



Protocadherin-1: epithelial barrier dysfunction in asthma and eczema

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A role for protocadherin-1 in loss of epithelial integrity in both asthma and eczema

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Asthma and eczema result from the interaction between genetic susceptibility and environmental exposures. Over the last two decades, genetic studies have led to the identification of multiple susceptibility genes, increasing our understanding of the biological pathways leading to these diseases.

Many asthma and eczema susceptibility genes are expressed in epithelial cells of airway mucosa and skin [1]. The epithelium constitutes the first line of defence against allergens and invading microbes. Recently, loss of epithelial integrity has been implicated as an important mechanism leading to both asthma [2] and eczema [3].

In this issue of the *European Respiratory Journal*, MORTENSEN *et al.* [4] report the association of genetic variants in the gene encoding protocadherin-1 (*PCDH1*) with asthma, wheeze and eczema in (early) childhood in the Danish birth cohort study COPSAC (Copenhagen Studies on Asthma in Childhood). Their studies also suggest a gene–environment interaction of *PCDH1* with passive smoke exposure in early childhood in asthma development. These studies expand on previous data reporting a role for *PCDH1* polymorphisms in the susceptibility to both asthma [4–7] and eczema [4, 6, 8]. Given the replicated association of *PCDH1* with both diseases, this gene might contribute to a biological pathway that constitutes a shared cause for both diseases. Here, we will summarise the cumulative data on *PCDH1* polymorphisms in asthma and eczema, and make an attempt to interpret these genetic data in light of *PCDH1* expression and function as a contributor to epithelial integrity.

In 1993, *PCDH1* was originally identified by SANO *et al.* [9] as protocadherin-42 and was reported to induce cell adhesion upon ectopic membrane expression in a mouse fibroblast L-cell assay. *PCDH1* is a member of the $\delta 1$ subfamily of the nonclustered protocadherin genes. *PCDH1* is characterised by seven extracellular cadherin (EC) repeats, a transmembrane domain and three evolutionarily conserved motifs (CM1–3) in the intracellular tail that have been suggested to participate in intracellular signalling processes [10]. The *PCDH1* gene spans 25 kb on chromosome 5q31–33 and encodes two main transcripts that contain either three or five exons through alternative splicing. The three-exon isoform does not encode the cytoplasmic

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domains CM1–3 that are present in the five-exon isoform. Recently, a sixth exon and alternative expression of exon 1 were identified in bronchial epithelial cells [11], whereas skin epithelial expression of PCDH1 has not been extensively characterised to date.

In 2009, *PCDH1* was identified as a susceptibility gene for bronchial hyperresponsiveness (BHR) and asthma in children and adults. Subsequently, PCDH1 was shown to be expressed in epithelial cells of the airway [5, 9] and the skin [12]. In four genetic association studies on *PCDH1* published to date, *PCDH1* gene variants were associated with BHR, asthma, eczema, nonallergic childhood asthma and transient early wheeze (table 1) [4–6, 8]. However, in large-scale genome-wide association studies of asthma, such as the GABRIEL consortium study [7], *PCDH1* was not reported to be associated, at a genome-wide significance level, with asthma defined as a self-reported “asthma ever” diagnosis. In our opinion, these contradictory results may be explained by asthma heterogeneity.

Asthma is a chronic heterogeneous disease of the airways with different disease subtypes, characterised by specific onset of respiratory symptoms, such as cough and wheeze. MORTENSEN *et al.* [4] report, in a longitudinal analysis of specific disease subtypes, that *PCDH1* gene variants were associated with specific temporal episodes of wheezing and coughing symptoms, and with the onset of asthma. Moreover, characteristic respiratory symptom patterns in early childhood, such as transient early respiratory symptoms and recurrent troublesome symptoms, were associated with different *PCDH1* polymorphisms. These specific associations with subphenotypes of the disease may well have been missed in an association analysis with “Have you ever had asthma?” as an outcome parameter.

In summary, while different association analyses identify a variety of genetic signals in the *PCDH1* gene, the general picture that starts to emerge indicates that *PCDH1* gene function is associated with asthma, BHR and eczema in early life. Importantly, gene by passive smoking interactions were found to be relevant for the association of *PCDH1* with asthma in two populations [4, 5]. These data suggest that the contribution of

TABLE 1 Associations of *PCDH1* SNPs with asthma and eczema

SNP	Location and function	MAF	Risk allele	Population/cohort	Phenotype	[Ref.]
rs6888135	Intron	C 0.467	A	COPSAC	Asthma (0–7 years of age)+transient TLS+ETS	[4]
rs10063472	Intron	T 0.198	T	COPSAC	Transient early respiratory symptoms phenotype (TLS early)+ETS	[4]
rs3822357	Exon 2, missense Ala514Thr	T 0.091	G	Children; Tucson, AZ, USA	BHR (methacholine)	[5]
			G	Southampton, UK	BHR (methacholine)	[5]
			G	Southampton, UK	BHR (methacholine)+ETS interaction	[5]
			A	PIAMA	Eczema protective based on ISAAC questionnaire	[8]
rs3797054	Exon 2, synonymous Ala750Ala	G 0.373	T	Dutch families	BHR (Histamine)	[5]
				Dutch families	Asthma (doctor diagnosis)	[5]
				Children; Tucson, AZ, USA	BHR (methacholine)	[5]
rs11167761	Intron	A 0.169	A	Vlagentwedde-Vlaardingen, the Netherlands	BHR (histamine)	[5]
				MAGICS	Non-atopic asthma	[6]
IVS3-116	Exon 3, 3' UTR	TTC 0.08 [#]	Del TTC	COPSAC	Atopic dermatitis	[4]
				Dutch families	BHR (histamine)	[5]
				Case-control, USA	BHR (methacholine)	[5]
				Case-control, USA	Asthma (doctor diagnosis)	[5]
				PIAMA	Eczema based on UKWP	[8]
				PIAMA	Eczema based on ISAAC questionnaire	[8]
rs7719391	Intron	C 0.344	C	KOALA and PIAMA	Eczema based on pooled ISAAC questionnaire	[8]
				MAGICS and ISAAC II	Asthma protective (doctor diagnosis)	[6]
rs2974704	Intron	T 0.065	A	MAGICS	Non-atopic asthma	[6]

PCDH1: protocadherin-1; SNP: single-nucleotide polymorphism; MAF: minor allele frequency; rs: reference SNP; COPSAC: Copenhagen Studies on Asthma in Childhood; TLS: troublesome lung symptoms; ETS: environmental tobacco smoke; BHR: bronchial hyperresponsiveness; MAGICS: Multicentre Asthma Genetics in Childhood Study; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; UKWP: UK Working Party; ISAAC: International Study of Asthma and Allergies in Childhood; Del: deletion. MAF was obtained from dbSNP. Risk allele tested for association varied in different cohorts. #: only in Dutch population.

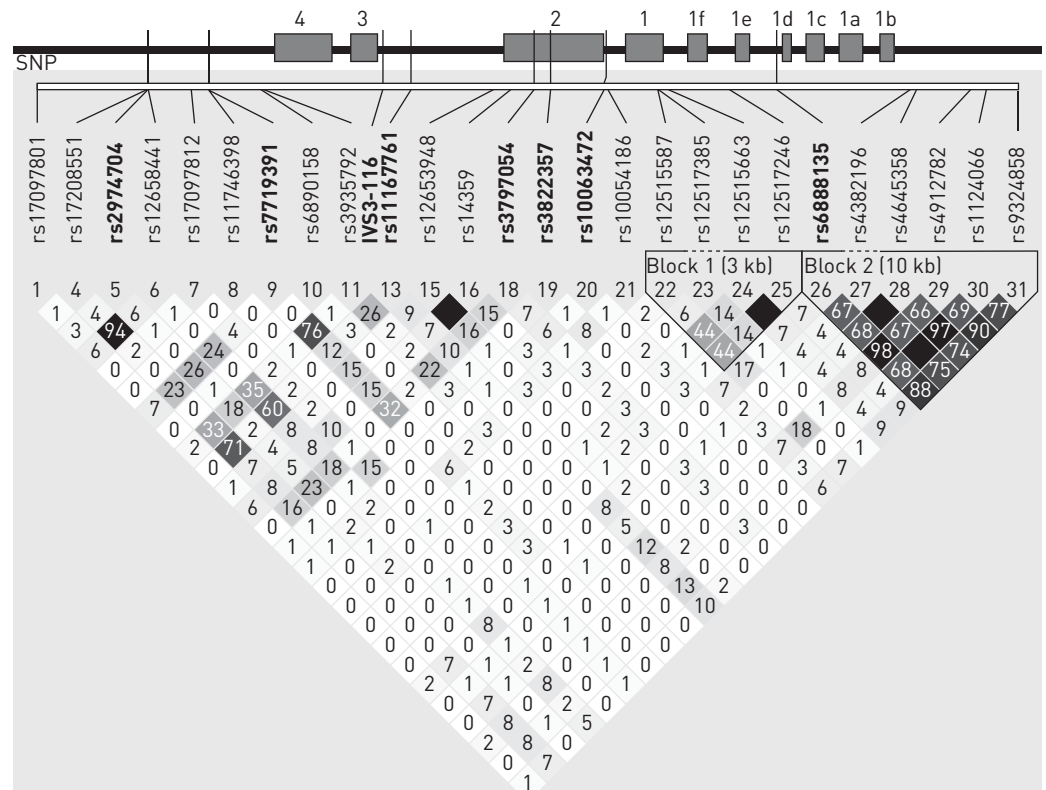


FIGURE 1 Protocadherin-1 gene, single-nucleotide polymorphism (SNP) positions and linkage disequilibrium (LD) pattern: position on reference contig NT_ AC094107.3 (*Homo sapiens*) based on genomic data. LD plots are displayed in r^2 values. The stronger the LD, the darker the squares; $r^2=0$ is white and $r^2=1$ is represented in black. LD plots were constructed with Haploview (version 4.2) software using SNP data from the HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>) for the CEU population. Bold type represents SNPs that also appear in table 1.

PCDH1 gene variants to asthma may become evident when studied in the right environmental context. Well-powered, collaborative meta-analyses of cohorts with specific asthma subphenotypes in early life are needed to provide more robust evidence of these associations (table 1). Finally, the lack of association with specific or total IgE levels or with allergic asthma [6] suggests that *PCDH1* is relevant to non-IgE-mediated mechanisms of disease susceptibility. Consequently, the contribution of *PCDH1* to asthma susceptibility appears to be IgE independent (table 1).

The cumulative data gathered to date indicate that *PCDH1* harbours a complexity of genetic signals associated with asthma and eczema that we do not yet fully understand, without a clear dominant polymorphism that is associated with asthma or eczema in most studies. In order to understand the interrelation of these associations observed for the different *PCDH1* single-nucleotide polymorphisms (SNPs), we considered the possibility of linkage disequilibrium (LD) between these SNPs. LD is defined as the preferential occurrence of specific combinations of SNPs in the population, due to their co-inheritance as a result of their close physical proximity. In a recent study in a German population, TONCHEVA *et al.* [6] investigated the possibility that associations of *PCDH1* with asthma were caused by LD with the nearby cytokine cluster on chromosome 5q31–33, but this was not the case. Our analysis of the LD pattern in Caucasian subjects did not reveal strong evidence for LD between the various SNPs (fig. 1), indicating that these SNPs do not represent a single genetic signal but apparently make individual, independent contributions to disease susceptibility.

Another explanation for the identification of multiple *PCDH1* SNPs to be independently associated with asthma and eczema in different studies is the presence of multiple SNPs within the *PCDH1* gene that have diverse functional effects. Firstly, very specific SNPs might interact with environmental factors such as passive smoke exposure. Secondly, other SNPs may have more generic effects, for instance, by regulating *PCDH1* gene expression. It is important to realise that the SNPs tested in the original study by KOPPELMAN *et al.* [5] were only situated in coding regions of the gene. Over the past years, it has become increasingly clear that noncoding SNPs can have important regulatory effects on gene expression (by acting as

expression quantitative trait loci (eQTLs)). The current publication by MORTENSEN *et al.* [4] adds the association of further noncoding *PCDH1* polymorphisms to asthma and eczema (table 1), whereas none of the initial associations were replicated in this study. Thus, eQTL studies on these *PCDH1* SNPs in lung and skin tissue are therefore urgently needed to assess their functional relevance. Thirdly, coding SNPs may have generic effects by affecting *PCDH1* protein function. The coding SNP Ala514Thr maps to an EC domain in the *PCDH1* protein, which is compatible with a potential effect on cell–cell adhesion that needs further study. So far, no data are available on the functional effects of any of the asthma- and eczema-associated SNPs. Finally, adding to the complexity of *PCDH1*, its expression is also probably regulated by epigenetic mechanisms that include gene methylation, given the identification of exon 1A as a CpG island that is potentially methylated [11].

So, how does *PCDH1* contribute to both asthma and eczema? We hypothesise that *PCDH1* dysfunction contributes to the defect in epithelial barrier function observed in both asthma and eczema by regulating the process of epithelial cell differentiation and/or repair. *PCDH1* mRNA and protein expression is strikingly upregulated during primary bronchial epithelial cell differentiation, suggesting a role for *PCDH1* in achieving or maintaining terminal differentiation of the airway epithelium [11]. The regulation of *PCDH1* expression in the skin during epidermal differentiation has not been characterised to date, although *PCDH1* expression is markedly induced in primary keratinocytes late during the repair process after scratch wounding [12]. A similar role in epidermal differentiation has been previously identified for multiple eczema genes in genetic studies. All in all, we propose a role for *PCDH1* in epithelial differentiation and repair in both asthma and eczema.

PCDH1 is capable of intracellular signalling. Downstream pathways or *PCDH1* interaction partners relevant to asthma or eczema pathogenesis have not been reported to date. Moreover, data on the differential expression of *PCDH1* in asthma or eczema *versus* healthy controls are still lacking. Future studies are needed that can validate the functional role of *PCDH1* in asthma and eczema by controlled experimental approaches, such as small interfering RNA or genetically engineered mouse models, that are currently being developed. These studies also need to address the possibility of environmental exposures affecting *PCDH1* expression, with cigarette smoke exposure being of particular interest. Clearly, more studies are needed to resolve the functional effects of *PCDH1* SNPs and their relevance for airway and skin epithelial cell biology. These studies will ultimately validate the initial genetic findings and perhaps provide clues for future novel therapeutic interventions in asthma and eczema.

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