

A Prospective Study of Filaggrin Null Mutations in Keratoconus Patients with or without Atopic Disorders

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Key Words

Filaggrin • Atopic dermatitis • Keratoconus

Abstract

Background: Atopic dermatitis (AD) is significantly associated with keratoconus (KC). An inherited component for KC has been suggested. Filaggrin (*FLG*) mutations are a strong genetic risk factor for AD. Since filaggrin is also expressed in the corneal epithelium, we hypothesized a common aetiology for ichthyosis vulgaris (IV), AD and KC. **Objectives:** We examined the prevalence of AD and IV in a KC population. We also studied the expression of filaggrin in normal and KC cornea and analysed 2 prevalent loss-of-function *FLG* alleles (R501X and 2282del4) in a KC population. Finally we examined whether the population with KC and *FLG* mutations had specific clinical characteristics. **Results:** Of 89 KC patients, 38 had current or a history of AD and/or IV. Five patients were carriers of at least 1 *FLG* mutant allele and had a clinical diagnosis of AD and IV with a severer KC. **Conclusion:** The low frequency of *FLG* mutations is surprising since 42.7% of our KC population had AD associated or not with IV; the expected frequency would have been 12–15%, based on our previous studies. Further studies are required to look at other possible *FLG* mutations or other candidate genes.

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Introduction

Keratoconus (KC) is a non-inflammatory corneal dystrophy characterized by progressive corneal thinning and corneal ectasia responsible for a loss of visual acuity. KC is usually bilateral and affects mainly young adults. The disease begins in adolescence and remains progressive until the third to fourth decade. The incidence of KC is estimated to be 1/500 to 1/2,000 per year with a prevalence rate of 54.4/100,000 eyes [1]. The pathogenesis of KC remains unclear but an inherited component has been suggested, with 8–10% of KC patients having a family history of KC [1].

KC is also associated with several diseases, especially those belonging to the atopic diathesis. Atopic disorders affect up to one third of the population in developed countries. They affect several epithelia including the skin (atopic dermatitis, AD), the respiratory tract (asthma, allergic rhinitis) and the eye (allergic conjunctivitis). Atopic diseases, especially AD, have been associated with KC in a number of uncontrolled studies with conflicting data according to the type of patient recruitment [2–7] (table 1).

A.T. and J.C. equally contributed to this work.

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Table 1. Association of KC with atopic disorders

Ophthalmology-based	Copeman [2], 1965	Gasset et al. [3], 1978	Harrison et al. [4], 1989	Bawazeer et al. [6], 2000	Kaya et al. [7], 2007
KC population studied	100	162	67	49	70
KC population with AD	32/100 (32%)	not studied	21/67 (31.3%)	10/49 (20.4%)	33/70 (47.1%)
KC population with asthma	not studied	29/162 (17.9%)	19/67 (28.4%)	not studied	not studied
Dermatology-based	Ohmachi et al. [5], 1994				
AD population studied	240				
Systematic ophthalmological examination: KC diagnosed	3/240 (1.3%)				

AD is typically the first clinical manifestation of atopy. It had been speculated that a genetically abnormal skin barrier would lead to increased atopen penetration and favour a Th2 response in predisposed individuals paving the way to AD and respiratory atopy [8]. Filaggrin genetic studies have confirmed that a primary defect in the skin barrier plays an important role in the pathophysiology of AD and atopic diseases. Filaggrin is a key protein in epidermal differentiation and maintenance of barrier function. Common filaggrin (*FLG*) gene null mutations (p.R501X and c.2282del4) were first identified as the molecular cause of ichthyosis vulgaris (IV) [9], which was known for being clinically associated with AD in a subset of patients. Indeed, Palmer et al. [10] demonstrated a strong association between the 2 prevalent *FLG* loss-of-function mutations found in IV and AD. Other studies in European Caucasian populations have confirmed this significant genetic predisposing factor to AD [9, 11–19]. *FLG* null mutations are an indicator of poor prognosis in AD, predisposing to a form of AD that starts in early infancy and persists into adulthood [14, 15, 17, 20–22]. These common mutations are associated with a risk of asthma in the context of AD [12, 13, 18, 21, 23]. The meta-analysis of 24 studies on *FLG* mutations has confirmed the association with asthma but only in the context of AD [22]. Some studies have also found significantly higher median serum IgE levels among carriers of *FLG* mutations [11, 17, 21].

One study has demonstrated the expression of epithelial barrier proteins including filaggrin in the cornea and has identified filaggrin expression by different modalities including immunofluorescence staining on cornea sections and Western blot analysis of primary cultured corneal epithelial cells; filaggrin is present in the central and peripheral corneal epithelium and the limbal epithelium

[24]. This suggests that filaggrin plays a major role in the corneal epithelial barrier [24]. Hence, the corneal epithelium and epidermis share biochemical similarities. In addition, both are stratified and have a similar polarity of differentiation from basal to superficial layers.

Based on such similarities, we hypothesized a common dysfunction of the cornea and skin barriers linked to *FLG* gene mutations which could explain the clinical association of KC with AD and possibly the clinical characteristics of KC associated with atopic disease. The main objectives of this study were: (1) to investigate the prevalence of AD and IV in adults and children with a diagnosis of KC, (2) to study the expression of filaggrin in normal and KC corneas, (3) to evaluate the frequency of the 2 common loss-of-function *FLG* mutations (p.R501X and c.2282del4) in a KC population with or without AD, and (4) to better delineate clinically the population with KC and *FLG* mutations.

Patients and Methods

Patients

This study was carried out prospectively between April 2008 and June 2009 among Caucasian children and adults consulting for KC at the Department of Ophthalmology (National Reference Centre for Keratoconus, Bordeaux, France). All the patients were seen at the same visit by a dermatologist (C.D.) and an ophthalmologist (D.T.).

For the diagnosis of AD, we used the UK Working Party criteria [25]. AD severity was assessed using the SCORAD [26]. All patients were questioned about the history of AD (onset of the disease, duration), asthma, allergic rhinoconjunctivitis, family history of atopy and atopen sensitization. Concomitantly, clinical markers of IV (squames on legs, marked palmar hyperlinearity, skin xerosis) were evaluated, and the diagnosis of IV was made based on an overall clinical judgement (yes/no). The diagnosis of KC was made by a senior ophthalmologist (D.T., J.C.). Age at di-

agnosis, family history of KC, corneal topography, Krumeich severity stage and course of disease were also recorded.

Immunohistochemistry and Immunofluorescence on Epidermis and Cornea

Biopsy specimens of corneas were obtained from 6 patients with KC treated by a corneal graft. Cutaneous biopsy specimens from patients with AD were obtained during flares for diagnostic purposes. Filaggrin was detected by immunoperoxidase using antifilaggrin (FLG01, Santacruz) and revealed by the Envision system (Dako, Trappes), and by immunofluorescence using antifilaggrin (FLG01, Santacruz) and revealed with Alexa Fluor 555 (Invitrogen). Nuclei were visualized using 4,6-diamidino-2-phenylindole (blue).

Western Blot on Cornea

Fifty micrograms of total extract of cornea or whole skin were subjected to 10% SDS-PAGE. The separated proteins were transferred to an Immobilon-P transfer membrane and blotted with antifilaggrin (ab24584, Abcam, Cambridge) or anti- β -actin (A5441, Sigma-Aldrich, Saint-Quentin-Fallavier), detected with ECL+ (RNP2106, GE Healthcare, Orsay) and quantified with LAS 3000 (Fujifilm). β -Actin served as a loading control.

Total IgE Serum Levels

The total IgE serum level was determined by using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Total IgE serum levels higher than 150 kU/l were considered as abnormal.

Genetic Analysis

Written consent for genetic analysis was obtained from all patients included in the study and control individuals. DNA was extracted from peripheral blood of patients. DNA samples were screened for the 2 prevalent loss-of-function *FLG* alleles (p.R501X and c.2282del4).

DNA was extracted using the Wizard genomic kit (Promega, France). The genotypes were determined by restriction fragment length polymorphism (RFLP) analysis (p.R501X) or by fluorescent fragment size analysis (c.2282del4) using an automated gene analyser and the Gene mapper software (Applied Biosystems 3130). The RFLP analyses were performed as described by Palmer et al. [10]. The fragment size analysis techniques were adapted from Palmer et al. The following sequences were used as amplimers: 5'-TCC CGC CAC CAG CTC C-3' (forward *FLG* 2282del4) and 5-TG GCT CTG CTG ATG GTG A-3' (6FAM-labelled reverse *FLG* 2282del4); 5'-ACA GCC TGA CTC TGC CCA TG-3' (forward *FLG* R501X) and 5'-GCA CTT CTG GAT CCT GAC TG-3' (reverse *FLG* R501X). Polymerase chain reaction (PCR) conditions were as follows: 100 ng genomic DNA, 2 mmol/l $MgCl_2$, 200 μ mol/l each dNTP, 200 nmol/l each amplimer, 1 $\times$ Taq Gold buffer and 1 unit Taq Gold enzyme (Perkin Elmer, France). Cycling conditions were as follows: 10 min at 95°C (initial denaturation), then 25 (*FLG* 2282del4) or 35 cycles (*FLG* R501X) including denaturation (30 s at 94°C), annealing (30 s at 60°C) and extension (30 s at 72°C). PCR products were run on a 1.5% agarose gel prior to RFLP or fragment size analysis. Amplicon sizes were 312 bp (*FLG* R501X) and 193–197 bp (*FLG* 2282del4), respectively. Restriction digests using *Nla*III (*FLG* R501X) were performed on 1/10 of the PCR product during 2 h at 37°C according to the sup-

plier's instructions (New England Biolabs, France) and run on a 3% agarose gel. The *FLG* R501X variant alleles exhibited a new restriction site for *Nla*III. For fragment size assays, 6FAM-labelled PCR products (*FLG* 2282del4) were diluted 1/4 to 1/40, and the Genescan Rox 350 fluorescent marker was added prior to analysis on the automated gene analyser.

Statistical Analysis

A descriptive analysis was made using SPSS version 17. Mean and median were calculated for continuous variables. Percentages were expressed for all variables.

Results

Demographic Characteristics of the Cohort

Eighty-nine patients with a diagnosis of KC were enrolled in this study. The sex ratio was 2.3 (27 females and 62 males). The median age was 27 years, ranging from 10 to 48 years. This population included 3 children (aged 10, 14 and 15) and 86 adults (table 2).

Prevalence of AD and IV in Adults and Children with KC

Of the 89 KC patients, 38 had a history of AD (42.7%). Among these patients, 19 had IV. Only 3 out of 38 AD patients had active AD with lesions of atopic eczema at enrolment in the study. Other atopic manifestations were present in 9 AD patients, namely asthma (n = 5), allergic rhinitis (n = 6) and allergic conjunctivitis (n = 4).

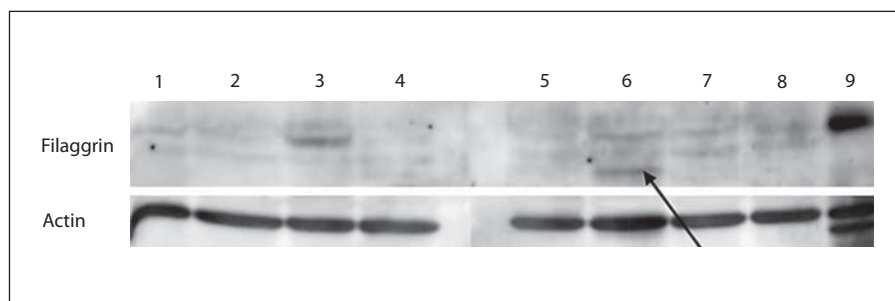
The median total IgE serum level was 170.5 kU/l for the whole KC population (n = 89), 76 kU/l (interquartile interval 2–556) for the KC population without a diagnosis of AD and/or other atopic conditions (n = 51), 254.5 kU/l (interquartile interval 14–6,800) for the KC population with a diagnosis of AD and/or other atopic conditions (n = 38).

A first-degree family history of KC was present in 17 patients (19%), including 11 patients with KC only and 6 patients with AD and/or other atopic disorders, whereas a family history of atopy was present in 49 patients (55%), namely 33 patients with AD and/or other atopic disorders and 16 patients with KC only.

Expression of Filaggrin in the Cornea

No filaggrin was detected by immunoperoxidase or by immunofluorescence in corneas of KC or control corneas (data not shown). However, Western blot confirmed the expression of filaggrin in the cornea (fig. 1). Filaggrin had a very low expression in the cornea compared to normal skin. No significant difference in filaggrin expression

Fig. 1. Detection of filaggrin in cornea and skin by Western blot. Control cornea: lanes 1 and 8; control skin: lane 9; KC cornea: lanes 2–7; note for lane 6 a different expression (arrow).



was observed between control and KC corneas. Interestingly, the KC patient featured in lane 6 presented a different pattern of filaggrin migration and was a heterozygous carrier of the p.R501X mutant allele (fig. 1).

Frequency of FLG Mutations (p.501X and c.2282del4) in KC Patients and Clinical Characteristics of Carriers of Mutant FLG Alleles

Five KC patients (5.6%) were carriers of 1 or more *FLG* mutated alleles: 4 patients were heterozygous for the mutated allele p.R501X, 1 patient was compound heterozygous (c.2282del4; p.R501X). The mutated allele frequency was 0.3% (6/178 screened alleles). All carriers of an *FLG* mutated allele and a KC had a clinical diagnosis of AD and IV (table 3).

The clinical characteristics of these 5 patients are described in table 3. The carriers of *FLG* mutated alleles and KC had a more severe KC clinical profile, i.e. earlier age at diagnosis (mean of 21 years at diagnosis in the patients carrying 1 mutation vs. a mean of 28.74 years for the other patients, $p = 0.02$); All patients carrying a mutated allele had progressive KC leading to intrastromal ring implantation (2 patients) or corneal graft considered as the last line of treatment (2 patients) (table 3).

Discussion

Atopic diseases, especially AD, have been associated with KC in a number of uncontrolled studies. Ophthalmology-based cohorts found a stronger link than dermatology-based studies (table 1). Our ophthalmology-based study confirms the strong clinical association of KC with a history of AD. In contrast, Ohmachi et al. [5] showed that a systematic ophthalmological examination of 240 children and adults with AD yielded a diagnosis of KC in 1.3% of patients, still more than the incidence of 1/2,000 of KC in the general population [1].

Table 2. Characteristics of 89 patients attending the Keratoconus Centre of Bordeaux between April 2008 and June 2009

	Number	Percent
Sex		
Female	27	30.3
Male	62	69.7
Age at KC diagnosis		
≤15 years	3	3.4
>15 years	86	96.6
History of AD		
Yes	38	42.7
No	51	57.3
Lesions of AD present at clinical examination		
Yes	3	7.9
No	35	92.1
Other atopic disorder present (asthma, allergic rhinitis and conjunctivitis)		
Yes	9	10.1
No	80	89.9
IV		
Yes	19	21.3
No	70	78.7
First-degree family history of atopy		
Yes	49	55.7
No	39	44.3
First-degree family history of KC		
Yes	17	19.3
No	71	80.7
	n = 178	Allele frequency
<i>FLG</i> gene analysis		
WT allele	172	96.63
p.R501X allele	5	2.81
c.2284del4 allele	1	0.56

The total number of subjects is not always equal to $n = 89$ because of missing data. WT = Wild type.

Table 3. Clinical characteristics of carriers of *FLG* mutants and KC

	Diagnosis	Genotype	Age, years	Age at KC diagnosis, years	Progressive KC disease	Treatment of KC
Patient 1	KC + AD + IV	WT/R501X	25	22	yes	glasses
Patient 2	KC + AD + IV	R501X/2282del4	28	–	yes	intrastomal ring implants
Patient 3	KC + AD + IV	WT/R501X	17	15	yes	intrastomal ring implants
Patient 4	KC + AD + IV	WT/R501X	17	12	yes	corneal graft
Patient 5	KC + AD + IV	WT/R501X	18	12	yes	corneal graft

WT = Wild type; – = no data available.

If the association between AD and KC has been clearly established, the pathophysiological background is still unclear. Copeman [2] has hypothesized that strong iterative eye rubbing might trigger KC. Bawazeer et al. [6] further supported this mechanical trigger. Beyond this hypothesis, we have looked for a common genetic background that may explain the association between AD and KC. We could demonstrate the weak presence of filaggrin in corneal protein extracts, and a variant migration profile (Western blot) in a sample carrying a *FLG* mutation. However, the low mutated allele frequency (6.8%) is overall surprising. Since 42.7% of our KC population had AD associated or not with IV, the expected frequency would have been 12–15%, based on our previous studies [16]. However, in our first study, all cases were symptomatic at inclusion, whereas 3 out of 38 AD patients were symptomatic in this study. Moreover, these 2 common mutations are mainly found in severe forms of AD [14, 17, 20], which was not the case in our KC population. Indeed the c.2282del4 mutation is supposed to be associated with a more severe phenotype [17]. However, in our previous study the SCORAD severity index was not statistically different between mutated and non-mutated cases [16]. *FLG* null mutations predispose to a form of AD that starts in early infancy and persists into adulthood [14, 15, 17, 20–22]. In our study, the 5 *FLG* mutated KC patients had AD starting in early infancy but persisting into adulthood only in 2 patients. The small sample size of our KC cohort, linked to the low prevalence of KC [1], may also explain the unexpected low *FLG* mutations.

One limitation of our study is that only the 2 prevalent *FLG* mutations have been studied in this KC cohort. To date, 26 *FLG* mutations have been identified in Caucasian populations with 5 considered as prevalent, i.e. c.2282del4, p.R501X, p.R2447X, c.3702delG and p.S3247X [20, 27, 28].

The more distal mutations limit the expression of pro-filaggrin with no production of functional filaggrin subunits implying severer AD [27]. Therefore, in our patients considered as expressing a less severe form of AD, it may be of interest to search for less common *FLG* mutations which may be found in the distal part of the gene.

Preliminary data have been obtained in 30 AD patients from both this and our previous AD cohort: none of the 3 recently described prevalent mutated alleles were identified in patients with a wild-type profile at positions p501 and c.2282. Thus the *FLG*-KC genetic paradox needs further studies to look at filaggrin expression in skin and cornea, other possible *FLG* mutations or other candidate genes in line with our working hypothesis.

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Disclosure Statement

The authors have no conflict of interest.

References

- 1 Rabinowitz YS: Keratoconus. *Surv Ophthalmol* 1998;42:297–319.
- 2 Copeman PW: Eczema and keratoconus. *Br Med J* 1965;ii:977–979.
- 3 Gasset AR, Hinson WA, Frias JL: Keratoconus and atopic diseases. *Ann Ophthalmol* 1978;10:991–994.
- 4 Harrison RJ, Klouda PT, Easty DL, Manku M, Charles J, Stewart CM: Association between keratoconus and atopy. *Br J Ophthalmol* 1989;73:816–822.
- 5 Ohmachi N, Sasabe T, Kojima M, Adachi J, Endo K, Fukuzumi T, Aoki T: Eye complications in atopic dermatitis. *Arerugi* 1994;43:796–799.
- 6 Bawazeer AM, Hodge WG, Lorimer B: Atopy and keratoconus: a multivariate analysis. *Br J Ophthalmol* 2000;84:834–836.
- 7 Kaya V, Karakaya M, Utine CA, Albayrak S, Oge OF, Yilmaz OF: Evaluation of the corneal topographic characteristics of keratoconus with orbscan II in patients with and without atopy. *Cornea* 2007;26:945–948.
- 8 Taieb A: Hypothesis: from epidermal barrier dysfunction to atopic disorders. *Contact Dermatitis* 1999;41:177–180.
- 9 Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al: Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337–342.
- 10 Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al: Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441–446.
- 11 Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al: Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006;118:214–219 (erratum published in *J Allergy Clin Immunol* 2006;118:b922, 2006;118:724).
- 12 Marenholz I, Nickel R, Rüschemdorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al: Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 2006;118:866–871.
- 13 Ruether A, Stoll M, Schwarz T, Schreiber S, Fölster-Holst R: Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. *Br J Dermatol* 2006;155:1093–1094.
- 14 Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, et al: Null mutations in the filaggrin gene (*FLG*) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol* 2007;127:564–567.
- 15 Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S: Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. *J Invest Dermatol* 2007;127:722–724.
- 16 Hubiche T, Ged C, Benard A, Léauté-Labrèze C, McElreavey K, de Verneuil H, Taieb A, et al: Analysis of SPINK 5, KLK 7 and FLG genotypes in a French atopic dermatitis cohort. *Acta Derm Venereol* 2007;87:499–505.
- 17 Weidinger S, Rodríguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, et al: Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2007;127:724–726.
- 18 Weidinger S, O'Sullivan M, Illig T, Baurecht H, Depner M, Rodriguez E, Ruether A, et al: Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008;121:1203–1209.
- 19 Hanifin JM: Filaggrin mutations and allergic contact sensitization. *J Invest Dermatol* 2008;128:1362–1364.
- 20 Brown SJ, McLean WH: Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009;129:543–552.
- 21 Greisenegger E, Novak N, Maintz L, Bieber T, Zimprich F, Haubenberger D, et al: Analysis of four prevalent filaggrin mutations (R501X, 2282del4, R2447X and S3247X) in Austrian and German patients with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2010;24:607–610.
- 22 Rodriguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown SJ, Cordell HJ, et al: Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic dermatitis. *J Allergy Clin Immunol* 2009;123:1360–1370.
- 23 Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et al: Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy* 2009;64:1758–1765.
- 24 Tong L, Corrales RM, Chen Z, Villarreal AL, De Paiva CS, Beuerman R, et al: Expression and regulation of cornified envelope proteins in human corneal epithelium. *Invest Ophthalmol Vis Sci* 2006;47:1938–1946.
- 25 Williams HC, Burney PG, Pembroke AC, Hay RJ: The UK Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994;131:406–416.
- 26 Kunz B, Oranje AP, Labrèze L, Stalder JF, Ring J, Taieb A: Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195:10–19.
- 27 Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, et al: Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis and atopic eczema. *Nat Genet* 2007;39:650–654.
- 28 Akiyama M: *FLG* mutations in ichthyosis vulgaris and atopic eczema: spectrum of mutations and population genetics. *Br J Dermatol* 2010;162:472–477.

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