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Common Filaggrin null mutations are associated with atopy but not with chronic rhinosinusitis

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Mutations in the *filaggrin* gene (*FLG*) are strongly associated with atopic dermatitis (AD) and skin barrier dysfunction (1, 2). *FLG* has been linked to asthma and asthma severity in subjects with and without atopic dermatitis (1, 3-5), and individuals with *FLG* null mutations are at increased risk of allergic sensitization and allergic rhinitis (5). However, despite evidence indicating a link between *FLG*, barrier dysfunction, and respiratory diseases (1), the importance of *FLG* in the pathogenesis of chronic rhinosinusitis (CRS) has never been evaluated.

We hypothesized that *FLG* mutations might cause mucosal barrier dysfunction and thereby be associated with CRS (6, 7). To test this hypothesis, we investigated the frequency of the two most common *FLG* null mutations (*R501X* and *2282del4* (3, 5)) that have been implicated in asthma and allergic rhinitis in a group of European American CRS and control subjects. Patients with CRS (determined using Sinus and Allergy Health Partnership

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Contribution: All of the authors listed in front page have contributed to this study; MM, JH have completed most of the genetic study and statistical analyses and have prepared the manuscript. JN, RD, PS, JS, LC, LS, AK and MM have contributed to the preparation of tissue, DNA extraction, sequencing and genetic analyses. AP, LG, BT, DC, RK have recruited the patients and extracted the tissue. PA, JP, CO and RPS have supervised the study and analyses. RPS is the senior author and has supervised the study and manuscript writing. All authors have accepted the manuscript's final version.

guidelines (8), and evaluated by nasal endoscopy and computerized tomography) were recruited from the Otolaryngology and Allergy-Immunology Clinics at Northwestern Feinberg School of Medicine (n=298; 137 CRS with nasal polyposis [CRSwNP] and 161 CRS without nasal polyposis [CRSsNP]). All subjects with CRS had medically refractory disease and underwent functional endoscopic sinus surgery, during which nasal tissue samples and blood were obtained. Control subjects without a history of CRS were recruited from the same Otolaryngology and Allergy-Immunology clinics (n=204). Atopy status for all subjects was determined by patient history based on positive skin prick test or specific IgE to aeroallergens. Race/ethnicity was categorized by self-report. The Northwestern Institutional Review Board approved this study and all subjects provided written informed consent.

DNA was extracted from either tissue, saliva or blood samples using a Nucleospin® Genomic DNA extraction kit (Machery-Nagel) following manufacturer's instructions. Genotyping of *2282del4* and *R501X* was performed by restriction fragment length polymorphism (RFLP) analysis and Taqman (Applied Biosystems)-based allelic discrimination assays (2, 9).

Logistic regression was used to examine the gender and age adjusted associations between *FLG* mutations and phenotype. Stratified analyses by atopic status were also conducted. Cubic exact solutions for the estimation of pairwise haplotype frequencies were used to assess linkage disequilibrium between *R501X* and *2282del4* (10). Statistical analyses were performed using SPSS version 21. A p value < 0.05 was considered statistically significant.

Null mutations *R501X* and *2282del4* were not in linkage disequilibrium ($r^2 = 0.006$). CRSwNP subjects were more likely to be atopic compared to controls and CRSsNP patients. *FLG* null mutations (at least one copy of *FLG* null mutation in either of the loci) were significantly associated with atopy both in the CRSsNP and control groups but not in the CRSwNP group (Table 1). Null mutations were not associated with CRSwNP or CRSsNP (Table 1), whether patients were stratified by atopy status or not.

The male to female ratio in the CRSwNP group was higher compared to control and CRSsNP groups, while the CRSsNP and control patients had similar male/female ratios. The mean age of CRSwNP patients was significantly higher than CRSsNP and control groups, while there was no significant difference between CRSsNP and controls. Subgroup analysis showed that CRSwNP non-atopic patients were older compared with both CRSsNP nonatopic and control non-atopic cases, while there was no significant difference among atopic cases of all groups in terms of age. All analyses were adjusted for gender and age as reported in table 1. Adjusting the data analysis for age and gender did not change any of the findings.

Our data confirm the association between null mutations in *FLG* and atopy first reported in a population-based study from Germany (5). This association was seen in both control subjects without CRS and in CRSsNP patients. Interestingly in CRSwNP patients, atopy was not associated with *FLG* mutations. As mentioned above the prevalence of atopy is significantly higher in CRSwNP patients compared to both control and CRSsNP patients.

Furthermore, the mean age of atopic CRSwNP patients is significantly higher than atopic control and CRSsNP patients. While skin barrier dysfunction in the setting of *FLG* mutations could be an important trigger for allergic sensitization in the control and CRSsNP group, in CRSwNP patients, other factors, perhaps limited to this group, might play a role in development of aeroallergen sensitization later in life.

An important conclusion of this report is that the present data do not support the hypothesis that *FLG* null mutations are associated with increased risk for either form of CRS (CRSwNP or CRSsNP). Our immunohistochemistry analysis failed to detect *FLG* in either uncinate or polyp tissue (not shown). It appears that despite their role in skin epithelial barrier dysfunction and allergic sensitization, even to aeroallergens, these *FLG* null mutations have no evident role in the upper respiratory epithelial barrier dysfunction present in CRS. This study had power of 67-70% for the various reported calculations. We did not examine other rare *FLG* variants (e.g., *R2447X*, *S3247X*, *3702delG*), choosing to focus on the two most common mutations identified in subjects of European ancestry (1, 3, 5).

In conclusion, this is the first study to our knowledge to assess whether *FLG* mutations are associated with CRS; we found no evidence for an association in our series of patients. This, along with lack of expression of filaggrin in sinus and polyp tissue, indicates that these mutations, and the atopic sensitization associated with them, are unlikely to make major contributions to the development of CRS. This conclusion is supported by other studies suggesting that CRS is not strictly a disease driven by aeroallergy. There was a significant association between *FLG* null mutations and atopy in controls and CRSsNP patients but not in CRSwNP, while CRSwNP patients had the highest prevalence of atopy. This could be suggestive of unique factors influencing the development of aeroallergen sensitization in CRSwNP.

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Table I

Association between <i>FLG</i> null mutations and atopy	Odds ratios (95% confidence intervals) for <i>FLG</i> null mutation, adjusted for gender and age		
	<i>R501X</i>	<i>2282del4</i>	<i>R501X</i> and/or <i>2282del4</i>
All subjects (221 atopic, 281 non-atopic)	2.06 (0.78, 5.44)	3.45 (1.55, 7.69)*	2.96 (1.55, 5.65)*
Controls (69 atopic, 135 non-atopic)	4.29 (1.17, 15.67)*	3.20 (0.86, 11.86)	4.26 (1.56, 11.61)*
CRS (152 atopic, 146 non-atopic)	1.26 (0.27, 5.81)	3.58 (1.25, 10.22)*	2.57 (1.07, 6.12)*
CRSsNP only (69 atopic, 92 non-atopic)	1.46 (0.19, 10.70)	5.18 (1.36, 19.77)*	3.73 (1.24, 11.19)*
CRSwNP only (83 atopic, 54 non-atopic)	2.16 (0.40, 11.48)	1.54 (0.13, 8.13)	1.72 (0.41, 7.21)
Association between <i>FLG</i> null mutations and CRS in atopic and non-atopic patients			
All CRS vs. controls (298 CRS, 204 controls)	1.33 (0.88, 6.15)	1.49 (0.68, 3.24)	0.89 (0.48, 1.65)
Atopic only (152 CRS, 69 controls)	0.31 (0.09, 1.05)	1.28 (0.47, 3.48)	0.82 (0.37, 1.77)
Non-atopic only (146 CRS, 135 controls)	1.26 (0.27, 5.89)	1.12 (0.29, 4.31)	0.94 (0.33, 2.68)
CRSsNP vs. controls (161 CRSsNP, 204 controls)	0.45 (0.13, 1.45)	0.63 (0.26, 1.49)	1.09 (0.54, 2.19)
Atopic only (69 CRS, 69 controls)	0.26 (0.05, 1.32)	0.56 (0.19, 1.65)	0.82 (0.35, 1.96)
Non-atopic only (92 CRS, 135 controls)	0.99 (0.15, 6.22)	1.02 (0.22, 4.77)	1.10 (0.32, 3.79)
CRSwNP vs. controls (137 CRSwNP, 204 controls)	0.39 (0.10, 1.47)	0.77 (0.28, 2.07)	0.80 (0.35, 1.81)
Atopic only (83 CRSwNP, 69 controls)	0.31 (0.07, 1.29)	1.11 (0.32, 3.76)	0.57 (0.22, 1.47)
Non-atopic only (54 CRSwNP, 135 controls)	1.63 (0.14, 8.58)	0.73 (0.12, 4.34)	1.60 (0.36, 7.08)

§ p<0.05 for comparing prevalence of atopy in controls vs. CRSwNP and p<0.05 for comparing prevalence of atopy in CRSsNP vs. CRSwNP

* p<0.05