Ectodermal dysplasia-skin fragility syndrome: Two new cases and review of this desmosomal genodermatosis

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Abstract

Background: Desmosomes are intercellular cadherin-mediated adhesion complexes that anchor intermediate filaments to the cell membrane and are required for strong adhesion for tissues under mechanical stress. One specific component of desmosomes is plakophilin 1 (PKP1), which is mainly expressed in the spinous layer of the epidermis. Loss-of-function autosomal recessive mutations in PKP1 result in ectodermal dysplasia-skin fragility (EDSF) syndrome, the initial inherited Mendelian disorder of desmosomes first reported in 1997.

Methods: To investigate two new cases of EDSF syndrome and to perform a literature review of pathogenic PKP1 mutations from 1997 to 2019.

Results: Sanger sequencing of PKP1 identified two new homozygous frameshift mutations: c.409_410insAC (p.Thr137Thrfs*61) and c.1213delA (p.Arg411Glufs*22). Comprehensive analyses were performed for the 18 cases with confirmed bi-allelic PKP1 gene mutations, but not for one mosaic case or 6 additional cases that lacked gene mutation studies. All pathogenic germline mutations were loss-of-function (splice site, frameshift, nonsense) with mutations in the intron 1 consensus acceptor splice site (c.203-1>A or G>T) representing recurrent findings. Skin fragility and nail involvement were present in all affected individuals (18/18), with most cases showing palmoplantar keratoderma (16/18), alopecia/hypotrichosis (16/18) and perioral fissuring/cheilitis (12/15; not commented on in 3 cases). Further observations in some individuals included pruritus, failure to thrive with low height/weight centiles, follicular hyperkeratosis, hypohidrosis, walking difficulties, dysplastic dentition and recurrent chest infections.

Conclusion: These data expand the molecular basis of EDSF syndrome and help define the spectrum of both the prototypic and variable manifestations of this desmosomal genodermatosis.

Keywords

desmosome, ectodermal dysplasia-skin fragility syndrome, Plakophilin 1
1 | INTRODUCTION

Desmosomes are specialised cadherin-mediated adhesion complexes that anchor intermediate filaments to the cell membrane, providing stability and rigidity to tissues under mechanical stress.\[^{1,2}\] Attachment of desmosomal cadherins (desmogleins and desmocollins) to intermediate filaments is facilitated by proteins from the armadillo (plakoglobin, plakophilin 1 and plakophilin 2) and plakin (desmoplakin, periplakin and envoplakin) superfamilies.\[^{1}\] Understanding the exact role of each component within these complexes has been difficult due to their intricate interactions and the heterogeneity of their molecular structure. The discovery of monogenic disorders involving dysfunction or absence of desmosomal components, however, has provided great insight into both the underlying loss of cell cohesiveness and importantly, the clinical consequences to skin and other tissues.\[^{3}\]

Plakophilin 1 (PKP1) is an armadillo protein that is found in desmosomes of stratified epithelia and localises primarily to the spinous layer of the epidermis.\[^{4,5}\] PKP1 has been shown to be a multifunctional protein that is inherently involved in the Wnt/β-catenin signalling cascade and promotes desmosomal cadherin stability and clustering.\[^{5,6}\] It enhances the recruitment of desmosomal proteins to the plasma membrane in cultured keratinocytes through direct and indirect interaction with plakoglobin and desmoplakin.\[^{4,5}\] Studies have shown that PKP1 interferes with plakoglobin binding to desmoplakin and promotes desmosomal clustering when bound to plakoglobin.\[^{4,5}\]

PKP1 also has a role in increasing cell survival following cell damage\[^{5,6}\] and in regulating keratinocyte proliferation and migration.\[^{1,7}\] There are two isoforms, PKP1a and PKP1b, which exist due to alternative splicing of the PKP1 gene. PKP1a is expressed in both desmosomes and nuclei, whilst PKP1b is exclusively nuclear.\[^{4,8}\] Both the desmosomal and nuclear isoforms are degraded by caspases during keratinocytic apoptosis, suggesting that PKP1 may be involved in remodelling of the cytoskeleton and cell integrity.\[^{9}\]

The clinical importance of PKP1 is demonstrated by the human disorder caused by autosomal recessive homozygous null and splice site mutations in the PKP1 gene. In 1997, McGrath et al, first reported that mutations in PKP1 result in the loss of connection between epidermal cells with skin fragility and blistering, and congenital ectodermal dysplasia (MIM #604536).\[^{10}\] The clinical features of the affected individual were termed “ectodermal dysplasia-skin fragility (EDSF) syndrome,” reflecting the combination of skin fragility, hair and nail pathologies that were present. Representing the first human inherited disorder of desmosomes, EDSF syndrome now is classified as a specific form of skin fragility disorder within the spectrum of epidermolysis bullosa.\[^{11}\]

Following the initial report of EDSF syndrome, other cases involving pathogenic mutations in the PKP1 gene have subsequently been described. Nevertheless, clinical diversity in EDSF syndrome has been noted prompting a need to review the clinical manifestations of the disorder. This article therefore reviews genotype-phenotype correlation for EDSF syndrome and PKP1 mutations.

2 | METHODS

Two unrelated cases of possible EDSF syndrome were investigated using Sanger sequencing of genomic DNA for PKP1 mutations and immunostaining of non-lesional skin, as described previously.\[^{12}\]

A comprehensive review of the PubMed database was undertaken for reports in English from October 1997 to November 2019. We used the (a) MeSH terms: ‘plakophilins/genetics’ AND ‘desmosomes/pathology’ OR ‘ectodermal dysplasia/genetics’, ‘mutation’ and (b) keywords “plakophilin 1”, “PKP1”, “ectodermal dysplasia-skin fragility syndrome”. Titles and abstracts were reviewed, and both references and citations of relevant studies were also examined for additional cases. The available data from each publication were investigated to identify patients with a genetic diagnosis involving a mutation in PKP1. Cases without a genetic diagnosis were excluded. For each case, demographics, mutation and protein analysis, and phenotypic features were recorded. For new, original cases that underwent genetic analysis by our laboratory, informed consent was obtained and in accordance with the Declaration of Helsinki principles, we performed Sanger sequencing on all coding exons and flanking intronic regions of PKP1 (GenBank NM_001005337.3) using genomic DNA extracted from peripheral blood.

3 | RESULTS

3.1 | Case 1

A 1-year-old Egyptian boy, born to consanguineous parents presented with a history of trauma-induced skin fragility since birth. On examination, he had hypotrichosis with scalp erosions, and erosions and crusts over the face and limbs (Figure 1A). He also displayed finger and toenail subungal hyperkeratosis with dystrophy and mild palmoplantar keratoderma (PPK). There was no perioral fissuring, hypohidrosis or mucous membrane involvement. There was no family history of skin fragility (Figure 1B). Histopathology and immunofluorescence microscopy showed widened intercellular spaces, hyperkeratosis and acantholysis, and staining for PKP1 showed complete absence compared to control skin (see Figure S1). Sanger sequencing revealed a homozygous insertion in exon 3 of the PKP1 gene (c.409_410insAC; p.Thr137Thrfs*61) in the proband, whereas both parents were found to be heterozygous carriers of the mutation (Figure 1C). The c.409_410insAC mutation maps upstream of the arm repeat domains of PKP1 (head domain) and therefore is predicted to result in severely truncated polypeptides lacking these arm-repeats.

3.2 | Case 2

An 11-month-old Egyptian boy, born to non-consanguineous parents presented similarly to case 1, with trauma-induced erosions and perioral bullae developing from 5 days of age and spreading to the upper and lower limbs (Figure 2A). He was noted to have...
FIGURE 1  Case 1 - A 1-year-old Egyptian male with a homozygous c.409_410insAC mutation in PKP1. A, Clinical phenotype including hypotrichosis with scalp erosions, moderate palmoplantar keratoderma of the hands and feet, with crusted lesions on both lower limbs. Hyperkeratotic and dystrophic finger/toenails are seen bilaterally. B, Pedigree structure and co-segregation analysis of the PKP1 c.409_410insAC mutation. C, Sanger sequencing chromatograms showing wild-type control DNA, heterozygous carrier status for the proband’s mother and the patient being homozygous for the c.409_410insAC mutation.
hypotrichosis with subungual hyperkeratosis and nail dystrophy. He had normal dentition, without hearing abnormalities or hypohidrosis. There was no family history of skin fragility (Figure 2B). Histopathology showed widened intercellular spaces, hyperkeratosis and acantholysis. Immunostaining for PKP1 showed almost complete absence compared to control skin (see Figure S1). Sanger sequencing revealed a homozygous deletion in exon 6 of the PKP1 gene (c.1213delA, p.Arg411Glufs*22) in the proband, whereas his unaffected brother and mother were found to be heterozygous.
TABLE 1  Summary of demographics and genetics of patients with bi-allelic germline PKP1 mutations

| Case | Age (yrs) | Sex (M/F) | Nationality | Parental consanguinity | Family history | cDNA mutation | Amino acid or RNA change | Amino acid change | Inheritance | Effect on PKP1 mRNA | Zygosity | Outcome | PKP1 protein expression | Reference |
|------|-----------|-----------|-------------|------------------------|----------------|---------------|------------------------|------------------|-------------|----------------------|----------|---------|------------------------|-----------|
| 1    | 6         | M         | British (Caucasian) | N | N | c.910C>T | p.Gln304* | p.Gln304* N/A | Paternal | Maternal | Nonsense | Frameshift | Compound heterozygote | PTC | Absent | McGrath et al., 1997[10] |
| 2    | 1.5       | M         | British (Caucasian) | N | N | c.203-1G>A | p.Tyr71* | N/A p.Tyr71* | Paternal | Maternal | Splice site | Nonsense | Compound heterozygote | PTC | Absent | McGrath et al., 1999[12] |
| 3    | 17        | M         | British (Caucasian) | N | N | c.1233-2A>T | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Whittock et al., 2000[13] |
| 4    | 42        | M         | Japanese | Y | N | c.2021+1G>A | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Reduced | Hamada et al., 2002[14] |
| 5    | 0.5       | F         | Arab       | Y | N | c.847-2A>G | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Sprecher et al., 2004[15] |
| 6    | 3         | F         | Arab       | Y | Y | c.203-1G>A | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Sprecher et al., 2004[15] |
| 7    | 33        | M         | Dutch      | N/A | N/A | c.1680+1G>A | Splice | N/A | N/A | Splice site | Homozygote | Reduced translated protein | Reduced | Steijien et al., 2004[16] |
| 8    | 3         | F         | Chinese    | N | N | c.1835-2A>G | Splice | N/A N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Zheng et al., 2005[17] |
| 9    | 6         | M         | Turkish | Y | Y | c.888delC | p.Arg297Alafs*42 | Paternal | Maternal | Frameshift | Homozygote | PTC | N/A | Ersoy-Evans et al., 2006[18] |
| 10   | 10        | M         | Brazilian | Y | Y | c.2014C>T | p.Arg672* | p.Arg672* | Paternal | Maternal | Nonsense | Homozygote | Reduced translated protein | Marked reduction | Tanaka et al., 2009[19] |
| 11   | 1.2       | F         | Iraqi      | Y | N | c.897del5 | p.Asn300Glufs*60 | Paternal | Maternal | Frameshift | Homozygote | Reduced translated protein | Marked reduction | Boyce et al., 2012[20] |
| 12   | 15        | M         | Spanish | N/A | N/A | c.1233-2A>G | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Hernandez-Martin et al., 2013[21] |
| 13   | 5         | F         | Egyptian | Y | N | c.203-1G>T | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | N/A | Abdalla & Has, 2014[22] |
| 14   | 4         | F         | Egyptian | Y | N | c.203-1G>T | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | N/A | Abdalla & Has, 2014[22] |
| 15   | 27        | F         | Turkish | Y | N | c.1411-9G>A | Splice | N/A | Paternal | Maternal | Splice site | Homozygote | PTC | Absent | Hsu et al., 2016[23] |
| 16   | 29        | M         | Turkish | Y | Y | c.1414_1415delTG | p.Val472Glyfs*28 | p.Val472Glyfs*28 | Paternal | Maternal | Frameshift | Homozygote | PTC | N/A | Alatas et al., 2017[24] |

(Continues)
carriers for that mutation (Figure 2C). The c.1213delA mutation maps on the armadillo arm domain 4.

Both PKP1 mutations result in frameshift and premature protein termination. Neither variant identified has been previously reported in the Human Gene Mutation Database, gnomAD (v3.0) or the 1000 Genomes Database (internationalgenome.org).

3.3 | Literature review

A total of 388 articles were identified, comprising mainly single case reports (Figure S2). Six cases with clinical features of EDSF syndrome were excluded due to lack of genetic diagnosis (Table S1). We identified 16 individual cases of EDSF syndrome caused by bi-allelic PKP1 germline mutations and 1 case caused by postzygotic mosaicism; we also include two further cases that are reported in this article (Tables 1 and 2). Consanguinity was present in 68.8% of the cases (11/16; not reported in 2 cases). None of the heterozygous carrier parents reported any skin or ectodermal abnormalities.

There were 21 pathogenic mutations identified comprising 3 nonsense, 6 frameshift, and 12 splice-site mutations (Figure 3). The 12 mutations affecting splicing were predominantly intronic, including 3 located at the +1 donor, 4 at the −1 acceptor, 4 at the −2 acceptor and 1 at the −9 acceptor positions. There was 1 case that involved post-zygotic mosaicism. In 15/18 cases, mutations were homozygous. Of the cases that reported PKP1 protein expression, all had either reduced skin labelling or a complete absence of immunostaining.

All germline mutation cases reported skin fragility (18/18) and nail involvement (18/18) in the clinical phenotype, with almost all cases reporting palmoplantar keratoderma (PPK) (16/18) and alopecia universalis. The phenotype exhibited perioral fissuring, marked PPK, with thickened nail plates and reduced growth centiles. Examination of other systems revealed hypohidrosis and unilateral astigmatism, with normal dentition, hearing and respiratory, gastrointestinal and genitourinary systems. Subsequent cases confirmed the core

| Case | Demographics | Mutation & protein analysis |
|------|--------------|-----------------------------|
| 17   | 1 M Egyptian | Y c.409_410insAC p.Thr137Thrfs*61 Paternal frameshift PTC Homozygote N/A This study - case 1 |
| 18   | 0.9 M Egyptian | N c.1213delA p.Arg411Glufs*22 Paternal frameshift PTC Homozygote N/A This study - case 2 |

Note: An additional case involving a frameshift mutation (c.638delT, p.Val213Glyfs*33) along with a post-zygotic somatic loss of PKP1 was also noted. This case presented with a reduced PKP1 protein expression only on lesional skin. Clinically, the presentation was mild with unilateral superficial erosions, hypogonadism, localized plaques and segmental hyperhidrosis. Swelling, teeth and scalp hair were normal. (Vázquez-Osorio et al., 2017).

Abbreviations: F, Female; M, Male; N, No; N/A, Not available; PKP1, Plakophilin 1; PTC, Premature termination codon; Y, Yes.

TABLE 1 (Continued)
### Table 2: Summary of clinical phenotype of patients with bi-allelic germline PKP1 mutations

| Case | Skin fragility | Palmo-plantar keratoderma | Alopecia/Hypotrichosis | Nail involvement | Hypo-hidrosis | Perioral fissuring/Chelitis | Mucous membrane involvement | Cardiac abnormalities | Additional observations                                                                                   | Reference                  |
|------|----------------|--------------------------|------------------------|------------------|---------------|-----------------------------|-----------------------------|--------------------------|---------------------------------------------------------------------------------|-----------------------------|
| 1    | Y              | Y                        | Y                      | Y                | Y             | Y                           | N                           | N                       | Small in stature. Astigmatism. No dental or hearing abnormalities. No respiratory, gastro-intestinal, cardiac or genito-urinary symptoms. Walking difficulties. | McGrath et al., 1997[10]    |
| 2    | Y              | Y                        | Y                      | Y                | Y             | Y                           | N                           | N                       | Poor weight gain. No dental or hearing abnormalities. No respiratory, gastro-intestinal or genito-urinary symptoms. | McGrath et al., 1999[12]    |
| 3    | Y              | Y                        | Y                      | Y                | N             | N/A                         | N                           | N                       | No dental, respiratory, gastrointestinal, genito-urinary or ocular symptoms. Scalp hair regrowth. Mild pruritus. Walking difficulties. | Whittock et al., 2000[13] |
| 4    | (late-onset)   | (late-onset) Y           | Y                      | Y                | Y             | N                           | N                           | N                       | Milder phenotype. Trauma-induced blisters from age 20. Extensive dental caries. | Hamada et al., 2002[14]     |
| 5    | Y              | N                        | Y                      | Y                | N             | N                           | Patent foramen ovale         | Pruritus                 |                                                                                   | Sprecher et al., 2004[15]   |
| 6    | Y              | Y                        | Y                      | Y                | N             | N/A                         | N                           | Right aortic arch         | Recurrent pneumonia, sepsis and marked failure to thrive. Pruritus.                        | Sprecher et al., 2004[15]   |
| 7    | Y              | Y                        | N                      | Y                | N             | N/A                         | N                           | N                       | Recurrent cutaneous infections. Stellate opacities in both eyes. Normal dentition. Follicular hyperkeratosis. Walking difficulties. | Steijten et al., 2004[16]   |
| 8    | Y              | Y                        | Y                      | Y                | N             | N                           | N                           | N                       | No developmental delay. No evidence of dental, respiratory or gastro-intestinal symptoms. Perianal erythema and erosions. | Zheng et al., 2005[17]      |
| 9    | Y              | Y                        | Y                      | Y                | Y             | Y - Loss of tongue papillae | N/A                         | N                       | No dental abnormalities. Low height and weight centiles. Chronic diarrhoea and pruritus. | Ersoy-Evans et al., 2006[18] |
| 10   | Y              | Y                        | Y                      | Y                | N             | N                           | N                           | N                       | Low height and weight centiles. Prominent perioral fissuring and chelitis.              | Tanaka et al., 2009[19]     |
| 11   | Y              | Y                        | Y                      | Y                | N             | N                           | N                           | N                       | Failure to thrive, small in stature. No developmental delay. Anal fissures with constipation. | Boyce et al., 2012[20]      |
| 12   | Y              | Y                        | Y                      | Y                | N             | N                           | N                           | N                       | Moderate pruritus. Wide scarring on cheeks. Maceration and deep fissuring in the perineal/inguinal folds. No developmental delay. Failure to thrive in infancy. | Hernandez-Martín et al., 2013[23] |
| Case | Phenotypic features | Additional observations | Reference |
|------|--------------------|------------------------|-----------|
| 13   | Y                  | Dysplastic dentition age 2, difficulty walking, recurrent chest infections, weight 3rd centile. No hearing abnormalities or gastrointestinal, genitourinary symptoms. | Abdalla & Has, 2014 [22] |
| 14   | Y                  | Dysplastic dentition age 2, difficulty walking, recurrent chest infections, weight 3rd centile. No hearing abnormalities or gastrointestinal, genitourinary symptoms. | Abdalla & Has, 2014 [22] |
| 15   | Y                  | Normal dentition. | Hsu et al., 2016 [23] |
| 16   | Y                  | Flat nasal bridge, occasional pruritus and skin infections. Normal dentition and hearing. No failure to thrive/developmental delay. No respiratory, gastrointestinal or genitourinary symptoms. | Alatas et al., 2017 [24] |
| 17   | Y                  | Normal dentition and hearing. No respiratory or gastrointestinal issues. Urethral stricture. Pruritus when hot. No recurrent upper respiratory tract infections. No developmental delay. | This study - case 1 |
| 18   | Y                  | Normal dentition and hearing. No respiratory, gastrointestinal or genitourinary issues. Pruritus on application of topical agents. No recurrent infections. Reduced weight and height centiles. | This study - case 2 |

Note: An additional case involving a frameshift mutation (c.638delT, p.Val213Glyfs*33) along with a post-zygotic somatic loss of PKP1 was also noted. This case presented with a reduced PKP1 protein expression only on lesional skin. Clinically, the presentation was mild with unilateral superficial erosions, hypopigmented plaques and segmental hyperkeratosis. Sweating, teeth and scalp hair were normal. (Vázquez-Osorio et al., 2017 [27]).

Abbreviations: N, No; N/A, Not available; PKP1, Plakophilin 1; Y, Yes.
features of skin fragility, nail dystrophy, alopecia/hypotrichosis, perioral fissuring and PPK. These features are consistent with the known cutaneous pathology of PKP1 deficiency due to a reduction in desmosomal stability.\cite{1,4,26} The EDSF syndrome extended phenotype now includes perianal and perineal erosions/fissuring and pruritus. Unlike the original report, no further cases of ocular abnormalities were observed, providing evidence that this original case presented an unrelated congenital astigmatism. Additionally, no ocular defects were noted in Pkp1 knockout mice, confirming a lack of ocular involvement with PKP1 mutations.\cite{27} Extracutaneous features such as failure to thrive/low height or weight centiles were a shared feature in some patients. A similar outcome was noted in Pkp1 knockout mice who were born at 25% of the Mendelian ratio with reduced subcutaneous adipose tissue.\cite{26} PKP1 deficient dogs were also noted to grow to only one-third the size and weight of others in the same litter.\cite{26,28} The exact mechanism is unknown, but it is hypothesised that this is due to alterations during in utero adipogenic signalling from the epidermis, reducing Wnt/β-catenin signalling, which is needed for initiation of proadipogenic pathways including insulin/growth factor signalling.\cite{24}

Dental abnormalities were only observed in 3/18 cases, but all three cases showed significant dysplastic dentition. It has been shown that PKP1 is highly expressed in the process of tooth development and is required for dynamic Wnt/β-catenin signalling.\cite{27} In mouse models, it was noted that PKP1 translocates and localises to the cell nucleus and contributes to inner dental epithelial cell differentiation, forming ameloblasts that secrete enamel matrix proteins for tooth development.\cite{27} Thus, even though the reported prevalence in this cohort is small, the risk of severe dental abnormalities should be considered significant. Although a patent foramen ovale and a right aortic arch were observed in our review, no major cardiovascular abnormality was identified, probably reflecting the tissue-specific pattern of expression of PKP1, which is not expressed in the heart or vasculature.\cite{29}

Hypohidrosis was noted in one-third of cases, although the link between loss of PKP1 expression and reduced sweating may be an indirect one. It has been shown that the Wnt/β-catenin–Eda–NF-kB–Shh cascade functions in a genetic relay for eccrine gland development.\cite{30} Sweat gland induction was noted to completely fail when canonical WNT signalling was blocked in skin epithelium.\cite{31} Thus, complete knockout of PKP1 protein expression may result in hypohidrosis, whilst a more mild form that produces a reduced PKP1 protein may result in partial function, allowing sweat production.

### 4.2 Histopathological findings

Histologically, the lesional skin of patients with EDSF syndrome is characterised by widening of the epidermal intercellular spaces in the suprabasal cell layer and detachment of keratinocytes in the mid and upper spinous-cell layer.\cite{10,13,32} These hallmark features usually result in intraepidermal clefts and blisters with a few acantholytic keratinocytes in the mid-epidermis or in a complete detachment of the epidermis above the upper spinous layers.\cite{33} Dyskeratotic keratinocytes were also noted particularly in the plantar keratoderma of patients.\cite{33} Electron microscopy has demonstrated loss of keratinocyte-keratinocyte adhesion, and desmosomes that are small, poorly formed and reduced in number.\cite{1,32} Lack of connections to the desmosomes is apparent due to disruption of keratin...
intermediate filament attachment to the intracellular desmosomal inner plaques.

Findings from hypotrichotic scalp skin demonstrated a mild decrease in the total number of hair follicles, without an increase in the number of vellus hair. It was also noted that there were no histological signs of inflammatory or scarring alopecia, but there was an increase in the number of catagen-telogen hair follicles as observed in chronic telogen effluvium.

4.3 Mutational analysis

It is noteworthy that 57.1% of PKP1 mutations affect splicing. In general, only about 10%-15% of genetic diseases described in humans are caused by splice-site mutations at exon-intron junctions, with most splice-site mutations occurring at the +1 or +2 positions for donor sites or the −1 or −2 positions for acceptor sites. Almost all splice-site mutations resulted in a premature termination codon and had similar clinical features to cases resulting from nonsense mutation. One exception was case 7, in which there was a partially functional protein expression due to a non-consensus cryptic splice-site mutation generating in-frame transcripts. This case presented with a relatively mild phenotype with plantar hyperkeratosis and nail involvement, but instead of alopecia or hypotrichosis, scalp hair was dark, thick and curly. Another case of interest (Tables 1 and 2—footnote) involved both a frameshift deletion mutation and a postzygotic somatic mutation, yet only presented with mild unilateral superficial erosions in a mosaic pattern, PPK and nail dystrophy. Immunofluorescence analysis in this case showed negative staining for anti-PKP1 antibodies in a mosaic pattern, but reduced immunoreactivity in surrounding, unaffected skin compared to control skin. The molecular pathology involved a heterozygous germline mutation that appeared to be homozygous in affected skin, possibly due to a postzygotic somatic mutation, yet only presented with mild unilateral superficial erosions in a mosaic pattern, PPK and nail dystrophy.

In summary, we have reviewed and expanded on the current literature of PKP1 mutations causing EDSF syndrome. We expand specifically on the extracutaneous effects of PKP1 mutation including, hypohidrosis, pruritus, perianal erosions and dysplastic dentition.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests. All authors contributed significantly, and all authors are in agreement with the contents of the manuscript.

AUTHOR CONTRIBUTION

BJD, JEM, AO and JAM contributed to the concept and design of the study, the analysis of the literature review, interpretation of the data and provided medical expertise for revision of the manuscript. NGS, MMF and NND performed clinical assessment of the two additional cases to the literature and revision of the manuscript. LL performed the genetic analysis of additional cases referred to the laboratory. All authors have read and approved the final manuscript.

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Additional supporting information may be found online in the Supporting Information section.

**Figure S1.** Ectodermal dysplasia-skin fragility syndrome skin pathology in case 1 and 2. (A, B) Histopathology from lesional skin biopsies of case 1 and case 2 patients, showing hyperkeratosis, widened intercellular spaces with acantholysis. Suprabasal intraepidermal clefts with detachment of the upper epidermal layers are observed (hematoxylin and eosin stain, x20 magnification). (C – E) Immunofluorescence analysis, (stained with FITC and DAPI) using antibodies against plakophilin 1 (Anti-PKP1-Ab, PP15C2). In normal control skin, there is PKP1 immunoreactivity at the keratinocyte cell peripheries throughout the epidermis. Both case 1 and 2 show almost complete absence of anti-PKP1 antibodies. (F – H) Immunofluorescence analysis, (stained with FITC and DAPI) using antibodies against desmoplakin (Anti-DSP-Ab, DP217). Control skin, case 1 and case 2 all show panepidermal desmoplakin immunoreactivity. (I – K) Immunofluorescence analysis, (stained with FITC and DAPI) using antibodies against plakoglobin (Anti-plakoglobin, 15F11). Control skin, case 1 and case 2 all show panepidermal plakoglobin immunoreactivity. Bar = 100 µm.

**Figure S2.** Flowchart demonstrating the literature search undertaken for the review of cases involving PKP1 gene mutations.

**Table S1.** Outline of additional cases involving clinical features of ectodermal dysplasia-skin fragility syndrome but lacking a genetic diagnosis.

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