

Accepted Manuscript

Functional genomics of CDHR3 confirms its role in HRV-C infection and childhood asthma exacerbations

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PII: S0091-6749(19)30418-X

DOI: <https://doi.org/10.1016/j.jaci.2019.01.052>

Reference: YMAI 13946

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 16 August 2018

Revised Date: 30 December 2018

Accepted Date: 30 January 2019

Please cite this article as: Everman JL, Sajuthi S, Saef B, Rios C, Stoner AM, Numata M, Hu D, Eng C, Oh S, Rodriguez-Santana J, Vladar EK, Voelker DR, Burchard EG, Seibold MA, Functional genomics of CDHR3 confirms its role in HRV-C infection and childhood asthma exacerbations, *Journal of Allergy and Clinical Immunology* (2019), doi: <https://doi.org/10.1016/j.jaci.2019.01.052>.

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Functional genomics of CDHR3 confirms its role in HRV-C infection and childhood asthma exacerbations

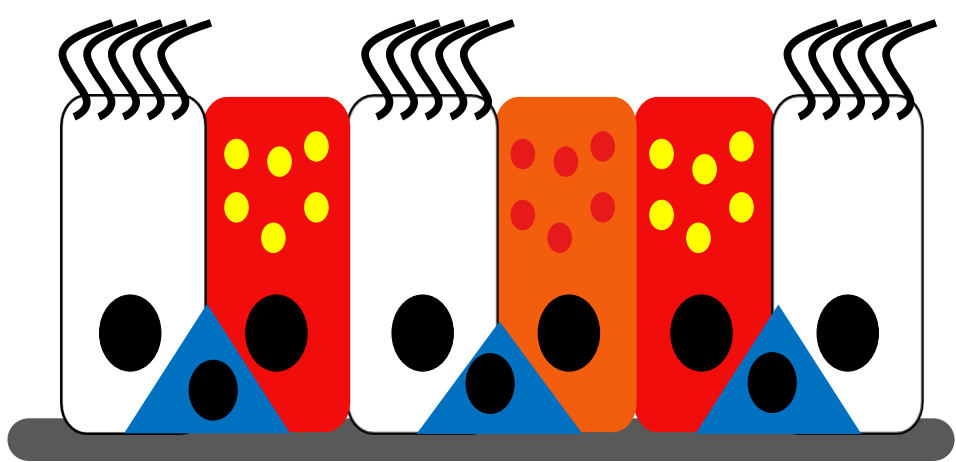
Role of CDHR3 in HRV-C15 infection of differentiated primary airway epithelium

CDHR3⁺ Ciliated Cells



↑ CDHR3
↑ HRV-C15 Infection

CDHR3 CRISPR Knockout Cells



↓ CDHR3
↓ HRV-C15 Infection

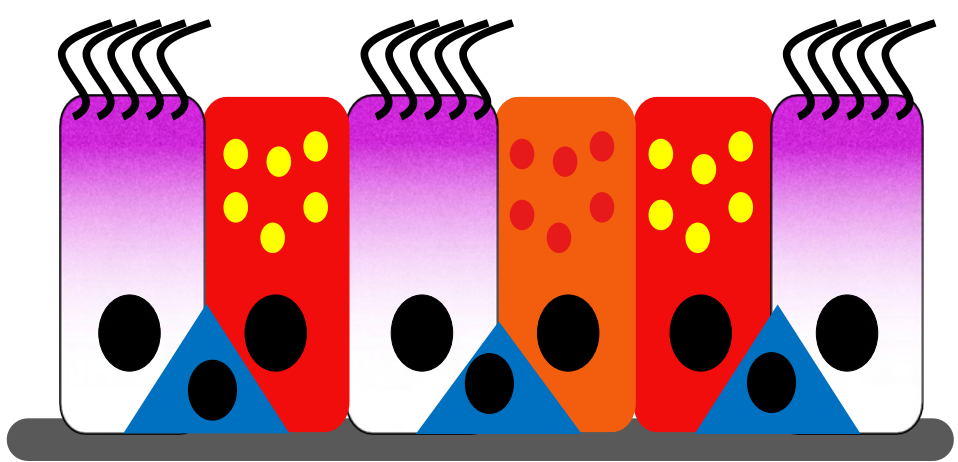
Coding variant association with *CDHR3* gene expression, HRV-C15 infection, and asthma hospitalization

CDHR3 rs6967330 [GG]



↑ *CDHR3* gene expression
↑ HRV-C15 Infection

CDHR3 rs6967330 [AA]



↑↑↑ HRV-C15 Infection
↑↑↑ *CDHR3* gene expression
↑ Asthma-induced hospitalization in minority children

Abbreviations

CDHR3: Cadherin-related family member 3; **HRV-C15**: Human Rhinovirus species C15

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Key Message

- Genetic deletion of CDHR3 in human mucociliary epithelial cultures strongly support involvement of CDHR3 in HRV-C infection of human airway epithelial cells .
- A CDHR3 coding SNP (rs6967330) increases HRV-C infection of airway epithelial cells and is associated with asthma hospitalization in minority children.

Capsule Summary

CRISPR-Cas9 studies of airway epithelial cells (AECs) confirm a role for CDHR3 in HRV-C infection and transepithelial resistance. A CDHR3 coding variant increases HRV-C infection and risk for asthma hospitalization in minority children.

Funding Sources:

This work was supported by the R01 HL135156, R01 MD010443, R01 HL128439, P01 HL132821, P01 HL107202 and U01 HL138626.

Conflict of Interest

MAS reports grants from the NIH during the conduct of the study, and grants from Medimmune, Department of Defense, Pfizer, Genentech outside the submitted work, Patent invention "Transcriptomic response of airway epithelial cells to IL-13", in process: File No. 2879-190-PROV-1 pending, Patent invention "Methods of diagnosing and treating subjects at risk of inflammation and/or exacerbation of a respiratory disease or condition", in process: File No. 2879-191-PROV-1 pending,

and invited lecture at Genentech. MAS and JLE report Patent invention “Methods of Identifying and treating subjects having inflammatory subphenotypes on asthma”, in process: File No. 2879-178-PROV pending. MN and DRV report grants from NIH and FAMRI during the conduct of this study focused upon mechanisms of anti-viral and anti-inflammatory actions of phospholipids, and are co-inventors of the Patent invention “Surfactant lipids, compositions thereof, and uses thereof” owned by National Jewish Health, 1400 Jackson St., Denver, CO 80206. The remaining authors report no relevant conflicts of interest for this study.

ABSTRACT

BACKGROUND: Research in transformed, immortalized cell lines indicates the cadherin-related family member 3 (CDHR3) protein serves as a receptor for human rhinovirus C (HRV-C). Similar experiments indicate the *CDHR3* coding variant, rs6967330, increases CDHR3 protein surface expression.

OBJECTIVE: To determine if CDHR3 is necessary for HRV-C infection of primary airway epithelial cells (AECs), and to identify molecular mechanisms by which CDHR3 variants confer risk for asthma exacerbations.

METHODS: CDHR3 function and influence on HRV-C infection was investigated by single cell transcriptomics, CRISPR-Cas9 gene knockout, and genotype-specific donor experiments performed in primary AECs. Nasal airway epithelium cis-eQTL analysis of *CDHR3* was performed followed by association testing for asthma hospitalization in minority children.

RESULTS: CDHR3 lung expression is exclusive to ciliated AECs and is associated with basal bodies during and after motile ciliogenesis. Knockout of CDHR3 in human AECs did not prevent ciliated cell differentiation but was associated with a decrease in transepithelial resistance, and an 80% decrease in HRV-C infection of the mucociliary epithelium. AECs from subjects homozygous for the risk-associated rs6967330 SNP exhibited greater HRV-C infection compared to cells homozygous for the non-risk allele. AEC cis-eQTL analysis indicated rs6967330 and other SNPs are eQTLs for *CDHR3*. Only the eQTL block containing the rs6967330 SNP exhibited a significant association with childhood asthma hospitalization.

CONCLUSIONS: Genetic deletion and genotype-specific studies in primary AECs indicate CDHR3 is critical to HRV-C infection of ciliated cells. The rs6967330 SNP confers risk of severe childhood asthma exacerbations likely through increasing HRV-C infection levels and protein surface localization.

Key Words

airway epithelium, CRISPR, rhinovirus, eQTL

Abbreviations

ALI – Air-Liquid Interface

KO – knockout

AEC – airway epithelial cell

GALA II – Genes-environments and Admixture in Latino Americans II

HRV – human rhinovirus

CDHR3 – cadherin-like family member 3

eQTL – expression Quantitative Trait Loci

ACT – acetylated alpha-Tubulin

TEER – transepithelial electrical resistance

GWAS – Genome Wide Association Study

SNP – Single Nucleotide Polymorphism

LD – Linkage Disequilibrium

RNA-seq – RNA sequencing

INTRODUCTION

Asthma is the most common chronic disease of childhood, with severe exacerbations representing the cause of most patient burden and associated economic costs (1, 2). Asthma exacerbations are the result of complex interactions between environmental exposures and genetic predisposition, which are poorly understood. The environmental factor most commonly associated with asthma exacerbations is viral upper respiratory tract infections, occurring in 80% of exacerbations (3). Specifically, human rhinovirus species are found in >60% of these virus-associated exacerbations (4). Multiple studies have established infection with HRV-A is much more strongly associated with asthma exacerbations than infection with HRV-B (5-7). More recently, a third species, HRV-C, was discovered which several studies have found to be even more strongly associated with severe asthma exacerbations than HRV-A infections (8, 9).

The viral entry receptor for the majority of HRV-A and all HRV-B serotypes is the intercellular adhesion molecule 1 (ICAM-1), and the low density lipoprotein receptor (LDLR) acts as the receptor for some HRV-A serotypes (10, 11). Structure binding studies revealed HRV-C does not use either ICAM-1 or LDLR as its entry receptor (12). Rather, a recent genomic study identified a set of genes that are differentially expressed between HRV-C susceptible versus non-susceptible cell/tissue types. Heterologous expression of these genes in HeLa cells (a non-susceptible cell type) revealed that the expression of the cadherin-related family member 3 (CDHR3) allowed viral entry and replication in these cells, providing strong evidence that CDHR3 serves as the receptor for HRV-C (13). Moreover, although CDHR3 expression has been confirmed in the human airway epithelium, specifically in ciliated cells cultured *in vitro* (14), it is unclear if other cells in the lung express CDHR3. Supporting the possible

expression in other lung cell types, a recent murine study described the mouse *CDHR3* ortholog as a marker of an alveolar progenitor cell type (15).

A genome-wide scan for severe childhood asthma exacerbations identified *CDHR3* among four genome-wide significant loci (16). The associated SNP, rs6967330, is a *CDHR3* coding variant that results in a cysteine to tyrosine amino acid substitution at position 529 in the amino acid sequence. Heterologous expression of these allelic forms of *CDHR3* in HeLa and HEK293T cells indicated that the asthma risk associated allelic form exhibited higher surface expression than the non-risk allelic form (13, 16). Taken together these data support a model where the rs6967330 variant increases asthma exacerbation risk by increasing surface expression of the *CDHR3* protein and thus risk and possibly level of an HRV-C respiratory infection and illness. Several genetic studies have now associated the rs6967330 SNP with risk of asthma-related illnesses, including in Danish and Japanese patient cohorts (16-19).

Despite these findings many questions remain including: (1) whether lung cells other than ciliated cells express *CDHR3*, (2) if, as a cadherin-like protein, *CDHR3* plays a role in cell adhesion or other ciliated cell functions, (3) if perturbation of *CDHR3* expression in human AECs modulates HRV-C infection levels, (4) whether the rs6967330 variant of *CDHR3* modifies HRV-C infection in AECs, and (5) whether rs6967330 or other cis-variants function as *CDHR3* expression quantitative trait loci (eQTL) and modify risk for childhood asthma exacerbations. Here, we use *CDHR3* CRISPR-Cas9 edited and *CDHR3* risk genotype-specific primary AECs for functional experiments, as well as a comprehensive *CDHR3* nasal airway epithelial eQTL analysis, and genetic association analysis for *CDHR3* functional variants with childhood asthma exacerbations in order to answer these questions.

ACCEPTED MANUSCRIPT

METHODS

Human Subject Information

Human lung cells for single cell RNA-sequencing and tracheal airway epithelial were isolated from de-identified lung donors whose lungs were not suitable for transplantation were obtained from International Institute for the Advancement of Medicine (Edison, NJ), and Donor Alliance of Colorado. The National Jewish Health Institutional Review Board (IRB) approved the research on lung cells under IRB protocol HS-3209. The tracheal airway epithelial cells were obtained in a de-identified fashion from the National Jewish Health (NJH) live cell core. The NJH Live Cell Core is an institutional review board-approved study (HS-2240) for the collection of tissues from consented patients for researchers at NJH. Nasal airway epithelial cells for culture and the eQTL study came from subjects recruited as part of the Genes-environments and Admixture in Latino Americans II (GALA II) childhood asthma cohort, which was approved by local institutional review boards (UCSF, IRB number 10-00889, Reference number 153543, NJH HS-2627). All subjects and their parents provided written informed assent and written informed consent, respectively (20, 21). Demographic and clinical variables for tissue donors used in this study are listed for all lung, tracheal, and nasal samples (Table E1) and for subjects in the genetic eQTL analysis (Table E2).

Single-Cell RNA Sequencing and Analysis

Single cell suspensions of elastase digested lung tissue was obtained as previously described (22). Cells were dispensed and imaged using the ICELL8™ Single-Cell System and samples sequenced with the Illumina HiSeq® 2500 System.

Culture of Primary Tracheal and Nasal Airway Epithelial Cells

Primary human tracheal and nasal AECs were expanded and differentiated at air-liquid interface (ALI) *in vitro* (23-26), and intact and dissociated cultures were harvested for Western blot, flow cytometry, immunofluorescence, and gene expression analyses (26).

Lentiviral CRISPR-Cas9 Gene Editing of Airway Basal Cells

The design of the CRISPR targeting guide sequences, addition of adaptors and cloning into the lentiCRISPR plasmid backbone, propagation and titration of lentivirus, and AEC transduction and selection were performed as previously described (23, 25).

Human Rhinovirus Infection

HRV-C15 virus was propagated using HeLa-E8 cells as previously described (12, 13).

Cultures were infected with virus at an MOI of 0.2 for 4 hours at 34°C, washed, and incubated for a total of 48 hours at 34°C prior to harvest.

Whole Transcriptome Analysis, Genotyping, and eQTL Analysis

Whole transcriptome libraries of 695 nasal brushes from the GALA II childhood asthma cohort were constructed using the Beckman Coulter FX^P automation system and samples sequenced using the Illumina HiSeq[®] 2500 system. DNA was genotyped using Affymetrix Axiom LAT 1 (World Array 4) and LAT plus HLA genome-wide arrays. Expression quantitative trait loci (eQTL) analysis followed the Genotype-Tissue Expression (GTEx) project protocol V7 for filtering and analysis (27).

Immunofluorescence Labeling

Intact ALI AEC culture inserts were fixed for 15 minutes at room temperature in 3.2% paraformaldehyde or with ice cold methanol at -20°C. Human lung tissue samples were fixed in 10% formalin prior to paraffin embedding and antibody labeling.

RESULTS

***CDHR3* is exclusively expressed by ciliated airway epithelial cells**

To identify *CDHR3* expressing cells within the human lung, we performed single cell RNA sequencing (RNA-seq) of whole lung cell suspensions. By t-SNE clustering of cells' gene expression we identified 13 distinct cell populations (Figure 1A). Differentially expressed genes between clusters were intersected with known cell type markers to determine the likely cell type identity of each cluster (Table E3). Cell types identified include airway epithelial cell (AEC) types (ciliated and secretory), alveolar type 1 and type 2 cells, lung immune cells, and endothelial cells. *CDHR3* was highly and exclusively expressed in ciliated cells (Figure 1B), which were clearly identified as such by high expression of genes contained in cilia gene ontology (GO) categories (Figure E1). Immunofluorescence labeling of lung airway sections confirmed this result at the protein level as *CDHR3* was only observed in ciliated AECs identified by acetylated alpha-Tubulin (ACT) and not secretory cells that are positive for MUC5AC (Figure 1C-D).

***CDHR3* protein is apically distributed and is most highly expressed in immature ciliated cells**

To study *CDHR3* function we differentiated basal AECs into a mucociliary epithelium using the air liquid interface (ALI) cell culture model. Using time course analysis of *CDHR3* gene and protein expression across mucociliary culture differentiation (Figure 2A), we found *CDHR3* gene expression was evident by ALI day 5 and protein expression by day 6, prior to the appearance of motile cilia at day 11. Gene and protein expression increased until day 18 and were maintained at this level through ALI day 30.

Co-labeling of CDHR3 and the cilia marker ACT in mature cultures (day 21) revealed the expected co-expression of these proteins (Figure 2B). Despite CDHR3 being a cadherin-related protein, it exhibited a punctate cytoplasmic rather than membrane localized staining pattern. Confirming this we co-labeled CDHR3 with E-cadherin, revealing no co-localization of these proteins. YZ-projection of confocal z-stacks revealed that CDHR3 puncta were localized in the cytoplasm, at the apical side of cells (Figure 2C). Given that CDHR3 is expressed early in ciliated cell development we also performed immunofluorescence labeling of day 14 cultures, which contain a mix of ciliating and mature ciliated cells. At ALI day 14, we identified many CDHR3⁺/ACT⁻ cells (32.9% of total cell count; Figure 2D). These cells were rare at day 21 (3.5% of total cell count; Figure 2B), which led us to hypothesize that these were ciliating cells expressing CDHR3 prior to the development of cilia. Moreover, there was an inverse relationship between the mean fluorescent intensity (MFI) of CDHR3 and ACT within double positive cells at day 14 ($r = -0.38$, $p = 0.0002$, Figure 2D). To support the higher expression of CDHR3 in ciliating rather than mature ciliated cells, we co-labeled day 14 cultures for CDHR3 and ezrin, a marker of the apical membrane in mature ciliated cells. The brightest CDHR3 labeled cells were among the cells weakest for ezrin labeling, as reflected by the strong inverse correlation between CDHR3 and ezrin MFI values ($r = -0.47$, $p < 0.0001$, Figure 2E). The YZ-projection images also indicate that CDHR3 does not co-localize with ezrin to the apical membrane, but is localized to the apical sub-membrane compartment of ciliated cells (Figure 2E). To further validate that higher CDHR3 expression is found in ciliating cells in the differentiating AECs, we co-labeled cultures for centrin, a component of the basal body, which forms the intracellular base that anchors the cilium to the apical cell surface. During ciliogenesis, basal bodies are generated in the cytoplasm, traffic to and dock

with the membrane, then elongate the cilium. Centrin has a well-defined pattern of basal body localization within ciliated cells during the course of ciliogenesis, which allows the identification of ciliating vs. mature ciliated cells (28). Image analysis revealed that cells with the strongest CDHR3 pattern co-expressed with centrin puncta in the cytoplasm indicative of ciliating cells with replicating or trafficking basal bodies. Alternatively, cells exhibiting centrin puncta evenly distributed at the apical surface to indicate the docked basal bodies of mature ciliated cells, exhibited dimmer expression of CDHR3 (Figure 2F).

CDHR3 gene knockout does not affect ciliated cell differentiation but results in reduced transepithelial membrane resistance

To investigate CDHR3 function in the airway epithelium we generated tracheal basal AECs knocked out for the *CDHR3* gene using our lentiviral mediated CRISPR-Cas9 gene editing system (n=3 donors) (23, 25). The CDHR3 edited cells (CDHR3 KO) appeared normal and proliferated in culture similarly to the control edited basal cells (control). To assess whether the edited cells exhibited a loss of CDHR3 protein we differentiated the CDHR3 KO and control basal cells at ALI for 21+ days and performed quantitative Western blot analysis. We found the ALI cultures generated from CDHR3 KO cells exhibited 94.57% lower CDHR3 protein expression (Figure 3A, B; SD +/- 4.18%; p<0.0001) compared to cultures generated from control cells. Immunofluorescent labeling of cytopins prepared from differentiated ALI cultures also showed a marked decrease in CDHR3 protein within ACT⁺ ciliated cells (Figure 3C), indicating a substantial knockdown of CDHR3.

Given the robust early ciliogenesis expression of CDHR3 we first wanted to determine if loss of CDHR3 expression affects the differentiation of ciliated cells. To address this, we

performed flow cytometry analysis for CDHR3 and ACT in cells isolated from mature ALI cultures differentiated from CDHR3 KO and control basal cells (Figure 3D, E). We found $12.9\% \pm 1.43$ of the control cells were $ACT^+/CDHR3^+$ versus only $2.34\% \pm 0.40$ of the CDHR3 KO cells, an $81.67\% \pm 2.81$ decrease with CDHR3 knockout (Figure 3E; $p = 0.0140$). In the CDHR3 KO cells we observed a corresponding $85.22\% \pm 1.33$ increase in $ACT^+/CDHR3^-$ cells, which represented $16.43 \pm 5.95\%$ of all cells versus only 2.65 ± 1.17 of all cells in the control population (Figure 3E; $p = 0.0032$). Importantly, we found the total percentage of ciliated cells (ACT^+) was unchanged by CDHR3 knockout (KO = $18.8 \pm 1.4\%$, Control = $15.6 \pm 1.2\%$, $p = 0.7531$; Figure 3E). Additionally, we performed RNA-seq analysis of ALI differentiated control and KO edited cells from these 3 donors to determine whether expression of gene markers of ciliated cells and transcription factor drivers of ciliogenesis were affected by CDHR3 KO (Table E4). Supporting our flow cytometry data expression of *FOXJ1*, *RFX2*, *RFX3*, *MYB*, *E2F4*, and *TP73* were not differentially expressed in the KO cells, despite significant downregulation of *CDHR3* expression ($p^{\text{adj}} = 4.6 \times 10^{-18}$, Table E4). Further quantification of immunofluorescence labeling of *FOXJ1*⁺ nuclei in ALI cultures demonstrated that CDHR3 KO does not affect the expression of *FOXJ1* or the ciliated cell fate of the airway epithelial cultures during differentiation (Figure E2). Moreover, club secretory and mucus secretory marker genes were unaffected by *CDHR3* KO, suggesting loss of CDHR3 function has no effect on secretory cell formation. We did however find that among the 27 differentially expressed genes in CDHR3 KO cells (5% FDR and log2 fold change of >0.5 or <-0.5) that the basal cell marker genes *KRT5*, *KRT14*, *NGFR* were all upregulated, supporting that loss of CDHR3 either increases basal frequency or basal cell marker gene expression (Table E4).

Although we did not detect junctional localization, as a cadherin-related protein we speculated that CDHR3 KO may impact apical junctional integrity. We evaluated transepithelial electrical resistance (TEER) in AECs and observed that it was reduced in the CDHR3 KO cultures. We found that immature CDHR3 KO cultures (day 14), exhibited a 12.56% decrease in TEER compared to control cultures (Figure 3F; $p < 0.0001$). Likewise, we found that mature differentiated ALI cultures from CDHR3 KO cells exhibited a 12.11% decrease in transepithelial resistance as compared to control cultures (Figure 3F; $p < 0.0001$). These data indicate that CRISPR-Cas9 knockout of CDHR3 in primary AECs does not affect the development or differentiation of ciliated cells in the airway epithelium. However, the effect of CDHR3 knockout on epithelial TEER indicates a potential effect on barrier function, ion transport, or other epithelial organization despite a lack of exclusive CDHR3 junctional localization.

Deficiency in CDHR3 protein downregulates HRV-C15 infection of AECs

Our CDHR3 KO AECs allowed us to directly test whether HRV-C infection is dependent on CDHR3 protein expression in well-differentiated ALI-cultured human AECs. We examined both viral binding at four hours post-apical infection and infection at 48 hours by qPCR. We found no difference in HRV-C15 viral binding between control and KO cells ($p = 0.1836$; data not shown). However, at 48 hours post-infection, the CDHR3 KO epithelium exhibited 80.42% lower HRV-C15 RNA levels compared to control infected cultures (Figure 3G; SD $\pm$ 2.30%; $p < 0.0001$). To test if this effect on HRV-C15 infection was specific to the Rhinovirus C species, we performed the same experiment on CDHR3 KO cells with the HRV-A16 virus. We found no difference in the HRV-A16 RNA levels between control and CDHR3 KO infected cultures (Figure 3G; $p = 0.9505$).

CDHR3 rs6967330 genotype is associated with increased HRV-C infection of AECs

Our CDHR3 knockout experiments are supportive of CDHR3 levels influencing HRV-C infection. The CDHR3 coding risk variant rs6967330 has been shown to increase CDHR3 protein surface localization when allelic forms of the protein are expressed heterologously in HeLa and 293T cells (13, 16). Therefore, we hypothesized that donor AECs natively expressing the CDHR3 variant (A) allele would exhibit higher HRV-C infection levels than donor AECs expressing the alternate CDHR3 (G) allele. To test this hypothesis, we compared HRV-C15 infection levels between nasal AEC ALI cultures generated from six pediatric donors homozygous for the G allele (poorer surface localization) versus six donors homozygous for the A allele (better surface localization). Following HRV-C15 infection, we found a 4.3-fold increase in viral RNA isolated from the AA donor cells as compared to the GG donors at four hours post-infection (Figure 4; $p = 0.0021$). Likewise, at 48 hours we observed a 7.5-fold higher infection level in the AA versus GG subjects (Figure 4; $p = 0.0390$). These results show for the first time in primary human AECs differentiated from donors with different alleles for the CDHR3 SNP rs6967330, that carriage of the homozygous [A] genotype increases the risk of HRV-C15 infection at both the early and late stages of rhinovirus infection of the nasal airway epithelium.

The rs6967330 variant is an eQTL associated with asthma hospitalization in minority children

We also considered whether the rs6967330 SNP might function as an expression quantitative trait loci (eQTL) in the airway epithelium to increase *CDHR3* expression levels and thus susceptibility to HRV-C infection. To investigate this, we extracted *CDHR3* eQTL data from

the ongoing, genome-wide nasal airway epithelial eQTL study of the Genes-environments and Admixture in Latino Americans (GALA II) cohort (unpublished data). In this study we are using whole transcriptome sequencing expression data on nasal brushings from 431 asthmatic and 242 healthy control children, paired with genome-wide genotype data to perform a cis-eQTL analysis. We tested all genic SNPs and those 1Mb up- and down-stream of the gene for association with *CDHR3* expression. We identified 11 independent eQTL loci for the *CDHR3* gene (Table E5). A locus zoom plot, reveals the gene localization and linkage disequilibrium (LD) structure for the top four loci, all with association p values of $<1 \times 10^{-10}$ (Figure 5A). The *CDHR3* eQTL SNP with the fourth strongest effect was part of an LD block which also included the rs6967330 coding risk SNP. We found the rs6967330 A allele, which we found was associated with increased HRV-C infection in primary AECs, was also associated with higher *CDHR3* expression levels (Figure 5B; $p = 2.16 \times 10^{-9}$). In fact, we found there was a linear relationship between the number of variant alleles (higher expressed allele) for these top four eQTL SNPs a subject carried and *CDHR3* gene expression levels (Figure 5C; $p = 2.86 \times 10^{-81}$). We tested the top four eQTL SNPs, as well as a variable for the total number of risk alleles carried, for association with asthma hospitalization risk in 670 Mexicans, 1153 Puerto Ricans, and 420 other Latino children with asthma by meta-analysis. We found only the rs73195680 SNP, located in the LD block with the rs6967330 loci, and rs6967330 itself were associated with asthma hospitalization risk ($p = 5.0 \times 10^{-3}$, 7.0×10^{-3} respectively, Table E6). These results reveal strong genetic regulation of the *CDHR3* gene expression, but that only the rs6967330 coding variant LD block confers greater risk of asthma hospitalization in minority children.

DISCUSSION

The translation of novel asthma genetics findings into better understanding of molecular disease mechanisms has been impeded by a lack of suitable research methodologies in human airway tissue and primary cells. We applied a series of novel approaches to human airway epithelial cells including single cell transcriptomics, CRISPR gene editing, genotype-specific primary tissue culture, and eQTL studies to better understand the role of *CDHR3* in viral induced asthma exacerbations.

Our single cell transcriptome data identifying most cell types in the human lung confirm a prior report of *CDHR3* expression being restricted to the ciliated cells within airway epithelium (14). A recent report demonstrated that the mouse *CDHR3* ortholog is expressed in a subset of alveolar cells, however, we did not detect *CDHR3* in alveolar cells, thus this is likely not a conserved pattern of expression (15). As *CDHR3* has been shown to be necessary for HRV-C infection, our data firmly establishes the tropism of HRV-C exclusively to ciliated cells in the human lung.

We leveraged the power of CRISPR-Cas9 technology to generate *CDHR3* KO AECs. While KO did not abolish HRV-C infection, it was reduced by 80%, strongly supporting a significant role for *CDHR3* in HRV-C infection. Surprisingly, we did not observe an effect of the KO on viral binding (although we did observe an effect of the *CDHR3* variant risk on HRV-C binding). This result could be related to the timing or technical limitations of the binding assay, as well as higher sensitivity of the binding assay to incomplete KO. However, the reduced but measurable level of HRV-C infection we observed may also suggest the existence of co-receptors or weak alternative receptors among the mature mucociliary

epithelium and/or other roles for CDHR3 in HRV-C infection including viral replication. In the future, development of an antibody against the extracellular domain of CDHR3 will allow a more complete investigation of the status of CDHR3 as the exclusive receptor for HRV-C in human AECs. Nonetheless, our CRISPR KO of CDHR3 allowed us to directly determine in primary well-differentiated AECs that robust HRV-C infection is dependent on expression of CDHR3.

As our data strongly support a role for CDHR3 in HRV-C infection of the human mucociliary airway epithelium, we addressed whether the rs6967330 asthma exacerbation risk variant could modulate HRV-C infection in well-differentiated AECs from genotyped donors. Our finding of a 7.5-fold increase in HRV-C infection among AECs derived from donors carrying the AA risk genotype strongly supports increased HRV-C infection as a mechanism for exacerbation risk associated with this variant. A prior study reported the CDHR3 protein carrying the rs6967330 risk allele (AA) results in greater protein surface localization than the non-risk allele (13, 16), providing a possible mechanism for our finding of increased HRV-C infection of AECs differentiated from donors with the AA genotype and consequently exacerbation risk. Unfortunately, the lack of an extracellular CDHR3 antibody has prevented formal confirmation of this increased surface localization of the endogenously expressed risk-associated rs6967330 allele in differentiated AECs.

Given that a large number of complex disease risk variants are regulatory in nature we decided to explore whether rs6967330 or other cis variants function as eQTLs for the *CDHR3* gene. Our comprehensive cis-eQTL analysis of the nasal airway epithelium from >600 children established that *CDHR3* gene expression modulated by genetic variation, with 11

independent eQTLs identified. This analysis allowed us to establish for the first time that the rs6967330 SNP is part of a *CDHR3* eQTL linkage disequilibrium block. Since the risk A allele of the rs6967330 SNP was associated with higher *CDHR3* gene expression, this presented the possibility that eQTL function rather than the previously suggested increase in *CDHR3* protein localization at the cell surface, could explain the increased HRV-C infection and exacerbation risk associated with the rs6967330 variant. If this hypothesis was correct we would have expected the rs6967330 LD block, which was the 4th strongest eQTL loci, and the 3 stronger *CDHR3* cis-eQTLs, to be associated with asthma exacerbation risk. Instead our finding that only the rs6967330 eQTL was associated with asthma hospitalization risk strongly supports an alternative mechanism for the variant risk, likely the reported change in surface localization. Moreover, our association between the rs6967330 variant and asthma-related hospitalization presents the first evidence that this variant confers risk in Hispanic populations. This extends a growing body of data implicating the rs6967330 variant in the risk of asthma exacerbations/hospitalization and related traits including early-life bronchiolitis, early-onset asthma, and chronic rhinosinusitis (16-19, 29).

Monitoring of gene and protein expression throughout AEC differentiation indicated robust *CDHR3* expression prior to the appearance of cilia. Our finding of *CDHR3* localization near the basal bodies in ciliating and mature ciliated cells, raised the possibility of its involvement in ciliogenesis. *CDHR3* KO AECs differentiated at ALI allowed us to conclusively determine that *CDHR3* loss does not block ciliated cell formation. We also localized *CDHR3* to the apical surface in the vicinity of ezrin, a protein that links the apical cytoskeleton to the apical membrane. We speculate that *CDHR3* may also be a component and possibly regulator of this network of actin and microtubules that is in constant contact with cilia, the cell surface,

and the apical cell junctions. Through these interactions, CDHR3 may impact many vital ciliated cell processes, including barrier function, which is supported by the consistent decrease in transmembrane resistance upon CDHR3 knockout. If the reduced transmembrane resistance is mediated by a change in barrier function, this could allow greater penetration of allergen, toxins, and microorganisms in the airway, providing a potential mechanism for CDHR3 function influencing asthma exacerbation risk. Further work will be needed to confirm an effect of CDHR3 on barrier function.

In summary, our work here has provided some of the first data on CDHR3 expression, biology, and molecular genetics in the human airway epithelium. We have revealed the likely mechanism underlying the risk of asthma exacerbations conferred by the CDHR3 rs6967330 variant, while extending this risk to minority children with asthma. These data form the basis for both further human genetics and molecular mechanistic studies of CDHR3 involvement in childhood asthma and asthmatic airway epithelium.

ACKNOWLEDGEMENTS

We would like to thank Shirley Sobus and Josh Loomis at the National Jewish Flow and Microscopy core for guidance with instrument usage and analysis.

References

1. Ivanova JI, Bergman R, Birnbaum HG, Colice GL, Silverman RA, McLaurin K. Effect of asthma exacerbations on health care costs among asthmatic patients with moderate and severe persistent asthma. *J Allergy Clin Immunol*. 2012;129(5):1229-35.
2. Nurmagambetov T, Kuwahara R, Garbe P. The Economic Burden of Asthma in the United States, 2008-2013. *Ann Am Thorac Soc*. 2018;15(3):348-56.
3. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ*. 1995;310(6989):1225-9.
4. Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol*. 1993;31(1):111-7.
5. Khetsuriani N, Lu X, Teague WG, Kazerouni N, Anderson LJ, Erdman DD. Novel human rhinoviruses and exacerbation of asthma in children. *Emerg Infect Dis*. 2008;14(11):1793-6.
6. Zhao M, Zhu WJ, Qian Y, Sun Y, Zhu RN, Deng J, et al. Association of Different Human Rhinovirus Species with Asthma in Children: A Preliminary Study. *Chin Med J (Engl)*. 2016;129(13):1513-8.
7. Chen WJ, Arnold JC, Fairchok MP, Danaher PJ, McDonough EA, Blair PJ, et al. Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults: rhinovirus among adults and children. *J Clin Virol*. 2015;64:74-82.
8. Bizzintino J, Lee WM, Laing IA, Vang F, Pappas T, Zhang G, et al. Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J*. 2011;37(5):1037-42.
9. Miller EK, Khuri-Bulos N, Williams JV, Shehabi AA, Faouri S, Al Jundi I, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. *J Clin Virol*. 2009;46(1):85-9.
10. Greve JM, Davis G, Meyer AM, Forte CP, Yost SC, Marlor CW, et al. The major human rhinovirus receptor is ICAM-1. *Cell*. 1989;56(5):839-47.
11. Hofer F, Gruenberger M, Kowalski H, Machat H, Huettinger M, Kuechler E, et al. Members of the low density lipoprotein receptor family mediate cell entry of a minor-group common cold virus. *Proc Natl Acad Sci U S A*. 1994;91(5):1839-42.

12. Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med*. 2011;17(5):627-32.
13. Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. *Proc Natl Acad Sci U S A*. 2015;112(17):5485-90.
14. Griggs TF, Bochkov YA, Basnet S, Pasic TR, Brockman-Schneider RA, Palmenberg AC, et al. Rhinovirus C targets ciliated airway epithelial cells. *Respir Res*. 2017;18(1):84.
15. Zacharias WJ, Frank DB, Zepp JA, Morley MP, Alkhaleel FA, Kong J, et al. Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. *Nature*. 2018;555(7695):251-5.
16. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet*. 2014;46(1):51-5.
17. Stenberg Hammar K, Niespodziana K, van Hage M, Kere J, Valenta R, Hedlin G, et al. Reduced CDHR3 expression in children wheezing with rhinovirus. *Pediatr Allergy Immunol*. 2018;29(2):200-6.
18. Kanazawa J, Masuko H, Yatagai Y, Sakamoto T, Yamada H, Kaneko Y, et al. Genetic association of the functional CDHR3 genotype with early-onset adult asthma in Japanese populations. *Allergol Int*. 2017;66(4):563-7.
19. Husby A, Pasanen A, Waage J, Sevelsted A, Hodemaekers H, Janssen R, et al. CDHR3 gene variation and childhood bronchiolitis. *J Allergy Clin Immunol*. 2017;140(5):1469-71 e7.
20. Neophytou AM, White MJ, Oh SS, Thakur N, Galanter JM, Nishimura KK, et al. Air Pollution and Lung Function in Minority Youth with Asthma in the GALA II (Genes-Environments and Admixture in Latino Americans) and SAGE II (Study of African Americans, Asthma, Genes, and Environments) Studies. *Am J Respir Crit Care Med*. 2016;193(11):1271-80.
21. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. *Am J Respir Crit Care Med*. 2013;188(3):309-18.
22. Kosmider B, Mason RJ, Bahmed K. Isolation and Characterization of Human Alveolar Type II Cells. *Methods Mol Biol*. 2018;1809:83-90.
23. Chu HW, Rios C, Huang C, Wesolowska-Andersen A, Burchard EG, O'Connor BP, et al. CRISPR-Cas9-mediated gene knockout in primary human airway epithelial cells reveals a proinflammatory role for MUC18. *Gene Ther*. 2015;22(10):822-9.

24. Reynolds SD, Rios C, Wesolowska-Andersen A, Zhuang Y, Pinter M, Happoldt C, et al. Airway Progenitor Clone Formation Is Enhanced by Y-27632-Dependent Changes in the Transcriptome. *Am J Respir Cell Mol Biol*. 2016;55(3):323-36.
25. Everman JL, Rios C, Seibold MA. Primary Airway Epithelial Cell Gene Editing Using CRISPR-Cas9. *Methods Mol Biol*. 2018;1706:267-92.
26. Everman JL, Rios C, Seibold MA. Utilization of Air-Liquid Interface Cultures as an In Vitro Model to Assess Primary Airway Epithelial Cell Responses to the Type 2 Cytokine Interleukin-13. *Methods Mol Biol*. 2018;1799:419-32.
27. Consortium GT, Laboratory DA, Coordinating Center -Analysis Working G, Statistical Methods groups-Analysis Working G, Enhancing Gg, Fund NIHC, et al. Genetic effects on gene expression across human tissues. *Nature*. 2017;550(7675):204-13.
28. Vladar EK, Stearns T. Molecular characterization of centriole assembly in ciliated epithelial cells. *J Cell Biol*. 2007;178(1):31-42.
29. Chang EH, Willis AL, McCrary HC, Noutsios GT, Le CH, Chiu AG, et al. Association between the CDHR3 rs6967330 risk allele and chronic rhinosinusitis. *J Allergy Clin Immunol*. 2017;139(6):1990-2 e2.

FIGURE LEGENDS

Figure 1: Single cell RNA-seq of the human lung reveals *CDHR3* is exclusively expressed in ciliated airway epithelial cells

A and **B**, RNA sequencing of single cells digested from a human lung identifies 13 distinct cell types using t-SNE analysis based on cell-specific gene markers (**A**) and demonstrates that *CDHR3* gene expression is highly specific to ciliated cells in the lung (**B**) (n=1,142 individual cells analyzed from 1 digested lung and representative of single cell data from 6 total human lungs). **C** and **D**, Immunofluorescence labeling of whole lung tissue histology sections illustrates that *CDHR3* localizes to the ciliated cells of the airway epithelium (**C**) but not to the MUC5AC⁺ secretory cells in the *in vivo* airway epithelium (**D**). Left panels – isotype control; Right panels – *CDHR3* antibody. Bars - 50µm; white dotted line designates the

basement membrane of the airway epithelium; image is representative of experiments completed using histology sections collected from 3 lung donors.

Figure 2: CDHR3 exhibits an apical, cytoplasmic expression pattern highest in immature ciliating epithelial cells

Tracheal basal AECs were differentiated *in vitro* at ALI for immunofluorescence labeling. **A**, qPCR and Western blot analyses were completed on cultures harvested over 30 days of differentiation for CDHR3 gene (n=3 donors) and protein expression (n=2 donors). **B** and **C**, ALI day 21 cultures were co-labeled for CDHR3 (green) and either ACT (red) (**B**) or E-cadherin (red) (**C**). Magnified inset panels indicate no co-localization of CDHR3 and E-cadherin at the cell-cell junction (**C** – far right panel). **D** and **E**, ALI day 14 cultures were co-labeled for CDHR3 (green) and either ACT (red) (**D**) or Ezrin (red) (**E**). MFI of each protein was quantified in individual cells in the culture for correlation analysis of intensities of CDHR3 and ACT or Ezrin (n=90 cells total) (**D** and **E** – far right panels). **F**, ALI day 21 cultures were co-labeled for CDHR3 (green) and centrin (red). Single cell panels illustrate the CDHR3 pattern in immature (top) and mature (bottom) cells as indicated by centrin pattern; single cell inset bars – 2µm. YZ projection images illustrate localization of proteins within the apical region of the epithelium (arrow indicates direction toward apical surface). Maximum projection bars - 20µm; images are representative of labeling performed in ALI cultures from 3 donors.

Figure 3: CDHR3 gene knockout decreases epithelium TEER and HRV-C infection

A - E, CDHR3 KO or control (scrb) tracheal basal AECs were differentiated at ALI for 21+ days. Dissociated cultures were harvested for CDHR3 protein expression by Western blot (**A**

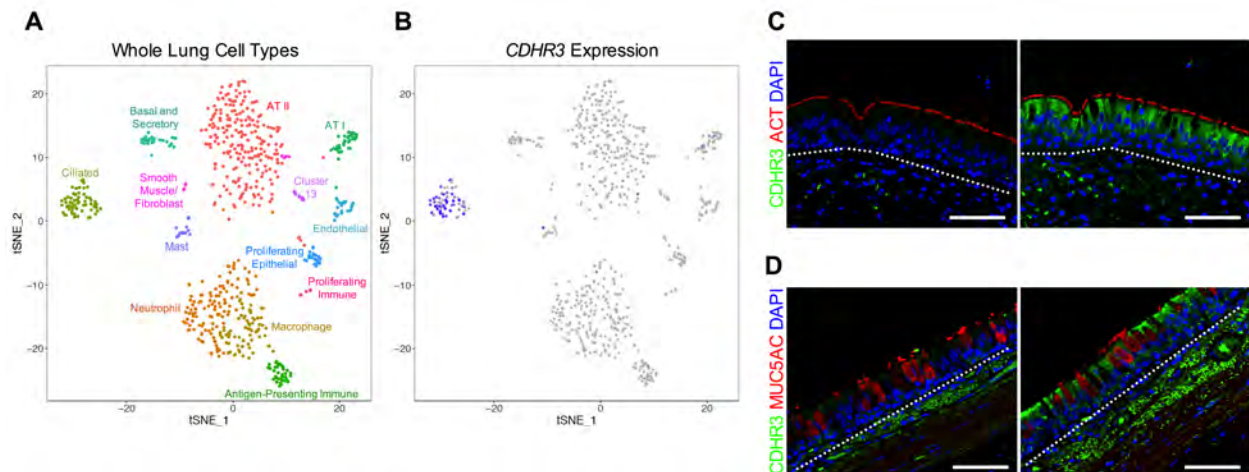
and B), immunofluorescence labeling for CDHR3 (green) and ACT (red), bars – 20µm (C), and flow cytometry analysis (D and E). **F**, Transepithelial electrical resistance (TEER) measurements were taken at day 14 and day 21+ of ALI differentiation. **G**, Cultures were infected with HRV-C15 or HRV-A16 virus at an MOI of 0.2 for a total of 48 hours at 34°C prior to harvest and quantification of viral load by qPCR. Data are representative of CDHR3 KO or control (scrb) cultures grown and analyzed in parallel (n=3 independent donors).

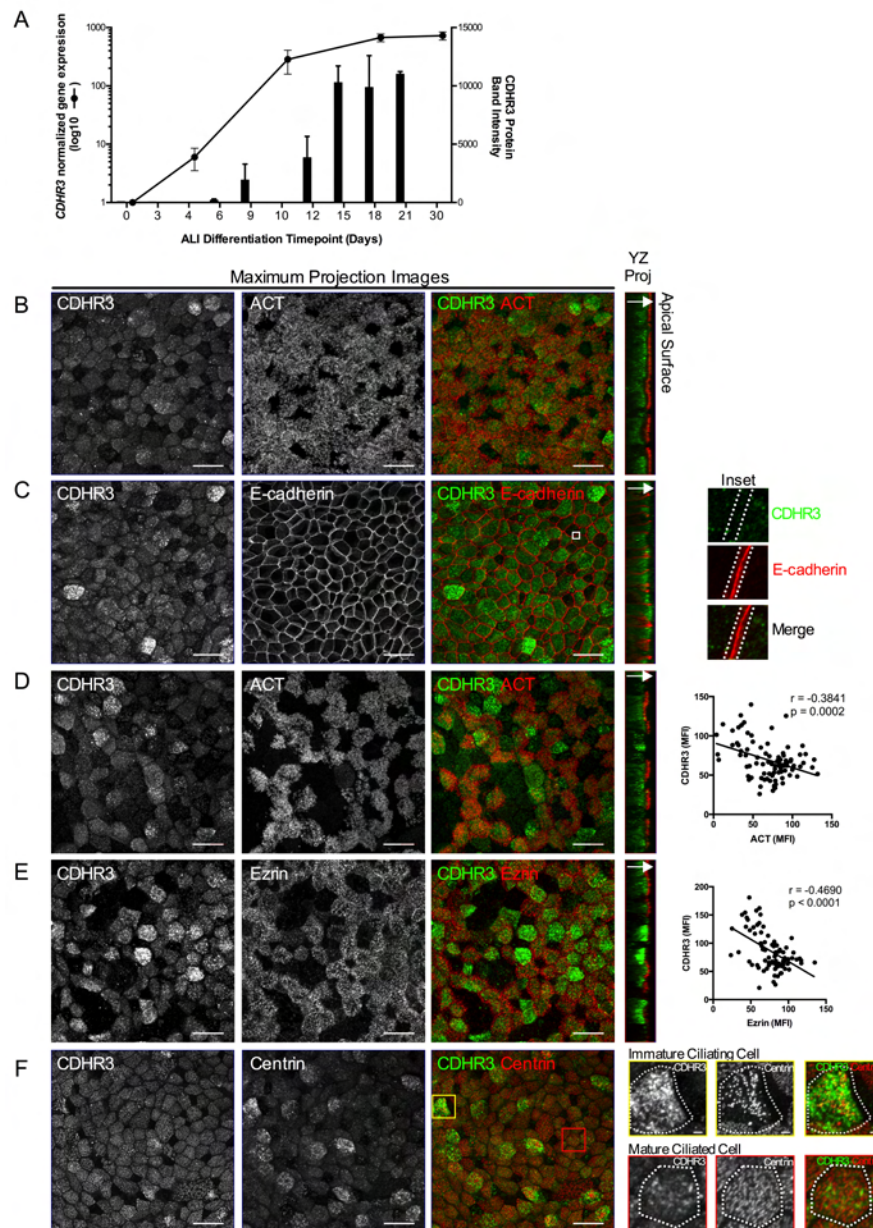
Figure 4: HRV-C15 infection level is higher in AECs expressing the risk-associated CDHR3 rs6967330 genotype

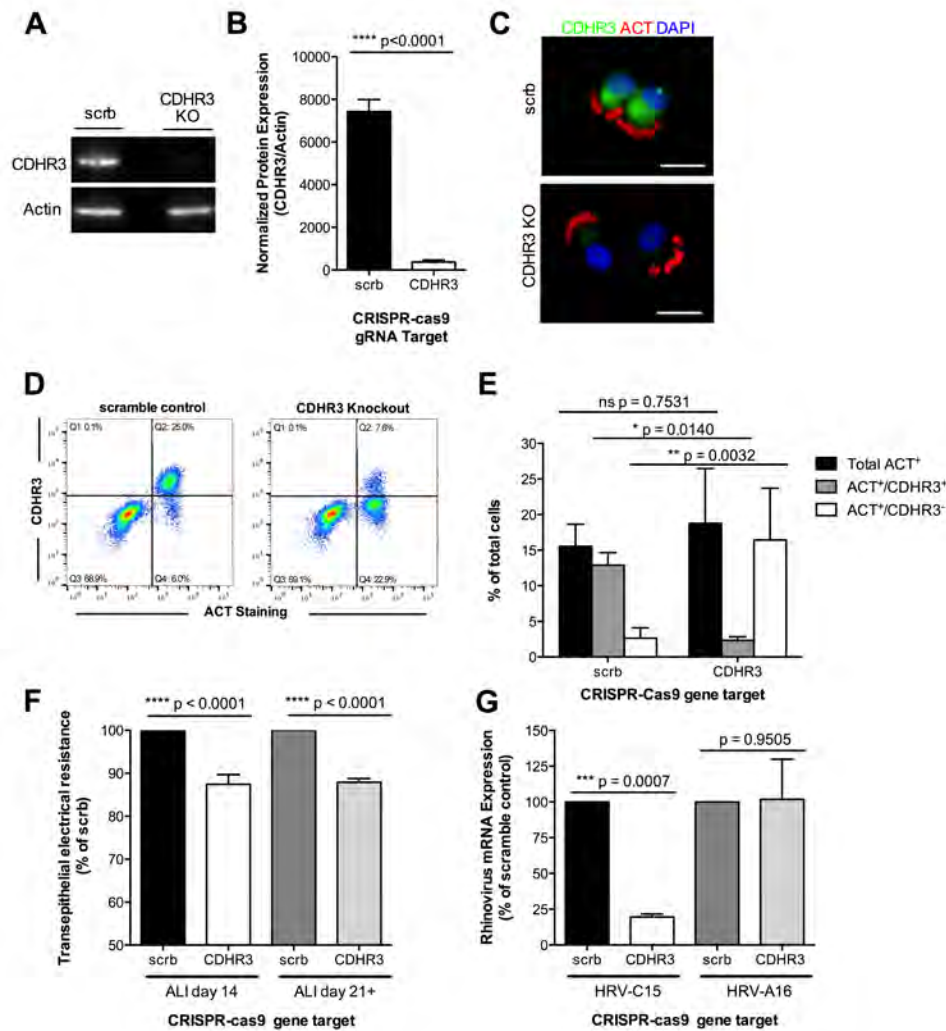
Nasal AECs homozygous for the rs6967330 G allele (n=6) or homozygous for the A allele (n=6) were differentiated at ALI. Cultures were infected with HRV-C15 at a MOI of 0.2 for 4 hours or a total of 48 hours at 34°C prior to quantitation of HRV-C15 by qPCR.

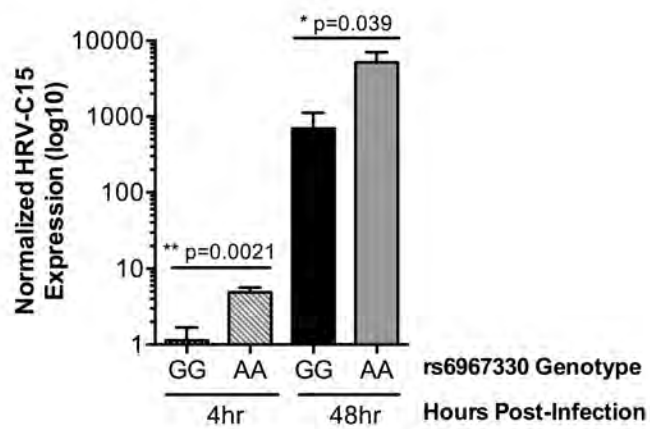
Figure 5: The rs6967330 risk variant and other cis-variants are eQTLs for the *CDHR3* gene in the nasal airway epithelium of children.

A, The top four independent cis-eQTLs for *CDHR3* are depicted in the *CDHR3* regional plot. **B**, The *CDHR3* risk variant rs6967330 acts as an eQTL for *CDHR3* gene expression. **C**, Additive effect of *CDHR3* cis-eQTL variants on *CDHR3* expression. Allele dosage was calculated as the sum of all eQTL associated (higher expression) effect alleles across the top four independent *CDHR3* cis-eQTL variants.

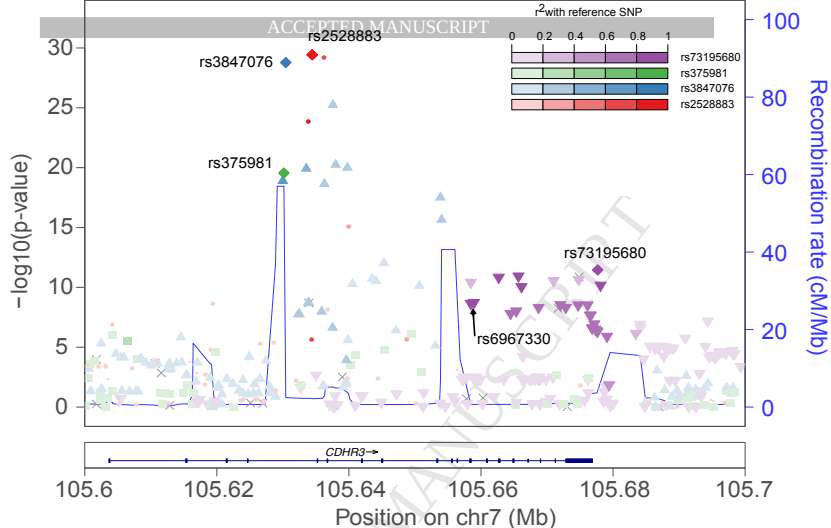




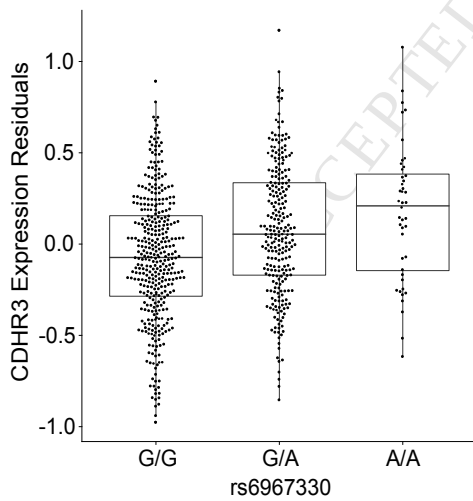




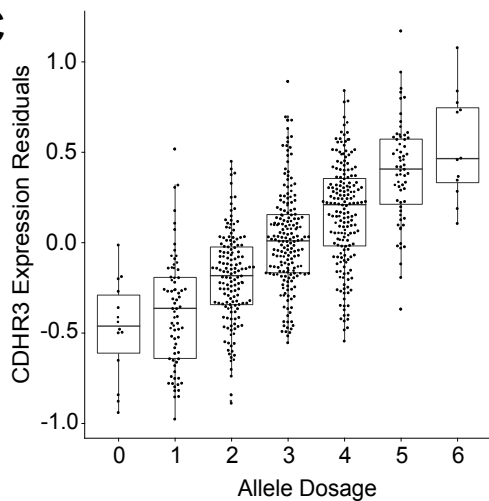
A

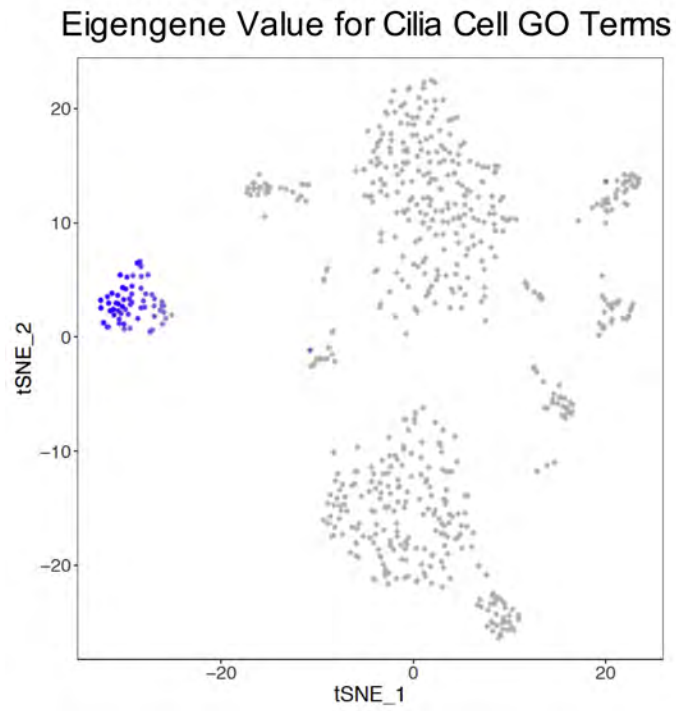


B



C





A

Donor	scrb	CDHR3 KO	p-value
T72	25.2% $\pm$ 5.32%	27.6% $\pm$ 8.68%	0.6806
T75	31.1% $\pm$ 6.71%	33.2% $\pm$ 4.18%	0.4604
T79	22.9% $\pm$ 6.54%	23.9% $\pm$ 4.07%	0.6795
All Donors	25.0% $\pm$ 6.31%	26.4% $\pm$ 7.80%	0.3782

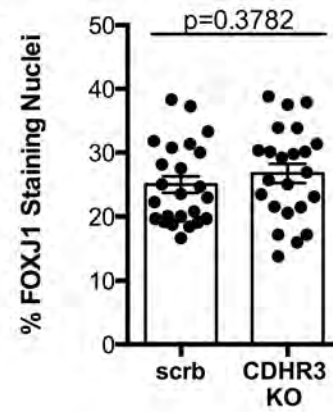
B

Table E1: Demographic and clinical variables for lung, tracheal, and nasal tissue samples

	Lung Donors	Tracheal Cells	Nasal Cells
Subjects (n)	6	7	12
Age (years)	45.0 ± 15.2	52.0 ± 17.7	13.8 ± 3.1
Sex (F, %)	2 (33.3)	3 (42.8)	6 (50.0)
Health Status (n, %)			
Healthy	6 (100.0)	7 (100.0)	0 (0.0)
Asthmatic	0 (0.0)	0 (0.0)	12 (100.0)
Asthma Status (n, %)			
Controlled	N/A	N/A	6 (50.0)
Not Well Controlled	N/A	N/A	2 (16.6)
Very Poorly Controlled	N/A	N/A	4 (33.3)
CDHR3 rs6967330 (AA, %)	0 (0.0)	0 (0.0)	6 (50.0)

Table E2: Demographic and clinical variables for GALA II subjects used in eQTL analysis

	Healthy	Asthmatic
Subjects (n)	242	431
Sex (F, %)	139 (57.4)	212 (49.0)
Age (years)	14.1 ± 3.1	14.0 ± 3.3
BMI (kg/m²)	22.9 ± 5.3	24.3 ± 6.9
Total IgE (kU/L)	330.1 ± 566.7	521.3 ± 687.0
Eosinophils (x10³/μL)	3.7 ± 2.9	4.9 ± 3.9
FeNO (ppb)	19.1 ± 16.2	30.5 ± 29.4
% FEV1	94.0 ± 13.7	88.2 ± 16.8

Table E3: Differentially expressed genes/cell type markers for identification of cell populations in single cell sequencing analysis of human lung cells (Top 50; adjusted p-value < 0.05)

Ciliated Cells		Basal/Secretory Cells		Alveolar Type I Cells	
Gene	P-value adj	Gene	P-value adj	Gene	P-value adj
ZBBX	4.07E-99	KRT5	2.84E-48	NTM	3.91E-108
DNAH11	9.67E-93	DSC3	1.42E-36	SPOCK2	6.18E-82
ERICH3	6.45E-92	GABRP	1.42E-36	AGER	1.88E-74
CCDC146	8.56E-92	KRT17	9.48E-35	RTKN2	3.16E-61
DNAH9	1.68E-89	MIR205HG	4.67E-34	CLIC5	5.62E-58
ARMC4	2.16E-87	AQP5	3.86E-29	NCKAP5	6.51E-55
HYDIN	2.31E-87	SERPINB13	7.48E-29	MAP2	2.01E-54
C1orf87	3.36E-87	SERPINB11	2.68E-27	FMO2	1.46E-49
ADGB	2.63E-85	CLCA2	6.34E-25	COL4A2	1.52E-49
DNAH12	1.71E-82	CXCL6	4.60E-22	TIMP3	1.60E-47
CFAP43	3.92E-82	SVOPL	4.97E-22	ST6GALNAC5	1.05E-42
DRC1	2.80E-81	SERPINB4	6.38E-22	SLC1A1	2.59E-42
DTHD1	9.24E-81	SERPINB5	3.24E-20	SEMA5A	6.36E-41
LRRIQ1	1.11E-80	TMPRSS4	3.37E-20	SCEL	7.15E-41
TSGA10	1.08E-79	SERPINF1	9.36E-20	GGTLC1	9.82E-41
DYNLRB2	1.30E-79	TP63	6.06E-19	CAV1	1.56E-37
LDLRAD1	2.84E-78	CHST9	1.82E-18	CAV2	1.52E-35
ARMC3	7.24E-78	GPX2	2.32E-17	WFS1	1.59E-33
CCDC170	1.28E-77	BMPRI1B	5.65E-17	MYRF	3.65E-33
NEK10	9.04E-77	NTS	2.16E-14	SERTM1	3.65E-33
SPAG17	2.72E-76	GRAMD2B	2.84E-14	ARHGEF26	1.34E-32
CFAP54	3.16E-76	CP	4.14E-14	ENSG00000279662	8.71E-32
WDR63	5.74E-76	HS3ST1	6.60E-14	CEACAM6	6.74E-31
ENKUR	1.70E-75	TACSTD2	1.45E-13	COL4A3	1.59E-30
NEK5	1.12E-74	PRSS23	2.09E-13	MS4A15	5.29E-30
CAPS	2.27E-74	ENSG00000223861	4.05E-13	ANKRD1	1.07E-29
FRMPD2	3.29E-74	KLK11	1.34E-12	ENSG00000257604	1.03E-28
TEKT1	6.84E-74	HES1	2.95E-12	COL4A1	2.76E-28
ECT2L	9.57E-73	RND3	3.59E-12	ABCA8	5.10E-28
CCDC39	1.53E-72	TSPAN8	6.16E-12	LAMC2	1.20E-25
DNAH7	7.26E-72	MDK	4.24E-11	MYL9	1.25E-25
C11orf88	3.83E-71	WEE1	7.99E-11	GPRC5A	1.49E-25
WDR66	7.19E-71	ADAM28	8.32E-11	COL12A1	2.30E-25
SPATA18	2.20E-70	IL33	1.69E-10	FAM167A	3.86E-24
DYDC2	4.75E-70	CAPN13	2.23E-10	COL8A1	4.50E-24
DNAH6	9.01E-70	S100A2	1.09E-09	SEMA3E	8.16E-24
DNAH10	1.87E-69	TUFT1	1.52E-09	EMP2	1.02E-22
FAM183A	1.89E-69	PTPRZ1	1.62E-09	EPB41L5	1.09E-22
RSPH1	3.97E-69	S100P	1.70E-09	LMO7	3.70E-22
DNAAF1	3.99E-69	SEMA3A	2.75E-09	COL4A4	8.91E-22
DCDC1	1.14E-68	ABCA13	8.12E-09	TSPAN13	1.23E-21
MS4A8	1.79E-68	CLDN1	1.36E-08	GOLGA8A	1.72E-21
BBOF1	5.71E-68	ECE1	2.26E-08	MSLN	3.16E-21
TSPAN19	2.05E-66	FMO2	2.34E-08	SCD5	1.51E-20
CCDC113	4.93E-66	SLC2A1	5.65E-08	ENSG00000276850	1.77E-20
CFAP157	5.07E-66	TFAP2C	1.59E-07	RIMKLA	2.93E-20
SNTN	5.78E-66	SCNN1G	1.63E-07	GPM6A	8.11E-20
EFCAB10	9.67E-66	TRIM29	3.27E-07	DST	1.05E-19
CDHR3	1.51E-65	FBXO32	4.42E-07	KRT7	1.11E-19
CFAP52	1.67E-65	MUC4	4.75E-07	ANKRD29	1.56E-19

Alveolar Type II Cells	
Gene	P-value adj
SFTPA1	1.48E-76
SFTPA2	1.49E-74
SFTPB	4.83E-74
SFTPC	4.84E-69
LRRK2	2.83E-61
SLC34A2	5.44E-61
NAPSA	2.85E-59
PGC	4.29E-49
MALAT1	7.81E-49
CTSH	3.33E-43
ROS1	2.45E-41
ADGRF5	6.95E-41
TTN	4.79E-40
SFTPD	1.16E-39
SFTA2	9.46E-38
ABCA3	1.12E-37
LAMP3	2.09E-37
SELENBP1	6.66E-37
HHIP	1.40E-36
SLPI	1.82E-36
NPC2	1.51E-35
PIGR	2.38E-34
MTUS1	1.38E-33
PTPN13	7.97E-33
C3	8.48E-33
SNX25	1.96E-31
TCIM	2.97E-31
HMGCS1	1.85E-30
MUC1	2.33E-30
CYB5A	7.09E-30
DHCR24	6.67E-29
CLDN18	9.01E-29
CCDC141	4.75E-28
MSMO1	6.91E-28
STEAP4	8.41E-28
PARM1	2.61E-27
NTN4	2.06E-25
DRAM1	3.25E-25
SNX30	4.12E-25
HOPX	3.05E-24
ZNF385B	6.42E-24
CXCL17	9.28E-23
MBIP	1.00E-22
PEBP4	4.26E-21
ALPL	9.09E-21
KIAA1324L	1.79E-19
SCD	3.39E-19
PDE4D	8.50E-19
CACNA2D2	1.56E-18
AKAP13	2.79E-18

Proliferating Epithelial Cells	
Gene	P-value adj
KIFC1	1.04E-42
PBK	6.03E-42
MKI67	2.95E-38
CKAP2L	2.26E-35
NDC80	1.23E-32
IQGAP3	1.24E-31
HMMR	3.20E-31
TTK	1.43E-29
FANCI	1.43E-28
NCAPG	1.84E-27
FANCD2	2.00E-27
DEPDC1	6.64E-27
NUSAP1	9.73E-27
CDC6	7.32E-26
KNL1	2.50E-25
DLGAP5	1.33E-24
ANLN	5.83E-24
KIF23	2.74E-23
SPAG5	3.19E-23
PCLAF	7.96E-23
CENPU	1.90E-22
BLM	2.03E-22
NUF2	2.67E-22
DIAPH3	6.56E-22
RACGAP1	1.23E-21
ASF1B	2.50E-21
TOP2A	3.14E-21
BRCA2	3.17E-21
RRM2	4.08E-21
TYMS	4.29E-21
HELLS	9.32E-21
CKAP2	1.12E-20
ARHGAP11A	5.54E-20
CDC20	6.60E-20
CDKN3	6.60E-20
ESCO2	1.39E-19
PRC1	1.30E-18
HIST1H2AM	4.44E-18
CENPF	4.50E-18
CDK1	5.48E-18
CENPW	1.62E-17
BIRC5	8.15E-17
SHCBP1	9.93E-17
TPX2	2.41E-16
KIF15	2.55E-16
ZWINT	2.87E-16
SPC25	3.27E-16
CDCA2	3.43E-16
RAD51AP1	8.18E-16
UBE2C	1.50E-15

Endothelial Cells	
Gene	P-value adj
PTPRB	1.55E-71
RGS5	5.47E-65
CLDN5	9.86E-61
CALCRL	1.17E-60
PCDH17	1.73E-47
C7	3.50E-46
VWF	2.01E-45
GJA5	7.15E-42
MCAM	1.89E-41
SRPX	2.84E-39
EMCN	3.54E-39
ACKR1	5.01E-39
NOS1	2.29E-37
NRN1	4.11E-35
SELP	7.64E-34
PLCXD3	2.83E-31
CYYR1	2.83E-31
JAM2	4.13E-31
NUAK1	6.24E-31
TMEM100	1.46E-30
CLEC3B	2.28E-29
IL33	2.01E-28
TEK	6.64E-28
PTGIS	1.00E-27
MMRN2	2.18E-26
CLEC14A	2.89E-25
RAMP2	4.68E-25
ERG	5.45E-25
NPR3	1.17E-24
FLT1	1.32E-24
CDH5	1.44E-24
PCAT19	8.02E-24
CPE	7.20E-23
GPX3	1.75E-22
TLL1	6.57E-22
CD93	1.62E-20
NOTCH4	3.65E-20
ST8SIA6	3.69E-19
MGP	2.37E-18
FAT4	3.12E-18
MMRN1	1.71E-17
FCN3	1.82E-17
LAMB1	2.38E-17
SLCO2A1	5.39E-17
SPARCL1	8.60E-16
CTNNAL1	9.56E-16
LEPR	2.70E-15
VCAM1	2.84E-15
PLA1A	3.20E-15
RAPGEF4	4.32E-14

Smooth Muscle Cells	
Gene	P-value adj
SPON1	9.30E-58
GFPT2	1.24E-43
OGN	6.88E-41
PCOLCE	4.28E-34
COL6A1	1.60E-31
COL14A1	4.98E-30
COL6A2	4.98E-30
FBLN2	5.53E-30
SYNPO2	5.53E-30
MEG8	6.14E-30
PDGFRB	7.79E-30
ENSG00000271811	8.94E-30
COL5A1	5.38E-27
PLA2G2A	4.75E-26
ADH1B	5.21E-26
PAPPA	3.43E-23
THBS2	1.14E-22
SFRP2	1.24E-22
MXRA5	1.24E-22
LUM	5.54E-21
FBLN1	4.03E-20
ENPP1	1.04E-19
MEG3	3.65E-19
SCARA5	1.23E-18
ISLR	1.23E-18
PTGDS	1.62E-18
NR2F1	4.24E-18
LAMA2	5.26E-18
ELN	1.52E-17
PIEZO2	1.62E-17
DDR2	2.40E-17
IGFBP6	4.15E-16
COL5A2	7.81E-16
SPON2	1.51E-15
TMEM45A	1.87E-14
PTGIS	2.57E-14
C1S	5.10E-14
TRAF3IP2-AS1	5.69E-14
PDGFRA	2.05E-13
SLC16A2	5.84E-13
ZNF521	7.66E-13
PHLDB2	2.18E-12
LAMA4	3.00E-12
BICC1	7.23E-12
FBN1	7.25E-12
MIR100HG	3.30E-11
NES	3.73E-11
SPARC	8.12E-11
ITGA1	1.61E-10
CCDC80	1.84E-08

Macrophage Cells	
Gene	P-value adj
CYBB	1.31E-26
CTSS	1.21E-24
CD68	1.52E-24
GPNMB	3.04E-24
CTSB	1.71E-23
FTL	5.91E-23
MRC1	6.47E-23
OLR1	8.80E-22
NCF2	1.11E-21
CTSD	1.42E-20
PSAP	1.92E-20
APOE	7.48E-20
LAPTM5	2.55E-19
FTLP3	3.15E-19
VIM	7.74E-19
LYZ	2.69E-18
ZEB2	7.78E-18
ACP5	8.98E-18
FTH1	1.83E-17
MS4A7	9.45E-17
CTSZ	9.73E-17
CD163	1.48E-16
CCDC88A	1.53E-16
S100A4	5.06E-16
SLC11A1	1.81E-15
TMSB10	3.62E-15
FCGR3A	3.99E-15
PLXDC2	5.61E-15
SLFN11	8.73E-15
IFI30	3.74E-14
LHFPL2	9.34E-14
EVI2B	1.42E-13
PPT1	1.71E-13
FPR3	1.77E-13
ITGB2	1.77E-13
FTH1P11	2.53E-13
TGFBI	2.56E-13
MARCO	2.83E-13
CTSL	4.28E-13
CCL18	4.88E-13
TMSB4X	5.50E-13
FTH1P10	6.20E-13
LGALS1	8.07E-13
APOC1	2.07E-12
CD53	2.79E-12
VCAN	3.36E-12
LCP1	3.96E-12
CSTB	7.83E-12
PTAFR	8.92E-12
CPVL	2.16E-11

Neutrophil Cells	
Gene	P-value adj
IL1B	1.30E-37
ZEB2	5.42E-37
EREG	4.80E-33
CCL3	7.70E-32
F13A1	1.83E-30
IER3	1.26E-29
CXCL8	7.48E-29
ACTB	8.26E-28
FOS	9.11E-28
LCP1	3.83E-27
PLAUR	1.22E-26
OLR1	3.79E-26
PLEK	7.43E-25
SAMSN1	1.50E-24
GPR183	1.95E-24
NLRP3	2.39E-24
CYBB	2.39E-24
CXCL3	3.94E-24
SRGN	1.18E-23
VCAN	2.14E-23
NAMPT	2.80E-23
VIM	1.62E-22
AIF1	1.70E-22
FYB1	2.86E-22
NFKBIA	4.30E-22
FTH1	5.06E-21
C5AR1	9.58E-21
COTL1	5.13E-20
TMSB10	6.88E-20
CTSB	1.13E-19
CCL3L1	1.81E-19
CCL4	2.72E-19
PTPRC	4.53E-19
LYZ	6.02E-18
CD84	9.76E-18
FGL2	1.22E-17
CD300E	1.68E-17
MRC1	3.08E-17
PDE4B	3.93E-17
C15orf48	6.50E-17
MS4A6A	8.51E-17
TGFBI	1.02E-16
MNDA	1.10E-16
CLEC5A	1.48E-16
CCR1	2.23E-16
KLF6	3.22E-16
CCL4L2	5.06E-16
SOD2	1.05E-15
ZFP36	1.16E-15
TYROBP	2.85E-15

Mast Cells	
Gene	P-value adj
TPSB2	9.98E-139
TPSAB1	7.25E-73
KIT	2.76E-66
IL1RL1	7.46E-64
CPA3	6.48E-62
SLC18A2	3.33E-58
HPGDS	3.13E-52
MS4A2	1.82E-42
GCSAML	1.00E-41
GATA2	3.20E-41
RGS13	2.95E-24
MAOB	3.96E-19
CD22	1.46E-16
CD69	7.30E-16
VWA5A	6.63E-15
ADAM12	6.64E-11
LAX1	7.60E-11
RGS1	2.08E-08
IL18R1	9.84E-07
RAC2	2.33E-05
SAMSN1	6.39E-04
IKZF3	9.66E-04
SRGN	2.50E-03
ARHGEF6	1.83E-02

Antigen-Presenting Immune Cells	
Gene	P-value adj
INHBA	3.06E-59
GPD1	2.59E-52
ENSG00000256948	2.00E-46
FOLR3	1.32E-45
ITIH5	5.86E-45
CES1P1	1.53E-44
MME	6.44E-44
PPARG	3.98E-43
PCOLCE2	1.10E-40
CES1	3.84E-34
IFIT3	4.66E-34
FABP4	5.33E-32
RBP4	2.08E-30
IFI44L	3.88E-30
SLC19A3	3.58E-29
PARAL1	5.54E-29
FPR2	2.27E-28
C1QB	4.18E-28
MSR1	4.73E-28
ADTRP	1.88E-27
SPN	3.84E-27
MCEMP1	5.73E-27
CPE	1.38E-26
FHL1	1.42E-26
GPRIN3	3.72E-26
DOCK11	2.21E-25
HLA-DQA1	4.80E-25
GLDN	7.09E-24
STON1	2.62E-23
FN1	1.02E-22
SCIMP	1.86E-22
SECTM1	1.31E-21
C1QC	1.70E-21
MARCO	3.66E-21
BHLHE41	3.91E-21
HCAR2	3.93E-21
C8B	4.80E-21
C1QA	5.82E-21
ENSG00000259834	6.28E-21
FCGR3A	6.38E-21
FTH1P8	7.87E-21
ENSG00000260908	9.91E-21
SNX10	1.02E-20
LILRB1	1.33E-20
IFIT2	7.25E-20
PLBD1	8.03E-20
CYP27A1	1.08E-19
ARRDC4	1.84E-19
SIGLEC1	2.90E-19
ALOX5AP	3.10E-19

Proliferating Immune Cells	
Gene	P-value adj
FAM72B	2.76E-42
TYMS	4.55E-38
ENSG00000242282	2.31E-37
POLQ	1.39E-34
ARHGEF39	5.21E-33
ASPM	1.66E-31
ANLN	1.84E-30
SKA3	6.49E-29
SPC25	9.70E-29
KIF11	2.84E-28
NCAPG	9.91E-26
BUB1B	1.41E-25
KIF14	2.14E-24
CCNB2	2.46E-24
DLGAP5	5.05E-24
RAD51	8.59E-24
CDKN3	1.93E-23
CEP55	2.67E-22
CKAP2	1.11E-21
CDC45	2.12E-21
KIF4A	3.63E-21
NDC80	1.97E-19
ASF1B	2.60E-19
VASH1	3.96E-19
KNL1	5.75E-18
ZWILCH	6.59E-18
SHCBP1	1.50E-17
TROAP	5.46E-17
TEDC1	5.46E-17
NCAPH	1.08E-16
CIP2A	2.43E-16
TPX2	5.99E-16
CENPE	7.99E-16
PRC1	1.04E-15
MKI67	1.30E-15
HMMR	1.51E-15
BUB1	1.69E-15
TMEM51	3.64E-15
KIF18A	7.06E-15
CCNB1	1.50E-14
KIF15	1.67E-14
NRM	2.41E-14
TUBBP1	6.49E-14
PCLAF	7.04E-14
TTK	1.25E-13
KIF23	1.67E-13
ATAD5	1.75E-13
ZWINT	1.81E-13
CENPF	1.90E-13
LFNG	2.80E-13

Cluster 13 - Cell Identity Unclear	
Gene	P-value adj
PROX1	8.49E-125
CCL21	2.48E-66
PKHD1L1	3.91E-65
RELN	1.84E-52
BHMT2	2.15E-46
MMRN1	8.87E-42
SEMA6A	1.06E-40
PRELP	1.28E-40
GNG11	7.42E-37
ACKR2	2.11E-35
PDE1A	2.57E-35
FZD10-AS1	4.16E-34
FLT4	4.16E-34
CSGALNACT1	5.27E-31
CHRD1	9.61E-31
LYVE1	2.99E-29
SYNM	3.28E-29
ENSG00000278931	1.38E-27
LAMA4	2.44E-27
AKAP12	8.75E-27
TM4SF18	6.80E-25
KLHL4	1.02E-24
NR2F1	4.27E-23
RHOJ	3.14E-21
TSHZ2	4.74E-21
ENSG00000235505	5.26E-21
PODXL	2.74E-20
KBTBD11	3.23E-20
CAVIN2	4.20E-20
ART4	5.40E-19
SCN3B	5.92E-19
PGM5	4.60E-18
PPFIBP1	5.57E-18
CLDN5	2.23E-17
TIE1	1.16E-16
PLSCR4	1.41E-16
CALCRL	2.10E-15
GPRC5B	2.64E-15
TNXB	3.60E-15
PIEZO2	1.58E-14
NTS	7.94E-13
ENSG00000253816	1.32E-12
CDH13	1.50E-12
IGF1	1.34E-11
F8	3.70E-11
SEMA3D	1.40E-10
ERG	2.05E-10
PCAT19	3.99E-10
SEMA3A	1.46E-09
LDB2	1.72E-09

Table E4. RNA-seq analysis of differential gene expression between control and CDHR3 KO ALI cultures

DEGs (FDR<0.05; Log2 Fold Change >0.5 or <-0.5)			
Gene	log2 Fold Change	p-value	p-value adj
CDHR3	-0.53	7.27E-22	4.58E-18
VEGFA	0.56	1.71E-08	3.59E-05
TCN1	0.60	5.88E-07	7.41E-04
HLA-DQB1	-0.71	7.43E-07	7.81E-04
DSC3	0.64	1.13E-06	8.93E-04
NOTCH1	0.50	2.99E-06	1.89E-03
KRT14	2.25	2.72E-06	1.89E-03
COL12A1	0.71	4.23E-06	2.12E-03
ANKH	0.75	4.37E-06	2.12E-03
SERPINA1	-0.74	5.63E-06	2.54E-03
LTB4R	0.62	7.19E-06	2.83E-03
KRT15	1.19	1.26E-05	4.30E-03
TMEM2	-0.55	2.53E-05	6.95E-03
LTBP4	0.67	3.19E-05	7.73E-03
SEMA5A	0.55	8.57E-05	1.64E-02
FSCN1	0.57	9.74E-05	1.81E-02
ALPK3	-0.56	1.51E-04	2.27E-02
ARHGDIB	-0.52	2.48E-04	2.89E-02
TNS1	0.60	2.81E-04	3.16E-02
LIMCH1	-0.61	3.83E-04	3.41E-02
DCBLD2	-0.53	4.12E-04	3.47E-02
KRT5	0.87	4.41E-04	3.56E-02
NEDD9	-0.57	4.67E-04	3.57E-02
FAM213A	0.50	4.58E-04	3.57E-02
FOXQ1	0.53	5.65E-04	3.75E-02
NGFR	0.53	6.90E-04	3.92E-02
MIR205HG	0.50	7.51E-04	4.15E-02
Basal Cell Markers			
Gene	log2 Fold Change	p-value	p-value adj
KRT5	0.87	4.41E-04	3.56E-02
KRT14	2.25	2.72E-06	1.89E-03
NGFR	0.53	6.90E-04	3.92E-02
TP63	0.80	1.37E-03	5.49E-02
Mucus Secretory Cell Markers			
Gene	log2 Fold Change	p-value	p-value adj
MUC5AC	0.54	4.23E-01	7.60E-01
MUC5B	-0.39	1.71E-01	5.25E-01
Club Secretory Cell Markers			
Gene	log2 Fold Change	p-value	p-value adj
SCGB1A1	-0.19	4.42E-01	7.72E-01
BPIFB1	-0.51	1.67E-02	1.72E-01
WFDC2	-0.19	1.19E-02	1.44E-01
Ciliated Cell Transcription Factors			
Gene	log2 Fold Change	p-value	p-value adj
CDHR3	-0.53	7.27E-22	4.58E-18
FOXJ1	-0.13	2.45E-02	2.05E-01
FANK1	-0.09	3.33E-01	6.96E-01
RFX2	-0.16	2.30E-02	1.99E-01
RFX3	-0.06	5.65E-01	8.46E-01
MYB	-0.06	5.39E-01	8.31E-01
CCNO	-0.25	6.89E-03	1.10E-01
TP73	-0.02	7.79E-01	9.72E-01
E2F4	0.11	2.86E-01	6.55E-01

Table E5. Independent CDHR3 eQTL loci in the nasal airway epithelium of children

Top Model Derived Independent SNPs	Model eQTL Block	SNP Position (Chr 7)	Model SNP eQTL P Value	SNPs in LD ($R^2 > 0.4$)	SNP with strongest P Value in LD ($R^2 > 0.4$) with Model SNP	LD SNP P value	LD SNP Position
rs2528883	1	105993995	3.75E-30	3	rs2528883	3.75E-30	105993995
rs6973512	2	105989547	1.28E-19	2	rs3847076	1.66E-29	105989994
rs375981	3	105989710	2.80E-20	0	rs375981	2.80E-20	105989710
rs56235770	4	106025231	1.15E-11	22	rs73195680	3.49E-12	106037229
rs2526977	5	105995700	1.52E-02	1	rs2526977	1.52E-02	105995700
rs386716534	6	106010874	2.91E-02	2	rs386716534	2.91E-02	106010874
rs10263784	7	106251018	1.42E-04	36	rs138662748	1.26E-05	106224225
rs1024561	8	106094062	3.82E-09	2	rs1024561	3.82E-09	106094062
rs2528891	9	105961305	1.06E-04	27	rs2727755	3.56E-05	105964276
rs2519665	10	106374796	2.13E-02	2	rs2519665	2.13E-02	106374796
rs76910568	11	105758616	2.84E-03	0	rs76910568	2.84E-03	105758616

Table E6: Test for association of CDHR3 eQTL loci with asthma hospitalization in minority children

CHR	SNP	BP	A1	Mexicans			Puerto Ricans			Other Latino			Meta-analysis		
				Beta	SE	P	Beta	SE	P	Beta	SE	P	Beta.meta	SE.meta	P.meta
7	rs2528883	105634441	A	0.018	0.120	0.885	0.122	0.098	0.212	0.173	0.150	0.249	0.099	0.068	0.171
7	rs3847076	105630440	A	-0.074	0.136	0.587	-0.106	0.088	0.233	0.004	0.167	0.980	-0.080	0.068	0.280
7	rs375981	105630156	G	0.240	0.165	0.145	-0.006	0.104	0.958	-0.238	0.205	0.245	0.016	0.115	0.874
7	rs73195680	105677675	T	0.305	0.151	0.044	0.161	0.103	0.118	0.296	0.188	0.116	0.222	0.078	0.005
7	rs6967330	105658451	A	0.220	0.148	0.137	0.197	0.100	0.047	0.241	0.182	0.185	0.211	0.075	0.007
-	4 SNP Combination	-	-	0.083	0.064	0.199	-0.027	0.046	0.561	-0.072	0.082	0.381	-0.003	0.041	0.941

Supplemental Methods

Single-Cell RNA Sequencing

Single cell suspensions of elastase digested lung tissue (n=6) were obtained as previously described (1). Briefly, using an intact lung lobe, the airways were washed using HEPES/saline/EDTA buffer to remove excess alveolar macrophages and airway tissue was digested by an instillation of elastase enzyme followed by homogenization and DNaseI treatment of the lung tissue. Lung homogenates were sequentially filtered through cheesecloth followed by 100µm and 20µm nylon mesh resulting in single cell suspensions of the lung homogenate. Cell suspensions were pelleted at $330 \times g$ for 5 minutes at 4°C and washed twice with PBS. Cell concentrations were determined by hemocytometer and cell suspensions were prepared at 30 cells/µl, dispensed into 5184-well microchips (version 1), and imaged using the ICELL8™ Single-Cell System (WaferGen Bio-systems Inc., Fremont, CA, USA) per manufacturer guidelines. Nanowell images were evaluated using CellSelect™ Software parameters calibrated in-lab for epithelial cells, and manual review of results ensured that wells containing multiple cells or damaged cells were omitted from subsequent processing. The microchips were placed on a pre-frozen block on dry ice during manual review, quick thawed at room temperature for 5 minutes, and centrifuged at $3,000 \times g$ for 5 minutes at 4°C before reverse transcription (RT-PCR) mix dispense. Recovery of cDNA was carried out immediately following completion of the RT-PCR reaction to prevent evaporation of cDNA eluent. Complete sample workflow through the Nextera XT® sequencing library preparation was as outlined by the ICELL8™ Single-Cell Poly-(A)+ Transcriptome Amplification Hands-on Workflow Checklist and Preparation Guide (with Triton X-100 for Cell Lysis) (version D07-000040-003 rev 4) and the Nextera XT DNA Library Preparation Kit protocol. The Illumina MiSeq® System (Illumina, San Diego, CA, USA) served as a

sequencing quality control step before proceeding with Illumina HiSeq[®] 2500 System sequencing.

Single Cell Data Analysis: Dimensionality Reduction and Clustering

Highly variable genes used for dimensionality reduction were obtained by modeling the relationship between mean expression and the log of the ratio of expression variance to mean (dispersion). A total of 5,530 genes were selected for which fitted dispersion was > 0.5 and mean expression was > 0.0125 . These highly variable genes were subjected to principle components analysis (PCA) and then, based on visualizing a relative leveling off of the standard deviation across PCs, the top 12 PCs were selected for subsequent clustering and visualization. A two dimensional t-distributed neighborhood embedding (tSNE) map was constructed based on the top 12 PCs (perplexity = 30, number of iterations = 1,000).

Clustering of single cells expression was performed with a shared nearest neighbor (SNN) graph based on k-nearest neighbors calculated from the first 12 PCs of the scaled data ($k = 30$). The number of clusters was then determined using a modularity function optimizer based on the Louvain algorithm (resolution = 0.4).

ALI Culture of Primary Tracheal and Nasal Airway Epithelial Cells

Primary human tracheal epithelial cells were obtained from the Live Cell Core at National Jewish Health. Primary nasal airway epithelial brushes were obtained from subjects recruited as a part of the Genes-environments and Admixture in Latino Americans II (GALA II) childhood asthma cohort (2, 3). Basal cells were cultured for expansion using a modified Schlegel method as previously described (4-6). Basal cells were seeded onto 6.5mm 24-well

polyester Transwell inserts with 0.4µm pore size at 6.0×10^4 cells/cm² in ALI Expansion Medium supplemented with Y-27632 as previously described (5, 6), and were air-lifted upon development of an intact monolayer. Cultures were differentiated using PneumaCult ALI (PC-ALI) differentiation media (StemCell Technologies). For transepithelial electrical resistance (TEER), warm PBS was added to the apical chamber of each culture, resistance measurements were collected using a Millicell[®] ERS-2 Volt-Ohm Meter (Millipore Sigma), and measurements were normalized to a blank insert control.

Lentiviral CRISPR-Cas9 Gene Editing of Tracheal Airway Epithelial Cells

The design of the CRISPR targeting guide sequences, addition of adaptors and cloning into the lentiCRISPR plasmid backbone, propagation and titration of lentivirus, and AEC transduction and selection was performed as previously described (4, 7). The gRNA sequences were designed using the CRISPRdesign (version 1) tool offered by the Zhang lab at MIT (crispr.mit.edu) and chosen to target exon 1 of the *CDHR3* gene (forward *CDHR3*-exon1 guide 5'-CACCGCAGGAAGCAATCATTCTCC -3'; reverse *CDHR3*-exon 1 guide 5'-AAACGGAGAATGATTGCTTCCTGC-3'). A scrambled sequence was designed and used as a control in parallel for all CRISPR gene editing experiments (forward scramble guide 5' -CACCGCGTGCTCCGTTTCGCGCTTC-3'; reverse scramble guide 5'-AAACGAAGCGCGAACGGAGCACGC-3'). Screening primers were designed over the CRISPR-Cas9-targeted region of the *CDHR3* gene for high resolution melt curve analysis using MeltDoctor HRM Master Mix on a QuantStudio 6 Flex Real-Time PCR System (Life Technologies) and contain the appropriate adaptors for next-generation sequencing analysis as previously described (*CDHR3*-exon1 forward screening primer 5'-CTGCTGTACGCAGCGTTCAGGCTGTGGCTAATGTGC-3'; *CDHR3*-exon1 reverse

screening primer 5'- CCTCTCTATGGGCAGTCGGTGATTATTATGCAGACCAAGTAGG-3') (4, 7). Early passage primary tracheal airway epithelial cells were used for transduction in gene-editing experiments (n=3 donors). Following antibiotic selection, gene edited basal epithelial cells were seeded onto 6.5mm 24-well polyester Transwell inserts with 0.4µm pores at 3.0×10^5 cells/cm² in ALI Expansion Media supplemented with Y-27632, and were air-lifted upon development of an intact monolayer. Cultures were differentiated using PneumaCult ALI (PC-ALI) differentiation media (StemCell Technologies).

Dissociation of Epithelial ALI Cultures

Differentiated ALI cultures were grown for 21+ days prior to harvest for molecular analyses as previously described (6). Briefly, cultures were washed twice with warm PBS, followed by a wash with PBS supplemented with 10mM DTT to remove excess mucus from the cultures. For enzymatic dissociation, Accutase solution (Fisher Scientific) containing 10mM EDTA and 10mM EGTA was added to the apical chamber of each insert and incubated for 30 minutes at 37°C. Single cell suspensions were washed with warm PBS-10mM DTT solution prior to counting and harvest for cytopins, flow cytometry, or protein lysate collection as described below.

Human Rhinovirus Infection of ALI Epithelial Cultures

HRV-C15 virus was propagated by transfecting HeLa-E8 cells with RNA transcribed from the linearized plasmid pC15-Rz, as previously described (8). HRV-A16 was a gift from the Voelker lab at National Jewish Health. Prior to viral infections, the apical chamber of differentiated ALI cultures was washed twice with warm PBS. Virus was added to the apical chamber of each culture in X-Vivo 10 medium (Lonza) at an MOI of 0.2 for 4 hours at 34°C;

mock infections were conducted using media alone. The apical chamber of each infected and control culture was washed 3 times with warm PBS and cultures were incubated for a total of 48 hours at 34°C prior to harvest.

Whole Transcriptome Analysis of CRISPR-Cas9 gene-edited cultures

Primary tracheal airway basal cells were used for gene-editing experiments as described above. Control and CDHR3 KO cells were differentiated at ALI prior to harvest for RNA-seq experiments. RNA lysates were harvested from each mature culture and extracted using the Quick-RNA MiniPrep Kit (Zymo Research). RNA Normalization, library construction using KAPA Stranded mRNA-Seq Kit with KAPA mRNA Capture Beads (KAPA Biosystems), and library pooling were all performed on the Beckman Coulter FX^P automation system. Briefly, RNA samples were randomized over the normalization plate at 100ng per well, and barcodes were added using 12 cycles of amplification to generate sequencing libraries for Ion Torrent whole transcriptome sequencing. Sequencing reads were aligned and mapped to human reference genome GRCh38 with GSNAP (9). Transcript quantification of these aligned reads on iGenomes GTF file were conducted with HTSeq to generate a gene count matrix (10). Differential expression analysis using DESeq2 was based on RNA sequencing from 3 KO and 3 control culture replicates of 2 donors and a single KO and control replicate of a third donor.

Whole Transcriptome Analysis of the GALA II Cohort

Primary nasal airway epithelial brushes were obtained from subjects recruited as a part of the Genes-environments and Admixture in Latino Americans II (GALA II) childhood asthma cohort and placed into RLT lysis buffer for RNA and DNA extraction using the AllPrep

DNA/RNA MiniPrep Kit (Qiagen) (2, 11). RNA Normalization, library construction using KAPA Stranded mRNA-Seq Kit with KAPA mRNA Capture Beads (KAPA Biosystems), and library pooling for a total of 673 subjects were all performed on the Beckman Coulter FX^P automation system. Briefly, RNA samples were randomized over the normalization plate in alternating control and asthmatic conditions at 250ng per well. Illumina Dual-Index adapters (Integrated DNA Technologies) were used to barcode libraries using 12 cycles of amplification. Samples were pooled using equal concentrations of six libraries/pool and pair-end sequenced with Illumina HiSeq[®] 2500 System sequencing. Sequencing reads were trimmed with Skewer (--end-quality 15, --mean-quality 25, --min 30) (12). Trimmed reads were aligned and mapped to human reference genome GRCh38 with GSNAP (9). Transcript quantification of these aligned reads on iGenomes GTF file were conducted with HTSeq to generate a gene count matrix (10). Kallisto was used to generate Transcript per Million (TPM) values for all the subjects (13). A total of 17,039 genes with TPM > 0.1 and read counts > 6 for at least 20% of the subjects were subjected to downstream eQTL analysis.

Genotype Analysis of GALA II Cohort

A total of 2,243 asthma patients in the Genes-environments and Admixture in Latino Americans study (GALA II) were included in the analysis. The genotype and phenotype information analyzed in this manuscript is publicly available via dbGAP (dbGAP accession numbers: phs001274.v1.p1, phs001180.v1.p1, and phs00921.v1.p1). Samples were genotyped with Affymetrix Axiom LAT 1 (World Array 4) and LAT plus HLA genome-wide arrays (Affymetrix, Santa Clara, CA). SNPs were removed if they failed manufacturer's quality control, had genotyping call rates below 95%, and/or had a deviation from Hardy-Weinberg equilibrium ($p < 10^{-6}$) within controls. Samples were filtered if there is a discrepancy between genetic sex and reported gender. When cryptic relatedness (PI_HAT > 0.3) was

detected for a pair of samples, one of them was removed. Asthma hospitalization was defined as the patient having been hospitalized because of asthma before. Global ancestry was estimated using ADMIXTURE 1.3.0 for each individual assuming a 3-population model (European, Native American, and African ancestry) (14). Reference haplotypes were from the HapMap phase II CEU (European) and YRI (African), and 71 Native American individuals genotyped on the Axiom LAT1 array as described previously (15). The association of SNPs and asthma hospitalization was analyzed using logistic regression in Mexicans, Puerto Ricans, and other Latinos. The regression model was adjusted for age, sex, global African ancestry, and global Native American ancestry. Results from each population were combined in meta-analysis using METASOFT v2.0.1 (16).

CDHR3 eQTL Analysis

Expression quantitative trait loci (eQTL) analysis was performed following a pipeline similar to the Genotype-Tissue Expression (GTEx) V7 protocol (17). After initial filtering on minor allele frequency (>0.01), minor allele subject count (>10), and imputation quality score ($R^2 > 0.3$), a total of 8,089 SNPs within 1Mb of *CDHR3* TSS were tested for association with *CDHR3* gene expression level. *CDHR3* gene expression was TMM normalized with edgeR followed by inverse normalization (18, 19). Probabilistic Estimation of Expression Residuals (PEER) factors were computed with R package PEER (20). Admixture factors were estimated with the software Admixture (14). *CDHR3* eQTL mapping was performed with FastQTL (21), and adjusted for age, gender, body mass index (BMI), asthma status, 60 PEER factors, and 4 admixture factors. A stepwise regression approach implemented in QTLtools (22) was used to identify independent *CDHR3* eQTLs.

Immunofluorescence Labeling

Cytospins were prepared from single cell suspensions following ALI culture dissociation and fixed with 3.2% paraformaldehyde/1.5% sucrose in PBS fixation buffer for 20 minutes. Intact ALI culture inserts were fixed for 15 minutes at room temperature in 3.2% paraformaldehyde or with ice cold methanol at -20°C. Human lung tissue samples were fixed in 10% formalin prior to paraffin embedding and sectioning. Histology sections were stripped of paraffin using HistoChoice Clearing Agent (Sigma), rehydrated using a decreasing gradient of alcohol washes (100%, 90%, 70%, 50%, 30%, 0%), and antigen retrieval conducted using Citric Acid-based Antigen Unmasking Solution pH 6.0 (Vector Laboratories). Prior to labeling, all samples were blocked and permeabilized for 30 minutes using 3% BSA/0.1% Triton X-100 in tris-buffered saline (TBS). Primary labeling was conducted using 3% BSA/0.1% Triton X-100 in TBS for 1 hour with antibodies against acetylated α -tubulin (ACT; 1:10,000; Sigma), E-cadherin (1:100; ECM Biosciences), MUC5AC (1:200; ThermoScientific), CDHR3 (1:100; Sigma), Ezrin (1:200; Santa Cruz Biotechnology), Centrin (1:2000; Abnova) and FOXJ1 (1:100; ThermoScientific). Samples were washed twice using TBS/0.1% Triton X-100 (TBST) and secondary labeling was conducted using DAPI (1:1000), AlexaFluor488 or 594 goat anti-rabbit IgG and AlexaFluor488 or 594 goat anti-mouse IgG (1:500; ThermoScientific) for 30 minutes. Slides were washed twice in TBST and mounted with Vectashield HardSet Antifade Mounting Media (Vector Laboratories). Images were acquired using a Revolve microscope (Echo Labs) or a Zeiss LSM700 Confocal microscope. Images were analyzed using ImageJ and Zeiss ZEN Blue Lite analysis software.

SDS-PAGE and Western Blot Analysis

Differentiated ALI cultures were harvested by Accutase treatment as described above and dissociated cells were lysed in RIPA buffer containing protease inhibitor cocktail (Sigma).

Lysates were quantified by Bradford assay and 20µg of total protein was run on a BioRad TGX 4-20% SDS-PAGE gel at 150V for 1 hour and transferred to nitrocellulose at 90V for 2 hours. Membranes were blocked with 4% milk/TBS/0.1% Tween-20 buffer and immunoblots were conducted using a rabbit anti-CDHR3 (1:500; Sigma) and mouse anti-actin (1:1000; Millipore) in 4% milk/TBS/0.1% Tween-20 overnight at 4°C. Blots were probed with goat anti-mouse HRP or donkey anti-rabbit HRP-conjugated antibodies for 1 hour (1:10,000) and incubated with ClarityMax Western ECL Blotting Substrate for chemiluminescent detection of protein bands. Images were captured on a BioRad ChemiDoc, band intensity densitometry was measured using ImageJ, and CDHR3 bands were normalized to the actin loading control for quantitation.

Flow Cytometry

Differentiated ALI cultures were harvested as described above and single cell suspensions were fixed and permeabilized using the BD Fixation/Permeabilization Solution Kit (BD Biosciences) as per manufacturer's protocol. Primary labeling of cells was conducted using a rabbit anti-CDHR3 antibody (1:200; Sigma) or mouse anti-acetylated alpha-Tubulin (ACT) antibody (1:10,000; Sigma) for 30 minutes on ice. Cells were washed twice in 1x BD Perm/Wash buffer and secondary labeling was completed using AlexaFluor488 goat anti-mouse IgG and AlexaFluor594 goat anti-rabbit IgG antibodies (1:1000; ThermoScientific) for 30 minutes on ice in the dark. Labeled cells were washed, resuspended in 5% FBS/5mM EDTA in PBS FACS buffer, and samples analyzed on an LSR Fortessa instrument (BD Biosciences). Data analysis was conducted using FlowJo v10 software and data was analyzed using a ratio paired t-test to determine statistical significance.

Quantitative PCR Analysis

Epithelial samples were harvested in RNA/DNA Extraction Buffer supplemented with 40mM dithiothreitol (DTT). Samples were extracted using the Zymo MiniRNA Kit (Zymo Research) and cDNA was synthesized using the SuperScriptIII 1st Strand Synthesis SuperMix for qRT-PCR (Thermo Scientific). For qPCR analysis, cDNA samples were amplified and analyzed using a QuantStudio 6 Flex RealTime PCR System (Life Technologies). Assays were run using SYBR Green Master Mix (Qiagen) using the following cycling conditions: 50°C for 3 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C 30 seconds and 60°C for 1 minutes, and melt curve analysis completed from 60°C – 94°C. Primer sets used for SYBR Green qPCR analysis are as follows: PrimeTime qPCR Primer Assay for GusB (Hs.PT.56a.40743642; Integrated DNA Technologies); HRV-C15_Fwd 5'-CCT CCG GCC CCT GAA T-3'; HRV-C15_Rev 5'-AAA CAC GGA CAC CCA AAG TAG T-3' (8). TaqMan assays using PrimeTime qPCR 5' Nuclease probe assays for GusB (Hs.PT.51.2648420), CDHR3 (Hs.PT.58.28184603), FOXJ1 (Hs.PT.58.25051355), and Rhinovirus (Forward primer 5'- GGTCCCATCCCGCAATT-3', Reverse primer 5'- CCTCCGGCCCCTGAAT-3') were run using Brilliant III Master Mix (Agilent) using the following cycling conditions: 95°C for 3 minutes, followed by 40 cycles of 95°C 10 seconds and 60°C for 30 seconds. Expression of HRV-C15 was normalized to the airway epithelial housekeeping gene *GUSB* and genotype experiments were also normalized to the gene expression levels of *FOXJ1* to normalize for differences in differentiation across nasal cultures grown from all 12 subjects.

References

1. Kosmider B, Mason RJ, Bahmed K. Isolation and Characterization of Human Alveolar Type II Cells. *Methods Mol Biol.* 2018;1809:83-90.
2. Kumar R, Nguyen EA, Roth LA, Oh SS, Gignoux CR, Huntsman S, et al. Factors associated with degree of atopy in Latino children in a nationwide pediatric sample: the

Genes-environments and Admixture in Latino Asthmatics (GALA II) study. *J Allergy Clin Immunol.* 2013;132(4):896-905 e1.

3. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. *Am J Respir Crit Care Med.* 2013;188(3):309-18.
4. Chu HW, Rios C, Huang C, Wesolowska-Andersen A, Burchard EG, O'Connor BP, et al. CRISPR-Cas9-mediated gene knockout in primary human airway epithelial cells reveals a proinflammatory role for MUC18. *Gene Ther.* 2015;22(10):822-9.
5. Reynolds SD, Rios C, Wesolowska-Andersen A, Zhuang Y, Pinter M, Happoldt C, et al. Airway Progenitor Clone Formation Is Enhanced by Y-27632-Dependent Changes in the Transcriptome. *Am J Respir Cell Mol Biol.* 2016;55(3):323-36.
6. Everman JL, Rios C, Seibold MA. Utilization of Air-Liquid Interface Cultures as an In Vitro Model to Assess Primary Airway Epithelial Cell Responses to the Type 2 Cytokine Interleukin-13. *Methods Mol Biol.* 2018;1799:419-32.
7. Everman JL, Rios C, Seibold MA. Primary Airway Epithelial Cell Gene Editing Using CRISPR-Cas9. *Methods Mol Biol.* 2018;1706:267-92.
8. Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med.* 2011;17(5):627-32.
9. Wu TD, Nacu S. Fast and SNP-tolerant detection of complex variants and splicing in short reads. *Bioinformatics.* 2010;26(7):873-81.
10. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics.* 2015;31(2):166-9.
11. Wesolowska-Andersen A, Everman JL, Davidson R, Rios C, Herrin R, Eng C, et al. Dual RNA-seq reveals viral infections in asthmatic children without respiratory illness which are associated with changes in the airway transcriptome. *Genome Biol.* 2017;18(1):12.
12. Jiang H, Lei R, Ding SW, Zhu S. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. *BMC Bioinformatics.* 2014;15:182.
13. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol.* 2016;34(5):525-7.
14. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 2009;19(9):1655-64.
15. Torgerson DG, Gignoux CR, Galanter JM, Drake KA, Roth LA, Eng C, et al. Case-control admixture mapping in Latino populations enriches for known asthma-associated genes. *J Allergy Clin Immunol.* 2012;130(1):76-82 e12.
16. Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am J Hum Genet.* 2011;88(5):586-98.
17. Consortium GT, Laboratory DA, Coordinating Center -Analysis Working G, Statistical Methods groups-Analysis Working G, Enhancing Gg, Fund NIHC, et al. Genetic effects on gene expression across human tissues. *Nature.* 2017;550(7675):204-13.
18. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010;26(1):139-40.
19. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* 2012;40(10):4288-97.
20. Stegle O, Parts L, Piipari M, Winn J, Durbin R. Using probabilistic estimation of expression residuals (PEER) to obtain increased power and interpretability of gene expression analyses. *Nat Protoc.* 2012;7(3):500-7.

21. Ongen H, Buil A, Brown AA, Dermitzakis ET, Delaneau O. Fast and efficient QTL mapper for thousands of molecular phenotypes. *Bioinformatics*. 2016;32(10):1479-85.
22. Delaneau O, Ongen H, Brown AA, Fort A, Panousis NI, Dermitzakis ET. A complete tool set for molecular QTL discovery and analysis. *Nat Commun*. 2017;8:15452.

Supplemental Figure and Table Legends

Figure E1: Cilia cell gene ontology (GO) categories confirm ciliated cell cluster in single cell transcriptomics of human lung cell types.

GO terms for cilia and ciliated cell development (11 in total) were reduced to a single eigengene value and overlaid (purple) on the t-SNE feature plot of the identified cell types. 11 GO terms used for analysis are as following: GO:0044441: Ciliary Part, GO:0005929: Cilium, GO:0030286: Dynein Complex, GO:0044447: Axoneme Part, GO:0005874: Microtubule, GO:0005930: Axoneme, GO:0005875: Microtubule Associated Complex, GO:0005858: Axonemal Dynein Complex, GO:0005868: Cytoplasmic Dynein Complex, GO:0031514: Motile Cilium, GO:0036064: Ciliary Basal Body.

Figure E2: CDHR3 KO does not affect ciliated cell fate as measured by FOXJ1⁺ nuclei in differentiating ALI cultures

(A and B) Tracheal basal AECs were differentiated *in vitro* at ALI for immunofluorescence labeling. ALI day 14 cultures were co-labeled for FOXJ1 and DAPI and imaged by confocal microscopy. FOXJ1⁺ nuclei and total DAPI-labeled nuclei were quantified to determine the percentage of FOXJ1⁺ cells for each donor (A) and indicate no change ($p = 0.3782$) in the number of FOXJ1⁺ cells between differentiating control (scrb) and CDHR3 KO ALI cultures (A and B). Data are representative of the percentage of FOXJ1⁺ nuclei over 8 fields of view $\pm$ SD per CDHR3 KO or control (scrb) culture grown and analyzed in parallel ($n=3$ independent donors).

TABLE LEGENDS

Table E1: Demographic and clinical variables for lung, tracheal, and nasal tissue samples

Table E2: Demographic and clinical variables for GALA II subjects used in eQTL analysis

Table E3: Differentially expressed genes/cell type markers for identification of cell populations in single cell sequencing analysis of human lung cells (Top 50; adjusted p-value < 0.05)

Table E4: RNA-seq analysis of differential gene expression between control and CDHR3 KO ALI cultures

Table E5: Independent CDHR3 eQTL loci in the nasal airway epithelium of children

Table E6: Test for association of CDHR3 eQTL loci with asthma hospitalization in minority children