

University of Groningen

Breaking barriers: new insights into the contribution of the protocadherin-1 (PCDH1) gene in asthma

Martin-Faura Tellez, Grissel

DOI:
[10.33612/diss.565603673](https://doi.org/10.33612/diss.565603673)

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:
2023

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

Citation for published version (APA):

Martin-Faura Tellez, G. (2023). *Breaking barriers: new insights into the contribution of the protocadherin-1 (PCDH1) gene in asthma*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.565603673>

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.

General Introduction

Asthma: a heterogeneous inflammatory disease

Asthma is a heterogeneous disease characterized by chronic inflammation of the airways and considered a serious global health problem affecting 1-18% of the population worldwide (1). There has been a substantial increase in the prevalence of asthma over recent decades, especially in children. Asthma is characterized by airway hyperresponsiveness (AHR), an increased airway contractility in response to both direct and indirect stimuli. Asthma is also associated with mucus overproduction, airway obstruction and remodelling, and clinical symptoms such as wheezing, shortness of breath, chest tightness and cough, which can vary over time and in intensity (1).

Over the years, different phenotypes of asthma have been described. These asthma phenotypes are associated with different disease trajectories (2). Allergic asthma is the most recognized asthma phenotype (1). Most allergic asthma begins in childhood (95) and its symptoms are often triggered by factors such as exercise, change in weather, exposure to allergens such as house dust mite, pollen, cat or dog dander or viral respiratory infections (1). This type of asthma is defined by the presence of specific IgE antibodies in serum or a positive skin-prick test to common environmental allergens (3).

The pathophysiology of allergic asthma inflammation, characterized by inflammatory cells such as eosinophils, is dominated by a Th2-response upon exposure to allergens (4). Upon allergen exposure, airway epithelial cells are activated through pattern recognition receptors to produce chemokines (CCL17 and CCL22) and alarmins

(IL-25, IL-33 and TSLP). These factors activate cells of the innate immune system such as antigen-presenting dendritic cells (DCs) and ILC2 (type 2 innate lymphoid cells). Dendritic cells, having captured the allergens, will then relocate to the draining lymph node and initiate an allergen-specific adaptive immune response, leading to Th2 cell generation and B cell activation. Th2 cells produce the hallmark type-2 cytokines IL-4, IL-5, IL-9 and IL-13. IL-4 promotes IgE production by allergen-specific B cells. IL-5 plays a major role in the generation and activation of eosinophils, whereas IL-13 is an important mediator of smooth muscle hyperreactivity and goblet cell metaplasia leading to mucus hypersecretion. IL-9 promotes ILC2 and mast cell (MC) activation (5). Additionally, ILC2 cells activated by the epithelial alarmins IL33, TSLP or IL-25 will produce the cytokines IL-5 and IL-13, but not in an antigen-specific fashion. Furthermore, ILC2s and Th2 cells produce amphiregulin (Areg) that promotes epithelial repair after injury (5). Increased levels of these Th2 cytokines have been widely detected in the airways of asthma patients, and pathogenic Th2 cells were recently shown to be present in the airway wall of patients with childhood-onset asthma (6). Moreover, the airway epithelium in patients with asthma is more susceptible to allergen-induced damage and activation of a pro-inflammatory response.

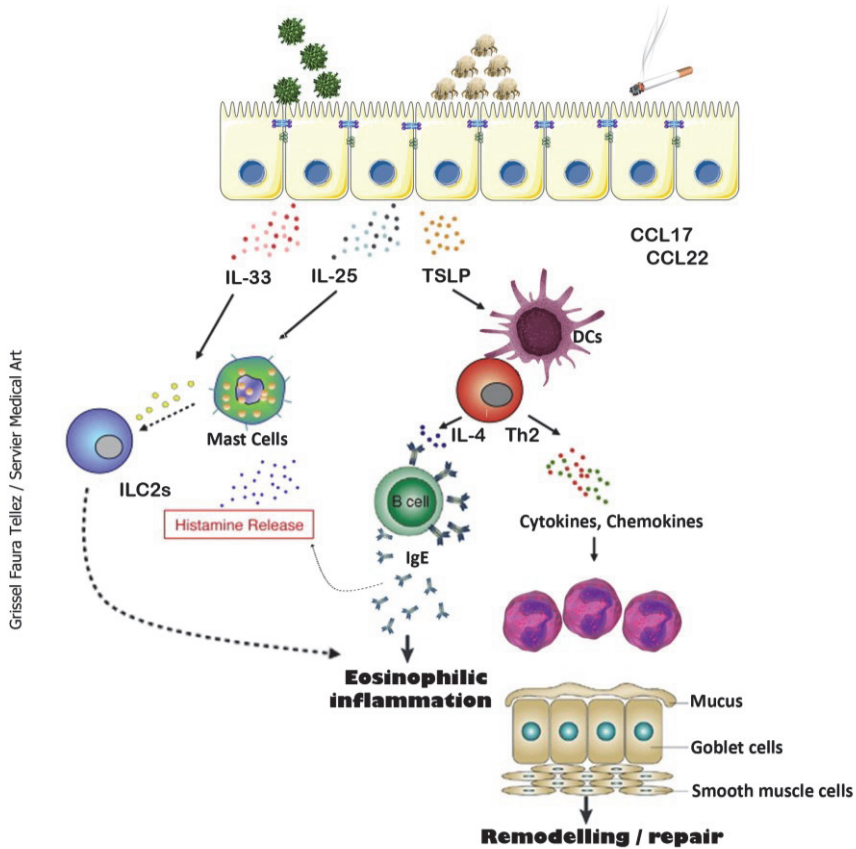


Figure 1. A simplified representation of the pathophysiological mechanisms in asthma is illustrated by the interactions between the airway epithelial cells, inflammatory cells and the external environment.

Airway epithelium and barrier function

The airway epithelium is located at the interface between the internal and external environment. It is the first line of defence in the airways, acting as a physical and chemical barrier and responsible for the mucociliary clearance that protects us from environmental insults

(7). When impaired, the airway epithelium is suggested to play an important role in the mechanisms of asthma (4,8). Since the importance of airway epithelial structure and function is increasingly appreciated in asthma, we will first review the structure and (adhesion) function of the airway epithelium.

The airway epithelium is formed by a variety of epithelial cell types: basal cells, columnar ciliated cells and secretory cells which are subdivided into Mucous goblet cells and club cells (previously known as Clara cells) (9,10) (Figure 2). In recent years, with the use of single-cell RNA sequencing, the cellular heterogeneity within the airways has been characterized in more detail. This resulted in the discovery of a new, previously unrecognized cell type, the pulmonary ionocyte, that has high expression of *CTFR* (the gene mutated in Cystic fibrosis) (11,12). This approach also refined the description of rare cell types in the airway epithelium, such as tuft cells (also called brush cells), Pulmonary neuroendocrine cells (PNECs), the hillock cell, an intermediate cell state in the squamous cell differentiation, and microfolds (or M) cells (11,13,14). Despite this, much remains to be discovered regarding the function of all these different cell types as both barrier epithelial cells and immune influencers.

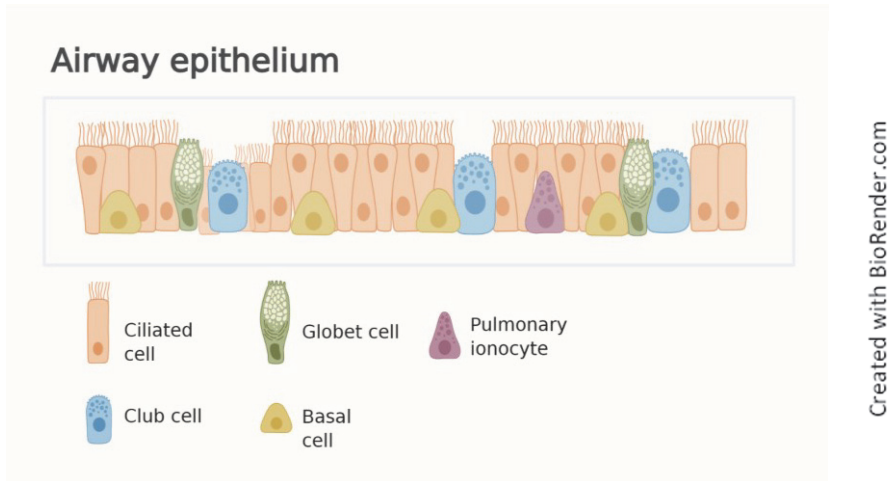


Figure 2. Cells and structure of the airway epithelium.

Basal cells and Club cells have stem-cell-like properties (15). These cells are capable to undergo (de)differentiation and proliferation, to facilitate epithelial regeneration after injury, but can also differentiate into goblet cells and/or ciliated epithelial cells (9). Club cells also represent the main progenitor of secretory cells characterized by the expression of the club cell secretory protein (CSSP also known as uteroglobin), the most abundant protein in the airway lining fluid (16,17). Goblet cells are mainly specialized in the secretion of mucus which sustains the protection mucus layer of the airway epithelium. These secretory cells can release several defence proteins into the airway trapping dust and foreign objects (18,19). Ciliated cells can originate from club cells or goblet cells, the most terminally differentiated cells in large airways (20). Typically, each (multi)ciliated cell possesses up to 300 hair-like cilia and their coordinated ciliary beating is essential for mucociliary clearance (MCC) of the airways. The role of the cilia in the respiratory tract is to

beat in a synchronized manner thereby propelling mucus as well as any particles trapped in the mucus towards the pharynx where the mucus can be swallowed, followed by degradation in the gastrointestinal tract (21). MCC is also an important innate defence mechanism of the airways against inhaled microbes and toxic particles from the environment.

Basal cells are small, cuboidal-shaped cells firmly connected to the basement membrane via hemidesmosomes and integrins thereby providing the foundation for the attachment of ciliated and goblet cells to the basement membrane. Additionally, the basal cells connect to the columnar cells via desmosomes. Lastly, all columnar cells are connected via tight and adherens junctions and the communication takes place via gap-junctions. The integrity of the healthy epithelium barrier relies on specialized cell junctions where tight and adherens junctions hold airway epithelial cells together, forming an ion- and size-selective barrier and maintaining the apical-basal polarity of the epithelial layer (22). The airway epithelium represents the first line of defence of the lungs acting as an immune and physical barrier (7). As an important part of the innate immune system, the epithelium regulates the innate and adaptive immune responses in the airways, governing the decision between the induction of immunity versus tolerance toward inhaled substances (23,24).

Epithelial cells are connected by different types of cell-cell adhesion and sealing junctional complexes which are formed by tight junctions (TJ), adherens junctions (AJ), gap junctions, and desmosomes (Figure

3). The tight junction is an intercellular junction between the apices of cells (apical cell-cell adhesion complexes), whereas the adherens junction is located basolaterally to the tight junction in the lateral membrane (15). Because tight junctions and adherens junctions are more tightly associated and often reside at the apical end of the lateral membrane, they are often referred to as the apical junctional complex (25).

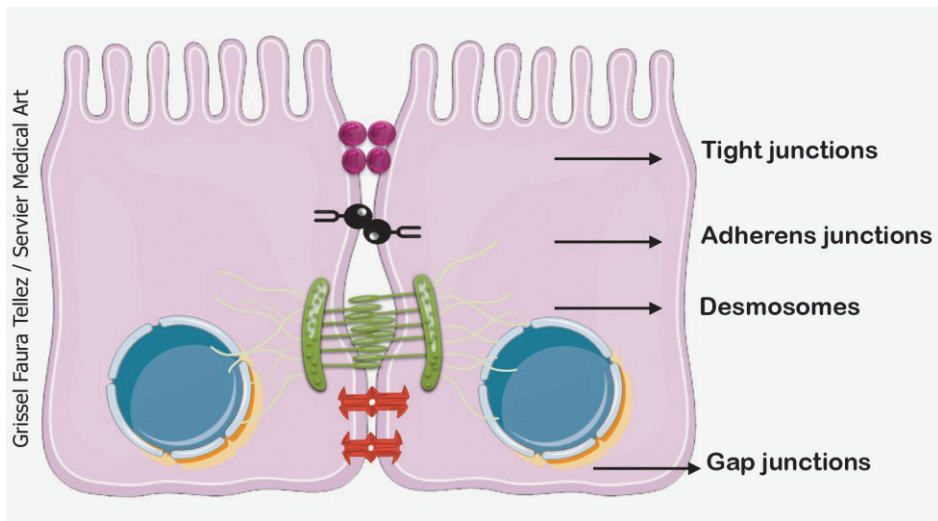


Figure 3. Junctional complexes in epithelial cells.

Tight junctions are the main contributors to the “fence” function in the epithelium creating a tight bond between the cell membranes of adjacent cells (4) which prevents lateral diffusion as well as intermixing of cell surface molecules in the apical membrane with those from the lateral membrane (26). It has been suggested that cells can regulate the composition of their apical and basolateral compartments by restricting the diffusion of both lipids and proteins in their membranes thereby implicating this fence function of the

apical junctional complex in the establishment and maintenance of cell polarity (25) during proliferation and differentiation of epithelial cells (27). The adherens junctions are important to initiate and maintain the cell-cell adhesion process by anchoring the junctional proteins in the lateral cell membrane to cytoplasmic actin filaments using a tightly controlled network of adaptor proteins. Formation of adherens junctions then leads to the assembly of tight junctions (23). However, after TJ formation, E-cadherin – which is critically required for AJ formation, is no longer required to maintain TJ organization (28).

Epithelial function and remodelling in asthma

Dysfunction of epithelial junctions has recently been linked to atopic diseases of the airway and skin. For example, loss of epithelial integrity has been implicated as an important mechanism leading to asthma (29). How could epithelial dysfunction contribute to the pathophysiology of asthma?

The airway epithelium in asthma has been shown to have several inherent defects, in both epithelial barrier function and immune activation (30). These epithelial alterations in patients with asthma include shedding of the epithelium with a detachment of ciliated cells, goblet cell hyperplasia, reduced expression of E-cadherin, increased epithelial permeability (8), aberrant epithelial repair responses, altered responses to viral infections and upregulation of growth factors, cytokines, and chemokines (31,32). In addition, previous

studies have shown that damage to airway epithelial cells coexists with increased AHR (33).

Injured epithelial cells and detachment of airway epithelial cells could lead to loss of barrier function, allowing inhaled allergens to reach the submucosa of the airways and activate the resident innate immune cells such as dendritic cells, leading to Type-2 immunity and allergic sensitization. Induced barrier dysfunction is also caused by many allergens and environmental molecules by damaging the epithelial junctions, independently of protease activity (22,34,35). Epithelial cells from asthma patients were more susceptible to this type of damage. Additionally, allergens might prevent the effective repair of the epithelial layer (4).

Hackett *et al.* showed that intrinsic defects of airway epithelial cells derived from patients with asthma also contribute to reduced barrier function, as airway epithelial cells from patients with asthma displayed reduced expression of the adherens junction molecule E-cadherin, associated with incomplete tight junction formation and increased permeability to macromolecules (36). In addition to this finding, the Davies lab showed that the physical barrier of the bronchial epithelium in asthma was disrupted, with evidence of loss of tight junction proteins (ZO-1) *in vivo* and *ex vivo* (29). Furthermore, a reduced expression of adherens junction proteins and a reduction in desmosome length was observed, which was paralleled by a leaky epithelial phenotype as evidenced by permeability for large molecules (dextran) (26,37). Cultured epithelial cells derived from

asthmatic donors also exhibit reduced transepithelial electrical resistance (TEER), consistent with reduced cell-cell adhesion in asthma (29,36).

An important feature in asthma is the remodelling of the airways due to increased proliferation, apoptosis and release of pro-inflammatory factors. Airway wall remodelling in asthma is evident from an increase in the smooth-muscle mass surrounding the airway wall, a deposition of collagen under the epithelial basement membrane which causes a thickened appearance, epithelial shedding but also an increase of mucus-producing goblet cells in the epithelium and submucosal glands (30). Airway remodelling and airway wall thickening are associated with airflow obstruction and airway hyper-responsiveness (AHR). In addition, these structural changes contribute to inflammatory processes through the release of cytokines, chemokines, growth factors and extracellular matrix components (8).

Ongoing inflammation and apoptosis due to the damaged and stressed epithelium can trigger cell-cell communication within the epithelial-mesenchymal trophic unit (EMTU) causing its (re)activation and leading to remodelling in part through epithelial to mesenchymal transition (EMT). EMT involves loss of the epithelial phenotype (characterized by reduced expression of epithelial markers (cytokeratins) and junctional proteins (E-cadherin, β -catenin, zonula occludens-1 and occludin)) but also an increase in the expression of mesenchymal-associated proteins such as α -smooth

muscle actin, vimentin, and/or fibronectin (38,39). The immediate outcome for EMT on epithelial reduced barrier function is an increased epithelial permeability that allows increased access of inhaled antigens to the submucosa, thus enabling their interaction with local immune cells. Although the initial induction of a mesenchymal phenotype via EMT is associated with tissue repair (8), the repetitive airway damage in response to chronic exposure by allergic insults can lead to persistent activation of airway epithelial repair processes and chronic absence of a well-differentiated airway epithelium supporting MCC (40). All the above, in combination with the increased production of mesenchymal factors, can contribute to the remodelling of the airways.

An important role in airway epithelial repair and remodelling has been identified for transforming growth factor- β (TGF- β) and the downstream signalling molecules SMAD2 and SMAD3. TGF- β isoforms are expressed under normal conditions in the airways. Yet, patients with severe asthma have reported to have increased expression of TGF- β_{1-3} and increased collagen deposition, compared to those with mild asthma (2). TGF- β is released from damaged epithelium, fibroblasts, smooth muscle cells and inflammatory cells. Increased amounts of TGF- β can also promote remodelling by inducing the expression of a wide range of extracellular matrix (ECM) components by fibroblasts, and by contributing to EMT.

Interestingly, in candidate gene studies, TGF- β gene polymorphisms have been associated with asthma susceptibility (41), asthma severity and markers of airway remodelling.

SMAD3 is a signal transduction molecule in TGF- β -induced signalling associated with EMT and epithelial repair (39). *SMAD3* is also an asthma susceptibility gene (42). Phosphorylation of Smad2 and Smad3 by TGF- β RI is a key event in TGF- β signalling. Increased TGF- β signalling as evidenced by higher expression levels of phospho-SMAD 2 was reported in bronchial biopsies specimens from asthmatic subjects. However, Smad3 signalling is required for the recognized allergen-induced airway remodelling and accumulation of myofibroblasts (43). The identification of the critical factors in this pathway, important for airway wall remodelling as well as asthma genes indicates that the susceptibility to the disease might regulate the airway epithelial responses thereby putting the airway epithelium at the forefront of asthma pathogenesis.

Asthma aetiology: Insights from Genetics and Epigenetics

The complexity of asthma is in part caused by the interaction between multiple genetic, epigenetic and environmental factors leading to disease inception (44). Based on twin studies, it has been estimated that heritability varies between 35% and 95% for asthma (45). Insights into the genetic predisposition to asthma can inform on the specific biological alterations during disease onset and therefore hold the promise of generating biological insights that can be used to develop new therapies and strategies to prevent disease. Over the last

40 years, significant progress has been made in uncovering the genetic basis of asthma by using unbiased approaches including large-scale and long-term follow-up of birth-cohort studies (45,46).

In the early days of asthma genetics, disease-associated genes were identified by positional cloning, where genes were selected based on chromosomal position by identification of risk alleles in studies using families displaying an asthma phenotype. These genetic studies relied on a positional analysis together with linkage analysis to detect genes associated with asthma. Examples of asthma genes identified by this approach are the genes encoding *A disintegrin and metalloprotease 33 (ADAM33)*, *GPRA*, *protocadherin-1 (PCDH1)*, *Serine protease inhibitor Kazal type-5 (SPINK5)*, *IL-1 receptor-associated kinase-M (IRAKM)*, *Dipeptidyl-peptidase 10 (DPP10)* and *HLA-G* (47).

Genetic studies then progressed from using linkage designs and candidate gene association studies to genome-wide association studies (GWAS). These genome-wide association studies use a hypothesis-free approach, with no prior assumptions regarding the identity of asthma genes, enabling the discovery of previously unknown new genes and pathways involved in disease pathogenesis. In 2007, Moffatt *et al.* reported the first asthma locus identified through GWAS, located at chromosome 17q12-21, and linked to childhood-onset asthma. The main limitation of GWAS studies is that they primarily detect common risk variants but are not well powered to detect rare risk variants (45). Moreover, most studies have been performed on populations of European descent, underrepresenting

the genetic diversity of the world's populations. Despite these limitations, many genetic variants have been discovered so far. A recent review by El-Husseini *et al.* documented a total of 128 independent asthma loci identified using GWAS (46) and a later GWAS meta-analysis of the UK Biobank and the TAGC consortium identified an additional 64 loci to be associated with asthma (48); bringing the total number of independent asthma loci to 172. The discussion of all of these loci is beyond the scope of this introduction, yet we will focus on the link between epithelial dysfunction in asthma and the possible genetically driven biological mechanisms.

Genetics alone does not fully account for the heritability of asthma. It is clear from many studies that both genetic and environmental risk factors play important roles in the development of asthma (47,49). Epigenetic changes refer to heritable modifications of the chromatin structure without altering the DNA sequence. These changes can switch genes on or off and determine which of these genes will become expressed (50). The most common epigenetic mechanisms, which are currently being investigated for asthma causality, are DNA methylation, histone modification and the expression of non-coding regulatory RNAs.

DNA methylation is the best studied epigenetic mark. DNA methylation can be influenced by the environment and result in changes to gene expression. During this process, DNA itself is chemically altered by the incorporation of a methyl group to a cytosine base in a cytosine-guanine dinucleotide by DNA

methyltransferase (DNMT) (49). Methylation of C residues occurs exclusively on cytosine nucleotide located next to a guanine nucleotide linked by a phosphate – so called CpG sites (51). Any CpG site can be subject to methylation, but the functional consequences thereof for gene expression are diverse. Approximately 60-70% of all CpG sites, denominated CpG islands, are found near the promoter region and generally remain unmethylated. DNA methylation in these regions is often associated with transcriptional repression and gene expression silencing through the formation of 5-methyl-cytosine (5mC). In contrast, DNA methylation in gene bodies is generally associated with high expression levels dependent on its genomic and cellular context (52,53).

DNA methylation is a common mechanism for maintaining tissue- or cell-type specific gene expression patterns. Interestingly, environmental factors such as cigarette smoke exposure, drugs, alcohol stress and pollution have also been shown to leave a "footprint" on the human genome in the form of DNA methylation of genes. This is the case for both active smoking (54) and passive smoke exposure, both postnatally (55) and *in utero* (56).

The associations between DNA methylation and diseases can be identified by epigenome-wide association studies (EWAS). Compared to the large number of GWAS reports, reports from EWAS in asthma are limited. Epigenetic signals reported in these studies are dependent on age, environment and cell or tissue type. The first consortium based EWAS for asthma was published by the

Mechanisms of the Development of Allergy (MEDALL) Consortium. These investigators showed reduced methylation at 14 CpG sites in whole-blood DNA to be genome-wide significantly associated with childhood asthma ($p < 1.14 \times 10^{-7}$) but did not show a significant association of these CpG sites in cord blood DNA at birth with subsequent early childhood asthma. These same 14 CpG sites also showed strongly reduced methylation levels within purified eosinophils. Five of these CpG sites were associated with asthma in respiratory epithelial cells, indicating cross-cell-type epigenetic effects (57).

These results were confirmed and extended through a larger EWAS meta-analysis by the Pregnancy and Childhood Epigenetics (PACE) consortium, which also identified significantly differential DNA methylation in relation to asthma in newborns' blood (58). Next to blood cells, DNA-methylation patterns in the nasal epithelium were investigated. Forno *et al.* showed that at many CpG sites, DNA methylation levels in nasal epithelium are significantly associated with atopic asthma and generated a 30 CpG panel that could accurately classify atopic asthma in children's populations with different ethnicities (59). These results appeared to be driven by changes in epithelial cells as well as an influx of immune cells, specifically T cells (60).

DNA methylation is highly cell-type specific, yet most EWAS are performed in samples consisting of a mixture of different cell types. Epigenetic alterations of lower airway epithelial cells in asthma

appear to be widespread (61). What is more, analysis interpreting multi-omics data obtained from DNA methylation, gene expression and its correlation with disease-associated genetic variants would be useful to improve our understanding of how genetic variation and epigenetic changes lead to asthma development.

Implications of asthma genetics: A focus on the epithelium

Many asthma susceptibility genes are expressed within the airway epithelium, highlighting the importance of epithelial cells in the pathology of the disease (96). For instance, IL1RL1/IL18R1, IL-33, and TSLP have emerged as important asthma genes, linking epithelial alarmins to the disease (26,42). Next to the important immune pathways, the pathway of cellular adhesion is strongly enriched amongst the allergy and asthma genes in the meta-analysis of allergic disease; especially genes with high expression in the skin when specifically analysing childhood-onset disease (62).

In line with this, several asthma genes associated with barrier function of the epithelium have been identified, including CDHR3, ORMDL3/GSDMB, MUC5AC, KIF3A and EFHC1 (8). In this thesis, we will focus on the link between epithelial dysfunction in asthma and the possible genetically driven mechanisms that contribute to such dysfunction. Selected asthma susceptibility genes identified through genetic studies and implicated in the epithelial cell dysfunction are summarized in **Table 1**.

The next challenge will be the systematic analysis of the precise

functions of these genes in the pathogenesis of asthma and their role in regulating epithelial barrier responses to environmental factors. Such studies may help to identify novel therapies targeting pathogenic mechanisms close to the origin of the disease. In this thesis, we will mainly focus on *Protocadherin 1 (PCDH1)*, an asthma susceptibility gene previously identified using a positional cloning approach (74).

Table 1. Examples of asthma genes susceptibility genes expressed in the airway epithelium identified by different genetic approaches and functional references.

Gene (s)	Mechanism	References
<i>FLG, GSDMB, KIF3A, EFHC1, PCDH1</i>	Reduce barrier	(63-65)
<i>MUC5AC, FOXA3</i>	Mucus hypersecretion	(66,67)
<i>ORMDL3, CDHR3, ICAM1</i>	Response upon viral infections	(68,69)
<i>IL33, IL1RL1, TSLP</i>	Immunomodulatory function, release of epithelial alarmins	(70)
<i>GSDMB, PCDH1, PLAUR, SMAD3</i>	Increased airway remodelling and airway hyperresponsiveness	(71-74)

*Adapted from Heijink et al, *Allergy* 2020; 75:1902-1917

Protocadherin-1: an asthma and airway hyperresponsiveness gene

Protocadherin-1 (PCDH1) was identified as a susceptibility gene for bronchial hyperresponsiveness (BHR), implying a role in the

pathogenesis of asthma (74). This finding was based on significant linkage of *PCDH1* gene variants with BHR in 200 Dutch families ascertained through a parent with asthma. Koppelman *et al.* identified a strong linkage signal of *PCDH1*, especially in families exposed to tobacco smoke. The linkage of the genetic region on chromosome 5q31-q33 (*PCDH1* gene) to BHR and asthma is limited to families with a significant smoking history of the proband (i.e. > 5 pack years), which is consistent with passive smoke exposure *in utero* and in the first years of life of the children. This association was replicated in a US family study (74,75). Interestingly, prenatal maternal smoke exposure is an important risk factor for asthma-related outcomes including increased BHR. The mechanisms by which prenatal cigarette smoke exposure mediates this effect, and whether it includes an effect on *PCDH1* gene expression is unknown. Until now, no DNA methylation differences in *PCDH1* have been identified (61). Noteworthy, *PCDH1* exon 1a is localised in a CpG island. In a recent publication from our group, Koning *et al.* reported downregulation of *PCDH1* expression upon cigarette smoke exposure *in vivo* in a mouse model (76), a treatment which is known to affect the methylation status of airway epithelial genes, thereby altering their expression patterns. Taken together there is enough evidence to support the hypothesis that *PCDH1* expression may be regulated by epigenetic mechanisms and further studies are warranted.

Several genetic studies have reported associations of single nucleotide polymorphisms (SNPs) in *PCDH1* with BHR and asthma. In these studies, *PCDH1* has been identified as a susceptibility gene for

different phenotypes of asthma (74,77) as well as for eczema (78,79). From these genetic studies of *PCDH1*, it is clear that so far, a single dominant *PCDH1* polymorphism associated with asthma has not been identified. Asthma and eczema often coexist in the same individuals, partly because of a shared genetic origin (80). Could it be that the *PCDH1* gene contributes to these diseases via a shared biological pathway?

Indeed, for the majority of allergy genes, it was shown that these contribute to asthma, eczema and hay fever, strongly supporting the concept of shared allergic disease genetic susceptibility (48,80).

PCDH1: expression, regulation and function

Protocadherins constitute the largest subfamily of the cadherin family of adhesion molecules, with more than 70 members in humans and mice (81). Most protocadherins were initially detected in the brain and later found to be also present in heart, kidney, lung and trachea (82). Furthermore, *PCDH19* and *PCDH1* have been reported to be expressed during mouse embryogenesis in a tissue-specific manner (83,84). *PCDH1* was originally described in 1993 (PC42) as an adhesion molecule localizing to cell-cell contact sites in mouse fibroblast L cell overexpression studies and was observed to mediate weak homophilic cell adhesion (85). *PCDH1* is located on 5q31-33 (86) and belongs to the delta-1 group of the non-clustered PCDHs group (87). This δ 1-protocadherin member has also been found to be essential for New World Hantavirus infection (88).

The *PCDH1* gene encodes three different mRNA transcripts. Two of these are translated into a transmembrane protein with putative cell adhesion properties (isoform 1 of 150 kDa and isoform 2 of 170 kDa). The third isoform lacks the extracellular domain but may retain signalling properties. *PCDH1* is expressed in the airway epithelium, endothelial cells and macrophages (74,89). *PCDH1* contains an extracellular domain with 7 cadherin repeats (EC1 to EC7 from N to C-terminus), a transmembrane domain and an intracellular domain containing several conserved serine and tyrosine residues in the membrane-proximal part as well as three highly conserved intracellular motifs (CM1, CM2 and CM3) in the more distal intracellular part. *Isoform-1* and *Isoform-2* are encoded by 3 or 5 exons, respectively, sharing the extracellular domain, the transmembrane domain and the membrane-proximal intracellular part, but differ in the presence of the distal intracellular domain (CM1/2/3). *PCDH1*- *Isoform-3*, the smaller protein product of 50 kDa, was recently identified in differentiated primary human bronchial epithelial cells (PBECS) by Koning *et al.* *PCDH1* isoform-3 has a relatively shorter transcript lacking exon 1 and part of exon 2 (89).

Interestingly, both isoforms 2 and 3 contain these evolutionarily conserved signalling motifs (CM1-3), indicating a possible role in signal transduction. Although the putative interaction partners of CM1 and CM2 are still unknown, the presence of the CM3 motif, RRVTF, in their cytoplasmic domain provides the ability of *PCDH1* to interact with protein phosphatase-1 α (PP1 α) (90,91) a phosphatase implicated in lung development.

The extracellular cadherin repeats are thought to mediate homophilic adhesion. A recent study using binding assays and x-ray crystal structures has revealed that PCDH1 EC repeats 1 to 4 is the minimum unit required for homophilic adhesion and the binding mode involves a twisted antiparallel overlap of repeats EC1 to EC4 respectively (92) similar to the observed in PCDH9 (93). In line with this finding direct recognition of PCDH1 EC1 has been recently shown to be critical for cellular attachment and entry of the hantaviruses glycoproteins. The exact role of PCDH1 in the airway epithelium and its contribution to both asthma susceptibility and hantavirus pulmonary syndrome is yet to be determined. Since the intercellular space can vary wildly among different types of cellular junctions, we carefully need to identify the localization of PCDH1 in the airway epithelium as well as the sub-cellular localisation of PCDH1 and its variants in order to get fundamental insights into the likely role of PCDH1 in the airway epithelium. Recent studies have found *PCDH1* expression to be strongly up-regulated during mucociliary differentiation of primary bronchial epithelial cells in ALI cultures (89). Yet, the PCDH1 adhesion specificity and the changes in PCDH1 expression during mucociliary differentiation remain to be characterized. Moreover, the presence of 3 conserved cytoplasmic domains (CM1-3) in the intracellular domain suggests that besides the possibility of differential intracellular localization there is also the chance to address PCDH1 role as a signalling molecule and to gain insight into the signalling pathways where it might be related.

The research described in this thesis aims to understand the role of PCDH1 into the mechanisms for airway epithelial repair under normal and chronic disease conditions. Shedding some light on the role of PCDH1 in epithelial barrier formation and repair is necessary to develop therapies that can prevent airway remodelling and promote normal epithelial repair.

Aims and outline of the thesis

In this thesis, we aim to investigate the role of PCDH1 in the airway epithelium. The *PCDH1* gene encodes a putative adhesion molecule of unknown function. We hypothesize that dysfunction of PCDH1 compromises the airway epithelial barrier of the asthmatic epithelium. Here, we investigate the potential mechanisms by which asthma-associated PCDH1 gene variants alter PCDH1 DNA methylation and expression; and address the potential role of cigarette smoke exposure, but also the localization and differential expression in airway epithelial cells in healthy and asthmatic epithelium and the role of PCDH1 as an adhesion molecule.

In Chapter 2, we review genetic studies of *PCDH1* polymorphisms that have been previously linked to bronchial hyperreactivity (BHR), asthma and eczema thoroughly to dissect the stronger and common *PCDH1* gene association signals. Recent publications were able to replicate some of these associations in different populations but also reported more associations. In addition, we made an attempt to interpret these genetic data in light of *PCDH1* expression and function

as a contributor to epithelial integrity in the lung and the skin.

In **chapter 3**, we aim to understand the association of *PCDH1* genetic and epigenetic variation with *PCDH1* gene expression. Therefore, we investigate the association of *PCDH1* genetic variants with DNA methylation and gene expression in lung tissue and nasal cells and also assess whether cigarette smoke exposure is associated with *PCDH1* gene methylation in airway epithelial DNA. To this end, we analyse a selection of lung tissue samples from smokers and non-smokers, the relationship between exposure, genetic polymorphisms, methylation and gene expression and compare these to results obtained in nasal samples.

In **chapter 4**, we describe the expression of different *PCDH1* isoforms in the airway epithelium. Likewise, given the differences in the gene and protein structure of *PCDH1* isoforms, we study the potential variations in differential intracellular localization and later on we address this possibility in their signalling properties. *PCDH1* differs from classical adhesion molecules as it does not display strong adhesion activity. Therefore, we attempt to pinpoint the exact subcellular location of *PCDH1* in relation to other classical adherens and tight junctions and ciliated bodies, by using a combination of high-resolution fluorescent microscopy and air-liquid interface cultures of asthmatic human primary bronchial epithelial cells (PBECS), in this chapter, we aim to investigate the localization, differential expression in airway epithelial cells in asthma and role of

PCDH1 as an adhesion molecule in the airway epithelium. Moreover, we explore the functional effects of PCDH1 on bronchial epithelial cell adhesion.

In chapter 5, we investigate the interaction of two asthma genes, Protocadherin-1 and SMAD-3. These asthma genes were reported to interact together at the protein level, using yeast two-hybrid screens (94). Here, we explore the possibility of PCDH1 and SMAD3 physically interacting in a single pathway in bronchial epithelial cells and investigate whether PCDH1 could influence TGF β 1 signalling by interacting with SMAD3. In addition, we evaluate the functional consequences for TGF- β -induced gene expression.

Finally, **in chapter 6**, we provide a summary and discussion of the previous findings, including suggestions for future studies.

References

1. GINA Main Report, Main Report. Global Strategy for Asthma Management and Prevention updated 2021. 2021.
2. Chung KF. Asthma phenotyping: a necessity for improved therapeutic precision and new targeted therapies. *J Intern Med* 2016;279(2):192-204.
3. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2014;16(1):45-56.
4. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol Int* 2018;67(1):12-17.
5. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity* 2015;43(1):29-40.
6. Vieira Braga FA, Kar G, Berg M, Carpaij OA, Polanski K, Simon LM, et al. A cellular census of human lungs identifies novel cell states in health and in asthma. *Nature medicine* 2019;25(7):1153-1163.
7. Vareille M, Kieninger E, Edwards MR, Regamey N. The airway epithelium: soldier in the fight against respiratory viruses. *Clin Microbiol Rev* 2011;24(1):210-229.
8. Heijink IH, Kuchibhotla V, Roffel MP, Maes T, Knight DA, Sayers I, et al. Epithelial cell dysfunction, a major driver of asthma development. *Allergy (Copenhagen)* 2020;75(8):1898-1913.
9. Tam A, Wadsworth S, Dorscheid D, Man SF, Sin DD. The airway epithelium: more than just a structural barrier. *Ther Adv Respir Dis* 2011;5(4):255-273.
10. Tata PR, Rajagopal J. Plasticity in the lung: making and breaking cell identity. *Development (Cambridge)* 2017;144(5):755-766.
11. Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* 2018;560(7718):319-324.
12. Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature (London)* 2018;560(7718):377-381.
13. Zaragosi LE, Deprez M, Barbry P. Using single-cell RNA sequencing to unravel cell lineage relationships in the respiratory tract. *Biochemical Society transactions* 2020;48(1):327-336.
14. Meyer KB, Wilbrey-Clark A, Nawijn M, Teichmann SA. The Human Lung Cell Atlas: a transformational resource for cells of the respiratory system. *Lung Stem Cells in Development, Health and Disease* 2021:158.
15. Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. *Tissue Barriers* 2013;1(4):e24997.
16. Davis JD, Wypych TP. Cellular and functional heterogeneity of the airway epithelium. *Mucosal immunology* 2021;14(5):978-990.
17. Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, et al. The Role of Scgb1a1 + Clara Cells in the Long-Term Maintenance and Repair of Lung Airway, but Not Alveolar, Epithelium. *Cell Stem Cell* 2009;4(6):525-534.
18. Whitsett JA. Airway Epithelial Differentiation and Mucociliary Clearance. *Annals of the American Thoracic Society* 2018;15(Supplement_3):S143-S148.
19. Frey A, Lunding LP, Ehlers JC, Weckmann M, Zissler UM, Wegmann M. More Than Just a Barrier: The Immune Functions of the Airway Epithelium in Asthma Pathogenesis. *Frontiers in immunology* 2020;11:761.
20. Ruiz García S, Deprez M, Lebrigand K, Cavard A, Paquet A, Arguel M, et al. Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development (Cambridge)* 2019;146(20).

21. Munkholm M, Mortensen J. Mucociliary clearance: pathophysiological aspects. *Clin Physiol Funct Imaging* 2014;34(3):171-177.
22. Georas SN, Rezaee F. Epithelial barrier function: at the frontline of asthma immunology and allergic airway inflammation. *Journal of allergy and clinical immunology* 2014;134(3):509-520.
23. Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol* 2011;32(6):248-255.
24. Schleimer RP, PhD, Kato A, PhD, Kern R, MD, Kuperman D, PhD, Avila PC, MD. Epithelium: At the interface of innate and adaptive immune responses. *Journal of allergy and clinical immunology* 2007;120(6):1279-1284.
25. Zihni C, Mills C, Matter K, Balda MS. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol* 2016;17(9):564-580.
26. Davies DE. Epithelial barrier function and immunity in asthma. *Ann Am Thorac Soc* 2014;11 Suppl 5:244.
27. Kast JI, Wanke K, Soyka MB, Wawrzyniak P, Akdis D, Kingo K, et al. The broad spectrum of interepithelial junctions in skin and lung. *J Allergy Clin Immunol* 2012;130(2):544-7.e4.
28. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta* 2008;1778(3):660-669.
29. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;128(3):549-12.
30. Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012;18(5):684-692.
31. Ordonez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in asthma: artifact or pathology? *Am J Respir Crit Care Med* 2000;162(6):2324-2329.
32. Montefort S, Roberts JA, Beasley R, Holgate ST, Roche WR. The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects. *Thorax* 1992;47(7):499-503.
33. Lauri A, Laitinen. 1. Damage of the Airway Epithelium and Bronchial Reactivity in Patients with Asthma. *Arerugi* 1995;44(8):826-827.
34. Heijink IH, van Oosterhout A, Kapus A. Epidermal growth factor receptor signalling contributes to house dust mite-induced epithelial barrier dysfunction. *The European respiratory journal* 2010;36(5):1016-1026.
35. Post S, Nawijn MC, Jonker MR, Kliphuis N, van den Berge M, van Oosterhout AJM, et al. House dust mite-induced calcium signaling instigates epithelial barrier dysfunction and CCL20 production. *Allergy (Copenhagen)* 2013;68(9):1117-1125.
36. Hackett TL, Singhera GK, Shaheen F, Hayden P, Jackson GR, Hegele RG, et al. Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 2011;45(5):1090-1100.
37. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol* 2008;86(3):105-112.
38. Pain M, Bermudez O, Lacoste P, Royer PJ, Botturi K, Tissot A, et al. Tissue remodelling in chronic bronchial diseases: from the epithelial to mesenchymal phenotype. *Eur Respir Rev* 2014;23(131):118-130.
39. Hackett TL, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky DV, Murray LA, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. *Am J Respir Crit Care Med* 2009;180(2):122-133.
40. Lopez-Rodriguez JC, Benede S, Barderas R, Villalba M, Batanero E. Airway Epithelium Plays a Leading Role in the Complex Framework Underlying Respiratory Allergy. *J Investig Allergol Clin Immunol* 2017;27(6):346-355.

41. Ierodiakonou D, Postma DS, Koppelman GH, Gerritsen J, ten Hacken NHT, Timens W, et al. TGF-beta 1 polymorphisms and asthma severity, airway inflammation, and remodeling. *Journal of Allergy and Clinical Immunology* 2013;131(2):582-585.
42. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363(13):1211-1221.
43. Al-Alawi M, Hassan T, Chotirmall SH. Transforming growth factor beta and severe asthma: a perfect storm. *Respir Med* 2014;108(10):1409-1423.
44. Blumenthal MN. Genetic, epigenetic, and environmental factors in asthma and allergy. *Ann Allergy Asthma Immunol* 2012;108(2):69-73.
45. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011;242(1):10-30.
46. El-Husseini ZW, Gosens R, Dekker F, Koppelman GH. The genetics of asthma and the promise of genomics-guided drug target discovery. *Lancet Respir Med* 2020;8(10):1045-1056.
47. Moheimani F, Hsu AC, Reid AT, Williams T, Kicic A, Stick SM, et al. The genetic and epigenetic landscapes of the epithelium in asthma. *Respir Res* 2016;17(1):119-4.
48. Han Y, Jia Q, Jahani PS, Hurrell BP, Pan C, Huang P, et al. Genome-wide analysis highlights contribution of immune system pathways to the genetic architecture of asthma. *Nature Communications* 2020;11(1):1776.
49. Stefanowicz D, Hackett TL, Garmaroudi FS, Gunther OP, Neumann S, Sutanto EN, et al. DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children. *PLoS One* 2012;7(9):e44213.
50. Kabesch M, Adcock IM. Epigenetics in asthma and COPD. *Biochimie* 2012;94(11):2231-2241.
51. Salam MT. Asthma epigenetics. *Adv Exp Med Biol* 2014;795:183-199.
52. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nature reviews. Genetics* 2012;13(7):484-492.
53. Lev Maor G, Yearim A, Ast G. The alternative role of DNA methylation in splicing regulation. *Trends Genet* 2015;31(5):274-280.
54. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, et al. Epigenetic Signatures of Cigarette Smoking. *Circ Cardiovasc Genet* 2016;9(5):436-447.
55. Reynolds LM, Magid HS, Chi GC, Lohman K, Barr RG, Kaufman JD, et al. Secondhand Tobacco Smoke Exposure Associations With DNA Methylation of the Aryl Hydrocarbon Receptor Repressor. *Nicotine & tobacco research* 2017;19(4):442-451.
56. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. *Am J Hum Genet* 2016;98(4):680-696.
57. Xu C, SÄ¶derhÅ¶ll C, Bustamante M, BaÅ¿ N, Gruzieva O, Gehring U, et al. DNA methylation in childhood asthma: an epigenome-wide meta-analysis. *The Lancet Respiratory Medicine* .
58. Reese SE, Xu C, den Dekker HT, Lee MK, Sikdar S, Ruiz-Arenas C, et al. Epigenome-wide meta-analysis of DNA methylation and childhood asthma. *Journal of Allergy and Clinical Immunology* 2019;143(6):2062-2074.
59. Forno E, Wang T, Qi C, Yan Q, Xu C, Boutaoui N, et al. DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *The lancet respiratory medicine* 2019;7(4):336-346.
60. Qi C, Jiang Y, Yang IV, Forno E, Wang T, Vonk JM, et al. Nasal DNA methylation profiling of asthma and rhinitis. *Journal of allergy and clinical immunology* 2020;145(6):1655-1663.

61. Nicodemus-Johnson J, Myers RA, Sakabe NJ, Sobreira DR, Hogarth DK, Naureckas ET, et al. DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight* 2016;1(20):e90151.
62. Pividori M, Schoettler N, Nicolae DL, Ober C, Im HK. Shared and distinct genetic risk factors for childhood-onset and adult-onset asthma: genome-wide and transcriptome-wide studies. *The lancet respiratory medicine* 2019;7(6):509-522.
63. Gao W, Gong J, Mu M, Zhu Y, Wang W, Chen W, et al. The Pathogenesis of Eosinophilic Asthma: A Positive Feedback Mechanism That Promotes Th2 Immune Response via Filaggrin Deficiency. *Frontiers in immunology* 2021;12:672312.
64. Geng G, Du Y, Dai J, Tian D, Xia Y, Fu Z. KIF3A knockdown sensitizes bronchial epithelia to apoptosis and aggravates airway inflammation in asthma. *Biomedicine & pharmacotherapy* 2018;97:1349-1355.
65. Marenholz I, Esparza-Gordillo J, Rüschemdorf F, Bauerfeind A, Strachan DP, Spycher BD, et al. Meta-analysis identifies seven susceptibility loci involved in the atopic March. *Nature communications* 2015;6(1):8804.
66. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, et al. Abnormalities in MUC5AC and MUC5B protein in airway mucus in asthma. *American journal of respiratory and critical care medicine* 2016;194(10):1296-1299.
67. Sajuthi SP, Everman JL, Jackson ND, Saef B, Rios CL, Moore CM, et al. Nasal airway transcriptome-wide association study of asthma reveals genetically driven mucus pathobiology. *Nature communications* 2022;13(1):1632.
68. Yang R, Tan M, Xu J, Zhao X. Investigating the regulatory role of ORMDL3 in airway barrier dysfunction using in vivo and in vitro models. *International Journal of Molecular Medicine* 2019;44(2):535-548.
69. Basnet S, Bochkov YA, Brockman-Schneider RA, Kuipers I, Aesif SW, Jackson DJ, et al. CDHR3 Asthma-Risk Genotype Affects Susceptibility of Airway Epithelium to Rhinovirus C Infections. *American journal of respiratory cell and molecular biology* 2019;61(4):450-458.
70. Saikumar Jayalatha AK, Hesse L, Ketelaar ME, Koppelman GH, Nawijn MC. The central role of IL-33/IL-1RL1 pathway in asthma: From pathogenesis to intervention. *Pharmacology & therapeutics (Oxford)* 2021;225:107847.
71. Das S, Miller M, Beppu AK, Mueller J, McGeough MD, Vuong C, et al. GSDMB induces an asthma phenotype characterized by increased airway responsiveness and remodeling without lung inflammation. *Proceedings of the National Academy of Sciences - PNAS* 2016;113(46):13132-13137.
72. Barton SJ, MSc, Koppelman, Gerard H., MD, PhD, Vonk JM, PhD, Browning CA, BSc, Nolte IM, PhD, Stewart CE, PhD, et al. PLAUR polymorphisms are associated with asthma, PLAUR levels, and lung function decline. *Journal of allergy and clinical immunology* 2009;123(6):1391-1400.e17.
73. DeVries A, Wlasiuk G, Miller SJ, Bosco A, Stern DA, Lohman IC, et al. Epigenome-wide analysis links SMAD3 methylation at birth to asthma in children of asthmatic mothers. 2016.
74. Koppelman GH, Meyers DA, Howard TD, Zheng SL, Hawkins GA, Ampleford EJ, et al. Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2009;180(10):929-935.
75. Colilla S, Nicolae D, Pluzhnikov A, Blumenthal MN, Beaty TH, Bleecker ER, et al. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immunol* 2003;111(4):840-846.
76. Koning H, van Oosterhout AJ, Brouwer U, den Boef LE, Gras R, Reinders-Luinge M, et al. Mouse protocadherin-1 gene expression is regulated by cigarette smoke exposure in vivo. *PLoS One* 2014;9(7):e98197.
77. Toncheva AA, Suttner K, Michel S, Klopp N, Illig T, Balschun T, et al. Genetic variants in Protocadherin-1, bronchial hyper-responsiveness, and asthma subphenotypes in German children. *Pediatr Allergy Immunol* 2012;23(7):636-641.

78. Koning H, Postma DS, Brunekreef B, Duiverman EJ, Smit HA, Thijs C, et al. Protocadherin-1 polymorphisms are associated with eczema in two Dutch birth cohorts. *Pediatr Allergy Immunol* 2012;23(3):270-277.
79. Mortensen LJ, Kreiner-Moller E, Hakonarson H, Bonnelykke K, Bisgaard H. The PCDH1 gene and asthma in early childhood. *Eur Respir J* 2014;43(3):792-800.
80. Ferreira MA, Vonk JM, Baurecht H, Marenholz I, Tian C, Hoffman JD, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet* 2017;49(12):1752-1757.
81. Lin J, Wang C, Redies C. Expression of delta-protocadherins in the spinal cord of the chicken embryo. *J Comp Neurol* 2012;520(7):1509-1531.
82. Wolverson T, Lalande M. Identification and characterization of three members of a novel subclass of protocadherins. *Genomics* 2001;76(1-3):66-72.
83. Gaitan Y, Bouchard M. Expression of the delta-protocadherin gene *Pcdh19* in the developing mouse embryo. *Gene Expr Patterns* 2006;6(8):893-899.
84. Redies C, Heyder J, Kohoutek T, Staes K, Van Roy F. Expression of protocadherin-1 (*Pcdh1*) during mouse development. *Dev Dyn* 2008;237(9):2496-2505.
85. Sano K, Tanihara H, Heimark RL, Obata S, Davidson M, St John T, et al. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J* 1993;12(6):2249-2256.
86. Kim SY, Yasuda S, Tanaka H, Yamagata K, Kim H. Non-clustered protocadherin. *Cell Adh Migr* 2011;5(2):97-105.
87. Morishita H, Yagi T. Protocadherin family: diversity, structure, and function. *Curr Opin Cell Biol* 2007;19(5):584-592.
88. Jangra RK, Herbert AS, Li R, Jae LT, Kleinfelter LM, Slough MM, et al. Protocadherin-1 is essential for cell entry by New World hantaviruses. *Nature* 2018;563(7732):559-563.
89. Koning H, Sayers I, Stewart CE, de Jong D, Ten Hacken NH, Postma DS, et al. Characterization of protocadherin-1 expression in primary bronchial epithelial cells: association with epithelial cell differentiation. *FASEB J* 2012;26(1):439-448.
90. Redies C, Vanhalst K, Roy F. delta-Protocadherins: unique structures and functions. *Cell Mol Life Sci* 2005;62(23):2840-2852.
91. Blevins CJ, Emond MR, Biswas S, Jontes JD. Differential expression, alternative splicing, and adhesive properties of the zebrafish delta1-protocadherins. *Neuroscience* 2011;199:523-534.
92. Modak D, Sotomayor M. Identification of an adhesive interface for the non-clustered delta1 protocadherin-1 involved in respiratory diseases. *Commun Biol* 2019;2:354-0. eCollection 2019.
93. Peng X, Emiliani F, Smallwood PM, Rattner A, Lei H, Sabbagh MF, et al. Affinity capture of polyribosomes followed by RNAseq (ACAPseq), a discovery platform for protein-protein interactions. *eLife* 2018;7.
94. Colland F, Jacq X, Trouplin V, Mouglin C, Groizeleau C, Hamburger A, et al. Functional proteomics mapping of a human signaling pathway. *Genome Res* 2004;14(7):1324-1332.
95. Holgate ST. Innate and adaptive immune responses in asthma. *Nature medicine* 2012;18(5):673-683.
96. Demenais F, Margaritte-Jeannin P, Barnes KC, Cookson WOC, Altmüller J, Ang W, et al. Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. 2018.

