

Specific Filaggrin Mutations Cause Ichthyosis Vulgaris and Are Significantly Associated with Atopic Dermatitis in Japan

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Mutations in the gene encoding filaggrin (*FLG*) have been identified as the cause of ichthyosis vulgaris (IV) and shown to be major predisposing factors for atopic dermatitis (AD). However, these studies have been mainly carried out in European populations. In early 2007, we identified two Oriental-specific *FLG* mutations in four Japanese families with IV and reported that filaggrin mutations were also significant predisposing factors for AD in Japan. However, the frequency of *FLG* mutations observed in our Japanese AD cohort (5.6%), was much lower than that seen in Europeans (up to 48%). Here, we studied a further seven Japanese families with IV and identified two additional nonsense mutations in *FLG*, S2889X, and S3296X. We found that more than 20% of patients in our Japanese AD case series carry *FLG* mutations, and there is significant statistical association between the four mutations and AD (χ^2 $P=8.4 \times 10^{-6}$; heterozygote odds ratio 7.57, 95% CI 2.84–23.03). These data emphasize that skin-barrier impairment due to reduced filaggrin expression plays an important role in the pathogenesis of AD and sheds further light on the genetic architecture of atopy in Japan.

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INTRODUCTION

Ichthyosis vulgaris (IV; OMIM no. 146700) is a very common genetic disorder of keratinization clinically characterized by scaling, especially on the extensor limbs, dry skin, and palmoplantar hyperlinearity (Sybert *et al.*, 1985; Smith *et al.*, 2006). IV symptoms are most pronounced in winter or in cold, dry climates. Keratosis pilaris is also a feature and, importantly, atopic dermatitis (AD) is commonly seen in IV (Mevorah *et al.*, 1985). Histologically, a decrease in the size and number or a complete absence of keratohyalin granules in the epidermis is typically characteristic of IV (Sybert *et al.*, 1985). Keratohyalin granules are mainly composed of a >400-kDa polypeptide, profilaggrin. During terminal differentiation of keratinocytes, profilaggrin is processed into

filaggrin, which plays an essential role for epidermal barrier formation and hydration (Irvine and McLean, 2006).

Recently, loss-of-function mutations in the gene encoding filaggrin (*FLG*) located on chromosome 1q21 have been identified as the cause of IV, and the disease was found to exhibit semidominant inheritance (Smith *et al.*, 2006). Although sequence analysis for the highly repetitive *FLG* gene has been considerably difficult, PCR protocols that permit routine and comprehensive sequencing of the *FLG* gene have recently been developed (Sandilands *et al.*, 2007). To date, 15 loss-of-function nonsense or frameshift mutations in *FLG*, including two that are Oriental-specific, have been identified by these sequencing methodologies (Nomura *et al.*, 2007; Sandilands *et al.*, 2007).

Interestingly, a strong association between these *FLG* null alleles and AD has also been firmly established (Palmer *et al.*, 2006). In Europe, this strong association has been extensively replicated in several independent studies and, importantly, no negative association study has been reported (Marenholz *et al.*, 2006; Ruether *et al.*, 2006; Weidinger *et al.*, 2006; Barker *et al.*, 2007; Morar *et al.*, 2007; Sandilands *et al.*, 2007; Stemmler *et al.*, 2007; Weidinger *et al.*, 2007), as reviewed recently (Irvine, 2007). In contrast, *FLG* variants in non-European populations have not been fully analyzed so far. The extent to which *FLG* mutations contribute to AD in a global context remains to be evaluated, as AD is undoubtedly a worldwide problem.

Early in 2007, we identified two Oriental-specific *FLG* mutations in four Japanese families with IV and reported that

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Abbreviations: AD, atopic dermatitis; *FLG*, filaggrin; IV, ichthyosis vulgaris

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the mutations were also predisposing factors for AD in Japan (Nomura *et al.*, 2007). This showed that the same gene is involved in AD susceptibility in a non-European population. However, the frequency of heterozygous, compound heterozygous, and homozygous *FLG* mutation carriers observed in our Japanese AD case series was only 5.6% (Nomura *et al.*, 2007), which was much lower than that seen in analogous European AD case series, where it is more than 40% (Barker *et al.*, 2007; Sandilands *et al.*, 2007). This suggested that there may be further prevalent mutations to be discovered in the Japanese population.

As the filaggrin gene is large and difficult to analyze and, furthermore, as AD is undoubtedly genetically heterogeneous, a useful strategy for identifying filaggrin mutations in a given population is to first concentrate on IV patients in whom likely filaggrin mutation carriers can be readily identified clinically. Once mutations are identified by sequencing, rapid genotyping tests can then be developed to study the overall population incidence and to allow case-control association studies in AD (Palmer *et al.*, 2006; Smith *et al.*, 2006; Sandilands *et al.*, 2007). Here, we have applied this strategy by studying seven Japanese families with IV, allowing identification of two additional *FLG* mutations. Subsequently, we have shown that these *FLG* mutations are also highly significant predisposing factors for AD in Japan.

RESULTS

Clinical and pathological features

In total, seven newly identified Japanese kindreds with IV were studied (Figure 1). The clinical phenotype consisted of variable scaling, dry skin, and palmoplantar hyperlinearity (Figure 2). All probands had a family history, but the clinical severity varied considerably among family members. Therefore, the inheritance pattern was consistent with semidominance, as reported previously (Sandilands *et al.*, 2006; Smith

et al., 2006). More than half of these IV patients had concomitant AD and several also had other symptoms of atopic disease, including asthma and allergic rhinitis (Figure 1).

In some cases, the diagnosis was also confirmed by skin biopsy. Light microscopy of the lesional skin samples from the proband in family 6 showed hyperkeratosis and markedly reduced keratohyalin granules. Immunohistochemically, the proband and his elder brother showed a marked reduction in staining for filaggrin, whereas normal control skin was stained strongly (Figure 2). Interestingly, the proband also showed a greater reduction in filaggrin staining intensity compared to his brother, who had a mild IV phenotype (Figure 2).

Further filaggrin mutations in Japanese IV families

Twenty-three individuals from seven unrelated Japanese IV families were screened for mutations within the *FLG* gene. Initially, all families were screened for the previously reported Japanese-specific *FLG* mutations 3321delA and S2554X by fluorescent PCR and restriction enzyme digestion, respectively, and then confirmed independently by direct DNA sequencing (Nomura *et al.*, 2007). We identified these two mutations in five out of the seven families studied here (Figure 1; families 1–5).

However, the probands from families 6 and 7 were wild type for both mutations, despite showing typical IV phenotypes. Thus, we carried out comprehensive sequencing of the *FLG* gene, using the recently developed overlapping PCR strategy that allows routine diagnostic sequencing of the entire filaggrin coding sequence (Sandilands *et al.*, 2007). This led to the identification of *FLG* mutation, S2889X, in the proband of family 6 (Figure 3). This mutation was shown to be the paternal mutation. In contrast, the proband's mother and brother were both wild type for this mutation, although

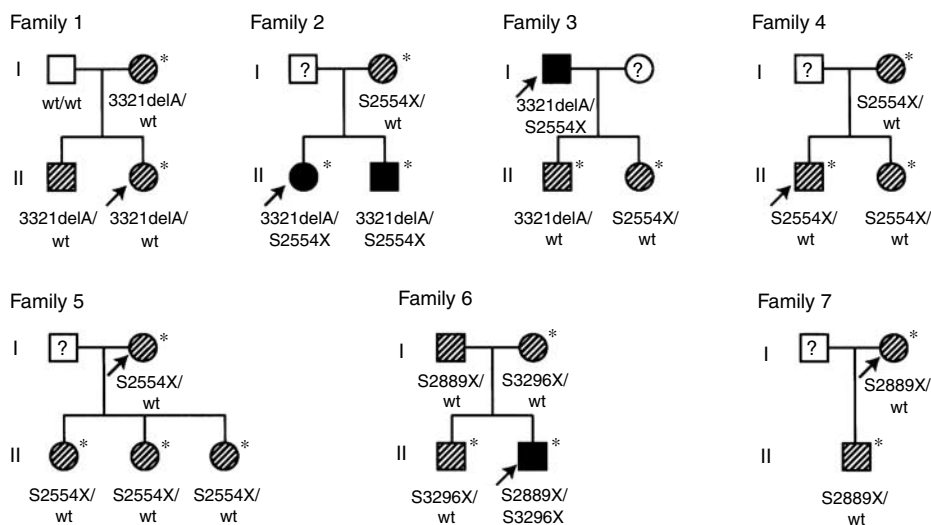


Figure 1. Pedigrees of IV families studied. Family trees of IV families show the semidominant inheritance pattern. Solid symbols refer to the marked IV presentation; cross-hatched symbols refer to the milder IV presentation. In addition, more than half of the IV patients had concomitant dermatologist-diagnosed AD (*). wt, wild type for *FLG* mutations.



Figure 2. Clinicopathological features of patients with IV in family 6. (a–d) Fine scaling clearly visible (a) on the proband's leg and (b) on his elder brother's leg. (c, d) Both of them also have apparent palmar hyperlinearity. The (a, c) proband showed more severe IV phenotype than (b, d) his elder brother. (e–g) Immunohistochemical staining using anti-filaggrin monoclonal antibody against an epitope conserved in all filaggrin peptides. The (e) proband and (f) his brother showed a marked reduction in staining for filaggrin, whereas (g) normal control skin was stained strongly. Scale bar = 50 μ m (e–g).

they also showed a typical IV phenotype. In addition, the proband in family 6 showed a much more severe phenotype than other affected family members and, furthermore, immunohistochemistry showed a greater reduction in epidermal filaggrin expression as compared to his more mildly affected brother (Figure 2). Thus, it was predicted that this severely affected proband was a likely compound heterozygote. Further sequencing of the *FLG* gene was carried out and another *FLG* mutation, S3296X, was identified. The proband was therefore compound heterozygous for S2889X and S3296X, and his mother and brother were heterozygous for S3296X (Figure 3). Mutations S2889X and S3296X lead to

premature termination of profilaggrin translation in filaggrin repeat domain 8.2 (one of the recently identified duplicated repeats; Sandilands *et al.*, 2007), and repeat domain 9, respectively.

The proband and his mother in family 7 were also found to be heterozygous carriers of S2889X, consistent with their milder IV phenotype. Both of these newly identified mutations were confirmed independently by restriction digestion (Figure 3). The Japanese IV families studied here were also confirmed to be wild type for all 13 *FLG* mutations found in European populations (Sandilands *et al.*, 2007), by direct DNA sequencing.

Prevalent *FLG* mutations are predisposing factors for AD in Japan

A total of 102 unrelated patients with AD were screened for mutations S2554X, S2889X, and S3296X by restriction digestion and for 3321delA by sizing of fluorescently labeled PCR fragments. The presence of the mutations was independently confirmed by direct DNA sequencing of PCR products. The *FLG* genotype data in the Japanese AD case series and an ethnically matched population control series are summarized in Table 1.

Interestingly, all the mutations found in the Japanese IV families were also identified in the Japanese AD population. Mutations S2554X, S2889X, S3296X, and 3321delA were found to be carried by 6 (5.9%), 10 (9.8%), 3 (2.9%), and 4 (3.9%) individuals in the Japanese AD case series, respectively. The combined minor allele frequency in the case series was observed to be 0.111 (95% confidence intervals 0.068–0.153). These mutations were also carried by 4 out of 133 unrelated general Japanese control individuals but at a much lower combined minor allele frequency of 0.015 (95% CI 0.000–0.030). These controls were not examined in relation to AD or IV status, that is, they are population controls, not “hypernormal” controls screened for the absence of IV/AD. This observed association between the combined *FLG* null variant genotypes and AD was statistically highly significant. Comparing the AD cohort and population cohort for the combined null alleles yielded a Pearson χ^2 *P*-value of 8.4×10^{-6} . Similarly, the Fisher exact test odds ratio for heterozygotes was highly significant (OR 7.57, 95% CI 2.84–23.03). The numbers of homozygotes/compound heterozygotes was too small in this study to allow calculation of a homozygote odds ratio. More than 20% of the individuals in the AD case series carried one or more of these four *FLG* mutations.

DISCUSSION

The human profilaggrin gene (*FLG*) is located on chromosome 1q21 and consists of three exons. Exons 1 and 2 are small, whereas exon 3 is unusually large (>12 kb) and encodes all filaggrin repeats that are almost 100% identical at the DNA level, making PCR and sequence analysis difficult (Sandilands *et al.*, 2007). Furthermore, some individuals show duplication of filaggrin repeat 8 and/or 10, which results in size variation of the *FLG* gene within the human population and further complicates routine sequence analysis

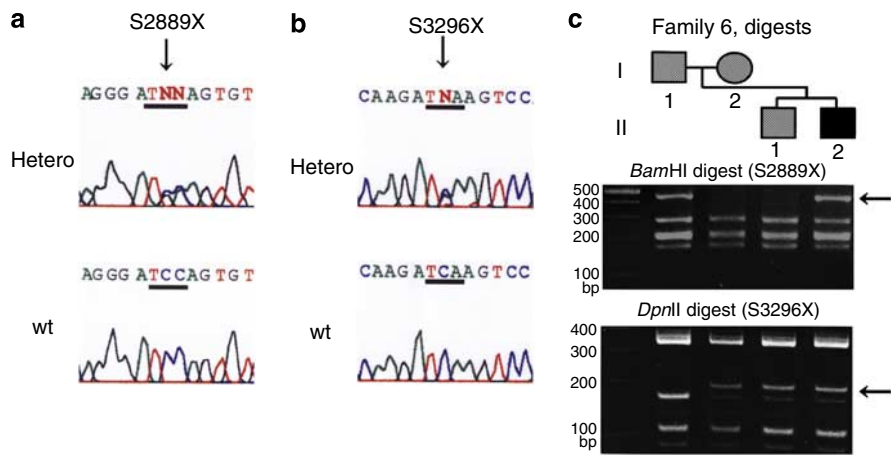


Figure 3. Mutation analysis of IV. (a) Two heterozygous transition mutations c.8666C→G and c.8667C→A were identified in the proband of family 6, resulting in S2889X. Normal control sequence from filaggrin repeat 8 in exon 3, corresponding to codons 8660–8672. Both transition mutations were confirmed to exist on the same allele by cloning and sequencing of PCR products. (b) A heterozygous transition mutation c.9887C→A was also identified in the proband of family 6, resulting in S3296X. Normal control sequence from filaggrin repeat 9 in exon 3, corresponding to codons 9881–9893. (c) Screening family 6 for the mutation S2889X by *Bam*HI restriction digest and S3296X by *Dpn*II restriction digest. Arrows indicate the additional bands specific to the mutant alleles. Individuals I-1 and II-2 were heterozygous for S2889X, whereas I-2 and II-1 were wild type. Similarly, individuals I-2, II-1, and II-2 were heterozygous for S3296X, whereas I-1 was wild type.

Table 1. Atopic dermatitis case–control association analysis for <i>FLG</i> null variants in Japan										
Genotypes	S2554X		S2889X		S3296X		3321delA		Combined	
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
AA	132	96	131	92	133	99	132	98	129	81
Aa	1	6	2	10	0	3	1	4	4	19
aa	0	0	0	0	0	0	0	0	0	2
Totals	133	102	133	102	133	102	133	102	133	102
χ^2	$P=0.022$		$P=0.004$		$P=0.047$		$P=0.095$		$P=8.4 \times 10^{-6}$	

(Sandilands *et al.*, 2007). Despite these difficulties, methods that enable sequencing of all coding regions of the *FLG* gene have been recently developed by identifying a restricted number of bases that are (a) specific to a particular repeat and (b) relatively non-polymorphic. These specific bases allow placement of primers for repeat-specific PCR and sequencing (Sandilands *et al.*, 2007). Using this methodology, we identified two additional *FLG* mutations, S2889X and S3296X, in Japanese individuals. These mutations are located in filaggrin repeats 8 and 9, respectively, and so truncated profilaggrin is likely to be produced from these mutant alleles. As previous immunoblot analysis of R501X/R2447X and R501X/11033del4 compound heterozygotes showed that truncated profilaggrin protein species are not processed into filaggrin (Sandilands *et al.*, 2007), we would therefore predict that S2889X and S3296X have similar pathogenicity to the common European repeat 1 mutations with respect to IV. Indeed, the proband in family 6, compound heterozygous for S2889X and S3296X identified here, showed a more clinically severe IV phenotype similar to that seen in individuals who harbor mutations in repeat 1 (Smith *et al.*, 2006; Sandilands *et al.*, 2007).

Immunohistochemistry also demonstrated a greatly reduced amount of profilaggrin granules in the epidermis (Figure 2). We also identified these *FLG* mutations in Japanese patients with AD. Notably, we demonstrate here that more than 20% of AD patients in our Japanese case series carry these four *FLG* mutations (3321delA, S2554X, S2889X, and/or S3296X), whereas 3% of ethnically matched control individuals carry these mutations (minor allele frequency=0.015; $n=266$). In the Japanese population, there is a statistically highly significant association between the four *FLG* mutations and AD (χ^2 $P=8.4 \times 10^{-6}$; heterozygote OR 7.57, 95% CI 2.84–23.03). Thus *FLG* mutations are shown to be major predisposing factors for AD in Japan as well as in Europe. In addition, all *FLG* mutations found in Japan are also shown to be prevalent in this ethnic population, analogous to the five prevalent mutations so far identified in Europeans (Sandilands *et al.*, 2007). This work brings the total number of prevalent filaggrin mutations so far identified in human populations studied to nine. Several large cohort studies in Europe demonstrated that *FLG* null mutations predispose to allergic phenotypes like asthma and allergic rhinitis involved in the atopic march

(Marenholz *et al.*, 2006; Weidinger *et al.*, 2006) and to early-onset AD phenotype persisting into adulthood (Barker *et al.*, 2007; Weidinger *et al.*, 2007). Recently, *FLG* null mutations have also been reported to be associated with increased asthma severity (Palmer *et al.*, 2007). Therefore, *FLG* null mutations are indicative of a more severe, persistent phenotype, and so screening for *FLG* mutations is emerging as a useful prognostic indicator in atopy.

In conclusion, we have identified two further prevalent *FLG* variants in the Japanese population. We also demonstrate that these four known *FLG* null variants are highly significant predisposing factors for AD in Japan. These data emphasize the importance of screening for *FLG* mutations in patients with IV and/or AD. It seems quite possible that *FLG* null variants are also predisposing factors for AD in most other races worldwide to differing degrees. Future analysis of *FLG* variants in other races will reveal the overall contribution of this gene to the pathogenesis of atopy and the subsequent global healthcare burden.

MATERIALS AND METHODS

Clinical materials

Blood samples were obtained from 23 patients with IV from 7 independent Japanese families and a further 102 unrelated Japanese AD patients. The diagnosis of IV and AD was performed clinically by experienced dermatologists. The diagnosis of AD was performed using the AD diagnostic criteria by Hannifin and Rajka (1980). For general controls, DNA samples from 133 unrelated Japanese individuals were included in the current study. Patients gave written informed consent, which complies with all the Declarations of Helsinki Principles. This study was approved by the Medical Ethical Committees of the Hokkaido University, Sapporo, Japan.

Filaggrin genotyping

FLG mutation analysis was performed in patients and their family members. Briefly, genomic DNA isolated from peripheral blood was subjected to PCR amplification, followed by direct automated sequencing using ABI PRISM 3100 or 3730 genetic analyzers (Applied Biosystems, Foster City, CA). Mutation S2554X was screened using restriction enzyme digestion of PCR products (Nomura *et al.*, 2007). Mutation 3321delA was screened by fluorescent PCR (Nomura *et al.*, 2007). Primers and PCR conditions were as described previously (Nomura *et al.*, 2007).

Mutation detection and screening for S2889X and S3296X

A 878-bp PCR product from repeat 8 of the *FLG* gene was amplified with forward primer 5'-GATGGACGGGGCCAGCACTA-3' and reverse primer 5'-CTTGGTGGCTCTGCTGATGGGA-3'. A total of 100 ng genomic DNA was amplified using High Fidelity PCR buffer (Roche Diagnostics Ltd., Lewes, UK) containing 1.5 mmol l⁻¹ MgCl₂, 4% (vol/vol) DMSO, and 1 U of Expand High Fidelity enzyme (Roche). PCR amplification conditions were as follows: 1 cycle of 94°C for 5 minutes, 32 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 1 minute; then 1 cycle of 72°C for 5 minutes. The 878-bp PCR fragment was sequenced with the amplification primers (above). The PCR products were also cloned into a plasmid vector (TA cloning kit, Invitrogen, Carlsbad, CA) and

sequenced to confirm that the two point mutations detected were on the same allele. Mutation S2889X abolishes a *Bam*HI restriction enzyme site. PCR products were digested with 20 U of *Bam*HI (New England Biolabs, Ipswich, MA) at 37°C for 1 hour. Digests were resolved on 3% (w/v) agarose gels. The mutant allele was resolved as 438-, 207-, 200-, and 33-bp fragments, whereas the wild-type allele yielded fragments of 274-, 207-, 200-, 164-, and 33-bp.

A 803-bp PCR product from repeat 9 of the *FLG* gene was amplified with forward primer 5'-TCTGTTTCAGGAGCAGTCAAGG-3' and reverse primer 5'-GCTTCATGGTGATCGACCA-3'. A total of 100 ng genomic DNA was amplified using High Fidelity PCR buffer (Roche) containing 1.5 mmol l⁻¹ MgCl₂, 4% (vol/vol) DMSO, and 1 U of Expand High Fidelity enzyme (Roche). PCR-amplification conditions were as follows: 1 cycle of 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute; then 1 cycle of 72°C for 5 minutes. The 803-bp PCR fragment was sequenced with the amplification primers (above). Mutation S3296X abolishes a *Dpn*II restriction enzyme site. PCR products were digested with 20 U of *Dpn*II (New England Biolabs) at 37°C for 1 hour. Digests were resolved on 3% (w/v) agarose gels. The mutant allele was resolved as 327-, 183-, 105-, 102-, and 86-bp fragments, whereas the wild-type allele gave fragments of 327-, 159-, 105-, 102-, 86-, and 24-bp.

Statistical analysis

In this study, case-control association analyses were performed by using Pearson χ^2 and Fisher exact test statistics, as described previously (Palmer *et al.*, 2006). All alleles were observed to be in normal Hardy-Weinberg equilibrium.

Immunohistochemical analysis

Immunoperoxidase staining of paraffin-embedded sections was performed by using the ChemMate Peroxidase/DAB system (Dako-Cytomation, Hamburg, Germany). Mouse mAb 15C10 (Novocastra, Newcastle, UK) was used to detect the human filaggrin repeat unit. Importantly, antigen retrieval of paraformaldehyde-fixed, paraffin-embedded sections was performed by heating sections under pressure for 10–15 minutes in 10 mmol l⁻¹ citrate buffer, pH 6.0.

CONFLICT OF INTEREST

Irwin McLean has filed patents relating to genetic testing and therapy development aimed at the filaggrin gene.

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REFERENCES

- Barker JN, Palmer CNA, Zhao Y, Liao H, Hull PR, Lee SP *et al.* (2007) Null mutations in the filaggrin gene (*FLG*) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol* 127:564–7

- Hannifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 92:44-7
- Irvine AD (2007) Fleshing out filaggrin phenotypes. *J Invest Dermatol* 127:504-7
- Irvine AD, McLean WHI (2006) Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. *J Invest Dermatol* 126:1200-2
- Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T *et al.* (2006) Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 118:866-71
- Mevorah B, Marazzi A, Frenk E (1985) The prevalence of accentuated palmoplantar markings and keratosis pilaris in atopic dermatitis, autosomal dominant ichthyosis and control dermatological patients. *Br J Dermatol* 112:679-85
- Morar N, Cookson WO, Harper JJ, Moffatt MF (2007) Filaggrin mutations in children with severe atopic dermatitis. *J Invest Dermatol* 127:1667-72
- Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K *et al.* (2007) Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 119:434-40
- Palmer CNA, Ismail T, Lee SP, Terron-Kwiatkowski A, Zhao Y, Liao H *et al.* (2007) Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol* 120:64-8
- Palmer CNA, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38:441-6
- Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R (2006) Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. *Br J Dermatol* 155:1093-4
- Sandilands A, O'Regan GM, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM *et al.* (2006) Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. *J Invest Dermatol* 126:1770-5
- Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM *et al.* (2007) Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 39:650-4
- Smith FJD, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y *et al.* (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 38:337-42
- Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S (2007) Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. *J Invest Dermatol* 127:722-4
- Sybert VP, Dale BA, Holbrook KA (1985) Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. *J Invest Dermatol* 84:191-4
- Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A *et al.* (2006) Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 118:214-9
- Weidinger S, Rodriguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T *et al.* (2007) Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 127:724-6