

Gene-environment interaction in atopic diseases: a population-based twin study of early life exposures

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Conflicts of interest: None of the authors have any conflicts of interest in relation to this manuscript.

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Abstract

Introduction: The development of atopic diseases early in life suggests an important role of perinatal risk factors.

Objectives: To study whether early life exposures modify the genetic influence on atopic diseases in a twin population.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/crj.12110

Methods: Questionnaire data on atopic diseases from 850 monozygotic and 2,279 like-sex dizygotic twin pairs, 3-9 years of age, from the Danish Twin Registry were cross-linked with data on prematurity, Caesarean section, maternal age at birth, parental cohabitation, season of birth, and maternal smoking during pregnancy, from the Danish National Birth Registry. Significant predictors of atopic diseases were identified with logistic regression and subsequently tested for genetic effect modification using variance components analysis.

Results: After multivariable adjustment, prematurity (gestational age below 32 weeks) (OR=1.93, CI=1.45-2.56); Caesarean section (OR=1.25, CI=1.05-1.49); and maternal smoking during pregnancy (OR=1.70, CI=1.42-2.04) significantly influenced the risk of asthma, whereas none of the factors were significantly associated with atopic dermatitis and hay fever. Variance components analysis stratified by exposure status showed no significant change in the heritability of asthma according to the identified risk factors.

Conclusion: In this population-based study of children there was no evidence of genetic effect modification of atopic diseases by several identified early life risk factors. The causal relationship between these risk factors and atopic diseases may therefore be mediated via mechanisms different from gene-environment interaction.

Key words: Asthma, twin study, genetics, environment.

Introduction

Twin studies have shown that up to 80% of the variation in the risk of developing atopic diseases can be ascribed to genetic effects, whereas environmental factors shared between family members do not, or only to a small extent, influence the variation in disease risk [1-6]. Contrary to this, several epidemiological studies of singleton populations have shown that environmental exposures shared between siblings during early life such as parental smoking, socioeconomic status, pet keeping, and dietary habits influence the development of atopic diseases [7-10]. This discrepancy between conclusions of epidemiological studies and twin studies can be ascribed to a number of factors; however, one probable cause is gene-environment interaction. In the classical twin study design interactions between genes and shared environment are included in the effect of genes [11]. This can underestimate the significance of shared environment and mask genetic effect modification [12].

The hypothesis of gene-environment interaction in atopic diseases is supported by several studies. For example, exposure to house dust mites modifies the risk of developing asthma in the presence of specific single nucleotide polymorphisms (SNPs) in the genes for *TGF- β 1* and *IL10* [13, 14]. Also, air pollution modifies the risk of developing asthma in the presence of specific variants in the arginase genes and the glutathione-S-transferase genes [15, 16]. Moreover, cat ownership increases the risk of eczema in the first years of life particularly in children with filaggrin loss-of-function mutations [17], whereas SNPs in the genes for *CARD4*, *CD14*, and Toll-like receptors are associated with a lower risk of asthma in children raised in a traditional farming environment [18-20]. Although several of these observations have not been replicated, they still suggest a gene-environment interaction. Furthermore, we recently found evidence of increased heritability of asthma over a relatively short time period from 1994-2003 indicating that

the increasing prevalence of asthma in the last decades is caused by environmental factors modifying the effect of susceptibility genes [21].

Twin studies offer a unique opportunity to examine gene-environment interactions since environmental risk factors shared between the two twins in a pair can be controlled for. Particularly, if a specific environmental factor interacts with genetics in the development of an atopic disease then the heritability of the atopic disease will differ in exposed twins compared with unexposed twins [22]. Accordingly, the twin design can be used to evaluate the modifying **effect** of environmental risk factors without knowing which genes are modified and can thereby guide future gene-environment interaction studies. The aim of this study was consequently to identify environmental risk factors that modify the genetic influence on atopic diseases.

Methods

Population

This study **was** based on all live born twins in Denmark between 1994 and 2000 registered in the Danish Twin Registry [23]. **In Denmark, all individuals have a unique personal identification number from birth, which allows complete track of the entire population. Consequently, there were no formal inclusion or exclusion criteria for this study since the entire population of twins born during these years was contacted.** In 2003, when the twins were **3-9** years of age, they were recruited for a questionnaire study about health and disease. The response rate to the questionnaire was 69% corresponding to 10,809 twin individuals of whom 5,399 were intact twin pairs. Among the intact pairs; 850 were monozygotic (MZ) and 2,279 were like-sex dizygotic (DZ) twin pairs. Zygosity was determined by four questions of similarity and mistaken identity between the twins, which determine zygosity correctly in more than 95% of the cases **compared with genetic marker information** [24].

Definition of diseases

Children with atopic diseases were identified by the questions: “Has your child ever had eczema in the folds of the elbows and knees?” **(this question diagnosed atopic dermatitis)**, “Has your child ever had asthma?” **(this question diagnosed asthma)**, and “Has your child ever had hay fever?” **(this question diagnosed hay fever)**. **These questions were adopted and modified from The International Study of Asthma and Allergies in Childhood (ISAAC) [25].**

Early life exposures

Information about gestational age, **type of delivery** (Caesarean section **vs. vaginal delivery**), maternal smoking during pregnancy, maternal age, and parental cohabitation at birth was collected from The Danish Medical Birth Registry **[26]. Selection of these potential these risk factors was based on previous knowledge of their relationship with atopic diseases. Furthermore, we chose these risk factors because they are shared between the two twin members of the pairs, in which case they can be entered into the analyses (see below).**

Statistical analysis

First, the risk (expressed as odds ratios, OR, with 95% confidence intervals, CI) of the three atopic diseases was calculated for the early life exposures using logistic regression with backwards model selection retaining factors with a p-value below 0.05 using SPSS 17.0 (SPSS Inc., Chicago, IL). However, due to the high number of early life exposures examined (six) and due to the fact that these were tested for an association with three different outcomes (atopic dermatitis, asthma, and hay fever) a conservative significance level of $0.05/18=0.003$ was employed to identify factors eligible for further genetic analysis. Second, the variation in the susceptibility to the atopic

diseases stratified by exposure status for these statistically significant early life factors was partitioned into components representing latent genetic (A), shared environmental (C), and non-shared environmental (E) factors, according to the classical twin method described by Neale and Cardon [27]. In these analyses, any shift in the relative influence of genetic factors on the susceptibility to the atopic diseases in exposed compared with unexposed twin pairs would signal a differential effect of genotypes under different environmental circumstances in which case we would speak of genetic effect modification or gene-environment interaction [22]. The statistical package Mx was used for the analyses [28].

Results

The lifetime prevalence of atopic dermatitis, asthma, and hay fever was 14.9%, 10.7%, and 3.8%, respectively. **A total of 24.9% had at least one atopic disease. There was an increased frequency of atopic dermatitis (24.8 vs. 13.7% (OR=2.08, CI=1.70-2.40), $p<0.001$), and hay fever (11.4 vs. 2.9%, OR=4.34 (CI=3.49-5.39), $p<0.001$) in children with asthma compared with children without asthma. The frequency of hay fever in children with atopic dermatitis was also increased compared with children without hay fever (9.0 vs. 2.9% (OR=3.35, CI=2.72-4.13), $p<0.001$).**

The association between early life exposures and atopic diseases is presented in Table 1. After multivariable adjustment and taking multiple testing into account, prematurity (gestational age below 32 weeks), (OR=1.93, CI=1.45-2.56); Caesarean section (OR=1.25, CI=1.05-1.49); and maternal smoking during pregnancy (OR=1.70, CI=1.42-2.04), significantly increased the risk of asthma, whereas none of the factors were significantly associated with atopic dermatitis and hay fever, except age group, which was significantly associated with hay fever (OR=2.46, CI=1.87-3.24).

Variance decomposition of the atopic diseases is shown in table 2. The overall contribution of genetic factors to the susceptibility to the atopic diseases was 94% (81-96%) for atopic dermatitis, 54% (40-68%) for asthma, and 43% (17-67%) for hay fever. Subsequent variance decomposition stratified by exposure status for the significant risk factors provided no evidence of genetic effect modification by prematurity on asthma (19% change in the heritability of asthma in children born at term compared with children born before 32 weeks gestation, $p=0.553$); by Caesarean section on asthma (16% change in the heritability of asthma in children delivered vaginally compared with children delivered by Caesarean section, $p=0.277$); or by maternal smoking during pregnancy on asthma (3% change in the heritability of asthma in children whose mothers smoked during pregnancy compared with children of non-smoking mothers, $p=0.851$). Age predicted a large change in the heritability of hay fever (42% change in the heritability of hay fever in children 7-9 years of age compared with children 3-6 years of age), however, this was not statistically significant, $p=0.099$.

Discussion

This study found that prematurity, Caesarean section, and **in utero exposure to passive smoking** were independent risk factors for asthma in 3-9-**year-old** children. However, as an extension to previous observations none of these early life exposures were found to significantly modify the influence of genetic effects on asthma. **This indicates** that their causal relationship with asthma is mediated via mechanisms different from gene-environment interaction.

Van Beijsterveldt et al are among few who have examined genetic effect modification in relation to atopic diseases in a twin study [29]. They found that the relative genetic contribution to the susceptibility to asthma in Dutch twins decreased significantly from 90% in children born at term, to 45% in children born at a gestational age of less than 32 weeks, and that

the relative contribution of shared environment increased from 0 to 52%. **This indicated** that the genetic susceptibility to asthma is modified by gestational age. We could not replicate this significant finding in our study. Although the Dutch population comprised almost 12,000 twin pairs the gestational age interval below 32 weeks only included 461 twin pairs (4.4% of the population), which may have led to chance allotment of DZ twin pairs concordant for asthma in this **group of very premature children** thereby lowering the heritability of the disease. In comparison, the number of twin pairs in our study born before 32 weeks gestation was 218 (7.0% of the population). However, a likely cause of discrepancy between the results of the Dutch study and our study in relation to gestational age is lack of power; our population comprised only about one fourth of the twins in the Dutch population.

Also, we did not find evidence of a significant genetic effect modification of Caesarean section on asthma. Caesarean section has been shown to increase the risk of asthma by 22% in a meta-analysis of observational studies [30] (we found an increased risk of asthma of 25% in children born after Caesarean section). Several theories have linked Caesarean section to asthma. Particularly, a change in the microbial stimulation of the newborn may favour a Th2 response in children delivered by Caesarean section leading to asthma [31, 32]. Moreover, asthma may arise in children born after Caesarean section due to physiological changes resulting from the lack of thoracic decompression of the newborn during delivery [33]. These theories support that direct causative mechanisms rather than genetic effect modification are responsible for the increased risk of asthma in children born after Caesarean section. Our data did not enable us to differentiate acute from elective Caesarean section. However, earlier studies have not convincingly documented a differential effect of these modes of delivery on the risk of asthma [34, 35].

Finally, we found that maternal smoking during pregnancy increased the risk of asthma by 70% in the offspring. However, this increased risk could not be ascribed to genetic

effect modification. This is somewhat in contrast to several earlier studies that have highlighted an interaction between maternal smoking during pregnancy and the genetic susceptibility to asthma in the offspring [36-39]. For example, it has been shown that there is an association between development of asthma in children and a SNP in the gene for *IL1RN* (interleukin-1 receptor antagonist) provided that the mother smoked during pregnancy [36], and also that *IL13* gene polymorphisms modify the effect of in utero exposure to tobacco smoke on the risk of persistent wheeze [37]. Moreover, children who are homozygous for the *Arg16* allele in the beta2-adrenergic receptor gene and who are exposed to maternal smoking during pregnancy are at a threefold increased risk of wheeze compared with children who are unexposed to smoking and who have at least one *Gly16* allele [38]. Likewise, in a study by Jaakkola and co-workers, children were divided into two groups based on their familial predisposition to atopic diseases [39]. Children from the group with a high genetic risk were shown to have an increased prevalence of asthma when exposed to tobacco smoke in the home in infancy. On the contrary, it was not possible to show an association between exposure to tobacco smoke and asthma for the group with a low genetic risk for developing asthma. Yet, non-genetic mechanisms may explain the harmful effects of in utero exposure to tobacco smoke. Notably, recent murine data suggest that prenatal nicotine exposure decreased forced expiratory flow in the offspring directly through effects on the $\alpha 7$ nicotinic receptor. More precisely, nicotine exposure was shown to increase airway length, to decrease airway diameter, and to increase the response to a methacholine challenge, even in the absence of allergic sensitization [40]. These observations fit with our conclusions and add to the body of evidence suggesting that the mechanisms by which maternal smoking during pregnancy increases the risk of asthma are multifactorial involving both direct effects as well as cross-talk with genetic make-up.

Even though we examined a large population of twins it was not possible to confirm a significant genetic effect modification regarding the early life exposures studied. **Still**, we cannot dismiss the significance of gene-environment interaction based on this study alone. It is most likely that examining specific genotypes instead of overall genetic effects could raise the sensitivity of the study. Furthermore, it is possible to increase the sensitivity by using clinical phenotypes. The atopic diseases were reported by the parents, which could have resulted in significant bias due to misreporting through omissions or false positives. Particularly, we determined whether a child had an atopic disease based on the question: ‘has your child ever had eczema in the folds of the elbows and knees/asthma/hay fever’, which may have a low diagnostic reliability. In further, the examined risk factors may be too unspecific as several of them do not inherently hold a biologically plausible model for gene-environment interaction, but presumably reflect one or more *genuine* risk factors. Finally, the population was followed for **3-9** years, and consequently exposure time varied from family to family, which could have induced bias. We found that age had a quite large impact on the heritability of hay fever. **Specifically, the** heritability was only 21% among **3-6-year-old** children, compared with 63% among **7-9-year-old** children, consistent with shared family environment accounting for a much higher proportion of variance in susceptibility to hay fever in the youngest age group. This could be regarded as evidence that the impact of the very early shared (intrauterine) environment on the risk of atopic diseases is strongest in the smallest children but weakens with age. However, the number of twins with hay fever was small in this age group, which made estimates less reliable.

In conclusion this study confirms several of the known risk factors for atopic diseases, primarily asthma, yet it was not possible to give proof of statistically significant genetic effect modifications for these factors. However, this does not preclude the existence of genetic effect modification in atopic diseases, and future studies could be designed to detect these effects if

they are present. Particularly, prenatal environmental factors influence the risk of asthma and atopic diseases in a complex and dynamic interplay with maternal and fetal immunogenetic factors. Consequently, future studies could address gene-environment interaction in atopic diseases in a genetic *expression* framework. For example, maternal folate supplementation resulted in hypermethylation (suppression) of regulatory genes and development of allergic disease in the offspring in a rodent model [41]. Also, maternal n-3 polyunsaturated fatty acid consumption during pregnancy may protect from subsequent infant allergic disease [42], possibly mediated via epigenetic mechanisms [43]. Epigenetics is an important area of future research where twin studies are expected to be particularly valuable [44]. Epigenetic mechanisms, which play an essential role in regulating transcription, are possibly capable of explaining the relatively high degree of discordance of atopic diseases in MZ twins. Studying tissue samples from MZ twin pairs discordant for atopic diseases therefore provides a unique opportunity for exploring the influence of epigenetic factors on disease expression.

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Table 1. Distribution of early life exposures and risk of atopic diseases in a sample of Danish twin pairs, 3-9 years of age.

	Atopic dermatitis			Asthma			Hay fever		
	N (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	N (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	N (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex									
Male	445 (13.6)	1.00	1.00	428 (13.1)	1.50 (1.28-1.76)	1.56 (1.32-1.85)**	143 (4.4)	1.43 (1.09-1.86)	1.51 (1.15-1.99)*
Female	460 (15.4)	1.15 (1.00-1.33)	1.19 (1.03-1.38)*	273 (9.1)	1.00	1.00	93 (3.1)	1.00	1.00
Age group									
3-6 years	527 (13.9)	1.00	-	388 (10.2)	1.00	1.00	98 (2.5)	1.00	1.00
7-9 years	378 (15.3)	1.12 (0.97-1.29)	-	313 (12.7)	1.27 (1.08-1.49)	1.30 (1.09-1.55)*	143 (5.8)	2.44 (1.87-3.18)	2.46 (1.87-3.24)**
Season of birth									
Spring	227 (14.2)	1.00	-	168 (10.5)	1.00	-	64 (4.0)	1.00	-
Summer	247 (14.2)	1.00 (0.83-1.22)	-	203 (11.7)	1.13 (0.91-1.40)	-	53 (3.1)	0.76 (0.52-1.09)	-
Autumn	225 (15.3)	1.09 (0.89-1.33)	-	163 (11.1)	1.06 (0.84-1.33)	-	54 (3.7)	0.91 (0.63-1.32)	-
Winter	206 (14.1)	0.99 (0.81-1.22)	-	167 (11.5)	1.10 (0.88-1.38)	-	65 (4.5)	1.12 (0.79-1.59)	-
Gestational age									
≥37 weeks	441 (14.5)	1.00	-	283 (9.3)	1.00	1.00	108 (3.6)	1.00	-
37-32 weeks	396 (14.3)	0.98 (0.85-1.14)	-	337 (12.2)	1.35 (1.14-1.59)	1.17 (0.98-1.40)	115 (4.2)	1.18 (0.90-1.54)	-
<32 weeks	62 (14.3)	0.98 (0.73-1.30)	-	79 (18.2)	2.16 (1.64-2.83)	1.93 (1.45-2.56)**	12 (2.8)	0.77 (0.42-1.41)	-
Caesarean section									
No	575 (15.0)	1.00	-	410 (10.7)	1.00	1.00	155 (4.0)	1.00	-
Yes	313 (13.3)	0.87 (0.75-1.01)	-	287 (12.2)	1.16 (0.99-1.37)	1.25 (1.05-1.49)**	81 (3.4)	0.85 (0.65-1.12)	-
Maternal age									
≥30 years	523 (13.7)	1.00	1.00	415 (10.9)	1.00	-	147 (3.8)	1.00	-
<30 years	382 (15.7)	1.17 (1.02-1.36)	1.18 (1.02-1.37)*	286 (11.8)	1.09 (0.93-1.28)	-	89 (3.7)	0.95 (0.73-1.24)	-
Maternal smoking									
No	698 (15.0)	1.00	1.00	463 (10.0)	1.00	1.00	176 (3.8)	1.00	-
Yes	158 (12.6)	0.82 (0.68-0.98)	0.80 (0.67-0.97)*	201 (16.1)	1.73 (1.45-2.07)	1.70 (1.42-2.04)**	50 (4.0)	1.06 (0.77-1.46)	-
Parents living together									
Yes	857 (14.5)	1.00	-	649 (11.0)	1.00	-	221 (3.7)	1.00	-
No	46 (14.7)	0.98 (0.71-1.35)	-	47 (15.0)	1.44 (1.04-1.98)	-	13 (4.2)	1.12 (0.63-1.98)	-

*P-value <0.05, **P-value <0.003 (significance level when adjusting for multiple testing).

Table 2. Variance decomposition of atopic diseases stratified by early life exposure status in a sample of Danish twin pairs, 3-9 years of age.

	A (%)	C (%)	E (%)	P-value*
Atopic dermatitis	94 (81-96)	0 (0-12)	6 (4-10)	
Asthma	54 (40-68)	41 (27-53)	5 (3-9)	
Sex				
Male	49 (30-69)	43 (25-60)	8 (4-14)	
Female	60 (41-82)	38 (16-56)	3 (1-7)	0.462
Gestational age				
≥37 weeks	61 (39-84)	35 (12-54)	4 (2-11)	
37-32 weeks	50 (31-71)	45 (25-62)	5 (2-10)	0.480
<32 weeks	42 (0-95)	43 (0-81)	15 (3-42)	0.553
Caesarean section				
No	47 (28-66)	46 (29-62)	7 (6-13)	
Yes	63 (42-86)	34 (11-53)	3 (1-9)	0.277
Maternal smoking				
No	56 (39-74)	38 (21-54)	6 (3-10)	
Yes	53 (27-82)	42 (14-64)	5 (1-13)	0.851
Hay fever	43 (17-67)	47 (25-66)	10 (4-20)	
Age group				
3-6 years	21 (0-57)	66 (34-87)	13 (4-30)	
7-9 years	63 (27-96)	28 (0-57)	9 (3-22)	0.099

Proportion of variance (95% confidence intervals) of disease susceptibility due to genetic factors (A), shared environmental factors (C), and non-shared environmental factors (E). Only exposures significantly associated with atopic diseases in multiple regression analysis are analysed above.

*Test for genetic effect modification (comparison of A (%) in exposed compared with unexposed children).