# Loss-of-Function Mutations in the Gene Encoding Filaggrin Are Not Strongly Associated with Chronic Actinic Dermatitis

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## TO THE EDITOR

Chronic actinic dermatitis (CAD) is an uncommon photosensitivity disease, characterized by a photoexposed site dermatitis that may be severe and disabling (Kerr and Ibbotson, 2006) (Supplementary Figure S1 online). Objective evidence of broadband photosensitivity to UVB and often also UVA and visible wavebands is evident and most patients also have multiple contact allergies (Frain-Bell *et al.*, 1974). The condition occurs worldwide, although it is more common in temperate climates and typically develops in middle-aged to elderly males. Most individuals have a history of a preceding dermatitis, either atopic, contact allergic, or seborrhoeic, although it can occur *de novo* (Frain-Bell *et al*, 1974; Hawk, 2004; Kerr and Ibbotson, 2006). Resolution of photosensitivity is reported in up to 50% of individuals after 15 years or more, with contact allergies persisting (Dawe *et al.*, 2000).

The pathogenesis of CAD is incompletely understood; cases are sporadic and genetic risk factors have not been identified. Histology and immunohistochemistry of UVR-induced lesional skin are consistent with an immunological delayed-type hypersensitivity reaction (Menage *et al.*, 1996). Given the associations with contact allergies and atopic eczema, we considered that epidermal barrier dysfunction might be a key factor in pathogenesis. The characteristic photosensitivity indicates aberrant cutaneous responses to UVR are also likely to be implicated. Filaggrin is an important structural protein in the epidermis (Smith *et al.*, 2006). Loss-of-function mutations in the gene encoding filaggrin (*FLG*) are associated with skin barrier dysfunction and increased risk of atopic eczema (Palmer *et al.*, 2006; Baurecht *et al.*, 2007). Filaggrin is degraded to release amino acids in the stratum corneum and *FLG* mutations lead to reduction in photo-absorbing amino acids, specifically urocanic acid (UCA), a chromophore for UVB that may protect against photodamage (de Fine Olivarius *et al.*, 1996; Mildner *et al.*, 2010; Barresi *et al.*, 2011). We aimed to test the hypothesis that *FLG* loss-of-function mutations are associated with increased risk of CAD, supporting a role for lack of intrinsic photoprotection and/or skin barrier dysfunction in the pathogenesis of CAD.

This study was conducted in accordance with the Declaration of Helsinki Principles; written informed consent was obtained from all participants. Sequential patients aged ≥18 years attending the National Photodiagnostic Service and diagnosed with CAD were invited to participate. The diagnosis was based on clinical and photobiological assessment (including monochromator phototesting) by a photodermatologist. Patient-reported history of atopic disease was recorded. Patch testing was performed as clinically indicated. Blood was taken for total IgE levels and DNA analysis. DNA samples were analyzed for *FLG* mutations within the CAD cases of white European ethnicity and in 100 Scottish population-matched controls. The four most prevalent *FLG* loss-of-function mutations in this population were screened (R501X, 2282del4, R2247X, and S3247X) as previously described (Kezic *et al.*, 2011). The $\chi^2$ and logistic regression analyses were used to test for association between CAD and *FLG* combined null genotype in Stata (version 13; StataCorp LP, College Station, TX).

A total of 55 patients were recruited; the clinical characteristics and investigation findings are summarized in Table 1. The mean age of onset of photosensitivity was 50.2 (range 21–77) years. The majority (35/55, 63.6%) had marked UVB sensitivity (<50% of lower limit of normal) and 25 patients (45.5%) had abnormal photosensitivity to UVB, UVA, and visible wavebands (Table 1). Photosensitivity had resolved by the time of recruitment in three cases (5.5%). Most patients (42/55; 76.4%) had positive patch testing, including 37 (88.1%) with multiple allergen reactions. Of those with contact allergy, the median number of allergens was 4 (range 2–31). A diagnosis of atopic disease (eczema, asthma, allergic rhinitis, or type I hypersensitivity reaction) was noted in 23 (41.8%) patients. Total IgE levels were elevated in the majority (Table 1).

Genotyping results were obtained for all four of the *FLG* mutations in 48/49 cases (Table 2 and Supplementary Figure S2 online), of whom 10 had a *FLG* mutation (20.4%). Of these 49 patients, 22 (44.9%) demonstrated atopic comorbidity and of the cases carrying a *FLG* mutation, 6/10 had a history of atopic eczema; in one patient the atopic status was not recorded. In the control group, 12/100 (12.0%) individuals were heterozygous for a *FLG* loss-of-function mutation but this was not significantly different from the prevalence seen in the cases ($\chi^2$ $P=0.16$, odds ratio (OR) = 1.92, 95% confidence interval (CI) = 0.77–4.85). The apparently higher prevalence of *FLG* mutations within the cases compared with controls may reflect the co-association

*Abbreviations: CAD, chronic actinic dermatitis; CI, confidence interval;* FLG, *gene encoding* filaggrin; *OR, odds ratio; UCA, urocanic acid*

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**Table 1. Demographic, clinical, and investigative characteristics of CAD cases and population controls**

| Characteristic | *N* (%) |
|---|---|
| *Cases* | Total 55 |
| Male | 28 (50.9) |
| Female | 27 (49.1) |
| Fitzpatrick skin type I–III (including 10 *FLG* heterogygotes)[1] | 49 (89.1) |
| Fitzpatrick skin type IV–VI (*FLG* genotype undefined) | 6 (10.9) |
| *Abnormal photosensitivity by waveband (at time of recruitment)* | 55 cases |
| UVB only (305 ± 5 nm) | 0 |
| UVA only (335/365 ± 30 nm) | 1 (1.8) |
| Visible only (400 ± 30 nm) | 0 |
| UVA and UVB | 22 (40.0) |
| UVA and visible | 4 (7.2) |
| All wavebands (UVB, UVA, and visible light) | 25 (45.5) |
| Photosensitivity resolved | 3 (5.5) |
| *Degree of UVB (305 ± 5 nm) sensitivity*[2] | |
| <10% Of normal | 17 (30.9) |
| 11–19% Of normal | 7 (12.7) |
| 20–49% | 11 (20.0) |
| 50–75% | 11 (20.0) |
| ≥76% | 9 (16.4) |
| *Atopic disease* | |
| Eczema, asthma, hay fever, or food allergy | 23 (41.8) |
| *Immunoglobulin E levels* (Ku $l^{-1}$) | |
| Normal (<100) | 13 (23.6) |
| 100–999 | 17 (30.9) |
| 1,000–5,000 | 10 (18.2) |
| >5,000 | 6 (10.9) |
| Unavailable | 9 (16.4) |
| *Positive reactions to contact allergens* | 42/55 cases (76.4%) |
| Single | 5 (11.9) |
| Multiple | 37 (88.1) |
| *Number of patch test series with positive reaction per patient* | |
| Single series | 19 (45.2) |
| Two series | 15 (35.7) |
| Three series | 7 (16.7) |
| Four series | 1 (2.4) |
| *Commonest positive patch test series* | |
| BCD standard | 38 (90.5) |
| Plants | 12 (28.6) |
| Sunscreens[3] | 7 (16.7) |
| Corticosteroids | 3 (7.1) |
| Medicaments | 3 (7.1) |
| *Controls* | Total 100 |
| Male | 50 (50.0) |
| Female | 50 (50.0) |

Abbreviations: BCD, british contact dermatitis; CAD, chronic actinic dermatitis; *FLG*, *filaggrin*.
[1]Details provided in Table 2.
[2]Abnormal UVB sensitivity (assessed by monochromator phototesting at 305±5 nm (half-maximum bandwidth)): the minimal erythemal dose (MED) expressed as a percentage of the lowest normal value for MED at this waveband in healthy volunteers of skin phototypes I–III. The lower the MED, the higher the level of abnormal photosensitivity. Thus, an MED of <10% of normal is the most abnormally photosensitive.
[3]Sunscreen patch testing does not represent photopatch testing.

of atopic eczema with CAD, and subgroup analysis of the small number of atopic CAD cases ($n=22$) showed a borderline but nonsignificant association with *FLG*-null genotype ($P=0.05$, OR = 2.93, 95% CI = 0.95–9.01). In contrast, the nonatopic cases ($n=26$) showed no association with *FLG* genotype ($P=0.95$, OR = 0.96, 95% CI = 0.25–3.67).

To assess whether the lack of association of *FLG*-null mutations with CAD is a true negative result or a reflection of the lack of statistical power, we performed a power calculation using Quanto 1.2 (http://biostats.usc.edu/software). The CAD prevalence is 1:2,000 within the Tayside area of Scotland (Dawe, 2005), and *FLG*-null allele frequency is 0.06 (Table 2), and therefore 48 cases and 100 controls provide >75% power to detect an OR of ≥3.2.

CAD generally occurs on the background of preexisting dermatitis, but the mechanisms triggering photosensitivity remain unclear. The chromophore(s) and the nature of UV-induced neoantigen initiation of a delayed hypersensitivity reaction remain unknown (Hawk, 2004). Profilaggrin and filaggrin are multifunctional proteins contributing to epidermal barrier formation and function (Brown and McLean, 2012), limiting allergen penetration and facilitating photoprotection. There is growing evidence that *FLG* mutations are strongly associated with delayed-type hypersensitivity (Thyssen *et al.*, 2013), and therefore *FLG* represents a candidate gene in CAD. The lack of association in this study has effectively excluded *FLG*-null genotype as playing a strong role (OR >3.2) in CAD pathogenesis, and further research is needed to define key pathomechanisms. Our data represent one of the largest collections of carefully phenotyped cases of this uncommon but highly symptomatic photodermatosis. Despite increasing clinical understanding of CAD, knowledge of mechanisms and thus of informed treatment options remains limited and further studies are indicated.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

**Table 2. *FLG* genotyping results for patients of white European ethnicity**

| | Cases, *n* (%) | Controls, *n* (%) |
|---|---|---|
| *Comorbid atopic disease* | 22 (44.9) | Undetermined |
| *FLG* wild-type genotype | 38 (77.6) | 88 (88.0) |
| *FLG* heterozygote genotype | 10 (20.4) | 12 (12.0) |
| *FLG* homozygote or compound heterozygote | 0 | 0 |
| Incomplete genotyping data | 1 (2.0) | 0 |
| Total | 49 (100.0) | 100 (100.0) |
| $\chi^2$ analysis | $P=0.16$ | |
| Odds ratio (95% CI) | 1.92 (0.77–4.85) | |

Abbreviations: CI, confidence interval; *FLG*, *filaggrin*.
Cases with Fitzpatrick skin type of IV–VI were excluded from the genetic analysis because *FLG* loss-of-function mutations remain ill defined in these ethnic groups.

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***Catriona P. Harkins[1], Alex Waters[1], Alastair Kerr[1], Linda Campbell[2], W.H. Irwin McLean[2], Sara J. Brown[3,4] and Sally H. Ibbotson[1,4]***

*[1]Photobiology Unit, Department of Dermatology, University of Dundee, Ninewells Hospital and Medical School, Dundee, Scotland, UK; [2]Dermatology and Genetic Medicine, Division of Molecular Medicine, College of Life Sciences and College of Medicine, Dentistry and Nursing, University of Dundee, Dundee, Scotland, UK and [3]Dermatology and Genetic Medicine, Division of Cancer Research, College of Medicine, Dentistry and Nursing, Ninewells Hospital and Medical School, Dundee, Scotland, UK*
*E-mail s.h.ibbotson@dundee.ac.uk*
*[4]Joint senior authors.*

## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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*See related commentary on pg 1714*

# Keloid Pathogenesis: Potential Role of Cellular Fibronectin with the EDA Domain

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## TO THE EDITOR

Fibronectins (FNs) are high molecular weight glycoproteins present in extracellular connective tissue matrices (ECM) and extracellular fluids, including blood plasma. The human FN gene consists of 45 exons, and the primary mRNA transcripts are alternatively spliced to form up to 20 different mRNA variants (White *et al.*, 2008). The FNs interact with other matrix macromolecules, such as collagens, glycosaminoglycans, and fibrin, as well as cell surface receptors, including

*Abbreviations: cFN, cellular fibronectin; ECM, extracellular matrix; EDA, extra domain A; FN, fibronectin; PBS, phosphate-buffered saline; TGF-β, transforming growth factor-β; TLR4, toll-like receptor 4; WT, wild type*

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