Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

PORTLAND PRESS

Review Article

The genetic and molecular basis of a connexin-linked skin disease

Sergiu A. Lucaciu[1,2] **and Dale W. Laird**[1,2]

[1]Department of Physiology and Pharmacology, University of Western Ontario, London, ON, Canada; [2]Department of Anatomy and Cell Biology, University of Western Ontario, London, ON, Canada

Correspondence: (dale.laird@schulich.uwo.ca)

Erythrokeratodermia variabilis et progressiva (EKVP) is a rare hereditary skin disorder characterized by hyperkeratotic plaques and erythematous patches that progressively worsen with age. This disorder has been associated with variants in three connexin encoding genes (*GJA1*, *GJB3*, *GJB4*) and four unrelated genes (*KRT83*, *KDSR*, *TRPM4*, *PERP*). Most cases of connexin-linked EKVP exhibit an autosomal dominant mode of inheritance, with rare autosomal recessive cases. Collectively, evidence suggests that connexin variants associated with EKVP elicit a plethora of molecular defects including impaired gap junction (GJ) formation, dysregulated hemichannel and/or GJ channel function, cytotoxicity, dominant disruption of co-expressed connexins, and/or altered turnover kinetics. Here, we review the progress made in understanding the genetic and molecular basis of EKVP associated with connexin gene variants. We also discuss the landscape of treatment options used for this disorder and the future directions for research into this rare condition.

Introduction

The human 21-member connexin family of gap junction (GJ) channel-forming proteins, classified into α, β, γ, δ, and ε subgroups based on sequence homology, all share a conserved structural topology (Figure 1A–C). All connexins have a cytoplasmic amino-terminal domain (NTD), four helical transmembrane domains (M1–M4), two extracellular loops (E1 and E2), a cytoplasmic loop (CL), and a cytoplasmic carboxy-terminal domain (CTD) (Figure 1B,C). This topology has been confirmed for at least one α-, β-, γ-, and δ-connexin through high-resolution structural analysis of Cx26 (*GJB2*) [2,3], Cx32 (*GJB1*) [4] Cx31.3 (*GJC3*) [5], Cx36 (*GJD2*) [6], Cx46/Cx50 (*GJA3* and *GJA8*, respectively) [7] and Cx43 (*GJA1*) [8,9] channels as revealed by x-ray crystallography and/or cryogenic electron microscopy. The CL and CTD vary the most between isoforms, while the NTD, transmembrane domains, and extracellular loops are highly conserved [10,11]. E1 and E2 are involved in GJ docking [12], with additional roles for E2 in determining heterotypic compatibility [12]. The NTD, M1, M2, E1, E2 and the CL contribute to forming the pore-lining region giving rise to a hydrophilic channel [2,13]. Meanwhile, M3 and M4 face the hydrophobic environment of the phospholipid bilayer [2]. Finally, the highly variable CL and CTD contain sites for various post-translational modifications and motifs for protein-protein interactions that regulate the fate of connexins in cells [14].

Connexins canonically form hemichannels (HCs) and GJ channels that directly couple cells with the extracellular milieu or adjoined cells, respectively (Figure 1D,E) [15]. Gap junctional intercellular communication (GJIC) conferred by connexins is a fundamental process that occurs in most cells of the body to maintain normal physiology and health [16–18]. In recent years, the physiological and pathophysiological roles of HCs in autocrine and paracrine signaling have also become apparent [15,17,19,20].

Received: 15 July 2024
Revised: 18 October 2024
Accepted: 23 October 2024

Version of Record published:
8 November 2024

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

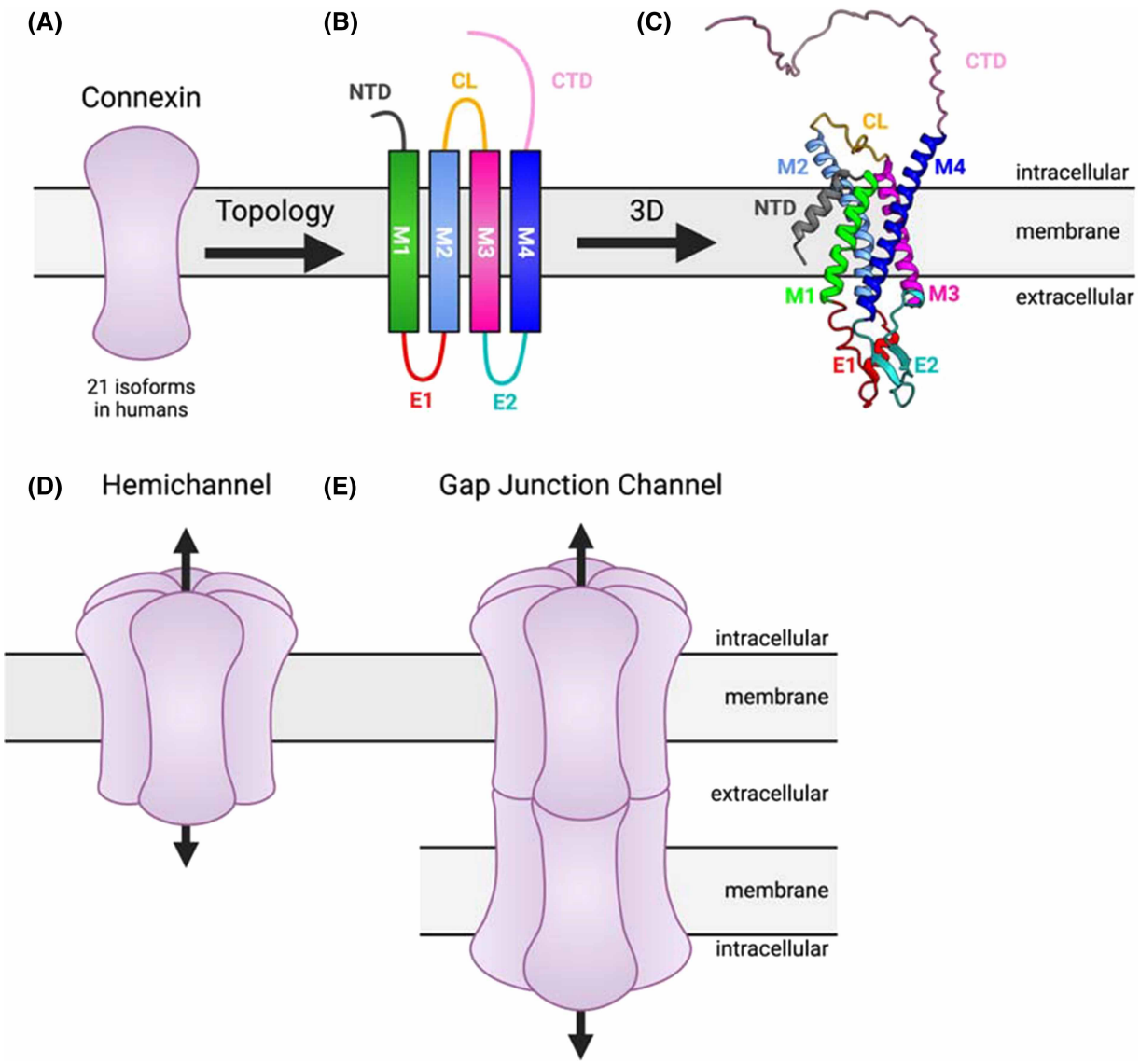

Figure 1. Connexin proteins are the protein subunits of HCs and GJ channels.

(**A**) The connexin protein family consists of 21 isoforms in humans. (**B**) Connexins are all thought to share a similar topology with four transmembrane domains (M1–M4), two extracellular loops (E1 and E2), a cytoplasmic loop (CL), and cytoplasmic amino terminal (NTD) and carboxy terminal (CTD) domains, also depicted in (**C**) the predicted 3-dimensional (3D) structure of a Cx30.3 monomer (AlphaFoldDB: Q9NTQ9) [1]. Note: the helical NTD inserts into the aqueous pore and is not embedded in the lipid bilayer. (**D**) Six connexins oligomerize to form a single membrane-spanning channel termed a HC (aka, connexon), with an aqueous pore connecting the cytoplasm and extracellular milieu. (**E**) Compatible HCs on opposing cell membranes can dock to form unique double membrane-spanning intercellular channels called GJ channels. The aqueous pore in these channels directly connect the cytoplasm of the adjoined cells. Both types of channels permit the passage of small metabolites and ions up to 1 kDa in size. Created using BioRender.

To fulfill these essential forms of intercellular communication, most cell types in the body express 1–3 connexin isoforms [15]. Interestingly, human epidermal keratinocytes differentially express at least eight so-called 'keratinocyte connexin' isoforms (namely Cx26, Cx30, Cx30.3, Cx31, Cx32, Cx40, Cx43 and Cx45) (Figure 2) during their short lifespan, making them uniquely enriched in connexins [21]. Granulosa cells are the only other human cell type known to express more connexin isoforms [15]. Keratinocyte connexins establish a vast communicative network through the avascular epidermis and are key regulators of keratinocyte proliferation, differentiation, and migration [22–24].

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

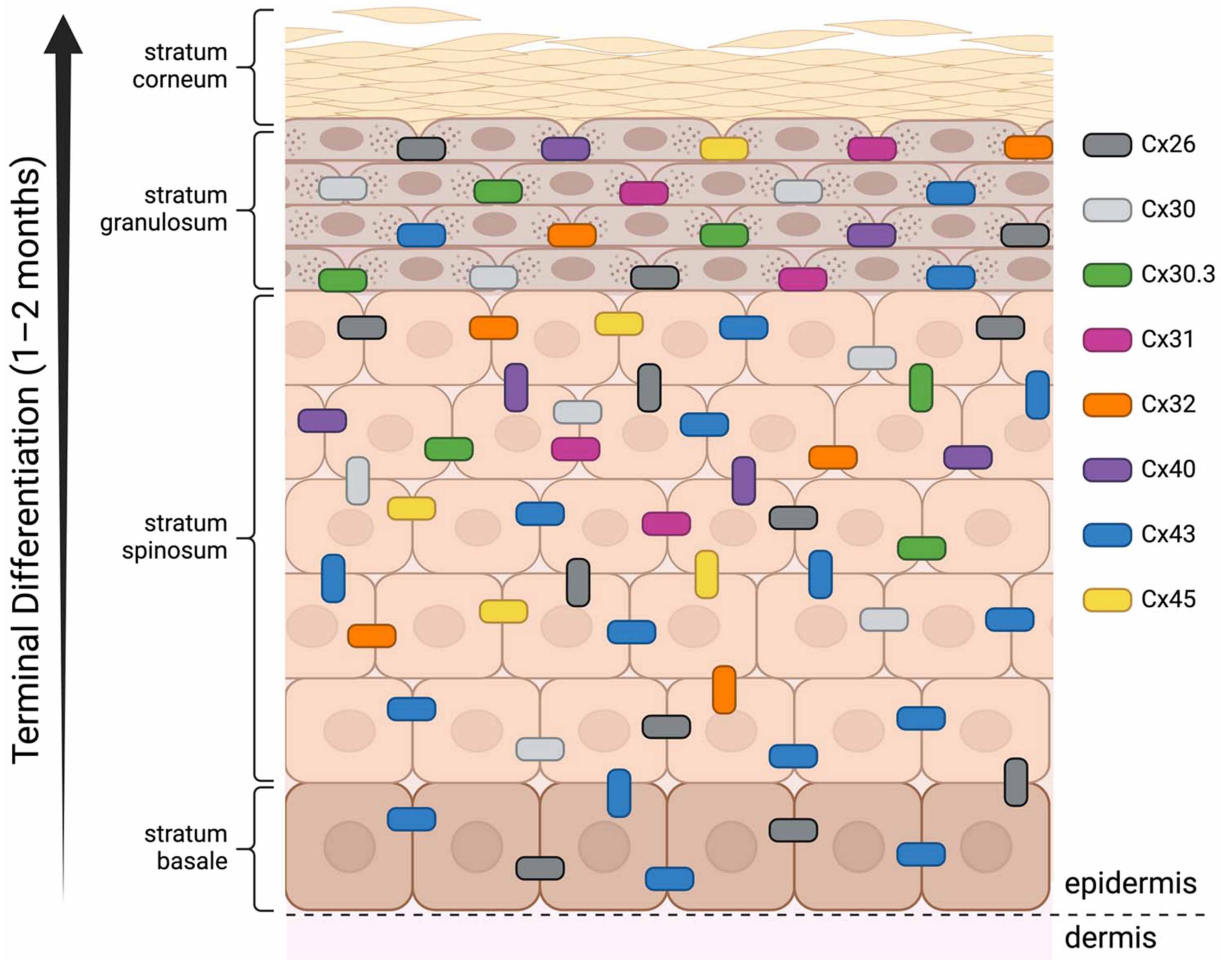

Figure 2. Keratinocytes are enriched in connexin expression.

Keratinocytes express at least eight connexin isoforms with distinct and overlapping spatiotemporal distribution patterns within the epidermis. For example, Cx43 (blue) is found in all living cell layers, while Cx30.3 (green) and Cx31 (magenta) are expressed in the upper stratum spinosum and stratum granulosum. Individual keratinocytes may express multiple connexins at a given time. Created using BioRender.

The crucial roles of these keratinocyte connexins within the epidermis are highlighted by gene ablation/attenuation studies and the association between connexin-encoding gene mutations and skin disease [25,26]. Variants in the genes encoding five of these keratinocyte connexins are clinically linked to at least seven cutaneous disorders with varying severity including some life-shortening conditions [25,26]. However, not all gene variants result in skin phenotypes, as Cx26 gene variants are the most common cause of sensorineural hearing loss that can occur with or without skin manifestations in a variant-dependent manner [26,27]. Similarly, the vast majority of Cx43 variants cause oculodentodigital dysplasia (ODDD), a rare congenital condition impacting various systems that rarely present with skin phenotypes [26,28,29].

Erythrokeratodermia variabilis (EKV) first described by da Costa in 1925 [30] is a rare hereditary skin disorder characterized by well-demarcated lesions consisting of fixed hyperkeratotic plaques and transient migratory erythematous patches that tend to progressively worsen with age [31]. This classical EKV phenotype is sometimes referred to as EKV of Mendes da Costa, while a similar clinical presentation with distinct circinate erythematous lesions is termed EKV Cram-Mevorah [32,33]. Another similar clinical presentation with static erythema is called progressive symmetric erythrokeratoderma (PSEK) [34]. In all cases, EKV and PSEK disease presentation is limited to the skin and occasionally its appendages (hair, nails), which enables clinical distinction between other conditions such as ODDD and keratoderma-hypotrichosis-leukonychia totalis syndrome [35]. Lesions typically present at birth or during infancy [36,37] and are subject to considerable variability even

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

between affected relatives [38]. This variability has been proposed to be due to trauma (i.e. mechanical irritation, UV exposure), stress, genetics, epigenetics and other factors [37].

Clinicians face a significant challenge in correctly differentiating between different keratodermas and erythrokeratodermas, as these disorders share many clinical and histological features. This has led to considerable debate and confusion over appropriate diagnoses and disease nomenclature. Some of this confusion may be due to the clinical ambiguity of the term 'keratoderma', which has led to the inaccurate clustering of drastically different clinical forms of keratoderma into a PSEK category [34]. This is especially apparent in many diagnoses of severe keratodermas or erythrokeratodermas linked to non-connexin encoding genes in which the clinical phenotype is clearly different from connexin-linked erythrokeratoderma [39–44]. Indeed, loricrin gene variants cause a range of phenotypic presentations, including PSEK-like keratodermas, thus the field adopted the terminology of 'loricrin keratoderma' to distinguish this disorder from other similar presenting diseases with distinct molecular etiologies [45]. Furthermore, since as early as 1991, it has been proposed that EKV and PSEK may represent different clinical manifestations of the same condition [46], since the main clinical difference between EKV and PSEK is that EKV lesions are well-demarcated and migratory, while PSEK lesions are non-migratory and symmetric [34]. It has been argued that the available clinical evidence does not support the existence of a non-migratory PSEK phenotype, rather the migratory erythemas in EKV are sometimes static and increasing in size, resembling the PSEK phenotype [34]. Once evidence emerged that the same *GJB4* variant (Cx30.3-G12D) may cause phenotypes consistent with a diagnosis of either EKV or PSEK in the same family, van Steensel and colleagues proposed adopting the new nomenclature of erythrokeratodermia variabilis et progressiva (EKVP) to encompass both the variable and progressive nature of the lesions [34,47]. For simplicity, we will use the term EKVP throughout this paper to collectively refer to these variable clinical manifestations, except in situations where the clinical phenotype demands distinguishing between EKVP and other erythrokeratodermas.

Here, we set out to review the known and putative underlying molecular causes of EKVP associated with connexin gene variants, what is known about the underlying pathogenic mechanisms, the landscape of treatment options, and the pressing questions that need to be addressed.

Heterogeneity of EKVP

Clinically, EKVP is heterogenous and has been most often linked to variants in the genes encoding Cx30.3 (*GJB4*; OMIM #617524) (Table 1, Figure 3A), Cx31 (*GJB3*; OMIM #133200) (Table 2, Figure 3B), and Cx43 (*GJA1*; OMIM #617525) (Table 3, Figure 3C) [31]. Rare cases of erythrokeratodermas with similar clinical presentations to EKVP or PSEK have also been linked to variants in the *KDSR* (OMIM #617526) [39], *KRT83* (OMIM #617756) [42], *TRPM4* (OMIM #618531) [43], and *PERP* (OMIM #619209) [41] genes. Variants in the connexin-encoding genes represent an overwhelming majority of the EKVP cases reported in the literature where genetic analyses were performed, of which *GJB3* and *GJB4* variants are the most common. Notably, there are several families with a history of EKVP over several generations [38,48,56], although direct genetic evidence to match the clinical diagnoses are only available for a limited number of these individuals. Therefore, the numbers presented here only include patients with direct genetic evidence (i.e. sequencing, confirmation via restriction endonucleases) and a confirmed diagnosis of EKVP by one or more clinicians.

In 1998, Richard et al. [56] were the first to identify pathogenic variants in *GJB3* that segregated with the EKVP phenotype in three families. However, since they were unable to identify *GJB3* variants in eight families with EKVP the authors speculated that EKVP might be heterogenous and proposed functional partners of Cx31 as candidate EKVP-linked genes. This notion was confirmed two years later when a *GJB4* gene variant was found to segregate in a family with EKVP for three generations [33]. Over time more variants in *GJB4* and *GJB3* were identified (summarized in Tables 1 and 2, respectively). Intriguingly, many of these variants impacted orthologous residues between the two isoforms [38]. While no clinical features could be identified to differentiate Cx31- from Cx30.3-linked EKVP, a more severe phenotype generally involving palmoplantar keratoderma (PPK) was associated with Cx31 variants. Furthermore, Cx30.3 variants sometimes cause erythema gyratum repens-like lesions [49]. Cases of EKVP have been reported in which patients carried mutations in both *GJB3* and *GJB4*, though these cases involved a pathogenic variant in one gene along with a benign polymorphism in the other [55]. However, families with EKVP continued to be identified that lacked pathogenic variants in *GJB3* and *GJB4* [38,55,89,90], pointing to a possible involvement of other genes in the pathogenesis of EKVP and/or to genetic changes in the non-coding regions of *GJB3* and *GJB4* [91]. In 2015, *GJA1* was identified as a third connexin-encoding gene associated with EKVP [75]. However, the pathogenicity of all Cx43

Table 1. EKVP-related Cx30.3 variants.

DNA change	Protein change (class)	No. of patients	EKVP components	Affected areas	PPK	Estimated severity	References
① 35G > A	G12D (I–IV)	① 5	① Symmetric hyperkeratosis. Transient erythema.	① Limbs, Trunk.	① nd	① 4	① [38]
② 35G > A		② 5	② Symmetric hyperkeratosis with erythematous borders.	② Limbs, Trunk, Buttocks, Axillae.	② nd	② 3	② [47]
③ 35G > A		③ 2	③ Fixed hyperkeratotic plaques. Erythema in females only.	③ Limbs, Trunk.	③ nd	③ 4	③ [48]
64G > A	R22H* (VIII)	2	Symmetric hyperkeratosis. Faint underlying erythema. Transient red patches.	Limbs.	nd	3	[38]
77C > A	S26Y (VIII)	1	Persistent hyperkeratosis. Transient erythema.	Body folds.	nd	4	[49]
109G > A	V37M (I, V)	1	Symmetric hyperkeratosis and erythema.	Limbs.	nd	3	[50]
182C > G	P61R (VIII)	1	Persistent hyperkeratosis. Transient erythema.	Dorsal feet.	Yes	4	[49]
253A > C	T85P (I–IV)	16	Fixed hyperkeratosis. Erythema only in childhood.	Wide distribution.	No	3	[38]
256T > A	C86S (VIII)	8	Symmetric hyperkeratosis. Diffuse migratory erythema.	Limbs, trunk, face, neck, ears.	No	3	[51]
292C > T	R98C (VIII)	2†	Fixed hyperkeratosis. Variable erythema.	Limbs, trunk, buttocks.	No	3	[52]
295G > A	E99K*‡ (VIII)	1	Hyperkeratosis with scales. Erythema gyratum repens	Buttocks, axillae, body folds.	No	3	[53]
389C > T	T130M* (VIII)	1	Hyperkeratosis. Transient erythema.	Limbs and face.	No	2	[54]
① 409T > C	F137L (I, II, V)	① 7	① Figurate hyperkeratosis. Erythema gyratum repens.	① Limbs, trunk, neck.	① No	① 3	① [33]
② 409T > C		② 3	② Figurate hyperkeratosis. Erythema.	② Trunk, feet.	② No	② 3	② [38]
③ 411C > A		③ 1	③ Symmetric hyperkeratosis. Erythema gyratum repens.	③ Trunk, legs and buttocks.	③ No	③ 4	③ [38]
④ 409T > C		④ 2	④ Hyperkeratosis. Erythema.	④ nd	④ nd	④ nd	④ [54]
566T > A	F189Y (I–IV)	9	Severe generalized hyperkeratosis.	Limbs, large body folds	No	4	[38]
568A > T	M190L* (VIII)	1	Fixed hyperkeratosis. Transient migratory erythema.	Limbs, trunk, face.	Yes	4	[55]

*Variant is reported in gnomAD.
†Patients were not related. Some features were not described (nd) in the case reports. Circled numbers refer to different case reports.
‡Autosomal recessive. Using the evidence in the literature, we estimated disease severity on a scale from 1 (mild) to 5 (severe) based on the estimated total body area affected (15–50% +1, >50% +2), presence of PPK (+1), erythema (+1), and hyperkeratosis (+1) at exam. Variants are classified according to information presented in Table 4.

variants identified to date remain to be fully established as all variants were *de novo* and thus lacked evidence of segregation with disease over several generations [75,76]. Since then, four additional genes (*KDSR, KRT83, TRPM4*, and *PERP*), all of which are unrelated to connexins, have been identified in patients diagnosed with a more severe erythrokeratoderma than patients with connexin-linked EKVP [39–43]. This is consistent with the hypothesis that, despite the clinical similarity, the etiology of EKVP and other erythrokeratodermas might be

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

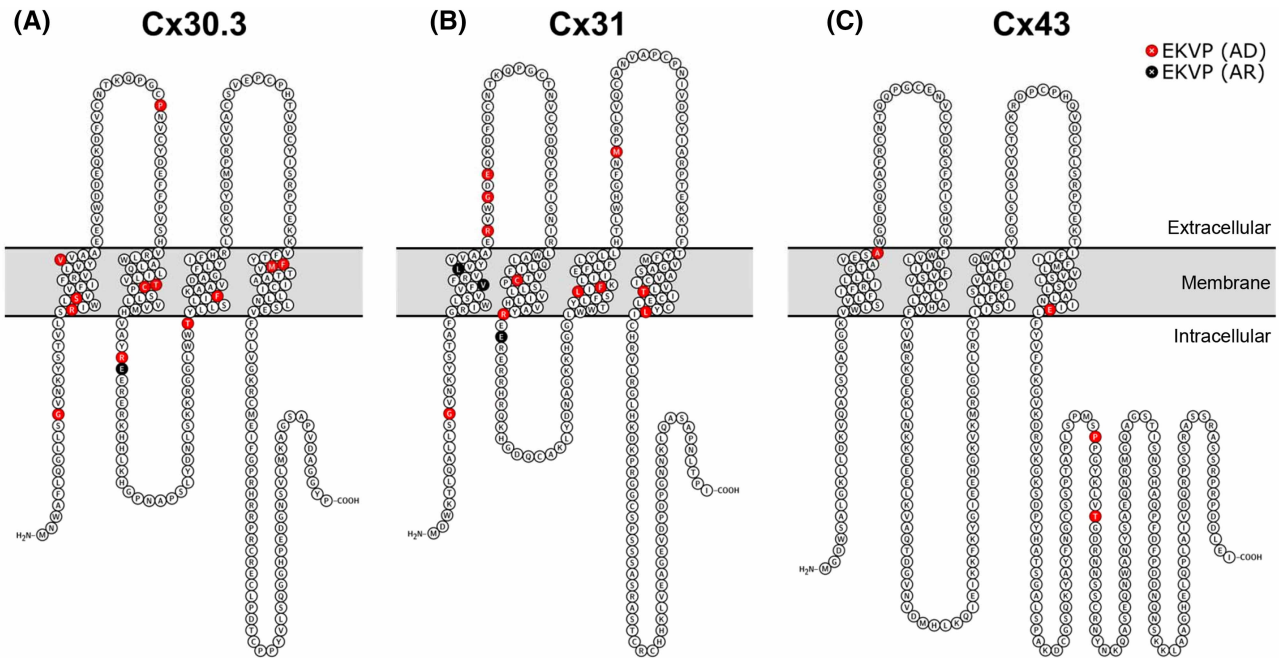

Figure 3. Overview of Connexin Variants Associated with EKVP.

The location of autosomal dominant (AD; red) or autosomal recessive (AR; black) variants associated with EKVP depicted on topological schematics of (**A**) Cx30.3, (**B**) Cx31, and (**C**) Cx43. Generated with Protter (https://wlab.ethz.ch/protter/start/).

distinct [91]. The heterogenous nature of EKVP could explain the range of phenotypic variability, even within relatives harboring the same genetic variant, pointing to the influence of individual genetic and non-genetic factors on disease presentation and severity [38].

EKVP associated with Cx30.3 variants

In humans, Cx30.3 is most highly expressed in suprabasal epidermal keratinocytes and found at low abundance in the lungs, cornea, anus, scrotum, and granulosa cells [15]. Interestingly, evidence suggests Cx30.3 may also be expressed in the intercalated discs of hypertrophic and hypertensive hearts, despite the fact Cx30.3 is not known to be normally expressed in healthy hearts [92]. Interestingly, the homozygous Cx30.3-E204A variant may be linked to hypertrophic cardiomyopathy, though other putative causes were not ruled out [92]. To date, there are 14 known single nucleotide substitutions in *GJB4*, encoding 13 Cx30.3 missense variants, associated with EKVP (Table 1, Figure 3A). These sites of amino acid substitution localize within the predicted NTD, all four transmembrane domains (M1-M4), the CL, and E1 of Cx30.3 (Figure 2A). Interestingly, all these residues, other than P61, are orthologous or proximal to residues that line an intracellular water pocket in Cx26 that is thought to be important for channel gating [93]. Residues orthologous to the mutational sites in Cx30.3, other than P61, are associated with disease in one or two other β-subtype connexins. Three residues in Cx30.3 — G12, C86, and F137 — appear to be mutational hotspots in β-subtype connexins, as mutations to the orthologous residues in Cx31, Cx32, and Cx26 are associated with EKVP, demyelinating disease and hearing loss, respectively. Curiously, two of the mutational hotspots (G12 and F137) and two other variants (Cx30.3-T130M and Cx31-L135V) fall within calmodulin binding sites found in the NTD and CL of connexins [94–96]. Though these have not been directly investigated to date, it is possible these variants all alter the functionality of these regulatory motifs. Nevertheless, the exact roles of these residues remain unclear, though they are evidently important for normal connexin biology.

Patients with Cx30.3 variants generally present with the classical features of EKVP, including fixed and usually symmetric hyperkeratosis and recurrent episodes of transient erythema that changes over the course of hours to days (Table 1). Some patients with Cx30.3 variants presented with distinct erythema-gyratum repens-like lesions [33,38,53], though most cases of Cx30.3-linked EKVP lacked this feature (Table 1). Disease severity is generally mild to moderate and most frequently affects the limbs, trunk and less commonly the face and

Table 2. EKVP-related Cx31 variants. Part 1 of 2

DNA change	Protein change (class)	No. of patients	EKVP components	Affected areas	PPK	Estimated severity	References
34G > C	G12R (I, IV)	13	Localized hyperkeratosis.	nd	nd	2	[56]
① 35G > A	G12D (I, II, IV)	① 2	① n.d.	① nd	① nd	① 2	① [56]
② 35G > A		② 2	② Symmetric hyperkeratosis. Transient figurate erythema.	② Limbs, trunk, neck	② No	② 5	② [55]
① 34G > A	① G12S*	① n.d.	① n.d.	① nd	① nd	① nd	① [57]
② 34G > A, 474G > A	② G12S*†, M158I† (VIII)	② 3	② Symmetric fixed hyperkeratosis. Transient erythema.	② Face, limbs, buttocks, trunk	② Yes	② 4	② [58]
88G > A	V30I*‡ (I)	3	Fixed hyperkeratosis. Transient figurate erythema.	Back, limbs	Yes	4	[59]
101T > C	L34P‡ (I)	2	Fixed hyperkeratosis. Transient figurate erythema.	Trunk, earlobes, limbs	No	3	[60]
① 125G > C	R42P (I, III, IV)	① 3	① Fixed hyperkeratosis. Transient erythema.	① Limbs, buttocks, trunk, neck	① Yes	① 5	① [37]
② 125G > C		② 3	② Figurate hyperkeratosis. Transient, variable erythema.	② Limbs, trunk	② Yes	② 4	② [61]
③ 125G > C		③ 1	③ Hyperkeratosis. Generalized transient erythema.	③ Generalized	③ Yes	③ 5	③ [62]
① 134G > A	G45E (I, IV)	① 1	① Hyperkeratosis. Erythema.	① Limbs, face	① Yes	① 3	① [63]
② 134G > A		② 2	② Generalized hyperkeratosis. Transient erythema.	② Trunk, limbs, face	② Yes	② 4	② [64]
③ 134G > A		③ 1	③ Diffuse ichthyotic hyperkeratosis. Transient erythema.	③ Generalized	③ Yes	③ 5	③ [65]
141G > C	E47D (VIII)	1	Scaly hyperkeratosis. Erythema.	Limbs, trunk	Yes	4	[66]
256T > A	C86S (I, IV)	① 4	① n.d.	① nd	① nd	① 2	① [56]
		② 1	② Persistent hyperkeratosis. Transient erythema.	② Limbs, trunk	② Yes	② 5	② [49]
293G > A	R98H* (VIII)	1–3	Annular hyperkeratosis. Erythema.	Limbs, trunk	Yes	3	[67]
298G > A	E100K (VIII)	1‡	Ichthyotic hyperkeratosis. Transient variable erythema.	Generalized	Yes	5	[68]
① 403C > G	L135V (VIII)	① 3	① Symmetric fixed hyperkeratosis. Transient erythema	① Trunk, limbs, neck, axillae	① No	① 4	① [54]
② 403C > G		② 1	② Hyperkeratosis.	② Limbs, trunk.	② No	② 2	② [52]
① 409T > C	F137L (I, II, IV, V)	① 1	① Severe hystrix-like hyperkeratosis. Erythema.	① Lower limbs.	① Yes	① 4	① [61]
② 411C > A		② 1	② Thick hyperkeratosis. Stable erythema.	② Lower limbs, buttocks, and back	② Yes	② 4	② [69]
③ 411C > G		③ 1	③ Localized hyperkeratosis. Extensive wavy erythema.	③ Limbs, buttocks	③ Yes	③ 5	③ [70]

Continued

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

Table 2. EKVP-related Cx31 variants. Part 2 of 2

DNA change	Protein change (class)	No. of patients	EKVP components	Affected areas	PPK	Estimated severity	References
605C > A	T202N* (VIII)	3	Variable and irregularly shaped erythema with scaling.	Trunk, limbs	Yes	5	[71]
① 625C > T	L209F (I)	① 3	① Fixed hyperkeratosis. Transient variable erythema.	① Limbs, buttocks, face	① Yes	① 4	① [72]
② 625C > T		② 8	② Symmetric hyperkeratosis. Variable erythema.	② Face, buttocks, limbs.	② Yes	② 4	② [73]
③ 625C > T		③ 3	③ Hyperkeratosis and erythema.	③ Limbs, face.	③ nd	③ 3	③ [55]
④ 625C > T		④ 1	④ Fixed symmetric hyperkeratosis. Transient erythema.	④ Limbs, trunk.	④ Yes	④ 4	④ [74]

*Variant is reported in gnomAD. Some features were not described (nd) in case reports. Circled numbers refer to different case reports.
†Only compound heterozygotes had EKVP.
‡Autosomal recessive. Disease severity was estimated as described in Table 1. Variants are classified according to information presented in Table 4.

other body parts (Table 1). A few exceptions of moderate-to-severe disease presentation characterized by a wider distribution of lesions, including generalized hyperkeratosis, have been reported in the case of the Cx30.3-G12D, Cx30.3-T85P, Cx30.3-R98C, Cx30.3-F137L, and Cx30.3-F189Y variants (Table 1) [33,38,52]. Disease presentation can also be highly variable, as in the case of a family with the Cx30.3-T85P variant where striking age-related differences were noted particularly in the erythematous component, which was evident in childhood and largely absent in adulthood [38]. This variability highlights the influence of age, environmental factors and individual genetics/epigenetics on disease presentation. PPK was absent in most patients with Cx30.3 variants, except for two spontaneous cases of EKVP linked to the Cx30.3-V37M and Cx30.3-M190L variants [50,55].

EKVP associated with Cx31 variants

Like Cx30.3, Cx31 is highly expressed in suprabasal epidermal keratinocytes. Cx31 is also found in sebaceous glands, hair follicles, lungs, tissues of the nose, cornea, scrotum, endocrine pancreas, and granulosa cells [15]. The clinical association between select Cx31 gene variants and hearing loss also points to its expression and

Table 3. EKVP-related Cx43 variants.

DNA change	Protein change (class)	No. of patients	EKVP components	Affected areas	PPK	Estimated severity	References
131C > T	A44V (III)	1	Transient erythema, darkening and thick scale.	Limbs.	Yes	4	[75]
681A > T	E227D (III)	2	Migratory transient figurate erythema, thick hyperkeratosis.	Limbs and frictional surfaces.	Yes	5	[75]
848C > T	P283L† (VI)	1	Symmetrical hyperkeratosis and erythema.	Limbs.	No	2	[76]
848C > T, 869C > A	P283L†, T290N (VI, VII)	1	Hyperkeratosis and erythema.	Face, neck, and limbs.	Yes	4	[76]

†Variant is reported in gnomAD. Disease severity was estimated as described in Table 1. Variants are classified according to information presented in Table 4.

role in the auditory system [71,97]. At the time of writing, 18 single nucleotide substitutions in *GJB3* encoding 16 Cx31 variants (three different DNA changes have been found in patients encoding the same Cx31-F137L variant) have been identified in patients diagnosed with EKVP (Table 2, Figure 3B). The sites of amino acid substitution associated with EKVP occur in all topological domains of Cx31 except for the C-terminal domain (Figure 3B) and are distinct from those linked to hearing loss. Most cases of Cx31-associated EKVP are autosomal dominant, except for three autosomal recessive variants: Cx31-V30I, Cx31-L34P, and Cx31-E100K (Figure 3B). Residues orthologous to all mutational sites in Cx31 are associated with disease in one to three other β-subtype connexins.

Generally, patients with Cx31 variants have more severe EKVP, affecting the same or more widespread areas, than patients with Cx30.3 variants (Table 2). However, disease severity can vary from mild to severe (Table 2). The trunk and limbs are most frequently impacted, with lesions less commonly affecting the face, buttocks, and body folds. Patients generally have both classical components of EKVP, though in some cases the presence or absence of the erythematous component was not described [56]. Two unrelated cases of advanced EKVP caused by the Cx31-F137L variant were amongst the most severe with hystrix-like hyperkeratosis, that reached 2 cm thick and became malodorous in at least one case [69]. Both cases showed partial clearance following retinoid therapy. A third, unrelated, case in a 3-year old patient had similar extent and distribution of lesions that had not reached such an advanced stage [70]. PPK is more prevalent in patients with Cx31 variants than those with Cx30.3 variants, as this feature was noted in patients with 11 of the 16 Cx31 variants known to date. However, the presence or absence of PPK did not correlate with any specific molecular defects observed in variants. Nevertheless, this observation may indicate that Cx31 plays a more important role in the thick skin of the palms and soles than Cx30.3.

EKVP associated with Cx43 variants

In humans, Cx43 is ubiquitously expressed in nearly all organs and cell types [15]. Given this range of tissue expression, it is not surprising that most of the ∼100 known disease-associated Cx43 variants cause multiorgan dysfunction as is the case for ODDD, which may on rare occasions present with disease phenotypes in the skin and its appendages [26,28,29]. Interestingly, select Cx43 variants involving non-ODDD associated amino acid residues are associated with at least five other disorders [26]. Of these variants, four dominant *de novo* Cx43 variants (Cx43-A44V, Cx43-E227D, Cx43-P283L, and Cx43-T290N) (Figure 3C, Table 3) have been suspected of being causal of EKVP suggesting these variants likely exhibit milder defects than ODDD-linked variants and/or manifest as pathologies only when found in specific cellular contexts [26,75,76]. Analysis of the cryogenic electron microscopy resolved structure of the C-terminal truncated Cx43 GJ channel (PDB ID 7F94) [8] revealed that these EKVP-associated residues occur in M1 (Cx43-A44V), M4 (Cx43-E227D), and the CTD (Cx43-P283L and Cx43-T290N) of the Cx43 polypeptide. As all four variants were *de novo*, there is no evidence of co-segregation with disease making it challenging to definitively state that these variants are causal of EKVP.

Clinical examination of patients with Cx43-linked EKVP revealed hyperkeratotic plaques and scaling most commonly impacting the limbs and frictional surfaces, with transient erythema or figurate erythema overlaying the hyperkeratotic lesions in most cases [75,76]. Disease onset began at 5–8 months of age in patients carrying the Cx43-A44V or Cx43-E227D variants, with lesions beginning at frictional surfaces and ultimately becoming generalized [75]. A patient carrying the Cx43-P283L variant had relatively mild disease presentation with symmetrical erythrokeratotic plaques on the hands, feet, wrists, and ankles with onset at 10 months of age [76]. Interestingly, an unrelated patient heterozygous for both the Cx43-P283L and Cx43-T290N variants had much more severe disease with earlier onset at 1 month of age. This patient presented with multiple hyperkeratotic plaques on the face, neck, elbows, wrists, hands, feet, limbs, and inguinal region [76]. This limited genotype-phenotype correlation could possibly suggest that the presence of two variant Cx43 alleles results in more severe disease than when a patient harbors only the Cx43-P283L variant, although this remains speculative. PPK and enlarged porcelain-white lunulae were also noted in all patients with the Cx43-E227D or Cx43-A44V variants [75] while PPK was also noted in the patient with Cx43-P283L/T290N [76].

Molecular mechanisms of EKVP associated connexin variants

Studies into 5 of the 13 known Cx30.3 variants have begun to elucidate the underlying molecular and cellular defects. The first Cx30.3 variant studied was Cx30.3-F137L, which showed impaired expression and GJ plaque

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

formation when stably expressed in HeLa cells [32]. Functional dye microinjection studies revealed decreased GJIC in cells stably co-expressing wildtype (WT) Cx31 and Cx30.3-F137L, consistent with a trans-dominant disruption of Cx31 GJ formation [32]. On the other hand, the Cx30.3-V37M variant was expressed at low levels and dominantly disrupted WT Cx30.3 protein levels in patient skin biopsies, either through impaired expression or enhanced degradation [50]. Finally, we recently reported that three Cx30.3 variants (Cx30.3-G12D, Cx30.3-T85P, and Cx30.3-F189Y) when expressed without a WT Cx30.3 counterpart, were GJIC incompetent due to a trafficking impairment resulting in their enriched localization within the endoplasmic reticulum [77]. Connexin-deficient rat epidermal keratinocytes expressing these variants were also more permeable to propidium iodide, which could reflect a HC gain-of-function and/or compromised membrane integrity due to cell death [77]. All three variants could be rescued into GJs by co-expressed connexins, though the functional status of intermixed channels was not tested [77]. While there are some subtle differences, all five Cx30.3 variants tested to date caused either partial or complete trafficking impairment, suggesting this may be a common mechanism underlying Cx30.3-linked EKVP. In addition, four variants (Cx30.3-G12D, Cx30.3-T85P, Cx30.3-F137L, and Cx30.3-F189Y) had deleterious effects on HC function and/or cell death, the former being a mechanism frequently associated with connexin-linked skin disorders [25,26,98–100]. Paracrine signaling via the release of ATP and/or other factors could drive inflammatory signaling and subsequent hyperproliferation of keratinocytes [101] thereby underlying the two classical components of EKVP. The Cx30.3-G12D, Cx30.3-T85P, Cx30.3-F137L, and Cx30.3-F189Y variants are also well documented to co-segregate with disease suggesting they are most likely causal of EKVP, while Cx30.3-V37M lacks evidence of segregating with disease and its molecular characterization has been more limited. All other Cx30.3 variants either have limited or no evidence of segregation with disease and/or have yet to be characterized further *in vitro*. For these reasons, the pathogenicity of Cx30.3-V37M and the other Cx30.3 variants remains ambiguous and awaits the genotyping of more related individuals with EKVP harboring these variants.

The molecular mechanisms associated with nine Cx31 variants have been interrogated *in vitro* and/or *in vivo*. All variants studied to date (Cx31-G12R, Cx31-G12D, Cx31-V30I, Cx31-L34P, Cx31-R42P, Cx31-G45E, Cx31-C86S, Cx31-F137L, and Cx31-L209F) exhibit some degree of trafficking impairment likely resulting in a reduction or loss of GJIC [55,60,78–82,84,86]. In the case of Cx31-R42P, this trafficking impairment was also evident in the stratum granulosum in lesional biopsies [78], while a different study showed this variant also formed constitutively active HCs and led to necrotic cell death [84]. Interestingly, Cx30.3-G12D, Cx31-G12R, Cx31-G12D, and Cx32-G12S all displayed trafficking/assembly defects pointing to the importance of G12 in β-subtype connexins [77,78,102,103]. Many, but not all, Cx31 variants induced cell death either downstream of unfolded protein response activation [81,84] or through other unidentified mechanisms [77,86]. It has been speculated that variant-induced cell death may activate a wound-healing response in surviving cells leading to hyperproliferation [78]. In addition, cell death could activate inflammatory signaling that could underlie the erythematous component of EKVP. However, the transient nature of the erythematous lesions could reflect that connexin variants have varying effects on different keratinocyte populations [78]. In addition to *in vitro* models, one transgenic mouse model heterozygous for Cx31-F137L partially re-capitulated an EKVP-like phenotype characterized by hyperproliferative skin but lacked the erythematous component [83]. A similar EKVP-like phenotype was observed in a mouse model overexpressing Cx31 in the skin, possibly suggesting that excessive Cx31 expression may dysregulate epidermal physiology [82]. Dye transfer assays, that report on the level of GJIC, in embryonic stem cells from transgenic mice and wound healing experiments in the same genetically-modified mice suggested the Cx31-F137L variant may trans-dominantly disrupt Cx43 [83]. The Cx31-G45E variant also disrupted Cx43 leading to its entrapment within the endoplasmic reticulum possibly reflecting a direct interaction between these isoforms [86]. Overall, there is strong evidence for the pathogenicity of five Cx31 variants (Cx31-G12R, Cx31-G12D, Cx31-R42P, Cx31-G45E, and Cx31-C86S), based on their co-segregation with disease and molecular manifestation *in vitro*. Most notably all five variants induced cell death and impaired GJ plaque formation in cultured cells. Although Cx31-F137L has been identified in only three unrelated patients to date, not only was the disease presentation strikingly similar across the patient cohort but this variant also partially modeled EKVP in transgenic mice, collectively supporting a pathogenic classification for this variant. Two remaining variants, Cx31-L135V and Cx31-T202N, co-segregate with disease, and are therefore more likely to be pathogenic, but the molecular mechanisms associated with these variants remain unknown. All other Cx31 variants lack evidence of co-segregation with disease, and thus cannot be definitively classified as pathogenic until more familial patients are identified, and a detailed genotype characterization is performed.

In EKVP patient skin sections, Cx43 primarily localized to intracellular compartments [75,76]. However, Cx43 occasionally localized to the cell membrane in skin sections from patients with the Cx43-P283L and Cx43-P283L/Cx43-T290N variants [76]. Cx43-A44V and Cx43-E227D tagged with hemagglutinin were detected within intracellular compartments with a sub-population co-localizing with the *cis*-Golgi marker GM130 when expressed in HeLa cells [75]. Interestingly, the expression of untagged Cx43-A44V and Cx43-E227D in HeLa cells led to the formation of clearly identifiable GJs [85]. In line with this, both variants formed functional GJs with similar conductance and voltage gating as WT Cx43 in *Xenopus laevis* oocytes [85]. Furthermore, *Xenopus* oocytes expressing either Cx43-A44V or Cx43-E227D exhibited pathologically augmented HC activity [85], a phenomenon linked to inflammatory diseases and connexin-linked skin disorders [19,99,104]. This HC gain-of-function possibly suggests a role for Cx43 HCs in nail physiology, as prominent white lunulae were observed in patients with the Cx43-A44V and Cx43-E227D variants.

In another study, we found GFP-tagged Cx43-P283L and Cx43-T290N, expressed alone or in various combinations, formed functional GJs in Cx43-ablated REKs and otherwise appeared benign aside from a modest change in turnover kinetics for the Cx43-P283L variant [88]. A similar delay in the Cx43 lifecycle due to phosphorylation of Cx43 on S368 contributes to the dysregulated connexin expression that occurs in psoriasis [105]. Under all tested conditions, no robust defects were detected for the Cx43-T290N variant except when co-expressed with Cx43-P283L, indicating Cx43-T290N may be a benign variant. Overall, augmented Cx43 HC function seems to correlate with moderate-to-severe disease phenotype induced by Cx43 variants, while variants that do not alter HC function may cause less severe disease. Collectively, *in vitro* data points to the pathogenicity of the Cx43-A44V and Cx43-E227D variants, while the effect of the Cx43-P283L variant remains ambiguous, and Cx43-T290N is unlikely to be pathogenic, based on available evidence. In all cases, establishing the pathogenicity of these variants requires more clinical evidence of co-segregation with disease.

Classification of EKVP-related connexin variants

The existing data supports the classification of connexin variants associated with EKVP into one of eight classes (Table 4), as has been done for connexin variants associated with deafness [106]. These classes

Table 4. Classification of connexin variants associated with EKVP as revealed by *in vivo* and/or *in vitro* interrogation.

Class	Connexin variant	References
I	**Cx30.3**: G12D, V37M, T85P, F137L, F189Y	[32,50,77]
	Cx31: G12R, G12D, V30I, L34P, R42P, G45E, C86S, F137L, L209F.	[55,60,78–82]
II	**Cx30.3**: G12D, T85P, F137L, F189Y	[32,50,77]
	Cx31: G12D, F137L	[83]
III	**Cx30.3**: G12D*, T85P*, F189Y*	[77]
	Cx31: R42P	[84]
	Cx43: A44V, E227D	[85]
IV	**Cx30.3:** G12D*, T85P*, F189Y*	[77]
	Cx31: G12R, G12D, R42P, G45E, C86S, F137L	[80,81,84,86,87]
V	**Cx30.3:** V37M, F137L	[32,50]
	Cx31: G45E, F137L	[83,86]
VI	**Cx43:** P283L	[88]
VII	**Cx43:** T290N	[88]
VIII	**Cx30.3:** R22H, S26Y, P61R, C86S, R98C, E99K, T130M, M190L	[38,49,51–55]
	Cx31: G12S, E47D, R98H, E100K, L135V, M158I, T202N	[52,54,57,58,66–68,71,76]

Class I variants exhibit impaired GJ formation. Class II variants form GJs with altered GJIC. Class III variants form leaky or hyperactive hemichannels. Class IV variants elicit cytotoxic effects when expressed in cells. Class V variants dominantly disrupt other connexins. Class VI variants have altered turnover properties. Class VII variants have no known deleterious properties or effects when expressed in cells. Class VIII variants have not been thoroughly studied.*Variants that may not fully belong to assigned class.

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

encompass variants with impaired GJ formation (Class I), altered GJIC (Class II), HC gain-of-function (Class III), cytotoxic effects (Class IV), dominant effects on co-expressed connexins (Class V), altered turnover (Class VI), no known deleterious effects (Class VII), and variants that have not been studied *in vitro* (Class VIII). Importantly, variants may exhibit multiple defects and can thus be categorized into more than one category. This classification system will be useful in guiding future research into the treatments aimed at alleviating the specific molecular defects that underlie EKVP.

Current and future treatments for EKVP

In five of the Cx30.3-associated EKVP case reports the authors discussed various attempts to treat patients including using topical (keratolytics, emollients, corticosteroids, retinoids) and systemic (retinoids) therapies [47,50,51,53,55]. It is important to note that these studies only report anecdotal responses to treatment in limited patient populations as, to the best of our knowledge, no clinical trials have been carried out. For example, topical keratolytics and emollients partially alleviated hyperkeratosis in a patient with the Cx30.3-V37M variant [50]. Furthermore, the retinoids etretinate and acitretin provided satisfactory symptomatic relief in patients harboring the Cx30.3-G12D and Cx30.3-C86S variants, although the extent of disease clearance was not described [47,51]. Encouragingly, acitretin treatment led to a complete clearance of skin disease in a patient with the Cx30.3-M190L variant [55]. It is unclear if ongoing treatment is required to maintain disease clearance in this patient, as significant disease relapse has been reported in at least one case following the discontinuation of retinoid treatment [69]. Despite these promising results in a limited patient cohort these treatments are not always effective as topical (corticosteroids, retinoids) and systemic (retinoids) treatments all failed to achieve long term clearance of disease in a patient homozygous for the Cx30.3-E99K variant [53].

Ten clinical case reports on Cx31-associated EKVP discussed various therapeutic strategies and their efficacy in patients [37,55,59,61,62,68–70,73,74]. Wilgoss et al. [37] reported that retinoid (etretinate or acitretin) treatment nearly cleared all clinical manifestations of EKVP linked to Cx31-R42P, while acitretin and/or etretinate showed partial to complete resolution of hyperkeratosis and mixed relief of erythema in six other studies involving ten patients harboring Cx31-G12D, Cx31-V30I, Cx31-R42P, Cx31-E100K or Cx31-F137L variants [55,59,61,69,73,74]. Responses to treatment in patients with Cx31-R42P [37,62] and Cx31-F137L [61,69] ranged from limited efficacy to near complete resolution. While the exact mechanism of action of retinoids is unknown, one putative mechanism is through the down-regulation of Cx31 by retinoids [107]. While this may indeed be useful in cases of cytotoxic Cx31 variants, caution is advised as at least one Cx30.3 variant is thought to cause EKVP through the dominant disruption of Cx31, and possibly other co-expressed connexins [32]. Thus, it is more likely that retinoids act independent of their effects on Cx31 expression. In another example, various therapies (topical corticosteroids, calcineurin inhibitors, and emollients) had limited efficacy in a 3 year old patient with EKVP [70]. Finally, therapeutic options for patients harboring Cx43 variants are virtually non-existent with evidence limited to reduced hyperkeratosis following acitretin treatment in a single patient harboring the Cx43-A44V variant [75].

Although retinoids represent the most promising treatment option for EKVP, these drugs are teratogens with long biological half-lives (~120 days for etretinate) that may have serious consequences including negatively affecting bone growth in children [108]. For these reasons, there is a pressing need to identify or develop new therapeutic options for EKVP patients. Learning points may be obtained from advances in other inheritable genetic diseases. As an example, the FDA has recently approved gene therapy approaches to cure transfusion-dependent β-thalassemia [109] and severe sickle cell disease [110]. These exciting developments provide promise that gene therapy approaches may 1 day provide a cure for other rare inherited disorders such as EKVP. For instance, ablation of a cytotoxic connexin variant or correction of a missense connexin variant could theoretically cure EKVP. However, significant financial hurdles and therapeutic delivery approaches will continue to hinder the development of genetic approaches to treat or cure EKVP. Alternatively, repurposing existing FDA-approved drugs may have utility in the treatment of EKVP. For instance, based on the finding that some Cx31 variants exhibit impaired trafficking and defects in GJ formation that could be overcome via temperature changes [82] drugs that improve protein folding (i.e. pharmacological or chemical chaperones) may be efficacious in treating EKVP. This treatment approach may also alleviate ER stress and cell death associated with some Cx31 variants. Indeed, chemical chaperones have successfully restored the trafficking of select disease-linked Cx26 [111] and Cx50 [112] variants *in vitro*. One important caveat to consider is that the restored trafficking of variants to the plasma membrane may still exhibit impaired GJIC or reveal additional

defects such as hyperactive HC function. To that end, EKVP caused by augmented HC function may be amenable to treatment with FDA-approved mefloquine and flufenamic acid that serve to block the HC channel pore [26,113,114]. In addition, a novel antibody capable of blocking hyperactive Cx30 HCs showed promising effects in a mouse model of Clouston syndrome, reducing both epidermal hyperproliferation and sebaceous gland hypertrophy [115]. Overall, the results from various pre-clinical studies are beginning to pave the way for novel therapeutic strategies that may effectively control or cure EKVP.

Future questions

Tremendous strides have been made over the years in understanding the consequences of EKVP-associated connexin variants both *in vitro* and, less commonly, *in vivo*. There is a growing body of evidence that augmented HC function may underpin connexin-linked disorders [19,98,104,114–116]. Hyperactive or leaky HC may be closely associated with eventual keratinocyte death. Future studies utilizing well characterized systems should continue to discern connexin variant-dependent HC dysfunction and HC-independent cell death. In addition, deeper insight is needed to understand the consequences of connexin variant expression on keratinocyte proliferation, differentiation and viability in transgenic mice and/or organotypic epidermis [117,118]. Once established and validated, these models will complement two-dimensional cell cultures as platforms to test the efficacy of future therapeutics that might include chemical chaperones, antibodies, or gene editing strategies.

Conclusion

EKVP is a complex heterogenous disorder in terms of its underlying genetics and pathogenic mechanisms. Significant progress has been made in characterizing the underlying molecular and cellular mechanisms associated with the known pathogenic variants, paving the way for the future development of treatments targeting specific underlying defects.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

D.W.L. was supported by the Canadian Institutes of Health Research (148630) and Natural Sciences and Engineering Research Council (03869). S.A.L. was supported by an Ontario Graduate Scholarship.

CRediT Author Contribution

Dale W. Laird: Supervision, Funding acquisition, Project administration, Writing — review and editing. **Sergiu A. Lucaciu**: Conceptualization, Resources, Data curation, Software, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing — original draft.

Acknowledgements

We would like to acknowledge Mengxi Sun who created an internal reference database of all known connexin gene variants linked to disease. The authors also thank Dr. Ruxandra-Maria Bogdan who helped estimate disease severity as presented in Tables 1–3.

Abbreviations

CL, cytoplasmic loop; CTD, carboxy-terminal domain; EKVP, erythrokeratodermia variabilis et progressiva; EKV, erythrokeratodermia variabilis; GJ, gap junction; GJIC, gap junctional intercellular communication; HC, hemichannel; ODDD, oculodentodigital dysplasia; PPK, palmoplantar keratoderma; PSEK, progressive symmetric erythrokeratoderma; WT, wildtype.

References

1 Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O. et al. (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 https://doi.org/10.1038/s41586-021-03819-2

2 Maeda, S., Nakagawa, S., Suga, M., Yamashita, E., Oshima, A., Fujiyoshi, Y. et al. (2009) Structure of the connexin 26 gap junction channel at 3.5 A resolution. *Nature* **458**, 597–602 https://doi.org/10.1038/nature07869

3 Brotherton, D.H., Savva, C.G., Ragan, T.J., Dale, N. and Cameron, A.D. (2022) Conformational changes and CO_2-induced channel gating in connexin26. *Structure* **30**, 697–706 e4 https://doi.org/10.1016/j.str.2022.02.010

Biochemical Journal (2024) **481** 1639–1655
https://doi.org/10.1042/BCJ20240374

4 Qi, C., Lavriha, P., Bayraktar, E., Vaithia, A., Schuster, D., Pannella, M. et al. (2023) Structures of wild-type and selected CMT1X mutant connexin 32 gap junction channels and hemichannels. *Sci. Adv.* **9**, eadh4890 https://doi.org/10.1126/sciadv.adh4890
5 Lee, H.J., Jeong, H., Hyun, J., Ryu, B., Park, K., Lim, H.H. et al. (2020) Cryo-EM structure of human Cx31.3/GJC3 connexin hemichannel. *Sci. Adv.* **6**, eaba4996 https://doi.org/10.1126/sciadv.aba4996
6 Lee, S.N., Cho, H.J., Jeong, H., Ryu, B., Lee, H.J., Kim, M. et al. (2023) Cryo-EM structures of human Cx36/GJD2 neuronal gap junction channel. *Nat. Commun.* **14**, 1347 https://doi.org/10.1038/s41467-023-37040-8
7 Myers, J.B., Haddad, B.G., O'Neill, S.E., Chorev, D.S., Yoshioka, C.C., Robinson, C.V. et al. (2018) Structure of native lens connexin 46/50 intercellular channels by cryo-EM. *Nature* **564**, 372–377 https://doi.org/10.1038/s41586-018-0786-7
8 Lee, H.J., Cha, H.J., Jeong, H., Lee, S.N., Lee, C.W., Kim, M. et al. (2023) Conformational changes in the human Cx43/GJA1 gap junction channel visualized using cryo-EM. *Nat. Commun.* **14**, 931 https://doi.org/10.1038/s41467-023-36593-y
9 Qi, C., Acosta Gutierrez, S., Lavriha, P., Othman, A., Lopez-Pigozzi, D., Bayraktar, E. et al. (2023) Structure of the connexin-43 gap junction channel in a putative closed state. *Elife* **12**, RP87616 https://doi.org/10.7554/eLife.87616
10 Mese, G., Richard, G. and White, T.W. (2007) Gap junctions: basic structure and function. *J. Invest. Dermatol.* **127**, 2516–2524 https://doi.org/10.1038/sj.jid.5700770
11 Bai, D., Wang, J., Li, T., Chan, R., Atalla, M., Chen, R.C. et al. (2021) Differential domain distribution of gnomAD- and disease-linked connexin missense variants. *Int. J. Mol. Sci.* **22**, 7832 https://doi.org/10.3390/ijms22157832
12 Bai, D., Yue, B. and Aoyama, H. (2018) Crucial motifs and residues in the extracellular loops influence the formation and specificity of connexin docking. *Biochim. Biophys. Acta Biomembr.* **1860**, 9–21 https://doi.org/10.1016/j.bbamem.2017.07.003
13 Alexander, D.B. and Goldberg, G.S. (2003) Transfer of biologically important molecules between cells through gap junction channels. *Curr. Med. Chem.* **10**, 2045–2058 https://doi.org/10.2174/0929867033456927
14 Leithe, E., Mesnil, M. and Aasen, T. (2018) The connexin 43 C-terminus: a tail of many tales. *Biochim. Biophys. Acta Biomembr.* **1860**, 48–64 https://doi.org/10.1016/j.bbamem.2017.05.008
15 Lucaciu, S.A., Leighton, S.E., Hauser, A., Yee, R. and Laird, D.W. (2023) Diversity in connexin biology. *J. Biol. Chem.* **299**, 105263 https://doi.org/10.1016/j.jbc.2023.105263
16 Laird, D.W. (2006) Life cycle of connexins in health and disease. *Biochem. J.* **394**, 527–543 https://doi.org/10.1042/BJ20051922
17 Lampe, P.D. and Laird, D.W. (2022) Recent advances in connexin gap junction biology. *Fac. Rev.* **11**, 14 https://doi.org/10.12703/r/11-14
18 Aasen, T. (2021) Connexins, innexins, and pannexins: from biology to clinical targets. *Biomolecules* **11**, 155 https://doi.org/10.3390/biom11020155
19 Peng, B., Xu, C., Wang, S., Zhang, Y. and Li, W. (2022) The role of connexin hemichannels in inflammatory diseases. *Biology* **11**, 1–20 https://doi.org/10.3390/biology11020237
20 Lillo, M.A. and Contreras, J.E. (2021) Opening the floodgates: an emerging role for Connexin-43 hemichannels in the heart. *Cell Calcium* **97**, 102410 https://doi.org/10.1016/j.ceca.2021.102410
21 Di, W.L., Rugg, E.L., Leigh, I.M. and Kelsell, D.P. (2001) Multiple epidermal connexins are expressed in different keratinocyte subpopulations including connexin 31. *J. Invest. Dermatol.* **117**, 958–964 https://doi.org/10.1046/j.0022-202x.2001.01468.x
22 Martin, P.E., Easton, J.A., Hodgins, M.B. and Wright, C.S. (2014) Connexins: sensors of epidermal integrity that are therapeutic targets. *FEBS Lett.* **588**, 1304–1314 https://doi.org/10.1016/j.febslet.2014.02.048
23 Faniku, C., Wright, C.S. and Martin, P.E. (2015) Connexins and pannexins in the integumentary system: the skin and appendages. *Cell. Mol. Life Sci.* **72**, 2937–2947 https://doi.org/10.1007/s00018-015-1969-0
24 Au, A., Shao, Q., White, K.K., Lucaciu, S.A., Esseltine, J.L., Barr, K. et al. (2020) Comparative analysis of Cx31 and Cx43 in differentiation-competent rodent keratinocytes. *Biomolecules* **10**, 1–22 https://doi.org/10.3390/biom10101443
25 Lilly, E., Sellitto, C., Milstone, L.M. and White, T.W. (2016) Connexin channels in congenital skin disorders. *Semin. Cell Dev. Biol.* **50**, 4–12 https://doi.org/10.1016/j.semcdb.2015.11.018
26 Laird, D.W. and Lampe, P.D. (2022) Cellular mechanisms of connexin-based inherited diseases. *Trends Cell Biol.* **32**, 58–69 https://doi.org/10.1016/j.tcb.2021.07.007
27 Laird, D.W., Naus, C.C. and Lampe, P.D. (2017) Snapshot: connexins and disease. *Cell* **170**, 1260–1260.e1 https://doi.org/10.1016/j.cell.2017.08.034
28 Paznekas, W.A., Boyadjiev, S.A., Shapiro, R.E., Daniels, O., Wollnik, B., Keegan, C.E. et al. (2003) Connexin 43 (*GJA1*) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. *Am. J. Hum. Genet.* **72**, 408–418 https://doi.org/10.1086/346090
29 Paznekas, W.A., Karczeski, B., Vermeer, S., Lowry, R.B., Delatycki, M., Laurence, F. et al. (2009) *GJA1* mutations, variants, and connexin 43 dysfunction as it relates to the oculodentodigital dysplasia phenotype. *Hum. Mutat.* **30**, 724–733 https://doi.org/10.1002/humu.20958
30 da Costa, M. (1925) Erythro-et keratodermia variabilis in a mother and a daughter. *Acta Derm. Venerol.* **6**, 255
31 Ishida-Yamamoto, A. (2016) Erythrokeratodermia variabilis et progressiva. *J. Dermatol.* **43**, 280–285 https://doi.org/10.1111/1346-8138.13220
32 Plantard, L., Huber, M., Macari, F., Meda, P. and Hohl, D. (2003) Molecular interaction of connexin 30.3 and connexin 31 suggests a dominant-negative mechanism associated with erythrokeratodermia variabilis. *Hum. Mol. Genet.* **12**, 3287–3294 https://doi.org/10.1093/hmg/ddg364
33 Macari, F., Landau, M., Cousin, P., Mevorah, B., Brenner, S., Panizzon, R. et al. (2000) Mutation in the gene for connexin 30.3 in a family with erythrokeratodermia variabilis. *Am. J. Hum. Genet.* **67**, 1296–1301 https://doi.org/10.1016/S0002-9297(07)62957-7
34 van Steensel, M. (2004) Does progressive symmetric erythrokeratoderma exist? *Br. J. Dermatol.* **150**, 1043–1045 https://doi.org/10.1111/j.1365-2133.2004.05965.x
35 Duchatelet, S. and Hovnanian, A. (2015) Erythrokeratodermia variabilis et progressiva allelic to oculo-dento-digital dysplasia. *J. Invest. Dermatol.* **135**, 1475–1478 https://doi.org/10.1038/jid.2014.535
36 Hendrix, Jr, J.D. and Greer, K.E. (1995) Erythrokeratodermia variabilis present at birth: case report and review of the literature. *Pediatr. Dermatol.* **12**, 351–354 https://doi.org/10.1111/j.1525-1470.1995.tb00200.x
37 Wilgoss, A., Leigh, I.M., Barnes, M.R., Dopping-Hepenstal, P., Eady, R.A., Walter, J.M. et al. (1999) Identification of a novel mutation R42P in the gap junction protein beta-3 associated with autosomal dominant erythrokeratoderma variabilis. *J. Invest. Dermatol.* **113**, 1119–1122 https://doi.org/10.1046/j.1523-1747.1999.00792.x

38 Richard, G., Brown, N., Rouan, F., Van der Schroeff, J.G., Bijlsma, E., Eichenfield, L.F. et al. (2003) Genetic heterogeneity in erythrokeratodermia variabilis: novel mutations in the connexin gene *GJB4* (Cx30.3) and genotype-phenotype correlations. *J. Invest. Dermatol.* **120**, 601–609 https://doi.org/10.1046/j.1523-1747.2003.12080.x
39 Boyden, L.M., Vincent, N.G., Zhou, J., Hu, R., Craiglow, B.G., Bayliss, S.J. et al. (2017) Mutations in *KDSR* cause recessive progressive symmetric erythrokeratoderma. *Am. J. Hum. Genet.* **100**, 978–984 https://doi.org/10.1016/j.ajhg.2017.05.003
40 Duchatelet, S., Boyden, L.M., Ishida-Yamamoto, A., Zhou, J., Guibbal, L., Hu, R. et al. (2019) Mutations in *PERP* cause dominant and recessive keratoderma. *J. Invest. Dermatol.* **139**, 380–390 https://doi.org/10.1016/j.jid.2018.08.026
41 Patel, N., Alkeraye, S., Alobeid, E., Alshidi, T., Helaby, R., Abdulwahab, F. et al. (2020) Confirming the recessive inheritance of *PERP*-related erythrokeratoderma. *Clin. Genet.* **97**, 661–665 https://doi.org/10.1111/cge.13699
42 Shah, K., Ansar, M., Mughal, Z.U., Khan, F.S., Ahmad, W., Ferrara, T.M. et al. (2017) Recessive progressive symmetric erythrokeratoderma results from a homozygous loss-of-function mutation of *KRT83* and is allelic with dominant monilethrix. *J. Med. Genet.* **54**, 186–189 https://doi.org/10.1136/jmedgenet-2016-104107
43 Wang, H., Xu, Z., Lee, B.H., Vu, S., Hu, L., Lee, M. et al. (2019) Gain-of-function mutations in *TRPM4* activation gate cause progressive symmetric erythrokeratodermia. *J. Invest. Dermatol.* **139**, 1089–1097 https://doi.org/10.1016/j.jid.2018.10.044
44 Hotz, A., Folster-Holst, R., Oji, V., Bourrat, E., Frank, J., Marrakchi, S. et al. (2024) Erythrokeratodermia variabilis-like phenotype in patients carrying *ABCA12* mutations. *Genes* **15**, 288 https://doi.org/10.3390/genes15030288
45 Ishida-Yamamoto, A. (2003) Loricrin keratoderma: a novel disease entity characterized by nuclear accumulation of mutant loricrin. *J. Dermatol. Sci.* **31**, 3–8 https://doi.org/10.1016/S0923-1811(02)00143-3
46 Macfarlane, A.W., Chapman, S.J. and Verbov, J.L. (1991) Is erythrokeratoderma one disorder? A clinical and ultrastructural study of two siblings. *Br. J. Dermatol.* **124**, 487–491 https://doi.org/10.1111/j.1365-2133.1991.tb00632.x
47 van Steensel, M.A., Oranje, A.P., van der Schroeff, J.G., Wagner, A. and van Geel, M. (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**, 657–661 https://doi.org/10.1002/ajmg.a.32744
48 de Oliveira, R.T.G., Christofolini, D.M., Criado, P.R., Lacaz Martins, E. and Kelsell, D. (2020) Machado Filho CDS. Clinical variability of the GJB4:c.35G > A gene variant: a study of a large Brazilian erythrokeratodermia pedigree. *Int. J. Dermatol.* **59**, 722–725 https://doi.org/10.1111/ijd.14894
49 Dai, S., Wang, H. and Lin, Z. (2020) Novel and recurrent mutations in *GJB3* and *GJB4* cause erythrokeratodermia variabilis et progressiva. *Indian J. Dermatol. Venereol. Leprol.* **86**, 87–90 https://doi.org/10.4103/ijdvl.IJDVL_926_18
50 Zhang, X., Xu, P., Lu, J., Ding, Y., Gu, J. and Shi, Y. (2022) Erythrokeratodermia variabilis et progressiva due to a novel mutation in *GJB4*. *Exp. Dermatol.* **31**, 594–599 https://doi.org/10.1111/exd.14490
51 Sbidian, E., Bousseloua, N., Jonard, L., Leclerc-Mercier, S., Bodemer, C. and Hadj-Rabia, S. (2013) Novel mutation in *GJB4* gene (connexin 30.3) in a family with erythrokeratodermia variabilis. *Acta Derm. Venereol.* **93**, 193–195 https://doi.org/10.2340/00015555-1436
52 Liu, H., Liu, H., Fu, X.A., Yu, Y.X., Zhou, G.Z., Lu, X.M. et al. (2012) Mutation analysis of GJB3 and GJB4 in Chinese patients with erythrokeratodermia variabilis. *J. Dermatol.* **39**, 400–401 https://doi.org/10.1111/j.1346-8138.2011.01314.x
53 Kokotas, H., Papagiannaki, K., Grigoriadou, M., Petersen, M.B. and Katsarou, A. (2012) Erythrokeratodermia variabilis: report of two cases and a novel missense variant in *GJB4* encoding connexin 30.3. *Eur. J. Dermatol.* **22**, 182–186 https://doi.org/10.1684/ejd.2011.1617
54 Scott, C.A., O'Toole, E.A., Mohungoo, M.J., Messenger, A. and Kelsell, D.P. (2011) Novel and recurrent connexin 30.3 and connexin 31 mutations associated with erythrokeratoderma variabilis. *Clin. Exp. Dermatol.* **36**, 88–90 https://doi.org/10.1111/j.1365-2230.2010.03945.x
55 Common, J.E., O'Toole, E.A., Leigh, I.M., Thomas, A., Griffiths, W.A., Venning, V. et al. (2005) Clinical and genetic heterogeneity of erythrokeratoderma variabilis. *J. Invest. Dermatol.* **125**, 920–927 https://doi.org/10.1111/j.0022-202X.2005.23919.x
56 Richard, G., Smith, L.E, Bailey, R.A, Itin, P., Hohl, D., Epstein, Jr, E.H. et al. (1998) Mutations in the human connexin gene *GJB3* cause erythrokeratodermia variabilis. *Nat. Genet.* **20**, 366–369 https://doi.org/10.1038/3840
57 Richard, G. (2000) Connexins: a connection with the skin. *Exp. Dermatol.* **9**, 77–96 https://doi.org/10.1034/j.1600-0625.2000.009002077.x
58 Deng, Y., Wang, H., Mou, Y., Zeng, Q. and Xiong, X. (2019) Exome sequencing identifies novel compound heterozygous mutations in GJB3 gene that cause erythrokeratodermia variabilis et progressiva. *Australas. J. Dermatol.* **60**, e87–e89 https://doi.org/10.1111/ajd.12887
59 Fuchs-Telem, D., Pessach, Y., Mevorah, B., Shirazi, I., Sarig, O. and Sprecher, E. (2011) Erythrokeratoderma variabilis caused by a recessive mutation in GJB3. *Clin. Exp. Dermatol.* **36**, 406–411 https://doi.org/10.1111/j.1365-2230.2010.03986.x
60 Gottfried, I., Landau, M., Glaser, F., Di, W.L., Ophir, J., Mevorah, B. 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**, 1311–1316 https://doi.org/10.1093/hmg/11.11.1311
61 Richard, G., Brown, N., Smith, L.E., Terrinoni, A., Melino, G., Mackie, R.M. et al. (2000) The spectrum of mutations in erythrokeratodermias–novel and de novo mutations in GJB3. *Hum. Genet.* **106**, 321–329 https://doi.org/10.1007/s004390051045
62 Ikeya, S., Urano, S., Sakabe, J.-i., Ito, T. and Tokura, Y. (2013) Erythrokeratodermia variabilis: first Japanese case documenting GJB3 mutation. *J. Dermatol.* **40**, 402–403 https://doi.org/10.1111/1346-8138.12101
63 Renner, R., Paasch, U., Simon, J.C., Froster, U.G. and Heinritz, W. (2008) A new mutation in the GJB3 gene in a patient with erythrokeratodermia variabilis. *J. Eur. Acad. Dermatol. Venereol.* **22**, 750–751 https://doi.org/10.1111/j.1468-3083.2007.02447.x
64 Wang, W., Liu, L.H., Chen, G., Gao, M., Zhu, J., Zhou, F.S. et al. (2012) A missense mutation in the GJB3 gene responsible for erythrokeratodermia variabilis in a Chinese family. *Clin. Exp. Dermatol.* **37**, 919–921 https://doi.org/10.1111/j.1365-2230.2012.04406.x
65 Takeichi, T., Sugiura, K., Hsu, C.K., Nomura, T., Takama, H., Simpson, M.A. et al. (2016) Erythrokeratoderma variabilis caused by p.Gly45Glu in connexin 31: importance of the first extracellular loop glycine residue for gap junction function. *Acta Derm. Venereol* **96**, 557–559 https://doi.org/10.2340/00015555-2307
66 Wang, Z.X., Lu, W.S., Li, H., Lin, D., Zhou, F.S., Sun, L.D. et al. (2011) A novel GJB3 (Cx31) missense mutation in a Chinese patient with erythrokeratodermia variabilis. *J. Eur. Acad. Dermatol. Venereol.* **25**, 113–115 https://doi.org/10.1111/j.1468-3083.2010.03691.x
67 Gao, Y., Zhang, Q., Zhang, S., Yang, L., Liu, Y., Liu, Y. et al. (2022) A connexin gene (*GJB3*) mutation in a Chinese family with erythrokeratodermia variabilis, ichthyosis and nonsyndromic hearing loss: case report and mutations update. *Front. Genet.* **13**, 797124 https://doi.org/10.3389/fgene.2022.797124

68 Terrinoni, A., Leta, A., Pedicelli, C., Candi, E., Ranalli, M., Puddu, P. et al. (2004) A novel recessive connexin 31 (GJB3) mutation in a case of erythrokeratodermia variabilis. *J. Invest. Dermatol.* **122**, 837–839 https://doi.org/10.1111/j.0022-202X.2004.22311.x
69 Glatz, M., van Steensel, M.A., van Geel, M., Steijlen, P.M. and Wolf, P. (2011) An unusual missense mutation in the GJB3 gene resulting in severe erythrokeratodermia variabilis. *Acta Derm. Venereol.* **91**, 714–715 https://doi.org/10.2340/00015555-1135
70 Imura, K., Ikeya, S., Ogata, T. and Tokura, Y. (2020) Erythrokeratodermia variabilis et progressiva with a rare GJB3 mutation. *J. Dermatol.* **47**, e111–e113 https://doi.org/10.1111/1346-8138.15206
71 Sugiura, K., Arima, M., Matsunaga, K. and Akiyama, M. (2015) The novel GJB3 mutation p.Thr202Asn in the M4 transmembrane domain underlies erythrokeratodermia variabilis. *Br. J. Dermatol.* **173**, 309–311 https://doi.org/10.1111/bjd.13641
72 Feldmeyer, L., Plantard, L., Mevorah, B., Huber, M. and Hohl, D. (2005) Novel mutation of connexin 31 causing erythrokeratoderma variabilis. *Br. J. Dermatol.* **152**, 1072–1074 https://doi.org/10.1111/j.1365-2133.2005.06561.x
73 Morley, S.M., White, M.I., Rogers, M., Wasserman, D., Ratajczak, P., McLean, W.H. et al. (2005) A new, recurrent mutation of GJB3 (Cx31) in erythrokeratodermia variabilis. *Br. J. Dermatol.* **152**, 1143–1148 https://doi.org/10.1111/j.1365-2133.2005.06610.x
74 Otaguchi, R., Kawakami, T., Matsuoka, M., Kimura, S., Soma, Y., Matsuda, M. et al. (2014) A sporadic elder case of erythrokeratodermia variabilis with a single base-pair transversion in GJB3 gene successfully treated with systemic vitamin A derivative. *J. Dermatol.* **41**, 1016–1018 https://doi.org/10.1111/1346-8138.12628
75 Boyden, L.M., Craiglow, B.G., Zhou, J., Hu, R., Loring, E.C., Morel, K.D. et al. (2015) Dominant de novo mutations in *GJA1* cause erythrokeratodermia variabilis et progressiva, without features of oculodentodigital dysplasia. *J. Invest. Dermatol.* **135**, 1540–1547 https://doi.org/10.1038/jid.2014.485
76 Li, C., Liang, J., Chen, P., Zeng, K., Xue, R., Tian, X. et al. (2019) Two de novo *GJA1* mutation in two sporadic patients with erythrokeratodermia variabilis et progressiva. *Mol. Genet. Genomic Med.* **7**, e670 https://doi.org/10.1002/mgg3.670
77 Lucaciu, S.A., Figliuzzi, R., Neumann, R., Nazarali, S., Del Sordo, L., Leighton, S.E. et al. (2023) *GJB4* variants linked to skin disease exhibit a trafficking deficiency en route to gap junction formation that can be restored by co-expression of select connexins. *Front. Cell Dev. Biol.* **11**, 1073805 https://doi.org/10.3389/fcell.2023.1073805
78 Di, W.L., Monypenny, J., Common, J.E., Kennedy, C.T., Holland, K.A., Leigh, I.M. et al. (2002) Defective trafficking and cell death is characteristic of skin disease-associated connexin 31 mutations. *Hum. Mol. Genet.* **11**, 2005–2014 https://doi.org/10.1093/hmg/11.17.2005
79 Rouan, F., Lo, C.W., Fertala, A., Wahl, M., Jost, M., Rodeck, U. et al. (2003) Divergent effects of two sequence variants of GJB3 (G12D and R32W) on the function of connexin 31 in vitro. *Exp. Dermatol.* **12**, 191–197 https://doi.org/10.1034/j.1600-0625.2003.120210.x
80 He, L.Q., Liu, Y., Cai, F., Tan, Z.P., Pan, Q., Liang, D.S. et al. (2005) Intracellular distribution, assembly and effect of disease-associated connexin 31 mutants in HeLa cells. *Acta Biochim. Biophys. Sin.* **37**, 547–554 https://doi.org/10.1111/j.1745-7270.2005.00080.x
81 Tattersall, D., Scott, C.A., Gray, C., Zicha, D. and Kelsell, D.P. (2009) EKV mutant connexin 31 associated cell death is mediated by ER stress. *Hum. Mol. Genet.* **18**, 4734–4745 https://doi.org/10.1093/hmg/ddp436
82 Tang, C., Chen, X., Chi, J., Yang, D., Liu, S., Liu, M. et al. (2015) Pathogenic Cx31 is un/misfolded to cause skin abnormality via a Fos/JunB-mediated mechanism. *Hum. Mol. Genet.* **24**, 6054–6065 https://doi.org/10.1093/hmg/ddv317
83 Schnichels, M., Worsdorfer, P., Dobrowolski, R., Markopoulos, C., Kretz, M., Schwarz, G. et al. (2007) The connexin31 F137L mutant mouse as a model for the human skin disease erythrokeratodermia variabilis (EKV). *Hum. Mol. Genet.* **16**, 1216–1224 https://doi.org/10.1093/hmg/ddm068
84 Chi, J., Li, L., Liu, M., Tan, J., Tang, C., Pan, Q. et al. (2012) Pathogenic connexin-31 forms constitutively active hemichannels to promote necrotic cell death. *PLoS One* **7**, e32531 https://doi.org/10.1371/journal.pone.0032531
85 Srinivas, M., Jannace, T.F., Cocozzelli, A.G., Li, L., Slavi, N., Sellitto, C. et al. (2019) Connexin43 mutations linked to skin disease have augmented hemichannel activity. *Sci. Rep.* **9**, 19 https://doi.org/10.1038/s41598-018-37221-2
86 Easton, J.A., Albuloushi, A.K., Kamps, M.A.F., Brouns, G., Broers, J.L.V., Coull, B.J. et al. (2019) A rare missense mutation in *GJB3* (Cx31G45E) is associated with a unique cellular phenotype resulting in necrotic cell death. *Exp. Dermatol.* **28**, 1106–1113 https://doi.org/10.1111/exd.13542
87 Diestel, S., Richard, G., Doring, B. and Traub, O. (2002) Expression of a connexin31 mutation causing erythrokeratodermia variabilis is lethal for HeLa cells. *Biochem. Biophys. Res. Commun.* **296**, 721–728 https://doi.org/10.1016/s0006-291x(02)00929-4
88 Lucaciu, S.A., Shao, Q., Figliuzzi, R., Barr, K., Bai, D. and Laird, D.W. (2022) Interrogation of carboxy-terminus localized *GJA1* variants associated with erythrokeratodermia variabilis et progressiva. *Int. J. Mol. Sci.* **23**, 1–16 https://doi.org/10.3390/ijms23010486
89 Arita, K., Akiyama, M., Tsuji, Y., Onozuka, T. and Shimizu, H. (2003) Erythrokeratoderma variabilis without connexin 31 or connexin 30.3 gene mutation: immunohistological, ultrastructural and genetic studies. *Acta Derm. Venereol.* **83**, 266–270 https://doi.org/10.1080/00015550310016517
90 Nakamura, M. (2007) Erythrokeratoderma variabilis without GJB3 or GJB4 mutation: a review of Japanese patients. *Br. J. Dermatol.* **157**, 410–411 https://doi.org/10.1111/j.1365-2133.2007.08023.x
91 Richard, G. (2005) Connexin disorders of the skin. *Clin. Dermatol.* **23**, 23–32 https://doi.org/10.1016/j.clindermatol.2004.09.010
92 Okamoto, R., Goto, I., Nishimura, Y., Kobayashi, I., Hashizume, R., Yoshida, Y. et al. (2020) Gap junction protein beta 4 plays an important role in cardiac function in humans, rodents, and zebrafish. *PLoS One* **15**, e0240129 https://doi.org/10.1371/journal.pone.0240129
93 Araya-Secchi, R., Perez-Acle, T., Kang, S.G., Huynh, T., Bernardin, A., Escalona, Y. et al. (2014) Characterization of a novel water pocket inside the human Cx26 hemichannel structure. *Biophys. J.* **107**, 599–612 https://doi.org/10.1016/j.bpj.2014.05.037
94 Zou, J., Salarian, M., Chen, Y., Veenstra, R., Louis, C.F. and Yang, J.J. (2014) Gap junction regulation by calmodulin. *FEBS Lett.* **588**, 1430–1438 https://doi.org/10.1016/j.febslet.2014.01.003
95 Peracchia, C. (2020) Calmodulin-mediated regulation of gap junction channels. *Int. J. Mol. Sci.* **21**, 485 https://doi.org/10.3390/ijms21020485
96 Peracchia, C. (2024) Gap junction channel regulation: a tale of two gates-voltage sensitivity of the chemical gate and chemical sensitivity of the fast voltage gate. *Int. J. Mol. Sci.* **25**, 982 https://doi.org/10.3390/ijms25020982
97 Kelsell, D.P., Di, W.-L. and Houseman, M.J. (2001) Connexin mutations in skin disease and hearing loss. *Am. J. Hum. Genet.* **68**, 559–568 https://doi.org/10.1086/318803
98 Srinivas, M., Verselis, V.K. and White, T.W. (2018) Human diseases associated with connexin mutations. *Biochim. Biophys. Acta Biomembr.* **1860**, 192–201 https://doi.org/10.1016/j.bbamem.2017.04.024
99 Cocozzelli, A.G. and White, T.W. (2019) Connexin 43 mutations lead to increased hemichannel functionality in skin disease. *Int. J. Mol. Sci.* **20**, 1–15 https://doi.org/10.3390/ijms20246186

100 Retamal, M.A., Reyes, E.P., Garcia, I.E. Pinto, B., Martinez, A.D. and Gonzalez, C. (2015) Diseases associated with leaky hemichannels. *Front. Cell. Neurosci.* **9**, 267 https://doi.org/10.3389/fncel.2015.00267
101 García-Vega, L., O'Shaughnessy, E.M., Jan, A., Bartholomew, C. and Martin, P.E. (2019) Connexin 26 and 43 play a role in regulating proinflammatory events in the epidermis. *J. Cell. Physiol.* **234**, 15594–15606 https://doi.org/10.1002/jcp.28206
102 Deschênes, S.M., Walcott, J.L., Wexler, T.L., Scherer, S.S. and Fischbeck, K.H. (1997) Altered trafficking of mutant Connexin32. *J. Neurosci.* **17**, 9077–9084 https://doi.org/10.1523/jneurosci.17-23-09077.1997
103 Bailey, R.A., Beahm, D.L. and Skerrett, I.M. (2021) The complex and critical role of glycine 12 (G12) in beta-connexins of human skin. *Int. J. Mol. Sci.* **22**, 1–15 https://doi.org/10.3390/ijms22052615
104 Levit, N.A. and White, T.W. (2015) Connexin hemichannels influence genetically determined inflammatory and hyperproliferative skin diseases. *Pharmacol. Res.* **99**, 337–343 https://doi.org/10.1016/j.phrs.2015.07.015
105 O'Shaughnessy, E.M., Duffy, W., Garcia-Vega, L., Hussey, K., Burden, A.D., Zamiri, M. et al. (2021) Dysregulation of connexin expression plays a pivotal role in psoriasis. *Int. J. Mol. Sci.* **22**, 6060 https://doi.org/10.3390/ijms22116060
106 Hoang Dinh, E., Ahmad, S., Chang, Q., Tang, W., Stong, B. and Lin, X. (2009) Diverse deafness mechanisms of connexin mutations revealed by studies using in vitro approaches and mouse models. *Brain Res.* **1277**, 52–69 https://doi.org/10.1016/j.brainres.2009.02.008
107 Grümmer, R., Hellmann, P., Traub, O., Soares, M.J., El-Sabban, M.E. and Winterhager, E. (1996) Regulation of Connexin31 gene expression upon retinoic acid treatment in rat choriocarcinoma cells. *Exp. Cell Res.* **227**, 23–32 https://doi.org/10.1006/excr.1996.0245
108 Zaenglein, A.L., Levy, M.L., Stefanko, N.S., Benjamin, L.T., Bruckner, A.L., Choate, K. et al. (2021) Consensus recommendations for the use of retinoids in ichthyosis and other disorders of cornification in children and adolescents. *Pediatr. Dermatol.* **38**, 164–180 https://doi.org/10.1111/pde.14408
109 Locatelli, F., Lang, P., Wall, D., Meisel, R., Corbacioglu, S., Li, A.M. et al. (2024) Exagamglogene autotemcel for transfusion-dependent beta-thalassemia. *N. Engl. J. Med.* **390**, 1663–1676 https://doi.org/10.1056/NEJMoa2309673
110 Frangoul, H., Locatelli, F., Sharma, A., Bhatia, M., Mapara, M., Molinari, L. et al. (2024) Exagamglogene autotemcel for severe sickle cell disease. *N. Engl. J. Med.* **390**, 1649–1662 https://doi.org/10.1056/NEJMoa2309676
111 Wang, D., Wang, H., Fan, L., Ludwig, T., Wegner, A., Stahl, F. et al. (2023) A chemical chaperone restores connexin 26 mutant activity. *ACS Pharmacol. Transl. Sci.* **6**, 997–1005 https://doi.org/10.1021/acsptsci.3c00056
112 Jara, O., Minogue, P.J., Berthoud, V.M. and Beyer, E.C. (2018) Chemical chaperone treatment improves levels and distributions of connexins in Cx50D47A mouse lenses. *Exp. Eye Res.* **175**, 192–198 https://doi.org/10.1016/j.exer.2018.06.015
113 Levit, N.A., Sellitto, C., Wang, H.Z., Li, L., Srinivas, M., Brink, P.R. et al. (2015) Aberrant connexin26 hemichannels underlying keratitis-ichthyosis-deafness syndrome are potently inhibited by mefloquine. *J. Invest. Dermatol.* **135**, 1033–1042 https://doi.org/10.1038/jid.2014.408
114 Sellitto, C., Li, L. and White, T.W. (2021) Connexin hemichannel inhibition ameliorates epidermal pathology in a mouse model of keratitis ichthyosis deafness syndrome. *Sci. Rep.* **11**, 24118 https://doi.org/10.1038/s41598-021-03627-8
115 Kuang, Y., Zorzi, V., Buratto, D., Ziraldo, G., Mazzarda, F., Peres, C. et al. (2020) A potent antagonist antibody targeting connexin hemichannels alleviates Clouston syndrome symptoms in mutant mice. *EBioMedicine* **57**, 102825 https://doi.org/10.1016/j.ebiom.2020.102825
116 Bruzzone, R. and White, T.W. (2020) Connexin hemichannel inhibition improves skin pathology in Clouston syndrome mice. *EBioMedicine* **57**, 102856 https://doi.org/10.1016/j.ebiom.2020.102856
117 Jakobsen, N.D., Kaiser, K., Ebbesen, M.F., Lauritsen, L., Gjerstorff, M.F., Kuntsche, J. et al. (2022) The ROC skin model: a robust skin equivalent for permeation and live cell imaging studies. *Eur. J. Pharm. Sci.* **178**, 106282 https://doi.org/10.1016/j.ejps.2022.106282
118 Maher, A.C., Thomas, T., Riley, J.L., Veitch, G., Shao, Q. and Laird, D.W. (2005) Rat epidermal keratinocytes as an organotypic model for examining the role of Cx43 and Cx26 in skin differentiation. *Cell Commun. Adhes.* **12**, 219–230 https://doi.org/10.1080/15419060500511818