inhibit nonspecific binding. Sections were stained with antibodies with the following specificities—pAkt^{Ser473}, total Akt, pmTOR^{Ser2448}, and total mTOR. Stained tissues were incubated with a secondary antibody followed by ABC reagents and DAB (Vector Lab, Burlingame, CA). Toludine blue was used to counterstain.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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A Deep-Intronic *FERMT1* Mutation Causes Kindler Syndrome: An Explanation for Genetically Unsolved Cases

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TO THE EDITOR

Kindler syndrome (KS) is a distinct type of epidermolysis bullosa (EB) defined by variable levels of skin cleavage and a progressive phenotype comprising skin blistering, photosensitivity, poikiloderma, mucocutaneous scarring, and malignancies (Has *et al.*, 2011). KS is caused by mutations in *FERMT1*, the gene encoding kindlin-1 (Jobard *et al.*, 2003). The particular features of KS may rely on the functions of kindlin-1, which is a member of the protein family of kindlins, essential integrin activators. Kindlin-1 is engaged in integrin β1 adhesion complexes, the focal adhesions, and regulates β1 activation, dynamics, and adhesion turnover (Harburger *et al.*, 2009; Margadant *et al.*, 2012). Besides, it acts as a linker between cell adhesion and regulation of the cell cycle (Patel *et al.*, 2013) and controls Wnt and transforming growth factor-β availability to regulate stem cell proliferation (Rognoni *et al.*, 2014).

Abbreviations: bp, base pair; EB, epidermolysis bullosa; FERMT1, gene coding for kindlin-1; KS, Kindler syndrome

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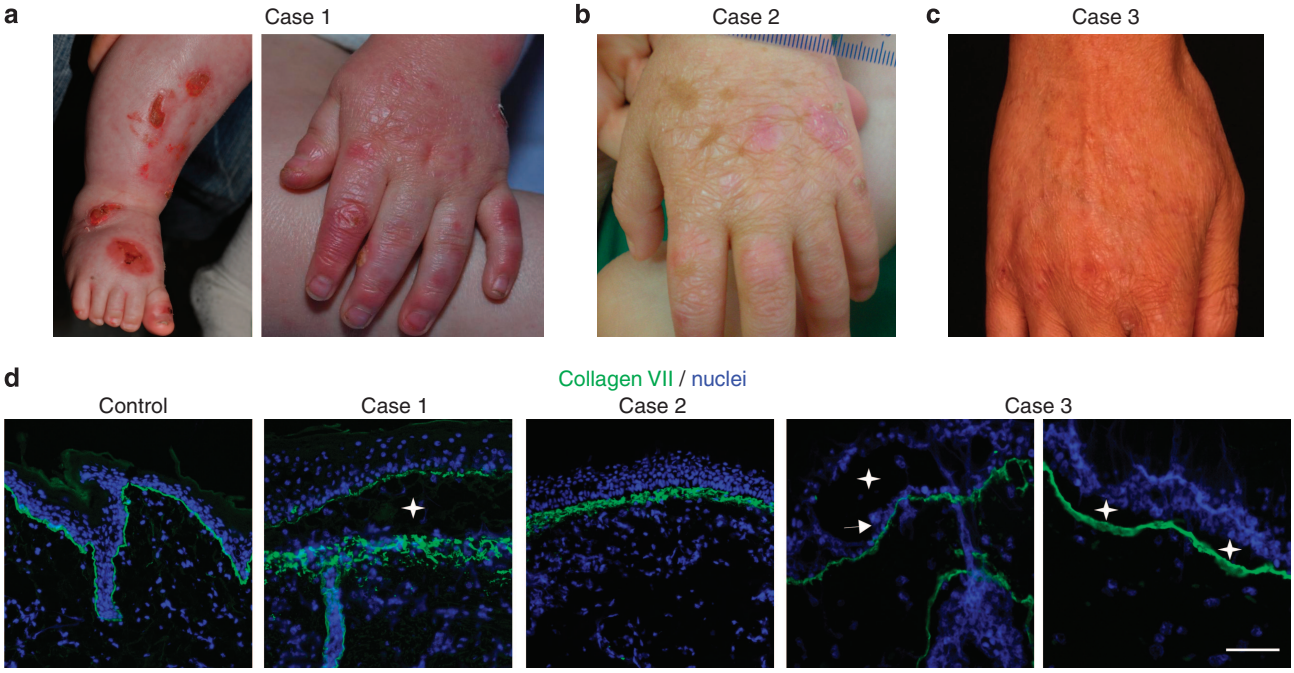

Figure 1. Clinical and skin morphological findings in patients with genetically unsolved KS. (**a**) Erosions were present at birth and skin atrophy at the age of 1.5 years in case 1. (**b**) Cigarette paper-like skin atrophy in case 2 at the age of 4 years. (**c**) Atrophy on the dorsal aspect of the hand of case 3 at the age of 27. (**d**) Immunofluorescence staining of the skin demonstrates irregular collagen VII in the patients, in contrast to the linear pattern at the dermal–epidermal junction in the control skin. Note a dermal level of skin cleavage in case 1, whereas in case 3 the split was both intraepidermal and junctional (stars). The arrow points to the nuclei of the basal keratinocytes on the base on the blister.

The evaluation of KS remains challenging, in particular in cases without full-blown clinical picture (Lai-Cheong *et al.*, 2008; Has *et al.*, 2014b). This is due to three factors: (i) the KS phenotype may overlap with those of other EB types; (ii) specific antibodies to kindlin-1 are not widely available and display faint immunostaining signals, even in normal human skin, probably because of the discrete distribution of "focal adhesion" equivalents in the tissue; and (iii) the broad spectrum of mostly private *FERMT1* mutations and mutational mechanisms (HGMD professional 2015.1, https://grenada.lumc.nl/LOVD2/) (Fuchs-Telem *et al.*, 2014; Youssefian *et al.*, 2015). Because of the long-term severe complications of KS, in particular aggressive squamous cell carcinomas, periodontitis, and mucosal strictures, accurate diagnosis is pivotal for the management of patients.

Here we extend the spectrum of *FERMT1* mutations showing that deep-intronic mutations may account for KS cases, which remain genetically unsolved with sequencing of the exons and exon–intron boundaries or gene-dosage analyses.

The patients included in this study were suspected with KS based on clinical and/or skin morphological findings (Figure 1). After written informed consent, EDTA-blood and skin biopsies were obtained. The study was approved and conducted according to the Declaration of Helsinki Principles. Mutational analysis of *FERMT1* and immunofluorescence mapping were performed as described (Has *et al.*, 2011). The mutation analysis could not or could only partially disclose the genetic basis of the disease. A new molecular mechanism was identified after employing analyses on RNA and the protein level in case 1.

Case 1 is a 2-year-old boy born to healthy non-consanguineous German parents. He had fragile skin at birth and developed incipient cigarette paper appearance of the skin over hands and feet, at the age 1.5 years (Figure 1a). The mutation c.676delC, p.Gln226Ser*fs**26, in *FERMT1* exon 5 was disclosed in a heterozygous state (Figure 2a), but the second mutation remained elusive. To the best of our knowledge, c.676delC has not been reported before, but the duplication, c.676dupC, in this same repeat of seven cytosines is recurrent (Martignago *et al.*, 2007). Screening for large deletions/duplications by quantitative real-time PCR (Borozdin *et al.*, 2004; Has *et al.*, 2006) excluded any rearrangements. Finally, sequencing of the promoter excluded any unreported variants (Has *et al.*, 2014a). To illuminate the genetic background, primary keratinocytes were isolated from the skin biopsy of case 1 and from control individuals and cultured in keratinocyte growth medium (Invitrogen, Karlsruhe, Germany). Cell lines were generated using retroviral particles containing human papillomavirus *E6E7* genes (gift of Dr Fernando Larcher). To validate *FERMT1* as the disease-causing gene, immunoblotting of lysates of keratinocytes was performed with the antibody KS4, which recognizes the N-terminus of kindlin-1 (Has *et al.*, 2009). This showed the absence of the kindlin-1 protein in the keratinocytes of case 1 (Figure 2b).

We reasoned that the undetected mutation must reside in regions not covered by the prior tests and isolated total RNA (Qiagen, Hilden, Germany) and performed reverse transcriptase –

PCRs to cover the entire *FERMT1* cDNA (Supplementary Table 1 online). All amplicons had the expected size, except for those spanning exons 5–11, which revealed an additional larger transcript in the patient (Figure 2c). Cloning into TOPOTA and sequencing showed that the larger transcript resulted from the insertion of 124 bp between exons 9 and 10 (Figure 2d and e). Blasting indicated that this was a part of intron 9, starting at the position IVS9+742. To determine the underlying genomic mutation, the intron 9 was sequenced, and a deep-intronic, unclassified variant was disclosed two positions upstream of the insertion, IVS9+740G>A, c.1139+740G>A, in a heterozygous state (Figure 2f). This variant is not referenced in databases (dbSNP142, exome variant server), and we excluded it from 202 chromosomes of a central European population. *In silico* prediction (http://www.cbs.dtu.dk/services/NetGene2/) indicated the generation of a new acceptor splice site (Figure 2e and f). Consequently, a 124-bp pseudo-exon containing a stop codon, p.P381H*fs**16, is integrated, whereby an existing cryptic donor splice site is used (Figure 2e).

Next, 15 patients suspected with KS but genetically unsolved were screened. We identified three more cases, all homozygous for IVS9+740G>A (Figure 2f). Case 2 is a 4-year-old male born to healthy unrelated Romanian parents. He had trauma-induced skin blistering since birth and mild photosensitivity. With time blistering susceptibility decreased, whereas poikiloderma appeared on sun-exposed areas (Figure 1b). In this case, also large rearrangements were excluded. Case 3, a 29-year-old woman, originated from Afghanistan and had two affected siblings. She had discrete poikiloderma, more pronounced on the extremities (Figure 1c), and esophageal stenosis. In both cases, immunofluorescence staining of the skin had suggested KS, based on the irregular staining of collagen VII or on multiple levels of skin cleavage, respectively (Figure 1d). Case 4, a 58-year old Romanian man, had had skin blistering on the extremities in childhood. Thereafter, he developed typical KS features, including poikiloderma on the neck and axillar folds, atrophy of the skin, pseudosyndactyly, periodontitis, tooth loss, ectropion, and urethral stenosis. These additional cases strongly support the disease-causing role of the mutation. Moreover, we excluded homozygosity in the family of case 2, which was available (Supplementary Figure online). The surrounding single-nucleotide polymorphisms suggest that the mutation is harbored on a common haplotype in cases 1, 2, and 4 but not in case 3 (Supplementary Table 2 online).

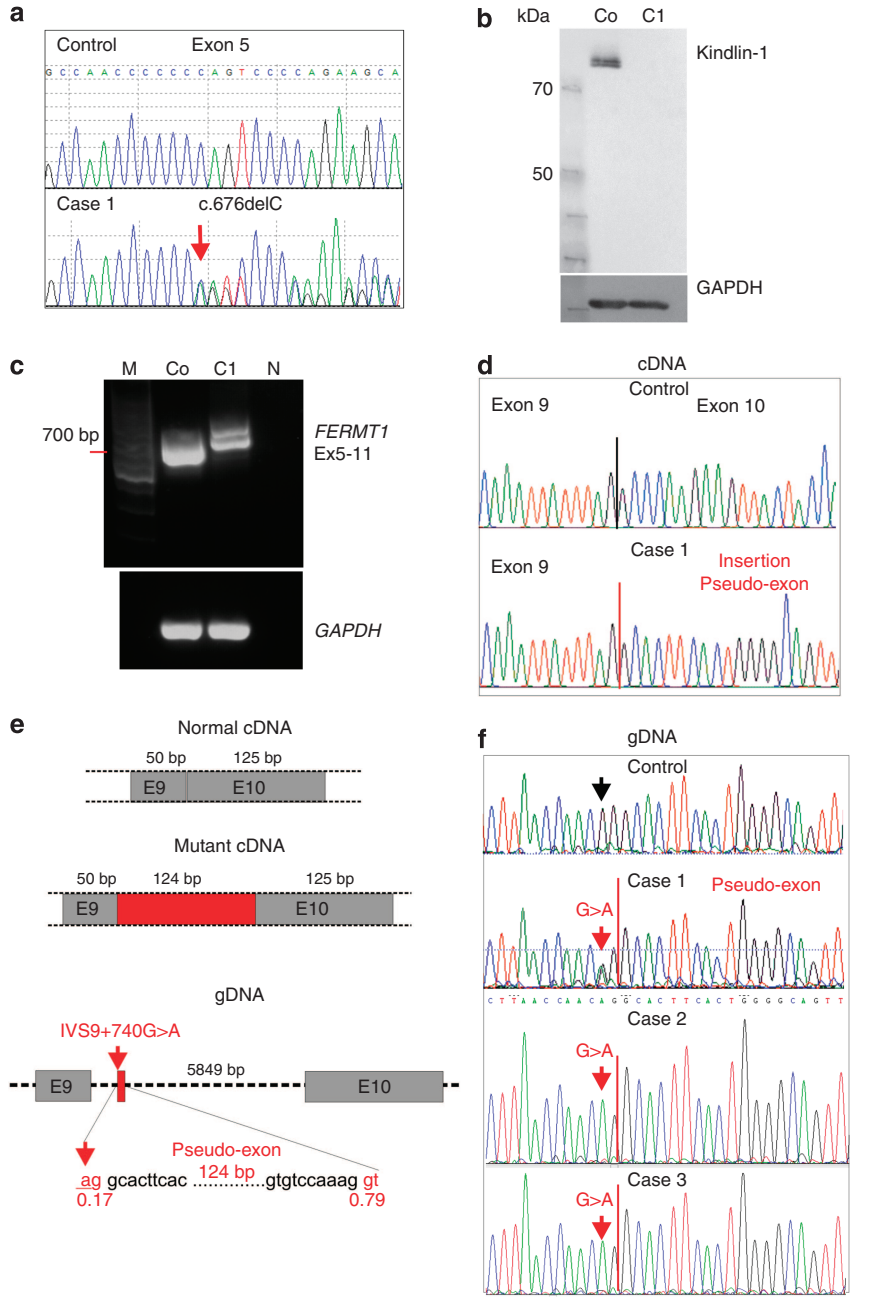

Figure 2. The consequences of the deep intronic *FERMT1* mutation, IVS9+740G>A. (**a**) Sequences of *FERMT1* exon 5 demonstrating the heterozygous frame shift mutation, c.676delC, in case 1 (arrow). (**b**) Immunoblot demonstrates the absence of kindlin-1 in keratinocytes lysates from case 1 (C1); Co, control. (**c**) Agarose gel electrophoresis demonstrating the expected band in the control and of an additional fragment in the patient. M, marker, N, negative control. (**d**) Analysis of the PCR products revealed the insertion of a part of intron 9 in case 1. (**e**) Schematic representation of the region spanning *FERMT1* exons 9 and 10 (E9, E10). The mutation IVS9+740G>A is depicted in red, as well as the pseudo-exon. (**f**) Sequencing of intron 9 revealed in all cases the mutation IVS9+740G>A.

Indeed, the surnames of cases 1 and 2 are of Slavic origin, suggesting an ancestral mutation propagated through Slavic migration to Northern Romania and Eastern Germany, where our patients are living. Nevertheless, the mutation affects a CpG dinucleotide, which has a high mutation rate from 5-methylated CG to TG and its complementary pair CA, suggesting that it could also be recurrent.

Altogether, we show that KS patients may harbor *FERMT1* deep-intronic mutations, which are missed in targeted and whole-exome sequencing, and require RNA analysis or whole-genome sequencing. Our results argue against a genetic heterogeneity of KS.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Expanding the Phenotypic Spectrum of Olmsted Syndrome

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TO THE EDITOR

Palmoplantar keratodermas (PPKs) are a group of genetically heterogeneous genodermatoses. Recently mutations in *TRPV3* were identified as a cause of the rare form of PPK, Olmsted syndrome (OS; OMIM 614594; Lai-Cheong *et al.*, 2012; Lin *et al.*, 2012; Danso-Abeam *et al.*, 2013; Kariminejad *et al.*, 2014; Duchatelet *et al.*, 2014b). OS was first reported in 1927 in an Italian American boy with painful palmoplantar keratoderma, deep fissures, pseudoainhum, curved thickened nails, and periorificial hyperkeratosis with fissuring (Olmsted, 1927). About 50 clinical cases of OS have been described, and all generally exhibit the features described by Olmsted as well as some additional features (Mevorah *et al.*, 2005).

In this study, we report the case of six families, referred to the Pachyonychia Congenita Project for the evaluation of painful plantar keratoderma, but lacking pseudoainhum or significant periorificial keratoderma. In each case, after no mutations were identified in the PC-associated keratin genes, *KRT6A, KRT6B, KRT6C, KRT16*, or *KRT17*, and in some cases, after other candidate genes including *GJB6, DSP, DSG1, KRT5*, and *KRT14* had been screened, we identified heterozygous missense mutations in *TRPV3*, thus greatly expanding the phenotypic

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