Loss of Function Mutations in *Filaggrin* are Associated with Multiple Contact Positive Reactions

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Background

Polysensitivity has been defined as the three or more positive patch tests. Patients with atopic dermatitis or other forms of eczema are more likely to develop polysensitivity. This suggests that defects in barrier function as caused by null mutations in *FLG* or other induced defects in barrier function may play a predisposing role. In this Canadian study we evaluated the association of loss of function mutations in *FLG* and polysensitivity.

Methods

Patients were ascertained over a 10 period from 3 University based patch test clinics at the Universities of Saskatchewan (PH), Ottawa (MP) and McGill University in Montreal (DS). Patients with 3 or more unrelated positive patch tests were included. In order to exclude skin barrier bypass, Nickel was excluded as were patients with metal implants, a prior history of hand dermatitis and stasis dermatitis with and without ulceration. The following prevalent European null mutations in *FLG* were sequenced, **R501X, 2282del4, R2447X and S3247X**. Sequencing of PCR products was performed by a core facility (DNA Sequencing and Services, University of Dundee, Dundee, United Kingdom) as well as at Dalhousie University. As prevalent mutations are not known for other ethnic groups in Canada, the study was confined to those with self identified European ancestry.

165 participants were included. In an additional 4 sequencing failed for all four mutations tested and they were excluded from the study. Single mutations failed in 2 cases (one each for R501X and S3247X).

Control Population

The control population were adult volunteers from Toronto, Ontario, whose DNA is commercially available. A total of **891** Canadian controls of Caucasian ethnicity were screened for the same *FLG* mutations. The presence or absence of coexisting atopic dermatitis is not known.

Patient Demographics

	Combined	FLG Wild Type	FLG Null Mutations
Female	71%	74%	62%
Average age	52	54	46
AD	34%	32%	46%
Asthma	22%	20%	35%
Hay fever	38%	37%	41%
Food allergies	18%	16%	29%
FH AD	37%	34%	29%
FH Asthma	33%	33%	53%
FH Food Allergies	17%	16%	21%
Average no of Positives	6	6	6
North American Series	4.5	4.5	4.7

References

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Results

Genotyping results and statistical analysis of *FLG* loss-of-function mutations in 165 patients with polysensitivity and 891 Canadian controls

	501X		2282del4		R2447X		S3247X		Combined	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Wild Type	134	859	135	846	136	883	136	884	137	793
One FLG Mutation	13	34	9	46	2	11	3	11	24	9
Compound Het									2	3
Homozygous	1		1					1	2	1
Percentage with Mutations	8.6%	3.8%	6.8%	5.2%	2.1%	1.2%	2.1%	1.1%	17%	9.9%
Fisher's Exact Test	P=0.005		P=0.426		P=0.698		P=0.4		P=0.036	
Odds Ratio (CI)	2.639596 (1.2719 to 5.2030)		1.3623 (0.5981 to 2.8190)		1.135027 (0.1210 to 5.2843)		1.95 (0.5725 to 6.6655)		1.6548 (1.0058 to 2.6513)	
Z Statistic Significance level	2.934 P=0.0033		0.856 P = 0.3917		0.858 P = 0.3912		1.005 P=0.3159		2.155 P= 0.0311	

Top 10 Positive Allergens

Combined	FLG Wild Type	FLG Null Mutations
Fragrance mix 1	Fragrance mix 1	Formaldehyde
Formaldehyde	Formaldehyde	Balsam of Peru
Quaternium 15	Quaternium 15	Cobalt
Balsam of Peru	Balsam of Peru	Chromate*
Nickel	Bacitracin	Quaternium 15*
Bacitracin*	Nickel	Lanolin*
Cobalt*	Colophony	Fragrance Mix 1*
Colophony**	Cobalt*	Neomycin
Neomycin**	Neomycin*	Colophony**
Lanolin	PPD	Nickel**
PPD	Lanolin	Propylene glycol**
*, ** same frequency		

Discussion.

Loss of function mutations are a major predisposing factor in atopic dermatitis. The role in allergic contact dermatitis is less clear. Loss of function mutations in *FLG* have been associated with irritant dermatitis, which in turn would damage barrier function and predispose to an allergic contact dermatitis.¹ Polysensitivity has been defined as the finding of 3 or more positive patch tests.² In a previous study, polysensitivity was not associated with *FLG* mutations.³ In this study, we examined this relationship, but excluded situations where the barrier may have been either by-passed or damaged.

Conclusion

In this Canadian group of patients, polysensitivity was significantly associated with loss of function mutations in *FLG*.