilst their mothers were in utero (n=691). This risk was elevated even when the mothers were non-smokers. Recently, the Norwegian Mother and Child cohort study, with much larger numbers of participants, produced a similar finding for asthma at 36 months and seven years of age.[17] In contrast, the Avon Longitudinal Study of Parents and Children found no increased risk to grandchildren from maternal exposure in smoking grandmothers[18]. They found, however, a suggestion of increased risk in the daughters of fathers who had been exposed to smoking grandmothers. Unlike the ALSPAC study, we found no differential effects according to the sex of the offspring and no evidence of modification by grandchild BMI. In all three studies, grandmaternal smoking was retrospectively reported by mothers (or other relatives) and is subject to recall bias. Additionally, although population based, these previous studies were subject to responder bias. Furthermore, none of the studies recorded the level of grandmaternal smoking.

The finding of distinct relationships with specific childhood asthma phenotypes is novel and highlights a potentially different mechanism for asthma development dependent upon whether the tobacco exposure was maternal or grandmaternal. We have previously shown that childhood wheeze phenotypes may be related to different early life exposures [23] and different long term consequences.[24] In contrast to our current findings, we had previously found late onset childhood asthma to be associated with parental tobacco use. Our previous data recorded symptomatic wheeze multiple times in early childhood rather than prescribed medication. In addition, using latent class analysis, the late onset wheeze group was aged greater than 4 years. Both of these differences may help to explain these apparently discrepant findings. Our current findings support the hypothesis that different phenotypes may be related to different exposures. Grand maternal exposure may result in epigenetic methylation of germline cells which may arguably have a greater effect than later environmental exposures, reflected in a persistent rather than transient asthma phenotype.

Recent reviews have helped to differentiate between transgenerational and intergenerational effects. [15, 25] Essentially, transmission can only be considered transgenerational when an exposure in grandmothers, (F0) has been shown to increase the risk in great grandchildren (F3). Exposures to the grandmothers while the mothers (F1) are in utero will also expose the primordial germ cells of the grandchildren (F2). Hence to

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avoid any suggestion of direct exposure, the F3 generation must be considered. Although there are no human studies which have addressed the impact on great grandchildren, there are compelling animal studies that suggest this is possible. In 2012 Rehan et al[26] demonstrated an inherited asthma phenotype in the second generation (F2) offspring of nicotine exposed grandmaternal mice. The grand dams had been given subcutaneous nicotine in a dose equivalent to habitual smokers (1mg/kg/day body weight) whilst the first generation (F1) offspring were in utero. Affected F2 generation rats had measured lung function changes including increased lung resistance and compliance which were reflected in pathological findings including increased contractile proteins and fibroblasts. Furthermore, they provided evidence of transferred germline epigenetic marks to the F1 generation. In 2013, an extension of this work was published, in which similar changes in the third (F3) generation (great grandchildren) were found. [27]

Although, none of the current human studies span four generations, epigenetic transmission of environmental risk has been demonstrated in other eukaryotic organisms and experimental transgenerational silencing of parts of the mammalian genome has also been shown[25]. Given these findings, it is plausible this could also be happening in humans and that the association in the current study may represent transgenerational transmission. The mechanism of transgenerational transmission is thought to be through inherited epigenetic change. There is some discussion around the ability of epigenetic marks to be transmitted between generations in humans providing a mechanism for the transmission of environmental risk, however there is good evidence from both plant and animal models. [15] In humans, epigenetic marks were thought until recently to be entirely removed after fertilization making this an unlikely mode of transmission. However, recent evidence supports the preservation of a proportion of epigenetic marks during this process. [28, 29]

The strengths of our study include the collection of large numbers of whole population data, prospective measurement of smoking data in pregnancy of both mothers and grandmothers and a measured level of smoking. A recent validation demonstrated very good agreement between reported smoking in the register and cotinine levels in maternal and cord blood from deliveries between 1982 and 2000.[20]. In utero smoke exposure was measured at only one time in the first trimester (10-12 weeks) which has previously been recognized as the most sensitive period for epigenetic change[30]. There is potential for misclassification of smoking status during the entire pregnancy if grandmothers smoked only in the second and third trimesters. This would lead to smoking grandmothers being classified erroneously as non-smoking grandmothers. As this information was collected prospectively, it is unlikely to be differential in terms of grandchild medication use. Additionally, if some participants stopped smoking after the first trimester then duration of smoking and potentially risk to the fetus may differ[30]. The likely result of both these misclassifications is to bias our findings towards the null. The found associations remain valid but are probably underestimates of the true effect.

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Additionally, our outcome measures, prescribed asthma medications and hospital admissions, are also objective and prospectively collected. Although the definition used to determine asthma will influence the prevalence estimate, and may thereby modify the relationship between an exposure and this outcome, it will not invalidate any relationship found. A more liberal definition of asthma (as defined by ISAAC questions rather than medication use), may increase the prevalence of asthma providing greater power to find an effect. In contrast, a highly specific but non-sensitive definition of asthma (medication use) may underestimate the degree of the true relationship. However, asthma medication use is more specific compared with questions on wheeze and the relationship found is more likely to reflect childhood asthma rather than transient early wheeze. Although access to and the costs of medication may be an issue in many countries, this is less relevant in Sweden, where there is high cost protection for purchasing children's medications. It is possible that some parents may not follow western medical practices. The misclassification of these children, however is unlikely to be differential, depending on whether or not grandmothers smoked, so would most likely cause a reduction in the effect size found. \We have no information concerning smoking behavior of grandparents other than the maternal grandmother. This lack of information, if non-differential, would result in reduced associations being seen in the current study, indicating that the true associations are likely greater than those found. We also have no information concerning fathers smoking behavior. Fathers smoking, however, occurs after and cannot plausibly influence grandmothers smoking so is not a true confounder. As our primary aim was to follow-up the grandchildren (F2) of our registry cohort of grandmothers (F0), we have inadvertently selected a younger population of mothers (F1 generation). It is possible that the effects of grandmaternal smoking may vary depending on the age at which the mothers (F1) had children. It is possible that a longer period of time between in -utero exposure of the mothers to grandmaternal smoking and conception of the grandchild generation may either increase the risk of asthma (if ageing of the primordial germ-cells increases the likelihood of sustained epigenetic change) or decrease the asthma risk, if epigenetic marks fade over time. Although this was a study based only on the Swedish population, the findings are likely to be relevant globally.

Many of the mother's variables may be mediators of the relationship between grandmaternal smoking and asthma in children. These include mother's: BMI, asthma, caesarian section, or pregnancy complications. Inclusion of maternal asthma in the models did not significantly change the associations (Supplement table 4) suggesting that this is unlikely to be a mediator. Although a formal mediation analysis has not been performed, inclusion of these variables in adjusted models may attenuate the found associations, underestimating the true association between grand maternal smoking and grandchild asthma.

Conclusion

Grandmaternal smoking during pregnancy is associated with an increased risk of early persistent asthma in grandchildren. These findings support possible epigenetic transmission of risk from environmental exposures in previous generations and will add further impetus to tobacco cessation campaigns with renewed

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focus on pregnant mothers. Additionally, taking preconception exposure into account when analyzing data in this area may help to produce more consistent results and untangle important environmental exposures for asthma risk. Understanding and investigating the possible transgenerational transmission of disease is applicable to a wide range of diseases.

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Declaration of interests

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No authors have relationships with organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Authorship

All authors on this paper fulfill the criteria for authorship. LB, DO and BF acquired the data. All authors contributed substantially to the conception, design and interpretation of the work. CL produced the initial draft which was revised following the input of all authors. All authors have approved the final version for publication.

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