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Filaggrin Null Mutations Are Not a Protective Factor for Acne Vulgaris

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TO THE EDITOR

Acne vulgaris is a very common skin disorder, affecting to some degree 88–94% of Singaporean adolescents (Tan *et al.*, 2007; Yosipovitch *et al.*, 2007). Genetic predisposition is a significant risk factor, as illustrated by familial and twin studies (Bataille *et al.*, 2002; Ghodsi *et al.*, 2009). The clinical features of acne include seborrhea, comedone formation, inflammatory pustules, nodules, and cysts, with resultant scarring. Important pathogenic mechanisms in acne include increased sebum production, hyperkeratinization and occlusion of the follicular duct, proliferation of *Propionibacterium acnes*, and an inflammatory reaction (Purdy and de Berker, 2006). *P. acnes* produces lipases, which liberate proinflammatory fatty acids from sebum and also triggers a cytokine response.

Filaggrin is expressed in terminally differentiating keratinocytes and has a key role in epithelial barrier formation. Immunostaining demonstrates increased filaggrin expression in the sebaceous duct and infundibulum of acne vulgaris skin (Kurokawa *et al.*, 1988), and *P. acnes* strains increase the expression of filaggrin and other differentiation-specific markers in normal human epidermal keratinocytes *in vitro* and in the suprabasal layers of human skin explants (Jarrousse *et al.*, 2007). Similarly, inflammatory cytokines resulted in increased filaggrin expression in sebaceous gland explants (Guy and Kealey, 1998). However, it is not known whether differences in filaggrin expression represent a primary or secondary effect in the pathogenesis of acne.

Null mutations in the filaggrin gene (*FLG*) result in reduced filaggrin expression and cause ichthyosis vulgaris

(Smith *et al.*, 2006). Such mutations are common in the general population, being carried by ~10% of Europeans and 7.3% of Singaporean Chinese (Sandilands *et al.*, 2007; Chen *et al.*, personal communication). This high carrier rate in different populations suggests a heterozygote advantage, and it has been proposed that a more permeable skin barrier may have been beneficial in evolutionary history (Irvine and McLean, 2006). The co-existence of *FLG* null mutations with other gene mutations that disrupt epidermal differentiation may increase phenotype severity (Liao *et al.*, 2007; Gruber *et al.*, 2009). It is also possible that heterozygosity for null mutations has other effects on skin physiology. Studying a cohort of 284 European dermatology patients not selected for dry skin (Sergeant *et al.*, 2009) raised the possibility that carriage of one *FLG* null mutation could provide a protective effect against acne vulgaris. In the Sergeant

Abbreviations: FLG, filaggrin gene

Table 1. Genotyping results and Fisher's exact test to investigate the association between *FLG* null mutations and acne vulgaris in a case-control study

<i>FLG</i> null mutation	Acne vulgaris cases and unselected population controls	Genotype ¹			Total ²	Fisher's exact test <i>P</i> -value
		AA	Aa	aa		
p.S406X	Cases	279	0	0	279	0.524
	Controls	438	2	0	440	
c.1249insG	Cases	276	4	0	280	0.023
	Controls	440	0	0	440	
c.2282del4	Cases	275	0	0	275	1.000
	Controls	436	1	0	437	
c.3321delA	Cases	277	4	0	281	0.582
	Controls	439	10	0	440	
p.S1302X	Cases	272	0	0	272	0.304
	Controls	434	4	0	438	
p.S1515X	Cases	275	1	0	276	1.000
	Controls	438	2	0	440	
c.6834del5 ³	Cases	280	1	0	281	0.390
	Controls	440	0	0	440	
c.6950del8	Cases	278	3	0	281	1.000
	Controls	436	4	0	440	
p.Q2417X	Cases	278	2	0	280	0.151
	Controls	440	0	0	440	
p.E2422X	Cases	278	2	0	280	0.151
	Controls	440	0	0	440	
c.7945delA	Cases	274	2	0	276	0.562
	Controls	439	1	0	440	
p.S2706X	Cases	276	1	0	280	0.160
	Controls	431	7	0	438	
c.8157delC ³	Cases	278	1	0	279	0.388
	Controls	440	0	0	440	
p.R4307X	Cases	278	1	0	279	0.488
	Controls	438	1	0	439	
Combined <i>FLG</i> null genotype	Cases	235	21	0	256	0.783
	Controls	402	31	1	434	

¹AA, homozygous wild type for *FLG* null mutation; Aa, heterozygous for the stated *FLG* null mutation or, in the combined null genotype, any one of the screened mutations; aa, compound heterozygous (i.e., an individual having two different *FLG* null mutations). The rationale for generating this combined null genotype is based on the fact that each mutation results in premature termination of the profilaggrin molecule and hence absence of processed filaggrin (Sandilands *et al.*, 2007).

²The figures in the total column vary because of incomplete genotyping results; all available data have been used for optimal analysis of each individual variant, but the combined *FLG* null genotype data include only those individuals for whom all 14 genotype results are available.

³Previously unreported *FLG* mutations.

study, the odds ratio of acne in the carrier group was 0.3 (95% confidence interval 0.1–1.0), but the difference between these individuals and the group without *FLG* mutations did not reach statistical significance ($P=0.08$). In addition, this study relied on a

recalled history of acne. We therefore aimed to test the hypothesis that *FLG* null mutations are protective against the development of acne vulgaris by studying a well-documented group of patients with acne, and comparing with a population control group.

A total of 287 Singaporean Chinese patients presenting with acne vulgaris to the National Skin Centre, a major dermatology outpatient facility in Singapore, were recruited: mean age 22 years (SD 4.8), range 14–50 (27% <20 years of age and 95% were under 30 years), 76.3%

were male. Acne vulgaris symptoms were reported for a mean of 5.6 years (SD 4.2), range from <1 to 32 years. Patients with polycystic ovarian syndrome were excluded from this study. Acne severity was assessed using the Global Severity Assessment Score (Lehmann *et al.*, 2002): 100 patients (34.8%) had mild acne, 129 (44.9%) had moderate acne, and 58 (20.3%) had severe acne. DNA samples from 440 unselected Singaporean Chinese population controls with a mean age of 44.6 years (SD 14.0), range 1–80, 44.1% male, for whom acne vulgaris status was unknown, were obtained from Singapore Bio-Bank, Singapore. This study was approved by the local domain specific ethical review board in accordance with the Declaration of Helsinki and all participants gave written, informed consent.

Cases and controls were screened for all 22 population-specific *FLG* null mutations as recently reported (Chen *et al.*, personal communication). In this group a total of 12 known *FLG* null mutations were detected: p.S406X, c.1249insG, c.2284del4, c.3321delA, p.S1302X, p.S1515X, c.6950del8, p.Q2417X, p.E2422X, c.7945delA, p.S2706X, and p.R4307X, plus two mutations: c.6834del5 and c.8157delC, which to our knowledge are previously unreported. Fisher's exact test and logistic regression analyses were used to compare the prevalence of *FLG* null mutations between cases and controls, using the statistical analysis package Stata (Version 9, Stata for Linux, StataCorp LP, College Station, TX). Power calculations were performed using Quanto version 1.2.4 (University of Southern California, <http://hydra.usc.edu/gxe/>).

In this study, 8.2% of Singaporean Chinese acne vulgaris cases carried one or more *FLG* null mutations compared with 7.3% of the control population, a non-significant difference (Fisher's exact test $P=0.783$, odds ratio 1.2, 95% confidence interval 0.7–2.1), shown in Table 1. It is unlikely that our failure to demonstrate an association has occurred because of lack of power, as analysis of 256 acne vulgaris patients and 434 controls, assuming a population prevalence of 88% for acne vulgaris of comparable severity (Tan *et al.*, 2007) would give a power of 99%

to detect an odds ratio of 0.3 (Sergeant *et al.*, 2009) for the combined *FLG* null genotype, with two-sided $P=0.05$. The equivalent calculation using a population prevalence of 23% (based on Singaporean teenagers reporting treatment for their acne (Yosipovitch *et al.*, 2007)) gives an estimated power of 90% for this study. Furthermore, the comprehensive screening of all 22 reported *FLG* null mutations from this carefully characterized Singaporean Chinese population means that the lack of association is unlikely to have occurred because of incomplete ascertainment of the *FLG* genotype.

Our finding, that in this Singaporean Chinese population the frequency of *FLG* null mutations in acne vulgaris patients is not statistically different from the ethnically matched controls, indicates that filaggrin haploinsufficiency is unlikely to have a generic protective effect in acne. It is likely that the overexpression of filaggrin in the pilosebaceous units reported in the disorder is a bystander effect, reflecting other alterations in keratinocyte differentiation, but not itself critical for acne pathogenesis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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