Dominant *De Novo* Mutations in *GJA1* Cause Erythrokeratodermia Variabilis et Progressiva, without Features of Oculodentodigital Dysplasia

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Genetic investigation of inherited skin disorders has informed the understanding of skin self-renewal, differentiation, and barrier function. Erythrokeratodermia variabilis et progressiva (EKVP) is a rare, inherited skin disease that is characterized by transient figurate patches of erythema, localized or generalized scaling, and frequent palmoplantar keratoderma. By using exome sequencing, we show that *de novo* missense mutations in *GJA1* (gap junction protein alpha 1) cause EKVP. The severe, progressive skin disease in EKVP subjects with *GJA1* mutations is distinct from limited cutaneous findings rarely found in the systemic disorder oculodentodigital dysplasia, also caused by dominant *GJA1* mutations. *GJA1* encodes connexin 43 (Cx43), the most widely expressed gap junction protein. We show that the *GJA1* mutations in EKVP subjects lead to disruption of Cx43 membrane localization and aggregation within the Golgi. These findings reveal a critical role for Cx43 in epidermal homeostasis, and they provide evidence of organ-specific pathobiology resulting from different mutations within *GJA1*.

Journal of Investigative Dermatology advance online publication, 8 January 2015; doi:10.1038/jid.2014.485

INTRODUCTION

Erythrokeratodermia variabilis (MIM 133200) is a rare, congenital skin disorder characterized by transient figurate patches of erythema on a background of localized or generalized scaling, with palmoplantar keratoderma present in nearly 50% of cases (Richard, 2000). Erythrokeratodermia variabilis is primarily inherited in an autosomal dominant manner, although rare autosomal recessive inheritance has been reported (Gottfried *et al.*, 2002; Fuchs-Telem *et al.*, 2011), and it shows marked phenotypic heterogeneity, even within kindreds bearing the same disease-causing mutation (van Steensel *et al.*, 2009). The term erythrokeratodermia variabilis et progressiva (EKVP) has been proposed to encompass the diversity of phenotypes, ranging from limited hyperkeratotic plaques and erythematous patches to severe progressive symmetric erythrokeratodermia, which can feature more generalized cutaneous involvement (van Steensel, 2004). Mutations in *GJB3* and *GJB4*, encoding connexins 31 and 30.3, respectively, have been reported to cause erythrokeratodermia variabilis/EKVP (Richard *et al.*, 1998; Macari *et al.*, 2000), although there is evidence of further genetic heterogeneity (Common *et al.*, 2005; Wei *et al.*, 2011).

Connexin proteins are named for their molecular weight in kDa (Pfenniger *et al.*, 2011), and they are phylogenetically classified into alpha and beta subgroups (Martin *et al.*, 2014), encoded by *GJA* and *GJB* genes. All connexin proteins have a conserved structure and topology, consisting of a cytoplasmic N terminus and C terminus, and four transmembrane domains connected by two extracellular loops and one cytoplasmic loop. After translation, connexins oligomerize to form hexameric connexons in the endoplasmic reticulum and Golgi, and they are transported to the membrane where they can either function as nonjunctional hemichannels between the cytoplasm and extracellular space or dock with partners in neighboring cells to form intercellular gap junctions. These channels provide both critical communication pathways, allowing passage of ions and small molecules, and structural support to tissues, accumulating to create large plaques. A diversity of potential permeabilities are possible via homomeric or heteromeric connexon configuration, and homotypic or heterotypic interactions between cells (Meşe *et al.*, 2007).

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Abbreviations: Cx43, connexin 43; EKVP, erythrokeratodermia variabilis et progressiva; GJA1, gap junction protein alpha 1; ODDD, oculodentodigital dysplasia

Received 25 July 2014; revised 15 October 2014; accepted 27 October 2014; accepted article preview online 14 November 2014

Gap junctions have diverse and important roles in normal physiology, and mutations in connexin genes cause a variety of pathological phenotypes including cardiovascular disease, myelin-related disease, skin disease, craniofacial disorders, and hearing loss (Pfenniger *et al.*, 2011; Scott *et al.*, 2012).

In recruiting subjects with EKVP-spectrum phenotypes, we identified a cohort without mutation in *GJB3* or *GJB4*, including kindreds 101–103. Each kindred consists of one affected subject born to unaffected parents.

RESULTS

Subject 101-1 is a 32-month-old boy who had no clinical phenotype until the age of 5 months. He then developed thick brown-gray scale on frictional surfaces (Figure 1a), as well as progressive darkening of his dorsal hands, arms, legs, and face. His palmoplantar surfaces and digits became pink and then markedly hyperkeratotic (Figure 2a), and migrating areas of transient figurate erythema overlay the generalized scaling (Figure 3a).

Subject 102-1 is a 6-year-old girl who was adopted from Guatemala at 6 months of age without notable skin disease (Supplementary Figure S1a online). At 8 months, she developed darkening and scaling of frictional surfaces (Figure 1b) and the dorsal hands, and progressive thickening of the palms and soles (Figure 2b). By the age of 13 months, hyperpigmented, hyperkeratotic plaques appeared, affecting the axillae, elbows, and inner thighs; these became confluent by the age of 2 years, with sparing of cheeks and upper chest (Figure 3b). She has intermittent annular red patches that fade within hours after onset.

Subject 103-1 is a 30-year-old woman who had normal skin through 6 months of age (Supplementary Figure S1b online), after which darkening of the inner thighs was noted, followed by development of a thick scale, which began on the knees, elbows, hands, and feet and then progressively spread up the legs and arms (Figures 1c and 2c). At the age of 10 years, pink to deep red transient erythematous patches, which were associated with a burning sensation, became prominent (Figure 3c). Oral acitretin therapy has reduced scale and improved keratoderma.

All subjects exhibit enlarged porcelain-white lunulae (Figure 2, Supplementary Figure S2 online) and darkening of periorificial areas (Supplementary Figure S3 online). Histology of the most severely affected skin in each case shows papillomatosis, acanthosis, hypergranulosis, and compact orthohyperkeratosis with retained nuclei (Figure 1d and Supplementary Figure S4 online). In less severely affected skin, acanthosis, papillomatosis, orthohyperkeratosis, and follicular plugging is found (Supplementary Figure S4 online).

Exome sequencing was performed from peripheral blood DNA of affected subjects (Supplementary Table S1 online). Sequences were aligned to the reference human genome, variants were annotated, and data were examined to identify novel coding mutations. Analysis revealed the same heterozygous missense mutation in gap junction protein alpha 1 (*GJA1),* E227D, in two affected subjects: 101-1 and 102-1. Another heterozygous *GJA1* mutation, A44V, was found in a third affected subject: 103-1 (Supplementary Figure S5 online). Sanger sequencing confirmed these mutations and revealed that E227D is not present in either parent of 101-1, and A44V is not present in either parent of 103-1; thus, these mutations arose *de novo* (Figure 4). Because subject 102-1 is adopted, we have no parental DNA, but her biological parents are documented to be unaffected by any skin disease. Neither E227D nor A44V was found in ~2500 control exomes or in public databases of human genetic variation. E227 is highly conserved in both orthologous and paralogous genes; A44 is highly conserved in orthologs (Figure 5, Supplementary Figure S6 online). *GJA1* encodes connexin 43 (Cx43), a widely expressed gap junction protein.

The *GJA1* E227D mutation in subjects 101-1 and 102-1 is at the intracellular boundary of the Cx43 fourth transmembrane domain, and it is within the region reported to be necessary for Cx43 phosphorylation and interaction with partner protein CIP150 (amino acids 227–242); Cx43 mutant protein lacking this region fails to localize to the membrane (Akiyama *et al.*, 2005). The observation of two identical, independent E227D mutations in unrelated EKVP subjects suggests a critical role for this site in the epidermal function of Cx43.

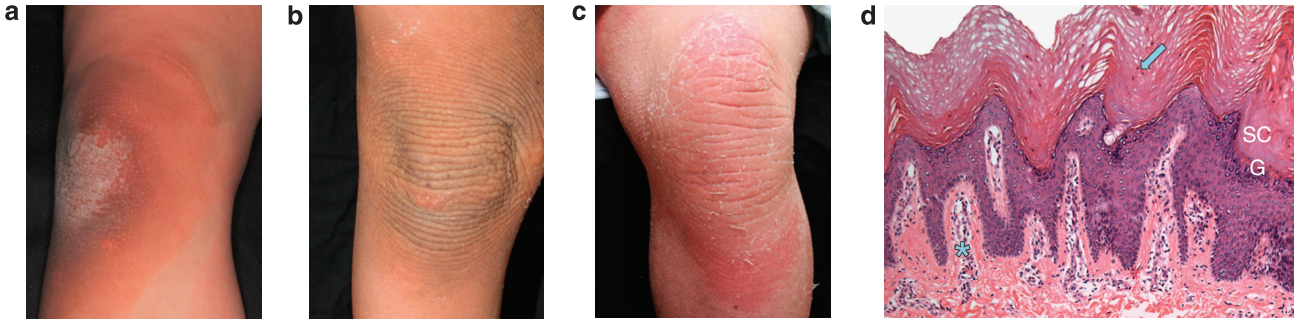

Figure 1. Clinical and histologic features of erythrokeratodermia variabilis et progressiva (EKVP) due to gap junction protein alpha 1 (*GJA1)* mutation. (**a**) The knee of 101-1 was normal at birth, but it became thickened and scaly when he began to crawl: shown at age 30 months. (**b**) The knee of 6-year-old 102-1 shows progression to corrugated, thickened, scaly skin, which has extended beyond sites of friction. (**c**) The knee of 30-year-old 103-1 shows marked hyperkeratosis with peeling scale; a patch of figurate erythema is present inferiorly. (**d**) Histology of affected leg skin of 102-1 shows a compact, thickened stratum corneum (SC) with retained nuclei (arrow), papillomatosis, a thickened granular layer (G), and a perivascular lymphocytic infiltrate (asterisk).

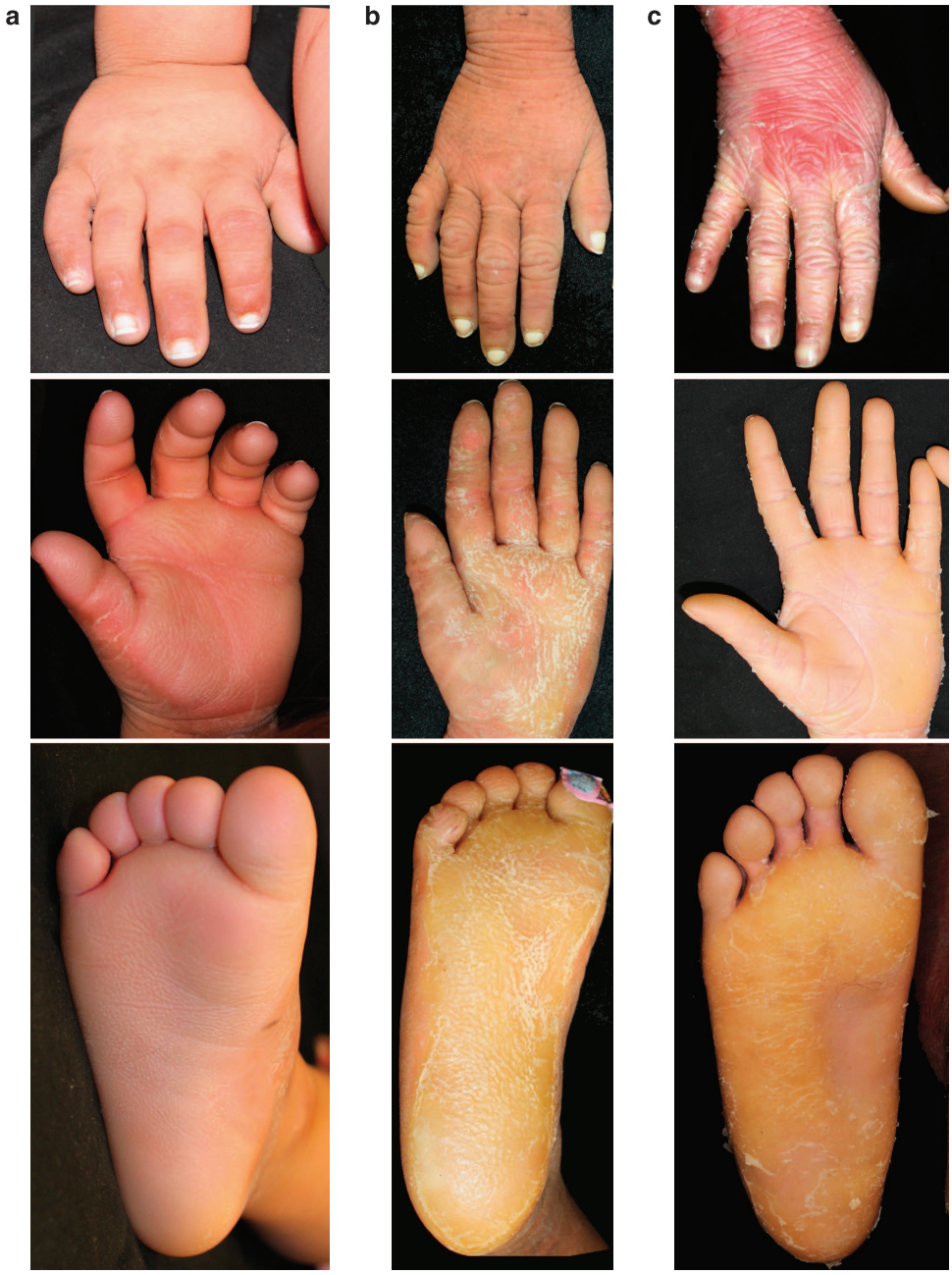

Figure 2. Prominent white nails and progressive keratoderma in erythrokeratodermia variabilis et progressiva (EKVP) due to gap junction protein alpha 1 (*GJA1*) mutation. All subjects show prominent porcelain-white proximal nails without dystrophy. Honeycombed thick hyperkeratosis is present on the palms, and similar thick hyperkeratosis is present on the feet. Keratoderma is progressive with 30-month-old 101-1 (**a**), showing less hyperkeratosis than 6-year-old 102-1 (**b**) or 30-year-old 103-1 (**c**). Subject 103-1 is on a systemic retinoid, accounting for peeling seen at lateral aspects of hand and foot and less exuberant hyperkeratosis.

The A44V mutation in subject 103-1 is at the extracellular boundary of the first transmembrane domain. In contrast to most other paralogs, which have valine at this position and alanine at a position three residues N-terminal, in Cx43 these residues are exchanged, with valine at aa41 and alanine at aa44. In Cx26, an A40V mutation causes keratitis–ichthyosis–deafness syndrome (Montgomery *et al.*, 2004), despite valine being wild-type at this site in Cx43 (Figure 5). These data suggest that these valine–alanine configurations are required for normal function across paralogs, including the role of Cx43 in the skin.

To examine the consequence of these mutations, we immunostained tissue sections from wild-type skin and affected skin of individuals 102-1 and 103-1 with an antibody to Cx43. In contrast to normal skin, in which Cx43 primarily localizes to intercellular junctions with faint, uniform cytoplasmic staining, both E227D (102-1) and A44V (103-1) mutants do not localize to the membrane, demonstrating

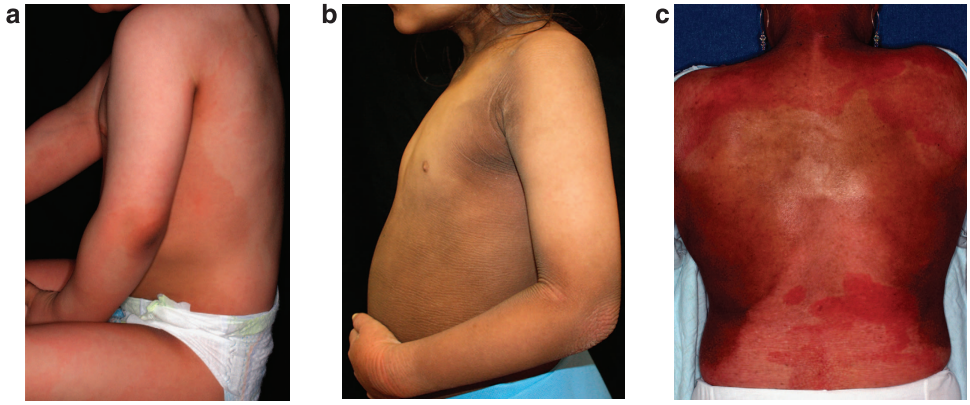

Figure 3. Figurate erythema and darkening of the skin in erythrokeratodermia variabilis et progressiva (EKVP) owing to gap junction protein alpha 1 (*GJA1*) mutation. All subjects report figurate erythema and have experienced progressive skin darkening. (**a**) In subject 101-1, figurate erythema is present on the back, flank, arm, and upper thigh. (**b**) Subject 102-1 shows thick, corrugated keratoderma with darkening prominent on the neck, forearm, axilla, and abdomen. There is a patch of erythema on the wrist. (**c**) In subject 103-1, figurate erythema is present on the upper and lower back. Notably, subject 103-1 reports that figurate erythema became more frequent and prominent at the age of 10 years, but it was present throughout childhood. Treatment with acitretin has reduced scaling, but hyperpigmentation is prominent on the flanks, shoulders, and neck. In all cases, erythema is induced by stress and warm conditions, and it can be accompanied by a stinging or burning sensation.

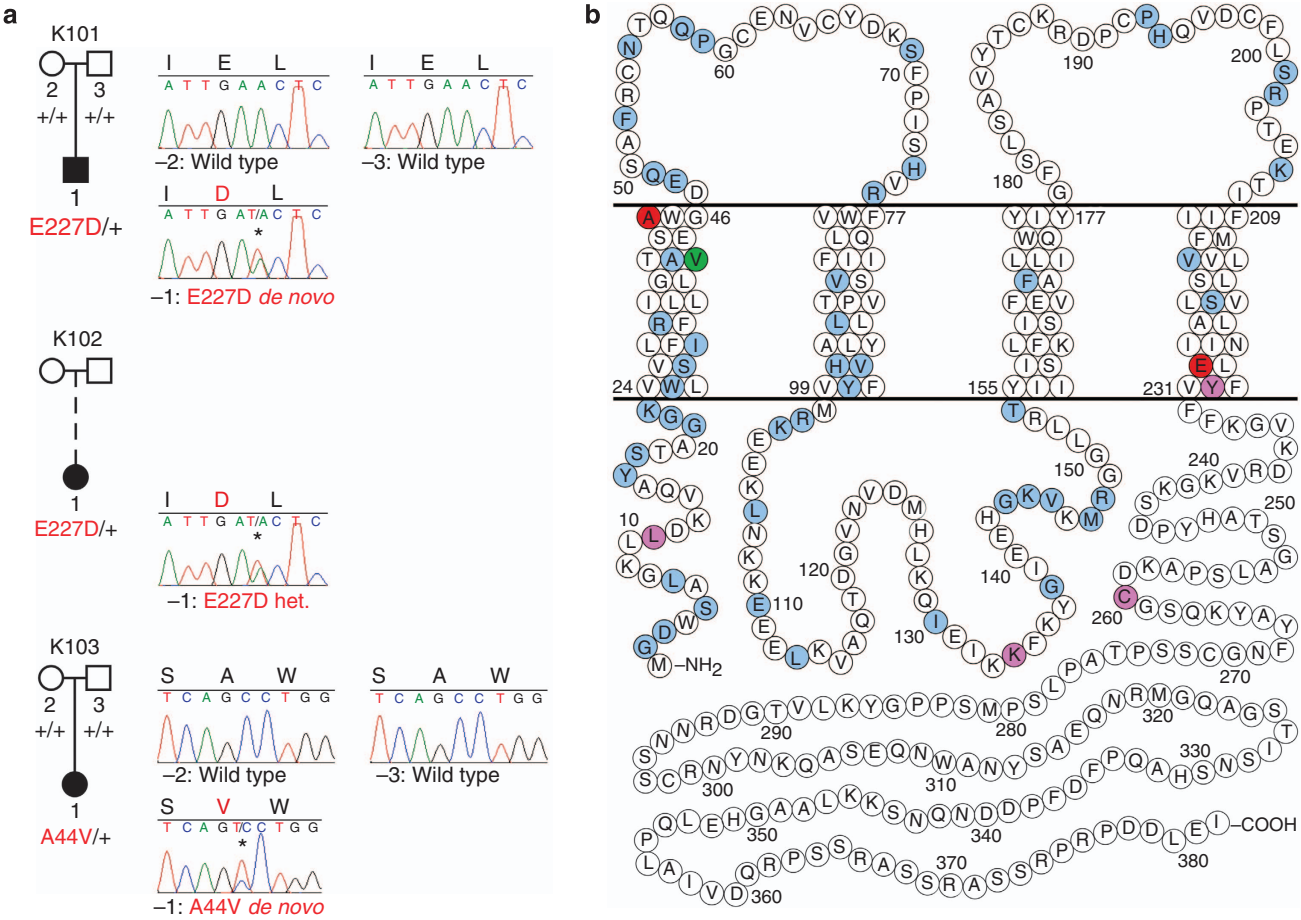

Figure 4. Gap junction protein alpha 1 (*GJA1*) mutations in erythrokeratodermia variabilis et progressiva (EKVP) kindreds. (**a**) Affected and unaffected subjects are denoted with black and white symbols, respectively; adoption is shown with a dashed line. *GJA1* alleles determined by sequencing of genomic DNA are denoted as '+' (wild-type) or by the amino acid substitution in red (mutant). To the right of each pedigree, Sanger sequence traces at *GJA1* mutation sites are shown for each subject and parents from whom DNA was available (mutant bases indicated with *). Amino acid sequences are shown at the top of each trace (mutant residues in red). Mutations are p.E227D, c.A681T (subjects 101-1 and 102-1), and p.A44V, c.C131T (subject 103-1) (NCBI RefSeq NM_000165). (**b**) Schematic model of connexin 43 (Cx43) shows locations of mutations in EKVP subjects reported here (red), and those reported in oculodentodigital dysplasia (ODDD) (blue), ODDD with palmoplantar hyperkeratosis (purple), and ODDD with Clouston syndrome (green).

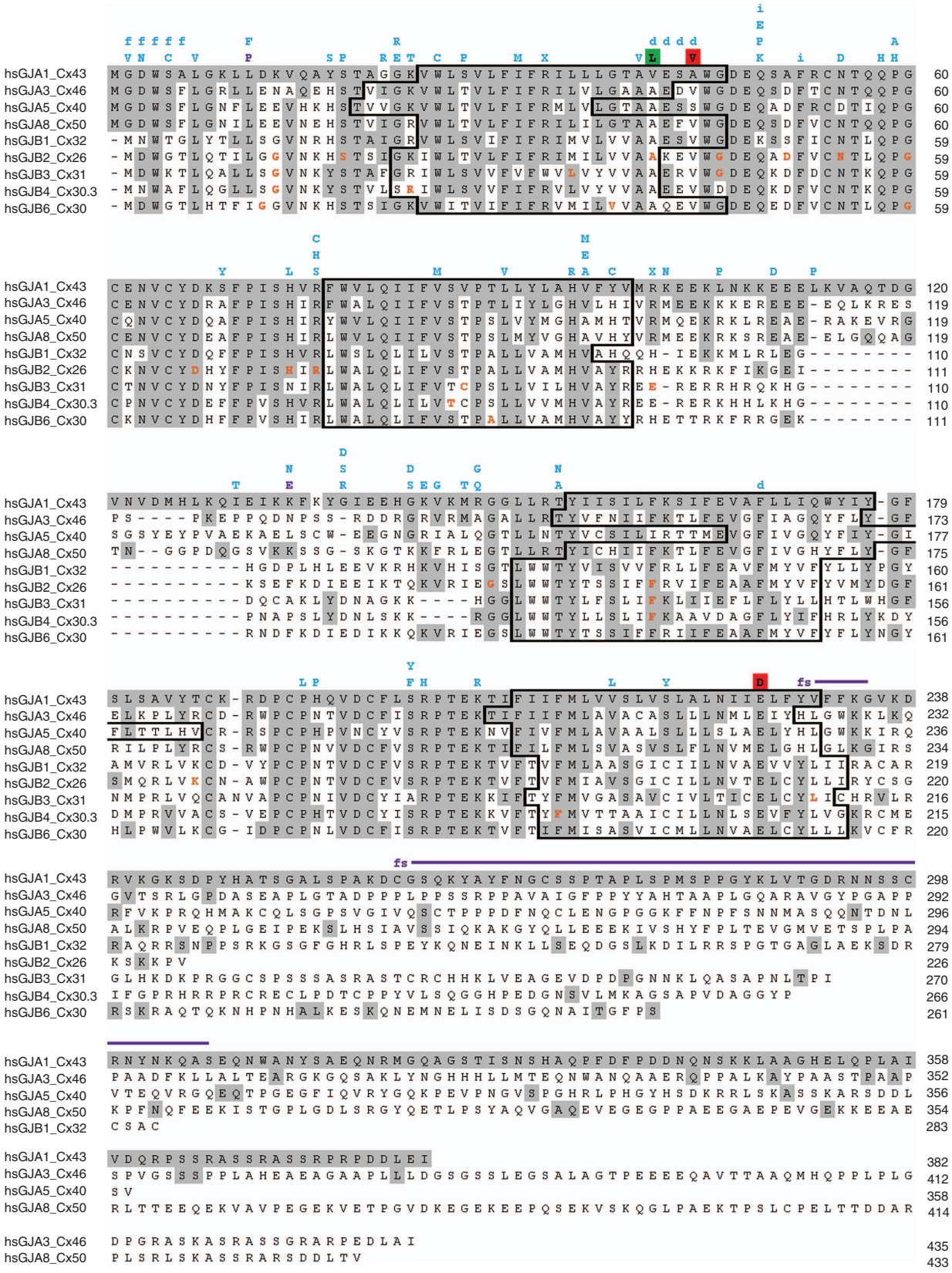

Figure 5. Connexin 43 (Cx43) and paralogs. Transmembrane domains are outlined; conserved residues are shaded gray. Disease-causing Cx43 mutations are shown above the alignment: erythrokeratodermia variabilis et progressiva (EKVP) reported here (red shade), oculodentodigital dysplasia (ODDD) (blue), ODDD with palmoplantar hyperkeratosis (purple), and ODDD with Clouston syndrome (green shade); frameshifts ('fs'), deletions ('d'), insertions ('i'). Mutation sites in paralogs reported to cause skin disease are in orange.

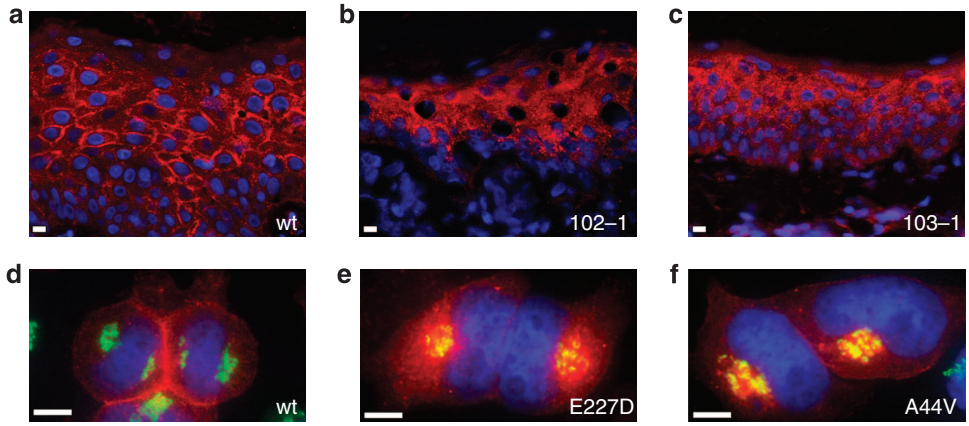

Figure 6. Connexin 43 (Cx43) is mislocalized in erythrokeratodermia variabilis et progressiva (EKVP). (**a**–**c**) Cx43 immunolocalization was performed on tissue sections from wild-type (wt) and affected skin (102-1: E227D, 103-1: A44V), with Cx43 antibody in red and 4′,6-diamidino-2-phenylindole (DAPI) nuclear counterstain in blue. (**a**) Wild-type tissue shows primarily intercellular membrane localization of Cx43. (**b**, **c**) Affected skin shows primarily cytoplasmic localization. (**d**–**f**) Wild-type (wt) and mutant Cx43 (E227D, A44V) were hemagglutinin-tagged and expressed in HeLa cells. Immunolocalization of Cx43 is red, *cis*-Golgi marker GM130 is green, and DAPI nuclear counterstain is blue. (**d**) Wild-type Cx43 localizes to intercellular junctions and does not colocalize with GM130. (**e**, **f**) The E227D and A44V mutants do not localize to intercellular junctions and accumulate in a subcellular compartment, partly colocalizing with GM130. Scale bars = 20 μm in all panels.

cytoplasmic localization (Figure 6a-c). To further explore this Cx43 localization defect, we generated expression constructs of wild-type, E227D, and A44V mutant Cx43 with a C-terminal hemagglutinin tag. Constructs were transfected into HeLa cells and immunostained with an anti-hemagglutinin antibody and an antibody to the *cis*-Golgi marker GM130. Although the wild-type protein primarily localized to intercellular junctions, both the E227D and A44V mutants aggregated in a subcellular compartment partly colocalizing with GM130, suggesting retention in the Golgi (Figure 6d-f).

DISCUSSION

There are 21 connexin-encoding genes identified in the human genome (Martin *et al.*, 2014). Given the variety of possible heteromeric connexon configurations, and heterotypic gap junction interactions between cells, a mutation in a single connexin gene has the potential to exert *trans*-dominant effects on a number of other connexins expressed in the same cell type. This may contribute to the spectrum of phenotypes seen with different mutations within a single connexin-encoding gene. Mutations in *GJB2* (Cx26) can cause sensorineural hearing loss, palmoplantar keratoderma (with and without deafness), and keratitis–ichthyosis–deafness syndrome. Phenotypes are correlated with mutation consequence, with pure sensorineural deafness resulting from loss of Cx26 channel function, and skin phenotypes from mechanisms including mislocalizaton, endoplasmic reticulum stress leading to abnormal epidermal differentiation, pathologically altered hemichannel or gap junction activity, and trans-dominant effects on localization or function of other connexins (Meşe *et al.*, 2007).

Previously described mutations in *GJA1* cause oculodentodigital dysplasia (ODDD) (Paznekas *et al.*, 2003; Laird, 2014), characterized by distinct facial anomalies including microcephaly, microphthalmia, and a thin nose with hypoplastic alae nasi, small anteverted nares, and a prominent bridge and columella. Features with wider phenotypic variability include other abnormalities of the eyes (microcornea, porous spongy irises, cataracts, glaucoma, optic atrophy), dentition (mandibular overgrowth, cleft palate, small or absent teeth, enamel hypoplasia, multiple caries, early tooth loss), and digits (syndactyly, short, bent, or flexed fingers and toes). Brittle nails and hypotrichosis may be present, and there are frequently neurological symptoms that can include hearing loss, lower body weakness, spasticity, and incontinence, often not presenting until adulthood. Mild mental retardation, cardiac abnormalities, and palmoplantar hyperkeratosis occur rarely. Our EKVP subjects with *GJA1* mutations exhibit none of these ODDD phenotypes, save mild dental enamel defects in 102-1 which are not present in 101-1, who shares an identical mutation, or 103-1 (Figure 2, Supplementary Figure S1 and S3 online), and mild clinodactyly (Figure 2), which can be found in ODDD but is also present in the general population (Skvarilová and Smahel, 1984).

Over 70 different mutations in *GJA1* cause ODDD, and almost all are heterozygous missense mutations or small in-frame deletions or insertions occurring before the C-terminal tail (Laird, 2014). Two kindreds have been reported with severe ODDD owing to homozygous or compound heterozygous early-termination mutations (Richardson *et al.*, 2006; Jamsheer *et al.*, 2010), suggesting a dominant-negative mechanism for the majority of ODDD cases. There are two frameshift mutations within the Cx43 tail (Y230fsX236 and C260fsX307) and two missense mutations (L11P and K134E) that are inconsistently associated with the additional feature of mild palmoplantar hyperkeratosis (Paznekas *et al.*, 2003; van Steensel *et al.*, 2005; Kelly *et al.*, 2006; Vreeburg *et al.*, 2007; Alao *et al.*, 2010), and a single case report describes a subject with a *GJA1* missense mutation (V41L) and a *GJB2* mutation who exhibits features of ODDD and Clouston syndrome with

alopecia, nail dystrophy, and well-demarcated, limited hyperkeratosis of extensor surfaces (Kellermayer *et al.*, 2005). Neither transient figurate erythema nor generalized erythrokeratodermia have been reported in ODDD subjects with *GJA1* mutations.

We have shown that previously unreported, dominant, *de novo* missense mutations in *GJA1* cause a consistent clinical phenotype of normal skin at birth, which develops hyperpigmentation and scale at sites of friction in childhood, with progression to near-confluent corrugated hyperkeratosis, palmoplantar keratoderma, and transient figurate erythema. Although mild palmoplantar hyperkeratosis and limited hyperkeratosis of extensor surfaces has been found in a small subset of ODDD patients, the widespread, severe phenotype described here is distinct. Subjects are without features of ODDD caused by over 70 other *GJA1* mutations, and the E227D mutation was observed in two out of three unrelated EKVP subjects, suggesting that EKVP pathology may be restricted to a small number of *GJA1* mutation sites.

Cx43 is the most widely expressed connexin (Laird, 2014). It is expressed throughout the epidermis, and it is the predominant connexin in basal proliferating cells (Martin *et al.*, 2014). Yet, Cx43 itself is not required for epidermal homeostasis, given that a human homozygous null allele causes ODDD without skin disease (Richardson *et al.*, 2006), although Cx43 is upregulated in chronic wounds, and knockdown of Cx43 speeds wound healing (Becker *et al.*, 2012; Scott *et al.*, 2012; Martin *et al.*, 2014). In this context, the induction of initial skin findings in our affected subjects at sites of friction with subsequent generalization is intriguing.

Our immunostaining shows that *GJA1* mutations A44V and E227D lead to Cx43 mislocalization. In normal skin, Cx43 localizes to intracellular junctions, whereas in the skin of our EKVP subjects Cx43 does not localize to the membrane, and cells transfected with either A44V or E227D mutant Cx43 demonstrate aggregation of Cx43 in the Golgi. This mislocalization of Cx43 with an A44V or E227D mutation stands in stark contrast to the localization observed with dominant ODDD mutations in Cx43. Of the twenty ODDD mutations that have been experimentally examined, the vast majority are expressed at the cell surface, but they form functionally impaired gap junctions, with a dominant-negative effect on wild-type Cx43 (Shao *et al.*, 2012). Whether the observed retention of A44V and E227D mutant Cx43 in the Golgi contributes to the previously unreported skin-specific EKVP phenotype we describe, either by trans-dominant effects on connexins or other proteins as observed for *GJB2* mutations in KID syndrome (Meşe *et al.*, 2007), or by other mechanisms, will require further experimental investigation.

MATERIALS AND METHODS

Human subjects

The Yale Human Investigation Committee approved the study protocol, consistent with the Declaration of Helsinki Principles, and subjects or their parents provided verbal and written informed consent, and consented to the publication of images as displayed. Each subject provided a blood sample and punch biopsies of affected skin from the upper thigh. De-identified, site-matched normal skin tissue discarded at the time of skin cancer excisions was obtained for immunostaining controls. Genomic DNA was isolated from blood using a standard phenol–chloroform protocol.

Whole-exome sequencing

Bar-coded DNA libraries were prepared and whole-exome capture was performed (EZ Exome 2.0, Roche, Basel, Switzerland) by the Yale Center for Genome Analysis. Illumina HiSeq 2000 and 2500 instruments (Illumina, San Diego, CA) were used for sequencing samples pooled 6 per lane, with 75-bp paired-end reads. The resulting reads were aligned to the human reference sequence (hg18 or hg19) with Efficient Large-scale Alignment of Nucleotide Databases software (ELAND, Illumina) and processed via a SAMtools-based Perl script to trim sequence to targeted intervals and remove PCR duplicates. Single-nucleotide variants and deletions and insertions (indels) were identified using the SAMtools software (Li *et al.*, 2009). Variants were annotated for functional impact using a Perl script and filtered in Excel to exclude frequent variants present in dbSNP (Build 140), 1000 Genomes, the NHLBI exome database (release ESP6500SI-V2), and in 2577 control exomes, and to examine coding mutations (missense, nonsense, and splice site single-nucleotide variants and indels) with SAMtools quality scores $\geqslant 50$ and coverage $\geqslant 8$. Aligned reads were examined with the Broad Institute Integrative Genomics Viewer to exclude single-nucleotide variants resulting from alignment error.

Sanger sequencing

Verification of *GJA1* mutations and sequencing of parental DNA was performed via PCR using Kapa 2G Fast polymerase (Kapa Biosystems, Woburn, MA) and Sanger sequencing. Primers were designed with ExonPrimer and SNPmasker to specifically amplify *GJA1* and not its pseudogene.

Expression construct generation

A *GJA1* complementary DNA clone was obtained (HsCD00332741, Harvard PlasmID, Brookline, MA) and subcloned into pcDNA3.1(−) Zeo (Invitrogen, Carlsbad, CA). Site-directed mutagenesis to introduce the A44V or E227D mutations into the wild-type complementary DNA was performed with QuikChange (Agilent, Santa Clara, CA). The entire complementary DNA sequence of resulting clones (wild-type, A44V, and E227D) was sequenced to ensure the absence of secondary mutation.

Transfections

HeLa cells (CCL-2, ATCC, Manassas, VA) were seeded at a density of 2×10^4 cells per well on 8-well culture slides (Thermo Fisher Scientific, Waltham, MA) in DMEM + 10% fetal bovine serum. Cells were transfected using Lipofectamine 2000 (Invitrogen) as per standard protocols and fixed for immunofluorescence studies at 24 hours post transfection.

Immunohistochemistry

5-μm sections from FFPE tissue were deparaffinized using a xylene–ethanol gradient, rehydrated, and rinsed in phosphate-buffered saline. Antigen retrieval was performed by immersion in a modified pH 6.0 citrate buffer for 20 minutes in a steamer. Slides were cooled and rinsed in phosphate-buffered saline. Tissue sections or transfected

cells were fixed with a 1:1 acetone-methanol mixture for 10 minutes at −20 °C. Blocking was performed with 10% donkey serum/1% BSA for 1 hour at room temperature. Slides were incubated for 1 hour with primary antibody, washed three times with 1X phosphate-buffered saline, incubated for 1 hour with secondary antibody, and again washed three times with 1X phosphate-buffered saline before mounting with Mowiol/1% n-propyl gallate (Polysciences, Warrington, PA). The following primary antibodies were used: 1:100 rabbit anti-Cx43, 1:100 goat anti-hemagglutinin, and 1:70 mouse anti-GM130 (ab11370, ab9134, ab169276; Abcam, Cambridge, England). The following secondary antibodies were used: 1:10,000 Cy3 donkey anti-rabbit IgG, 1:10,000 Cy3 donkey anti-goat IgG, and 1:200 Cy2 donkey anti-mouse IgG (711-165-152, 711-165-003, 711-225-150; Jackson ImmunoResearch, West Grove, PA).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank the EKVP subjects and their families for their invaluable contribution to this work; Jonathan Levinsohn, Young Lim, Leonard Milstone, and Haris Mirza for critical review of the manuscript; and Christopher Castaldi, Vincent Klump, Shrikant Mane, Peggy Myung, Carol Nelson-Williams, and Irina Tikhonova for technical assistance. This work was supported by a Doris Duke Charitable Foundation Clinical Scientist Development Award and a Research Grant from the Foundation for Ichthyosis & Related Skin Types (FIRST), funded by the Lennox Foundation to KAC; a Dermatology Foundation Pediatric Dermatology Fellowship, FIRST funds, and National Institutes of Health funds to BGC (NIH T32 AR007016); the Yale Center for Mendelian Genomics (NIH U54 HG006504); and the Yale Center for Clinical Investigation (NIH UL1 TR000142).

SUPPLEMENTARY MATERIAL

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

REFERENCES

Akicyama M, Ishida N, Ogawa T *et al.* (2005) Molecular cloning and functional analysis of a novel Cx43 partner protein CIP150. *Biochem Biophys Res Commun* 335:1264–71

Alao MJ, Bonneau D, Holder-Espinasse M *et al.* (2010) Oculo-dento-digital dysplasia: lack of genotype-phenotype correlation for GJA1 mutations and usefulness of neuro-imaging. *Eur J Med Genet* 53:19–22

Becker DL, Thrasivoulou C, Phillips AR (2012) Connexins in wound healing; perspectives in diabetic patients. *Biochim Biophys Acta* 1818: 2068–75

Common JE, O'Toole EA, Leigh IM *et al.* (2005) Clinical and genetic heterogeneity of erythrokeratoderma variabilis. *J Invest Dermatol* 125:920–7

Fuchs-Telem D, Pessach Y, Mevorah B *et al.* (2011) Erythrokeratoderma variabilis caused by a recessive mutation in GJB3. *Clin Exp Dermatol* 36:406–11

Gottfried I, Landau M, Glaser F *et al.* (2002) A mutation in GJB3 is associated with recessive erythrokeratodermia variabilis (EKV) and leads to defective trafficking of the connexin 31 protein. *Hum Mol Genet* 11:1311–6

Jamsheer A, Badura-Stronka M, Sowińska A *et al.* (2010) A severe progressive oculodentodigital dysplasia due to compound heterozygous GJA1 mutation. *Clin Genet* 78:94–7

Kellermayer R, Keller M, Ratajczak P *et al.* (2005) Bigenic connexin mutations in a patient with hidrotic ectodermal dysplasia. *Eur J Dermatol* 15:75–9

Kelly SC, Ratajczak P, Keller M *et al.* (2006) A novel GJA 1 mutation in oculo-dento-digital dysplasia with curly hair and hyperkeratosis. *Eur J Dermatol* 16:241–5

Laird DW (2014) Syndromic and non-syndromic disease-linked Cx43 mutations. *FEBS Lett* 588:1339–48

Li H, Handsaker B, Wysoker A *et al.* (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–9

Macari F, Landau M, Cousin P *et al.* (2000) Mutation in the gene for connexin 30.3 in a family with erythrokeratodermia variabilis. *Am J Hum Genet* 67:1296–301

Martin PE, Easton JA, Hodgins MB *et al.* (2014) Connexins: sensors of epidermal integrity that are therapeutic targets. *FEBS Lett* 588:1304–14

Meşe G, Richard G, White TW (2007) Gap junctions: basic structure and function. *J Invest Dermatol* 127:2516–24

Montgomery JR, White TW, Martin BL *et al.* (2004) A novel connexin 26 gene mutation associated with features of the keratitis-ichthyosis-deafness syndrome and the follicular occlusion triad. *J Am Acad Dermatol* 51:377–82

Paznekas WA, Boyadjiev SA, Shapiro RE *et al.* (2003) Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. *Am J Hum Genet* 72:408–18

Pfenniger A, Wohlwend A, Kwak BR (2011) Mutations in connexin genes and disease. *Eur J Clin Invest* 41:103–16

Richard G (2000) Connexins: a connection with the skin. *Exp Dermatol* 9:77–96

Richard G, Smith LE, Bailey RA *et al.* (1998) Mutations in the human connexin gene GJB3 cause erythrokeratodermia variabilis. *Nat Genet* 20:366–9

Richardson RJ, Joss S, Tomkin S *et al.* (2006) A nonsense mutation in the first transmembrane domain of connexin 43 underlies autosomal recessive oculodentodigital syndrome. *J Med Genet* 43:e37

Scott CA, Tattersall D, O'Toole EA *et al.* (2012) Connexins in epidermal homeostasis and skin disease. *Biochim Biophys Acta* 1818:1952–61

Shao Q, Liu Q, Lorentz R *et al.* (2012) Structure and functional studies of N-terminal Cx43 mutants linked to oculodentodigital dysplasia. *Mol Biol Cell* 23:3312–21

Skvarilová B, Smahel Z (1984) Clinodactyly—frequency and morphological implications. *Acta Chir Plast* 26:72–8

van Steensel M (2004) Does progressive symmetric erythrokeratoderma exist? *Br J Dermatol* 150:1043–5

van Steensel MA, Oranje AP, van der Schroeff JG *et al.* (2009) The missense mutation G12D in connexin30.3 can cause both erythrokeratodermia variabilis of Mendes da Costa and progressive symmetric erythrokeratodermia of Gottron. *Am J Med Genet A* 149A:657–61

van Steensel MA, Spruijt L, van der Burgt I *et al.* (2005) A 2-bp deletion in the GJA1 gene is associated with oculo-dento-digital dysplasia with palmoplantar keratoderma. *Am J Med Genet A* 132A:171–4

Vreeburg M, de Zwart-Storm EA, Schouten MI *et al.* (2007) Skin changes in oculo-dento-digital dysplasia are correlated with C-terminal truncations of connexin 43. *Am J Med Genet A* 143:360–3

Wei S, Zhou Y, Zhang TD *et al.* (2011) Evidence for the absence of mutations at GJB3, GJB4 and LOR in progressive symmetrical erythrokeratodermia. *Clin Exp Dermatol* 36:399–405