

University of Groningen

A search for missing pieces of the puzzle; the development of asthma and atopy

Reijmerink, Naomi Elizabeth

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Reijmerink, N. E. (2009). *A search for missing pieces of the puzzle; the development of asthma and atopy: innate immunity genes and environment*. [s.n.].

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

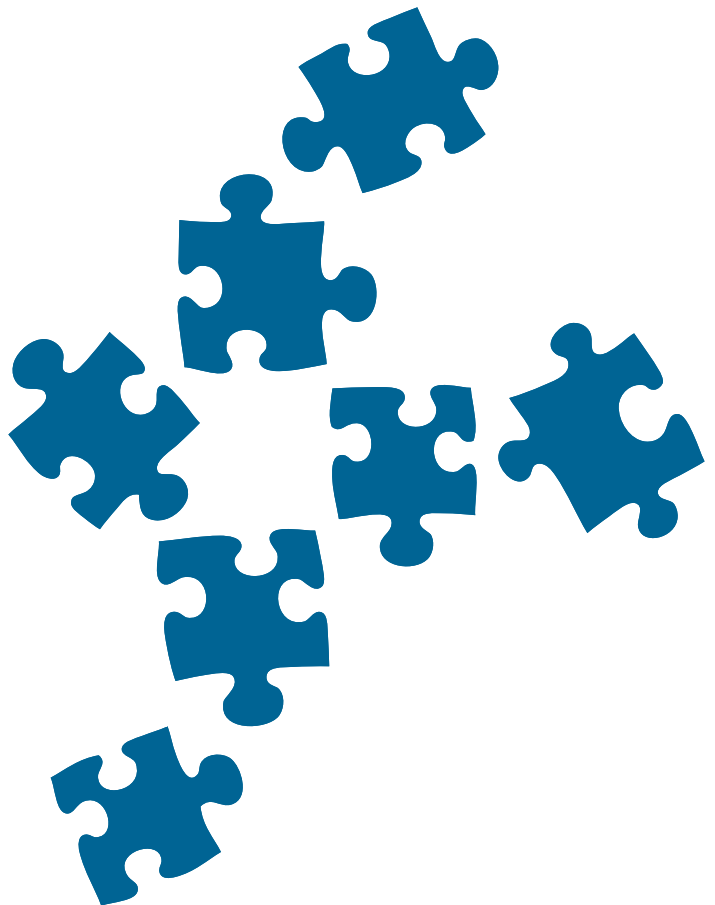
Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 7

Smoke exposure interacts with *ADAM33* polymorphisms in the development of lung function and hyperresponsiveness

Naomi E. Reijmerink
Marjan Kerkhof
Gerard H Koppelman
Jorrit Gerritsen
Johan C de Jongste
Henriette A. Smit
Bert Brunekreef
Dirkje S Postma

Allergy. 2009 jun;64(6):898-904



Abstract

Introduction: *ADAM33* is the first identified asthma gene by positional cloning, especially asthma combined with bronchial hyperresponsiveness (BHR). Moreover, *ADAM33* is associated with early life lung function and decline of FEV₁ in the general population. In utero and postnatal cigarette smoke exposure (CSE) is associated with reduced lung function, and development of BHR and asthma. We hypothesized that this may occur via interaction with *ADAM33*.

Aim: To replicate the role of *ADAM33* in childhood lung function and development of BHR and asthma. Furthermore, we investigated gene-environment interaction of *ADAM33* with in utero and postnatal CSE in the Dutch PIAMA cohort.

Methods: Six *ADAM33* SNPs were genotyped. Rint was measured at age 4 and 8 years, FEV₁ and BHR at age 8 years; asthma was based on questionnaire data at age 8.

Results: In the total cohort, the rs511898 A, rs528557 C and rs2280090 A alleles increased the risk to develop asthma (+BHR). There existed interaction between in utero, but not postnatal CSE and the rs528557 and rs3918396 SNPs with respect to development of BHR, the rs3918396 SNP with Rint at age 8 and the rs528557 SNP with FEV₁ % predicted.

Conclusions: We confirm associations between *ADAM33* and the development of asthma (+BHR). This is the first study suggesting that interaction of in utero CSE with *ADAM33* results in reduced lung function and the development of BHR, which needs further confirmation.

Introduction

Impaired lung function and bronchial hyperresponsiveness (BHR) in early childhood are well-known risk factors for the development of asthma later in life.¹⁻³ The origins of impaired lung function and BHR have not been elucidated so far, but it is plausible that biological processes that underlie in utero and early life lung development are important contributing factors. It is clear that asthma and BHR have strong genetic and environmental components. These environmental factors often start in pregnancy and early infancy, and include a.o. pre and postnatal cigarette smoke exposure (CSE).^{4,5}

ADAM33 is the first asthma gene identified by positional cloning.⁶ This gene was in particular found by combining a doctor's diagnosis of asthma with the presence of BHR. Interestingly, ADAM33 SNPs have also been associated with impaired early life lung function at age 3-5 years and with FEV₁ decline in both adult asthma patients and the general population.⁷⁻¹⁰

Multiple ADAM33 protein isoforms exist in human embryonic lung¹¹, as is the case in the lungs of adult asthma patients as well.^{11,12} Not only airway smooth muscle cells but also primitive mesenchymal cells of the fetal lung, forming a cuff at the end of the growing lung bud, stain with ADAM33.¹¹ Thus, ADAM33 may well play a role in orchestrating branching morphogenesis and polymorphic variations in the ADAM33 gene may influence the subsequent susceptibility of the lung to asthma.

Several epidemiologic studies have provided evidence that CSE in utero and early life is associated with reduced lung function and constitutes a risk factor for the development of asthma and BHR.^{13,14} It is thus conceivable that in utero and early life CSE interact with genes important in asthma development, thereby affecting in utero and early life lung development, airway remodelling, and subsequent development of early life lung function changes and asthma.

We studied the role of ADAM33 in early life lung function, BHR and asthma in a Dutch birth cohort (PIAMA) that allowed us to prospectively follow environmental exposure, symptoms and lung function, including BHR measurements up to 8 years of age. We analysed whether CSE in utero and in early life, in combination with ADAM33 genotypes influences early life lung function, BHR and asthma.

Methods

Study design

Prevention and Incidence of Asthma and Mite Allergy (PIAMA) is a birth cohort study that started in 1996. At baseline, 4146 children were included, 1327 high-risk children (with a positive maternal history for atopy) and 2819 children of non-allergic mothers. The children were recruited during the first trimester of pregnancy. Parents completed annual questionnaires derived from the International Study of Asthma and Allergies in Childhood (ISAAC)¹⁵ about allergic symptoms in the child, information about general health, indoor environment like CSE, socio-economic characteristics, lifestyle, breastfeeding and

demographic factors. All 1327 high risk children and a random sample of 663 low risk children were selected for medical examinations. Resistance interrupter technique (Rint; Micro Medical, Rochester, Kent, UK) measurements were obtained at age 4 yrs, and Rint as well as spirometry and BHR to methacholinebromide were measured at age 8 yrs. The Rint technique measures interrupter resistance, which is similar to airway resistance. It is a non invasive method that requires only tidal breathing. Therefore it can easily be performed in pre-school children.¹⁶⁻¹⁸ A further description of the study protocol has been published previously.¹⁹ All parents provided written informed consent.

DNA

Genomic DNA was extracted from buccal swabs or blood²⁰ at age 4 yrs (n=1037). Genetic studies were approved by local medical ethics committees of participating institutes. Six Single Nucleotide Polymorphisms (SNPs), i.e. rs511898, rs612709, rs3918396, rs528557, rs2280090 and rs2787094 were analysed. These SNPs were chosen since they were associated with asthma and lung function in the Dutch population.^{8,9,21} Children with a non-Caucasian ethnicity were excluded (n=51).

For all SNPs, primers and probes were ordered from Applied Biosystems TaqMan® SNP Genotyping Assays (Nieuwekerk aan de IJssel, The Netherlands), using the Assay-by-Design service for which we provided sequences, since the rs528557 SNP previously failed⁸ we used primers and probes with the Primer Express package and obtained primers from Biologio (Malden, The Netherlands) and probes from Applied Biosystems. Sequences of all primers and probes are shown previously.⁸ Reactions were performed in 5 µl volumes of 10 ng DNA, 1x Taqman Universal Mastermix (Applied Biosystems), 200 nM probe and 900 nM primer. Cycling conditions on the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems) were 10 min 95°C followed by 40 cycles of 15 seconds 95°C and 1 minute 60°C. End-point fluorescence was measured immediately after cycling. Alleles were assigned using SDS 2.1 software (Applied Biosystems). Genotyping call rates ranged from 89%-94.5%, comparable with the study by Schedel *et al.*²²

Phenotypes

Rint measurements were performed as described previously.²³ Rint measurements at age 4 and 8 yrs and FEV₁ % predicted at age 8 yrs were used as lung function variables. BHR was defined as a fall of ≥ 20% in FEV₁ after inhalation of a maximum of 0.62 mg methacholinebromide. We used two definitions of asthma; 1) Asthma at age 8, based on at least 1 episode of wheeze and/or dyspnea in the last year and/or the use of inhaled steroids; and 2) Asthma in combination with BHR.

In utero CSE was defined as a confirmative answer to the question “do you smoke cigarettes?” or “have you been smoking, and when did you quit smoking?” asked to the mother during the third trimester of pregnancy. CSE in the first year of life was defined as a confirmative answer to the question “does anyone smoke, at least once a week, in the home?” asked to the parents when the child was 1 year of age. Lung function, BHR, asthma and asthma with BHR were analyzed in the total group and in children with in utero CSE and CSE at age 1 year separately.

Statistical analysis

Lung function parameters were normally distributed and analyzed using analysis of variance (ANOVA) and linear regression. BHR, asthma and asthma with BHR were analysed using chi-squared tests and logistic regression. An interaction term of genotype and CSE (in utero and in the first year of life) was included into linear and logistic regression models. All analyses were corrected for gender. All genotypes were analysed in the best fitting, dominant model, i.e. by comparing children with one or two copies of the minor alleles with those carrying no copy. Analyses of ADAM33 in combination with CSE analyses were corrected for gender, atopy or asthma of the mother, atopy or asthma of the father, siblings, breastfeeding, pet and in utero CSE or CSE at age 1 year. Calculations were performed using SPSS 14.0 statistical software. Benjamini and Hochberg False Discovery Rate (FDR) was used to correct for multiple testing.²⁴

Results

DNA and Rint values at age 4 yrs were available in 768 children, DNA and spirometry at age 8 yrs were available in 746 children. Since inhalation medication taken before Rint measurement could influence the outcome, we excluded children who took inhalation medication <12 hours prior to Rint measurements (n=25 at 4 yrs and n=7 at 8 years).

General characteristics of the study population are shown in Table 1.

Table 1. Characteristics

| Characteristics | Genotyped Children |
|--|--------------------|
| Number | 1037 |
| Boys (%) | 532 (51.2) |
| Family history | |
| Atopy mother (%) | 690 (66.5) |
| Atopy father (%) | 327 (31.6) |
| Environmental exposures | |
| In utero smoke exposure (%) | 154 (15.0) |
| Smoke exposure in first year (%) | 230 (23.2) |
| Lung function | |
| number | 779 |
| FEV ₁ % predicted mean (SD) | 107.0 (11.5) |
| Methacholine Provocation | |
| number | 692 |
| BHR (%) | 313 (45.2) |
| Asthma (%) | 153 (16.5) |
| Asthma with BHR# (%) | 72 (10.2) |

Within children with complete methacholine provocation measurements

Genotyping

All SNPs were in Hardy Weinberg equilibrium ($P > 0.05$). There existed strong Linkage Disequilibrium (LD) between the SNPs rs511898, rs612709, rs3918396, rs528557 and rs2280090, ($D' > 0.8$), (Figure 1). Allele frequencies are described in Table 2.

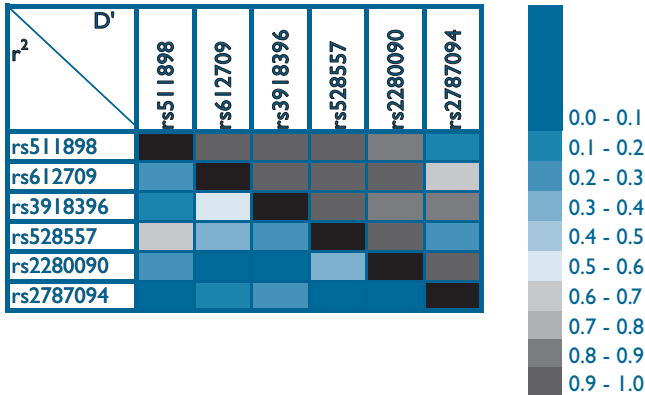


Figure 1.
LD structure of ADAM33 SNPs in the PIAMA cohort (r^2 below and D' above the diagonal)

Table 2. ADAM33 SNPs and their minor allele frequency in PIAMA cohort

| rs number | Alleles* | SNP** | Location | MAF*** |
|-----------|----------|-------|-----------------------|--------|
| rs511898 | G/A | F+1 | Catalytic domain | 0.35 |
| rs612709 | C/T | Q-1 | Cysteine rich domain | 0.13 |
| rs3918396 | G/A | S_1 | Trans membrane domain | 0.09 |
| rs528557 | G/C | S_2 | Trans membrane domain | 0.28 |
| rs2280090 | G/A | T_2 | Cytoplasmic domain | 0.14 |
| rs2787094 | C/G | V_4 | 3'UTR | 0.25 |

*First allele, wild type.

** SNP numbering as shown in previous publications^(6,8,9,21)

***Minor Allele Frequency

Association analysis

Lung function

No association was found between SNPs in ADAM33 and FEV₁ and Rint in the total cohort (data not shown).

BHR, asthma and asthma combined with BHR

No association was found between SNPs in ADAM33 and BHR in the total cohort. Children carrying the rs511898 A allele or rs528557 C allele were more likely to develop asthma compared to children with the rs511898 GG or rs528557 GG genotypes. Children carrying the rs528557 C allele or rs2280090 A allele were more likely to develop asthma in combination with BHR compared to the rs528557 GG or rs2280090 GG genotypes (Table 3).

Table 3. Association of ADAM33 SNPs with asthma and asthma combined with BHR in the PIAMA cohort

| SNP | Allele | N* | BHR | Asthma | Asthma with BHR |
|-----------|--------|-----|---------------|----------------------|-----------------------|
| rs511898 | GG | 397 | 1 | 1 | 1 |
| | GA | 397 | 1.1 (0.8-1.5) | 1.4 (1.0-2.1) | 1.5 (0.8-2.8) |
| | AA | 122 | 1.2 (0.8-2.0) | 1.7 (1.0-2.9) | 2.0 (0.9-4.3) |
| rs612709 | CC | 684 | 1 | 1 | 1 |
| | CT | 216 | 0.8 (0.5-1.2) | 1.2 (0.8-1.9) | 1.1 (0.6-2.1) |
| | TT | 11 | 0.2 (0.0-1.9) | 1.9 (0.5-7.4) | 1.2 (0.1-12.2) |
| rs3918396 | GG | 745 | 1 | 1 | 1 |
| | GA | 153 | 1.0 (0.6-1.5) | 1.4 (0.9-2.3) | 1.2 (0.6-2.4) |
| | AA | 9 | 0.4 (0.0-3.9) | 0.7 (0.8-5.3) | 1.3 (0.1-13.1) |
| rs528557 | GG | 458 | 1 | 1 | 1 |
| | GC | 330 | 1.1 (0.8-1.6) | 1.4 (0.9-2.0) | 1.8 (1.0-3.2) |
| | CC | 73 | 0.8 (0.4-1.5) | 1.9 (1.0-3.6) | 1.8 (0.1-4.6) |
| rs2280090 | GG | 659 | 1 | 1 | 1 |
| | GA | 219 | 1.2 (0.8-1.7) | 1.5 (1.0-2.3) | 1.7 (0.9-3.2) |
| | AA | 17 | 2.6 (0.8-8.9) | 1.0 (0.2-4.4) | 3.8 (0.6-23.9) |
| rs2787094 | CC | 520 | 1 | 1 | 1 |
| | CG | 334 | 0.9 (0.7-1.3) | (0.7-1.5) | 1.0 (0.6-1.8) |
| | GG | 59 | 1.1 (0.8-1.5) | 0.4 (0.1-1.0) | 0.9 (0.3-2.8) |

* numbers genotyped

Bold: P ≤ 0.05, in a dominant model **Odds ratios and (95% confidence intervals)**,

wild type is reference group.

BHR: Bronchial hyperresponsiveness

Interaction of ADAM33 genotypes and in utero smoke exposure

FEV₁: In children whose mother smoked during pregnancy, the rs528557 C allele was associated with lower FEV₁ % predicted compared to children with the GG genotype. In children whose mother did not smoke during pregnancy, the rs528557 C allele was associated with higher FEV₁ % predicted levels compared to children with the GG genotype (interaction term p=0.006, figure 2A). A similar trend was present for rs3918396 (smoke exposed children mean FEV₁ % predicted 104.0% (A allele) vs. 105.9% (GG genotype) and children not exposed to smoke in utero mean FEV₁ % 108.1% (A allele) vs. 106.9% (GG genotype))

Rint: A similar type of interaction as seen with FEV₁ was present for Rint at age 8 years and rs3918396 SNP, showing increased Rint values in children with the rs3918396 A allele in children whose mother smoked during pregnancy (interaction term p=0.02). A similar trend was present for rs528557. No interaction was present for Rint at age 4 yrs and in utero CSE.

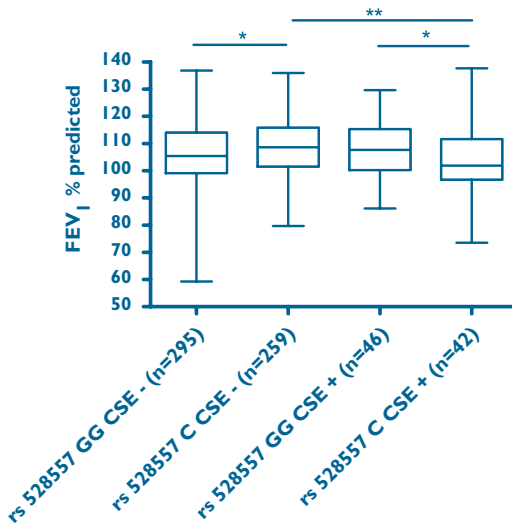


Figure 2A
Interaction of ADAM33 SNPs and in utero CSE in level of FEV₁ at 8 years of age

CSE + = Smoke exposure during pregnancy
CSE - = No smoke exposure during pregnancy
* P ≤ 0.05, **P ≤ 0.01

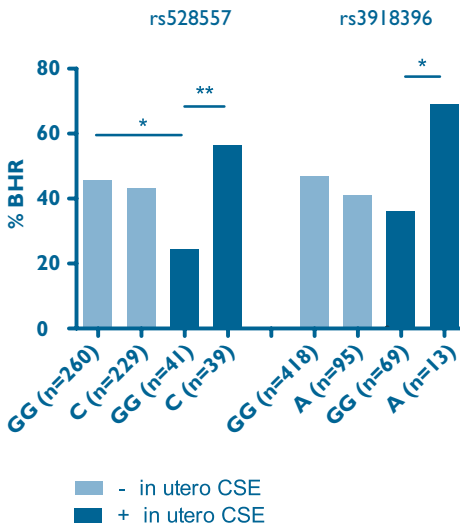


Figure 2B
Interaction of ADAM33 SNPs and in utero CSE in the development of BHR at 8 years of age

* P ≤ 0.05, **P ≤ 0.01

BHR: We found a significant interaction between in utero CSE and the ADAM33 SNPs rs528557 and rs3918396 with respect to the development of BHR. Within children whose mother smoked during pregnancy, the rs528557 C or rs3918396 A allele increased the risk for BHR development compared to rs528557 GG or rs3918396 GG (rs528557 Odds Ratio (OR) 4.0; 95% Confidence Interval (CI) 1.5-10.4 p-value interaction term = 0.004; rs3918396 OR 4.0; 95% CI 1.1-14.2, p-value interaction term = 0.02). This genotype effect was not found in children whose mothers did not smoke during pregnancy. Conversely, in children with the rs528557 GG or rs3918396 GG genotype in utero CSE decreased the risk of BHR, compared to all the children

who were not exposed to cigarette smoke (figure 2B). The same trend was present for the rs511898 SNP (data not shown).

The groups became too small to analyse an interaction between in utero CSE and ADAM33 genotypes with respect to asthma development.

Interaction of ADAM33 genotypes and smoke exposure in the first year of life

No significant interaction was found between CSE in the first year of life and the ADAM33 genotypes with respect to the development of BHR and level of lung function at the age of 4 and 8 years (data not shown).

Correction for multiple testing

None of the above described analyses reached significance after correction for multiple testing.

Discussion

Our results confirm the previously reported association of ADAM33 SNPs with asthma as well as asthma combined with BHR.⁶ We extend previous findings in that we provide data suggesting that gene-environment interaction of ADAM33 genotypes and in utero CSE exist with respect to the level of lung function and the development of BHR at age 8 years, an effect not observed with CSE in the first year of life.

ADAM33 is expressed in embryonic lung tissue on mesenchymal cells¹¹ which are responsible for branching, a lung developmental phase that continues until \pm 17 weeks of gestation.¹² The respiratory airways and future respiratory units develop during the third trimester. This is followed by an alveolar phase of lung development, which proceeds into the first 2-3 years of postnatal life, during which the number of alveoli increases.²⁵ ADAM33 may thus play a role especially in prenatal lung development, for instance by orchestrating branching morphogenesis. In this way it can affect the development of lung function, BHR, and asthma. Furthermore soluble ADAM33 in bronchial alveolar lavage fluid is increased in asthma patients and correlates with disease severity and reduced lung function.²⁶ A recent *in vitro* and *in vivo* study showed that soluble ADAM33 causes endothelial cell differentiation and affects angiogenesis in embryonic lung tissue.²⁷ Angiogenesis is considered an important aspect of tissue inflammation and remodelling. How would in utero CSE influence ADAM33 shedding? This may well run by increased levels of TGF- β by smoke exposure²⁸, since Puxeddu *et al* showed that TGF- β_2 enhances ADAM33 shedding from cells expressing ADAM33.²⁷

A recent report on another prospective birth cohort study, the MAAS cohort, showed at the age of 5 yrs that four SNPs in ADAM33 were associated with reduced FEV₁ (rs511898, rs3918395, rs2280091, and rs2280090; $p < 0.04$).⁷ Our study extends the latter observations. It appears that smoking by mothers during pregnancy is an important contributor to the effects of ADAM33 on lung function, since the rs528557 SNP was associated with reduced FEV₁ in children with in utero CSE, with an opposite

effect on FEV₁ in children not exposed. Interestingly, the percentage of children with in utero CSE in the MAAS cohort is much higher than in the PIAMA cohort (respectively 22.3% and 15%).²⁹ It is therefore plausible that the association of ADAM33 with reduced lung function in the MAAS cohort is predominantly present in the children whose mother smoked during pregnancy. In addition to the interaction of in utero CSE with ADAM33 SNPs on lung function we found interaction on the development of BHR as well. In utero CSE is known to influence lung function development and to increase the risk to develop asthma and BHR.^{13,30-32} Our data suggests that this association may at least in part be due to an interaction with ADAM33.

Of interest and requiring further functional study is the observation that the rs528557 GG and rs3918396 GG genotype have a protective effect on BHR when in utero smoke exposure occurred. It is yet unclear, and has to be determined whether this implies an effect on smooth muscle proliferation, epithelial integrity and/or airway branching.

The interaction of ADAM33 with in utero CSE and CSE in childhood was also analysed by Schedel *et al*²² in children from the ISAAC II and MAS cohorts. They did not find evidence for interaction of ADAM33 SNPs with asthma, BHR, or lung function. The difference in outcome between their and our study may be caused by, up to now unidentified environmental factors, or by methodological differences between the studies (e.g. methacholine provocation versus histamine and cold air provocation).

We replicate the initial association of ADAM33 genotypes with asthma and asthma with BHR as reported by van Eerdewegh⁶, thereby confirming the importance of ADAM33 in asthma. Not all studies have confirmed association of ADAM33 SNPs and asthma^{33,34}, and the studies that do replicate the results often find association with different SNPs or different alleles. There are several explanations for this discrepancy. First, there is variability in the definition of asthma used in the different studies. We defined asthma at age 8 based on at least 1 episode of wheeze or dyspnea in the last year and/or the use of inhaled steroids. Furthermore we analysed asthma in combination with BHR, since linkage became stronger combining asthma and BHR in the initial study by van Eerdewegh.⁶ Some studies with negative results analysed asthma solely based on a doctor's diagnosis.^{34,35} Secondly, a meta-analysis showed that ADAM33 has only a moderate effect on the development of asthma (odds ratio of 1.4 for SNPs known to date).³⁶ Therefore, as previously suggested^{22,36}, it is plausible that even with large datasets the chance of finding associations with such a moderate risk is limited. Thirdly, it may be possible that discrepant results between the studies are due to differences in environmental factors and population heterogeneity. We show in our study that the effects of ADAM33 polymorphisms on lung function and BHR in the total population were only present in combination with CSE, suggesting that ADAM33 is a gene that can be influenced by environmental factors. Finally, it is also possible that the associated SNPs are not the causative ones, but in linkage disequilibrium with the causative SNPs within ADAM33 or in genes located near ADAM33. This might also explain why different SNPs and alleles are associated in the studies. This phenomenon is also known as loose genotypic replication.³⁷

This study has some pitfalls, which should be acknowledged. First our study was

based on the selection of SNPs that were previously associated with lung function and asthma. We found strong LD between several genotyped SNPs, so it is plausible that the associations we observed with different SNPs and similar phenotypes is caused by one single SNP that is in strong LD with the other SNPs. Therefore a full description of ADAM33 in terms of polymorphic variations and LD structure is needed. Secondly, our results did not remain significant after correction for multiple testing. However, since this is a hypothesis testing study and (part of) the results are a replication of previously published data we feel that correction for multiple testing will underestimate the results. Notwithstanding this we do recognise that our results might be due to chance and that other studies have to replicate especially our new findings. Furthermore since mothers who are smoking during pregnancy are likely to smoke after pregnancy it is difficult to study the independent effect of CSE. However several studies have shown a strong effect of especially in utero smoke exposure on lung function and asthma development.³⁰⁻³² In our study, the majority of the children who were exposed in the first year of life (59%) were not exposed during pregnancy. CSE in the first year of life only did not show an interaction with ADAM33.

We conclude that ADAM33 genotypes increase the risk to develop asthma and asthma in combination with BHR. Furthermore, we have provided data that need confirmation, but suggest that gene-environment interaction of ADAM33 genotypes and in utero cigarette smoke exposure with the level of early life lung function and development of BHR. In combination with data in the literature, our study highlights that it is important to investigate cigarette smoking during pregnancy as a risk factor for early life lung function and BHR development, in interaction with genetic factors like ADAM33.

Reference list

1. Grol MH, Postma DS, Vonk JM, Schouten JP, Rijcken B, Koeter GH et al. Risk factors from childhood to adulthood for bronchial responsiveness at age 32-42 yr. *Am J Respir Crit Care Med* 1999; 160(1):150-6.
2. Haland G, Carlsen KC, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M et al. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; 355(16):1682-9.
3. Dezateux C, Stocks J. Lung development and early origins of childhood respiratory illness. *Br Med Bull* 1997; 53(1):40-57.
4. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. *Chest* 2005; 127(2):502-8.
5. Hofhuis W, de Jongste JC, Merkus PJ. Adverse health effects of prenatal and postnatal tobacco smoke exposure on children. *Arch Dis Child* 2003; 88(12):1086-90.
6. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418(6896):426-30.
7. Simpson A, Maniatis N, Jury F, Cakebread JA, Lowe LA, Holgate ST et al. Polymorphisms in a disintegrin and metalloprotease 33 (ADAM33) predict impaired early-life lung function. *Am J Respir Crit Care Med* 2005; 172(1):55-60.
8. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Schouten JP, Boezen HM. A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population. *Am J Respir Crit Care Med* 2005; 172(3):329-33.
9. Jongepier H, Boezen HM, Dijkstra A, Howard TD, Vonk JM, Koppelman GH et al. Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma. *Clin Exp Allergy* 2004; 34(5):757-60.
10. Gosman MM, Boezen HM, van Diemen CC, Snoeck-Stroband JB, Lapperre TS, Hiemstra PS et al. A disintegrin and metalloprotease 33 and chronic obstructive pulmonary disease pathophysiology. *Thorax* 2007; 62(3):242-7.
11. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Wilson SJ, Wilson DI et al. ADAM33 expression in asthmatic airways and human embryonic lungs. *Am J Respir Crit Care Med* 2005; 171(9):958-65.
12. Powell RM, Wicks J, Holloway JW, Holgate ST, Davies DE. The splicing and fate of ADAM33 transcripts in primary human airways fibroblasts. *Am J Respir Cell Mol Biol* 2004; 31(1):13-21.
13. Stick SM, Burton PR, Gurrin L, Sly PD, LeSouef PN. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. *Lancet* 1996; 348(9034):1060-4.
14. Cook DG, Strachan DP, Carey IM. Health effects of passive smoking. 9. Parental smoking and spirometric indices in children. *Thorax* 1998; 53(10):884-93.
15. Asher I. ISAAC International Study of Asthma and Allergies in Childhood. *Pediatr Pulmonol* 2007; 42(1):100.
16. Chowienczyk PJ, Lawson CP, Lane S, Johnson R, Wilson N, Silverman M et al. A flow interruption device for measurement of airway resistance. *Eur Respir J* 1991; 4(5):623-8.
17. Phagoo SB, Watson RA, Pride NB, Silverman M. Accuracy and sensitivity of the interrupter technique for measuring the response to bronchial challenge in normal subjects. *Eur Respir J* 1993; 6(7):996-1003.
18. Arets HG, Brackel HJ, van der Ent CK. Applicability of interrupter resistance measurements using the MicroRint in daily practice. *Respir Med* 2003; 97(4):366-74.
19. Brunekreef B, Smit J, de Jj, Neijens H, Gerritsen J, Postma D et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002; 13 Suppl 15:55-60.
20. Sambrook JaRD. Preparation of plasmid DNA by lysis with SDS. Cold Spring Harbour, New York: Laboratory press, 2001.

21. Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL et al. Association of a disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. *J Allergy Clin Immunol* 2003; 112(4):717-22.
22. Schedel M, Depner M, Schoen C, Weiland SK, Vogelberg C, Niggemann B et al. The role of polymorphisms in ADAM33, a disintegrin and metalloprotease 33, in childhood asthma and lung function in two German populations. *Respir Res* 2006; 7:91.
23. Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: measurement characteristics and reference values. *Am J Respir Crit Care Med* 2001; 163(6):1350-5.
24. Benjamini Y, and Hochberg Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society.* 57, 289-300. 1995.
25. Burri PH. Fetal and postnatal development of the lung. *Annu Rev Physiol* 1984; 46:617-28.
26. Lee JY, Park SW, Chang HK, Kim HY, Rhim T, Lee JH et al. A disintegrin and metalloproteinase 33 protein in patients with asthma: Relevance to airflow limitation. *Am J Respir Crit Care Med* 2006; 173(7):729-35.
27. Puxeddu I, Pang YY, Harvey A, Haitchi HM, Nicholas B, Yoshisue H et al. The soluble form of a disintegrin and metalloprotease 33 promotes angiogenesis: Implications for airway remodeling in asthma. *J Allergy Clin Immunol* 2008.
28. Ryder MI, Saghizadeh M, Ding Y, Nguyen N, Soskolne A. Effects of tobacco smoke on the secretion of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta from peripheral blood mononuclear cells. *Oral Microbiol Immunol* 2002; 17(6):331-6.
29. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001; 31(3):391-9.
30. Alati R, Al MA, O'Callaghan M, Najman JM, Williams GM. In utero and postnatal maternal smoking and asthma in adolescence. *Epidemiology* 2006; 17(2):138-44.
31. Gilliland FD, Li YF, Peters JM. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med* 2001; 163(2):429-36.
32. Gilliland FD, Berhane K, Li YF, Rappaport EB, Peters JM. Effects of early onset asthma and in utero exposure to maternal smoking on childhood lung function. *Am J Respir Crit Care Med* 2003; 167(6):917-24.
33. Raby BA, Silverman EK, Kwiatkowski DJ, Lange C, Lazarus R, Weiss ST. ADAM33 polymorphisms and phenotype associations in childhood asthma. *J Allergy Clin Immunol* 2004; 113(6):1071-8.
34. Lind DL, Choudhry S, Ung N, Ziv E, Avila PC, Salari K et al. ADAM33 is not associated with asthma in Puerto Rican or Mexican populations. *Am J Respir Crit Care Med* 2003; 168(11):1312-6.
35. Wang P, Liu QJ, Li JS, Li HC, Wei CH, Guo CH et al. Lack of association between ADAM33 gene and asthma in a Chinese population. *Int J Immunogenet* 2006; 33(4):303-6.
36. Blakey J, Halapi E, Bjornsdottir US, Wheatley A, Kristinsson S, Upmanyu R et al. Contribution of ADAM33 polymorphisms to the population risk of asthma. *Thorax* 2005; 60(4):274-6.
37. Holloway JW, Koppelman GH. Identifying novel genes contributing to asthma pathogenesis. *Curr Opin Allergy Clin Immunol* 2007; 7(1):69-74.

