Mutations in the SASPase gene (*ASPRV1*) are not associated with atopic eczema or clinically dry skin

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To the Editor

A key event during epidermal differentiation is the proteolytic breakdown of profilaggrin into "free" filaggrin monomers. A recent study has shown that the skin specific retroviral-like aspartic protease (SASPase) plays a key role in profilaggrin-filaggrin processing (Matsui *et al.*, 2011). SASPase cleaves the linker peptide between the individual filaggrin monomers of profilaggrin and on a *hairless* mouse background, loss of SASPase leads to dry, scaly skin with reduced stratum corneum hydration accompanied by accumulations of profilaggrin-filaggrin intermediates but an absence of filaggrin monomers (Matsui *et al.*, 2011). In this same study several missense mutations in the SASPase gene in atopic eczema patients and controls were identified, some of which were shown to have a detrimental effect on the ability of SASPase to cleave the profilaggrin linker peptide. Given the important role of filaggrin in skin barrier function and maintaining stratum corneum hydration (O'Regan and Irvine, 2010), these results prompted us to question whether aberrant profilaggrin-filaggrin processing due to altered SASPase activity could provide an alternative pathogenic mechanism for atopic eczema or clinically dry skin.

To answer this question, the entire coding region of the SASPase gene, *ASPRV1*, was amplified by PCR in a single fragment and fully sequenced. To maximize our chances of finding mutations that might be associated with atopic eczema or clinically dry skin we sequenced *ASPRV1* from three discovery cohorts; 96 paediatric atopic eczema cases from Ireland, 96 atopic eczema cases from the Cape Town region of South Africa (Xhosa people)

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and 99 cases of clinically dry skin from patients referred to dermatology clinics in Glasgow, Scotland. Atopic eczema in the Irish paediatric cases was diagnosed using the UK Diagnostic criteria (Williams *et al.*, 1994); atopic eczema in the Xhosa people was diagnosed by experienced dermatologists. Clinically dry skin was defined using a previously published scoring system (Sergeant *et al.*, 2009). Demographic and clinical data relating to the discovery cohorts are shown in Table 1.

Sequencing of the *ASPRV1* gene in the discovery cohorts identified a total of 5 non-synonymous mutations and 2 synonymous mutations (Table 2). None of the *ASPRV1* mutations identified in a previous Japanese study (Matsui *et al.*, 2011) were detected in our discovery cohorts. We then investigated some of these mutations further by screening an additional 259 Irish atopic eczema cases and 167 Scottish dry skin cases (which included the original 93 cases from the discovery cohort) using custom-designed TaqMan® allelic discrimination assays for the mutations V74I, G87R and S333F (Supplementary Table 1). The G87R mutation was identified only in a single case of atopic eczema and the V74I and S333F mutations were not detected in any of these additional cases. In the 167 cases of dry skin, the G87R and S333F mutations were found only in single cases (ie. in the original discovery cohort) and the V74I mutation was not found in any of the cases. We then carried out two independent case-control studies to investigate any association between the T49A mutation and atopic eczema and clinically dry skin (Supplementary Table 2). 442 Irish atopic eczema cases (which included the original 92 cases from the discovery cohort) and 458 Irish population controls were screened using a TaqMan® allelic discrimination assay. There was no association between the T49A mutation and atopic eczema in the Irish study: chi-square p=0.415, odds ratio 0.98 (95% confidence interval 0.81–1.18). Similarly, screening of 167 clinically dry skin cases and 100 Scottish population controls failed to reveal any association between the T49A mutation and dry skin: p=0.479, odds ratio 0.90 (0.63–1.28). Power calculations showed that the eczema case-control study had >80% power to detect an odds ratio of 1.5 or above and the dry skin case-control study study had >70% power to detect an odds ratio of 2.0 (Quanto 1.2.4, University of Southern California, http:// hydra.usc.edu/gxe/). Since *FLG* null mutations are known to have such a strong effect on eczema risk, it is possible that the effect of *ASPRV1* mutations may only be apparent in *FLG* wild-type individuals. Therefore the four most prevalent *FLG* null mutations (R501X, 2282del4, R2447X and S3247X) were screened in each of the cases and controls using methods described previously (Kezic *et al.*, 2011; Sandilands *et al.*, 2007). The statistical analyses for each study were repeated after excluding individuals carrying *FLG* null mutations, but there was still no evidence of association between *ASPRV1* mutation T49A and eczema or clinically dry skin (Supplementary Table 3).

With the exception of T49A and to a lesser extent L325L, the remaining *ASPRV1* mutations that we identified were rare (<1%) and therefore unlikely to be significant on a population level, although it is still possible that these rare mutations could contribute significantly to individual disease risk. Mutations P206P and L325L result in synonymous changes and are therefore unlikely to be pathogenic. All of the non-synonymous mutations we identified (Supplementary Figure 1) affect amino acid residues outside the active protease site of SASPase (Bernard *et al.*, 2005), however the effect of these mutations on SASPase activity remains to be determined experimentally.

Finally, we used custom-designed TaqMan® allelic discrimination assays (Supplementary Table 1) to screen for the V187I and V243A mutations which reduce and abolish SASPase-mediated profilaggrin cleavage respectively (Matsui *et al.*, 2011). However we failed to detect these mutations in any of the discovery cohorts, nor in the Irish atopic eczema cohort, indicating that these mutations are likely to be specific to the Japanese population.

Although our results failed to find an association between *ASPRV1* gene mutations and atopic eczema or clinically dry skin in the European populations that we studied, they do not exclude the possibility that an association exists in other ethnicities. In the populations that we studied, other factors which modulate SASPase activity could contribute instead, such as the actions of protease inhibitors which provide a powerful counterbalance against excessive protease activities (Hewett *et al.*, 2005). Profilaggrin-filaggrin processing is a tightly regulated process involving not just SASPase but multiple proteases such as elastase 2 (Bonnart *et al.* 2010) and the serine proteases matriptase/MT-SP1 (List *et al.*, 2003) and prostasin (Leyvraz *et al.*, 2005). A greater understanding of the proteases and inhibitors involved in profilaggrin-filaggrin processing will be required to fully appreciate their contribution to skin barrier dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic and clinical data relating to eczema and dry skin cases and population controls

Demographic and clinical features	South African atopic eczema cases	Irish atopic eczema cases	Irish population controls	Scottish dry skin cases	Scottish population controls
Total number (% male)	102 (51.0)	442 (63.6)	460 (30.7)	178 (34.8)	100 (50.0)
Age in years mean (SD) and range	8.9 (9.9) 0.7 to 50.9	3.3 (11.8) 0.25 to 17.0	35.4 (9.2) 19 to 69	45.5 (48.1) 18 to 86	18
Eczema severity score, mean (SD) and range	10.4 (2.7) 5 to 15	11.3 (6.4) 3 to 15	0	NA	NA
Reported childhood eczema (%)	NA	NA	NA	16.3	NA

All patient studies conformed to the Declaration of Helsinki Principles and written informed consent was obtained. Irish population controls represent healthy adults from the population-based Trinity Biobank control samples; Scottish population controls are derived from adults attending hospital for haematologic investigations; eczema severity is scored using the Nottingham Eczema Severity Score (Emerson et al., 2000); dry skin was defined using a previously reported scoring system (Sergeant et al., 2009) and corresponds to visible fine scale (noted by a trained observer) on one/more body sites, self-reported use of a moisturizer more than once weekly or self-reported dry skin 'moderately' to 'a lot'. SD, standard deviation; NA, not applicable.

Table 2

dbSNP minor allele frequencies of *ASPRV1* polymorphisms identified in the discovery cohorts

ASPRV1 mutation	dbSNP minor allele	rs number	Irish atopic eczema cases (n=92)	South African atopic eczema cases (n=90)	Scottish dry skin cases (n=93)
c.145 A>G * p.T49A	A	rs3796097	A=0.418	A=0.083	A=0.435
c.155 G>A p.R52Q	NA	rs151323610	A=0.005	0	0
c.220 G>A p.V74I			A=0.005	0	0
c.259 G>A p.G87R	NA	rs148290351	0	0	A=0.005
c.618 C>T p.P206P	T	rs114182672	0	T=0.005	0
c.973 C>T p.L325L	T	rs115036001	0	T=0.111	0
c.998 C>T p.S333F			0	0	T=0.005

n indicates the number of fully sequenced samples NA: minor allele not ascertained.

The *ASPRV1* gene was amplified for sequencing using forward primer 5′ -ATGTGGTAGGAGGCTCAGTACACATGTAAAC-3′ and reverse primer 5′ -AGAAGAGCAAGAGTTGATAAAGCAGACTG-3′ to generate a 1532bp product. 50ng of genomic DNA was amplified in a 25μl reaction using 0.5U AmpliTaq Gold® polymerase (Applied Biosystems). For PCR amplification, an annealing temperature of 65°C and a 3 minute extension at 72°C was used (35 cycles). PCR products were purified and sequenced using overlapping primers in both directions: Forward 1 5′ - TTCCTTCACTGGCTGATGAC -3′; Forward 2 5′ -TTGCTGCTGAGGTTCCAGAG -3′; Forward 3 5′ -TCACTGATGGCGATCTGGAC -3′ and Reverse 1 5′ - AGAAGAGCAAGAGTTGATAAGC-3′; Reverse 2 5′ - CCCAGGATCTTCATTTCAGC-3′ Reverse 3 5′ -GATGACTTCAAAGCTGTGCAG-3′ .

* G is the ancestral allele and A is the designated minor allele for this mutation.