Letter to the Editor

Association between the *CDHR3* rs6967330 risk allele and chronic rhinosinusitis

To the Editor:

Rhinoviruses (RVs) are the most frequent virus isolated in chronic rhinosinusitis (CRS)[1] and asthma exacerbations.[2] CRS and asthma are both heterogeneous inflammatory disorders of the airway; however, their frequent co-occurrence suggests a unified airway endotype with a common pathophysiology. A recent genome-wide association study identified a missense single nucleotide polymorphism in the Cadherin-related family member 3 gene *(CDHR3)* that results in a cysteine to tyrosine substitution at position 529 (rs6967330). This rs6967330 risk allele (A) was found to be a significant additive risk factor for acute hospitalizations secondary to asthma in the first 6 years of life.[3] Further mechanistic studies identified CDHR3 as the receptor for RV-C, an especially virulent subtype of RV highly correlated with severe airway disease.[4] Given that sinonasal epithelia is the primary site for RV infection,[5] we hypothesized that the combination of the rs6967330 genetic single nucleotide polymorphism with RV-C infections could be responsible for the development of CRS in adults.

We performed a retrospective case-control study of non-Hispanic white adults recruited from 2 tertiary academic rhinology centers—the University of Arizona (UofA) and the University of Pennsylvania (UPenn)—with diagnosed CRS (UofA n = 76, UPenn n = 267) and healthy controls (UofA n = 52, UPenn n = 337). Further details are available in this article's Methods section in the Online Repository at www.jacionline.org and the participant's characteristics are presented in Table E1 in this article's Online Repository at www.jacionline.org. In the UPenn group, those with CRS were significantly older and more likely to be males compared with the controls. At both UofA and UPenn, the prevalence of asthma was approximately 2-fold greater in the CRS group compared with the control group.

DNA was extracted from buccal swabs or saliva samples collected from all study participants and rs6967330 was genotyped. In the control group for both populations, the allele frequency of the minor A allele was similar to that seen in the general non-Hispanic white population.[3] There were no significant differences in subjective or objective measures of CRS including nasal polyp status in individuals with CRS related to CDHR3 genotype. We calculated the risk for CRS associated with rs6967330, stratified by location and adjusted for both sex and age in additive and dominant models. Additive models included genotype as an ordinal variable with 3 categories (0, 1, and 2 for GG, AG, and AA genotypes) and dominant models included genotype as a binary variable (0, 1 for GG vs AA + AG genotypes). Using an additive model, rs6967330 significantly increased the odds for CRS by 2.08 at the UofA (95% CI, 1.02-2.41; $P = .043$) for each additional "A" allele and remained significant when adjusting for sex and age. Using a dominant model, rs6967330 significantly increased the odds for CRS by 1.5 at UPenn (95% CI, 1.0-2.1; $P = .022$) if an individual carried 1 or 2 copies of the minor A allele of rs6967330. Meta-analysis estimates of the UofA and UPenn populations demonstrated significantly increased risk for CRS in both additive and dominant models, which remained essentially unchanged after adjustment for age and sex (Table I). Excluding adults with co-occurring asthma did not significantly change this risk (meta odds ratio [mOR], 1.55; 95% CI, 1.11-2.17; $P = .011$), suggesting that this association was not confounded by the presence of asthma (Fig 1).

RV infections are the most frequent cause of upper respiratory infections (URIs), otherwise known as the common cold. In most individuals, URIs resolve within 5 to 7 days. However, it is unknown why in some adults, symptoms persist and can progress into CRS or trigger asthma exacerbations. The primary site of RV infection is the sinonasal epithelia. Until recently, the only known RV subtypes (A and B) bound to the low-density lipoprotein receptor[5] and the intercellular adhesion molecule-1 receptors expressed on the apical surface of ciliated airway epithelia.[6] In 2006, a third subtype, RV-C, was discovered using molecular detection techniques,[4] and found to be associated with more severe airway disease. RV-C isolates were identified with significantly higher frequency in the nasal washes of individuals with CRS,[1] and determined to be a trigger for acute wheezing lower respiratory illnesses (WLRIs) and the subsequent development of childhood asthma.[7]

CDHR3 was first identified as a candidate gene for early childhood asthma with severe exacerbations in a large genome-wide association study.[3] The CDHR3 protein is highly expressed in both upper and lower airway epithelia, and when plasmid DNA containing the mutation domain 5 ($C_{529}Y$) was transfected into 293T cells, it resulted in a marked increase in CDHR3 trafficking to the cell membrane compared with the wild-type (G) major allele. Moreover, CDHR3 was identified as the putative receptor for RV-C, with the homozygous presence of the rs6967330 risk allele increasing RV-C binding by 10-fold in a transduced HeLA cell line stably expressing CDHR3-Y_{529} compared with control HeLa cells.[8]

In summary, we have determined that carriers of rs6967330 are at significantly increased risk for CRS. The association between rs6967330 and airway disease was first reported in a study of acute WLRIs in young children.[3] It is now well established that WLRIs caused by rhinovirus in this age group are strongly associated with the subsequent development of asthma.[7] Similarly, CRS usually starts with acute URI episodes, most often caused by rhinoviruses, and progresses into a disease characterized by quiescent periods followed by acute flare-ups. CDHR3 rs6967330 may be a common genetic risk factor between viral-associated childhood asthma and CRS.

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TABLE I. Association between rs6967330 and CRS at UofA and UPenn

	Unadjusted					Adjusted for sex and age				
Participants	**n**	**OR**	**95% CI**	***P* value**	**P_{Het}***	**n**	**Adjusted OR**	**95% CI**	***P* value**	**P_{Het}***
All participants										
Additive model										
UofA	128	2.08	1.02-4.21	.043		126	2.14	1.04-4.42	.039	
UPenn	604	1.31	0.97-1.78	.077		595	1.28	0.91-1.80	.162	
Meta	*732*	*1.41*	*1.07-1.86*	*.015*	*NS*	*721*	*1.40*	*1.03-1.92*	*.032*	*NS*
Dominant model										
UofA	128	2.00	0.94-4.23	.071		126	2.06	0.95-4.46	.068	
UPenn	604	1.50	1.06-2.11	.022		595	1.46	0.99-2.17	.057	
Meta	*732*	*1.57*	*1.15-2.15*	*.005*	*NS*	*721*	*1.57*	*1.11-2.23*	*.012*	*NS*
Participants without asthma										
Additive model										
UofA	95	2.09	0.93-4.72	.076		93	2.05	0.86-4.78	.095	
UPenn	442	1.48	1.02-2.15	.038		435	1.43	0.95-2.15	.087	
Meta	*537*	*1.57*	*1.12-2.20*	*.009*	*NS*	*528*	*1.53*	*1.06-2.21*	*.023*	*NS*
Dominant model										
UofA	95	2.04	0.88-4.74	.095		93	2.01	0.84-4.81	.115	
UPenn	442	1.85	1.22-2.80	.004		435	1.77	1.12-2.81	.014	
Meta	*537*	*1.88*	*1.30-2.73*	*.001*	*NS*	*528*	*1.82*	*1.22-2.74*	*.004*	*NS*

OR, Odds ratio.
*Heterogeneity *P* value based on χ^2 test (NS indicates no heterogeneity between studies).

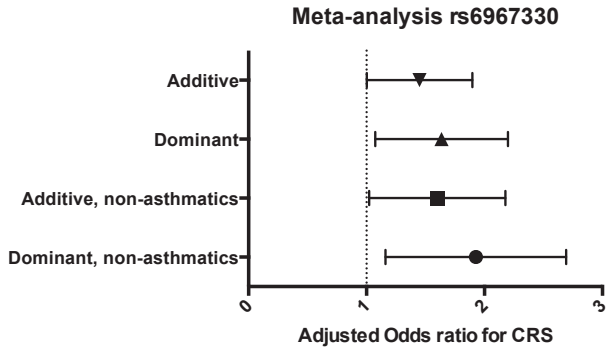


FIG 1. Meta-analysis of rs6967330 from UofA and UPenn. The rs6967330 single nucleotide polymorphism is significantly associated with CRS in both additive and dominant models. This association remains significant in those without self-reported asthma (non-asthmatics). Meta odds ratio (mOR) was adjusted for sex and age.

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This study was supported by the National Institutes of Health (grant no. K08 DE021413 to E.H.C., grant no. P30 DC011735 to D.R.R., grant no. R01DC013588 to N.G.C., and grant no. R01 HL132523 to E.H.C., S.G., and F.D.M.).

Disclosure of potential conflict of interest: E. H. Chang receives research support from the National Institutes of Health (NIH) and serves as a consultant for Olympus. A. L. Willis and S. Guerra receive research support from the NIH. D. R. Reed receives research support from the NIH and is an employee of Monell. F. D. Martinez receives grant support from the NIH and Johnson & Johnson and serves as a consultant for Copeval and Commense, Inc. The rest of the authors declare that they have no relevant conflicts of interest.

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http://dx.doi.org/10.1016/j.jaci.2016.10.027

METHODS

Subjects

All participants were self-reported non-Hispanic whites who gave their written informed consent and institutional review boards at the respective institutions approved the study. Chronic rhinosinusitis was physician-diagnosed according to the criteria set forth by the European Position Paper on Rhinosinusitis and Nasal Polyps[E1] and the American Academy of Otolaryngology.[E2] Patients with known etiologies for CRS including cystic fibrosis, immunodeficiency, aspirin-sensitive allergic disease, allergic fungal rhinosinusitis, and sinonasal tumors were excluded from the study. The prevalence of asthma was self-reported in questionnaires, or previously diagnosed by a physician.

Healthy controls from the UofA were evaluated by otolaryngologists for benign pituitary lesions or nasal septal disorders, and all controls had a negative history of CRS, negative endoscopic examination, and negative sinus computed tomography scans. Controls from the University of Pennsylvania were collected from volunteers who (1) had not been treated by a doctor with antibiotics for a sinus infection, (2) had not had sinus surgery for CRS, and (3) had absence of symptoms or history of CRS via the sino-nasal outcome test 20 (SNOT-20) questionnaire. Buccal swabs or salivary samples were collected from all participants for DNA extraction.

Definitions of CRS

We obtained a complete medical and surgical history of all individuals with CRS. This included subjective symptom scoring by the SNOT-20 questionnaire, a validated 5-point scale based on 20 questions related to sinonasal disease. All individuals underwent endoscopic examination of the sinonasal cavity for the presence of mucus, edema, or nasal polyposis. High-resolution sinus computed tomography scans were performed, and the Lund-Mackay scoring system was used to grade the degree of mucosal opacification of each sinonasal unit (0, no disease; 1, <50%, 2; >50% mucosal opacification) with a total possible score of 24.

Participant genotyping

Genomic DNA was extracted from buccal swabs or salivary samples using the Qiagen Blood and Tissue extraction kit and then sequenced for the presence of the rs6967330 single nucleotide polymorphism by a Taqman-based or restriction fragment length polymporphism assay. Taqman: Genomic DNA was diluted to 10 ng/uL and Taqman allele-specific probes and primers purchased from Life Technologies (Grand Island, NY; Catalog #: C__29286131_10) with the StepOne Plus or QuantStudio 12K Flex instruments from Life Technologies (formerly Applied Biosystems, Foster City, Calif) to genotype rs6967330. For genotyping quality control, we included 8 technical replicates (genomic DNA from 8 participants, included in every assay) and 57 biological replicates (2 independently collected saliva samples from 57 participants). All genotypes matched in all cases. Restriction fragment length polymporphism: a portion of the *CDHR3* gene was amplified using primers (forward 5′-ATTCCTCCAGCCAGAACCCG-3′, reverse 5′-TGTTTCTCACCACATCCGCAG-3′). The PCR product was then digested using the *HpyCH4III* restriction enzyme and fragments of the digestion were visualized in agarose gel electrophoresis.

Statistical analysis

Association between CDHR3 allele status and categorical variables (presence or absence of sinus disease) was calculated using χ^2 testing. The relation of subjective and objective CRS features to CDHR3 genotype was tested either by χ^2 testing or 2-sample *t* tests. Unadjusted and adjusted dominant and additive logistic regression models were assessed using STATA (version 13, StataCorp LP). Meta-analysis of the association between CDHR3 genotype and CRS was performed using estimates from each study to compute the combined estimate of risk. Forest plot and graphs were generated by GraphPad Prism version 6.0 (GraphPad Software Inc, La Jolla, Calif). For all analyses, the level for statistical significance was set at $P < .05$.

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TABLE E1. CDHR3 rs6967330 genotype frequencies (% and (N)) and demographic information for participants with CRS and healthy controls at UofA and UPenn

Center	Group	n	rs6967330 Genotype frequencies			% Female	Age (y), mean ± SD	% Asthma
			GG	AG	AA			
UofA	Controls	52	71.1 (37)	28.8 (15)	0.0 (0)	55.8	51 ± 19	19.2
	CRS	76	55.3 (42)	40.8 (31)	3.9 (3)	55.3	56 ± 17	32.9*
UPenn	Controls	337	72.4 (244)	24.3 (82)	3.3 (11)	54.0	40 ± 15	16.0
	CRS	267	63.7 (170)	34.1 (91)	2.2 (6)	39.0*	54 ± 14*	40.0*

*$P < .05$.