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The role of *CDHR3* in susceptibility to otitis media

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Code Availability: Not Applicable

Ethics Approval: Human studies were approved by these institutional review boards (IRB): Bahauddin Zakariya University; Colorado Multiple IRB; Helsinki University Hospital; University of Maryland Baltimore; University of Minnesota; University of Texas Medical Branch; University of Virginia; University of Washington. Informed consent was obtained from all study participants. Mouse studies were approved by the Institutional Animal Care and Use Committees of the Veterans Affairs Medical Center, San Diego.

Web Resources

ANNOVAR, annovar.openbioinformatics.org

Burrows-Wheeler Aligner, bio-bwa.sourceforge.net

Combined Annotation Dependent Depletion, cadd.gs.washington.edu

dbNSFP, sites.google.com/site/jpopgen/dbNSFP

Explicet, www.explicet.org

Genetic Power Calculator, www.bwh.harvard.edu/gpc/

Genetic Variant Interpretation Tool, www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/

Genome Aggregation Database, gnomad.broadinstitute.org

Genome Analysis Toolkit, gatk.broadinstitute.org

Genotype-Tissue Expression (GTEx) Portal, gtexportal.org

InterPro, www.ebi.ac.uk/intepro/

Likelihood Ratio Test, www.genetics.wustl.edu/jflab/lrt_query.html

MutationAssessor, mutationassessor.org/r3/

MutationTaster, www.mutationtaster.org

Online Mendelian Inheritance in Man, www.omim.org

PolyPhen-2, genetics.bwh.harvard.edu/pph2/

PROVEAN, provean.jcvi.org

silva, www.arb-silva.de

Seurat, satijalab.org/seurat/

UCSC Genome Browser, genome.ucsc.edu

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Abstract

Otitis media (OM) is common in young children and can cause hearing loss and speech, language and developmental delays. OM has high heritability, however little is known about OM-related molecular and genetic processes. *CDHR3* was previously identified as a locus for OM susceptibility, but to date studies have focused on how the *CDHR3* p.Cys529Tyr variant increases epithelial binding of rhinovirus-C and risk for lung or sinus pathology. In order to further delineate a role for *CDHR3* in OM, we performed: exome sequencing using DNA samples from OM-affected individuals from 257 multi-ethnic families; Sanger sequencing, logistic regression and transmission disequilibrium tests for 407 US trios or probands with OM; 16S rRNA sequencing and analysis for middle ear and nasopharyngeal samples; and single-cell RNA sequencing and differential expression analyses for mouse middle ear. From exome sequence data, we identified a novel pathogenic *CDHR3* splice variant that co-segregates with OM in US and Finnish families. Additionally, a frameshift and six missense rare or low-frequency variants were identified in Finnish probands. In US probands, the *CDHR3* p.Cys529Tyr variant was associated with absence of middle ear fluid at surgery, and also with increased relative abundance of *Lysobacter* in the nasopharynx and *Streptomyces* in the middle ear. Consistent with published data on airway epithelial cells and our RNA-sequence data from human middle ear tissues, *Cdhr3* expression is restricted to ciliated epithelial cells of the middle ear and is downregulated after acute OM. Overall these findings suggest a critical role for *CDHR3* in OM susceptibility.

Keywords

CDHR3; expression; microbiome; middle ear; otitis media; rs6967330

Introduction

Otitis media (OM), defined as inflammation of the middle ear (ME) that is usually due to viral or bacterial infection, affects 46% of children <1-year-old, with ~30% having ≥3 episodes by 3 years old [1,2]. OM can cause hearing loss in young children, leading to speech and learning difficulties during a sensitive time period for cognitive development [3]. Often the ME fluid (MEF) and hearing loss resolve spontaneously or after antibiotic treatment. However, 4-10% of children with OM require tympanostomy tube (TT) surgery due to more severe forms of recurrent acute (RA)OM or chronic OM with effusion (COME) [4,5]. These OM types and TT surgery are associated with at least 4× risk of permanent hearing loss [4]. OM is a multifactorial disease due to: (1) environmental factors -- secondhand smoke exposure, upper respiratory infections (URI), older siblings, daycare attendance, and lack of breastfeeding; (2) clinical factors -- craniofacial abnormalities, syndromes, and allergies; and (3) family history and genetic factors [2,6-8]. Several studies have delved into the genetic component of OM, with heritability estimates of 22-74% [9-11].

A genome-wide association study (GWAS) involving >120,000 European-descent individuals identified the synonymous variant rs114947103 within *CDHR3* (MIM 615610) as a protective locus against childhood ear infections [OR=0.93; 95%CI:0.91,0.96; $p=5.4x10^{-9}$] [12,13]. This OM locus is in linkage disequilibrium (LD) with the known variant *CDHR3* c.1586G>A (p.Cys529Tyr; rs6967330), which increases risk of chronic rhinosinusitis and asthma exacerbations in childhood [12-15]. *CDHR3* encodes a protein

from the cadherin superfamily of transmembrane calcium-dependent adhesion proteins and is involved in cell adhesion, epithelial polarity, cell-cell interaction, and differentiation [16]. Unlike other cadherins which are diffusely expressed in many cell types, CDHR3 is limited to the apical membrane of ciliated epithelial cells in the respiratory tract, and is expressed primarily in cilia [15,17-19]. CDHR3 is the predominant receptor for rhinovirus-C (RV-C) [18]. After RV-C binds to CDHR3 and adheres to cilia, there is diffuse apical shedding of intact epithelial cells, which is hypothesized to be due to patchy cellular lysis within the epithelium, which in turn may lead to impaired barrier function and bacterial superinfection [18]. *In vitro*, CDHR3-knockout (KO) airway epithelial cells (AEC) of the trachea appeared normal and proliferated similarly to control basal cells [17]. However, CDHR3-KO AECs had reduced transepithelial electrical resistance, likely indicating an effect on junction barrier function [17]. Alternatively, in cells transfected with or derived from carriers of the p.Cys529Tyr variant, surface expression of CDHR3 was increased, and binding and replication of RV-C also increased [15,17,19-20]. Because the *CDHR3* p.Cys529Tyr variant facilitates binding and replication of RV-C, this variant has undergone balancing selection in human populations [21]. In chimpanzees with the ancestral CDHR3-Y_{529} variant, infection with human RV-C caused an 8.9% mortality rate [22]. It is now established that the increased risk for hospitalization among p.Cys529Tyr carriers with early-onset asthma is due to acute exacerbations from RV-C infection in both children and adults [15,23].

Except for the OM GWAS finding, to date there are no reports of additional OM-related *CDHR3* variants or studies. To further define the role of *CDHR3* in OM susceptibility, we examined our multi-omic data, including DNA sequence and microbiome data from families with OM, and transcriptomic data from mouse ME. Our goal was to determine if *CDHR3* variants are associated with OM susceptibility and microbiota shifts in the ME or nasopharynx (NP), and if *Cdhr3* is expressed in the ME mucosal epithelium.

Materials and Methods

Subject Ascertainment

The study was approved by the following institutional review boards (IRB): Bahauddin Zakariya University; Colorado Multiple IRB; Helsinki University Hospital; University of Maryland Baltimore; University of Minnesota; University of Texas Medical Branch; University of Virginia; University of Washington. Informed consent was obtained from adult participants and parents of children enrolled in the study. Approval was also obtained from the Institutional Animal Care and Use Committee of the Veterans Affairs Medical Center, San Diego, California.

Families from different cohorts were first identified upon referral of a child or proband for OM surgery, except for the Pakistani families (Online Resource 1). DNA samples were obtained from two established family cohorts for OM, namely Minnesotan [24] and Finnish families [11]. In Minnesota, all family members were examined by an otolaryngologist and tested by tympanometry for ME function, then they were considered affected if ≥2 data sources -- whether otoscopy, tympanometry, medical records or personal history -- were OM-positive. For Finnish families, clinical data, including history, risk factors for OM and details from otolaryngologic surgery, were obtained. Finnish individuals were considered

OM-positive if they had TT surgery, COME with MEF >2 months, or RAOM, i.e. >3 episodes of acute infection in 6 months or >4 episodes in 12 months. Of the 245 Finnish and Minnesotan families with exome data, 116 (47%) were trios, 64 (26%) were proband-parent pairs, and the rest had >1 OM-affected child.

For this study, 316 Texan and 91 Coloradan trios were enrolled (Online Resource 1). Each trio included a child with OM and both biological parents who provided saliva samples using Oragene DNA collection kits (DNAgenotek, Ontario, Canada), and from which DNA was isolated. The Pakistani families were identified from communities in which families were known to have multiple children with discharging ears. Twelve families with OM were enrolled from the southern Punjab province of Pakistan (Online Resource 1). Age-at-onset and recurrence or chronicity of OM episodes were determined from medical history and OM status by otoscopy. DNA was extracted from blood samples provided by family members. For all cohorts, individuals with known genetic, craniofacial and immunodeficiency syndromes were excluded.

Human DNA Sequencing

DNA samples from OM-positive individuals from 217 Finnish, 28 Minnesotan, and 12 Pakistani families were submitted for exome sequencing as previously described [25]. In brief, sequence capture was performed using the Roche NimbleGen SeqCap EZ Human Exome Library v.2.0 (37Mb) for Finnish and Minnesotan families, while the Agilent SureSelect Human Expanded All Exon V5 (62Mb) was used for Pakistani families. Exome sequencing was performed to an average depth of ~60× using an Illumina HiSeq. Burrows-Wheeler Aligner [26] and the Genome Analysis Toolkit [27] were used to generate .bam and .vcf files, respectively.

Sanger sequencing for *CDHR3* (NM_152750.4) was performed for: (1) four common missense variants in 399 US trios; and (2) selected variants in Minnesotan, Finnish, and Pakistani families (Table 1, Table 2). Sanger sequencing was performed on Finnish and Minnesotan families with ≥2 OM-positive individuals to test whether the *CDHR3* variant co-segregated with OM (Figure 1, Online Resource 2, Online Resource 3).

Bioinformatic and Statistical Analyses

ANNOVAR [28] was used to annotate .vcf files, with data retrieved from hg19 databases refGene, gnomad_exome [29] and dbNSFP41a [30]. Frameshift, missense, and splice variants were selected for further examination (Table 1). In US trios, the transmission disequilibrium test (TDT) was performed for *CDHR3* variants (Table 2) [31]. The TDT is robust against population stratification, substructure and admixture, allowing the inclusion of multi-ethnic US trios in TDT analysis [31].

To define the OM sub-phenotype due to carriage of *CDHR3* variants, the clinical records of 91 Coloradan probands were reviewed for: demographic data -- age, sex, ethnicity, breastfeeding history, family history of OM, daycare attendance; clinical data -- comorbidities, antibiotic use, surgical findings, diagnoses of RAOM and/or COME; and genotypes for two OM-related variants *FUT2* p.Trp154* and *PLG* p.Asp472Asn (Table 3) [25,32]. Bivariate analysis using standard statistical methods (chi-square, t-test) was

conducted with each variable against presence of MEF. Multivariate logistic regression was performed using the glm function (family=binomial) from the MASS package in R with presence of MEF as outcome variable, and age, sex, ethnicity, daycare attendance, and the p.Cys529Tyr variant as determinant variables (Table 4).

16S rRNA Sequencing and Microbiome Analysis

Ninety-three ME samples (43 swabs, 50 aspirates) and 80 NP swabs from Coloradan probands were submitted for 16S ribosomal RNA gene sequencing and analysis, as previously described [25]. Each batch of 16S rRNA gene PCR and sequencing included ≥3 negative process controls in order to test for contaminating bacterial DNA in reagents. After sequence alignment and filtering, 37 ME and 40 NP samples were used for biodiversity and taxa abundance analyses. Essential details of the sequencing and analyses steps include: (a) generation of amplicons using primers targeting ~300bp of the V1-V2 variable regions of the 16S rRNA gene; (b) paired-end sequencing on an Illumina MiSeq; (c) exclusion of paired-end sequences that aligned to the human reference hg19 data; (d) assembly, trimming, chimera removal, and alignment and classification of paired reads with SINA (1.3.0-r23838) [33] using the bacterial sequences in Silva 115NR99 [34]; and (e) clustering of sequences with identical assignments of Silva taxonomy to generate operational taxonomic units (OTUs). PERMANOVA tests using Bray-Curtis dissimilarity scores with 10^6-10^7 permutations were performed according to carriage of the p.Cys529Tyr variant. Standard measures of alpha-diversity (Chao1, Shannon H/H_{max}, Shannon diversity) were calculated according to carriage of the p.Cys529Tyr variant. For OTUs, data analysis and generation of figures were performed using R and Explicet v2.10.8 [35].

Expression of Cdhr3 mRNA in Normal and Infected Mouse ME

Cdhr3 expression was analyzed using microarray mRNA data from ME tissues of 60-90-day-old wildtype C57/WB F1 hybrid mice (Jackson Lab, Bar Harbor, ME) that were inoculated with the human otopathogen non-typeable *Haemophilus influenzae* (NTHi) to induce AOM [36]. The ME bullae of control mice were inoculated with placebo. ME tissues were harvested at 0h (control), 3h, 6h, 1d, 2d, 3d, 5d and 7d after inoculation to obtain two independent biological replicates of the OM time course. The expression of *Cdhr3* was normalized and compared to that in the control (untreated) ME tissues.

Three single-cell samples were generated from C57-BL6 mice. For each sample, ME mucosal tissue was harvested from both ears of six young adult mice. The tissue was enzymatically digested and triturated into single-cell suspensions. Cell viability, assessed by trypan blue exclusion, was >95%. Single-cell libraries were generated using the 10X Genomics (Pleasanton, CA, USA) Chromium Single Cell 3' Reagent Kit V2. cDNA synthesis, barcoding, and library preparation were carried out on a 10X Genomics Chromium Controller. cDNA library quality was validated by electrophoresis and the libraries were sequenced on an Illumina HiSeq 2500 with a read length of 26 bp for read 1 (cell barcode and unique molecule identifier (UMI)), 8 bp i7 index read (sample barcode), and 98 bp for read 2 (actual RNA read). Reads were demultiplexed using 10X Genomics Cellranger 2.0.2 and mkfastq in conjunction with bcl2fastq 2.17.1.14 (Illumina) and aligned to the murine reference genome (mm10 with annotations from Ensembl, release 84). 10X

Genomics Cellranger aggr and Seurat [37] were used to generate principal components (PC) clustering. The expression of well-recognized marker genes identified 17 distinct cell types. Linearized relative expression levels for *Cdhr3* were log-transformed from single-cell mRNA copy numbers, normalized, and scaled for each cell type [38]. Clustering was visualized using 10X Genomics cLoupe, based on 10X Genomics CellRanger PC and expression level analysis.

Results

Identification of potential risk variants conferring OM susceptibility

From exome sequence data, we identified 17 *CDHR3* variants (Table 1), of which five were common (minor allele frequency or MAF>0.05 in any population in the Genome Aggregation Database or gnomAD), seven were low-frequency (MAF=0.01-0.05) and five were rare (MAF<0.01). Of the 17 variants, ten single nucleotide variants, including the common variant p. Val55Met and the low-frequency splice variant c.1653+3G>A, had scaled Combined Annotation Dependent Depletion (CADD) [39] scores >10 and/or were predicted to be deleterious by at least one bioinformatic prediction tool, suggesting that these are potential risk variants for OM.

Three putative loss-of-function (LOF) *CDHR3* variants were identified: the frameshift variant c.1310delC (p.(Pro439Leufs*12)) and non-canonical splice variants c.862+30G>A and c.1653+3G>A. Of these LOF variants, the c.1310delC and c.1653+3G>A variants were predicted to be pathogenic by MutationTaster [40]. The c.1310delC variant was identified in a family with one OM-affected child (Table 1). On the other hand, the c.1653+3G>A variant was heterozygous in all affected siblings in each of two families UMN123 and FIN142 (Figure 1) and in the affected probands of six additional Finnish families (Table 1). In one family UMN48, the c.1653+3G>A variant was present in only two out of three OM-affected siblings; however family UMN48 has a known *PLG* c.112A>G (p.Lys38Glu) variant that co-segregates with OM (Figure 1) [32].

The c.862+30G>A variant and missense variants p.Ala476Thr, p.Arg603Cys and p.Pro752Thr were Sanger-sequenced using DNA samples from Minnesotan and Pakistani families, but these variants did not co-segregate with OM, suggesting that they are not OM-related (Online Resource 2, Online Resource 3). In contrast, six rare or low-frequency, deleterious missense variants – p.Val208Met, p.Pro355Leu, p.Pro379Ser, p.Leu506Phe, p.Tyr729His and p.Ala798Ser – were identified, but only in 1-2 probands per variant (Table 1). Taken together, based on co-segregation with RAOM+COME in multiple families, the c.1653+3G>A variant is a novel, LOF variant that confers risk for OM susceptibility. This splice variant is classified as pathogenic by the American College of Medical Genetics and Genomics (ACMG) / Association for Molecular Pathology (AMP) Guidelines [41]. On the other hand, the c.1310delC and six missense variants were identified as variants of unknown significance (VUS).

Elucidation of the role of the CDHR3 p.Cys529Tyr variant in OM susceptibility

In previous studies, the common variant p.Cys529Tyr was shown to increase susceptibility to RV-C in AECs [17,19-20], but is in LD with an intronic *CDHR3* variant that was deemed protective against OM in previous GWAS [12]. To potentially delineate the role of common missense *CDHR3* variants in OM susceptibility, four variants were Sanger-sequenced in 316 Texan and 83 Coloradan trios with OM (Table 1, Table 2). In these trios, 286 (81.5%) were diagnosed to have RAOM while the rest had COME. In the combined trio cohort, OM probands carrying the p.Cys529Tyr and/or p.Thr532Ser variants were more likely to have RAOM than COME (p=0.02). However, in these 399 trios, the TDT results were not significant (Table 2).

Although all the Coloradan children (n=91) had a history of RAOM or COME, 30 (33.0%) children did not have MEF at the time of surgery (Table 3). In patients with MEF, 36 (39.6%) had serous or mucoid and 15 (16.5%) had purulent MEF. Another 10 children had cholesteatoma and mixed type of MEF. Absence of MEF was associated with carriage of the p.Cys529Tyr variant (p=0.04) and RAOM diagnosis (p=0.004; Table 3), both of which remained significant after adjustment for multiple variables (Table 4).

In the ME and NP samples from the Colorado cohort, there were no differences in alpha- or beta-diversity according to *CDHR3* p.Cys529Tyr genotype (data not shown). Additionally, relative abundance of known otopathogens was not increased. However, the relative abundance of *Streptomyces* was greater in ME samples (nominal-p=0.04) and *Lysobacter* in the NP swabs from carriers of the p.Cys529Tyr variant (nominal-p=0.01; Figure 2).

To summarize, the p.Cys529Tyr variant was not significant in TDT and was not associated with increased biodiversity nor bacterial otopathogens in the ME or NP. On the contrary, this variant was associated with increased relative taxa abundance of commensals in the ME and NP and with lack of MEF at surgery.

Cdhr3 Expression in Mouse ME

Single-cell RNA sequencing of non-infected mouse ME revealed that *Cdhr3* expression is restricted to ciliated epithelial cells of mouse ME (Online Resource 4), a finding that is consistent with expression in lung [17] and other ciliated epithelial tissues [GTEx]. In wildtype mouse ME epithelium, *Cdhr3* is downregulated 3 hours after inoculation with NTHi (Figure 3; Table 5) [36], further supporting a role for *CDHR3* in OM pathology.

Discussion

CDHR3 was previously implicated in OM and other respiratory phenotypes through GWAS and cell transfection studies with mixed results, with the p.Cys529Tyr variant being implicated in RV-C infection thereby increasing risk of asthma and chronic sinusitis [14,15], while being in LD with an intronic variant that was deemed protective against OM in GWAS [12,13]. In this report, our mouse expression data strongly suggests a role for *Cdhr3* in OM, with selective expression in ciliated epithelial cells in the ME (Online Resource 4). Additionally, *Cdhr3* is significantly downregulated in NTHi-induced AOM (Figure 3,

Table 5), which is consistent with our previous finding of lower *CDHR3* expression in human cholesteatoma compared to ME mucosal tissue within the context of chronic OM (transcriptome-wide-adj-p=0.004) [42]. Because CDHR3 is involved in epithelial integrity, reduced CDHR3 levels could lead to increased permeability of the airway epithelium and therefore increased vulnerability to infection [17]. In CDHR3(+) human bronchial AECs, inoculation with RV-C decreased the intensity of CDHR3 staining [18]. It was hypothesized that this reduced CDHR3 staining upon RV-C infection is due to viral shutoff of host protein synthesis, down-regulation after internalization of the RV-C-CDHR3 receptor complex, and/or lysis of infected cells [18].

Using exome sequence data, we identified novel *CDHR3* variants in families and probands with OM (Table 1), including a pathogenic splice variant c.1653+3G>A. Of the 17 *CDHR3* variants that we identified, only two variants p.Val55Met and p.Gln61His lie within the extracellular domain 1 (EC1) which is known to interface with RV-C; however these two variants do not affect amino acid residues that directly interact with RV-C [43,44], which might explain why these variants were not significant in TDT analyses (Table 2). Interestingly, LOF variants such as the frameshift variant c.1310delC and splice variant c.1653+3G>A are predicted to cause loss of the EC6 and transmembrane (TM) domains, which would affect protein folding and transcellular adhesion. Four missense VUS -- p.Val208Met, p.Pro355Leu, p.Pro379Ser and p.Leu506Phe -- lie within cadherin repeat domains EC2, EC4 or EC5, while two VUS – p.Tyr729His and p.Ala798Ser – respectively lie within the transmembrane domain and cytoplasmic tail, indicating the potential deleteriousness of these variants to CDHR3 protein structure [15,43,44].

The p.Cys529Tyr variant occurs at the interface of the EC5 and EC6 domains, which help stabilize Ca^{++} binding and protein folding, thereby enabling the CDHR3 protein to be trafficked from the endoplasmic reticulum to the Golgi apparatus [43]. Remarkably while either cysteine or tyrosine at position 529 facilitates Golgi transport, the p.Cys529Tyr variant allows for longer cell surface expression of CDHR3, which in turn facilitates RV-C binding [15,43]. Previously the *CDHR3* p.Cys529Tyr variant was associated with risk for asthma exacerbations and rhinosinusitis [14,15]. However, based on our findings, the role of this common variant in OM is more nuanced. While the previous GWAS showed that the variant that is in LD with p.Cys529Tyr is protective against OM, we did not identify a significant association between p.Cys529Tyr variant with OM in our trios, whether as a risk or protective variant (Table 2). Subsetting by RAOM or COME diagnoses also did not lead to significant TDT results (data not shown). We did find associations between the p.Cys529Tyr variant and (a) lack of MEF at surgery (Table 4) and (b) non-otopathogenic taxa in the ME and NP (Figure 2). These findings fit best with a clinical profile of viral RAOM in children who carry the p.Cys529Tyr variant. Alternatively, because *Streptomyces* and *Lysobacter* are known to produce antibiotic metabolites that may help prevent infection by other opportunistic bacteria [45-47], our findings might also suggest the *CDHR3* p.Cys529Tyr variant may protect against bacterial rather than viral OM. This phenomenon, in which genetic variants may shift the microbiome by facilitating specific microbes while decreasing relative abundance of other taxa, has been demonstrated for *FUT2*-related OM [25]. If spontaneous resolution of MEF prior to OM surgery is observed in carriers of the

p.Cys529Tyr variant, the need for TT surgery or antibiotic treatments in the absence of bacterial superinfection after viral URI or AOM may be obviated.

The limitations of this study include: the finding of rare variants in 1-2 trios rather than in families with multiple OM-affected relatives, precluding testing of co-segregation of variants with OM; the inability to assess viruses due to microbial collection technique; and limited sample sizes for analysis which are likely the cause for failure to reach significance for some experiments (e.g. relative taxa abundance after correction for multiple testing using the Benjamini-Hochberg procedure for false discovery rate). Given MAF 0.07-0.20 for the common variants tested, our trio cohort has sufficient power to detect an association using TDT for genotypic relative risks ≥1.55 [48], but not for effect sizes ≤1.5. The increase in non-transmitted alleles for the p.Gln61His and p.Cys529Tyr in the Texan trios might suggest that these variants are protective against OM (Table 2), however the TDT for these variants were non-significant.

To summarize, we identified novel rare and low-frequency *CDHR3* variants that confer risk for OM in a multi-ethnic cohort of families, which is supported by restricted expression of *Cdhr3* in ciliated epithelial cells of the ME. Knowledge of these variants will help identify individuals who are at risk for RAOM plus COME. On the other hand, the p.Cys529Tyr variant was associated with absence of MEF even after adjustment for multiple covariates, suggesting that the clinical and microbial effects we observed were not due to OM diagnosis or antibiotic use. The unnecessary use of antibiotics may have effects on other organ systems and promote antibiotic resistance. By screening OM patients for the common *CDHR3* p.Cys529Tyr variant, we may be able to select patients who might have less benefit from standard treatments (TT insertion, antibiotics) due to spontaneous MEF resolution and may instead be targeted for rhinoviral or *CDHR3*-based therapies or vaccines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Chonmaitree T, Trujillo R, Jennings K, Alvarez-Fernandez P, Patel JA, Loeffelholz MJ, Nokso-Koivisto J, Matalon R, Pyles RB, Miller AL et al. (2016) Acute Otitis Media and Other Complications of Viral Respiratory Infection. Pediatrics 137:e20153555. [PubMed: 27020793]
2. Brennan-Jones CG, Whitehouse AJ, Park J, Hegarty M, Jacques A, Eikelboom RH, Swanepoel DW, White JD, Jamieson SE (2015) Prevalence and risk factors for parent-reported recurrent otitis media during early childhood in the Western Australian Pregnancy Cohort (Raine) study. J Paediatr Child Health 51:403–409. [PubMed: 25303240]
3. Su JY, Guthridge S, He VY, Howard D, Leach AJ (2020) Impact of hearing impairment on early childhood development in Australian Aboriginal children: A data linkage study. J Pediatr Child Health 56: 1597–1606.
4. Beyea JA, Cooke B, Rosen E, Nguyen P (2020) Association of tympanostomy tubes with future assistive hearing devices-a population based study. BMC Pediatr 20:76. [PubMed: 32070307]
5. Bhattacharyya N, Shay SG (2020) Epidemiology of Pediatric Tympanostomy Tube Placement in the United States. Otolaryngol Head Neck Surg 163:600–602. [PubMed: 32284005]
6. de Jong T, Toll MS, de Gier HHW, Mathijssen IM (2011) Audiological profile of children and young adults with syndromic and complex craniosynostosis. Arch Otolaryngol Head Neck Surg 137:775–778. [PubMed: 21844411]
7. Zhang Y, Xu M, Zhang J, Zeng L, Wang Y, Zheng QY (2014) Risk factors for chronic and recurrent otitis media-a meta-analysis. PLoS One 9:e86397. [PubMed: 24466073]
8. Kaur R, Morris M, Pichichero ME (2017) Epidemiology of acute otitis media in the postpneumococcal conjugate vaccine era. Pediatrics 140:e20170181 [PubMed: 28784702]
9. Casselbrant ML, Mandel EM, Fall PA, Rockette HE, Kurs-Lasky M, Bluestone CD, Ferrell RE (1999) The heritability of otitis media: a twin and triplet study. JAMA 282:2125–2130. [PubMed: 10591333]
10. Casselbrant ML, Mandel EM, Rockette HE, Kurs-Lasky M, Fall PA, Bluestone CD, Ferrell RE (2004) The genetic component of middle ear disease in the first 5 years of life. Arch Otolaryngol Head Neck Surg 130:273–278. [PubMed: 15023832]
11. Hafren L, Kentala E, Jarvinen TM, Leinonen E, Onkamo P, Kere J, Mattila PS (2012) Genetic background and the risk of otitis media. Int J Pediatr Otorhinolaryngol 76:41–44. [PubMed: 22018929]
12. Pickrell JK, Berisa T, Liu JZ, Segurel L, Tung JY, Hinds DA (2016) Detection and interpretation of shared genetic influences on 42 human traits. Nat Genet 48:709–717. Supplement file: NIHMS780506-supplement-8.pdf [PubMed: 27182965]
13. Tian C, Hromatka BS, Kiefer AK, Eriksson N, Noble SM, Tung JY, Hinds DA (2017) Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nat Commun 8:599. Supplement file: 41467_2017_257_MOESM1_ESM.pdf [PubMed: 28928442]
14. Chang EH, Willis AL, McCrary HC, Noutsios GT, Le CH, Chiu AG, Mansfield CJ, Reed DR, Brooks SG, Adappa ND et al. (2017) Association between the CDHR3 rs6967330 risk allele and chronic rhinosinusitis. J Allergy Clin Immunol 139:1990–1992 e1992. [PubMed: 27923563]
15. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, den Dekker HT, Husby A, Sevelsted A, Faura-Tellez G et al. (2014) A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet 46:51–55. [PubMed: 24241537]
16. Hulpiau P, van Roy F (2009) Molecular evolution of the cadherin superfamily. Int J Biochem Cell Biol 41:349–369. [PubMed: 18848899]
17. Everman JL, Sajuthi S, Saef B, Rios C, Stoner AM, Numata M, Hu D, Eng C, Oh S, Rodriguez-Santana J et al. (2019) Functional genomics of CDHR3 confirms its role in HRV-C infection and childhood asthma exacerbations. J Allergy Clin Immunol 144:962–971. [PubMed: 30930175]
18. Griggs TF, Bochkov YA, Basnet S, Pasic TR, Brockman-Schneider RA, Palmenberg AC, Gern JE (2017) Rhinovirus C targets ciliated airway epithelial cells. Respir Res 18::84 [PubMed: 28472984]

19. Basnet S, Bochkov YA, Brockman-Schneider RA, Kuipers I, Aesif SW, Jackson DJ, Lemanske RF Jr., Ober C, Palmenberg AC, Gern JE (2019) CDHR3 Asthma-Risk Genotype Affects Susceptibility of Airway Epithelium to Rhinovirus C Infections. Am J Respir Cell Mol Biol 61:450–458. [PubMed: 30916989]
20. Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, Palmenberg AC, Gern JE (2015) Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad Sci U S A 112:5485–5490. [PubMed: 25848009]
21. O'Neill MB, Laval G, Teixeira JC, Palmenberg AC, Pepperell CS (2019) Genetic susceptibility to severe childhood asthma and rhinovirus-C maintained by balancing selection in humans for 150,000 years. Hum Mol Genet 29:736–744.
22. Scully EJ, Basnet S, Wrangham RW, Muller MN, Otali E, Hyeroba D, Grindle KA, Pappas TE, Thompson ME, Machanda Z et al. (2018) Lethal Respiratory Disease Associated with Human Rhinovirus C in Wild Chimpanzees, Uganda, 2013. Emerg Infect Dis 24:267–274. [PubMed: 29350142]
23. Kanazawa J, Masuko H, Yatagai Y, Sakamoto T, Yamada H, Kaneko Y, Kitazawa H, Iijima H, Naito T, Saito T et al. (2017) Genetic association of the functional CDHR3 genotype with early-onset adult asthma in Japanese populations. Allergol Int 66:563–567. [PubMed: 28318885]
24. Sale MM, Chen WM, Weeks DE, Mychaleckyj JC, Hou X, Marion M, Segade F, Casselbrant ML, Mandel EM, Ferrell RE et al. (2011) Evaluation of 15 functional candidate genes for association with chronic otitis media with effusion and/or recurrent otitis media (COME/ROM). PLoS One 6:e22297. [PubMed: 21857919]
25. Santos-Cortez RLP, Chiong CM, Frank DN, Ryan AF, Giese APJ, Bootpetch Roberts T, Daly KA, Steritz MJ, Szeremeta W, Pedro M et al. (2018) FUT2 Variants Confer Susceptibility to Familial Otitis Media. Am J Hum Genet 103:679–690. [PubMed: 30401457]
26. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. [PubMed: 19451168]
27. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303. [PubMed: 20644199]
28. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164. [PubMed: 20601685]
29. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. [PubMed: 27535533]
30. Liu X, Wu C, Li C, Boerwinkle E (2016) dbNSFP v3.0: A One-Stop Database of Functional Predictions and Annotations for Human Nonsynonymous and Splice-Site SNVs. Hum Mutat 37:235–241. [PubMed: 26555599]
31. Spielman RS, McGinnis RE, Ewens WJ (1993) Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516 [PubMed: 8447318]
32. Bootpetch TC, Hafren L, Elling CL, Baschal EE, Manichaikul AW, Pine HS, Szeremeta W, Scholes MA, Cass SP, Larson ED et al. (2020) Multi-omic studies on missense PLG variants in families with otitis media. Sci Rep 10:15035. [PubMed: 32929111]
33. Pruesse E, Peplies J, Glockner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. [PubMed: 22556368]
34. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–596. [PubMed: 23193283]
35. Robertson CE, Harris JK, Wagner BD, Granger D, Browne K, Tatem B, Feazel LM, Park K, Pace NR, Frank DN (2013) Explicet: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data. Bioinformatics 29:3100–3101. [PubMed: 24021386]

36. Hernandez M, Leichtle A, Pak K, Webster NJ, Wasserman SI, Ryan AF (2015) The transcriptome of a complete episode of acute otitis media. BMC Genomics 16:259. [PubMed: 25888408]
37. Satija R, Farrell JA, Gennert D, Schier AF, Regev A (2015) Spatial reconstruction of single-cell gene expression data. Nat Biotechnol 33:495–502. [PubMed: 25867923]
38. Ryan AF, Nasamran CA, Pak K, Draf C, Fisch K, Webster N, Kurabi A (2020) Single-cell transcriptomes reveal a complex cellular landscape in the middle ear and differential capacities for acute response to infection gene expression by cell types in the middle ear. Front Genet 11:358. [PubMed: 32351546]
39. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46:310–315 [PubMed: 24487276]
40. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D (2010) MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 7:575–576. [PubMed: 20676075]
41. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–423. [PubMed: 25741868]
42. Baschal EE, Larson ED, Bootpetch Roberts TC, Pathak S, Frank G, Handley E, Dinwiddie J, Moloney M, Yoon PJ, Gubbels SP et al. (2020) Identification of Novel Genes and Biological Pathways That Overlap in Infectious and Nonallergic Diseases of the Upper and Lower Airways Using Network Analyses. Front Genet 10:1352. [PubMed: 32010199]
43. Watters K, Palmenberg AC (2018) CDHR3 extracellular domains EC1-3 mediate rhinovirus C interaction with cells and as recombinant derivatives, are inhibitory to virus infection. PLoS Pathog 14:e1007477. [PubMed: 30532249]
44. Lee W, Frederick RO, Tonelli M, Palmenberg AC (2021) Solution NMR Determination of the CDHR3 Rhinovirus-C Binding Domain, EC1. Viruses 13:159. [PubMed: 33499226]
45. Liras P (1999) Biosynthesis and molecular genetics of cephamycins. Cephamycins produced by actinomycetes. Antonie Van Leeuwenhoek 75:109–124. [PubMed: 10422584]
46. Xie Y, Wright S, Shen Y, Du L (2012) Bioactive natural products from Lysobacter. Nat Prod Rep 29:1277–1287. [PubMed: 22898908]
47. Ye Y, Xia Z, Zhang D, Sheng Z, Zhang P, Zhu H, Xu N, Liang S (2019) Multifunctional Pharmaceutical Effects of the Antibiotic Daptomycin. Biomed Res Int 2019:8609218. [PubMed: 31263709]
48. Purcell S, Cherny SS, Sham PC (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19:149–150. [PubMed: 12499305]

Key Messages

- Novel rare or low-frequency *CDHR3* variants putatively confer risk for otitis media
- Pathogenic variant *CDHR3* c.1653+3G>A was found in nine families with otitis media
- *CDHR3* p.Cys529Tyr was associated with lack of effusion and bacterial otopathogens
- *Cdhr3* expression was limited to ciliated epithelial cells in mouse middle ear
- *Cdhr3* was downregulated 3 hours after infection of mouse middle ear

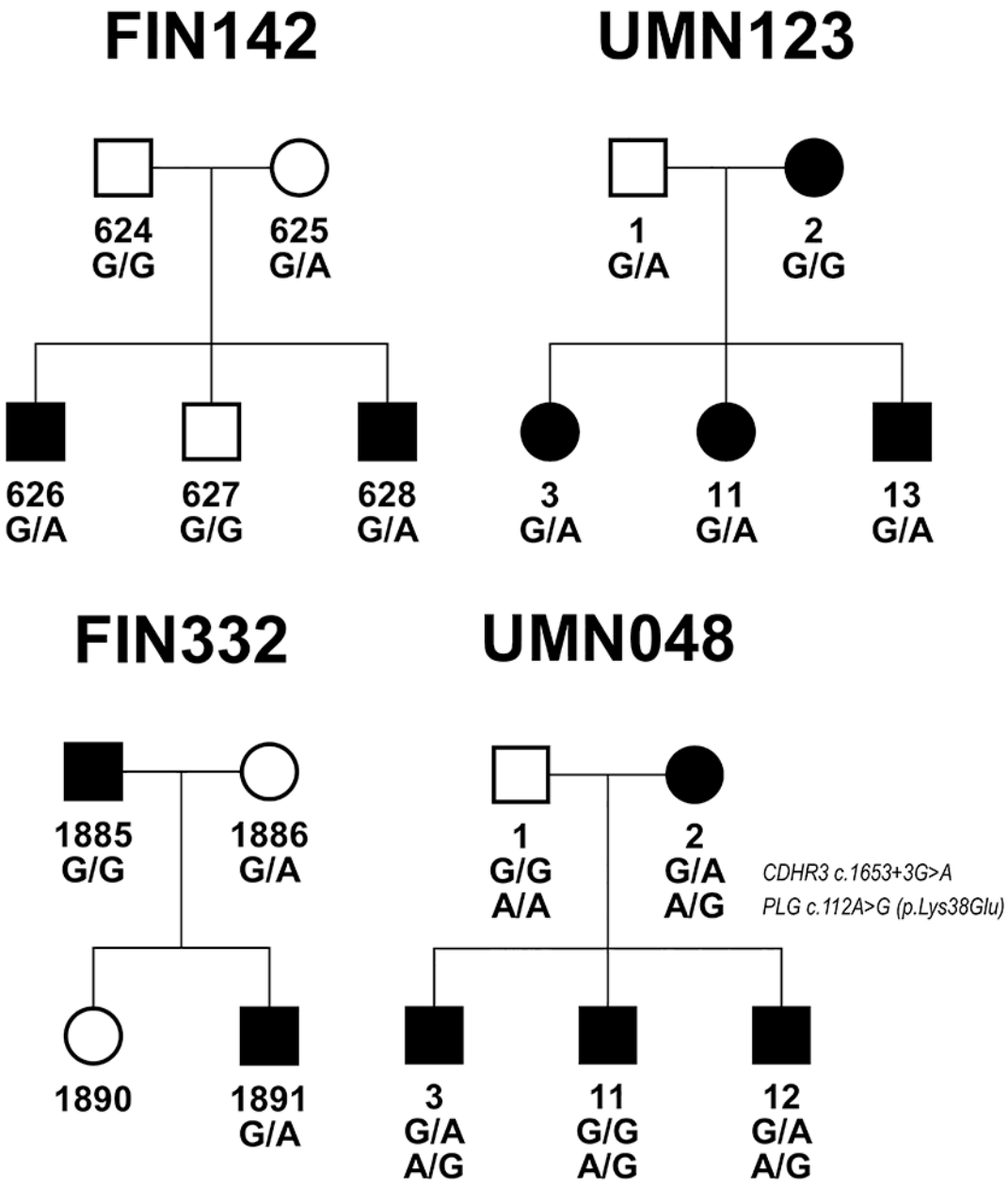

Figure 1.

Three European-descent families with co-segregation of the same *CDHR3* splice variant c.1653+3G>A (rs117797654) variant with otitis media (OM). *Black symbols*, OM-affected; *clear symbols*, no diagnosis of OM. Note that parents were ascertained for OM as adults, which does not completely rule out OM in early childhood. Alternatively, the variant may be inherited in an autosomal dominant pattern with reduced penetrance. Five additional Finnish probands (from two trios and three parent-proband pairs) are heterozygous for this splice variant (Table 1). A Minnesotan family UMN48 has three out of four OM-affected relatives that are heterozygous for the *CDHR3* splice variant, however family UMN48 also has the known *PLG* variant c.112A>G (p.Lys38Glu, rs73015965) co-segregating with otitis media

[32]. Probands from eight of the nine families with the splice variant, including the four families in the figure, have both RAOM and COME diagnoses

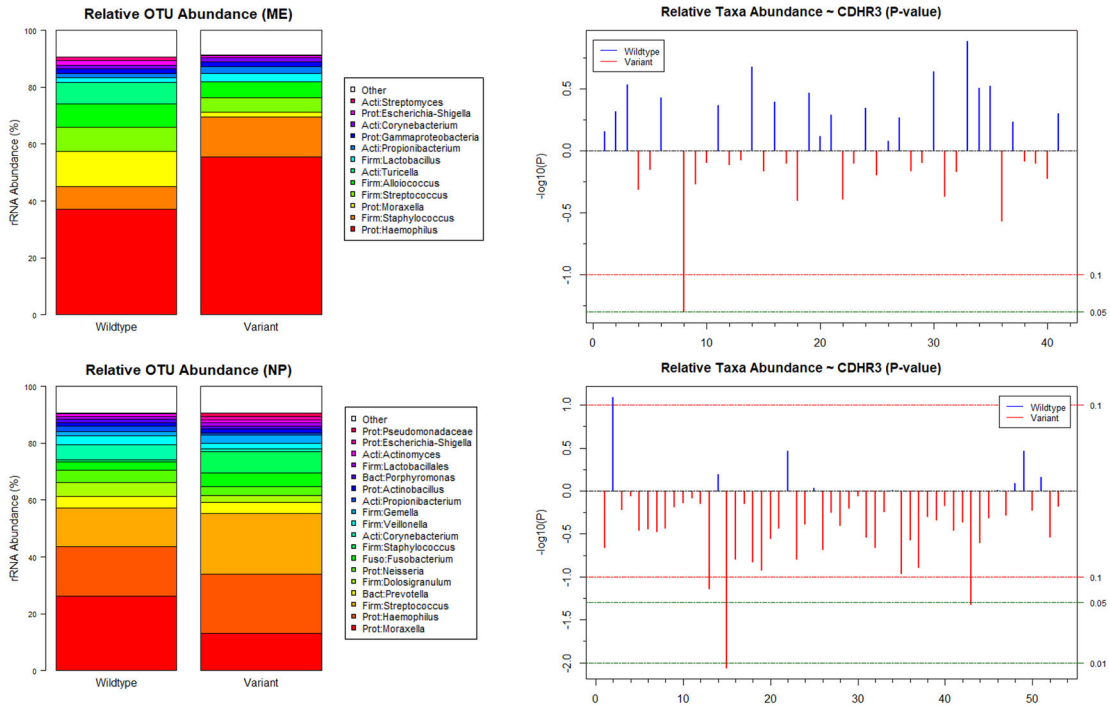

Figure 2.

Relative taxa abundance in the middle ear (ME) and nasopharynx (NP). *Left panels* summarize average relative abundances (RA) of predominant bacterial genera (taxa with RA <0.5% were collapsed into the "Other" group). *Right panels* show the results of non-parametric Wilcoxon rank sum tests, which plot -log(nominal-p-values) for each genus along the x-axes. *Blue lines* indicate taxa that were of greater RA in the microbial samples from wildtype individuals vs. carriers of the *CDHR3* p.Cys529Tyr variant, whereas *red lines* indicate taxa that were of greater RA in carriers of the p.Cys529Tyr variant compared with wildtype. *Horizontal lines* mark p-values equal to 0.1, 0.05, or 0.01. The *CDHR3* p.Cys529Tyr variant was nominally associated with increased relative abundance of *Streptomyces* (p=0.04) in the ME and *Lysobacter* (p=0.01) in the NP

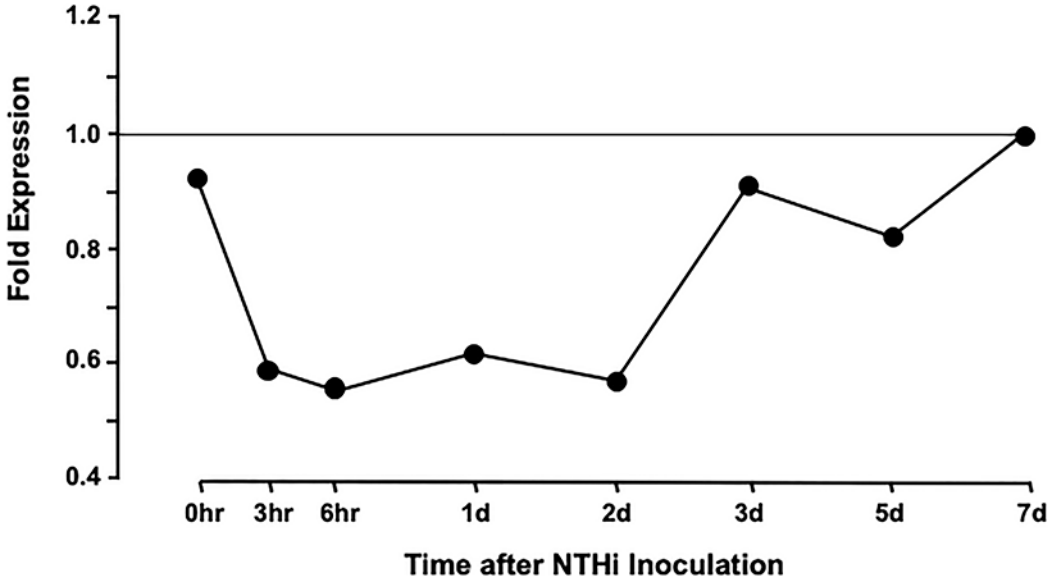

Figure 3.

Relative *Cdhr3* expression after inoculation of wildtype mouse middle ears with non-typeable *Haemophilus influenzae* (NTHi). *Cdhr3 i*s significantly downregulated three hours post-infection (Table 5)

Table 1.

CDHR3 (NM_152750.4) variants identified in exome data from Finnish, Minnesotan and Pakistani families

Family ID	hg19 Position	cDNA Variant	Amino Acid Variant	rsID	Damaging Prediction[a]	CADD	gnomAD NFE MAF	gnomAD FIN MAF	gnomAD SAS MAF
72 FIN families, 16 UMN families	105615406	c.163G>A	p.Val55Met	rs35008315	LRT,MA,PP2,SI	23.0	0.20	0.18	0.14
72 FIN families, 16 UMN families	105615426	c.183G>C	p.Gln61His	rs34426483	--	12.1	0.20	0.18	0.14
FIN182, FIN250	***105636709***	***c.622G>A***	***p.Val208Met***	***rs200019471***	***MA,mLR,mSVM,MT,PP2,SI***	***23.4***	***0.0003***	***0.002***	***0***
PKOM3, PKOM10	105642086	c.862+30A>C	Intronic-near-splice	rs10229260	--	5.0	0.01	0.002	0.18
FIN204	***105653317***	***C.1064C>T***	***p.Pro355Leu***	***rs202204966***	***MA,MT,PR,SI***	***15.9***	***0.00001***	***0.001***	***0***
FIN100	***105653388***	***C.1135C>T***	***p.Pro379Ser***	***rs200102793***	***LRT,MA,MT,PP2,PR,SI***	***23.2***	***0.0001***	***0.013***	***0***
FIN352	***105655642***	***c.1310delC***	***p.Pro439Leufs*12***	***rs778071363***	***MT (NMD)***	--	***0.0001***	***0.002***	***0***
FIN85, FIN307, UMN233, UMN9338	105656471	c.1426G>A	p.Ala476Thr	rs143495908[b]	PP2,SI	23.0	0.005	0.002	0.001
FIN152, FIN342	***105658381***	***C.1516C>T***	***p.Leu506Phe***	***rs76067797*** [b]	***FA,PP2,SI***	***14.2***	***0.006***	***0.004***	***0.0008***
120 FIN families, 14 UMN families, PKOM14	**105658451**	**c.1586G>A**	**p.Cys529Tyr**	**rs6967330**	--	**14.3**	**0.17**	**0.30**	**0.20**
26 FIN families, 9 UMN families, PKOM14	105658460	c.1595C>G	p.Thr532Ser	rs73195662	--	5.4	0.07	0.08	0.14
UMN123, FIN142, FIN332, 2 FIN trios, 3 FIN PPP, UMN48 [c]	**105658521**	**c.1653+3G>A**	**Splice**	**rs117797654**	**MT**	**19.5**	**0.03**	**0.009**	**0.005**
UMN8014	105660972	C.1807C>T	p.Arg603Cys	rs144905888	LRT,MA,MT,PP2,PR,SI	28.4	0.004	0.001	0.0006
FIN261, FIN636	***105664935***	***c.2185T>C***	***p.Tyr729His***	***rs201969268***	***SI***	***16.2***	***0.0003***	***0.005***	***0***
FIN7, FIN280, FIN321, UMN9161	105665004	c.2254C>A	p.Pro752Thr	rs117406926[b]	SI	6.1	0.02	0.004	0.004
FIN258	***105672877***	***c.2392G>T***	***p.Ala798Ser***	***rs140818720*** [b]	***LRT,MA,MT,SI***	***18.8***	***0.008***	***0.008***	***0.002***
FIN278, FIN300, FIN329	105672985	c.2500A>G	p.Arg834Gly	rs184921567	--	10.4	0.0003	0.01	0

Abbreviations: CADD, scaled Combined Annotation-Dependent Depletion score; FA, FATHMM; FIN, Finnish; gnomAD, genome aggregation database v3.1.1; LRT, likelihood ratio test; MAF, population-matched minor allele frequency; mLR, MetaLR; mSVM, MetaSVM; MT,

MutationTaster; NFE, gnomAD non-Finnish European; NMD, predicted nonsense-mediated decay; PKOM, Pakistani family; PP2, PolyPhen2 HVAR; PPP, parent-proband pair; PR, PROVEAN; SAS, gnomAD South Asian; SI, SIFT; UMN, Minnesotan family. MAF estimates were rechecked in gnomAD v.3.1.1.

[a]Damaging prediction from bioinformatics tools in dbNSFP41a. Variants in *bold* have additional supporting information on a role in OM susceptibility. Variants in *bold italics* are of unknown significance due to occurrence in 1-2 trio probands with no additional co-segregation data. **Note that 81% of probands with pathogenic variants or variants of unknown significance have both RAOM and COME diagnoses, while the rest have RAOM only.**

[b]The p.Ala476Thr and p.Leu506Phe variants have low-frequency Middle Eastern MAF=0.019. The p.Pro752Thr and p.Ala798Ser variants have low-frequency Amish MAF=0.03.

[c]The c.1653+3G>A splice variant is carried by only 3 out of 4 OM-affected relatives in family UMN48 (Figure 1). However the family also co-segregates a known *PLG* c.112A>G (p.Lys38Glu) variant [32], indicating multiple alleles conferring OM susceptibility within the same family.

Table 2.

Transmission disequilibrium tests for *CDHR3* variants in 399 US trios

Variant	White	Hispanic	Mixed/ Other	T	NT	TDT *p*
		Texas				
p.Val55Met (rs35008315)	197	65	36	125	122	0.85
p.Gln61His (rs34426483)	195	65	36	115	118	0.84
p.Cys529Tyr (rs6967330)	208	68	40	86	107	0.16
p.Thr532Ser (rs73195662)	207	68	40	41	39	0.82
		Colorado				
p.Cys529Tyr	62	9	12	26	24	0.78
p.Thr532Ser	62	9	12	10	12	0.67
		Combined				
p.Cys529Tyr	270	77	52	117	135	0.26
p.Thr532Ser	269	77	52	53	52	0.92

All four variants are in Hardy-Weinberg equilibrium (total cohort or per ethnicity).

T, transmitted; NT, non-transmitted; TDT, transmission disequilibrium test.

Table 3.

Study variables for Coloradan children with otitis media by occurrence of middle ear fluid (MEF) at surgery[a]

Variable[b]	(+)MEF (n=61)[c]	(−)MEF (n=30)[c]	p-value[d]
***CDHR3* p.Cys529Tyr**	**14 (23.0)**	**15 (50.0)**	**0.035***
FUT2 p.Trp154*	42 (68.9)	22 (73.3)	1
PLG p.Asp472Asn	31 (50.8)	16 (53.3)	0.82
Age	3.4 (3.7)	3.3 (2.6)	0.86
Male	42 (68.9)	18 (60.0)	0.55
Not White non-Hispanic	16 (26.2)	7 (23.3)	0.97
Family history of OM	36 (59.0)	22 (73.3)	0.27
Breastfed	55 (90.2)	26 (86.7)	0.88
Attended daycare	47 (77.0)	18 (60.0)	0.15
Exposure to cigarette smoke	7 (11.5)	5 (16.7)	0.74
History of allergies	13 (21.3)	11 (36.7)	0.21
History of asthma	4 (6.6)	3 (10.0)	0.87
History of allergic rhinitis	21 (34.4)	16 (53.3)	0.13
History of sinusitis	9 (14.8)	8 (26.7)	0.28
Antibiotic use	53 (86.9)	28 (93.3)	0.57
RAOM	**38 (62.3)**	**28 (93.3)**	**0.004***

[a] All tests were performed with chi-square analyses except for age, which was performed with student's t-test.

[b] All three variants tested are in Hardy-Weinberg equilibrium within the Coloradan cohort. Previous antibiotic use was also associated with the *CDHR3* p.Cys529Tyr variant (p=0.03). RAOM, recurrent acute otitis media.

[c] Numbers indicate counts (%), except for age which denotes average age in years (SD).

[d] Statistically significant variables are in *bold font*.

Table 4.

Multivariate logistic regression analyses for presence of middle ear fluid (MEF) in Coloradan children with otitis media

Variables	n (%)	Bivariate OR (95%CI)[a]	p-value	Multivariate OR (95%CI)[b]	p-value
CDHR3 p.Cys529Tyr (rs6967330)	29 (31.9)	0.33 (0.12,0.84)	0.02*	0.32 (0.10,0.94)	0.04*
Age (years)	91 (100)	1.01 (0.89,1.17)	0.87	0.97 (0.81,1.16)	0.74
Male	60 (65.9)	1.47 (0.59,3.66)	0.40	1.25 (0.41,3.74)	0.69
Not White non-Hispanic	23(25.3)	1.17 (0.43,3.40)	0.77	--	--
Attended daycare	65 (71.4)	2.24 (0.87,5.80)	0.09	1.73 (0.52,5.86)	0.37
Allergic rhinitis (AR)	37 (40.7)	0.46 (0.19,1.11)	0.09	0.46 (0.15,1.31)	0.15
RAOM	66 (72.5)	0.12 (0.02,0.44)	0.006**	0.10 (0.01,0.44)	0.007**

[a]Bivariate analyses using presence of MEF at surgery as outcome variable and each listed variable as independent determinant. Average age is 3.4 years. RAOM, recurrent acute otitis media.

[b]Model for multivariate analysis: MEF at surgery ~ rs6967330 + age + sex + daycare + AR + RAOM. For ethnicity, White non-Hispanic is the reference group in logistic regression, while RAOM was compared to chronic otitis media.

*≤0.05

**≤0.01

Table 5.

Expression of *Cdhr3* (1110049B09Rik, Probe ID:1439147_at) during acute otitis media induced by middle ear inoculation of non-typeable *Haemophilus influenzae*

Time	Fold Change (range)	*p-value*
0h	0.92 (0.61 to 1.39)	0.87
3h	**0.59 (0.59 to 0.60)**	**0.009**
6h	0.57 (0.40 to 0.83)	0.38
1d	0.61 (0.58 to 0.65)	0.07
2d	0.58 (0.46 to 0.72)	0.24
3d	0.90 (0.73 to 1.11)	0.70
5d	0.79 (0.74 to 0.86)	0.20
7d	0.99 (0.95 to 1.02)	0.73