

Identification of *PCDH1* as a Novel Susceptibility Gene for Bronchial Hyperresponsiveness

Gerard H. Koppelman¹, Deborah A. Meyers², Timothy D. Howard², S. Lilly Zheng², Greg A. Hawkins², Elizabeth J. Ampleford², Jianfeng Xu², Henk Koning^{1,3}, Marcel Bruinenberg⁴, Ilja M. Nolte⁵, Cleo C. van Diemen⁵, H. Marika Boezen⁵, Wim Timens⁶, Paul A. Whittaker⁷, O. Colin Stine⁸, Sheila J. Barton⁹, John W. Holloway^{9,10}, Stephen T. Holgate⁹, Penelope E. Graves¹¹, Fernando D. Martinez¹¹, Antoon J. van Oosterhout³, Eugene R. Bleeker², and Dirkje S. Postma¹²

¹Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, ³Medical Biology, ⁴Genetics, ⁵Epidemiology, ⁶Pathology, and ¹²Pulmonology, University Medical Center Groningen, University of Groningen, The Netherlands; ²Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, North Carolina; ⁷Respiratory Disease Area, Novartis Institutes for Biomedical Research, Horsham, United Kingdom; ⁸Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland; ⁹Infection, Inflammation, and Immunity Division, ¹⁰Human Genetics Division, School of Medicine, University of Southampton, Southampton, United Kingdom; ¹¹Arizona Respiratory Center, University of Arizona, Tucson, Arizona

Rationale: Asthma is a chronic inflammatory airway disease that affects more than 300 million individuals worldwide. Asthma is caused by interaction of genetic and environmental factors. Bronchial hyperresponsiveness (BHR) is a hallmark of asthma and results from increased sensitivity of the airways to physical or chemical stimulants. BHR and asthma are linked to chromosome 5q31-q33.

Objectives: To identify a gene for BHR on chromosome 5q31-q33.

Methods: In 200 Dutch families with asthma, linkage analysis and fine mapping were performed, and the *Protocadherin 1* gene (*PCDH1*) was identified. *PCDH1* was resequenced in 96 subjects from ethnically diverse populations to identify novel sequence variants. Subsequent replication studies were undertaken in seven populations from The Netherlands, the United Kingdom, and the United States, including two general population samples, two family samples, and three case-control samples. *PCDH1* mRNA and protein expression was investigated using polymerase chain reaction, Western blotting, and immunohistochemistry.

Measurements and Main Results: In seven out of eight populations (n = 6,168) from The Netherlands, United Kingdom, and United States, *PCDH1* gene variants were significantly associated with BHR (P values, 0.005–0.05). This association was present in both families with asthma and general populations. *PCDH1* mRNA and protein were expressed in airway epithelial cells and in macrophages.

Conclusions: *PCDH1* is a novel gene for BHR in adults and children. The identification of *PCDH1* as a BHR susceptibility gene may suggest that a structural defect in the integrity of the airway epithelium, the first line of defense against inhaled substances, contributes to the development of BHR.

Keywords: bronchial hyperresponsiveness; asthma genetics; protocadherin-1; cell adhesion; airway epithelium

(Received in original form October 20, 2008; accepted in final form August 31, 2009)

The Dutch studies were supported by the Netherlands Organization for Scientific Research (ZON-Mw VENI grant number 91656091), the Netherlands Asthma Foundation (AF 95.09, AF 98.48, AF 3.2.02.51.), Stichting Astma Bestrijding, the National Institutes of Health (NIH RO1/HL48341), and Novartis Pharmaceuticals. The Tucson study was supported by grants from the National Heart, Lung and Blood Institute.

Correspondence and requests for reprints should be addressed to Gerard H. Koppelman, M.D., Ph.D., Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands. E-mail: g.h.koppelman@bkk.umcg.nl

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 180, pp 929–935, 2009

Originally Published in Press as DOI: 10.1164/rccm.200810-1621OC on September 3, 2009
Internet address: www.atsjournals.org

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Asthma and bronchial hyperresponsiveness (BHR) are caused by multiple genetic and environmental factors. Linkage studies suggest the presence of one or more genes for asthma and bronchial hyperresponsiveness on chromosome 5q31-q33.

What This Study Adds to the Field

This study reports *Protocadherin-1* as a novel gene for bronchial hyperresponsiveness on chromosome 5q31-q33. This gene encodes an adhesion molecule expressed in airway epithelium, which may implicate that a structural defect in the integrity of the airway epithelium contributes to the development of BHR.

Asthma is a chronic inflammatory airway disease that affects more than 300 million individuals worldwide (1). It is characterized by respiratory symptoms, variable airway obstruction, and bronchial hyperresponsiveness (BHR) and is caused by multiple genetic and environmental factors that may interact (2). BHR is a hallmark of asthma and is due to increased sensitivity of the airways to physical or chemical stimulants (cold air and cigarette smoke) and to pharmacological agents, such as methacholine and histamine. BHR has a considerable genetic component (3) and constitutes a risk factor for asthma development, even in subjects without respiratory symptoms (4). Furthermore, BHR is increased in children who are exposed to environmental tobacco smoke (ETS) in utero and in the first years of life (5).

Our initial report of linkage of BHR to chromosome 5q31-q33 in Dutch families with asthma (6) has been confirmed in six other populations for asthma-associated phenotypes (7–12). This finding has been extended by showing that chromosome 5q31-q33 interacts with ETS exposure in utero and/or early childhood in the development of BHR and asthma (13, 14). This region of chromosome 5q31-q33 contains a large number of candidate genes for allergy and asthma, such as *Interleukin-13*, *Interleukin-9*, *Interleukin-4*, *CD14*, *IRF-1*, *GM-CSF*, *TIM-1*, and the β_2 -adrenoceptor (15). These genes have been reported to be associated with asthma-associated phenotypes, which strongly suggests that chromosome 5q31-q33 contains multiple asthma susceptibility genes that each contribute to the observed asthma linkage.

In this article, we report the identification of protocadherin-1 (*PCDH1*) as a novel gene for BHR on chromosome 5q31-q33. We used positional cloning to identify this gene in the Dutch population, subsequently identified novel sequence variants, performed genetic replication studies in seven independent populations in The Netherlands, the United States, and the United Kingdom, and showed that *PCDH1* mRNA and protein expression is present in airway epithelium, the first line of defense against inhaled allergens and toxic substances known to contribute to asthma development.

METHODS

Study Populations

Recruitment and clinical characterization of all study samples are described in detail in the online data supplement. The primary population consists of 200 families (1,259 individuals) ascertained through probands with asthma who were initially studied between 1962 and 1975 at Beatrixoord Hospital, Haren, The Netherlands (see Table E1 in the online supplement) (16). The Dutch replication samples include an asthma trio population of 407 trios (17), and the longitudinal Vlagtweede/Vlaardingen cohort study, which includes subjects from the general population who have been tested for BHR at one or more time points during follow-up ($n = 418$) (Table E2) (18).

Replication samples from the United States include the prospective Children's Respiratory Study (Tucson, AZ), in which children were tested for BHR to methacholine at ages 11 and 16 ($n = 329$) (Table E2) (19). Furthermore, three U.S. case-control populations were recruited from three ethnically diverse US populations (African-American [$n = 522$], Hispanic [$n = 246$], and white [$n = 665$]) as part of the Collaborative Study on the Genetics of Asthma (20, 21). BHR measurements to methacholine or reversibility to albuterol were performed in the cases (Table E2) (21).

The replication sample from the United Kingdom is an asthma family study from Southampton, United Kingdom, which includes 341 affected sib pair families with asthma ($n = 1508$) (22). Bronchial responsiveness was measured in subjects with a baseline FEV₁ of greater than 70% predicted using inhaled methacholine (Table E2) (22).

Local medical ethics committees approved these studies, and all participants provided written informed (parental) consent.

Genotyping and Sequence Analysis

Genotyping methods and marker selection are described in the online supplement. Briefly, 22 microsatellite markers were identified on chromosome 5q31-q33 and genotyped in the Dutch family study (14). Subsequently, 103 single nucleotide polymorphisms (SNPs) were selected for fine mapping and genotyped in the Dutch family study (Table E3). The *PCDH1* gene (2 kb of the putative promoter region, all exons, and the complete 3' untranslated region [UTR]) was resequenced in 96 subjects as described previously (23).

Statistical Analysis

Linkage analysis of the microsatellite fine screen markers on chromosome 5q31-33 was performed using GENEHUNTER-PLUS, using the same model as described previously (14).

Association analysis of Dutch families, trios, and UK families was done with the family-based association test (FBAT) using an additive model, using the -e option in case of linkage (24). Case-control analysis was performed using Chi-square tests and analysis of variance, if appropriate. In the Vlagtweede/Vlaardingen cohort study, the association between *PCDH1* SNPs and the occurrence of becoming BHR-positive over time was investigated longitudinally using a Cox regression model (SPSS 14.0), with adjustment for smoking, age at investigation, and FEV₁ at baseline. All statistical tests were performed two sided.

PCDH1 expression was investigated using polymerase chain reaction in mRNA obtained from an epithelial cell line 16HBE, and human cells, e.g., blood cells, brain, lung fibroblasts, and cultured air-

way epithelial cells from patients with asthma. *PCDH1* protein expression was investigated using two different antibodies (a polyclonal and a monoclonal antibody) using western blotting and immunohistochemistry as described in the online data supplement.

RESULTS

Linkage analysis using a dense map of 29 microsatellite markers in the linked region on chromosome 5q31-q33 in 200 Dutch families ($n = 1,259$) revealed significant LOD scores in two regions: HLOD was 3.88 at marker 5q31-33_36 and 3.84 in a region flanked by markers D5S2117 and *IL9*. In 95 ETS-exposed families, a peak HLOD score of 4.36 was flanked by markers 5q31-33_56 and *FGF α* (Figure E1).

Using the approach previously used to identify *DPP10* and *PHF11*, two positionally cloned genes for asthma (25, 26), allelic association of microsatellite markers and BHR was tested. One allele of marker 5q31-33_40 was significantly associated with BHR using probands and spouses in a case-control design ($P = 0.0029$). This marker is located next to the peak LOD score in the ETS-exposed families at 142.13 cM; its physical position is at 136,413,897-136,414,296 on the reference sequence. The 250-kb region surrounding this marker was investigated, based on the extent of linkage disequilibrium between markers on 5q in the Dutch population. One hundred three haplotype tagging and functional SNPs were genotyped and FBAT identified significant associations of SNPs in *PCDH1* with BHR, and specifically with rs3797054, which encodes a synonymous SNP (Ala750Ala) in *PCDH1* ($P = 0.005$) (Figure 1).

Resequencing of *PCDH1* in 96 subjects of ethnically diverse populations revealed 22 SNPs and two insertion deletion polymorphisms (Table E4).

Potential functional SNPs significantly associated with BHR were genotyped in seven replication populations from the Netherlands (adults), the United States (adults and children), and the United Kingdom (children), displaying significant associations in two Dutch, one UK, and three US populations (Table 1). First, the association found between rs3797054 (T allele) and BHR was consistently observed in the parents of the Dutch families (using the probands with asthma as cases and unaffected spouses in a case-control design) and in the children of the Dutch families (using FBAT). The association of rs3797054 with BHR was replicated in participants of the population-based Children's Respiratory Study in Tucson, Arizona, who were investigated for BHR at ages 11 and 16 years (19). Moreover, rs3797054 was associated with the development of BHR in adults from a longitudinal adult population-based study in the Netherlands (Figure 2) (18). Second, a 3-bp insertion/deletion polymorphism (IVS3-116) in the 3' UTR region of exon 3 was associated with BHR in two Dutch populations and with asthma and BHR in two US case-control populations. Third, the major allele of rs3822357, which encodes Ala514Thr localized in the fifth cadherin repeat, was associated with BHR in the US Children Respiratory Study (19), and with BHR in a UK population of 341 families ascertained through affected siblings with asthma (22). Interestingly, *PCDH1* Ala514Thr was associated with BHR when ETS exposure in utero and the first years of life was taken into account in the latter population (Table 1). However, there was no strong evidence for gene by smoking interaction in the Dutch or the United States Tucson populations.

In addition to BHR, we investigated *PCDH1* SNPs in asthma defined by an algorithm (14) and observed significant association of rs3797054 and asthma ($P = 0.003$) in the 200 Dutch families. Haplotype analysis did not further improve these results.

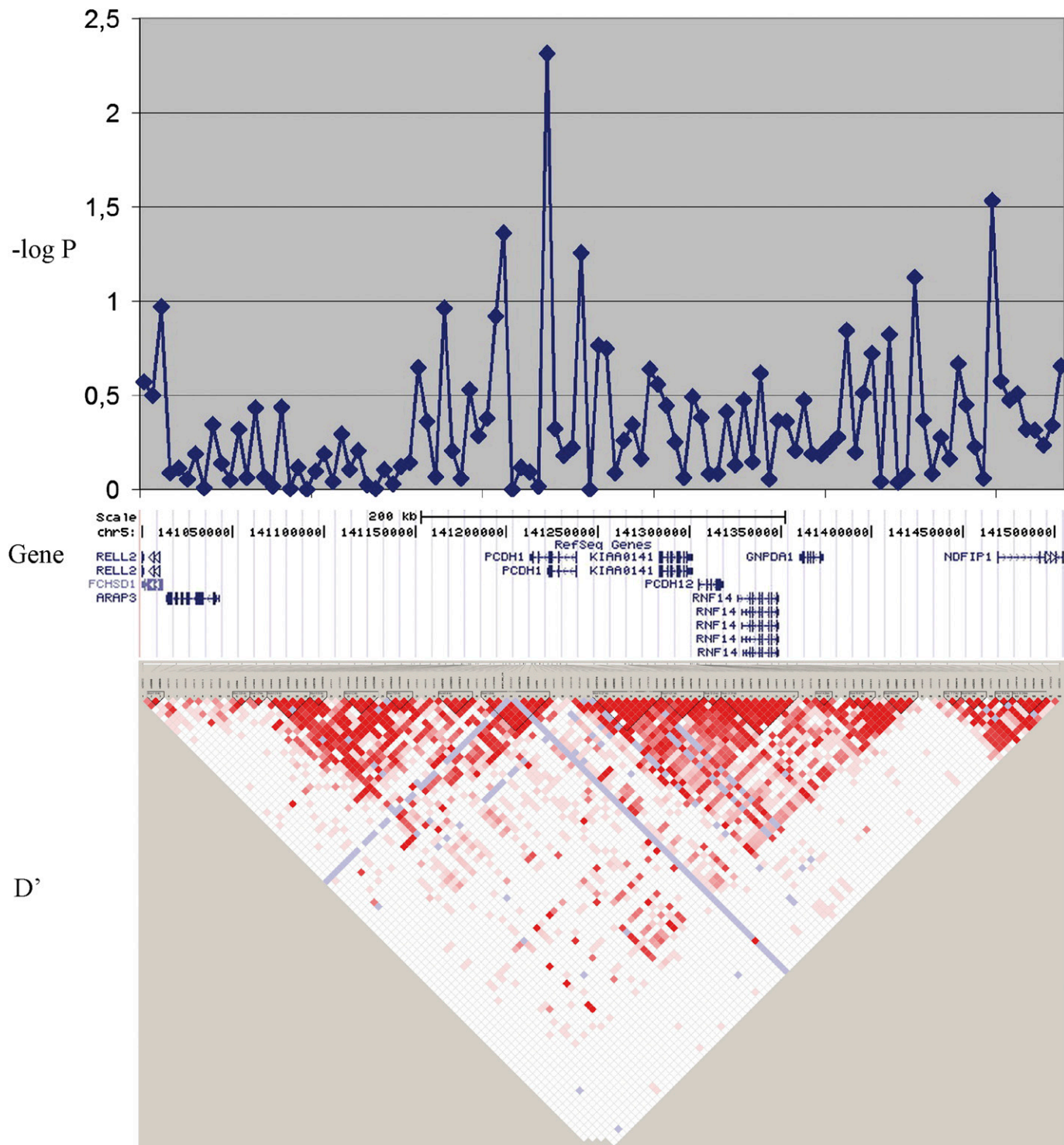


Figure 1. Linkage disequilibrium (D') and association of single nucleotide polymorphisms at chromosome 5q31-q33 and bronchial hyperresponsiveness in the Dutch family study.

By polymerase chain reaction, we identified high *PCDH1* mRNA expression in human brain, airway epithelial cell lines (16HBE), and in primary epithelial cell cultures of patients with asthma. Weak mRNA expression was observed in airway fibroblasts, peripheral blood mononuclear cells, and granulocytes (Figure 3). Western blot analysis showed consistent and specific *PCDH1* protein expression in bronchial epithelial cell line 16HBE and primary epithelial cell cultures of patients with

asthma using different polyclonal and monoclonal antibodies. Proteins of 150–160 kD were identified with a protein weight consistent with previous observations (Figure 4) (27). Immunohistochemistry using the *PCDH1* polyclonal antibody showed a specific expression pattern of *PCDH1* protein in the apical part of differentiated airway epithelial cells lining the airway lumen and in the membrane of macrophages in lung resection specimens of a patient with bronchitis as well as airway wall

TABLE 1. ASSOCIATION OF *PCDH1* GENE VARIANTS WITH BRONCHIAL HYPERRESPONSIVENESS IN EIGHT POPULATIONS (N = 6,168)

SNP	rs Number	Risk Allele (All Populations)	Allele Frequency in Dutch Families	Netherlands 200 Families with Asthma	Netherlands 200 Parents with Asthma (Case-Control)	Netherlands 407 Asthma Trios	Netherlands General Population, Adults	Tucson, AZ General Population, Children Aged 11 and 16 y	Southampton, UK 341 Families with Asthma, Children	US-White (W); Hispanic (H) and African American (A), Case-Control	
N				1259	401	1221*	418	318 [†]	329 [†]	1508	W: 338 cases, 327 controls H: 116 cases, 130 controls A: 222 cases, 300 controls
Analysis				FBAT	Chi-square	FBAT	Cox regression	ANOVA	ANOVA	FBAT	Chi-square, ANOVA
-1191	RS1335929	A	0.03	0.45	0.31	0.09	—	—	—	—	Ns
Ala514Thr	RS3822357	G	0.92	0.47	0.11	0.72	0.58	0.07	0.005[†]	0.009	Ns
Ala750Ala	RS3797054	T	0.67	0.005	0.02	0.49	0.05	0.04[‡]	0.28	0.19	Ns
IVS3+164	RS14359	C	—	—	0.45	0.93	0.09	0.37	1.0	0.18	Ns
IVS3-116	—	Del TTC	0.08	0.04	0.25	0.05	0.95	§	§	0.58	0.02 (C, BHR)

Definition of abbreviations: ANOVA = analysis of variance; BHR = bronchial hyperresponsiveness; Del = deletion; FBAT Family-Based Association Test; rs = Ref SNP accession ID; SNP = single nucleotide polymorphism.

Significant findings appear in bold. US case-control populations: BHR was analyzed in cases only, as BHR was not measured in the controls. Due to low numbers of Hispanic cases, power was low for BHR analysis and therefore asthma was analyzed ($P = 0.02$ for IVS3_116). In all populations, the same risk allele was tested for association.

* Number represents total number of subjects of these trios, including 407 asthma probands.

[†] Only children with BHR measurements were included in these analyses.

[‡] Dominant model (P value for ANOVA 2 df = 0.13).

§ Data not shown due to differences in allele frequency of Hispanic and Caucasian populations.

biopsies in patients with asthma (Figure 5). *PCDH1* expression was also observed between epithelial cells at the apical side of the epithelium.

DISCUSSION

This study identified *PCDH1* as a novel gene for BHR in children and adults. Interestingly, we have provided evidence that *PCDH1*, particularly rs3797054, is important in the development of BHR in a sample of families with asthma. In addition, *PCDH1* is associated with BHR in two population-based samples from the Netherlands and the United States, irrespective of the presence of asthma. Moreover, we present strict replication of a second gene variant in *PCDH1*, a 3-bp insertion-deletion, in three additional study samples ascertained for asthma. Finally, loose replication (28) was observed in the Tucson cohort and a UK family study for a coding SNP not associated with BHR in the primary Dutch population. Of importance for the relevance of the *PCDH1* gene for BHR and asthma, *PCDH1* mRNA and protein expression was shown in macrophages and airway epithelial cells from subjects with asthma and control subjects.

To interpret these findings, several strengths and limitations need to be considered. First, to our knowledge this is the first gene that is identified by positional cloning for an intermediate phenotype of asthma, BHR. *PCDH1* gene variants are associated with BHR in families with asthma as well as two general populations not ascertained for asthma. Previous epidemiological data have shown that BHR is a risk factor for asthma, even in subjects without respiratory symptoms (4). Based on these data, we hypothesize that mechanisms related to the function of *PCDH1* contribute to susceptibility to BHR and subsequent asthma development. This may implicate that *PCDH1* dysfunction plays an early role in asthma pathogenesis.

Second, in all study populations, a direct agonist of BHR was used (either histamine or methacholine). Our findings suggest that *PCDH1* function is not specific for BHR induced by either methacholine or histamine. Previous work has indicated that BHR to histamine and methacholine is highly correlated (29).

We suggest that further work should be performed on the role of *PCDH1* in indirect measures of BHR, such as exercise or adenosine-monophosphate.

Third, we used the positional cloning approach that has been previously used to identify *DPP10* and *PHD11* (25, 26). This approach is based on identification of a genetic association with a microsatellite marker used for linkage analyses, to subsequently fine map the region that is in possible linkage disequilibrium (LD) with this marker and the gene of interest. Our fine mapping results indicate that we have adequately screened the region for SNPs in other genes that may be in LD with *PCDH1*, given the extent of LD of about 100 kb in this region in the Dutch population (Figure 1).

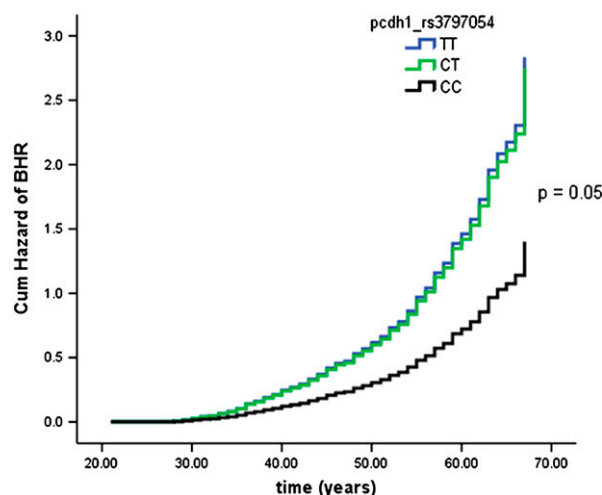


Figure 2. Longitudinal analysis of *PCDH1* Ala750Ala in the general Dutch population. Hazard function of developing bronchial hyperresponsiveness (BHR) in the general Dutch population of Vlagtwedde-Vlaardingen over time. *PCDH1* CT or TT carriers are at increased risk of becoming hyperresponsive over time compared with *PCDH1* Ala750Ala CC homozygotes ($P = 0.05$).

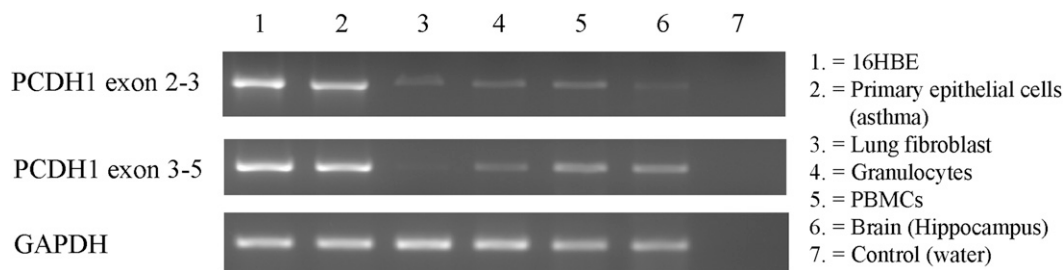


Figure 3. mRNA expression of *PCDH1* in various tissues and cells. GAPDH = glyceraldehyde 3 phosphate dehydrogenase; PBMC = peripheral blood mononuclear cell.

Fourth, we identified *PCDH1* in a primary family population, in which linkage to BHR was reported (6, 14). We did not correct for multiple testing, but rather performed extensive replication studies of significant and/or functional SNPs in seven independent populations. We interpret the consistent signal in parents and offspring in the Dutch families as internal validation of this genetic association in the Dutch families. Moreover, the strict replication of two *PCDH1* gene variants with the same risk allele being associated with BHR in the same direction in four independent populations provides strong support for a role of *PCDH1* in BHR (28). Specifically, we found strict replication with regard to phenotype and genotype for Ala750Ala and IVS3_116. Loose replication with regard to genotype was observed for Ala514Thr. The reasons for this allelic heterogeneity are yet to be determined, but include the presence of multiple functional SNPs, other SNPs that are in linkage disequilibrium with the associated SNPs, or gene by environment interaction. Because gene variants on 5q31-q33 have been shown to interact with ETS exposure in utero/early life, we investigated the association of *PCDH1* with BHR in ETS exposed and nonexposed children in four populations. These gene-environmental interaction analyses (Table E3) in the study cohorts revealed evidence of gene-environment interaction for *PCDH1* in the UK family cohort only, but not in the other populations. However, the power to detect such an interaction was low.

We suggest characterizing the functional role of these gene variants in *PCDH1*. Ala750Ala and IVS3_116 are localized in the 3'UTR of exon 3 and may affect mRNA stability or splicing, whereas Ala514Thr is localized in the fifth cadherin repeat of the extracellular domain and may affect cell-cell adhesion.

The *Protocadherin 1* gene (*PCDH1*, *PC42*) has five exons and encodes multiple mRNA isoforms through alternative splicing (Table E5). There are two annotated isoforms: a three-exon isoform and a five-exon isoform. The three-exon isoform lacks the major part of the cytoplasmic domain,

which encodes conserved signaling sequences CM1, CM2, and CM3 (22). Protocadherins are believed to play an important role in homologous cell adhesion and organ development, in particular within the neural system (30). Overexpression of *PCDH1* induces calcium-dependent cell-cell adhesion and membrane expression of *PCDH1* in a mouse fibroblast L cell assay (27). Protocadherin 1, like *PCDH*, belongs to the $\delta 1$ -protocadherin family of transmembrane proteins. $\delta 1$ -Protocadherins are characterized by six or seven cadherin repeats in the extracellular region, and three conserved regions designated CM1, CM2, and CM3 in the intracellular domain (31). The conserved region CM2 interacts with protein phosphatase 1 α (PP1 α) in *PCDH7* (31). Interestingly, PP1 α plays an important role in lung development, as inhibition of PP1 α in a mouse model led to impaired lung development and branching morphogenesis (32). Finally, in a skin keratinocyte wounding model, *PCDH1* mRNA was significantly up-regulated 24 hours after wounding, compatible with a role of *PCDH1* in epithelial repair (33).

The expression pattern of *PCDH1* is consistent with expression in the apical adhesion complex of airway epithelial cells. We therefore hypothesize that *PCDH1* plays a role in epithelial integrity of the airways and that loss of function of *PCDH1* is associated with increased BHR, which may lead to symptomatic asthma (34). It is tempting to speculate that *PCDH1* dysfunction may provide a functional explanation for the observed epithelial vulnerability and increased epithelial shedding in asthma (35). Further investigations will address the functional relevance of genetic variations in *PCDH1* in epithelial cell adhesion and its interaction with environmental tobacco smoke exposure. Moreover, these findings may be relevant for other diseases in which the integrity of the epithelium is a potential pathogenetic mechanism, such as atopic dermatitis (36) and celiac disease (37). We therefore suggest performing genetic studies of *PCDH1* in these diseases.

In conclusion, this is the first report of a gene specifically identified for HR, an important hallmark of asthma. Further investigations in *PCDH1* function may provide novel insight

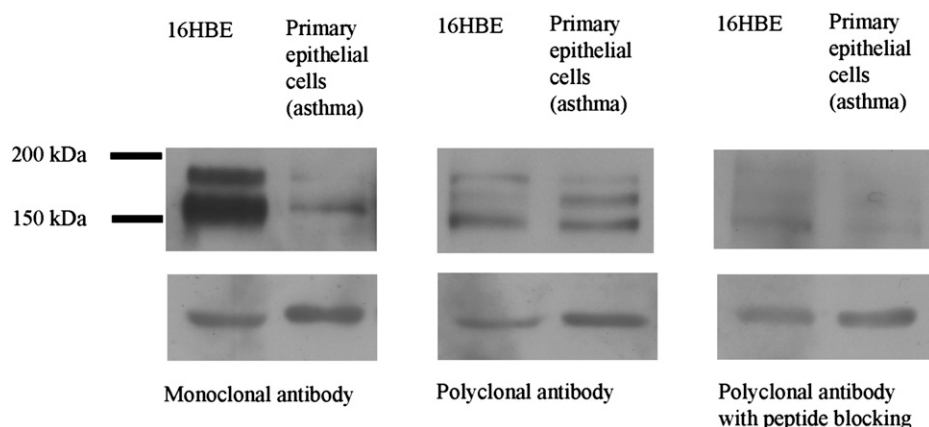


Figure 4. Western blot of epithelial cell line 16 HBE, and primary epithelial cell culture of a patient with asthma using a monoclonal and a different polyclonal antibody. Upper lane, *PCDH1*, lower lane, β -actin. One experiment of $n = 4$ is shown. Protein expression was identified using two different antibodies, a monoclonal (Abnova, Taiwan) (left panel) and a polyclonal antibody (Eurogentec, Liege, Belgium) (middle panel). The signal of the polyclonal antibody was blocked by preincubation with peptide (right panel).

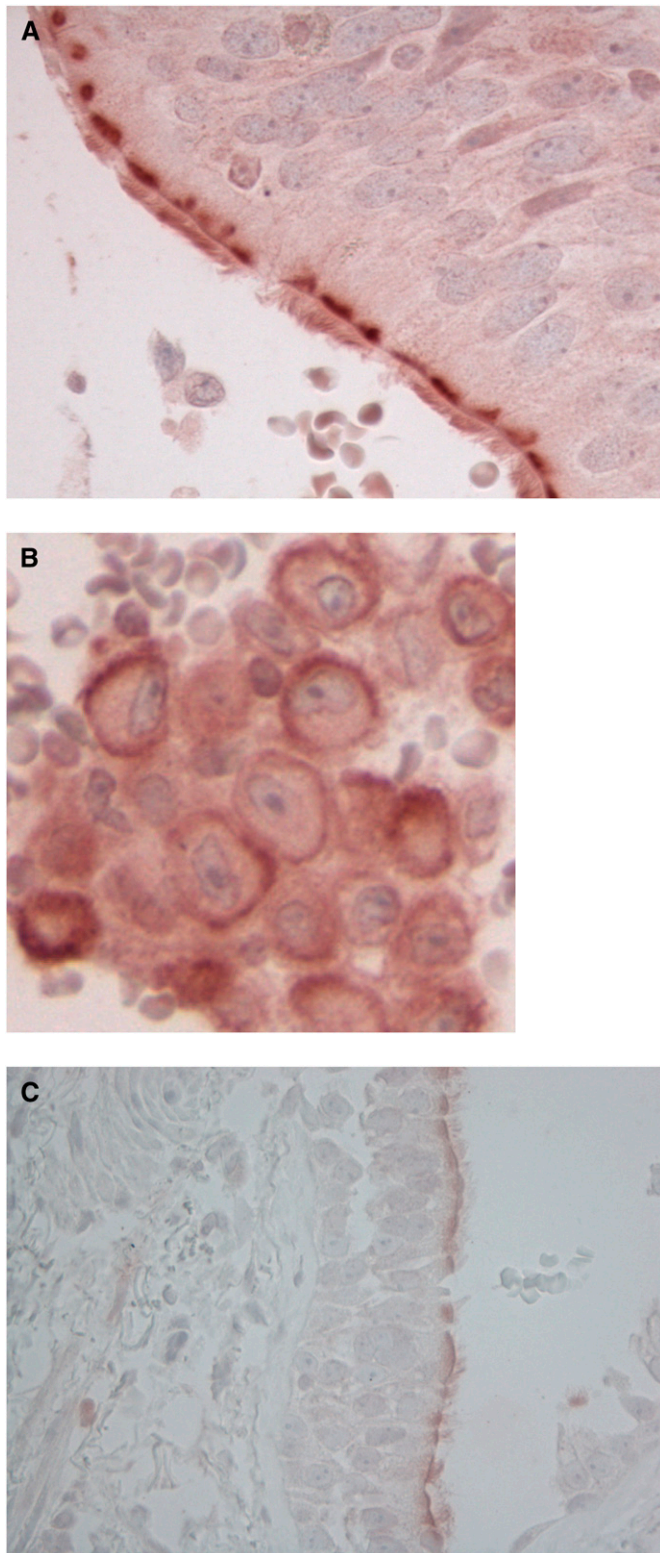


Figure 5. Protein expression of PCDH1 in airway epithelial cells and macrophages on (A, B) lung, and (C) airway wall biopsy of patient with asthma. Expression is observed (A, C) at the apical part of airway epithelial cells, and (B) in macrophages.

into its role in the integrity of the airway epithelium in BHR and asthma development.

Conflict of Interest Statement: G.H.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.A.M.

declares that a patent on PCDH1 has been filed as US patent 7122311 and PCT patent application WO 03/008640 A2 and assigned to Novartis Pharmaceuticals. T.D.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.L.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.A.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.J.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.X. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. I.M.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.C.v.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.M.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. W.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.A.W. is an employee of Novartis and has stock ownership and options in the company. O.C.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.J.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.W.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.T.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.E.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. F.D.M. in the last 3 years has served on both Merck and MedImmune Advisory Boards, and as consultant for GlaxoSmithKline and MedImmune. F.D.M. received lecture fees from speaking at symposia sponsored by Merck and Genentech. A.J.v.O. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.R.B. declares that a patent on PCDH1 has been filed as United States patent 7122311 and PCT patent application WO/03/008640 A2 and assigned to Novartis Pharmaceuticals. D.S.P. received up to \$1,000 from AstraZeneca, \$1,000 from GlaxoSmithKline, and \$1,000 from Nycomed for serving on an advisory board, \$1,001–\$5,000 from GlaxoSmithKline, \$1,001–\$5,000 from AstraZeneca, and \$1,001–\$5,000 from Nycomed in lecture fees, more than \$100,001 from GlaxoSmithKline, \$50,001–\$100,000 from AstraZeneca, and \$50,000–\$100,000 from Nycomed in industry-sponsored grants.

Acknowledgment: The authors thank all participants of all studies, and the lung function department of Beatrixoord Hospital who assisted in the clinical testing. They also thank the collaborating Frisian pulmonologists for their help in patient recruitment. They also thank G. van der Steege, G.J. te Meerman, D. de Jong, M.N. Hylkema, A. Blacquire, M. Geerlings, M. Nawijn, M.T. Hanley, A. Bench, and J. Cakebread for advice and technical assistance.

References

- Masoli M, Fabian D, Holt S; Global Initiative for Asthma (GINA) Program. Global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004;59:469–478.
- Koppelman GH. Gene by environment interaction in asthma. *Curr Allergy Asthma Rep* 2006;6:103–111.
- Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J Allergy Clin Immunol* 1984;73:265–270.
- Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR. Outcome in adulthood of asymptomatic airway hyperresponsiveness in childhood: a longitudinal population study. *Pediatr Pulmonol* 2002;34:164–171.
- Cook DG, Strachan DP. Parental smoking, bronchial reactivity and peak flow variability in children. *Thorax* 1998;53:295–301.
- Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, Meyers DA, Levitt RC. Genetic susceptibility to asthma–bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894–900.
- Noguchi E, Shibasaki M, Arinami T, Takeda K, Maki T, Miyamoto T, Kawashima T, Kobayashi K, Hamaguchi H. Evidence for linkage between asthma/atopy in childhood and chromosome 5q31–q33 in a Japanese population. *Am J Respir Crit Care Med* 1997;156:1390–1393.
- Yokouchi Y, Nukaga Y, Shibasaki M, Noguchi E, Kimura K, Ito S, Nishihara M, Yamakawa-Kobayashi K, Takeda K, Imoto N, et al. Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31–q33 near the interleukin 12 B locus by a genome-wide search in Japanese families. *Genomics* 2000;66:152–160.
- Walley AJ, Wiltshire S, Ellis CM, Cookson WO. Linkage and allelic association of chromosome 5 cytokine cluster genetic markers with atopy and asthma associated traits. *Genomics* 2001;72:15–20.

10. Shek LP, Tay AH, Chew FT, Goh DL, Lee BW. Genetic susceptibility to asthma and atopy among Chinese in Singapore—linkage to markers on chromosome 5q31–33. *Allergy* 2001;56:749–753.
11. Holloway JW, Lonjou C, Beghe B, Peng Q, Gaunt TR, Gomes I, Hall IP, Dewar JC, Wilkinson J, Thomas NS, *et al.* Linkage analysis of the 5q31–33 candidate region for asthma in 240 UK families. *Genes Immun* 2001;2:20–24.
12. Haagerup A, Bjerke T, Schiøtz PO, Binderup HG, Dahl R, Kruse TA. Asthma and atopy – a total genome scan for susceptibility genes. *Allergy* 2002;57:680–686.
13. Colilla S, Nicolae D, Pluzhnikov A, Blumenthal MN, Beaty TH, Bleecker ER, Lange EM, Rich SS, Meyers DA, Ober C, *et al.* Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immunol* 2003;111:840–846.
14. Meyers DA, Postma DS, Stine OC, Koppelman GH, Ampleford EJ, Jongepier H, Howard TD, Bleecker ER. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol* 2005;115:1169–1175.
15. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006;7:95–100.
16. Panhuysen CI, Bleecker ER, Koeter GH, Meyers DA, Postma DS. Characterization of obstructive airway disease in family members of probands with asthma. An algorithm for the diagnosis of asthma. *Am J Respir Crit Care Med* 1998;157:1734–1742.
17. Jongepier H, Koppelman GH, Nolte IM, Bruinenberg M, Bleecker ER, Meyers DA, te Meerman GJ, Postma DS. Polymorphisms in SPINK5 are not associated with asthma in a Dutch population. *J Allergy Clin Immunol* 2005;115:486–492.
18. 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;172:329–333.
19. Stein RT, Holberg CJ, Morgan WJ, Wright AL, Lombardi E, Taussig L, Martinez FD. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. *Thorax* 1997;52:946–952.
20. Basehore MJ, Howard TD, Lange LA, Moore WC, Hawkins GA, Marshik PL, Harkins MS, Meyers DA, Bleecker ER. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol* 2004;114:80–87.
21. Xu J, Meyers DA, Ober C, Blumenthal MN, Mellen B, Barnes KC, King RA, Lester LA, Howard TD, Solway J, *et al.* Genomewide screen and identification of gene-gene interactions for asthma-susceptibility loci in three US populations: collaborative study on the genetics of asthma. *Am J Hum Genet* 2001;68:1437–1446.
22. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, Torrey D, Pandit S, McKenny J, Braunschweiler K, *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;418:426–430.
23. Hawkins GA, Amelung PJ, Smith RS, Jongepier H, Howard TD, Koppelman GH, Meyers DA, Bleecker ER, Postma DS. Identification of polymorphisms in the human glucocorticoid receptor gene (NR3C1) in a multi-racial asthma case and control screening panel. *DNA Seq* 2004;15:167–173.
24. Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype–phenotype associations. *Eur J Hum Genet* 2001;9:301–306.
25. Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, Edser P, Bhattacharyya S, Dunham A, Adcock IM, *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat Genet* 2003;34:181–186.
26. Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, Bhattacharyya S, Tinsley J, Zhang Y, Holt R, *et al.* Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003;35:258–263.
27. Sano K, Tanihara H, Heimark RL, Obata S, Davidson M, St JT, Taketani S, Suzuki S. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J* 1993;12:2249–2256.
28. Holloway JW, Koppelman GH. Identifying novel genes contributing to asthma pathogenesis. *Curr Opin Allergy Clin Immunol* 2007;7:69–74.
29. Sekerel BE, Saraclar Y, Kalayci O, Cetinkaya F, Tuncer A, Adalioglu G. Comparison of four different measures of bronchial responsiveness in asthmatic children. *Allergy* 1997;52:1106–1109.
30. Frank M, Kemler R. Protocadherins. *Curr Opin Cell Biol* 2002;14:557–562.
31. Redies C, Vanhalst K, Roy F. delta-Protocadherins: unique structures and functions. *Cell Mol Life Sci* 2005;62:2840–2852.
32. Hormi-Carver KK, Shi W, Liu CW, Berndt N. Protein phosphatase 1alpha is required for murine lung growth and morphogenesis. *Dev Dyn* 2004;229:791–801.
33. Fitisialis G, Chassot AA, Turchi L, Dayem MA, LeBrigand K, Moreillon C, Meneguzzi G, Busca R, Mari B, Barbry P, *et al.* Transcriptional signature of epidermal keratinocytes subjected to in vitro scratch wounding reveals selective roles for ERK1/2, p38, and phosphatidylinositol 3-kinase signaling pathways. *J Biol Chem* 2007;282:15090–15102.
34. Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J* 2007;29:793–803.
35. Ohashi Y, Motojima S, Fukuda T, Makino S. Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. *Am Rev Respir Dis* 1992;145:1469–1476.
36. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, Carrick T, Evans AT, Liao H, Zhao Y, *et al.* Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007;39:650–654.
37. Monsuur AJ, de Bakker PI, Alizadeh BZ, Zhernakova A, Bevova MR, Strengman E, Franke L, van't Slot R, van Belzen MJ, Lavrijsen IC, *et al.* Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. *Nat Genet* 2005;37:1341–1344.