Lees s.v.p. zorgvuldig de handleiding.
Klik bovenaan de 1e pagina in de cel 'Coordinating investigator 1'. Gebruik de tab-toets om binnen pagina 1 naar een ander veld te gaan. Klik bovenaan de volgende pagina weer in de 1e cel (vraag 1.3) om verder te gaan. Gebruik daarna weer de tab-toets.

Final report

LF 2016

Projectnr:
3.2.09.055

1. General information

1.1	Coordinating investigator 1	Prof.dr. G.H. Koppelman
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1.3	Title of project:	
	English	Protocadherin-1 expression in airway epithelium: Investigations into a novel cause of bronchial hyperresponsiveness and asthma
	Dutch	Protocadherine-1 expressie in het luchtwegepitheel: onderzoek naar een nieuwe oorzaak van luchtwegovergevoeligheid en astma
	Project number	3.2.09.055
1.4	Time schedule:	
	Start of project	01-04-2011 (dd-mm-yyyy)
	Duration of project	48 (months)
	Period of funding	from 01-04-2011 (dd-mm-yyyy) till 01-04-2017 (dd-mm-yyyy) With the approval of the Lung Foundation, the time schedule of this project changed and was extended due to change in personnel who carried out the work on this project: B Willemse (postdoc), U. Brouwer (technician), and finally D. van Gosliga (technician).
1.5	Grant	€ 250.000
1.6	Short description of the project for public information (in Dutch, see guidelines) (max. 250 words):	

Astma is een veel voorkomende chronische ontstekingsziekte van de luchtwegen en hangt samen met luchtwegovergevoeligheid: de luchtwegen vernauwen zich na inademing van prikkels zoals koude lucht, rook of mist. Luchtwegovergevoeligheid wordt deels door erfelijke aanleg bepaald; in 2009 ontdekten we dat een gen genaamd Protocadherine-1 (PCDH1) hiervoor mede verantwoordelijk is.

Het doel van dit onderzoeksproject was het beschrijven van de expressie, regulatie en functie van PCDH1 in de luchtwegen.

De belangrijkste bevindingen van dit project zijn:

1. Het PCDH1 eiwit bevindt zich op de contactpunten tussen de cellen die de bekleding van de luchtwegen vormen (luchtwegepitheel), waarbij naast 2 bekende vormen van PCDH1 nog een nieuwe, derde vorm ontdekt is.
2. De mate waarin PCDH1 tot expressie komt hangt af van de mate van uitrijping (differentiatie) van luchtwegepitheelcellen.
3. PCDH1 expressie wordt sterk geremd door sigarettenrook, dit zou kunnen bijdragen aan de toegenomen luchtwegovergevoeligheid bij rokers.
4. PCDH1 codeert voor een adhesiemolecuul, een eiwit dat er voor zorgt dat cellen aan elkaar hechten. De eerste functie van PCDH1 is dat het bijdraagt aan de integriteit van het luchtwegepitheel, aan het herstel van de beschermende barrière functie na beschadiging ervan. PCDH1 heeft een tweede functie bij de signaleringsroute van TGFβ in de epitheelcel. TGFβ is verhoogd aanwezig in de luchtwegen van astma patiënten en activeert de cel via SMAD3. PCDH1 kan aan SMAD3 binden en deze signaal route remmen.
5. PCDH1 bindt naast SMAD3 nog een aantal eiwitten, waarmee het mogelijk de mate van ontsteking in de luchtwegen kan reguleren.

2. Report

2.1 Summary:

Title	Protocadherin-1 in the airway epithelium: The two faces of an airway hyperresponsiveness gene.
Authors	Gerard H. Koppelman (1) and Martijn C. Nawijn (2)
Dept./Institute(s)	(1) Dpt of Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital and (2) EXPIRE laboratory, Dpt of Pathology and Medical Biology, GRIAC Research Institute, University Medical Center Groningen
Keywords (max. 6)	Airway hyperresponsiveness, Genetics, Epithelial Integrity, TGFβ, remodeling

Abstract (max. 250 words):

Asthma is a common, chronic respiratory disease that is accompanied by airway hyperresponsiveness (AHR). AHR is defined as the exaggerated response of the airways to nonspecific stimuli. AHR is caused by genes and the environment (such as cigarette smoke). In 2009, we discovered the first AHR gene: *Protocadherin 1* (*PCDH1*).

The aim of this research project was to define the expression, regulation, and function of PCDH1 in the airway epithelium.

The main findings of this project are:

1. PCDH1 is expressed in airway epithelial cells. Next to two known isoforms, a third intracellular isoform was discovered that may contribute to cellular signaling. PCDH1 localizes to the cell membrane, basolateral from but proximal to the Adherens Junction.
2. The expression of PCDH1 is induced during airway epithelial differentiation.
3. PCDH1 mRNA and protein expression is reduced by inhalation of cigarette smoke.
4. PCDH1 has a dual function:
   (i) adhesion molecule contributing to both airway epithelial integrity and restoration of barrier after damage.
   (ii) regulation of TGFβ signaling through binding to SMAD3 and inhibition of its activity in airway epithelial cells, linking PCDH1 to airway remodeling in asthma. The asthma genes PCDH1, TGFβ and SMAD3 likely contribute to a shared pathophysiological mechanism in inception of disease.
5. PCDH1 also binds to other signalling intermediates (such as NF-kB regulators), and the cap-independent translation initiation complex, relevant to viral protein translation. These data identify PCDH1 as a potential regulator of innate inflammatory responses and viral replication in airway epithelial cells.

2.2 Description of original question/aim (max. 150 words):

The *central hypothesis* of this research project was that PCDH1 plays a role in epithelial integrity of the airways and that an altered function of PCDH1 is associated with BHR development, which may lead to symptomatic asthma.

The specific aims of this project were to:

1. To investigate if the PCDH1 gene variants that are associated with BHR and asthma result in altered PCDH1 isoform expression.
2. To investigate the PCDH1 mRNA and protein expression in airway epithelial cells.
3. To address if environmental factors, such as cigarette smoking, alter PCDH1

	expression in the lung. 4. To investigate the role of PCDH1 in the sensitivity of airway epithelial cells to injury in asthma. 5. To investigate intracellular signal transduction of PCDH1 in airway epithelium using co-immunoprecipitation and perform an unbiased proteomic approach to identify novel interaction partners of PCDH1.
2.3	Results (max. 2500 words, please submit a maximum of 4 figures and diagrams separately): Please see addendum 1 for this document.
2.4	Did the study solve the original question? Yes (explain) (max. 250 words): This project has provided novel insights into the gene expression of PCDH1 in the airway epithelium with the confirmation of the expression of isoform 1 and 2 and the discovery of a novel isoform 3 in mouse and man. We also show that human *PCDH1* and mouse *Pcdh1* are highly homologous, making the mouse a good model to study PCDH1 function. Moreover, we describe how PCDH1 expression is upregulated during epithelial differentiation and downregulated upon cigarette smoke exposure in a mouse model. We show that PCDH1 has a dual role: It contributes to epithelial barrier function and repair, but also plays a role in TGFβ induced SMAD3 signalling and may therefore affect remodelling of the airways. Moreover, novel interaction partners have been found, that include regulators of the NF-κB pathway, the eIF3/eIF5B cap-independent translation initiation complex and the complement pathway, which all will be subject of validation studies in our laboratory. Several questions, however, are still not fully answered: we found relatively weak expression Quantitative Trait Locus effects of *PCDH1* gene variants in airway epithelium and therefore extended our analysis to a potential regulatory effect of these genetic variants on gene methylation. Moreover, since expression of the transmembrane isoforms of PCDH1 in airway wall biopsies was not found to be different between asthma and healthy controls, the specific isoform that affects its function, as well as the subgroup of asthmatics for which PCDH1 dysfunction is important remains to be elucidated. This will be subject of further investigation in our laboratory.
3	**Papers (see instructions)**
3.1	All publications (published or submitted peer-reviewed manuscripts): Koning H, Sayers I, Stewart CE, de Jong D, Ten Hacken NH, Postma DS, van Oosterhout AJ, Nawijn MC, Koppelman GH. Characterization of protocadherin-1 expression in primary bronchial epithelial cells: association with epithelial cell differentiation. FASEB J. 2012 Jan;26(1):439-48 Koning H, van Oosterhout AJ, Brouwer U, den Boef LE, Gras R, Reinders-Luinge M, Brandsma CA, van der Toorn M, Hylkema MN, Willemse BW, Sayers I, Koppelman GH, Nawijn MC. Mouse protocadherin-1 gene expression is regulated by cigarette smoke exposure in vivo. PLoS One. 2014 Jul 3;9(7):e98197. Faura Tellez G, Nawijn MC, Koppelman GH. Protocadherin-1: Epithelial barrier dysfunction in asthma and eczema. Eur Respir J. 2014 Mar;43(3):671-4 Faura Tellez G, Vandepoele K, Brouwer U, Koning H, Elderman RM, Hackett TL, Willemse BW, Holloway J, Van Roy F, Koppelman GH, Nawijn MC. Protocadherin-1 binds to SMAD3 and suppresses TGF-β1-induced gene transcription. Am J Physiol Lung Cell Mol Physiol. 2015 Oct 1;309(7):L725-35. Faura Tellez G, Willemse BW, Brouwer U, Nijboer-Brinksma S, Vandepoele K, Noordhoek JA, Heijink I, de Vries M, Smithers NP, Postma DS, Timens W, Wiffen L, van Roy F,

	Holloway JW, Lackie PM, Nawijn MC, Koppelman GH. Protocadherin-1 Localization and Cell-Adhesion Function in Airway Epithelial Cells in Asthma. PLoS One. 2016 Oct 4;11(10):e0163967 Dissertation: Characterization and regulation of Protocadherin-1: a novel gene for asthma. H. Koning 21-11-2012. Thesis, Rijksuniversiteit Groningen.
3.2	All publications (not peer-reviewed like abstracts, newspapers, websites, etc.): *Abstracts:* Regulation Of Protocadherin-1 Expression In Mouse Lung And Experimental Asthma Henk Koning, Uilke Brouwer, Machteld N. Hylkema, Dirkje S. Postma, Antoon van Oosterhout, Gerard Koppelman, Martijn C. Nawijn. American Journal of Respiratory and Critical Care Medicine 2011;183:A1347. Poster presentation ATS 2011 Airway epithelial protocadherin-1 expression is regulated by house-dust mite and cigarette smoke exposure in mice. Martijn Nawijn, Henk Koning, Uilke Brouwer, Lisette den Boef, Machteld Hylkema, Dirkje Postma, Gerard Koppelman, Antoon van Oosterhout. European Respiratory Journal Sep 2011, 38 (Suppl 55) p770. Poster presentation ERS 2011 Expression Of Protocadherin-1, A Novel Asthma Susceptibility Gene, Is Regulated By Cigarette Smoke Exposure In Vivo: A Role For CpG Methylation? Martijn C. Nawijn, Henk Koning, Uilke Brouwer, Lisette E. den Boef, Antoon J.M. van Oosterhout, Gerard H. Koppelman. American Journal of Respiratory and Critical Care Medicine 2012;185:A3491. Poster presentation ATS 2012 Localisation of protocadherin-1 splice variants in airway epithelial cells. Tellez, G. Faura; Koppelman, G. H.; Holloway, J.W.; et al. CLINICAL AND EXPERIMENTAL ALLERGY. Volume: 42 Issue: 12 Pages: 1824-1825 Published: DEC 2012 Protocadherin-1 binds to SMAD3 in airway epithelial cells and regulates sensitivity to TGF-ß: A novel pathway to asthma? Martijn C Nawijn, Uilke Brouwer, Henk Koning, Brigitte WM Willemse, Antoon JM van Oosterhout en Gerard H Koppelman. American Journal of Respiratory and Critical Care Medicine 2013;187:A3563. Poster Presentation ATS 2013 Protocadherin-1: Localization In Airway Epithelial Cells. Grissel Faura Tellez, Uilke Brouwer, Brigitte W.M. Willemse, Martijn C. Nawijn, Peter Lackie, John W. Holloway, and Gerard H. Koppelman American Journal of Respiratory and Critical Care Medicine 2014;189:A4894. Poster presentation ATS 2014 PCDH1: Localization and cell-adhesion in airway epithelium in asthma. Grissel Faura Tellez, Uilke Brouwer, Brigitte Willemse, Jacobien Noordhoek, Irene Hejink, Maaike Vries de, Natalie Smithers, Dirkje Postma, Wim Timens, Laura Wiffen, John Holloway, Peter Lackie, Martijn Nawijn, Gerard Koppelman. European Respiratory Journal Sep 2015, 46 (suppl 59) PA920; Poster presentation ERS 2015. *Poster Presentation* NRS Nationale Longdagen, April 2013, Utrecht, the Netherlands. Protocadherin-1 regulates airway epithelial cell sensitivity to TGF-ß by binding to SMAD3 A novel pathway to asthma? Martijn C Nawijn, Uilke Brouwer, Henk Koning, Brigitte WM Willemse, Antoon JM van Oosterhout en Gerard H Koppelman *News message / General public / Websites*: 2012: RuG news on the thesis of Henk Koning: http://www.rug.nl/news/2012/11/39_koning?lang=en

	- Nationale Longdagen. Van astma-gen naar geen astma. Koppelman GH. 24 April 2014 (invited lecture for the general public). - Medische publieksacademie UMCG. Koppelman GH. Astma bij kinderen. Publiekslezing. Maart 2015. https://www.umcg.nl/NL/UMCG/medische_publieksacademie/eerdere%20jaargangen%20mpa/Paginas/Astma-bij-kinderen.aspx. Zie ook : http://www.dvhn.nl/archief/'Vroeger-hingik-aan-de-kapstok-20854874.html - Publieksvoorlichting voor kinderen: Website en Filmpje op Kennis in zicht, publieksvoorlichting UMCG. https://kennisinzicht.umcg.nl/Paginas/ademen-door-een-rietje.aspx
4.	**Implementation (see instructions)**: In this project, we validated the mouse *Pcdh1* as a good model for human *PCDH1*, given its high homology. We developed a Pcdh1 mouse model, that exhibits AHR, validating our observations in human. We believe that this mouse model is an important breakthrough in fundamental research in asthma, since it is generated based on a genetic cause of AHR found in man. Follow up grant support from the lung foundation to study the inception of asthma was obtained (Lung Foundation Consorium grant together with Prof L Bont, UMCU and Prof B Lambrecht (Ghent University). Moreover, two additional grants were obtained from the Tetri foundation on PCDH1 function. We have discussed with an international pharmaceutical company if PCDH1 is druggable. Given its known function as an adhesion molecule (and thus not a classical drug target), this has not received priority for further testing in their pipeline. However, we interpret the interaction with SMAD3 as a novel clue for intervention, since this may be a method to start treating TGF-β induced remodelling in the airways using the interaction domains of PCDH1. We will first validate this idea in our *Pcdh1* knockout model.

Ondertekening	
Datum: 12 juli 2017	Handtekening aanvrager: