



Letter

Association of common filaggrin null mutations with atopy but not chronic rhinosinusitis

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 individuals 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 among *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 2 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 patients with CRS and controls. Patients with CRS (determined using Sinus and Allergy Health Partnership guidelines⁸ and evaluated by nasal endoscopy and computed tomography) were recruited from the otolaryngology and allergy-immunology clinics at Northwestern Feinberg School of Medicine (n = 298; 137 patients with CRS with nasal polypsis [CRSwNP] and 161 patients with CRS without nasal polypsis [CRSsNP]). All patients with CRS had medically refractory disease and underwent functional endoscopic sinus surgery, during which nasal tissue samples and blood were obtained. Controls without a history of CRS were recruited from the same otolaryngology and allergy-immunology clinics (n = 204). Atopy status for all patients was determined by patient history based on positive skin prick test result or specific IgE to aeroallergens. Race/ethnicity was categorized by self-report. The Northwestern Institutional Review Board approved this study, and all participants provided written informed consent.

DNA was extracted from tissue, saliva, or blood samples using a Nucleospin Genomic DNA extraction kit (Machery-Nagel, Bethlehem, Pennsylvania) following the manufacturer's instructions.

Drs Mahdavinia and Hsu contributed equally to this study.

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Genotyping of 2282del4 and R501X was performed by restriction fragment length polymorphism analysis and Taqman-based allelic discrimination assays (Applied Biosystems).^{2,9}

Logistic regression was used to examine the sex- and age-adjusted associations between *FLG* mutations and phenotype. Stratified analyses by atopy 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 statistical software, version 21. $P < .05$ was considered statistically significant.

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

The male to female ratio in the CRSwNP group was higher compared with the control and CRSsNP groups, whereas the CRSsNP patients and controls had similar male to female ratios. The mean age of CRSwNP patients was significantly higher than that of the CRSsNP and control groups, whereas there was no significant difference between the CRSsNP patients and controls. Subgroup analysis revealed that CRSwNP nonatopic patients were older compared with both CRSsNP nonatopic patients and nonatopic controls, whereas no significant difference was found among atopic cases of all groups in terms of age. All analyses were adjusted for sex and age as reported in Table 1. Adjusting the data analysis for age and sex 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 controls 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 with controls and CRSsNP patients. Furthermore, the mean age of atopic CRSwNP patients is significantly higher than atopic controls and CRSsNP patients. Although skin barrier dysfunction in the setting of *FLG* mutations could be an important trigger for allergic sensitization in the control and CRSsNP groups, 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 immunohistochemical analysis failed to detect *FLG* in

Table 1
Association between *FLG* null mutations and atopy and CRS

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

Abbreviations: CI, confidence interval; CRS, chronic rhinosinusitis; CRSsNP, CRS without nasal polyposis; CRSwNP, chronic rhinosinusitis with nasal polyposis; OR, odds ratio.

^aORs are adjusted for sex and age.^b*P* < .05.

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% to 70% for the various reported calculations. We did not examine other rare *FLG* variants (eg, R2447X, S3247X, 3702delG), choosing to focus on the 2 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 of 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 patients, whereas CRSwNP patients had the highest prevalence of atopy. This could be suggestive of unique factors that influence the development of aeroallergen sensitization in CRSwNP patients.

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