The *GSDMB* rs7216389 SNP is associated with chronic rhinosinusitis in a multi-institutional cohort.

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Abstract

Background: Chronic rhinosinusitis (CRS) is a multifactorial disease with a high co-occurrence with asthma. In this multi-cohort study, we test if single nucleotide polymorphisms (SNPs) associated with childhood asthma and rhinovirus-associated disease have an increased susceptibility to adult CRS in a multi-cohort retrospective case-control study.

Methods: Participants at two tertiary academic rhinology centers, University of Arizona (UofA) and University of Pennsylvania (UPenn) were recruited. Cases were defined as those with physician diagnosed CRS (UofA n=149, UPenn n=250), and healthy controls were those without CRS (UofA n=66, UPenn n=275). Genomic DNA was screened for the *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP. Gene dosage, or the number of combined risk alleles in a single subject was calculated. Meta-analysis of the association between *GSDMB* or *CDHR3* genotypes and CRS was performed and additive gene dosage effect for each population calculated using a p-trend.

Results: A meta-analysis revealed a combined increased risk for CRS in subjects with the *GSDMB* rs7216389 SNP (OR=1.40, 95%CI:1.16, 1.76, p=0.004). Both the UofA (OR=1.73, 95%CI:1.23, 2.43, p=0.002) and UPenn (OR=1.27, 95%CI:1.02, 1.58, p=0.035) populations showed a significant positive association between the number of combined risk alleles of *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP and risk for CRS.

Conclusions: Carriers of the *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP are at increased susceptibility for CRS. This data suggest that therapeutic approaches to target aberrant responses to RV infection may play a role in the treatment of unified airway disease.

Introduction:

Chronic rhinosinusitis (CRS) affects nearly 12% of the adult population in the United States with direct costs of $60 billion yearly.[1] CRS is characterized by a minimum of 12 consecutive weeks of symptoms including nasal congestion, nasal discharge, facial pain/pressure, and decreased smell.[2] Severity of disease is commonly measured by the Lund-Mackay score (LMS), a radiologic scoring system of mucosal sinus disease on CT scan.[3] CRS can be characterized into two clinical phenotypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).[2] CRSwNP is generally a more severe disease phenotype than CRSsNP and one that shares features of inflammation and remodeling with asthma.[1]

Airway researchers have been interested in the relationship between CRS and asthma due to an increasing number of studies demonstrating their frequent co-occurrence within the same patient. In a survey performed by Jarvis, et al., patients identified as asthmatics demonstrated a 3-fold higher risk of also having CRS symptoms. This same survey indicated an 11-fold higher risk association between self-reported asthma and CRS symptoms with the presence of coexisting allergic rhinitis.[4] Many other clinical studies have confirmed this trend.[5-7] Furthermore, radiological severity of CRS—measured by LMS—correlates with asthma severity.[8,9] These findings suggest that CRS and asthma not only frequently co-occur, but also have a tendency to demonstrate parallel disease burden.

Pathophysiological commonalities exist between CRS and asthma and help further elucidate the link between lower and upper airway disease. In patients with persistent CRS, findings within sinonasal tissues reveal several of the same major histological effects seen in asthma, including neutrophilic and eosinophilic inflammation, epithelial shedding, and basement membrane thickening.[10,11] Moreover, rhinovirus infections can induce upper and lower airway exacerbations in CRS and asthma and have been linked to the pathogenesis of unified airway disease. Common histological and immune responses between CRS and asthma—as well as the

corresponding incidence of disease burden—suggest the possibility of regional manifestations of the same disease processes.[12]

Using genome-wide association studies (GWAS), scientists have identified 17q21 as a childhood-onset asthma susceptibility locus[13-15] containing—but not limited to—the following genes: *IKZF3*, *ZPBP2*, *GSDMB*, *ORMDL3*, and *GSDMA*. Within these five genes are several single nucleotide polymorphisms (SNPs) which comprise an expression quantitative trait locus (eQTL), or a region of the genome which influences the expression levels of one or more genes.[16] In particular, five of these SNPs are in near perfect linkage disequilibrium, with rs7216389 serving as a representative surrogate with the strongest asthma association.[17] Interestingly, the rs7216389 variant has been associated with increased susceptibility and response to human rhinovirus (HRV) infections, resulting in childhood wheezing illnesses and the development of childhood-onset asthma.[17] Similarly, the rs6967330 SNP within the *CDHR3* gene is the receptor for rhinovirus-C and is a known risk factor for both severe childhood asthma and adult CRS.[18,19] Given the common findings of RV-induced childhood asthma exacerbations, we hypothesized that the *GSDMB* rs7216389 SNP would be significantly associated as a genetic risk factor for CRS and persons with both the *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP might have an increased risk for CRS.

Methods:

Subjects. All participants self-reported as non-Hispanic Caucasian and gave their written informed consent. This study was evaluated and approved by the institutional review board at the University of Arizona (IRB#1502660530) and at the University of Pennsylvania (IRB#701426, 800614). A subset of these subjects had been screened for the rs6967330 SNP as previously reported.[20] Chronic rhinosinusitis was physician-diagnosed according to the criteria set forth by the European Position Paper on Rhinosinusitis and Nasal Polyps and the American Academy of Otolaryngology. 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 University of Arizona (UofA) were evaluated by otolaryngologists for benign pituitary lesions or nasal septal disorders. All controls had a negative history of CRS, negative endoscopic examination, and negative sinus computed tomography scans. Healthy controls from the University of Pennsylvania (UPenn) were collected from volunteers who (1) had not been treated by a doctor with antibiotics for a sinus infection, (2) had never undergone sinus surgery for CRS, and (3) had absence of symptoms 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 self-reported symptom scoring through 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 all CRS samples had a Lund-Mackay score > 5.

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 *GSDMB* rs7216389 and *CDHR3* rs6967330 single nucleotide polymorphisms by Taqman-based and/or restriction fragment length polymorphism (RFLP) assays. For genotyping quality control, technical replicates were included in each assay. All genotypes matched in all cases.

Taqman: Genomic DNA was diluted to 10 ng/uL as template in order to genotype alleles using Taqman allele-specific probes and primers purchased from Life Technologies (Assay IDs: C__29062108_10 and C__29286131_10).

Restriction fragment length polymorphism (RFLP) assays: A portion of the *GSDMB* gene and a portion of the *CDHR3* gene were amplified using gene-specific primers (*GSDMB*: forward 5′-AAG AAG TAG GAG CCC CAG CC -3′, reverse 5′- GGG TGG CAA CTG ACT CAG AA -3′; *CDHR3*: forward 5'-ATTCCTCCAGCCAGAACCCG -3', reverse 5'-TGTTTCTCACCACATCCGCAG -3'). The PCR products were then digested using restriction enzymes (*GSDMB*: *NspI*, *CDHR3*: *HpyCH4III*) and fragments of the digestion were visualized using agarose gel electrophoresis by previously published protocols.[21]

Statistical Analysis. The association between *GSDMB* rs7216389 SNP or *CDHR3* rs6967330 SNP and categorical variables (presence or absence of sinus disease) was assessed using χ^2 testing on both the UofA and UPenn populations. The relation of *GSDMB* and *CDHR3* genotypes to subjective and objective CRS features was assessed either by χ^2 testing or 2-sample *t*-tests. SNP genotypes were tested in additive models using multivariate logistic regression. This same approach was used for the genetic risk score as described above. We used a fixed-effect model for a meta-analysis between these two studies because a test for heterogeneity of effect estimates between the two cohorts was non-significant. Meta-analysis of the association between *GSDMB* rs7216389 SNP or *CDHR3* rs6967330 SNP and CRS was performed using estimates from each study to compute the combined meta-analyzed estimate

of additive risk. For all analyses, the level for statistical significance was set at $P < .05$. STATA was used for all analyses (version 13, StataCorp LP).

Gene Dosage. The *GSDMB* rs7216389 SNP is a C→T risk mutation and *CDHR3* rs6967330 SNP is a G→A risk mutation for sinus disease. Counts of *GSDMB* rs7216389 SNPs and *CDHR3* rs6967330 SNPs were combined to create a genetic risk score and characterized as follows: (# of combined risk alleles = *GSDMB* genotype + *CDHR3* genotype): 0 = CC+GG; 1 = CT+GG or CC+AG; 2 = CC+AA, CT+AG, or TT+GG; 3 = CT+AA or TT+AG; and 4 = TT+AA.

Results:

The UofA population consisted of 149 patients with CRS and 66 controls (Table 1). Asthma was significantly more prevalent in the CRS patients (38.3%) compared to controls (7.6%), p<0.001. Both groups had a similar proportion of females and mean age (Table 1). The UPenn population consisted of 250 CRS patients and 275 controls. Similar to the UofA sample, asthma was significantly more prevalent in the CRS patients (40.0%) compared to controls (16.4%), p<0.001. In contrast however, the UPenn CRS patients were significantly older and less likely to be female compared to the controls, p<0.001 for both comparisons (Table 1).

***GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP.**

In the UofA population, the homozygous TT risk genotype for *GSDMB* rs7216389 was significantly less common in the controls (13.6%) and more common in patients with CRS (32.2%), p=0.017 (Table 2). The TT genotype was also higher in the UPenn CRS patients but the difference in prevalence compared to the controls was not significant (p=0.19). Using an additive model, each addition of the *GSDMB* rs7216389 SNP significantly increased the likelihood of CRS in the UofA population (Table 3). When asthmatics were excluded, the relation between *GSDMB* rs7216389 SNP and CRS in the UofA population remained significant, while the UPenn population risk diminished. We tested for heterogeneity of effect between the two cohorts and it was found to be non-significant. This allowed us to perform a meta-analysis of the two cohorts using a fixed-effects model, revealing a combined increased risk for CRS with a higher significance value than either population individually. Meta-analyses aggregate results from similar cohorts thereby increasing power, and testing for a potential statistical effect. In smaller groups, the magnitude of the OR will be similar for both populations but because of sample size limitations may not be significant for one population. After adjusting for age and sex, each additional *GSDMB* rs7216389 SNP was associated with significantly increased odds for CRS in the meta-analysis (OR=1.40, 95%CI:1.16, 1.76, p=0.004). We have previously

reported on the significant association between the *CDHR3* rs6967330 SNP in a meta-analysis in the same UofA and UPenn populations in both additive and dominant models in all subjects, as well as in patients without asthma.[20] We then tested to see if there was an association between the *GSDMB* rs7216389 SNP or the *CDHR3* rs6967330 SNP to specific CRS phenotypes including CRSwNP or CRSsNP. We were unable to find a statistical association to either group.

Gene Dosage Effect. Both the UofA and UPenn populations showed a significant positive association between the number of combined risk alleles of *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP and risk for CRS (Table 4). After adjusting for age and sex, each additional SNP was associated with significantly increased odds for CRS in both the UofA (OR=1.73, 95%CI:1.23, 2.43, p=0.002) and UPenn (OR=1.27, 95%CI:1.02, 1.58, p=0.035) populations.

188 **Discussion:**

189 CRS and asthma are complex heterogeneous diseases that frequently co-occur. Large
190 genome-wide association studies for asthma have identified several genetic risk factors.
191 However, their relative contribution to asthma development has been hampered by the extreme
192 heterogeneity of the disease and likely reflects the interaction between genetic risk and
193 environmental exposures. Longitudinal cohort studies have identified a specific asthma
194 phenotype associated with severe childhood asthma exacerbations and RV infections in early
195 life. Focused analyses of this asthma subtype have identified a high association with the
196 *GSDMB* rs7216389 SNP[13-15] and the *CDHR3* rs6967330 SNP[18,22]. This study suggests a similar
197 association between these genetic risk factors and CRS, suggesting that genetic risk and RV-
198 induced disease may play a role in the development of CRS.

199 We first determined if the *GSDMB* rs7216389 SNP was associated with adult CRS in a multi-
200 institutional cohort. The *GSDMB* rs7216389 SNP, located in a non-coding intron region of
201 *GSDMB*, has been shown to influence transcript levels of both *ORMDL3* and *GSDMB.*[17,23]
202 *ORMDL3* is involved in endoplasmic reticulum-mediated calcium homeostasis and the unfolded
203 protein response (UPR)[24,25] which are thought to play a role in the development of chronic
204 inflammatory diseases.[26] Changes in calcium signaling correlate with changes in Th-2 cytokine
205 levels, thereby contributing to asthma susceptibility.[27-29] The function of *GSDMB* remains largely
206 unknown, but it is highly expressed in the bronchial epithelium of asthmatic human lungs and—
207 when overexpressed in bronchial epithelium—increases expression of genes involved in airway
208 remodeling and hyperresponsiveness.[30] Caliskan et al. found that RV challenges to peripheral
209 mononuclear blood cells of patients increased the expression of *ORMDL3* and *GSDMB* and was
210 highly associated and specific to rhinovirus induced wheezing in childhood onset asthma.[31]
211 Together, this data suggest that the *GSDMB* rs7216389 SNP may contribute to an aberrant
212 response to RV and thereby exacerbate both upper and lower airway disease.

We have previously reported that *CDHR3* rs6967330 SNP[18,22] was highly associated with adult CRS. CDHR3 is the only known receptor for RV-C and Basnet et al. determined that the rs6967330 genotype increased *CHDR3* expression in airway epithelial cells with a subsequent increase in RV-C binding and replication.[32] Our group also recently reported that RV-C infections were common in those with symptomatic adult CRS exacerbations.[33]

Interestingly, we were unable to find any statistical association between the *GSDMB* rs7216389 SNP and *CDHR3* rs6967330 SNP to either CRSwNP or CRSsNP. One possibility could be due to small sample sizes and lack of power. However, another possibility is that our current CRS phenotypes do not reflect underlying pathophysiologic process. CRS endotypes, in which inflammatory markers are used to distinguish specific subtypes of disease, may help elucidate common pathophysiologic processes associated with genetic risk.[34] Given that rhinovirus infections are potent interferon stimulators, genes related to an aberrant innate immune response could result in childhood asthma exacerbations and potentiate the pathogenesis of variations of CRS. As an example, Wang et al. recently identified that decreased expression of stimulator of interferon genes (STING) and resulting decreased interferon production was associated with the eosinophilic CRS with nasal polyposis phenotype.[35] Although we attempted to reduce the genetic heterogeneity of this study by focusing on non-Hispanic Caucasians, a significant limitation is that these findings may not be applicable to other ethnic groups. Moreover, identifying genetic risk factors to specific CRS endotypes may elucidate the function of these genes in the pathophysiology of CRS.

In summary, we identified a novel association with genes associated with RV-induced childhood asthma and CRS that are related to RV-induced airway disease. Further longitudinal clinical studies according to CRS endotypes and mechanistic studies in sinonasal airway cell lines are required to identify the genome-virome interactions that underly the pathophysiology of CRS.

Our results suggest that therapeutic approaches to target aberrant responses to RV infection may play a role in the treatment of unified airway disease.

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Table 1: Demographics comparison between the UofA and UPenn populations

Location	Population	n	% Female (n)	Mean Age (SD)	% Asthma (n/N)	% Nasal Polyps (n)
UofA	**Controls**	66	48.5 (32)	56.8 (18.4)	7.6 (5/66)	N/A
	CRS	149	45.6 (68)	54.6 (17.54)	38.3 (57/149)	52.4 (78)
			p= 0.70	p= 0.40	p<0.001	N/A
UPenn	**Controls**	275	56.0 (154)	40.4 (15.2)	16.4 (45/274)	N/A
	CRS	250	38.0 (95)	54.8 (13.8)	40.0 (100/250)	42.8 (107)
			p<0.001	p<0.001	p<0.001	N/A

Table 2: *GSDMB* rs7216389 and *CDHR3* rs6967330 genotype frequencies at the UofA and UPenn

***GSDMB* rs7216389**			**Genotype Frequencies**		
Location	Population	n	% CC (n)	% CT (n)	% TT (n)
UofA	Controls	66	25.8 (17)	60.6 (40)	13.6 (9)
	CRS	149	19.5 (29)	48.3 (72)	32.2 (48)
UPenn	Controls	275	26.6 (73)	48.4 (133)	25.1 (69)
	CRS	250	22.4 (56)	45.6 (114)	32.0 (80)
***CDHR3* rs6967330**			**Genotype Frequencies**		
Location	Population	n	% GG (n)	% AG (n)	% AA (n)
UofA	Controls	65	72.3 (47)	27.7 (18)	0.0 (0)
	CRS	147	54.4 (80)	42.9 (63)	2.7 (4)
UPenn	Controls	275	72.4 (199)	24.0 (66)	3.6 (10)
	CRS	250	64.8 (162)	32.8 (82)	2.4 (6)

Table 3: Association between *GSMDB* rs7216389 and CRS at UofA and UPenn using an additive model in all participants and among non-asthmatics

***GSDMB* rs7216389**		**Unadjusted for sex and age**				**Adjusted for sex and age**			
Location	**n**	**OR**	**95%CI**	**P**	**PHet***	**OR**	**95%CI**	**P**	**PHet***
Including asthmatics									
UofA	215	1.70	1.10, 2.62	0.016		1.72	1.11, 2.65	0.015	
UPenn	525	1.23	0.97, 1.56	0.082		1.29	0.99, 1.69	0.063	
Meta	740	1.33	1.08, 1.64	0.007	0.201	1.40	1.16, 1.76	0.004	0.278
Excluding asthmatics									
UofA	153	2.02	1.21, 3.37	0.007		2.04	1.22, 3.41	0.007	
UPenn	379	1.03	0.77, 1.37	0.863		1.04	0.75, 1.43	0.819	

*Heterogeneity p-value based on chi-squared (PHet value > 0.05 indicates no heterogeneity between studies), PHet was significant when asthmatics were excluded, therefore meta-analysis was not performed

Table 4: Combined genotype effect of risk alleles (gene dosage effect) at the UofA and UPenn

			Risk Allele Dosage					
Location	**Population**	**n**	**% 0 alleles (n)**	**% 1 allele (n)**	**% 2 alleles (n)**	**% 3 alleles (n)**	**% 4 alleles (n)**	**P***
UofA	**Controls**	65	18.5 (12)	53.9 (35)	21.5 (14)	6.2 (4)	0 (0)	0.002
	CRS	147	12.2 (18)	37.4 (55)	29.9 (44)	18.4 (27)	2.04 (3)	
UPenn	**Controls**	275	20.4 (56)	40.4 (111)	29.8 (82)	8.0 (22)	1.5 (4)	0.027
	CRS	250	13.6 (34)	35.6 (89)	41.2 (103)	9.2 (23)	0.4 (1)	

*P-value calculated from nonparametric test for trend across ordered groups

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