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Molecular Studies of Ichthyosis Vulgaris in Pakistani Families

Azam Jah Samdani¹, Naghma Naz^{2,3} and Nuzhat Ahmed³

ABSTRACT

Objective: To target and amplify a 1.5 kb FLG gene fragment known to carry R501X mutation responsible for causing ichthyosis vulgaris.

Study Design: A case series.

Place and Duration of Study: Centre for Molecular Genetics, University of Karachi and Dermatology Department, Jinnah Postgraduate Medical Centre (JPMC), Karachi, from October 2007 to December 2008.

Methodology: Clinically examined seven ichthyosis vulgaris families were included in this study. The 1.5 kb FLG gene fragment was located in the genomic DNA of both the affected (patients) and unaffected (normal, controls) members of the families by PCR amplification using known primers FilF3 and RPTIP6.

Results: Amplification of 1.5 kb FLG gene fragment was successful in four families while one family showed amplification of the gene fragment in 3 members (one affected and two normal). Two families showed no amplification.

Conclusion: The results obtained during this study suggested the possibility of the R501X mutation as being one of the major causes of ichthyosis vulgaris in Pakistan. In addition, the study also revealed the possibility of the presence of novel FLG gene mutations in our population.

Key words: *Ichthyosis vulgaris. Filaggrin gene (FLG). R501X mutation. PCR amplification.*

INTRODUCTION

Ichthyoses are a group of inherited diseases caused by the abnormal skin cornification. One of these diseases known as ichthyosis vulgaris is a commonly occurring disease in many populations with a reported incidence in between 1:250 and 1:5300.¹⁻⁴ The phenotypic characters of the disease include generalized fine scaling which is more intense on the extensor surfaces of the extremities including lower abdomen, arms and legs.⁵ Other clinical features include hyperkeratosis and hyperlinearity of the palms and soles, atopic dermatitis, asthma, hypohydrosis, keratosis pilaris, and allergic rhinoconjunctivitis.⁵⁻⁸ The biochemical studies have shown defective processing of filaggrin, a filament aggregating protein in ichthyosis vulgaris patients.⁹ Filaggrin (FLG) is an approximately 37 kDa protein which is formed by proteolytic cleavage of the precursor molecule profilaggrin when the granular cells undergo terminal differentiation.¹⁰ The protein plays a key role in the formation of stratum corneum (cornified cell envelop) during epidermal differentiation. The cornified cell envelope forms the outermost barrier layer of the skin which not only prevents water loss but also provides

protection against allergens and infectious agents.^{10,11} Filaggrin was found absent or significantly reduced in the skin and keratinocytes of ichthyosis vulgaris patients as demonstrated by immunological studies.^{9,12,13} In addition, decreased filaggrin mRNA has also been observed in cases of ichthyosis vulgaris.¹⁴

Genetic studies of the disease have shown the involvement of a mutation R501X in the exon 3 of the gene encoding filaggrin (FLG) on chromosome 1q21.¹⁵⁻¹⁷ The mutation has been reported to be inherited in a semidominant pattern that is the homozygotes show a severe phenotype and heterozygotes show a mild to moderate phenotype.¹⁷ The mutation has been studied in European, American and other populations.¹⁷⁻¹⁹

A 1.5 kb FLG gene fragment is known to carry the R501X mutation and its presence may indicate the possibility of the same mutation as being one of the genetic defects involved in causing the disease in Pakistani population. The objective of this study was to target the 1.5 kb FLG gene fragment by PCR amplification in seven ichthyosis vulgaris families.

METHODOLOGY

This case series was carried out at the Centre for Molecular Genetics, University of Karachi and Dermatology Department, Jinnah Postgraduate Medical Centre (JPMC), Karachi, from October 2007 to December 2008. Sixteen affected and 19 unaffected (control) members belonging to 7 families with the family history of ichthyosis vulgaris were included in this study. The ichthyosis vulgaris families were given the codes IV1, IV2, IV3, IV4, IV5, IV6 and IV7 (Table I). The clinical

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examination of both the affected and unaffected members was carried out by the experienced consultant dermatologists. Five millimeter punch skin biopsies were obtained from affected patients and processed for routine histopathological H and E examination. To exclude X-linked recessive ichthyosis, male patients

were tested for steroid sulfatase (STS) deficiency by lipid electrophoresis. Blood samples (5 ml) were collected after informed consent, both from the affected and normal members of the families. Pedigrees (Figure 1) of the families were drawn and analyzed by using the software CYRILLIC.

Table I: Details of the Pakistani ichthyosis vulgaris families included in the study.

Family code	Total number of family members included in the study	Affected	Normal
IV1	8	5	3
IV2	5	3	2
IV3	5	2	3
IV4	5	1	4
IV5	2	1	1
IV6	5	2	3
IV7	5	2	3

Genomic DNA was prepared using 500 µl blood samples. DNA was isolated using FlexiGene DNA preparation kit (QIAGEN) according to the manufacturer's guidelines. DNA was stored at -20°C. A 1.5 kb PCR fragment was amplified from human genomic DNA using primers¹⁷ FilF3 (5' – GCT GAT AAT GTG ATT CTG TCT G - 3') and RPTIP6 (5' – ACC TGA GTG TCC AGA CCT ATT G - 3'). PCR was performed in a total volume of 50 µl containing 1 x PCR buffer with added 1.5 mmol MgCl₂, 5 pmol of each primer, 0.2 mmol of each of the four deoxynucleotide triphosphates (dNTPs), 10 ng genomic

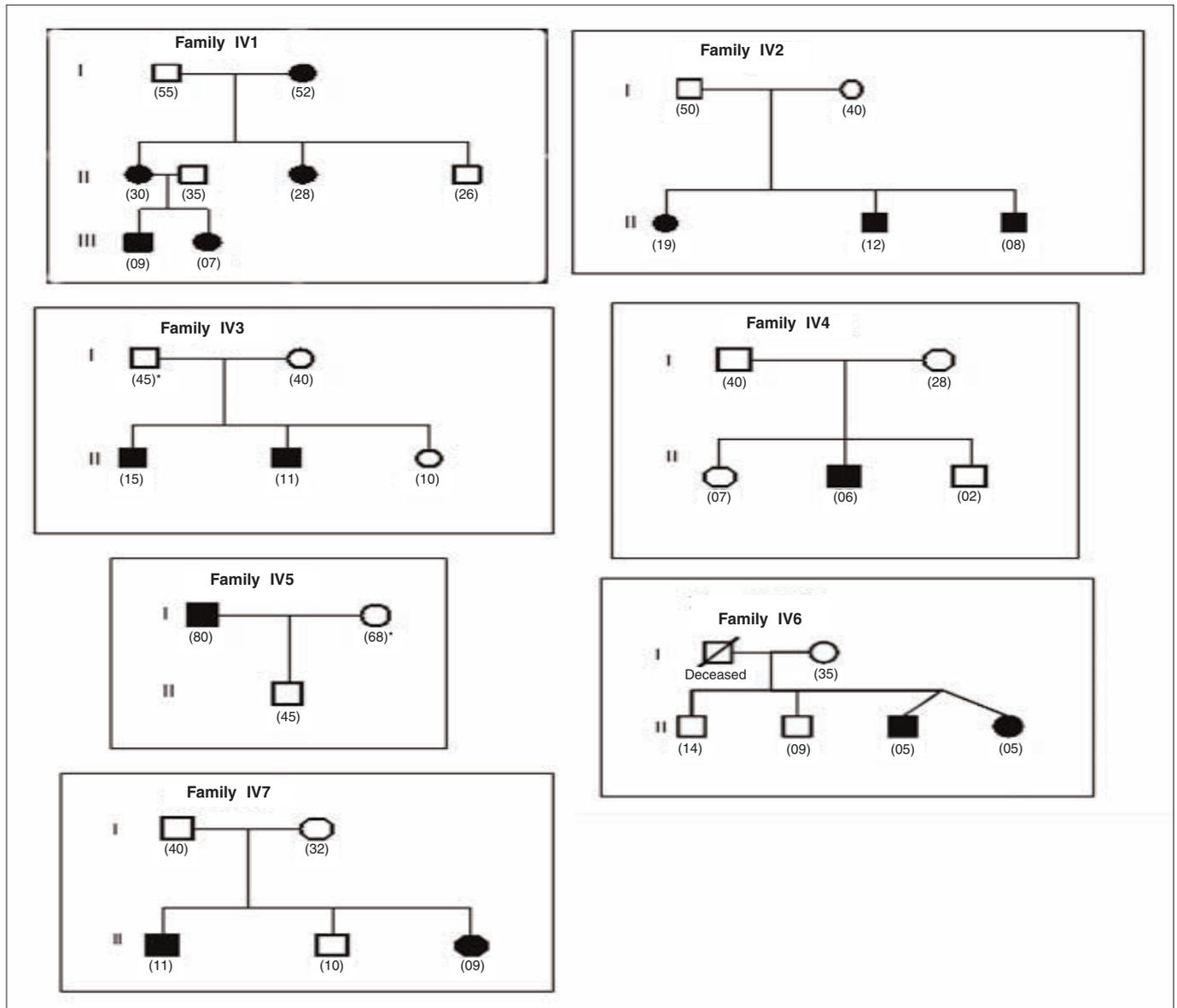


Figure 1: Pedigrees of ichthyosis vulgaris families studied. Roman numerals refer to generations. Individuals in a generation are numbered from left to right, as per convention. Numbers in parentheses indicate age of the individual. *, Blood sample of the individual not available. ■ Affected male. ● Affected female. □ Unaffected male. ○ Unaffected female.

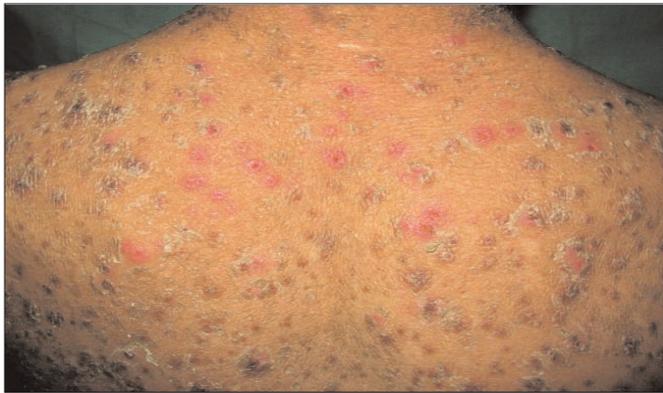


Figure 2: A photograph showing the severe lesions on the back of a 15 years old ichthyosis vulgaris male patient of the family IV3.

DNA and 0.5 U DNA Polymerase (KOD XL DNA Polymerase, Novagen). The amplification conditions were as follows: (94°C, 5 minutes) x 1 cycle; (94°C, 30 seconds, 55°C, 1 minute, 72°C, 2 minutes) x 30 cycles; and a final extension at 72°C for 5 minutes. The PCR products (10 µl) were analyzed on 1% agarose gels. Electrophoresis of DNA was carried out at 80 V using TAE buffer (40 mM Tris-acetate, 5 mM sodium acetate, 0.1 mM Na₂-EDTA, pH-7.8). Amplification results were described in as amplification or otherwise.

RESULTS

A total of 35 members of seven ichthyosis vulgaris families were studied for the presence of 1.5 kb FLG gene

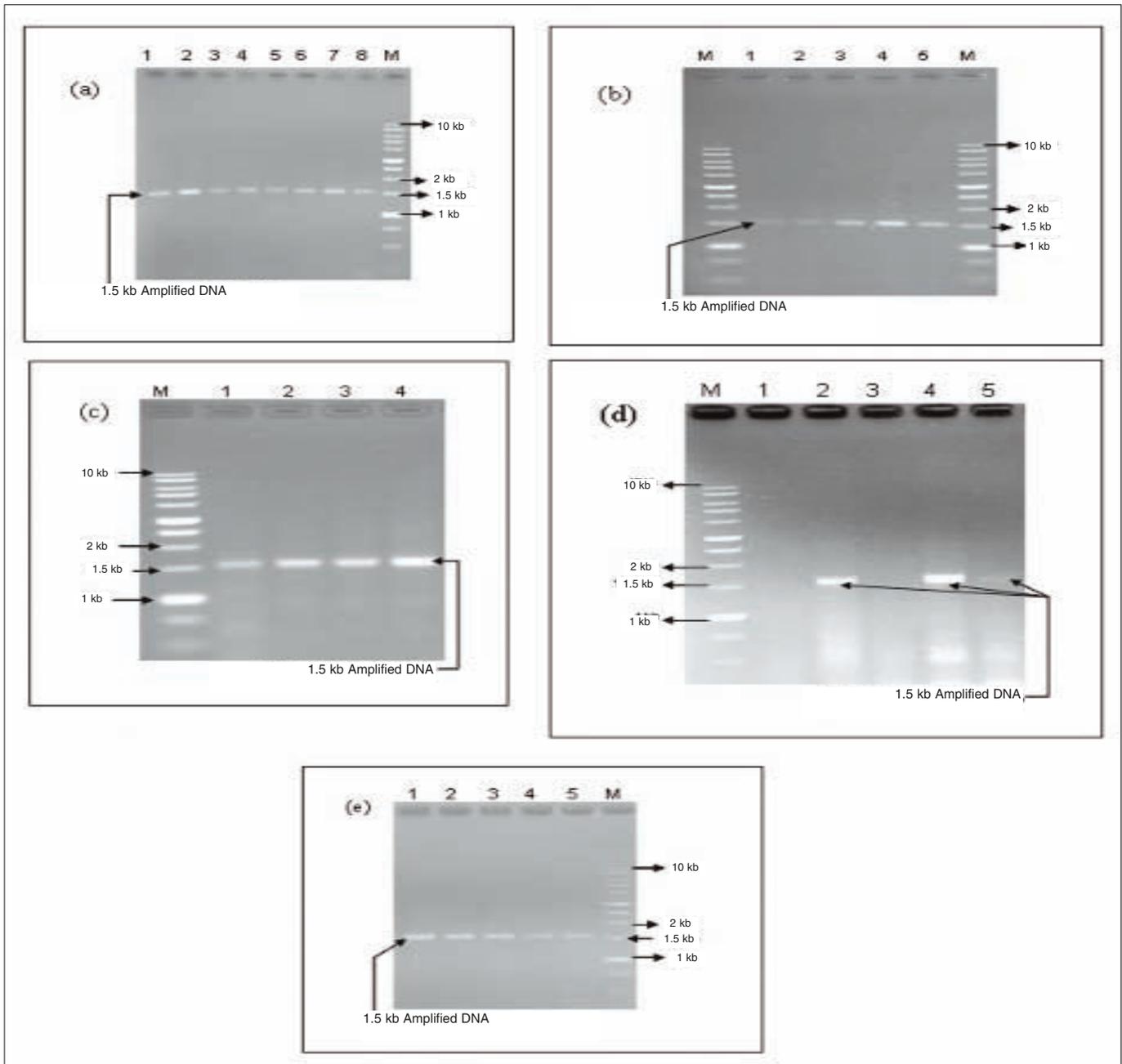


Figure 3: PCR amplification of 1.5 kb FLG gene fragment using primers FilF3 and RPTIP6. Ichthyosis vulgaris families : IV1 (a), IV2 (b), IV3 (c), IV4 (d), and IV6 (e); Numbered lanes: amplified DNA; M: Molecular Weight Marker (Genecraft - 1 kb DNA ladder).

fragment. All the families were of Pakistani origin. The families were given the codes IV1, IV2.....IV7 (Table I). The family IV1 comprised of three generations while the rest of the families comprised of two generations (Figure 1).

There were eight family members (aged 7-55 years) in the family IV1 (Figure 1) of which five were affected and three were normal (controls). In this family, patients were found in all the three generations and included both the male and female members. Family IV2 (Figure 1) included five members of 8-50 years of age. Both the parents were normal but all their children including one daughter and two sons were affected. Family IV3 (Figure 1) comprised of five members aged 10-45 years. Again, both the parents were normal and out of their three children, one daughter was normal and 2 sons were severely affected. Figure 2 shows the back of one of the affected boys of the family with severe lesions. Family IV4 (Figure 1) consisted of 5 members of 2-40 years of age. Both the parents were normal and out of their three children, two were normal (a boy and a girl) and one (a boy) was affected. Family IV5 (Figure 1) included three members with an affected 80 years old father, while the mother and her 45 years old son were normal. Family IV6 (Figure 1) comprised of 6 members of age 5 to 35 years. In this family, father had died. Both the parents and two boys were normal, however, 5 years old twins (a boy and a girl) were affected. Family IV7 (Figure 1) included 5 members of 9-40 years of age. Both the parents and a boy were normal but the other 2 kids (one boy and one girl) were affected.

Genomic DNA prepared from the blood samples (of both the affected and normal individuals of the seven ichthyosis vulgaris families) was used as a template for the amplification of 1.5 kb FLG gene fragment using the primers FilF3 and RPTIP6. Amplification was found successful in all the members of the families IV1 (Figure 3a), IV2 (Figure 3b), IV3 (Figure 3c) and IV6 (Figure 3e). In the family IV4 (Figure 3d), amplification was found successful in three male members (two normal and one affected). The rest of the two female members (normal mother and daughter) of the family showed no amplification. Amplification of the 1.5 kb FLG gene fragment was also not detected in any of the members of the families IV5 and IV7.

DISCUSSION

The disease ichthyosis vulgaris is characterized by scaling of the body involving trunk and usually more marked on the extensor surfaces of the limbs. Interestingly, the major flexures and neck are spared in this condition. The lesions in patients in this part of the world are more marked during winter as compared to summer. Profilaggrin is one of the major proteins of keratohyalin granules in the cells of the epidermal layer.

It has been observed that loss or reduction of this major structural protein lead to varying degrees of impaired keratinization.^{9,12,13} Filaggrin is, therefore, a key protein which helps in facilitating epidermal differentiation and maintenance of barrier function.¹⁰ In this study, clinical examination of the patients revealed that the disease has the onset since childhood. Patients showed the generalized scaling phenotype. The ichthyosis vulgaris patients of the families IV3, IV4, IV5 and IV6 showed more severe clinical symptoms including hypohydrosis, hyperkeratosis, hyperlinearity, atopic dermatitis and asthma. The pedigrees of the families showed that the disease is not X-linked since both the males and females were found affected in most of the families.

Smith *et al.*, identified homozygous or compound heterozygous mutations R501X and 2282del4 in the gene encoding filaggrin (FLG) as a genetic cause of moderate or severe ichthyosis.¹⁷ The two mutations have been studied in Irish, Scottish and European-American populations.^{17,18} During this study, the amplification of the 1.5 kb FLG gene fragment known to carry R501X mutation in ichthyosis vulgaris patients of European-American origin suggested the possibility of the presence of the same mutation in Pakistani ichthyosis vulgaris patients. In this current study, amplification of the 1.5 kb FLG gene fragment was not detected in the genomic DNA of two members of the family IV4 and in any of the members of the families IV5 and IV7. These results suggested the involvement of some novel FLG gene mutations other than R501X in causing the disease. New filaggrin gene mutations, 3702delG²⁰ and 3321delA²¹ have been reported in Irish and Japanese families respectively in addition to the R501X mutation which is common in European population.^{17,18}

Consanguineous marriages are common in Pakistan. There is a little or no awareness in the majority of our population regarding the harmful effects of extensive inbreeding within a family as a result of which alleles of the disease genes unite and come into expression. Therefore, it is important to study the genetic basis of diseases in order to provide proper guidance and genetic counseling to people to minimize the chances of the transfer of disease genes to the next generations. The results of this study will also provide useful insight for further research in understanding the molecular basis of ichthyosis vulgaris in Pakistan. DNA sequencing of the amplified PCR products is in progress. The sequencing results will further confirm whether R501X or some novel mutations are involved in causing the disease in our population.

CONCLUSION

The presence of 1.5 kb FLG gene fragment in five out of the seven ichthyosis vulgaris families suggested the

involvement of R501X mutation as one of the causes of ichthyosis vulgaris in our population. The two families that did not show the presence of 1.5 kb FLG gene fragment suggested the involvement of some novel mutations other than the reported R501X as being the cause of the defective FLG gene expression and hence responsible for the disease.

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REFERENCES

1. Al-Zayir AA, Al-Amro Alakloby OM. Primary hereditary ichthyosis in the Eastern Province of Saudi Arabia. *Int J Dermatol* 2004; **43**:415-9.
2. Cuevas-Covarrubias SA, Diaz-Zagoya JC, Rivera-Vega MR, Beirana A, Carrasco E, Orozco E, *et al.* Higher prevalence of X-linked ichthyosis vs. ichthyosis vulgaris in Mexico. *Int J Dermatol* 1999; **38**:555-6.
3. Okano M, Kitano Y, Yoshikawa K, Nakamura T, Matsuzawa Y, Yuasa T. X-linked ichthyosis and ichthyosis vulgaris: comparison of their clinical features based on biochemical analysis. *Br J Dermatol* 1988; **119**:777-83.
4. Wells RS, Kerr CB. Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. *BMJ* 1966; **1**: 947-50.
5. Shwayder T, Ott F. All about ichthyosis. *Pediatr Clin North Am* 1991; **38**:835-57.
6. Uehara M, Hayashi S. Hyperlinear palms: association with ichthyosis and atopic dermatitis. *Arch Dermatol* 1981; **117**:490-1.
7. Rabinowitz LG, Esterly NB. Atopic dermatitis and ichthyosis vulgaris. *Pediatr Rev* 1994; **15**:220-6.
8. Mevorah B, Marazzi A, Frenk E. The prevalence of accentuated palmoplantar markings and keratosis pilaris in atopic dermatitis, autosomal dominant ichthyosis and control dermatological patients. *Br J Dermatol* 1985; **112**:679-85.
9. Sybert VP, Dale BA, Holbrook KA. Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. *J Invest Dermatol* 1985; **84**:191-4.
10. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005; **6**:328-40.
11. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009; **122**:1285-94.
12. Fleckman P, Holbrook KA, Dale BA, Sybert VP. Keratinocytes cultured from subjects with ichthyosis vulgaris are phenotypically abnormal. *J Invest Dermatol* 1987; **88**:640-5.
13. Pena Penabad C, Pérez Arellano JL, Becker E, Gutierrez de Diego J, García Salgado MJ, Valle FJ, *et al.* Differential patterns of filaggrin expression in lamellar ichthyosis. *Br J Dermatol* 1998; **139**:958-64.
14. Nirunskisiri W, Presland RB, Dale BA, Fleckman P. Decreased profilaggrin expression in ichthyosis vulgaris is a result of selectively impaired post-transcriptional control. *J Biol Chem* 1995; **279**:871-6.
15. Compton JG, DiGiovanna JJ, Johnston KA, Fleckman P, Bale SJ. Mapping of the associated phenotype of an absent granular layer in ichthyosis vulgaris to the epidermal differentiation complex on chromosome 1. *Exp Dermatol* 2002; **11**:518-26.
16. Zhong W, Cui B, Zhang Y, Jiang H, Wei S, Bu L, *et al.* Linkage analysis suggests a locus of ichthyosis vulgaris on 1q21. *J Hum Genet* 2003; **48**:390-2.
17. 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. *Nature Genet* 2006; **38**:337-42.
18. Gruber R, Janecke AR, Fauth C, Utermann G, Fritsch PO, Schmuth M. Filaggrin mutations p.R501X and c.2282del4 in ichthyosis vulgaris. *Eur J Hum Genet* 2007; **15**:179-84. Epub 2006 Dec 13.
19. Hamada T, Sandilands A, Fukuda S, Sakaguchi S, Ohyama B, Yasumoto S, *et al.* De novo occurrence of the filaggrin mutation p.R501X with prevalent mutation c.3321delA in a Japanese family with ichthyosis vulgaris complicated by atopic dermatitis. *J Invest Dermatol* 2008; **128**:1323-5. Epub 2007 Nov 15.
20. Sandilands A, O'Regan GM, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM, *et al.* Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. *J Invest Dermatol* 2006; **126**:1770-5. Epub 2006 Jun 29.
21. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, *et al.* Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 2007; **119**:434-40.

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