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Identification of a novel partial deletion of *STS* associated with pre-Descemet corneal dystrophy and X-linked ichthyosis

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Purpose: Pre-Descemet corneal dystrophy (PDCD) with X-linked ichthyosis (XLI) is associated with mutations in or deletions of the steroid sulfatase gene (*STS*). As only three cases of genetically confirmed PDCD associated with XLI have been reported, we sought to expand our understanding of the genetic basis of PDCD by screening *STS* in two previously unreported families.

Materials and Methods: The affected individuals underwent cutaneous and slit-lamp examinations. Saliva samples collected from each affected individual served as a source of DNA for the amplification of the 10 coding exons of *STS* and flanking DNA markers.

Results: The slit-lamp examination of three affected men (two of whom were brothers) from two families revealed bilateral punctate posterior corneal stromal opacities anterior to the Descemet membrane. Cutaneous examination demonstrated dry, coarse, scaly ichthyotic changes characteristic of XLI in all individuals. Genetic examination of the *STS* locus on the X chromosome in Case 1 revealed a deletion that spanned across DNA markers DXS1130–DXS237, which includes all the coding exons (exons 1–10) of *STS*. Genetic screening of Cases 2 and 3 revealed a partial deletion of the *STS* locus involving exons 1–7 and flanking DNA marker DXS1130 on the X chromosome.

Conclusions: PDCD with XLI may be associated with either partial or complete deletion of *STS*. Despite the identification of point mutations, partial deletion, and complete deletion of *STS* in different affected families reported to date, there was no apparent difference in the affected phenotype between the families, suggesting that the identified variants likely all resulted in loss of function of steroid sulfatase.

With an estimated prevalence of 1:1500–1:6000 male individuals worldwide, X-linked ichthyosis (XLI) is an inherited skin disorder with a typical onset soon after birth that initially presents with large, thin, translucent scales, which are replaced later in life by polygonal, dark lesions restricted to the extremities, trunk, and neck [1–4]. Located on chromosome Xp22.3 and spanning an approximately 135 kb genomic region with 10 exons, the steroid sulfatase (*STS*) gene encodes a membrane-bound enzyme called steroid sulfatase. *STS* mutations are believed to cause XLI by impairing steroid sulfatase activity, ultimately leading to the retention of hyperkeratosis and impaired skin permeability [5,6]. Approximately 90% of reported XLI patients who underwent genetic screening demonstrated deletions of the entire *STS* gene and flanking sequences, while point mutations or partial deletions accounted for the remaining 10% of cases [7]. Larger deletions involving contiguous genes may result in syndromic conditions that involve other tissues and organs, resulting in cryptorchidism, corneal opacification, and neurologic deficits [3]. Nonsyndromic ocular disorders associated with XLI, in addition to corneal opacification, include recurrent corneal epithelial erosions, corneal ulcers, posterior embryotoxon, and deuteranopsia [8].

Pre-Descemet corneal dystrophy (PDCD) is associated with multiple small, gray, deep stromal punctate opacities; in mild cases, opacities can be seen not only immediately anterior to the Descemet membrane but also involving the posterior stroma [9]. Approximately 25% of female carriers of XLI will demonstrate the characteristic pre-Descemet opacities of PDCD, which are thought to be due to the accumulation of cholesterol sulfate anterior to the Descemet membrane and serve to distinguish male individuals with XLI from other types of ichthyosis, as well as to identify female carriers [10].

Similar to the majority of individuals with XLI without corneal manifestations, the majority of individuals with both XLI and PDCD have demonstrated deletions spanning the entire *STS* gene locus [11–13]. While partial deletions of *STS* have been reported nearly two dozen times in association

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with XLI without PDCD, a partial deletion of *STS* has never been reported previously in association with PDCD. Here, we report three individuals from two previously unreported families with PDCD and XLI associated with deletions in the *STS* locus, including the first report of PDCD associated with a partial *STS* deletion.

METHODS

Participant enrollment and clinical evaluation: This study followed the guidelines established by the Declaration of Helsinki for the treatment of all subjects, and all research performed was compliant with HIPAA guidelines. Informed written consent was obtained from all participants and was approved by the Institutional Review Board at the University of California, Los Angeles (UCLA IRB#11–000020). Ophthalmologic evaluation of the affected individuals was performed using slit-lamp biomicroscopy. The diagnosis of PDCD was based on the presence of punctate opacities in the posterior corneal stroma immediately anterior to the Descemet membrane in both eyes.

DNA collection and genetic evaluation by PCR: After informed consent was obtained from all participating individuals, saliva samples were collected using an Oragene Saliva Collection Kit (DNA Genotek, Inc., Ottawa, CA). Genomic DNA was then isolated using the Oragene PrepIT-L2P Kit (DNA Genotek, Inc.). Previously published primers were used to amplify exons 1–10 of *STS* (NG_021472.2) and DNA markers that flank *STS* [14,15]. Each PCR was performed in a 25-µl reaction mixture consisting of 50 ng of genomic DNA template, 12.5 µL of GoTaq® DNA Polymerase (Promega, Madison, WI), 10 µM forward and reverse primer (Integrated DNA Technologies, Coralville, IA), and 10.3 µl of nuclease free water. Amplification reactions were performed under the following conditions: denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for *STS* exons 1–10 and 58 °C for *STS* flanking markers for 30 s, elongation at 72 °C for 35 s, and a final extension step at 72 °C for 5 min. The PCR-amplified sequences used in this study are arranged on the X chromosome as follows: telomere-DXS89-DXS996-DXS1139-DXS1130-*STS* exons 1–10-DXS1131+-DXS1133-DXS237-DXS1132-DXF22S1-DXS278-DXS1134-centromere. All PCR-amplified products were separated on a 1.25% (wt/vol) agarose gel with ethidium bromide and imaged using the INGenius3 system (Syngene, Frederick, MD).

RESULTS

Case 1: A 43-year-old man who presented to the Hoopes Vision Research Center for a refractive surgery consultation had an uncorrected visual acuity of 20/200 in the right eye and 20/250 in the left eye, with a corrected distance visual acuity (CDVA) of 20/20 in each eye after correcting for myopic astigmatism. Slit-lamp biomicroscopy was significant only for numerous tiny, gray-white opacities in the posterior corneal stroma anterior to the Descemet membrane, primarily involving the central and midperipheral cornea bilaterally (Figure 1A). The corneal endothelium was normal in both eyes, and the central corneal thickness measured 475 µm OD and 487 µm OS. The remainder of the ocular examination was unremarkable. The patient was noted to have dry, coarse skin that was characteristic of ichthyosis, with excessive skin flaking predominantly over the forearms and lower extremities (Figure 2A). The patient reported having excessive flaking and scaling since early childhood, which he self-treated with topical moisturizers, but he had not seen a dermatologist and had not been diagnosed with ichthyosis. He had no immediate family history of XLI or other dermatologic disorders.

Case 2: A 34-year-old man presented to the Malayan Ophthalmologic Center in Yerevan, Armenia, for ophthalmic evaluation. His uncorrected visual acuities measured 20/20 in each eye. Slit-lamp examination demonstrated diffuse, punctate, gray posterior stromal corneal opacities diffusely distributed across the cornea in each eye (Figure 1B). The remaining ocular examination was unremarkable. External examination revealed excessive flaking, scaling, and thickening of the skin over his hands, bilateral lower extremities, and lower back (Figure 2B). The patient was first noted to have abnormal skin findings at age 5 and was subsequently diagnosed with XLI at age 18. His family history was significant for his maternal grandfather and brother having XLI.

Case 3: A 33-year-old man, the brother of the Case 2 patient, also presented to the Malayan Ophthalmologic Center for ophthalmic evaluation. The patient's uncorrected visual acuities measured 20/100 OD and 20/25 OS, improving to 20/20 OU with refraction. Slit-lamp biomicroscopy demonstrated numerous tiny gray-white opacities in the posterior stroma bilaterally, with an otherwise unremarkable ocular exam (Figure 1C). Cutaneous examination revealed a dry, scaly appearance of the skin consistent with XLI, which had been diagnosed at age 18 (Figure 2C).

Genetic evaluation of the STS locus: PCR amplification of *STS* exons 1–10 in Case 1 failed to produce any amplicons, while each of the 10 exons was amplified in two unrelated, healthy controls with the same primers and PCR conditions

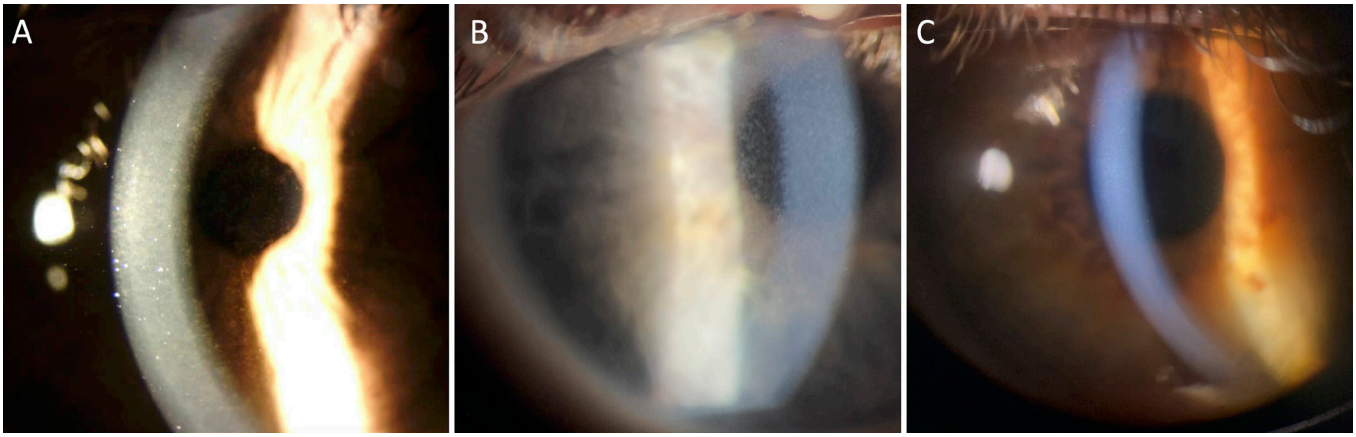

Figure 1. Slit-lamp photomicrographs of pre-Descemet corneal dystrophy. Slit-lamp examination of Case 1 (**A**, right eye), Case 2 (**B**, right eye), and Case 3 (**C**, left eye) demonstrating diffuse, punctate, white-gray opacities in the posterior stroma anterior to the Descemet membrane.

(Figure 3A), indicating the deletion of all *STS* exons in Case 1. To identify the extent of the X chromosome deletion in Case 1, PCR amplification of flanking DNA markers was performed, which demonstrated that the boundaries of the ~1.2 Mb deletion were DNA markers DXS1139 and DXS1132 (Figure 3B). Genetic screening of the *STS* locus in Cases 2 and 3 both failed to produce amplicons for *STS* exons 1–7, while producing amplicons for exons 8–10, indicating a partial deletion of *STS* (Figure 3A). PCR amplification of flanking markers demonstrated that the boundaries of the ~0.7 Mb deletion in both Cases 2 and 3 were DNA markers DXS1139 and DXS1131+ (Figure 3B).

DISCUSSION

PDCD is characterized by punctate corneal opacities anterior to the Descemet membrane, with histopathological studies being rare due to the opacities not affecting visual acuity. The corneal stromal deposits in PDCD usually arise in early adulthood, and previous studies have not shown a correlation between the number of corneal deposits and age

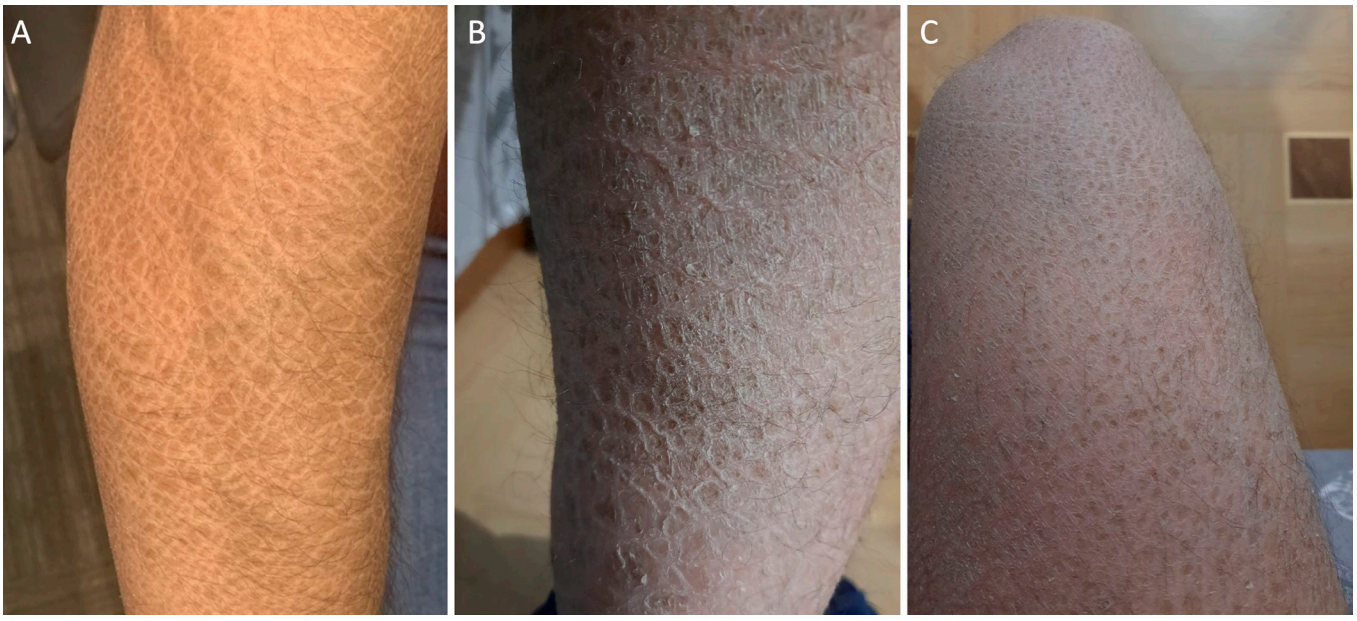

Figure 2. Dermatologic findings of X-linked ichthyosis. External examination of Case 1 (**A**, forearm), Case 2 (**B**, leg), and Case 3 (**C**, thigh) with characteristic ichthyotic changes, including thickened, dry, coarse scaling skin.

[12,16]. Histopathologic studies of the corneal button from an individual with PDCD with associated XLI showed electron dense, polymorphic material in the pre-Descemetic, posterior corneal stroma, suggestive of cholesterol sulfate deposits [17]. *In vivo* confocal microscopic studies of an individual with genetically confirmed PDCD associated with XLI demonstrated an abundance of hyperreflective aggregates in the cell bodies of posterior stromal keratocytes, likely representative of focal accumulations of cholesterol sulfate, while nongenetically confirmed cases of PDCD similarly showed both intracellular and extracellular hyperreflective particles in mid and deep stromal keratocytes [13,18–20].

In the majority of individuals with XLI with or without PDCD, the genetic basis is a complete deletion of *STS*, likely as a result of recombination due to homologous sequences and low copy repeats flanking *STS* [15,21]. To our knowledge, this is the first report of PDCD associated with XLI associated with a partial deletion of *STS*. The corneal and cutaneous features in the individuals with a partial *STS* deletion were indistinguishable from those of the individual with the complete *STS* deletion and from previously reported individuals with XLI and PDCD [11–13], with development in early childhood in all cases. Previous studies examining individuals with isolated XLI secondary to *STS* mutations and partial and complete *STS* deletions have also failed to show a genotype–phenotype correlation [7,22–24]. Therefore, the partial deletion of *STS* that we report in two individuals with PDCD likely results in a complete loss of expression of *steroid sulfatase*, similar to the complete deletion of *STS*.

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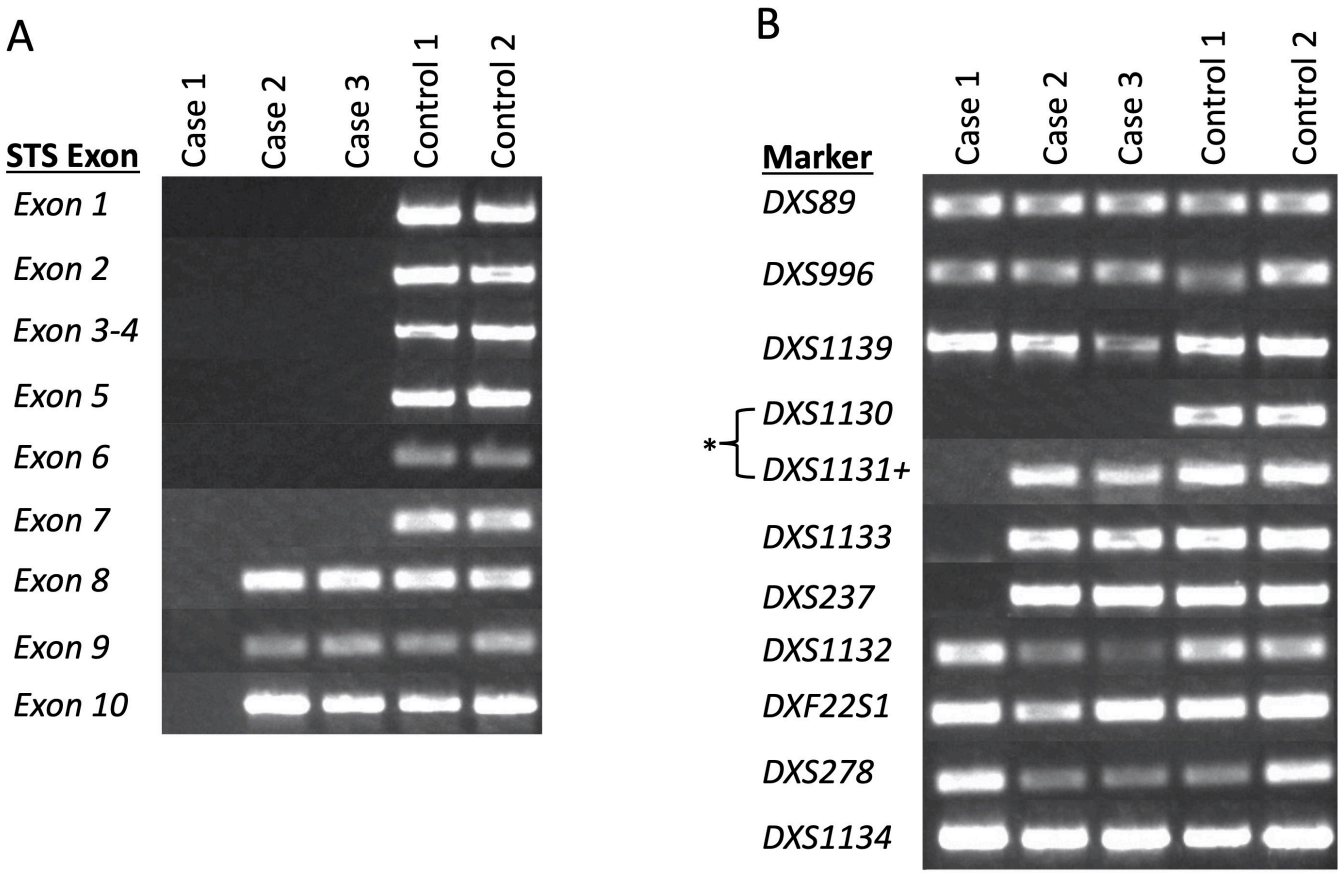

Figure 3. PCR analysis of the *STS* gene and flanking DNA markers. **A**: PCR analysis of *STS* exons 1–10 in Cases 1–3 and two unrelated healthy controls. **B**: PCR analysis of flanking DNA markers DXS89–DXS1134 in Cases 1–3 and two unrelated healthy controls. The asterisk (*) indicates the two DNA markers, DXS1130 and DXS1131+, located on either side of and closest to the *STS* gene.

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