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Original Article

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Does parental farm upbringing influence the risk of asthma in offspring? A three-generation study

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Abstract

Background: A farm upbringing has been associated with lower risk of asthma and methylation of asthma-related genes. As such, a farm upbringing has the potential to transfer asthma risk across generations, but this has never been investigated. We aimed to study the generational effects from a parental farm upbringing on offspring asthma.
Methods: Our study involved three generations: 5759 participants from the European Community Respiratory Health Survey (ECRHS) study (born 1945–1971, denoted G1), their 9991 parents (G0) and their 8260 offspring (G2) participating in RHINESSA

(Respiratory Health In Northern Europe, Spain and Australia). Questionnaire data were collected on G0 and G1 from G1 in 2010 and on G2 from themselves in 2013. The parental/grandparental place of upbringing was categorized: (i) both parents from farm; (ii) mother from farm, father from village/city; (iii) father from farm, mother from village/city; (iv) both parents from village or one parent from village and one from city; (v) both parents from city (reference group). Grandparental upbringing was equivalently categorized. Offspring asthma was self-reported and data were analysed using Cox-regression models with G2 age as the time scale.

Results: A parental farm upbringing was not associated with offspring asthma when compared with city upbringing [hazard ratio (HR) 1.12, 95% confidence interval (CI) 0.74–1.69]. Findings remained similar when stratified by offspring upbringing and asthma phenotypes. Quantitative bias analyses showed similar estimates for alternative data sources. A grandparental farm upbringing was not associated with offspring asthma in either the maternal (HR 1.05, 95% CI 0.67–1.65) or paternal line (HR 1.02, 95% CI 0.62–1.68).

Conclusions: This multigenerational analysis suggests no evidence of an association between parental/grandparental farm upbringing and offspring asthma.

Key words: Asthma, ECRHS, RHINESSA, farm upbringing, generation study, generational effects

Key Messages

- A farm upbringing has been suggested to reduce the risk of asthma and potentially induce epigenetic changes related to asthma, suggesting that a farm upbringing has the potential to transfer asthma risk across generations, but this has never been investigated.
- In our three-generation study, we observed no evidence of an association between farm upbringing in previous generations and offspring asthma, either for parental or grandparental upbringing.
- These null findings were consistent when stratified by the offspring's own upbringing or by asthma phenotypes.
- A quantitative bias analysis showed that the results were similar regardless of whether the information on upbringing was provided by the parent themselves or as second-hand information by their offspring.

Background

Asthma prevalence has risen steeply during the last decades and several hypotheses have been proposed for this increase.[1] One of the most promising explanations is the Hygiene Hypothesis, subsequently modified to the Microbial Diversity Hypothesis, which suggests the development of a compromised immune system due to low exposure to microbes in early life.[2–4] Studies suggest that farm exposure in early life reduces the risk of asthma, which has been attributed to greater microbial diversity, i.e. from stable dust and unpasteurized farm milk,[5–9] leading to immunomodulatory changes.

Early exposure to a farm environment has been found to influence methylation in asthma-related genes at age 4.[10] Furthermore, one study indicated that the CD14-promoter region was differently methylated in placentae among mothers living on a farm compared with mothers not living on a farm.[11] This suggests that exposure to a farming environment might cause intergenerational effects through the induction of changes to gene expression. Whereas there is accumulating evidence that adverse exposures, such as smoking, prior to conception might play a role in the aetiology of asthma,[12,13] there is little evidence on the potential effect of microbial exposure as a preconception protective factor for offspring asthma.[14]

Evidence for generational effects from farming on asthma mainly arise from epigenome studies in animals[14] but, as of yet, this has never been investigated in an epidemiological study. Such information may help us to identify critical exposure periods and, in the long run, enable targeted intervention strategies for individuals at high risk of

subsequent asthma development. Therefore, the aim of this study was to investigate the generational effect of early farm exposure on asthma in offspring in an international multicentre and generational study.

Methods

Study population

The present study is nested within two cohort studies: the ECRHS (European Community Respiratory Health Survey) and RHINESSA (Respiratory Health in Northern Europe, Spain and Australia). The ECRHS collected information from the parents (G1) of the offspring (G2) who were investigated within RHINESSA (Figure 1).

In 1988–1992, the ECRHS randomly included a population-based sample of 1500 men and 1500 women born in 1945–1973 from each of the participating study centres across Europe.[15] The RHINESSA study included ECRHS participants (G1), their parents (G0) and their offspring (G2) in the following ECRHS centres: Denmark (Aarhus), Norway (Bergen), Sweden (Gothenburg, Umeå, Uppsala), Iceland (Reykjavik), Estonia (Tartu), Spain (Albacete, Huelva) and Australia (Melbourne).

Data collection and definitions

G1 provided information via the ECRHS questionnaire in 2010 and G2 provided information via the RHINESSA questionnaire in 2013. G1 started as a population-based study and did not include spouses of the participants. Therefore, we collected information on the spouse of G1 participants via G2. G2 also provided information on the place of upbringing of G0 via the RHINESSA questionnaire and G1 provided information on G0 smoking and asthma via the ECRHS questionnaire.

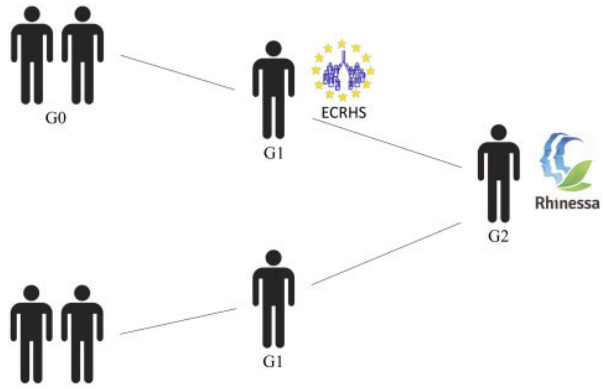

Figure 1. Three generations G0, G1 and G2 derived from the two cohorts ECRHS (European Community Respiratory Health Survey) and RHINESSA (Respiratory Health In Northern Europe, Spain and Australia).

G2 asthma status was defined as an affirmative answer to *'Do you have or have you ever had asthma?'* and a reported age of onset. G1 provided information on their own and their parents' (G0) asthma status via the same question. G2 hay fever was defined as an affirmative answer to *'Do you have any nasal allergies including hay fever?'*. Information on parental place of upbringing was reported by the ECRHS parent themselves (G1) and by their offspring (G2) via the question '*What term best describes the place you (your father, your mother) lived most of the time before the age of five years?'*, with response categories: (i) farm with livestock, (ii) farm without livestock, (iii) village in a rural area, (iv) small town, (v) suburb of city and (vi) inner city. G2 also gave information on grandparental (G0) place of upbringing. The groups were merged as follows: a + b as 'farm', c + d as 'village' and e + f as 'city', assuming the exposure level to be reasonably similar within the merged groups.

As the initial analyses showed similar estimates for maternal and paternal upbringing separately, these two variables were merged into a combined parental exposure variable. Parental (G1) place of upbringing was categorized as five groups after merging father's and mother's upbringing: (i) both parents from farm; (ii) mother from farm, father from village/city; (iii) father from farm, mother from village/city; (iv) both parents from village or one parent from village and one from city; (v) both parents from city (reference group). The grandparental place of upbringing was analysed in the same way.

Statistical analyses

Data were analysed in Cox-regression models with G2 age as the time scale and presented as hazard ratios (HRs) with the corresponding 95% confidence intervals (CIs) adjusted for potential confounders taking clustering within families into account via robust standard errors. Although the data collection is cross-sectional, the information given by the subjects specifies the exact time of exposure and duration and age at onset of the outcome, which provided the opportunity for longitudinal data analysis. Subjects were assumed to be at risk from birth and censored at the time of asthma onset or at the end of follow-up, whichever appeared first. Thereby, the Cox models account for the fact that the study participants (G2) are participating with different follow-up times according to their different ages, which is a more robust method of investigating the relevant associations.

A minimum set of confounders was identified using Directed Acyclic Graphs (DAGs) via the software DAGitty 2.3. Adjustment for this set of confounders blocks any known backdoor paths between the exposure and the

outcome. Factors such as smoking, asthma status, socioeconomic status, gene expression (as an epigenetic marker), microbial exposure, etc. were included in the DAG. From this, the minimal adjustment set for the association between parental (G1) place of upbringing and offspring (G2) asthma were identified to be: G0 asthma, G0 place of upbringing and G0 smoking (Supplementary Figure 1, available as Supplementary data at *IJE* online). As the prevalence of farm upbringing varies markedly between study centres, an a priori decision was made to adjust for study centre. Analyses on parental (G1) place of upbringing and offspring (G2) asthma were presented as crude and adjusted estimates in two models. *Adj1* was adjusted for centre and place of upbringing, available for all four G0 grandparents, and *adj2* was adjusted for centre and all confounders identified in our hypothesized DAG. However, whereas data on the place of upbringing was available for all G0 grandparents, information on smoking and asthma was only available for half of the G0 grandparents.

A separate analysis on grandparental (G0) place of upbringing and offspring (G2) asthma was also performed, but no adjustments were made due to a lack of data on the great-grand generation.

Secondary analyses included stratification by G2 place of upbringing and analyses on subjects with hay fever to specifically investigate the allergic-asthma phenotype. Furthermore, we conducted a quantitative bias analysis to investigate the potential bias from using second-hand information on parental place of upbringing from the offspring (G2) instead of direct information from the parent

Table 1. Characteristics of the study population: participants in the European Community Respiratory Health Survey (ECRHS, G1), their parents (G0) and their offspring participating in Respiratory Health In Northern Europe, Spain and Australia (RHINESSA, G2)

	Parental (G1) place of upbringing					
	Both parents from farm	Mother from farm, father from village/city	Father from farm, mother from village/city	Both parents from village or one from village and one from city	Both parents from city	Missing
Offspring (G2), *N* (%)	405 (5%)	790 (10%)	866 (10%)	3553 (43%)	2246 (27%)	400 (5%)
Offspring (G2) age, mean ± SD	31.95 ± 7.33	31.17 ± 7.63	31.12 ± 7.68	30.21 ± 7.69	30.39 ± 7.57	29.13 ± 7.90
Offspring (G2) sex, *N* (%F)	218 (54%)	474 (60%)	504 (58%)	2069 (58%)	1283 (57%)	233 (58%)
Offspring (G2) smoking status						
Never smoker, *N* (%)	283 (70%)	538 (68%)	590 (68%)	2343 (66%)	1476 (66%)	174 (43%)
Current smoker, *N* (%)	42 (10%)	90 (11%)	99 (11%)	448 (12%)	307 (14%)	59 (15%)
Ex-smoker, *N* (%)	79 (20%)	160 (20%)	172 (20%)	739 (21%)	456 (20%)	58 (15%)
Missing, *N* (%)	1 (0%)	2 (0%)	5 (1%)	23 (1%)	7 (0%)	109 (27%)
Offspring (G2) asthma, *N* (%)	79 (20%)	146 (18%)	171 (20%)	628 (18%)	415 (18%)	51 (13%)
Missing, *N* (%)	2 (0%)	2 (0%)	1 (0%)	15 (0%)	11 (0%)	109 (27%)
Centre						
Aarhus (DK), *N* (%)	35 (9%)	100 (13%)	98 (11%)	349 (10%)	265 (12%)	45 (12%)
Albacete (ES), *N* (%)	3 (1%)	6 (1%)	4 (0%)	53 (1%)	8 (0%)	1 (0%)
Bergen (NO), *N* (%)	69 (17%)	178 (23%)	166 (19%)	698 (19%)	523 (23%)	125 (31%)
Gothenburg (SE), *N* (%)	18 (4%)	49 (6%)	50 (6%)	378 (11%)	423 (19%)	21 (5%)
Huelva (ES), *N* (%)	4 (1%)	0 (0%)	3 (0%)	54 (2%)	7 (0%)	2 (0%)
Melbourne (AU), *N* (%)	6 (1%)	7 (1%)	11 (1%)	54 (2%)	106 (5%)	7 (2%)
Reykjavik (IS), *N* (%)	22 (5%)	93 (12%)	117 (14%)	432 (12%)	477 (21%)	52 (13%)
Tartu (EE), *N* (%)	56 (14%)	73 (9%)	71 (8%)	157 (4%)	124 (6%)	63 (16%)
Umeaa (SE), *N* (%)	144 (36%)	162 (20%)	222 (26%)	676 (19%)	63 (3%)	33 (8%)
Uppsala (SE), *N* (%)	48 (12%)	122 (15%)	124 (14%)	702 (20%)	250 (11%)	51 (13%)
Grandparental (G0) smoking[a]						
No grandparents smoke, *N* (%)	157 (39%)	258 (33%)	272 (31%)	986 (28%)	487 (22%)	90 (23%)
One grandparent smoke, *N* (%)	139 (34%)	266 (34%)	276 (32%)	1099 (31%)	664 (30%)	112 (28%)
Both grandparents smoke, *N* (%)	30 (7%)	139 (18%)	155 (18%)	802 (23%)	520 (23%)	76 (19%)
Don't know, *N* (%)	15 (4%)	16 (2%)	28 (3%)	101 (3%)	70 (3%)	17 (4%)
Missing, *N* (%)	64 (16%)	111 (14%)	135 (16%)	565 (15%)	505 (22%)	105 (26%)

[a]Reported by the ECRHS participant and therefore only available for the grandparents on the ECRHS participants' side.

him/herself (G1). This was in order to establish the likelihood of differential recall of parental place of upbringing according to offspring vs parental reports.[16]

Statistical analyses were performed in Stata 15 (Stata Corp., College Station, TX, USA).

Results

Basic characteristics of the G2 study population in RHINESSA ($N = 8260$) are shown in Table 1.

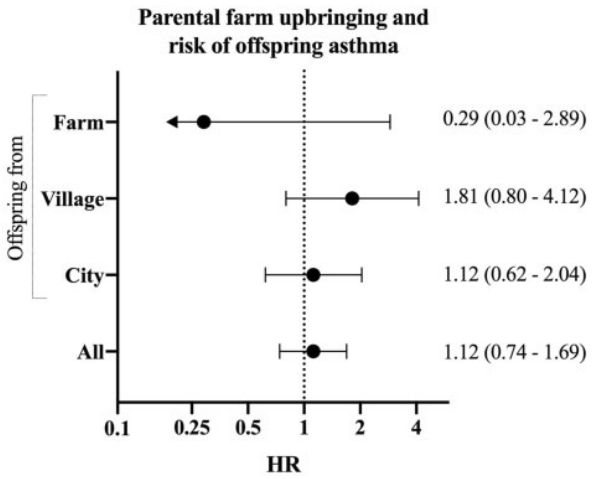

Figure 2 Hazard ratios (HR) with 95% confidence intervals (CI) for offspring asthma according to parental farm upbringing (both parents from farm VS. both parents from city) among offspring in RHINESSA (Respiratory Health In Northern Europe, Spain and Australia) adjusted for centre, grandparental asthma, grandparental upbringing and grandparental smoking (adj2 model), for all ($N = 4279$) and stratified by offspring upbringing (Table 2 and Supplementary Material Table S2).

The number in each exposure group was unevenly distributed, ranging from 405 (5%) offspring with both parents from farms to 2246 (27%) offspring with both parents from cities. This was even more pronounced when dividing by study centres. Offspring (G2) were comparable with regard to age, sex, smoking status and asthma across parental-place-of-upbringing categories (Table 1). Grandparental smoking ranged from 39% G0 non-smokers in the group with both parents from farms to 22% non-smokers in the group with both parents from cities.

In Cox-regression models, parental (G1) farm upbringing was not associated with offspring (G2) asthma when compared with city upbringing, either among all offspring ($_{adj2}$HR 1.12, 95% CI 0.74–1.69) or among the subgroup of offspring born and raised in the city themselves ($_{adj2}$HR 1.12, 95% CI 0.62–2.04) (Table 2A and Figure 2). Similar findings were observed when investigating only allergic asthma ($_{adj2}$HR 0.96, 95% CI 0.54–1.70) (Table 2B). Centre-specific estimates showed some variation in the association, especially for Tartu (EE), although with very wide CIs (Supplementary Figure 2, available as Supplementary data at *IJE* online).

The quantitative bias analyses comparing the association between maternal/paternal (G1) upbringing and offspring (G2) asthma showed similar results when using either G2 or G1 as the source of information (Table 3).

Grandparental (G0) farm upbringing was not associated with offspring (G2) asthma either in the maternal line (HR 0.89, 95% CI 0.73–1.08) or in the paternal line (HR 1.05, 95% CI 0.86–1.29) (Supplementary Table 1, available as Supplementary data at *IJE* online). The results persisted when investigating the subgroup of offspring and parents,

Table 2A Hazard ratios and corresponding 95% confidence intervals for offspring asthma according to parental place of upbringing

	All			Offspring from city		
	$N = 7795$	$N = 5799$	$N = 4279$	$N = 5096$	$N = 3679$	$N = 2522$
Parental upbringing	Crude	Adj1	Adj2	Crude	Adj1	Adj2
Both parents from farm	1.03 (0.81–1.33)	1.06 (0.75–1.49)	1.12 (0.74–1.69)	0.92 (0.64–1.34)	0.99 (0.62–1.59)	1.12 (0.62–2.04)
Mother from farm, father from village/city	0.99 (0.81–1.21)	1.01 (0.77–1.33)	1.09 (0.78–1.51)	0.90 (0.70–1.16)	0.89 (0.62–1.26)	0.96 (0.62–1.49)
Father from farm, mother from village/city	1.03 (0.85–1.25)	1.07 (0.82–1.39)	1.13 (0.81–1.56)	1.04 (0.82–1.33)	0.96 (0.69–1.35)	0.96 (0.62–1.50)
Both parents from village or one parent from village and one from city	0.95 (0.84–1.09)	0.93 (0.78–1.11)	0.98 (0.79–1.23)	0.91 (0.78–1.06)	0.90 (0.74–1.10)	0.90 (0.71–1.16)
Both parents from city (ref)	1	1	1	1	1	1

Adj1, adjusted for centre and grandparental place of upbringing for all four grandparents.

Adj2, adjusted for centre, grandparental asthma (two grandparents), grandparental place of upbringing (four grandparents) and grandparental smoking (two grandparents).

Table 2B Hazard ratios and corresponding 95% confidence intervals for a subgroup of offspring with hay fever investigating allergic asthma according to parental place of upbringing

	All		
	$N = 2250$	$N = 1708$	$N = 1237$
Parental upbringing	Crude	Adj1	Adj2
Both parents from farm	1.14 (0.81–1.60)	1.04 (0.66–1.66)	0.96 (0.54–1.70)
Mother from farm, father from village/city	1.16 (0.89–1.51)	1.08 (0.76–1.55)	1.20 (0.76–1.87)
Father from farm, mother from village/city	0.92 (0.70–1.22)	0.85 (0.60–1.21)	0.82 (0.52–1.31)
Both parents from village or one parent from village and one from city	0.98 (0.82–1.17)	0.98 (0.78–1.24)	1.04 (0.77–1.40)
Both parents from city (ref)	1	1	1

Adj1, adjusted for centre and grandparental place of upbringing for all four grandparents.

Adj2, adjusted for centre, grandparental asthma (two grandparents), grandparental place of upbringing (four grandparents) and grandparental smoking (two grandparents).

Table 3 Quantitative bias analyses—hazard ratios with corresponding 95% confidence intervals for offspring asthma according to maternal and paternal place of upbringing, respectively, adjusted for grandparental asthma, grandparental place of upbringing and grandparental smoking

	Village vs city	Farm vs city
Mother's place of upbringing		
Own reports	1.12 (0.88–1.44)	0.83 (0.59–1.16)
Offspring reports	1.14 (0.85–1.55)	1.13 (0.77–1.67)
Father's place of upbringing		
Own reports	0.99 (0.76–1.29)	0.95 (0.67–1.34)
Offspring reports	0.90 (0.69–1.18)	1.08 (0.73–1.60)

who were born in the city themselves: maternal line (HR 1.05, 95% CI 0.67–1.65) and paternal line (HR 1.02, 95% CI 0.62–1.68) (Supplementary Table 1, available as Supplementary data at *IJE* online).

Discussion

Key results

To the best of our knowledge, this is the first study to investigate the generational effects of farm exposure on asthma. In this three-generation study, parental farm upbringing was not associated with offspring asthma among all offspring or when stratified by the offspring's own upbringing or asthma phenotype. A quantitative bias analysis showed that these estimates were similar regardless of whether the information was provided by G1 themselves or as second-hand information by their offspring (G2). Furthermore, grandparental farm upbringing was not associated with offspring asthma in either the maternal or the paternal line. Therefore, this study does not support the hypothesis suggesting generational effects from farm exposure in previous generations on offspring asthma development.

Strengths and limitations

The key strength of this study is the three-generation study design. Whereas few other studies in this field have focused on the pregnancy period or the time just before conception, this study includes information on exposures long before conception and for both mothers/grandmothers and fathers/grandfathers. However, a clear limitation of this study is that information on both exposure and outcome data is questionnaire-based and therefore may be subject to recall error. However, this error is unlikely to be differential and therefore would have skewed our estimates towards the null. In addition, some information is given on behalf of relatives. We anticipate that offspring are able to report their own place of upbringing correctly; however, a study investigating the agreement in offspring and parental reports on parental upbringing in RHINESSA showed that offspring tend to report incorrectly about their parents if their parents were born and raised in a different setting than the offspring themselves.[17] Our study did not investigate the patterns of offspring misclassification when reporting about their grandparents, but we suspect that misclassification is present in this case as well. Nevertheless, the quantitative bias analyses in the present paper showed that estimates were consistent when using offspring and parental reports on parental upbringing, respectively, suggesting that any misclassification from this source is unlikely to influence the results.

Studies have shown that exposure levels and diversity of microbes are higher on farms than in urban homes.[6,18] However, in our study, we consider the place of upbringing as a crude measure for early-life microbial exposures. Furthermore, based on results from a previous study on the urban–rural gradient in asthma, we merged farms with and without livestock in our analyses, although farms with livestock still accounted for the majority in this group.[9] In addition, the response categories in the questionnaires were

not accompanied by any objective indicators and the interpretation was left open to the participant. This may have caused some random error in the measurement of exposure. A further limitation of this study is the lack of biological material from the subjects. This makes us unable to detect any biological pathways related to the effects of farm exposure in prior generations, including epigenetic methylation patterns, and also hindered objective markers for atopic disposition. In addition, the stability of epigenetic markers in parents from early-life farm exposure is unknown. However, a recent study on prenatal smoking exposure found that the methylation markers were stable throughout childhood and into adulthood.[19] If we assume a more transient epigenetic effect, the place where the parents lived closest to conception or the place where they spend most of their life are of more importance when investigating trans- or intergenerational effects on asthma. Unfortunately, our study did not have data to further investigate this.

A study on gene–environment interactions in asthma suggested that influences on genetic susceptibility may not be sufficient to develop asthma unless an appropriate environmental stimulus is also present.[20] This is further supported by a hypothesis-generating study suggesting that a farm upbringing may be an effect modifier in the association between different toll-like receptors and early-onset asthma.[21] In our study, we could not distinguish between inter- and transgenerational effects, as defined by Krauss-Etschmann *et al.*[22] as either effects in the intrauterine environment affecting the germ line of the foetus or effects transmitted across generations that cannot be explained by direct environmental exposures.

A study in the Danish National Birth Cohort compared three methods of measuring asthma and found self-report to pose a higher prevalence when compared with the hospitalization registry (12% vs 7%) and lower prevalence when compared with the prescription registry (32%).[23] As there is no consensus about a 'gold standard' for asthma diagnosis in epidemiological studies, we cannot rule out an overestimated asthma prevalence in our study (18%). It would have been useful to have included information on asthma symptoms in the analyses to assess asthma severity but, as we did not have the time of onset for these data, they were not suitable for the Cox-regression models. However, in a post-hoc comparison of the proportion of offspring with at least three symptoms of asthma, we found the same distribution as for 'ever asthma' within the different exposure groups of upbringing. Despite the limitations in the outcome measurement, two other studies in RHINESSA have found an increased risk of offspring asthma after preconception smoking exposure.[13,24]

The dropout in the ECRHS population (G1) has been substantial (~50%) and, in addition, only a third of the invited offspring participated in RHINESSA (G2). A non-response analyses in the Nordic part of ECRHS, named the RHINE cohort, showed a similar prevalence of asthma among baseline responders (4.7%) and long-term participants in RHINE 3 (4.6%) but, as we do not have information on offspring asthma from other sources, i.e. registries, we were not able to investigate whether the prevalence of asthma varied between participants and non-participants in RHINESSA.[25] However, based on the results from the RHINE cohort, we do not expect asthma status to influence the likelihood of participation in RHINESSA.

The original ECRHS population were sampled in and around larger cities in all study centres and this may have resulted in an overrepresentation of urban-dwellers in our study population. In addition, two non-response analyses from Denmark and Belgium showed that the risk of non-participation is higher among urban residents.[26,27] Thus, we believe that dropout is related to exposure (parental upbringing) but probably not outcome (offspring asthma), and is unlikely to have skewed our results. Dropout could be associated with other asthma-relevant variables, e.g. parental smoking status, but, due to the similar prevalence of asthma among participants and non-participants in RHINE, we do not think this is of major concern.[25] Furthermore, in the relatively small group with both parents from a farm (5% of the study population), the statistical power is limited, although, as we see consistent results across a number of different analyses, we believe this is robust.

Interpretation

A few studies investigating farm exposure *in utero* suggest that it may protect against asthma in the offspring,[28,29] but there is very little evidence on the effect of parental farm exposure before conception.[28] Also, some animal studies suggest that perinatal farm exposure is positively associated with epigenetic changes, reducing the risk of asthma. In a murine study from 2011, Brand *et al.* showed that prenatal exposure to the farm-derived gram-negative bacterium *A. lwoffii F78* caused alternation in histone acetylation in specific genomic loci and prevented the development of an asthmatic phenotype in the offspring.[30] Another study assessed DNA methylation in 10 genes related to asthma and found a change in the methylation patterns in DNA from farmers' children compared with non-farmers' children.[10] These epigenetic changes clustered in genes highly associated with asthma (*ORMDL family*) and IgE regulation (*RAD50*, *IL13* and *IL4*), but not in T-regulatory genes (*FOXP3* and *RUNX3*). Both studies support the Hygiene Hypothesis by indicating that

exposure to microbes and farm environments protects against asthma development in childhood through epigenetic mechanisms and, in this cohort, we previously showed that parents' place of upbringing is negatively associated with their own asthma status.[9]

Adverse exposures such as particulate air pollution in cities or cigarette smoke are known to increase the risk of asthma development. In a three-generation study from 2018, Accordini *et al.* found that maternal smoking during pregnancy was significantly associated with a higher risk of asthma in the offspring.[24] A murine study by Gregory *et al.* showed that exposure to diesel exhaust particles and concentrated urban air particles led to the generational maternal transmission of increased risk of asthma.[29] Also, Baiz *et al.* investigated the impact of maternal exposure to air pollutants before and during pregnancy on the newborn's immune cells.[28] The relative distribution of NK cells and T-lymphocytes including CD4+CD25+ regulatory T-cells in cord blood were found to be significantly altered when exposed to ambient air pollutants. These studies all indicate that both parental and grandparental exposure to smoking or air pollutants is positively associated with an increased risk of asthma in the offspring. Compared with a farm upbringing, smoking is a more direct and often long-term exposure. Therefore, we did not expect to find effects that are of comparable magnitude to smoking in this study. However, among other covariates, we adjusted our analyses for grandparental place of upbringing and grandparental smoking (Adj2 model). The similar results of the adjusted and unadjusted HRs indicate that the bias induced through these factors is minimal (Table 2B).

Conclusion

This study does not support the hypothesis that parental or grandparental upbringing has an important effect on the risk of offspring asthma. Further human studies that address the limitations in our study and provide a more precise measurement of exposure and the means to investigate possible mechanisms, i.e. a change in gene expression due to epigenetic effects, are needed.

Supplementary data

Supplementary data are available at *IJE* online.

Author Contributions

Vivi Schünssen, Cecilie Svanes and Signe Timm had the original idea for the study and, with all co-authors, carried out the design. Christer Janson (SE), Cecilie Svanes (NO), Randi Jacobsen Bertelsen (NO), Mathias Holm (SE), Lennart Bråbäck (SE), Rain Jogi (EE), Shyamali Dharmage (AU), Jose Luis Sanchez-Ramos (ES), Jesus Martinez-Moretalla (ES) and Vivi Schlünssen (DK) were responsible for recruitment and follow-up of study participants. Signe Timm, Morten Frydenberg and Ane Johannessen were responsible for data cleaning and carried out the analyses. Torben Sigsgaard, Brittany Campbell, Marie Kjær Madsen, Nils Oskar Jogi, Linus Schiöler and Julia Dratva provided feedback during the process due to their expert knowledge in this field. Signe Timm drafted the manuscript, which was revised by all authors. All authors read and approved the final manuscript.

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Conflict of interest

None declared.

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