INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 6, p. 10-20, 2019

RESEARCH PAPER OPEN ACCESS

Novel and recurrent mutations in *FLG, ALOXE3* and *STS* genes underlying different forms of hereditary ichthyosis

Hina Mir[1,2*], Abdul Haleem Shah[1] Ayesha Haleem Shah[1], Wasim Ahmad[2]

[1]*Department of Biological Sciences, Faculty of Sciences, Gomal University, D. I. Khan, Pakistan*

[2]*Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan*

Key words: Ichthyosis, recessive,*FLG, ALOXE3, STS, VCX.*

http://dx.doi.org/10.12692/ijb/14.6.10-20 Article published on June 16, 2019

Abstract

Ichthyoses are group of genetic disorders of cornification with abnormal differentiation and desquamation of the epidermis. Clinically ichthyosis can be distinguished into syndromic ichthyosis and non-syndromic ichthyosis (limited to the skin only). This study presents clinical and molecular characterization of three urelated Pakistani families displaying different forms of hereditary ichthyosis. The objective of the study was to search for pathogenic mutations in *FLG, ALOXE3* and *STS* genes in three Pakistani families with different forms of recessive hereditary ichthyoses. DNA samples of all available affected and unaffected individuals were PCR amplified using microsatellite markers and further analyzed by DNA sequencing. DNA sequence analysis revealed a novel and two previously reported mutations in the genes *FLG, ALOXE3* and *STS*. Deletion mapping showed a deletion of about 1.67Mb region having genes *VCX3A, HDHD1, STS, VCX* and *PNPLA4* in one family with recessive X-linked ichthyosis. This study expandsspectrum of mutations in the genes *FLG, ALOXE3* and *STS*.

* **Corresponding Author:** Hina Mir ✉ hinamir12@yahoo.com

Introduction

Ichthyoses are group of genetic disorders of cornification with abnormal differentiation and desquamation of the epidermis, clinically characterized by scaling or hyperkeratosis of the skin or both. Scaling is usually associated with thickening of the cornified layer. Ichthyoses can be inherited or acquired, presenting at birth or later in life associated with autoimmune, metabolic, infectious, and inflammatory diseases or medication. The mode of inheritance of ichthyoses may be, autosomal dominant, autosomal recessive, X-linked dominant or X-linked recessive (Oji *et al.*, 2010).

The ichthyoses are both clinically and etiologically enormously heterogeneous resulting in considerable difficulties in their classification. Clinically ichthyosis can be distinguished into syndromic ichthyosis (involvement of skin with other organs) and non-syndromic ichthyosis (limited to the skin only). Non-syndromic ichthyoses are further classified into common ichthyoses including ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (RXLI); autosomal recessive congenital ichthyosis (ARCI) that include lamellar ichthyosis (LI), congenital ichthyosiform erythroderma (CIE), harlequin ichthyosis (HI), pleomorphic2 ichthyosis (PI); and keratinopathic ichthyosis (Oji *et al.*, 2010; Vahlquist, 2010).

Ichthyosis vulgaris (MIM 146700) is a relatively common genetic keratinization disorder, accounting for more than 95% of ichthyosis cases. Individuals with ichthyosis vulgaris commonly display dry skin with mild generalized fine scaling especially on the flexor limbs and lower abdomen, palmoplantar hyperlinearity and keratosis pilaris (Sybert*et al.*, 1985). Symptoms usually manifest within the first year of life and become more severe with age. Smith *et al.* (2006) first demonstrate that loss of function mutations in *FLG*(Fillagrin) gene on chromosome 1q21.3, underlie ichthyosis vulgaris.

The X-linked recessive ichthyosis (MIM 308100) is a disorder of cutaneous keratinization, which results due to deficiency of an enzyme steroid sulfatase (STS), encoded by the *STS* gene located on chromosome Xp22.31 (Webster., *et al* 1978). RXLI is usually evident during the first few weeks of life as polygonal, loosely adherent translucent scales in a generalized distribution that desquamate widely. These are then quickly replaced by large, dark brown, tightly adherent scales occurring primarily on the extensor surfaces of the lower limbs, trunk, neck and scalp (Høyer *et al.*, 1986). The palms and soles are usually spared. The face is usually free of scales, except in the preauricular areas (Wells and Jennings, 1967). Most patients with RXLI (>90%), have deletion of the entire *STS* gene and flanking sequences. Xp22.3 is rich in low-copy repeats (LCRs), having multiple recombination hot spot motifs. These repeats are responsible for the microdeletions in this region due to non-allelic homologous recombination (NAHR) (Van-Esch *et al.*, 2005).

Autosomal recessive congenital ichthyosis (ARCI) are heterogeneous disorders of the skin. ARCI is divided into lamellar ichthyosis, congenital ichthyosiform erythroderma, harlequin ichthyosis and pleomorphic ichthyosis. Lamellar ichthyosis (LI) is characterized by the presence of large dark, plate like scales with mild to moderate erythema. Congenital ichthyosiform erythroderma (CIE) represents fine white scales with variable erythroderma. Harlequin ichthyosis is the most severe form of ARCI, patients with HI are born encased in thick collodion membrane that gradually disappears during the first weeks of life and is replaced by large, thick, plate like scales. A new type of ARCI, pleomorphic ichthyosis (PI); is characterized by marked cutaneous hyperkeratosis at birth and later develop mild skin symptoms of ichthyosis (Oji *et al.*, 2010; Vahlquist, 2010).

Nine genes for ARCI have been identified to date, including five LI associated genes; *TGM1*(Transglutaminase 1) (MIM 242300) on chromosome 14q11, *CYP4F22*(Cytochrome P4F22) (MIM 604777) on chromosome 19p12-q12, *NIPAL4*(NIPA like domain containing 4) (MIM 612281) on chromosome 5q33, *PNPLA1*(Patatin like phosolipase domain containing 1) (MIM 612121) on

chromosome 6p21.31, *LIPN*(Lipase N) (MIM 613924) on chromosome 10q23.31; three CIE associated genes *ALOX12B*(Arachidonate 12-lipoxygenase) (MIM 603741) and *ALOXE3*(Arachidonate lipoxygenase 3) (MIM 607206) on chromosome 17p13, and *CERS3*(ceramide syntase 3) (MIM 615276) on chromosome 15q26.3; and a single HI associated gene; *ABCA12*(ATP binding cassette subfamily A member 12) (MIM 601277) onchromosome 2q34-q35 (Huber *et al.*,1995; Russell *et al.*, 1995; Jobard*et al.*, 2002; Lefevre*et al.*, 2003, 2004, 2006; Akiyama *et al.*, 2005; Natsuga*et al.*, 2007; Israeli *et al.*, 2011; Grall*et al.*, 2012; Radner*et al.*, 2013).

In the present study, we have investigated three unrelated Pakistani families segregating different forms of ichthyosis. Genotyping using microsatellite markers showed linkage of family A to *FLG* gene and family B to *ALOXE3*. DNA sequence analysis revealed a novel mutation in *FLG* gene and a recurrent mutation in the *ALOXE3* gene. Sequence analysis of *STS* gene in the third family with X-linkedichthyosis revealed complete deletion of the *STS* gene.

Materials and methods

Subjects

For this study three families (A, B and C), demonstrating various forms of hereditary ichthyoses, were recruited from different regions of Pakistan **(**Fig. 1). Approval of the study was obtained from the Institutional Review Board (IRB) of Quaid-i-Azam University, Islamabad and Gomal University D. I. Khan, Pakistan. Both affected and unaffected members of all the three families were informed about research methodology and objectives of this study. Pedigree drawings of the families were based upon detailed question/answer sessions conducted with affected and elders of the families.

Genomic DNA (deoxyribonuclease) was extracted from peripheral blood samples, collected from 9 affected and 11 unaffected members of the three families, by GenElute™ blood genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). DNA was quantified by Nanodrop-1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA) measuring its optical density (OD) at 260nm and diluted to 40–50 ng/µl for amplification by polymerase chain reaction (PCR).

Genotyping

Considering the features observed in affected members and mode of inheritance of the phenotype, linkage in two families (A and B) was tested by genotyping microsatellite markers linked to gene *FLG* on chromosome 1q21.3 (D1S2715, D1S305, D1S1153, D1S2624, D1S1653, D1S398, D1S1167, D1S2768), *TGM1* on chromosome 14q11 (D14S1430, D14S581, D14S972, D14S264, D14S1041, D14S1032, D14S275), *ALOX12B* and *ALOXE3* on chromosome 17p13 (D17S906, D17S960, D17S1353, D17S1812, D17S1844), *NIPAL4* on chromosome 5q33 (D5S1978, D5S2012, D5S1507, D5S2852, D5S820, D5S412), *CYP4F22* on chromosome 19p12–q12 (D19S840, D19S226, D19S929, D19S588, D19S199) and *ABCA12* on chromosome 2q34–q35 (D2S371, D2S2322, D2S2319, D2S1345,D2S2382). Family C representing X-linked ichthyosis, *STS* gene mapped on Xp22.3 was sequenced directly in both affected and unaffected members.

PCR-amplification of the microsatellite markers was performed according to standard procedure as described by Mir *et al.* 2012. The PCR-amplified products were resolved on 8% non-denaturing polyacrylamide gel, stained with ethidium bromide and genotypes were assigned by visual inspection. Allele size for respective microsatellite markers was determined using 5-, 10- and 20-bp DNA ladders (MBI Fermentas®, Life Sciences, York, UK). Order of markers was based on Rutgers combined linkage-physical map of the human genome (Matise*et al.*, 2007).

Sequencing

Standard sequences of the genes including *FLG, ALOXE3, STS, PNPLA4,HDHD1, VCX3A, VCX, VCX2* and *VCX3B* were obtained from Ensembl Genome Browser(http://www.ensembl.org/Homo_sapiens/Gene). Using Primer3 version 0.4.0 software (Rozen and Skaletsky, 2000), forward and reverse primers

for PCR amplification of coding exons, splice junction sites, 5' UTR (untranslated region) and 3' UTR of the genes were designed. The PCR amplification conditions used were 95°C for 5 min, followed by 30 cycles of 95°C for 30 sec, 59°C for 30 sec, and 72°C for 4 min with a final extension at 72°C for 10 min. Amplified PCR products were analyzed on 2.5% agarose gel under UV transilluminator (Biometra, Germany). Fragment size of each amplicon was determined using 100 bp DNA ladder (MBI, Fermentas, UK). Purification of the PCR-amplified products wasperformed with a commercially available kit (Marligen Bio-sciences, Ijamsville, MD, USA). DNA Sequencing of the amplified PCR products was performed with Big Dye Terminator v3.1 Cycle Sequencing Kit together with an ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA, USA). The sequence of each amplicon was then aligned with reference sequence by using Bioedit sequence alignment tool (editor version 6.0.7, Ibis, Biosciences, CA, USA).

Results

Clinical features

Affected members of the three families (A, B and C) were clinically examined by dermatologists at the local government hospitals. Affected individuals of family A, presented here, displayed characteristic features of ichthyosis vulgaris, having severe dry, desquamated skin with generalized fine to dark scales especially on the face, flexor limbs and abdomen (Fig. 2a, b).

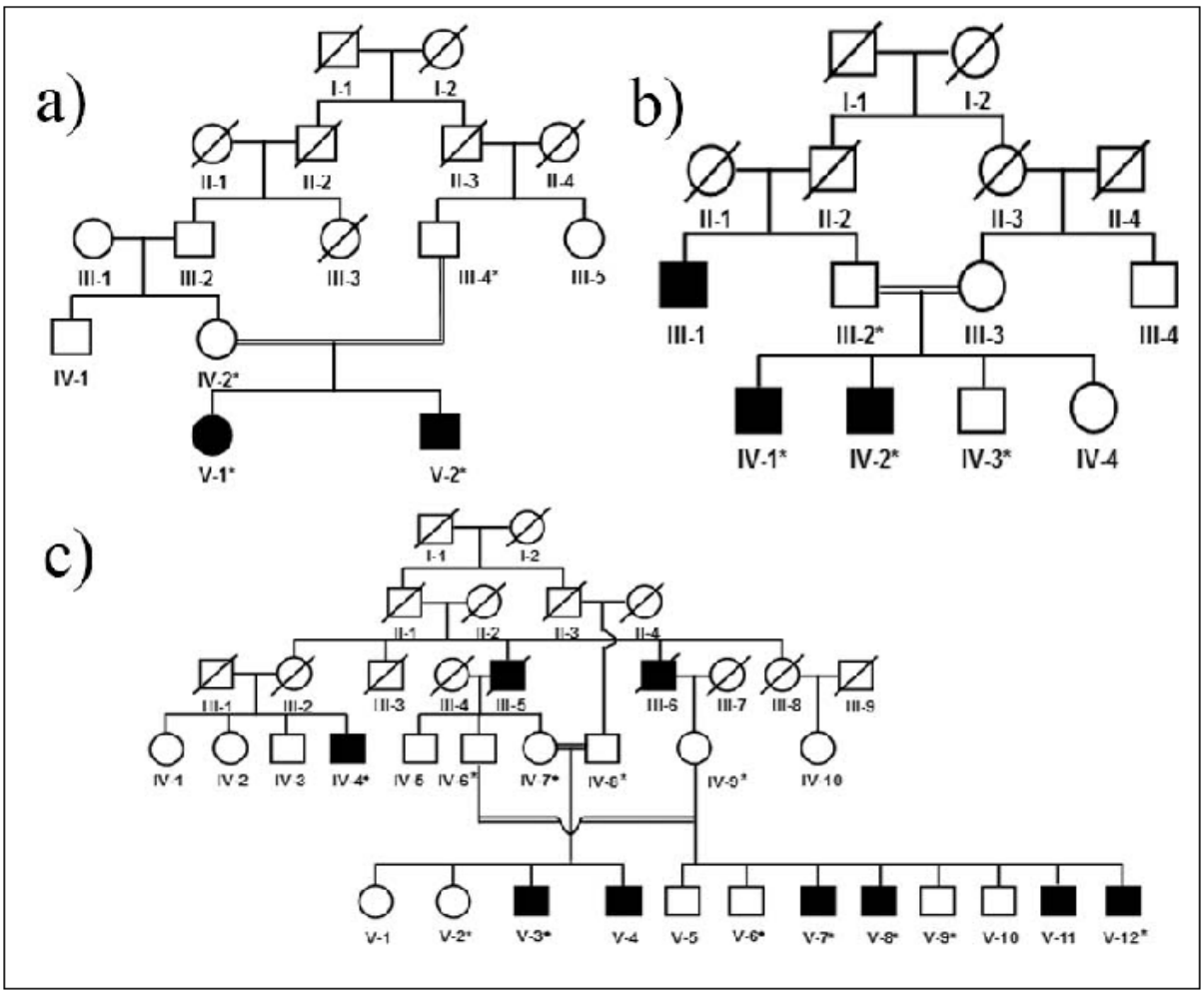

Fig. 1. Pedigree drawings of three Pakistani families with different forms ofrecessive hereditary ichthyoses. Circles and squares represent females and males, respectively. Clear symbols represent unaffected individuals while filled symbols represent affected individuals. Symbols with crossed lines represent deceased individuals. Symbols with a star represent the samples that were available for the study.

In family B, affected members exhibited congenital ichthyosiform erythroderma with fine white ichthyotic scales that are more severe on back, arms and legs. Affected individual IV-2 has highly furfuraceous skin on back (Fig. 2c). Affected members in family C showed typical features of recessive X-linked ichthyosis (RXLI) with mild erythroderma and polygonal, loosely adherent translucent scales developed few weeks after birth that later on become larger and dark brown. No scales were found on palms and soles.Severity of RXLI phenotype was observed on the lower extremities of affected individuals with tightly adherent brown to black scales (Fig. 2d). The patients have complaints of bleeding from scaly skin, which occurs mostly in severe cold conditions.

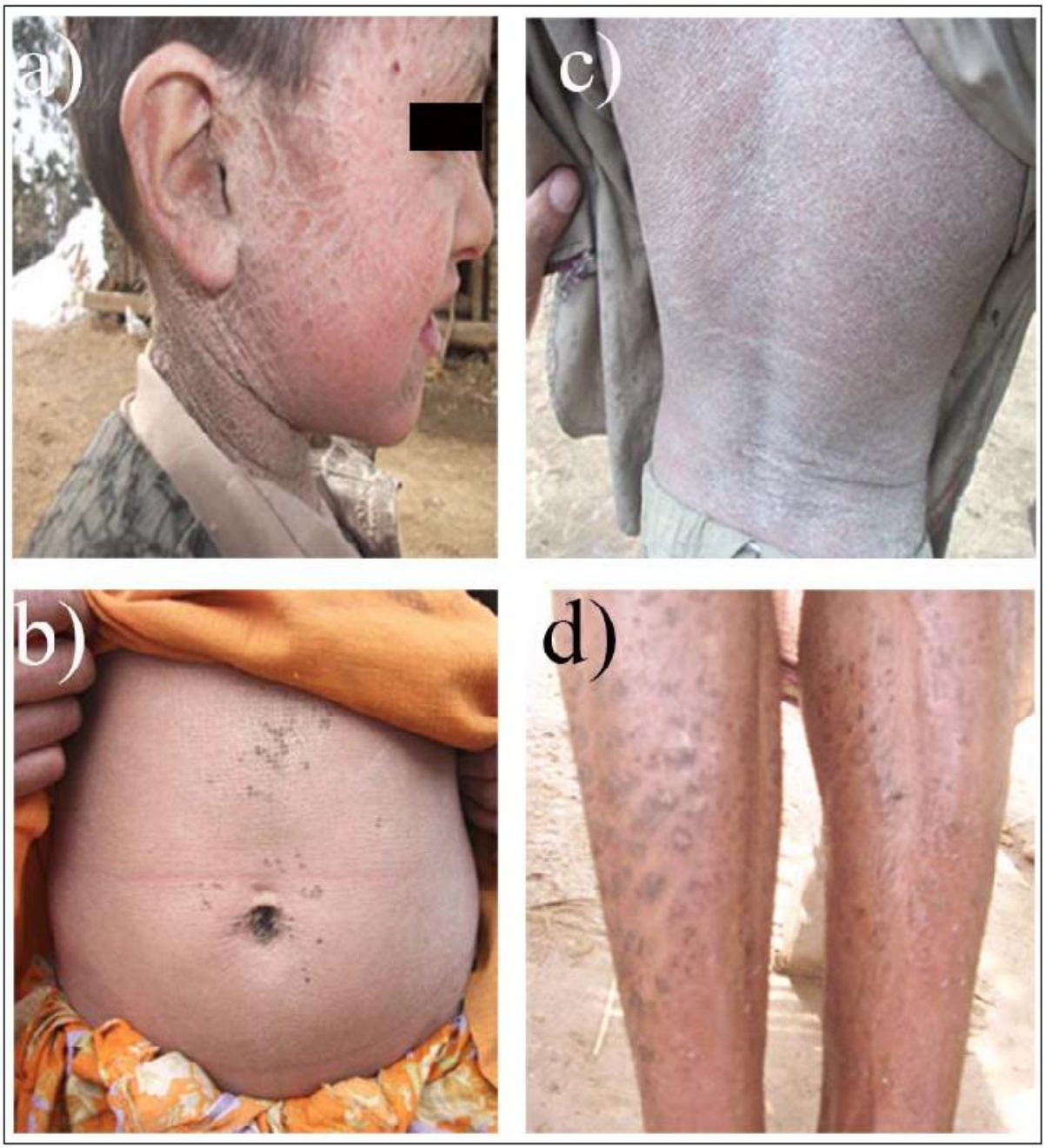

Fig. 2. Clinical presentation of ichthyosis vulgaris**a,b:** severe dry skin with finewhite to dark scales on the face and trunk of affected individual (V-1 and V-2) in family A. **c:** ARCI, Fine white ichthyotic scales on the back of 14 year old affected individual (IV-2) of family B. **d:** X-linked ichthyosis, thick, large, tightly adherent, brown scales on the front of the lower extremities of a 36 years old affected individual (V-4) of family C.

Height, growth, vision, dentition and mental condition were normal in affected members of the three families. Ectodermal abnormalities of hair, nail, sebaceous glands and sweat glands were not associated with the disease phenotype in affected individuals. Ectropion and eclabium were not found in any affected member of the three families. Heterozygous carrier individuals had normal skin, and were clinically indistinguishable from genotypically unaffected individuals of the respective families.

Genotyping and mutation analysis

Linkage in two families (A and B) was tested, using microsatellite markers, specific to genes including *FLG* (1q21.3), *TGM1* (14q11), *ALOX12B* (17p13), *ALOXE3* (17p13), *NIPAL4* (5q13), *CYP4F22* (19p12–q12), and *ABCA12* (2q34–q35). Haplotype analysis showed linkage in the family A to the gene FLG and family B to *ALOXE3* and *ALOX12* genes.

In family A, sequence analysis of exon 3 of the *FLG* gene detected a novel single base-pair homozygous duplication (c.358dupG) leading to a frameshift and premature termination codon 40 bp downstream (p.Glu120Glyfs*14) (Fig-3a). In family B, sequence analysis of exon 4 of *ALOXE3* gene revealed a recurrent homozygous nonsense mutation involving C to T transition at nucleotide position 418 (c.418C>T) of the gene. This resulted in substitution of a codon for arginine at amino acid position 140 to become a stop codon (p.Arg140*) (Fig-3b). The sequence variants, identified here, were found in the heterozygous state in the obligate carriers and segregated with the disease in the respective families. To exclude the possibility that the mutations identified in the present families, do not represent non-pathogenic polymorphisms, a panel of 100 unaffected unrelated ethnically matched control individuals were screened for the frameshift and nonsense mutations identified in family A and B, respectively.

In family C, PCR results analysis showed complete deletion of the *STS* gene in the affected individuals (Fig-3c). To define the deletion breakpoints in families with RXLI, we designed primer pairs for the neighboring genes *HDHD1* and *PNPLA4* which were also found deleted in all affected members. We then used primer pairs as described by Van-Esch*et al.* (2005). Affected individuals of family showed amplification of primer pairs VCX3A-dis and VCX2-prox but no amplification was obtained in primer pairs VCX3A-prox and VCX2-dis (Fig-3d), thus representing a deletion of about 1.67Mb region involving *VCX3A, HDHD1, STS, VCX* and *PNPLA4* genes. To further confirm these results PCR analysis of RU1region of the *VCX* genes was carried out. In affected individuals of the three families, we obtained two fragments of 163bp and 523bp corresponding to RU1 region of *VCX2* and *VCX3B* genes respectively, while the normal individuals of the three families and control showed amplification of four fragments (523bp, 403bp, 343bp and 163bp) representing RU1 region of all the four *VCX* genes (Fig-3e). These results demonstrates that recombination may took place between the 1Kb repeat unit 2 (RU2) regions of *VCX3A* and *VCX2* genes that share >95% identity, by the mechanism of NAHR and results in the deletion of *VCX3A,HDHD1, STS, VCX* and *PNPLA4* genes leaving *VCX2* intact.

Discussion

In the present investigation we have described three Pakistani families affected with different forms of recessive hereditary ichthyoses. The affected members of family A showed characteristic features of ichthyosis vulgaris having severe dry skin with dark scales. Affected members of family B exhibited erythroderma with fine white ichthyotic scales, exhibiting phenotypes of autosomal recessive congenital ichthyosis. Family C was associated with recessive X-linked ichthyosis presenting polygonal, loosely adherent translucent scales on the body of the affected members after births, which are then replaced by large, dark brown, tightly adherent scales.

Screening of the genes linked to the respective families with different forms of recessive hereditary ichthyoses revealed a novel and two recurrent

mutations. DNA sequence analysis identified a novel homozygous duplication mutation (c.358dupG) in *FLG* gene leading to a frameshift and premature termination codon 40 bp downstream (p.Glu120Glyfs*14) in family A with ichthyosis vulgaris, a recurrent homozygous nonsense mutation involving C to T transition at nucleotide position 418 (c.418C>T) in *ALOXE3* gene, resulting in substitution of a codon for arginine at amino acid position 140 to stop codon (p.Arg140*) in family B with ARCI. Family C showed a deletion of about 1.67 Mb region having *VCX3A, HDHD1, STS, VCX* and *PNPLA4* genes.

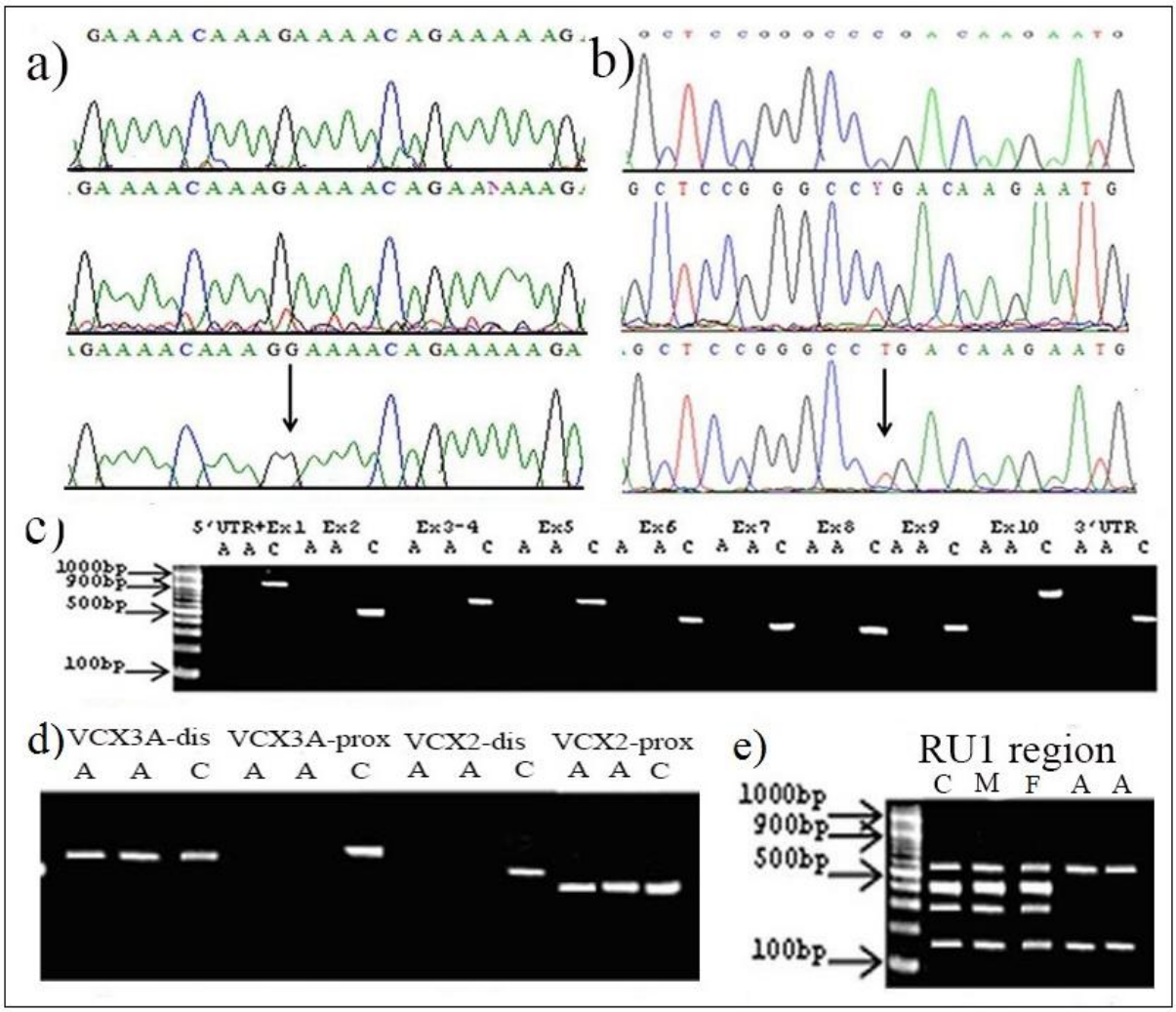

Fig. 3. (a) Sequence analysis of a novel duplication mutation c.358dupG in FLGgene in family A and **(b)** a recurrent nonsense mutation c.418C>T in *ALOXE3* gene, in family B. The upper panels represent the nucleotide sequences in the control unaffected individual, the middle panels in the heterozygous carrier and thelower panels in the affected individual. Arrows indicate position of mutations in the affected individuals. **(c)** Deletion mutations analysis of STS gene showing amplification of all exons, 5'UTR and 3'UTR of STS gene sequences only in a carrier individual of family C. No amplification has been observed in any of the affected individual. **(d)** Affected individuals of family C showed amplification of primer pairs VCX3A-dis and VCX2-prox but no amplification was obtained in primer pairs VCX3A-prox and VCX2-dis. **(e)** Amplification of the RU1 region of the VCX genes showed four fragments (523bp, 403bp, 343bp and 163bp) representing RU1 region of all the four VCX genes in control, Mother and Father of affected individuals while in affected individuals, we obtained two fragments of 163bp and 523bp corresponding to RU1 region of VCX2 and VCX3B genes respectively. 100 bp DNA ladder in the left column of the panel indicate size of the PCR amplified products.UTR untranslated region, Ex exon, A affected, C control, bp base pair, M mother, F father.

FLG gene (MIM 135940) comprises three exons, spans ~25 kb of DNA and located in the epidermal differentiation complex (EDC) on chromosome 1q21.3. The 4061 amino acids protein consists of S100 calcium binding domain, B domain, 10-12 filaggrin repeats and C-terminal domain. Filaggrin (filament aggregating protein) plays an important role in the epidermal barrier function by aggregating keratin intermediate filaments in the granular cell layer to form the stratum corneum (Steinert*et al.*, 19881; Dale *et al.*, 1985). In addition, the degradation products of filaggrin contribute to moisture retention in the cornified layers (Akiyama, 2011). To date, more than 40 different population-specific FLG mutations have been identified, each resulting in a truncated profilaggrin geneproduct, which is not processed into functional FLG monomers? The novel mutation (p.Glu120Glyfs*14) identified in family A is located in the Leader Peptide of S100 calcium binding domain, thus results in loss of function of the Flg protein. The truncated protein so formed, loses major portion of the Flg protein require for aggregating keratin intermediate filaments to form the stratum corneum.

ALOXE3 gene (MIM 607206) has fifteen exons, spaning about 22 kb of genomicDNA on chromosome 17p13.1. The 843 amino acids protein contains PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain or LH2 (Lipoxygenase homology 2) domain and a large lipoxygenase domain. To date, 19 pathogenic mutations in *ALOXE3* gene, comprising 6 missense, 5 nonsense, 4 splice-site, 3 small deletion and 1 gross deletion mutations. Lipoxygenase-3 encoded by *ALOXE3* gene, is non-heme iron-containing dioxygenase, highly expressed insupra basal layer of epidermis. This enzyme participate in lipid metabolism of lamellar granule content or intercellular lipid layer, by acting as hydroperoxide isomerase (epoxyalcohol synthase) using product of *ALOX12B*, 12R -HPETE, into a specific epoxy alcohol product, 8 R -hydroxy-11R ,12 R -epoxyeicosa-5 Z ,9 E ,14 Z -trienoic acid (Krieg *et al* ., 2001; Yu *et al.*, 2003). The recurrent mutation (p.Arg140*), identified in family B is located in the lipoxygenase domain, results in the production of truncated protein.

STS gene (MIM 300747) consists of 10 exons and spans about 146 kb onchromosome Xp22.31, encoding 583 amino acids protein. This protein contains two sulphatase domains. Deficiency of steroid sulphatase encoded by *STS* gene accumulates cholesterol sulphates in the outer epidermis thereby inhibiting the production of cholesterol (Epstein and Williams, 1981; Elias *et al.*, 1984; Bergner and Shapiro, 1988;). This also results a delay in corneodesmosme degradation, thus disrupting the lamellar membrane architecture, accounting for the barrier abnormality in RXLI (Zetterste*et al.*, 1988; Elias *et al.*, 2004). The large number of deletion mutations reported in *STS* gene is probably due to several variable number of tandem repeats (VNTR) sequences flanking the gene. Some of these VNTR sequences are recombinogenic and stimulate nonallelic homologous recombination (NAHR) (Wahls*et al.*, 1990; Li *et al.*, 1992; Van *et al.*, 2005). The 1.67 Mb microdeletions in family C may result in deletion of RU2 of *VCX3A* and *VCX2* genes as described in (Van-Esch*et al.*, 2005).

The common mechanism among all forms of ichthyoses is the disruption of the epidermal barrier. Any abnormality in the formation, processing, or transportation of lipids including cholesterol esters, epoxy alcohols and others, alter the stability of the skin barrier, leading to ichthyoses with the severity depending on where the disruption occur.

Conclusion

In this study, we have identified novel and previously reported mutations in FLG, ALOXE3, and STS genes in three Pakistani families with different forms of hereditary ichthyoses. All the mutations were loss of function mutations either may impair the enzyme activity or ablating protein synthesis, thus confirming the crucial role played by these genes during epidermal barrier formation.

Acknowledgment

We highly appreciate invaluable cooperation and

participation of the three family members in the present study. This work was financially supported by Higher Education Commission (HEC), Islamabad, Pakistan. Hina Mir was supported by indigenous PhD fellowship from HEC, Islamabad, Pakistan.

Conflicts of interest

None declared.

References

Akiyama M. 2011. Updated molecular genetics and pathogenesis of ichthyosis. Nagoya Journal of Medical Sciences **73,** 79-90.

Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M,Arita K, Tsuji-Abe Y, Tabata N, Matsuoka K, Sasaki R, SawamuraD, Shimizu H. 2005. Mutations in ABCA12 in harlequin ichthyosis and functional rescue by corrective gene transfer. Journal of Clinical Investigations **115,** 1777-1784.

Bergner EA, Shapiro LJ. 1988. Metabolism of 3H-dehydroepiandrosterone sulphate by subjects with steroid sulphatase deficiency. Journal of Inherited Metabolic Diseases**11,** 403-415.

Dale BA, Resing KA, Lonsdale-Eccles JD. 1985. Filaggrin: a keratin filament associated protein. Annals of New York Academy of Sciences **455,** 330-342.

Elias PM, Crumrine D, Rassner U, Hachem JP, Menon GK, Man W, Choy MH, Leypoldt L, Feingold K, Williams ML. 2004. Basis for abnormal desquamation and permeability barrier dysfunction in RXLI. Journal of Investigative Dermatology **122,** 314-319.

Elias PM, Williams ML, Maloney ME, Bonifas JA, Brown BE, Grayson S, Epstein EH Jr. 1984. Stratum corneum lipids in disorders of cornification. Steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. Journal of clinical Investigations **74,** 1414-1421.

Epstein EH Jr, Williams ML. 1981. Steroid sulfatase, X-linked ichthyosis, and stratum corneum cell cohesion. Archives of Dermatology **117,** 761-763.

Grall A, Guaguère E, Planchais S, Grond S, Bourrat E, Hausser I, Hitte C, Le-Gallo M, Derbois C, Kim GJ, Lagoutte L, Degorce-Rubiales F, Radner FP, Thomas A, Küry S, Bensignor E, FontaineJ, Pin D, Zimmermann R, Zechner R, Lathrop M, Galibert F, AndréC, Fischer J. 2012. PNPLA1 mutations cause autosomal recessive congenital ichthyosis in golden retriever dogs and humans. Nature Genetics **44,** 140-147.

Hoyer H, Lykkesfeldt G, Ibsen HH, Brandrup F. 1986. Ichthyosis of steroid sulphatasedeficiency.Clinical study of 76 cases. Dermatologica**172,** 184-190.

Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponec M,Bon A, Lautenschlager S, Schorderet DF, Hohl D. 1995. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. Science **267,** 525-528.

Israeli S, Khamaysi Z, Fuchs-Telem D, Nousbeck J, Bergman R, Sarig O, Sprecher E. 2011. A Mutation in LIPN, Encoding Epidermal Lipase N, Causes a Late-Onset Form of Autosomal-Recessive Congenital Ichthyosis. American Journal Human Genetics **88,** 482–487.

Jobard F, Lefèvre C, Karaduman A, Blanchet-Bardon C, Emre S,Weissenbach J, Ozgüc M, Lathrop M, Prud'homme JF, Fischer J. 2002. Lipoxygenase-3 (ALOXE3) and 12(R)-lipoxygenase (ALOX12B) are mutated in nonbullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. Human Molecular Genetics **11,** 107-113.

Krieg P, Marks F, Fürstenberger G. 2001. A

gene cluster encoding human epidermis-type lipoxygenases at chromosome 17p13.1: cloning, physical mapping, and expression. Genomics **73,** 300-323.

Lefèvre C, Bouadjar B, Ferrand V, Tadini G, Mégarbané A, Lathrop M, Prud'homme JF, Fischer J. 2006. Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. Human Molecular Genetics**15,** 767-776.

Lefèvre C, Bouadjar B, Karaduman A, Jobard F, Saker S, Ozguc M, Lathrop M, Prud'homme JF, Fischer J. 2004. Mutations in ichthyin; anew gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. Human Molecular Genetics **13,** 2473-2482.

Lefévre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, Blanchet-Bardon C, Heilig R, Foglio M, Weissenbach J, Lathrop M, Prud'homme JF, Fischer J. 2003. Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. Human Molecular Genetics **12,** 2369-2378.

Li XM, Yen PH, Shapiro LJ. 1992. Characterization of a low copy repetitive element S232 involved in the generation of frequent deletions ofthe distal short arm of the human X-chromosome. Nucleic Acids Research **20,** 1117-1122.

Matise TC, Chen F, Chen W, De-La-Vega FM, Hansen M, He C,Hyland FC, Kennedy GC, Kong X, Murray SS, Ziegle JS, Stewart WC, Buyske S. 2007. A second-generation combined linkage physical map of the human genome. Genome Research **17,** 1783-1786.

Mir H, Khan S, Arif MS, Ali G, Wali A, Ansar M, Ahmad, W. 2012. Mutations in the gene phospholipase C, delta-1 (PLCD1) underlying hereditary leukonychia. European Journal of Dermatology **22,** 736-739.

Natsuga K, Akiyama M, Kato N, Sakai K, Sugiyama-Nakagiri Y,Nishimura M, Hata H, Abe M, Arita K, Tsuji-Abe Y, Onozuka T, Aoyagi S, Kodama K, Ujiie H, Tomita Y, Shimizu H. 2007. Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. Journal of Investigative Dermatology**127,** 2669-2673.

Oji V, Tadini G, Akiyama M, Blanchet-Bardon C, Bodemer C, Bourrat E, Coudiere P, DiGiovanna JJ, Elias P, Fischer J, Fleckman P, Gina M, Harper J, Hashimoto T, Hausser I, Hennies HC, Hohl D, Hovnanian A, Ishida-Yamamoto A, Jacyk WK, Leachman S, Leigh I, Mazereeuw-Hautier J, Milstone L, Morice-Picard F, Paller AS, Richard G, Schmuth M, Shimizu H, Sprecher E, Van-SteenselM, Taïeb A, Toro JR, Vabres P, Vahlquist A, Williams M, Traupe H. 2010. Revised nomenclature and classification of inherited ichthyoses:Results of the First Ichthyosis Consensus Conference in Sore`ze 2009. Journal of American Academy of Dermatology **63,** 607-641.

Radner FP, Marrakchi S, Kirchmeier P, Kim GJ, Ribierre F, Kamoun B, Abid L, Leipoldt M, Turki H, Schempp W, Heilig R, Lathrop M, Fischer J. 2013. Mutations in CERS3 cause autosomal recessive congenital ichthyosis in humans. PLoS Genetics **9,** e1003536 p.

Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods in Molecular Biology **132,** 365-386.

Russell LJ, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG, Bale SJ. 1995. Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. Nature Genetics **9,** 279-283.

Smith FJ, Irvine AD, Terron-Kwiatkowski A,

Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-JonesS, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WH. 2006. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nature Genetics **38,** 337-342.

Steinert PM, Cantieri JS, Teller DC, Lonsdale-Eccles JD, Dale BA. 1981. Characterization of a class of cationic proteins that specifically interact with intermediate filaments. Proceedings of the National Academy of Sciences USA **78**, 4097-4101.

Sybert VP, Dale BA, Holbrook KA. 1985. Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. Journal of Investigative Dermatology **84,**191-194.

Vahlquist A. 2010. Pleomorphic ichthyosis: proposed name for a heterogeneous group of congenital ichthyoses with phenotypic shifting and mild residual scaling. Acta Dermato Venereologica **90,** 454-460.

Van-Esch H, Hollanders K, Badisco L, Melotte C, Van-Hummelen P,Vermeesch JR, Devriendt K, Fryns JP, Marynen P, Froyen G. 2005. Deletion of VCX-A due to NAHR plays a major role in the occurrence of mental retardation in patients with X-linked ichthyosis. Human Molecular Genetics**14,** 1795-1803.

Wahls WP, Wallace LJ, Moore PD.1990. Hypervariable minisatellite DNA is a hotspot for homologous recombination in human cells. Cell **60,** 95-103.

Webster D, France JT, Shapiro LJ, Weiss R. 1978. X-linked ichthyosis due to steroid sulfatase deficiency. The Lancet **1,** 70-72.

Wells RS, Jennings MC. 1967. X-linked ichthyosis and ichthyosis vulgaris. Clinical and genetic distinctions in a second series of families. JAMA**202,** 485-488.

Yu Z, Schneider C, Boeglin WE, Marnett LJ, Brash AR. 2003. The lipoxygenase gene ALOXE3 implicated in skin differentiation encodes a hydroperoxide isomerase. Proceedings of the National Academy of Sciences USA **100,** 9162-9167.

Zettersten E, Man MQ, Sato J, Denda M, Farrell A, Ghadially R, Williams ML, Feingold KR, Elias PM. 1998. Recessive x-linked ichthyosis: role of cholesterol-sulfate accumulation in the barrier abnormality. Journal of Investigative Dermatology **111,** 784-790.