The PCDH1-Gene and Asthma in Early Childhood.

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Authors Contributions
The guarantor of the study is HB who has been responsible for the integrity of the work as a whole, from conception and design to conduct of the study and acquisition of data, analysis and interpretation of data and writing of the manuscript. LJM drafted the manuscript. EKM, KB contributed to data acquisition, analysis and interpretation. All co-authors have contributed substantially to the analyses and interpretation of the data, and have provided important intellectual input and approval of the final version of the manuscript.
The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.
**Abbreviations**

PCDH1:  protocadherin-1  
COPSAC:  The Copenhagen Prospective Studies on Asthma in Childhood  
BHR:  bronchial hyperresponsiveness  
TLS:  troublesome lung symptoms  
ICS:  inhaled corticosteroid  
ETS:  environmental exposure to tobacco smoke  
OR:  odds ratio  
CI:  confidence interval  
TDT:  transmission disequilibrium test  
CEU:  Utah residents with Northern and Western European ancestry; a reference population from the Hapmap project
**ABSTRACT**

**Rationale**

Previous studies suggest that variants in the protocadherin-1 (PCDH1) gene, important for cell-cell adhesion, are associated with asthma, bronchial hyperresponsiveness and atopic dermatitis in school-children.

**Objective**

To associate common variants of the PCDH1-gene to longitudinally assessed asthma-phenotypes and atopic dermatitis in early childhood.

**Method**

We analyzed 8 SNPs in PCDH1 from 411 children born to asthmatic mothers from the Copenhagen Prospective Studies on Asthma in Childhood birth-cohort.

Asthma and atopic dermatitis were diagnosed prospectively to age seven and asthma was categorized by temporal pattern: transient early respiratory symptoms, persistent symptoms, and late-onset symptoms. Bronchial responsiveness was measured at age six. We used additive genetic models.

**Main Results**

Kaplan-Meier plots revealed early onset for hetero- and homozygote for the rs10063472-T allele.

Significant association was observed between the transient early phenotype and rs10063472-T (transient early vs. all: OR=1.91, CI: 1.21-3.01, p = 0.0058; transient early vs. asymptomatic: OR=2.00, CI: 1.23-3.25, p = 0.0053). No association was observed for other symptom patterns or bronchial responsiveness.

Significant association was observed for atopic dermatitis and rs11167761-A (OR = 1.85, CI: 1.24-2.75, p = 0.0026).

**Conclusion**
Common variations in *PCDH1* increase the risk of developing both transient early asthma and atopic dermatitis in early childhood.

*(word count abstract: 200)*
INTRODUCTION

Asthma is the most common chronic disease in children and it is a major cause of emergency room visits, hospitalizations, and school absences\(^1,2\). Incidence of childhood asthma seems to be increasing despite improved treatments over the last few decades\(^1\). The development of asthma is known to be a complex interaction between environmental factors and genetics\(^3\). It is defined as a chronic inflammatory disorder with bronchial hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning and it is usually associated with widespread, but variable, airflow obstruction within the lungs, which is reversible either spontaneously or with treatment\(^4\). The different respiratory symptoms - phenotypes in early childhood, especially under the age of three, vary significantly over time and are associated with different risk factors\(^5,6\). New insight in the disease pathogenesis and the finding of new genes might improve management through improved understanding of sub-phenotypes.

Several genes are reported to have an effect on the development of asthma\(^7\). Previous research has shown that polymorphisms in the gene of protocadherin-1 (PCDH1) are associated with bronchial hyperresponsiveness, asthma among adults and school children and development of atopic dermatitis among children\(^8,9\). Interactions with in utero exposition to tobacco smoke have been proposed to affect development of BHR in children\(^8\). \textit{PCDH1} belongs to a large group of cell-cell-adhesion molecules. It is expressed in several tissues, including airway epithelium and skin keratinocytes\(^10,11\). It shows intracellular signalling abilities and is thought to play a role in cell-repair and is expressed during foetal lung development of mice\(^12\text{-}15\). The \textit{PCDH1}-gene is 25 kb long positioned on chromosome 5q31-q33. It consists of 5 exons and through alternative splicing, two annotated protein-isoforms\(^16\).
Children with asthmatic symptoms are at risk of developing atopic dermatitis, and vice versa and the diseases are therefore believed to share some common background, genetically and environmentally\textsuperscript{17}. Genetic variations altering epithelial cell-repair and/or epithelial barrier-function are plausible of causing development of both asthma and atopic dermatitis.

The aim of this study was to associate common variants of the \textit{PCDH1}-gene to different asthma phenotypes and atopic dermatitis in young children followed since birth.

**METHOD**

\textit{Study population} 

Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) is a single-centre, prospective clinical birth-cohort study of 411 children born to asthmatic mothers, with the objective of investigating the gene-environment phenotype relationship in the development of atopic diseases\textsuperscript{18}. The study was approved by the Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754).

**CLINICAL ASSESSMENT AND DIAGNOSING**

\textit{Recurrent episodes of troublesome lung symptoms} 

Troublesome lung symptoms (TLS) were defined as breathlessness, wheeze, recurrent cough severely affecting, sleep, activity and/or the well-being of the child. “Recurrent TLS” were defined as three consecutive days of TLS, five times within six months, TLS for four consecutive weeks or one episode of severe asthmatic symptoms requiring hospitalization\textsuperscript{18}.
**Recurrent TLS phenotypes**

Children with recurrent TLS, where categorized, in three groups, by symptom pattern\textsuperscript{19}. *Transient early respiratory symptoms*: debut of symptoms, and symptom remission within the first three years of life. *Late-onset respiratory symptoms*: debut of symptoms after the age of three. *Persistent respiratory symptoms*: debut of symptoms before the age of three, continuing after the age of three.

**Asthma diagnosis**

Doctors at the research unit diagnosed asthma according to a predefined algorithm, based on “recurrent TLS” and relapse after discontinued ICS-treatment. Algorithm is previously described in details\textsuperscript{18}.

**Atopic dermatitis**

Skin rash on the child was evaluated according to Hanifin and Rajka criteria\textsuperscript{18,20}.

**Bronchial hyperresponsiveness**

Bronchial hyperresponsiveness was determined in the sixth year of life as the dose of metacholine required to induce a 20\% fall in FEV1, measured by spirometry\textsuperscript{21}.

**Tobacco smoke exposure**

Environmental tobacco smoke exposure (ETS) in utero was dichotomized by maternal smoking pattern as *having smoked during 3rd trimester of pregnancy, or not*.

**Genotyping and SNP analysis**
DNA was purified from blood cells from probands and parents and multiple single-nucleotide-polymorphisms (SNP) were genotyped genome-wide, using high throughput Illumina Infinium II HumanHap550 BeadChip technology \(^{22,23}\).

Linkage-disequilibrium calculation and visualization of \(r^2\) values was performed in Haploview v4.2 \(^{24,25}\).

Quality control of genotyping included missingness per SNP, per individual and Hardy-Weinberg equilibrium. Correct familial relations were double checked by Mendel-error rates and Identity-by-descent analyses. All quality control was performed using PLINK \(^{26,27}\).

We aimed to analyze all regulatory elements by including a distance of +/-10 kb from transcription start site. The selected region was furthermore based on recombination-rates from CEU population, made available by the International HapMap-project \(^{28,29}\).

Allele frequencies and linkage disequilibrium-pattern in study population and CEU population were compared. Finally, we annotated the SNPs for functional effects.

**Statistical analysis**

SNPs were analyzed for association with recurrent TLS, asthma, atopic dermatitis, and bronchial hyperresponsiveness respectively, performed in PLINK, in an additive-genetic model by logistic- or linear regression and also in a family based method, transmissions-disequilibrium-test (TDT). In the TDT the untransmitted alleles are set as reference group, for further information on TDT, please see PLINK webpage\(^{26}\). The three phenotypes of recurrent TLS were analyzed separately using the remaining phenotypes and/or asymptomatic children as controls.

We investigated whether PCDH1-variants showed interaction with children’s exposure to environmental tobacco smoke in utero, resulting in severe lung symptoms in childhood.
We used a Bonferroni corrected alpha level ($0.05 / 8 = p < 0.00625$) as statistical significance threshold. Development of asthma and atopic dermatitis was visualized in Kaplan-Meier plots using R-project $^{30}$. Groups with one or two minor alleles were merged due to low number of minor-allele-homozygous probands (asthma Kaplan-Meier: 15 homozygous probands; atopic dermatitis Kaplan-Meier: 10 homozygous probands)

**RESULTS**

**Baseline**

*Study population:* 411 infants, one month of age, were included in the study. At three years of age, 345 children were still adhering to the study protocol, and 336 at seven years of age.

*Genotyping:* blood samples for genotyping of parents and infants were collected at the beginning of the study. After performing quality control of the genotype data (described below), and after removing one of the siblings in each sib-pair (total of 15 probands), 362 children, and 552 parents were left for analyses.

*Diagnoses:* Recurrent TLS was diagnosed longitudinally throughout the study. Of the initial 411 children, 139 children were categorized as having one of the three recurrent TLS phenotypes and 151 children were asymptomatic (127 of the genotyped had a recurrent TLS phenotype and 136 where asymptomatic). Asthma was also diagnosed longitudinally. By age seven, 70 children had, at some point during childhood, had an asthma diagnosis (66 of the genotyped children). Atopic dermatitis as well, was diagnosed longitudinally and by age seven, 174 children had, at some point during childhood, had atopic dermatitis (154 of the genotyped children).
Current asthma by age 7 was determined in 47 children (45 of the genotyped children). Bronchial hyperresponsiveness was measured by PD20 in 253 children (230 of the genotyped children) by age 6 1/2. (See Online Data Supplement 1).

**Genotyping**

The final region chosen for analysis (141,205,502 bp – 141,248,128 bp, build 36) included the 10 kb upstream region and approximately 7 kb of the downstream region, thus excluding a peak in recombination (see Online Data Supplement 2). The region of analysis contained 8 SNPs: 3 positioned downstream, 0 positioned upstream and 5 positioned within transcription area (see Online Data Supplement 3). The closest upstream SNP was placed more than 21 kb away from transcription start site and was due to the distance not included in the region of interest.

Allele frequencies in the CEU-HapMap population and the study cohort were comparable (see Online Data Supplement 6).

The linkage disequilibrium pattern in study population revealed low correlation between all 8 SNPs expressed as $r^2$ was between 0.00 and 0.62 (see Online Data Supplement 4), as well as in the CEU-HapMap population (see Online Data Supplement 5).

Subsequently 15 siblings were removed in order to avoid bias in association analyses. Final analyses included 914 individuals in total, consisting of 362 probands and 552 parents, containing 234 complete trios (proband + mother + father). The call rate for all individuals in the final dataset was $\geq$ 99%, and the call rate for all SNPs in the final dataset was $\geq$ 95%. All analyzed SNPs were in Hardy-Weinberg Equilibrium (p > 0.1).

Functional annotation of the SNPs, showed that function of the variants were: synonymous (rs3797054), non-synonymous
(rs3822357), Intronic (rs11167761, rs10063472, rs6888135) and outside the gene or unknown (rs17208551, rs2974704, rs17097812) \(^{31}\) (see Online Data Supplement 6).

**GENETIC VARIATIONS AND ASSOCIATIONS WITH ATOPIC DISEASE**

*Recurrent troublesome lung symptoms in early life*

A significant association was observed between the transient early respiratory symptoms phenotype and rs10063472 in logistic model \([\text{transient early vs. all: OR} = 1.91 \text{ per T allele (95\% CI: 1.21 to 3.01) } p = 0.0058; \text{transient early vs. asymptomatic: OR} = 2.00 \text{ per T allele (95\% CI: 1.23 to 3.25) } p = 0.0053]\) (see table 1). Using the family based method: \([\text{transient early vs. all: OR} = 1.93 \text{ per T allele (95\% CI: 1.01 to 3.68) } p = 0.042; \text{transient early vs. asymptomatic: OR} = 1.93 \text{ per T allele (95\% CI: 1.01 to 3.68) } p = 0.042]\). None of the other SNPs were associated with transient early phenotype and no SNPs were associated with the late-onset- or persistent phenotype.

*Asthmatic symptoms*

Kaplan-Meier plot revealed that children with the rs10063472, T allele, either heterozygote or homozygote, had an earlier onset of disease (see figure 1), consistent with the association to the transient early phenotype. Asthma at any point from birth until seven years of age, or cross-section of asthma at seven years of age, explored by logistic regression in an additive-genetic model showed no statistical significance. Cox regression and/or log rank was not performed since we did not observe a constant effect over time.

*Environmental Tobacco Exposure Interaction*

Interaction between environmental tobacco exposure in utero and the SNPs and development of asthmatic symptoms were tested by logistic regression with two different symptom-outcomes;
having asthma at any point from birth until seven years of age, and belonging to the phenotype, transient early respiratory symptoms. The interaction term for having asthma was significant for rs6888135 [OR = 3.56, per A allele (95% CI: 1.37 to 9.28) p = 0.028], and also for having recurrent troublesome lung symptoms in the first three years of life [OR = 3.67, per A allele (95% CI: 1.23 to 10.95) p = 0.020] (see table 1).

We furthermore repeated the association analysis on the transient early phenotype, but now stratified on exposure/no-exposure to environmental tobacco smoke in utero. We found no association between rs6888135 and transient early respiratory symptoms in any of the groups (15 cases, 41 controls in the exposed group; 44 cases, 163 controls in the unexposed group). We did rediscover the association between transient early respiratory symptoms and rs10063472 in the unexposed group.

**Atopic Dermatitis**

Children diagnosed with atopic dermatitis during the first seven years of life were compared to children with no atopic dermatitis in the same period. A significant association was seen between rs11167761 and the development of atopic dermatitis, using logistic regression [OR = 1.86, per A allele (95% CI: 1.20 to 2.86) p = 0.0053] (see table 1). The TDT showed a comparable result but at a lower significance level [OR = 1.59 (95% CI: 1.00 to 2.53) p = 0.050]. This increased risk of developing atopic dermatitis, per A allele, was visualized by Kaplan-Meier, stratifying on number of effect alleles (see figure 2).

The allele increasing the risk of developing transient recurrent troublesome lung symptoms, rs10063472 T allele, showed an atopic dermatitis risk-reduction [OR = 0.60 per T allele (95% CI: 0.41 to 0.89) p = 0.011], though this was non-significant at the Bonferroni threshold, time-to-onset
of atopic dermatitis, visualized by Kaplan-Meier, showed at trend towards the risk-reduction (see figure 3).

Furthermore, the allele that showed interaction with maternal smoking and increased development of lung symptoms in the child (rs6888135 A allele) also showed risk-reduction in relation to atopic dermatitis [OR = 0.67 per A allele (95% CI: 0.48 to 0.93) p = 0.016]. This was not significant at the Bonferroni threshold, (see figure 4). The two SNPs associated to atopic dermatitis, had very low correlation, \( r^2 = 0.03 \) (see Online Data Supplement 4).

**Bronchial hyperresponsiveness**

Bronchial hyperresponsiveness, PD20, was tested by linear regression and showed no significant association to any of the SNPs (see Online Data Supplement 7).

**Table 1:**

<table>
<thead>
<tr>
<th></th>
<th>Rs10063472</th>
<th>Rs6888135</th>
<th>Rs11167761</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of recurrent TLS in early life</td>
<td>OR = 2.00, per T allele (95% CI: 1.23 to 3.25) p = 0.0053**</td>
<td>OR = 0.73, per A allele (95% CI: 0.47 to 1.16) p = 0.18</td>
<td>OR = 0.58, per A allele (95% CI: 0.30 to 1.13) p = 0.11</td>
</tr>
<tr>
<td>Interaction term between in utero-ETS and the SNP for the outcome recurrent TLS</td>
<td>OR = 0.46, per T allele (95% CI: 0.13 to 1.68) p = 0.24</td>
<td>OR = 3.67, per A allele (95% CI: 1.23 to 10.95) p = 0.020*</td>
<td>OR = 0.35, per A allele (95% CI: 0.038 to 3.27) p = 0.36</td>
</tr>
<tr>
<td>Development of atopic dermatitis</td>
<td>OR = 0.60, per T allele (95% CI: 0.41 to 0.89) p = 0.011*</td>
<td>OR = 0.67, per A allele (95% CI: 0.48 to 0.93) p = 0.016*</td>
<td>OR = 1.86, per A allele (95% CI: 1.20 to 2.86) p = 0.0053**</td>
</tr>
</tbody>
</table>
Table 1 legend:
Result table: showing results for three SNPs of interest.
TLS: Troublesome lung symptoms. ETS: Environmental exposure to tobacco smoke.
*: Nominal significant results. **: Bonferroni significant associations.

DISCUSSION

Key findings
Common variations in the gene of protocadherin-1 increase risk of having recurrent troublesome lung symptoms during the first three years of life, accelerates time-to-onset of asthma during that same period, and is associated with the development of atopic dermatitis.

Strengths and Limitations of the Study
Strengths: The COPSAC study is a single centre clinical birth cohort. Asthma as well as atopic dermatitis was diagnosed according to standard operating procedure: all children were diagnosed and monitored at the clinic and not by general practitioner ensuring standardized diagnosis, treatment and regular evaluation.

Limitations: this is a high-risk study population of asthmatic mothers. This has been accommodated - first by comparison of allele frequencies and linkage disequilibrium, which show similarity to background population; second controls are all from within the cohort, thus being at the same risk of developing atopic diseases as cases.

The genotyping, for this study, of multiple single-nucleotide-polymorphisms in probands and parents was performed before 2012, when the study by Koning et al. and the study by Toncheva et al. was published. We were for that reason, unfortunately not able to analyse the polymorphisms rs7719391 and IVS3_116 in this present study.
**Meaning of this study**

We found an association between the SNP rs10063472 (T allele increased risk) and recurrent troublesome lung symptoms before age three. Asthma onset was accelerated by the T allele of this SNP. This is a novel finding not previously reported. The previously reported findings of association between asthma and the rs3797054 did not replicate in our study. This previous report, by Koppelman et al., included one main population, consisting of 200 families ascertained through probands with asthma, who were characterized between 1962 and 1975 and seven heterogeneous replication populations (1: Dutch trios, probands with asthma, adults – mean age 33.8 years. 2: Caucasian general cohort, adults – mean age 51.7 years. 3: Caucasians, Hispanic, Other, general cohort, teenagers – mean age 10.7 years. 4: UK citizens, probands with asthma, children – mean age 11.4 years. 5,6,7: Caucasians, Hispanics, African-Americans, case/control-designs, adults – mean age 27.1, 17.4 and 24.4 years respectively.). In the main study population, asthma was diagnosed by algorithm combining symptoms, bronchial hyperreactivity and reversibility. Two paediatric cohorts were analyzed by Koning et al. in a subsequent report, showing no association between rs3797054 and rs3822357 and asthma (‘doctor diagnosed’ by any doctor or use of ICS), which is in agreement with our study. In the most recent study, Toncheva and colleagues found an association between a SNP, not included in our genotyping, rs7719391 and a decrease in asthma in school children; and furthermore in a subdivision of phenotypes an association between rs2974704 and a decrease in non-atopic asthma in school children, and rs11167761 and an increase in non-atopic asthma in school children. No SNP’s where associated to atopic asthma in their study. Toncheva et al. pooled two German/Austrian child-populations, all asthma cases from the MACIG study and randomly chosen children from the ISAAC II study. They included 1311 children (651 asthmatics, 652 controls, 8 missing status) and analysed 14 tagging-SNPs. In the ISAAC II population, all children were age 9-11. Asthma was assessed based on parents’ reports of a
physician diagnosis of asthma and/or recurrent spastic or asthmatic bronchitis and atopy was defined as a positive skin prick test. In the MACIG population children had a mean age of 11 years (3-17) and asthma was diagnosed by a paediatric pulmonologist and atopy by a positive RAST blood test. Toncheva et al. did not find an association between rs10063472 and development of asthma in school children. We were not able to replicate their findings of an association between rs11167761 and asthma sub-phenotypes in school children, in our cohort of preschool children.

We observed a significant interaction between the rs6888135 polymorphism and in utero tobacco exposure. Our findings are only borderline significant, and previous studies on this interaction are inconsistent. In the aforementioned study, Koppelman et al. found an interaction between a different polymorphism (rs3822357) and environmental tobacco smoke in utero, during the first year of life and development of BHR in childhood (age not reported), in a single high-risk family population (UK citizens, n = 340), but this was not replicated in the other populations included in this study. This indicates a possible interaction between smoking and SNPs in this locus, but needs further confirmation. In our cohort rs6888135 and rs3822357 were low correlated ($r^2 = 0.03$). Neither Koning and colleagues nor Toncheva and colleagues assessed interactions with environmental tobacco smoke in utero and development of lung symptoms in the children.

We found an association between the SNP rs11167761 and development of atopic dermatitis not previously investigated. Koning et al. studied two paediatric cohorts and found an association between a three base pair deletion (IVS3-116) and atopic dermatitis. They also investigated rs3797054 and rs3822357. The first was not found statistical significant, but the latter was in their high-risk study cohort (n = 967), but not in the general cohort (n = 1560), and also not in their pooled analysis. Toncheva et al. assessed atopic eczema in 734 (131 cases) school children from the
ISAAC II study. They found no significant association with any $PCDH1$ polymorphisms. In details rs3822357 and IVS3_116 previously reported by Koning et al. where, in line with our study, insignificant; and so where rs11167761, found to be associated with development of atopic dermatitis in preschool children, in this present study. It is worth to note that Toncheva et al. on the other hand, found a significant association between rs11167761 and an increased the risk of non-atopic asthma in school children.

One unpredicted discovery, we did, was the risk reduction in relation to atopic dermatitis, of the allele increasing the risk of asthma. We are currently not able to offer a biologic explanation for this observation.

We found no association between bronchial hyperresponsiveness (age six) and any $PCDH1$ associated SNP in our study. This is in contrast to the study by Koppelman et al., reporting an association between rs3797054 and BHR in 4 populations (two high-risk, two general) including adult or teenagers. However they did not replicate this association in two family-populations with asthma or three adult case/control studies. Rs3822357 was associated to BHR in two cohorts, one general population of teenagers and one high-risk family-cohort, but this association did not replicate in the remaining 7 populations. Koppelman et al. also found the base pair deletion, IVS3-116, to be associated with BRH in three populations (two high-risk adult populations, one case/control with adults)\(^8\).

In agreement with our findings Koning et al., found no association between any $PCDH1$ polymorphism and the BHR (age eight) in two paediatric cohorts (one high-risk, one general)\(^9\). Also in agreement with our findings, Toncheva et al. assessed BHR in 344 (71 cases) school children, age 9-11, from the ISAAC II study, and found no significant association between any
genetic variants in \textit{PCDH1} and BHR. More specifically, rs3797054, rs3822357 and IVS3\_116, reported by Koppelman et al. where found insignificant.

Our negative findings regarding rs3797054, rs3822357 and asthma at school age is in line with previous findings. The study by Koppelman et al. (eight populations; child + adult); the study by Koning et al. (two child-populations) and finally the study by Toncheva et al. (two school age child-populations) all included del IVS3-116, rs3797054 and rs3822357 in their analyses. In those studies, only inconsistent associations were seen for these variations. In particular, discrepancies between adult and child populations were apparent. Associations with any \textit{PCDH1} polymorphisms and both asthma and BHR failed replication in the child cohorts by Koning et al. and association with any \textit{PCDH1} polymorphisms and asthma also failed replication in the child- and family cohorts by Koppelman et al., finally associations with del IVS3-116, rs3797054 and rs3822357 and asthma and BHR failed replication by Toncheva et al in their child-populations.

Our novel discovery associates asthmatic symptoms to a SNP not previously reported to effect asthma development. This is the first time asthmatic symptoms in preschool children is associated with variation in \textit{PCDH1}. Our study is the first to investigate this particular SNP in relation to onset of asthma in children this early in life. The effect of the variation is specifically observed in children much younger than those in previous studies, which makes these results essential to the contribution of the overall understanding of \textit{PCDH1} and its influence on asthma development. Particularly, the thorough endotyping as well as the early identification of asthmatic symptoms in our cohort enabled us to distinguish the specific group of children affected by this variation. In accordance with our novel results, as well as previously well-illustrated results, it seems apparent that common genetic polymorphisms in the \textit{PCDH1} gene do influence the development of asthma.
and atopic dermatitis. We are witnessing yet another clue to how various underlying mechanisms, lead to the heterogeneous disease, asthma – and this supports the relevance of subdividing it into clinically characteristic phenotypes with distinct symptom pattern, prognosis and in the future perhaps even treatment.

Conclusion

Common variations in PCDH1 increase the risk of developing both asthma and atopic dermatitis in early life.

This study supports the overall hypothesis that PCDH1 plays a role in development of atopic diseases, and furthermore offers new insight to its role in early childhood.

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externally-funded). The funding agencies did not have any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**SUMMARY POIN BOX**

*What is already known on this topic?*

Common variations in the PCDH1 gene have previously been associated with adult and school age asthma.

*What this study adds!*

Our novel findings associate variations not previously reported to affect asthma development with a distinct asthma sub-phenotype visibly affecting infants and preschool children.

**Twitter feed @ERSpublishations**

(116 characters)

Common variations in the *PCDH1*-gene increase the risk of developing both asthma and atopic dermatitis in early life.
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Figure legends

Figure 1 heading:
Increased risk of asthma development having rs10063472 T allele

Figure 1 legend:
Kaplan-Meier plot illustrating time-to-onset of asthma when having one or two rs10063472 risk-alleles (T allele), opposed to having none. Due to low number of probands being minor allele homozygous (TT), groups with one or two minor alleles were merged.
Figure 2 heading:
Increased risk of atopic dermatitis development having rs11167761 A allele

Figure 2 legend:
Kaplan-Meier plot illustrating time-to-onset of atopic dermatitis when having one or two
rs11167761 risk-alleles (A allele), opposed to having none. Due to low number of probands being
minor allele homozygous (AA), groups with one or two minor alleles were merged.
Figure 3 heading:
Risk reduction in atopic dermatitis development having rs10063472 T allele

![Kaplan-Meier plot illustrating the risk-reduction of time-to-onset of atopic dermatitis when having one or two rs10063472 minor-alleles (T allele), opposed to having none. Due to low number of probands being minor allele homozygous (TT), groups with one or two minor alleles were merged.](image)

Figure 3 legend:
Kaplan-Meier plot illustrating the risk-reduction of time-to-onset of atopic dermatitis when having one or two rs10063472 minor-alleles (T allele), opposed to having none. Due to low number of probands being minor allele homozygous (TT), groups with one or two minor alleles were merged.
Figure 4 heading:
Risk reduction in atopic dermatitis development having rs6888135 A allele

rs6888135 Atopic Dermatitis

Figure 4 legend:
Kaplan-Meier plot illustrating the risk-reduction of time-to-onset of atopic dermatitis when having one or two rs6888135 minor-alleles (A allele), opposed to having none. Due to low number of probands being minor allele homozygous (AA), groups with one or two minor alleles were merged in visualization.

Furthermore; rs6888135 A allele shows interaction with exposure to tobacco smoke, increasing risk of developing recurrent troublesome lung symptoms in child (interaction not shown here).
Online Data Supplement

Figure legends, tables and table legends

Figure 1, Online Data Supplement heading:
Flow chart

Figure 1, Online Data Supplement legend:
Distribution of children from recruitment until seven years of age. 411 children were enrolled at 1 month of age, 345 children had full follow-up at three years of age and 336 children at seven years of age. Clinical assessment of asthma, recurrent troublesome lung symptoms (TLS) and atopic dermatitis was done longitudinally. Bronchial hyperresponsiveness (BHR) measured as PD20 was done in the sixth year of life, and current asthma was determined at age seven.

Figure 2, Online Data Supplement heading:
Recombination in $PCDH1$-area

Figure 2, Online Data Supplement legend:
Area of analysis in region of $PCDH1$. Recombination expressed as cM/Mb in chromosome 5 based on HapMap-CEU population. Overall recombination rates in chromosome 5 in the HapMap CEU-population, had a mean recombination rate of 1,37 cM/Mb and a maximum recombination of 100,9 cM/Mb. The $PCDH1$-region revealed a recombination rate of approximately 40 cM/Mb in a large area.
Figure 3, Online Data Supplement heading:

PCDHI-gene: inter-/intragenic distribution of analysed SNPs

Figure 3, Online Data Supplement legend:

Illustration of analysis area including the location of SNPs. Gene is transcribed in reverse.


Figure 4, Online Data Supplement heading:

LD-plot of COPSAC cohort

Online Data Supplement, figure E4 legend:

Linkage disequilibrium plot based on COPSAC-cohort SNP genotypes. Correlation expressed as $r^2$. Plot shows all analyzed SNPs. Low to intermediate linkage is observed in entire region ($r^2 = 0.00$ to 0.62).

Figure 5, Online Data Supplement heading:

LD-plot of CEU-population

Figure 5, Online Data Supplement legend:

Linkage disequilibrium plot based on HapMap-CEU population SNP genotypes. Correlation expressed as $r^2$. Plot shows the SNPs included in analyses. Low to intermediate linkage is observed in entire region ($r^2 = 0.00$ to 0.76).
### Table 1, Online Data Supplement heading:

Details on SNPs included in analyses, including functional annotation of the SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Base pair position (build 36)</th>
<th>Effect allele (minor allele)</th>
<th>MAF COPSAC</th>
<th>MAF CEU</th>
<th>SNP position</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17208551</td>
<td>141207154</td>
<td>G</td>
<td>0.224</td>
<td>0.217</td>
<td>Intergenic downstream</td>
<td>NA</td>
</tr>
<tr>
<td>rs2974704</td>
<td>141207226</td>
<td>T</td>
<td>0.078</td>
<td>0.150</td>
<td>Intergenic downstream</td>
<td>NA</td>
</tr>
<tr>
<td>rs17097812</td>
<td>141209073</td>
<td>T</td>
<td>0.076</td>
<td>0.083</td>
<td>Intergenic downstream</td>
<td>NA</td>
</tr>
<tr>
<td>rs11167761</td>
<td>141218527</td>
<td>A</td>
<td>0.168</td>
<td>0.175</td>
<td>Intron</td>
<td>Intron</td>
</tr>
<tr>
<td>rs3797054</td>
<td>141223830</td>
<td>C</td>
<td>0.329</td>
<td>0.375</td>
<td>Exon 3: syn. Ala -&gt; Ala</td>
<td>Synonymous</td>
</tr>
<tr>
<td>rs3822357</td>
<td>141224540</td>
<td>A</td>
<td>0.065</td>
<td>0.067</td>
<td>Exon 3: miss. Ala -&gt; Thr</td>
<td>Non-Synonymous</td>
</tr>
<tr>
<td>rs10063472</td>
<td>141226830</td>
<td>T</td>
<td>0.216</td>
<td>0.267</td>
<td>Intron</td>
<td>Intron</td>
</tr>
<tr>
<td>rs6888135</td>
<td>141234247</td>
<td>A</td>
<td>0.468</td>
<td>0.517</td>
<td>Intron</td>
<td>Intron</td>
</tr>
</tbody>
</table>

### Table 1, Online Data Supplement legend:

SNPs included in analyses. SNP base-pair position on chromosome 5. Frequencies of effect (minor-) alleles in study population (COPSAC) and reference population (CEU). SNP intra/intergenic location. MAF = minor allele frequency. CEU = Utah residents with Northern and Western European ancestry; a population.
Table 2, Online Data Supplement heading:

Results of bronchial hyperresponsiveness, PD20, and association to the SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Beta</th>
<th>CI 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs17208551</td>
<td>-0.2795</td>
<td>-1.44 to 0.8816</td>
<td>0.6375</td>
</tr>
<tr>
<td>Rs2974704</td>
<td>-0.8463</td>
<td>-2.903 to 1.21</td>
<td>0.4207</td>
</tr>
<tr>
<td>Rs17097812</td>
<td>-0.3074</td>
<td>-2.393 to 1.778</td>
<td>0.7729</td>
</tr>
<tr>
<td>Rs11167761</td>
<td>-0.3235</td>
<td>-1.597 to 0.9499</td>
<td>0.6191</td>
</tr>
<tr>
<td>Rs3797054</td>
<td>0.1897</td>
<td>-0.8768 to 1.256</td>
<td>0.7277</td>
</tr>
<tr>
<td>Rs3822357</td>
<td>-0.3362</td>
<td>-2.45 to 1.777</td>
<td>0.7555</td>
</tr>
<tr>
<td>Rs10063472</td>
<td>0.7622</td>
<td>-0.441 to 1.965</td>
<td>0.2157</td>
</tr>
<tr>
<td>Rs6888135</td>
<td>0.5257</td>
<td>-0.5256 to 1.577</td>
<td>0.3281</td>
</tr>
</tbody>
</table>

Table 2, Online Data Supplement legend:

The analysis showed no association between the SNPs and bronchial hyperresponsiveness tested by linear regression.