



## Transgenerational Influences

**Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study**

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## Abstract

**Background:** Whereas it is generally accepted that maternal environment plays a key role in child health, emerging evidence suggests that paternal environment before conception also impacts child health. We aimed to investigate the association between children's asthma risk and parental smoking and welding exposures prior to conception.

**Methods:** In a longitudinal, multi-country study, parents of 24 168 offspring aged 2–51 years provided information on their life-course smoking habits, occupational exposure to welding and metal fumes, and offspring's asthma before/after age 10 years and hay fever. Logistic regressions investigated the relevant associations controlled for age, study centre, parental characteristics (age, asthma, education) and clustering by family.

**Results:** Non-allergic early-onset asthma (asthma without hay fever, present in 5.8%) was more common in the offspring with fathers who smoked before conception {odds ratio [OR] = 1.68 [95% confidence interval (CI) = 1.18–2.41]}, whereas mothers' smoking before conception did not predict offspring asthma. The risk was highest if father started smoking before age 15 years [3.24 (1.67–6.27)], even if he stopped more than 5 years before conception [2.68 (1.17–6.13)]. Fathers' pre-conception welding was independently associated with non-allergic asthma in his offspring [1.80 (1.29–2.50)]. There was no effect if the father started welding or smoking after birth. The associations were consistent across countries.

**Conclusions:** Environmental exposures in young men appear to influence the respiratory health of their offspring born many years later. Influences during susceptible stages of spermatocyte development might be important and needs further investigation in humans. We hypothesize that protecting young men from harmful exposures may lead to improved respiratory health in future generations.

**Key words:** asthma, epidemiology, smoking, occupational exposure, epigenesis

### Key Messages

- Smoking and occupational welding in young men appeared to independently increase asthma risk in their offspring born many years later.
- Early puberty might possibly be a critical vulnerable period.
- The findings provide an unexpectedly strong argument for focusing on the adverse environmental exposures of adolescent males.

## Introduction

It is well known that a mother's environment plays a key role in child health. The hypothesis that health and disease originate early in life has dramatically increased our understanding of this issue and is guiding public health policy. However, emerging evidence suggests that there are mechanisms whereby the father's environment before conception could also impact the health of future generations.<sup>1</sup>

Environmental factors such as smoking and occupational exposures can cause genetic and epigenetic changes in sperm that are transmissible to offspring.<sup>2–9</sup> There is evidence for inheritable effects of food restriction through the male line from human studies, suggesting particular effects on offspring when exposure occurs during prepuberty.<sup>10–13</sup> The recent development of hypotheses regarding 'windows of susceptibility' to environmental insults during sperm

development suggests an urgent need to investigate exposures occurring in adolescent men with potential impacts for their offspring.<sup>2</sup> Such investigation requires large, long-term, epidemiologic studies of multiple generations.

One outcome in which paternal exposures might play an important role is childhood asthma. Despite major scientific efforts and mounting evidence that asthma aetiology starts as early as the intra-uterine period, knowledge of the causal factors remains incomplete. There is some evidence that paternal *in utero* exposure to cigarette smoke is associated with his children's asthma<sup>14</sup> but, to our knowledge, there are no studies of asthma investigating fathers' exposures beyond this, including exposures prior to starting a family. Existing knowledge of inheritable spermatogenic effects make this an appealing target for investigating windows of susceptibility in fathers. In addition, occupational environmental exposures in young men prior to reproduction need to be investigated, as the workplace provides an important source of potentially harmful exposures.<sup>2</sup>

The Respiratory Health In Northern Europe (RHINE) study provides a unique data set including detailed information on smoking habits and occupational exposure to welding and metal fumes from large population-based cohorts in multiple centres and countries, followed over a 20-year period. RHINE thus provides a rare opportunity for detailed investigation of parental environmental exposures and potential impact on asthma risk in their children. The most recent follow-up included information on asthma prevalence in participants' offspring (27 120). Welding is a relevant occupational exposure implicated in a variety of respiratory health problems. It has also been shown to have effects on sperm quality.<sup>15–17</sup>

In this study, we first aimed to investigate whether parental exposure to smoking and welding, including exposure prior to conception, influenced asthma risk in offspring, with a particular focus on exposures in fathers. Second, we aimed to identify susceptible windows during male reproductive development by addressing whether potential pre-conception effects were related to exposure age, exposure duration and time from quitting exposure until conception.

## Methods

### Study population

RHINE III is the second follow-up of a population-based cohort from seven northern European centres (Supplementary Figure 1, available as Supplementary data at *IJE* online). Random population samples of men and women born in 1945–73 completed postal questionnaires

in 1991–93, 1999–2001 and 2010–12. In 2010–12, participants were asked about their children, including each child's birth year, asthma and allergies.<sup>18</sup> The parent study (ECRHS) recruited random samples from population-based registers in each country. The intention of this sampling was to recruit a population-based sample but not 'families' or 'married/cohabiting couples'. Therefore, we have information on women who became mothers and men who became fathers—but not 'couples who became parents. Thus, pre-conception information was available only for one of the offspring's parents, the one that did participate in the parent study'; 1880 reported having no children and the remaining participants reported a total of 27 120 children aged 0–51 years (mean age 22 years), with mean family size 1.9 siblings.

The studies were approved by medical research ethics regional committees according to national legislations. All participants gave written informed consent.

### Parental smoking

RHINE participants provided information about smoking habits, including year of smoking debut and quitting. Parental smoking exposure was defined in three groups by relating this information to birth year for each offspring: (i) 'Smoking only prior to conception': parent quit smoking  $\geq 2$  years prior to child's birth year; (ii) 'Smoking including period around birth': parent smoked in the period around child's birth; most smoked before, during and after pregnancy; (iii) 'Smoking started after birth'. Fathers' age of starting smoking was categorized as before or after 15 years, the mean age of completed puberty (mean age of voice break 14.5 years, first nocturnal emission 14.8 years).<sup>19</sup>

### Parental welding

In the first follow-up (1999–2001), prior to collecting information about offspring (2010–11), parents were asked about occupational exposure to welding or metal fumes ('Have you worked with welding or in any other way in your work been exposed to metal fumes/welding fumes?'). Those answering 'yes' were asked about years of starting and quitting. Median age for starting welding was 19 years (interquartile range 17–22) among those who started welding before conception.

### Asthma phenotypes in offspring

Asthma was defined based on the following question: 'Please write the years when your children were born, and tick "YES" if they have had any of the following: Asthma

before 10 year; Asthma after 10 years; Hayfever/rhinitis; Atopic eczema/skin allergies'. Asthma before 10 years defined 'early-onset asthma' and asthma after age 10 years defined 'later-onset asthma'. 'Allergic asthma' was defined by a positive answer to asthma and to hay fever; 'non-allergic asthma' as having asthma but not hay fever. Age 10 years was set as the cut-off point to separate childhood asthma from asthma with onset in early puberty or later in both sexes.

Infants under 2 years of age were excluded from analyses, as asthma diagnosis in this age group is unclear ( $n = 175$  excluded, two with parent-reported asthma).

### Statistical analysis

Associations of parental smoking patterns and occupational welding with asthma phenotypes in offspring were analysed using logistic regressions adjusting for age, study centre and parental characteristics (age, parent asthma before age 10, education level) and clustering by family using multilevel modelling. Analyses of welding included adjustment for smoking. Grandparent education, number of siblings and type of dwelling were considered as potential confounders in all analyses, but were not included in the final models, as they did not alter the effect estimates by  $\geq 5\%$ .

Analyses were structured as follows:

- i. We examined the association between maternal and paternal smoking during different periods (smoked only prior to conception; smoked including period around birth; smoked only after child's birth) and asthma phenotypes (early-onset and later-onset asthma, further characterized as allergic and non-allergic asthma).
- ii. For the time periods and phenotypes where there was evidence of an association between smoking and asthma, we next tested for dose-response effects by examining age of smoking debut, smoking duration, amount and years before birth that smoking stopped.
- iii. Finally, we investigated whether the association between paternal smoking and asthma in offspring differed according to paternal grandmothers' smoking status (a marker of fathers' *in utero* smoking exposure). For this analysis, we created five mutually exclusive groups of paternal smoking: never smoked; smoking debut before age 15; smoking debut after age 15 and duration less than 10 years before conception; smoking debut after age 15 and duration more than 10 years before conception; smoking debut after conception.

The relationship between paternal welding (including age started and duration) with offspring asthma was analysed using similar definitions as for smoking (welding before or after age 15 and for less or more than 10 years). Only 40 fathers started welding before age 15; early-onset welding was thus not analysed. Welding in mothers was uncommon and not analysed.

Meta-analysis was conducted using both fixed effects and random effects models.<sup>20</sup> Given that there was almost no heterogeneity and both models gave identical results, we have presented the fixed effect models in the manuscript.

Sensitivity analyses were conducted to consider the effect of missing data and differential recall based on child's reported asthma status (see [Supplementary data](#), available at *IJE* online).

Stata Statistical Software was used to analyse the data (StataCorp. 2013, *Stata Statistical Software: Release 13*, College Station, TX, StataCorp LP).

## Results

### Characteristics of the cohort

Of 26 945 offspring over 2 years old, asthma status was reported for 24 168 (90%); these were included in the analyses ([Supplementary Table 1](#), available as [Supplementary data](#) at *IJE* online). Early- and later-onset asthma were present in 8.5% (allergic 2.6%; non-allergic 5.8%) and 4.2%, respectively ([Supplementary Table 1](#), available as [Supplementary data](#) at *IJE* online). [Supplementary Table 2a](#) (available as [Supplementary data](#) at *IJE* online) describes characteristics and outcomes by parental smoking history (never smoked 48%, smoked only prior to conception 10%, other smoking patterns 40%, smoked only after birth of child 1.6%). [Supplementary Table 2b](#) (available as [Supplementary data](#) at *IJE* online) describes characteristics and outcomes by paternal welding (never welded 79%, welded prior to conception 18%, welded only after birth of child 4%).

### Relationship between paternal smoking and offspring asthma phenotypes

Individuals with a father who smoked only prior to conception had more early-onset asthma than those whose father never smoked ([Table 1](#)). This was due to an association with non-allergic early-onset asthma. There was no association with late-onset asthma ([Table 1](#)), including when late-onset asthma was further divided into allergic or non-allergic asthma (data not shown). Maternal smoking around pregnancy and birth was associated with increased early-onset asthma in offspring, most pronounced for the

**Table 1.** Offspring asthma as associated with parental smoking patterns among 24 168 offspring aged 2–51 years from seven northern European study centres

	Father				Mother			
	N	% asthma	OR* (95%CI)	P-value	N	% asthma	OR* (95%CI)	P-value
<b>Early-onset asthma</b>								
Parent never smoked	4715	7.5	1 (ref)		5488	8.3	1 (ref)	
Smoked only prior to conception	1148	9.5	1.37 (1.01–1.86)	0.044	1423	11.0	1.14 (0.88–1.47)	0.32
Smoked including period around birth	3777	7.2	1.17 (0.93–1.48)	0.19	4542	10.2	1.48 (1.22–1.78)	<0.001
Started after child's birth	142	5.8	1.01 (0.42–2.43)	0.99	259	4.9	0.67 (0.33–1.38)	0.28
<b>Early-onset allergic asthma†</b>								
Parent never smoked	3822	3.1	1.0		4221	3.8	1.0	
Smoked only prior to conception	909	1.9	0.60 (0.31–1.20)	0.15	1113	4.5	1.29 (0.80–2.08)	0.31
Smoked including period around birth	3046	2.3	0.84 (0.54–1.31)	0.45	3407	3.9	1.17 (0.82–1.68)	0.39
Started after child's birth	113	2.4	0.67 (0.13–3.46)	0.63	212	3.8	0.99 (0.34–2.89)	0.99
<b>Early-onset non-allergic asthma‡</b>								
Parent never smoked	3944	5.9	1.0		4364	6.7	1.0	
Smoked only prior to conception	985	9.3	1.68 (1.18–2.41)	0.004§	1164	9.2	1.11 (0.81–1.52)	0.51
Smoked including period around birth	3184	6.1	1.27 (0.96–1.69)	0.097	3610	9.0	1.65 (1.31–2.07)	<0.001
Started after child's birth	116	4.7	1.03 (0.35–3.05)	0.96	208	2.2	0.33 (0.10–1.03)	0.057
<b>Later-onset asthma‡</b>								
Parent never smoked	3627	2.8	1.0		4429	3.6	1.0	
Smoked only prior to conception	801	3.1	1.23 (0.73–2.04)	0.44	1046	2.9	0.79 (0.49–1.27)	0.33
Smoked including period around birth	3243	2.5	0.97 (0.69–1.38)	0.87	3903	4.3	1.13 (0.86–1.50)	0.37
Started after child's birth	130	2.1	0.69 (0.19–2.53)	0.57	246	2.6	0.57 (0.22–1.44)	0.23

OR adjusted for offspring age, parent characteristics (age, sex, early-onset asthma, education) study centre, cluster by family.

† Baseline group for comparison was those with no asthma and no hay fever. Excludes those who developed asthma after age 10 years.

‡ Excludes those with early-onset asthma and those under age 10 years.

non-allergic phenotype (Table 1). Paternal or maternal smoking only after the child's birth was not associated with offspring asthma.

### Dose–response effect of paternal pre-conception smoking and non-allergic early-onset asthma in offspring

Both a father's early smoking debut and a father's longer smoking duration before conception increased non-allergic early-onset asthma in offspring, even with mutual adjustment and adjusting for number of cigarettes and years since quitting smoking (Table 2). Early smoking debut was associated with more non-allergic early-onset offspring asthma even if the smoking stopped at least 5 years before the child's birth [odds ratio (OR) = 2.68, 95% confidence interval (CI) 1.17–6.13]. A father's smoking debut before age 11 years (102 fathers) showed the greatest increased risk (OR = 3.95, 95% CI = 1.07–14.60), followed by smoking debut ages 11–14 (OR = 1.75, 95% CI 1.07–1.86) and smoking debut after age 15 (OR = 1.37, 95% CI 1.00–1.86). Longer duration of smoking was also associated with an increased risk, up to 1.8-fold for those smoking for more than 10 years (OR 1.76, 95% CI 0.96–3.25). Time of quitting before conception was

not independently associated with non-allergic early-onset offspring asthma.

### Relationship between paternal exposure to tobacco smoke *in utero* (grandmaternal smoking) and offspring non-allergic early-onset asthma

Grandmaternal smoking status was associated with risk of non-allergic early-onset asthma in offspring (OR 1.3, 95% CI 1.0–1.6) and also modified the association between the father's own smoking status and asthma in his offspring. If the paternal grandmother did not smoke, the father's own smoking before age 15 was associated with more non-allergic early-onset asthma in his children (Table 3), but this association was not present if the paternal grandmother smoked ( $P_{\text{interaction}} = 0.02$ ). In contrast, long smoking duration was associated with increased offspring asthma irrespective of whether or not the paternal grandmother smoked.

### Relationship between paternal occupational exposure to welding prior to conception and non-allergic early-onset asthma in offspring

Paternal welding starting prior to conception, but not welding starting after conception, was associated with



**Table 2.** Patterns of paternal smoking prior to conception in relation to early-onset non-allergic asthma in their children

	OR* (95% CI)	P-value
<b>Age of smoking debut</b>		
≥15 years	1.0	
<15 years	3.24 (1.67, 6.27)	0.001
<b>Duration (per 2 year increase)<sup>a</sup></b>		
	1.14 (1.03–1.26)	0.014
<b>Amount of smoking</b>		
<10 cig/day	1.0	
10–20 cig/day	1.42 (0.57–3.55)	0.45
>20 cig/day	0.97 (0.36–2.64)	0.96
<b>Years before birth without smoking (per 2 years)<sup>b</sup></b>		
	0.90 (0.78–1.04)	0.14

OR from analyses with mutual adjustment for the four smoking characteristics, and adjusted for offspring age, parent characteristics (age, early-onset asthma, education) study centre, cluster by family.

<sup>a</sup>OR is per 2-year increase in smoking duration, with the baseline category set as those who smoked less than 1 year and a single upper category for those who smoked ≥ 21 years.

<sup>b</sup>OR is per 2-year increase in time before birth at which smoking stopped, with the baseline category set as those who stopped within 3 years of the child's birth and a single upper category for those who stopped >15 years before the child's birth.

increased non-allergic early-onset asthma in offspring (Table 4). Welding increased offspring asthma risk even if the welding stopped prior to conception [adjusted odds ratio (aOR) 1.56, 95% CI 0.99–2.46]. Welding duration of at least 10 years was associated with a greater increase in asthma (aOR 2.10, 95% CI 1.36–3.22) than welding for less than 10 years (aOR 1.56, 95% CI 0.98–2.48).

Smoking and welding independently increased offspring asthma risk and mutual adjustment did not alter the estimates of either. Offspring had more asthma if fathers were exposed to both smoking and welding (OR 3.6, 95% CI 1.7–7.6 for both smoking and welding for at least 10 years) (Supplementary Table 3, available as Supplementary data at *IJE* online). Welding duration for at least 10 years was associated with more non-allergic early-onset asthma in offspring irrespective of grandmaternal smoking (no grandmaternal smoking: OR 1.9, 95% CI 1.01–3.43; grandmaternal smoking OR 2.8, 95% CI 1.34–5.67,  $P_{\text{interaction}} = 0.38$ ).

### Heterogeneity between study centres

There was no evidence of heterogeneity between study centres for the effects of either paternal pre-conception smoking or welding prior to conception (Figure 1a and b).

## Discussion

Smoking and occupational welding in young men were associated with asthma risk in their children, even if these exposures occurred many years prior to conception. We identified several potential susceptibility windows for paternal environmental exposures, with the highest risk related to fathers' smoking before completing puberty. Figure 2 shows our findings in the context of existing knowledge from laboratory and animal studies regarding vulnerability windows in sperm development.<sup>2</sup> For smoking and welding starting after puberty, exposure duration appeared to be the most important determinant. These findings were consistent across geographical regions with different smoking habits and occupational exposures, and with different offspring asthma prevalence. These novel results suggest that environmental exposures in young men can have important impact on their future offspring's respiratory health, and interventions aimed at adolescent men might have large public health consequences.

To our knowledge, this is the first study investigating a father's environment prior to starting a family and the resulting asthma risk in his children. Our findings confirm an effect of fathers' *in utero* exposure (grandmothers' smoking).<sup>14,21</sup> Consistently with Miller and colleagues,<sup>14</sup> we find that paternal exposure to his own mother's smoking increased his children's asthma risk. However, we also found effects of a father's own pre-conception smoking.

Concerning a mother's smoking, we found more offspring asthma if the mother smoked around pregnancy, consistently with previous studies.<sup>22,23</sup> However, no effect of maternal smoking prior to conception was identified. This has not previously been investigated, to our knowledge. Male and female germ cells develop differently. Further, if there is a prepuberty susceptibility window in females, this would be at a younger age than in boys, and at an age when the girls have not yet started smoking. This could explain why we find an effect of pre-conception smoking in men but not in women.

Several lines of evidence suggest that the observed effects could be mediated through influences on spermatozoa development. The fathers' but not the mothers' pre-conception exposure was associated with offspring risk, suggesting effects through male germ cells. Smoking is known to cause genetic and epigenetic damage to spermatozoa, which are transmissible to offspring and have the potential to induce developmental abnormalities.<sup>4,5,7,9,24–29</sup> *In utero* smoking exposure of fathers could influence primordial germ cell development.<sup>3</sup> A prepubertal vulnerability window could be related to *de novo* DNA methylation occurring during primordial germ cell differentiation to spermatogonia.<sup>30</sup> Such a window is indicated by the

**Table 3.** Father's age of smoking debut and smoking duration prior to conception in relation to early-onset non-allergic asthma in their children, stratified by paternal grandmother's smoking

	Paternal grandmother did not smoke			Paternal grandmother smoked			<i>P</i> <sub>interaction</sub> <sup>a</sup>
	N	% asthma	OR (95%CI)*	N	% asthma	OR (95%CI)*	
Father never smoked	2902	5.5	1.0	1433	6.8	1.0	
Father smoked before age 15	466	9.0	3.14 (1.68, 5.84)	405	7.7	0.94 (0.44, 1.99)	0.022
Father smoked after age 15 and smoked <10 years pre-conception	902	4.8	1.15 (0.65, 2.03)	456	7.5	0.96 (0.47, 1.94)	0.49
Father smoked after age 15 and smoked ≥ 10 years pre-conception	1220	7.5	1.75 (1.09, 2.80)	795	8.3	1.58 (0.93, 2.68)	0.98

OR adjusted for offspring age, paternal characteristics (age, education, smoking status), grandparent education (grandmaternal and grand-paternal), study centre, cluster by family.

<sup>a</sup>Interaction *P*-values calculated using the likelihood ratio test.

**Table 4.** Father's exposure to occupational welding in relation to early-onset non-allergic asthma in their children

	N	% asthma	OR* (95%CI)	<i>P</i> -value
<b>Early-onset asthma</b>				
Father never welded	6773	7.0	1.0	
Welding started prior to conception	1549	10.4	1.64 (1.25, 2.15)	<0.001
Welding started after conception	305	7.5	1.14 (0.63, 2.05)	0.66
<b>Early-onset allergic asthma<sup>a</sup></b>				
Father never welded	5521	2.6	1.0	
Welding started prior to conception	1232	3.1	1.31 (0.75, 2.28)	0.35
Welding started after conception	243	2.5	1.06 (0.34, 3.36)	0.92
<b>Early-onset non-allergic asthma<sup>a</sup></b>				
Father never welded	5706	5.8	1.0	
Welding started prior to conception	1317	9.3	1.80 (1.29, 2.50)	<0.001
Welding started after conception	254	6.7	1.15 (0.55, 2.38)	0.71
<b>Later-onset asthma<sup>b</sup></b>				
Father never welded	5473	2.7	1.0	
Welding started prior to conception	1192	3.6	1.42 (0.93, 2.19)	0.11
Welding started after conception	282	3.2	1.03 (0.45, 2.38)	0.94

OR adjusted for offspring age, paternal characteristics (age, education, smoking status), study centre, cluster by family.

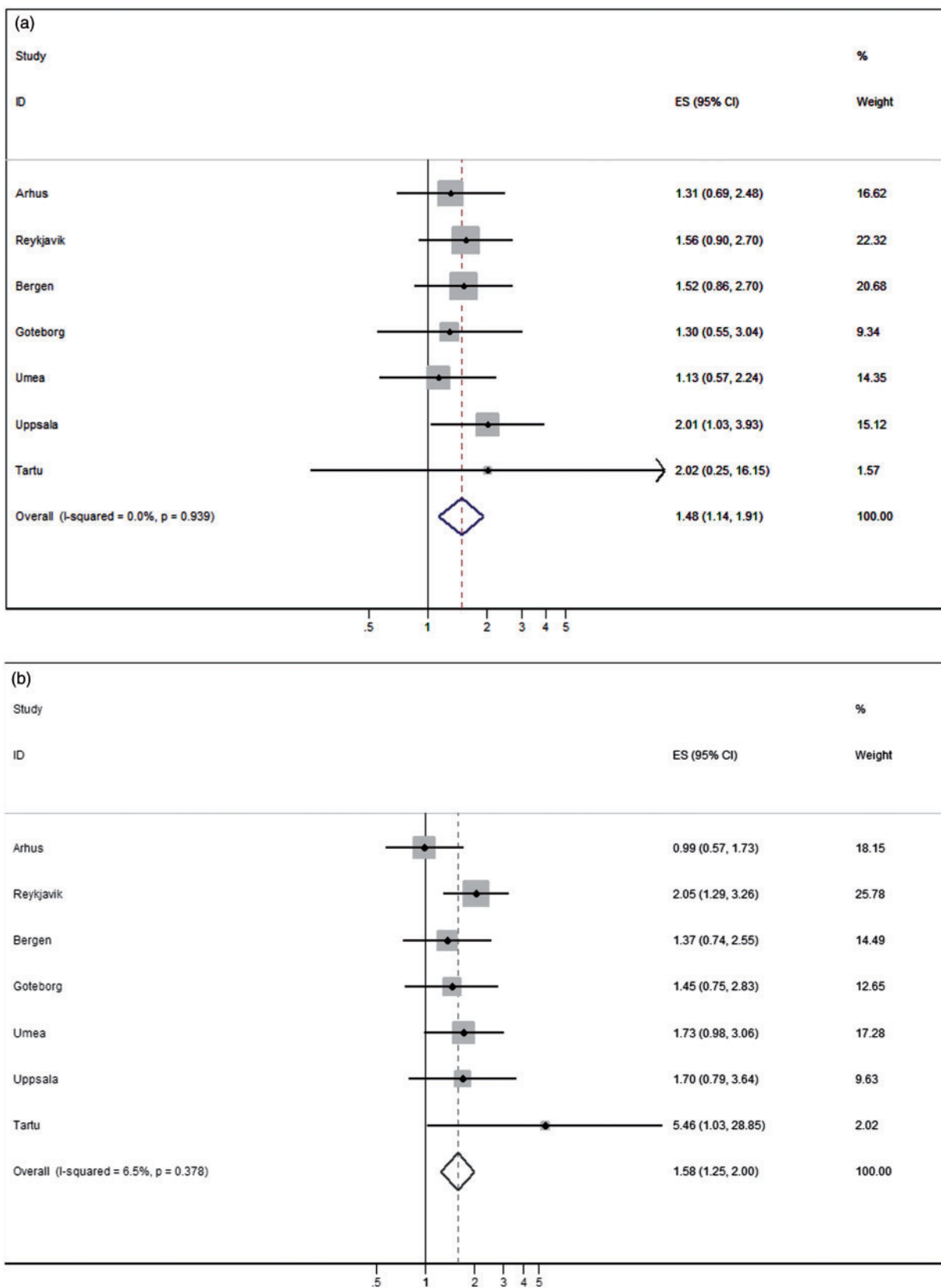
<sup>a</sup>Baseline group for comparison was those with no asthma and no hay fever. Excludes those who developed asthma after age 10.

<sup>b</sup>Excludes those with early-onset asthma and those under 10 years.

observation of increased body fat in sons of fathers who smoked before age 11 years.<sup>10</sup> There was higher offspring risk if the smoking started before completed puberty, but not in the case where fathers' own mother smoked, suggesting that *in utero* exposure of primordial germ cells could induce adaptive responses, preventing further damage to this cell stage during differentiation to spermatogonia in prepuberty, possibly by inducing specific maintenance mechanisms. Alternatively, there could be a threshold for damage of a certain exposure to this cell stage that is already reached by *in utero* exposure. After puberty, DNA methylation patterns are kept up through cycles of spermatogenesis. This could constitute another vulnerability window, requiring long exposure duration; our findings

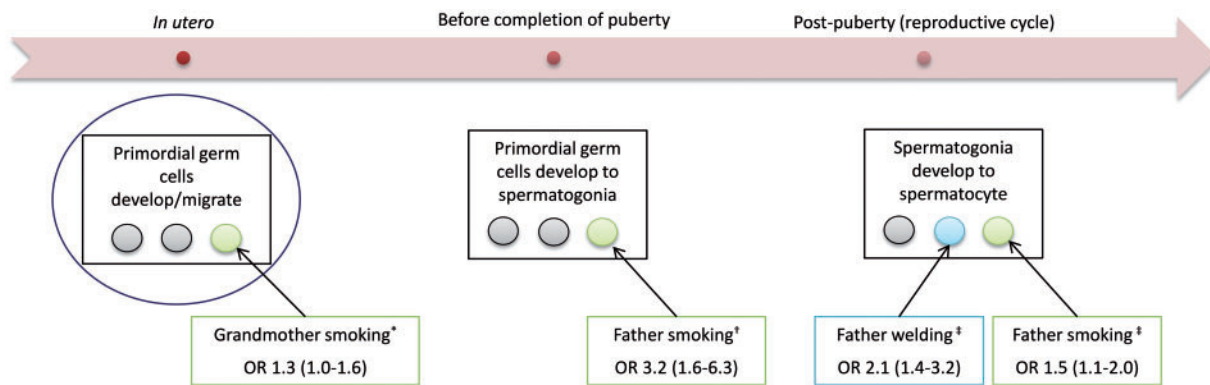
agree with findings of higher inheritable mutation frequency in murine spermatogonial stem cells with a longer duration of smoking exposure.<sup>7,31</sup> We found little benefit from quitting smoking prior to conception, in agreement with findings of impaired DNA repair mechanisms in smoking exposed spermatozoa.<sup>32</sup>

Smoking causes hormonal disruption, affecting hormonal and metabolic profiles. We found that men with early smoking debut were shorter and heavier than never-smokers and men with later smoking debut. However, adjustment for paternal height, weight, or body mass index (BMI) did not attenuate the association with offspring asthma (data not shown), suggesting that early smoking effects on offspring asthma were not mediated through the



**Figure 1.** Meta-analysis of the association between paternal (a) pre-conception smoking and (b) preconception welding with early-onset non-allergic asthma in offspring by study centre, adjusted for offspring age and paternal characteristics (age, education, smoking status). Boxes show odds ratios and horizontal lines show 95% confidence intervals for each study centre, box size is proportional to number of study subjects, diamond shows combined estimate for all centres with 95% confidence interval from fixed effects meta-analysis.





**Figure 2.** A hypothesized model of our findings in the context of existing knowledge regarding vulnerability windows in sperm development

\* Information on grandmother's smoking does not specify whether smoking took place during pregnancy

† Smoking starting before age 15

‡ Smoking or welding which started after 15 years of age and persisted for at least 10 years prior to conception. Circles represent stages in sperm development and colours represent an effect of an exposure on this cell stage.

impact on the father's own growth and metabolic development.

The findings relating to occupational welding support the concept that young men's environmental exposures impact their offspring's health. Welding represents a complex, mixed exposure that is associated with cancer and respiratory diseases. In addition, welding may cause heating of the groin region that can affect spermatozoa. Animal studies have shown effects on sperm, which could affect subsequent generations.<sup>15-17</sup>

A father's pre-conception exposures were related to a non-allergic asthma phenotype in offspring, suggesting that the effect is on lung function development rather than allergy (early frequent infections might be implicated). One may question whether growth and other chronic diseases in offspring might also be affected by a father's environmental exposures before parenthood.

The strengths of the present study include repeated detailed smoking assessment in a large cohort, allowing analyses of effect modification and restricted subgroups (i.e. smoked only before conception). A limitation is the lack of information on smoking for the non-RHINE participating parent. As smokers' spouses more often smoke themselves, this would attenuate observed differences between mothers' and fathers' smoking effects; thus, true differences by parent gender may be larger than observed. Asthma status and birth year were missing for 1961 subjects with data on fathers' smoking; sensitivity analyses suggested that this did not influence the results (see [Supplementary data](#), available at *IJE* online). Lacking information on the offspring's own smoking is unlikely to have affected results on early-onset asthma.

Misclassification of offspring asthma is not likely to be differential with regard to parental smoking debut and duration. As parents might not be aware of their children's

asthma status after childhood, late-onset asthma in particular could be underreported. Reporting bias is an unlikely explanation for the results, as similar associations were seen when using exposure data collected 10 years prior to collecting information about offspring (see [Supplementary data](#), available at *IJE* online). The lack of detail with regard to asthma phenotype in the offspring is a limitation of the study. Confounding by socio-economic status did not seem to explain our findings, as these changed little with adjustment for parental education, grandparental education and type of dwelling, and persisted in persons with tertiary educated parents (see [Supplementary data](#), available at *IJE* online). An association between paternal smoking and offspring asthma was stronger in those who quit smoking prior to conception (higher educational level, less potential for recall bias) than in those who continued smoking after child birth (possibly more recall bias). We did not have information about the use of personal preventive equipment in relation to welding, but we controlled for paternal age at survey, which is likely to account for potential changes in exposures over time. The finding that smoking and welding starting after the child's birth had no association with offspring asthma also supports a potentially causal effect. In general, whereas negative findings could be related to error, it is unlikely that systematic error has caused the observed associations. The potential role for unmeasured confounding in our positive findings must still be kept in mind.

In conclusion, our results suggest that a father's smoking and welding exposure prior to conception increased the risk for non-allergic early-onset asthma in his offspring and that early smoking debut and long exposure duration substantially increased the risk, even if the father quit exposure years before conception. These results come from a

large, population-based, multicentre study from northern Europe and are consistent between areas with different smoking and work habits and asthma prevalence. To our knowledge, this is the first study on asthma risk in humans that investigates in detail the effects of paternal exposures prior to conception. Future research should attempt to replicate these findings in other populations. Mechanistic studies are now important to understand the detrimental effect of paternal pre-conception smoking and welding, as well as potential effect modification by *in utero* exposure. Such investigations might provide targets for intervention in terms of inducing protective mechanisms. Furthermore, we believe that it is urgent to investigate other potentially harmful exposures in young men, where occupational exposures may be particularly important in high-income as well as in low-income societies. Our findings should not lessen efforts to improve maternal health, which is crucial for offspring health, but they provide an unexpectedly strong argument for also focusing on the environment of young men.

## Supplementary Data

Supplementary data are available at *IJE* online.

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## Authors' contributions

CS, AJ, BB, BF, TG, MH, CJ, RJ, EL, FM, AM, LM, DN, EO, EWS, TS, TDS, KT, VS and FGR carried out the

studies included in the present paper, and participated in study design, coordination and data collection. JK and CS performed the statistical analyses and drafted the manuscript together with SMS and FGR. SMS, AJ, RB, LB, AEC, SD, JD, TG, JH, MH, CJ, DJ, SKE, TS, ØS and FGR participated with study design, development of ideas, analytical design and interpretation of the findings. CT contributed to analyses. All authors read and approved the final manuscript. CS and JK contributed equally to this manuscript.

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