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Exacerbation of X-linked ichthyosis phenotype in a female by inheritance of filaggrin and steroid sulfatase mutations

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ABSTRACT

Background: X-linked ichthyosis (XLI) is a relatively common, recessive condition caused by mutations in the steroid sulfatase (STS) gene. Common loss-of-function mutations in the filaggrin gene (FLG) cause ichthyosis vulgaris and predispose individuals to atopic eczema.
Objective: To test the hypothesis that co-inheritance of FLG mutations can act as a genetic modifier in XLI.
Methods: An unusually severe XLI phenotype in addition to eczema and mild childhood asthma was investigated in a female Indian patient by fluorescent *in situ* hybridization (FISH) for the common STS gene deletion. Direct sequencing of the entire FLG gene was also performed.
Results: FISH analysis revealed that the proband was homozygous for the common STS genomic deletion mutation. Further investigation revealed a frame-shift mutation 3672del4 in the gene encoding filaggrin (FLG), leading to premature termination of profilaggrin translation. Interestingly, her father, who had a very typical mild presentation of XLI, did not carry this FLG mutation in addition to his STS deletion. Her mother was a heterozygous carrier of the FLG mutation and consistent with this, had mild symptoms of ichthyosis vulgaris; she was also a heterozygous carrier of the STS deletion.
Conclusion: This is the second reported case of the modifying effects of FLG null alleles on XLI and strengthens the hypothesis that filaggrin defects can synergize with STS deficiency to exacerbate the ichthyosis phenotype.

1. Introduction

X-linked ichthyosis (XLI) is a relatively common genodermatosis that affects 1 in 6000 males [1]. It is caused by a deficiency in steroid sulfatase (also known as aryl sulfatase C), encoded by the *STS* gene, which causes accumulation of epidermal cholesterol sulfate and with decreased stratum corneum cholesterol [2]. This is thought to result in disturbed epidermal permeability barrier function through impaired lamellar/nonlamellar phase separation and abnormal epidermal desquamation. Cutaneous manifestations typically present in the first weeks of life with symmetrical adherent brown scales, most notably on the extensor surfaces of the lower limbs and lateral aspects of the trunk with relative sparing of the flexural sites [3]. The *STS* gene is located on the distal short arm of the X-chromosome at the Xp22.3 locus [4]. About 90% of XLI patients have large deletions involving the entire *STS* gene however less frequent point mutations have also been reported [5,6]. Reported extracutaneous manifestations include corneal opacities (10–15%), cryptorchidism and an increased risk of attention deficit hyperactivity disorder and autism [7].

Loss-of-function mutations in the gene encoding filaggrin (*FLG*) have been shown to underlie ichthysosis vulgaris (IV), which is inherited as a semidominant trait where heterozygotes may show a very mild or sub-clinical phenotype compared with homozygotes who present with a moderate to severe ichthyosis [8]. Recently it has been reported that *FLG* mutations can adversely modify the phenotype in X-linked ichthyosis [6]. We report a female affected with X-linked recessive ichthyosis due to homozygosity for the *STS* deletion mutation, who has also inherited a novel *FLG* mutation.

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2. Subjects and methods

2.1. Subjects

The proband presented at age 11 years with symmetrical scaling, that was more pronounced on the lateral aspects of the trunk and extensor surfaces of the limbs, together with large brown, tightly adherent polygonal scales (Fig. 1a). There was flexural sparing and the palms and soles were unaffected (Fig. 1c). These cutaneous signs were evident shortly after birth following a prolonged labour but an otherwise normal pregnancy. She has suffered from mild flexural atopic dermatitis and asthma although there is no family history of atopic disease. Slit lamp ophthalmic examination revealed a faint hazy dot opacity scattered through the posterior stroma of both corneas. The proband is the second child born to consanguineous Indian parents who are first cousins. The father has a similar but less severe cutaneous phenotype (Fig. 1b) and the mother has dry skin with fine scales on her limbs and hyperlinearity of the palms (Supplementary Fig. 1). The elder sister is unaffected. In the extended family there are 7 affected males having a similar phenotype to that of the father (Fig. 2). There are no other known females affected by ichthyosis. Of note, the proband's parents are first cousins (Fig. 2). The proband's ichthyosis has been treated with emollients and topical tazarotene 0.05% gel and the atopic dermatitis with moderately potent topical corticosteroids.

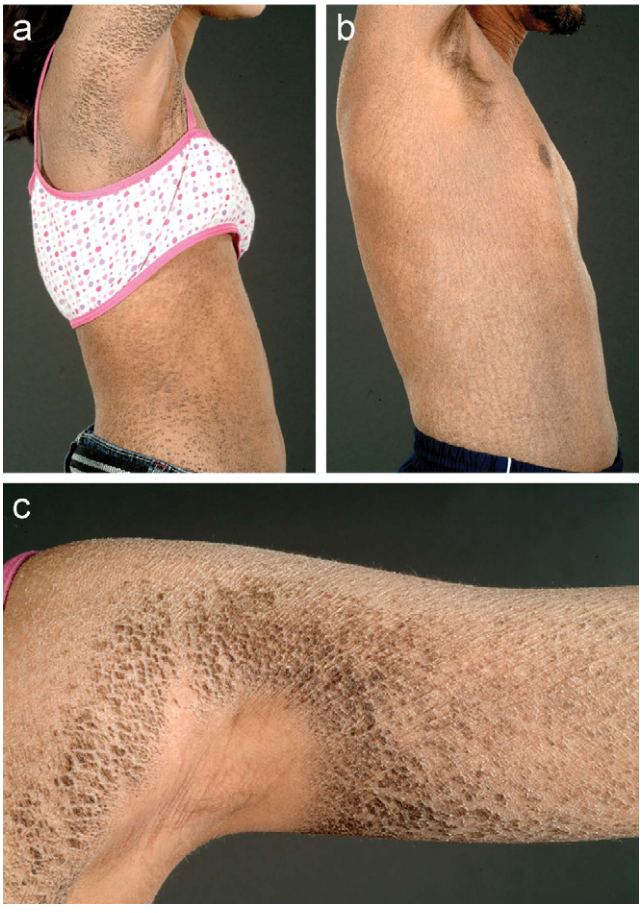

Fig. 1. Clinical appearance of mild and severe XLI in a family of Indian ancestry. (a and c) The proband had a quite sever, widespread and hyperpigmented ichthyosis despite systemic retinoid treatment in contrast to her father (b), who has a much milder presentation. Both father and daughter are hemizygous and homozygous, respectively, for complete deletion of the steroid sulfatase gene. The daughter also carries a heterozygous filaggrin loss-of-function mutation, which was inherited from her mother and not carried by the father. (c) Close-up of the hyperkeratotic scaling in the proband, with flexural sparing.

2.2. Clinical material

Blood samples from family members were obtained with written informed consent in full accordance with the Helsinki Guidelines. Genomic DNA was extracted by standard methods.

2.3. STS deletion analysis

FISH analysis for the *STS* gene was performed using the Vysis LSI Steroid Sulfatase Microdeletion system in combination with the Vysis CEPX control probe for the X-chromosome (Abbott Laboratories Ltd., Maidenhead, UK), according to the maker's recommended methodology.

2.4. Filaggrin sequencing and genotyping

FLG mutation analysis was performed using primers and conditions that have been previously reported [9]. Further specific details are available on request. All PCR products were sequenced using an ABI PRISM 3730 genetic analyzer (Applied Biosystems, Foster City, CA). Mutation 3672del4 was genotyped in 113 ethnically matched control population using fluorescent PCR, using the methods described previously [10], with the following primers: 5′-^{FAM}GTT TCT TGC AAG CAG ACA AAC TCG TAA G-3′ and 5′-CAG ACA CCT CTC GGA GTC G-3′.

3. Results

3.1. Homozygous STS deletion causing XLI in a female

The proband's white blood cell steroid sulfatase activity was 0.9 nmol/mg protein/h (normal range 1.5–4.4 nmol/mg protein/h) consistent with a diagnosis of XLI. The mother had low levels of steroid sulfatase activity (1.5 nmol/mg protein/h) consistent with carrier status. The proband had a normal karyotype, however, fluorescence *in situ* hybridization analysis of cultured lymphocytes detected a homozygous deletion of the X region p22.3 from both X chromosomes (Supplementary Fig. 2). The father was hemizygous for the STS deletion but because the proband had a markedly more severe phenotype, *FLG* mutation analysis was undertaken.

3.2. Unique heterozygous FLG mutation exacerbates the XLI phenotype

Sequencing of the *FLG* gene in the proband identified a novel 4 base-pair deletion mutation, designated 3672del4 in exon 3 of the *FLG* gene (Fig. 3). This frameshift mutation leads to a premature termination codon 1444-bp downstream, consistent with other filaggrin loss-of-function mutations reported in ichthyosis vulgaris [8,9]. This particular mutation is located in filaggrin repeat 3. The proband's mother was found to be heterozygous for the same *FLG* mutation consistent with her phenotype of palmar hyperlinearity and mild ichthyosis vulgaris (Supplementary Fig. 1), whereas her father did not carry the *FLG* mutation (data not shown). A fluorescent PCR genotyping assay was developed for this mutation and 113 ethnically matched population controls (with no phenotypic data linkage) were screened. This mutation was excluded from 113 controls and is therefore a rare variant.

4. Discussion

Males are almost exclusively affected in X-linked recessive disorders and transmission occurs through unaffected female carriers to their sons. Although rare, X-linked recessive disorders in females have been reported and can arise through X-chromosome abnormalities (e.g. Turner's syndrome 45 X0), X-autosomal