Genetic Heterogeneity of KID Syndrome: Identification of a Cx30 Gene (GJB6) Mutation in a Patient with KID Syndrome and Congenital Atrichia

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Connexins are integral membrane proteins forming aqueous gap junction channels that allow the diffusional exchange of ions and small metabolites between cells, thus coordinating metabolic activities in multicellular tissues. Dominant mutations in the Cx26 gene GJB2 have been shown to cause keratitis-ichthyosis-deafness (KID) syndrome, palmoplantar keratoderma associated with hearing loss, and Vohwinkel syndrome. Missense mutations in the closely related Cx30 gene GJB6 underlie Clouston syndrome (autosomal dominant hidrotic ectodermal dysplasia). We report a 6-y-old boy with phenotypic characteristics of KID syndrome as well as atrichia. In contrast to other KID syndrome patients, molecular analysis of the connexin gene GJB2 did not disclose a pathogenic mutation, although the patient was homozygous for a common polymorphism (V27I) in the coding sequence of Cx26. Nevertheless, screening of GJB6 revealed a heterozygous missense mutation (V37E) predicted to alter sequence and charge of the first transmembrane helix of Cx30, which was previously implicated in Clouston syndrome (Smith et al, 2002). The presence of a pathogenic Cx30 mutation and the lack of a pathologic molecular change in Cx26 in this patient, whose clinical features predominantly resemble KID syndrome, suggest genetic heterogeneity of KID syndrome and underscore that mutations in Cx30, similar to those in Cx26 or Cx31, can cause different phenotypes. Based on our results, connexin gene mutations should be considered in patients presenting with congenital sensorineural hearing loss and disorders of cornification, and screening of several connexin genes with known cutaneous phenotype, such as those for Cx26, Cx30, Cx30.3, and Cx31, may be required.

Key words: connexin-30/connexin-26/hearing loss/palmoplantar keratoderma/gap junctions


Keratitis-ichthyosis-deafness (KID) syndrome (OMIM 148210) and Clouston syndrome (hidrotic ectodermal dysplasia; OMIM 129500) are rare autosomal dominant ectodermal dysplasias due to germline mutations in the connexin genes GJB2 and GJB6, respectively, which cluster at chromosome 13q11 and encode the closely related gap junction proteins Cx26 and Cx30 (Lamartine et al, 2000; Richard et al, 2002; van Steensel et al, 2002). KID and Clouston syndrome share a few overlapping features, such as nail dystrophy, hair loss, and palmoplantar keratoderma. Although the disorders are usually readily distinguishable on clinical grounds in patients with a full-blown picture, the differentiation can be challenging in others.

KID syndrome is a rare, mostly sporadic disorder with a broad spectrum of cutaneous features, including erythrokeratoderma, follicular hyperkeratoses, psoriasiform or verrucous plaques, acanthosis, and palmoplantar keratoderma (PPK) often described as reticulated or pitted (reviewed in Caceres-Rios et al, 1996; Sundaram et al, 2003). Between 90% and 93% of affected individuals have prelingual sensorineural hearing loss (SNHL) (Caceres-Rios et al, 1996; Sundaram et al, 2003), which is often bilateral and severe to profound (Sybert, 1997; Szymko-Bennett et al, 2002). Corneal symptoms often manifest during childhood with photophobia, punctate keratitis, and progressive corneal vascularization that may later result in visual decline and blindness (Caceres-Rios et al, 1996). Many KID syndrome patients were reported to have sparse or lusterless hair, but only approximately 10%–23% have congenital absence of hair (Caceres-Rios et al, 1996; Sundaram et al, 2003). Other findings that are variably present include nail dystrophy, dental anomalies, hypohidrosis, growth delay, increased susceptibility to cutaneous infections, and a propensity for developing squamous cell carcinomas (Caceres-Rios et al, 1996). KID syndrome is caused by autosomal dominant missense mutations in the connexin gene GJB2. A common mutation that has been reported in 12 of 15 unrelated patients tested results in the replacement of aspartic acid 50 with asparagine (D50N) in the first extracellular loop of Cx26 (Richard et al, 2002; van Geel et al, 2002; van Steensel et al, 2002; Alvarez et al, 2003; Yotsumoto et al, 2003). This sequence motif is highly conserved among all connexins and any change in composition and charge is likely detrimental for the formation and function of gap junction channels (Rubin et al, 1992; White et al, 1995). Other mutations cluster in the intracellular amino terminus of this gap junction protein (Richard et al, 2002).
Clouston syndrome is an autosomal dominant ectodermal dysplasia particularly common among the French–Canadian population due to a founder effect (Lamartine et al., 2000). The principal features include nail dystrophy, hair loss, and PPK. Nail abnormalities are usually present in all patients and may range from almost normal appearing nails to micro- or anonychia (Clouston, 1929; Clouston, 1939; Rajagopalan and Tay, 1977; Hassed et al., 1996). Paronychia and nail infections are common (Clouston, 1929; Kibar et al., 1996). Hair abnormalities manifest at birth or later as atrichia or hypotrichosis with brittle, slow-growing hair, and may be progressive. Some but not all patients develop diffuse PPK and discrete hyperpigmentations over joints, whereas strabismus and cataracts are rare (Clouston, 1929; Clouston, 1939; Hazen, 1980). Sweat function and teeth are normal (Rajagopalan and Tay, 1977; Hassed et al., 1996; Sybert, 1997). Unlike in KID syndrome, patients with Clouston syndrome lack vascularizing keratitis and SNHL. The latter has only been described in families with autosomal recessive onychodystrophy (Feinmesser and Zelig, 1961) or autosomal dominant onychodystrophy with additional abnormalities (conical teeth, hypodontia, syndactyly, and polydactyly) without PPK and hair involvement, suggestive of a distinct syndrome (see OMIM 124480) (Robinson et al., 1962). Lamartine et al. (2000) identified two different missense mutations in the Cx30 gene, G11R and A88V, each of which segregated with Clouston syndrome in 12 families of French–Canadian and other backgrounds. In addition, a de novo mutation in the first transmembrane domain of Cx30, V37E, has been detected in a patient with sporadic Clouston syndrome (Smith et al., 2002).

Here, we report a young boy with features of KID syndrome and congenital atrichia, who was found to carry a deleterious missense mutation in the Cx30 gene (GJB6). Our findings expand the phenotypic spectrum of mutations in GJB6, and further underscore the overlapping nature of syndromes thus far attributed to the four connexins with cutaneous manifestations.

Results

Clinical features The proband is a 6-y-old boy born to non-consanguineous, unaffected parents after an uneventful pregnancy. The maternal family history did not reveal evidence for hearing impairment or ectodermal dysplasia. The paternal family history could not be obtained. Since birth, he has had dry, leathery skin; short and thickened dystrophic nails; no scalp hair, eyebrows and eyelashes; and severe photophobia. His sweating has been impaired, which was confirmed with a bromophenol test at 6.5 mo of age. In early childhood, his growth and weight remained below the 5th percentile and his motor development was slightly delayed. In recent years, he has caught up to the 5th–10th percentile for these growth parameters. Audiological testing revealed prelingual bilateral mild to moderate SNHL but his speech has improved significantly with the use of hearing aids since the age of 5 y. Mild keratitis and corneal vascularization were evident on ophthalmologic examination at the age of 5 y.

At 6.5 mo of age, dermatological examination revealed generalized erythema and thickened skin with a cobblestone appearance, sparing only the diaper area. In addition, a verrucous, hyperkeratotic plaque on his left antecubital fossa was noted. Six years later (Fig 1), the patient's skin continued to show slight erythrodema and was covered with innumerable spiny papules with accentuation on the posterior neck, lower back, knees, and elbows. Only the glans penis and sandal strap regions of the dorsal feet were spared. He also had diffuse PPK with a cobblestone or cerebriform surface. In addition, a few scattered, verrucous, and fissured papules up to 10 mm in diameter were noted in the perineal area. The patient had complete absence of body and scalp hair, eyebrows, and eyelashes. He had mild conjunctival injection and corneal neovascularization, but no blepharitis, ectropion, eyelid adhesions, or nystagmus. His primary teeth were normal in number and shape, although he had a few dental cavities. The oral mucosa was unremarkable. Several of his fingernails had been shed, and the remaining ones, similar to the toenails, were thickened, short, and demonstrated distal onycholysis. He had mild heat intolerance during the summer months. The past medical history also revealed recurrent episodes of nail infections as well as an unrelated peanut allergy.

Histology At 6.5 mo of age, a skin biopsy of the patient's left posterior arm revealed an abundance of eccrine sweat glands and ducts, which could be age-related, whereas only a few small, abortive hair follicles without hair shafts were identified (Fig 1). The ostia of appendages showed dilatation and plugging with orthokeratotic hyperkeratosis and a prominent granular layer. Sebaceous glands were present.

Mutation analysis reveals a heterozygous missense mutation in GJB6 (Cx30) The presence of all key features of KID-syndrome prompted initial mutation analysis of the GJB2 (Cx26) gene. The proband was found to be homozygous for a G to A transition at nucleotide 79 (from ATG start site) in codon 27, changing a hydrophobic residue, valine (GTC), with another, isoleucine (ATC) (V27I). This substitution, also found in unaffected controls in homozygous or heterozygous state, represents a common polymorphism with a frequency of the minor (A) allele of 0.005–0.52 in different populations (Kelley et al, 1998; Abe et al, 2000; Kudo et al, 2000; Park et al, 2000). In our Northern European control cohort (156 chromosomes), the allele frequency was 0.013. Since no other sequence variants were detected in GJB2, the connexin genes GJB6 as well as GJB3, GJB4, GJB5, and GJA1 were scrutinized. The proband harbored a heterozygous 110T — A transversion in GJB6, which was not present in his mother's DNA sample (Fig 2). His father was unavailable for testing. This point mutation is predicted to lead to a non-conservative replacement of valine 37 (GTG) with a negatively charged glutamic acid (GAG) (V37E) in the first transmembrane helix of Cx30. The nucleotide change was not detected in a cohort of 50 unrelated individuals of Northern European origin without genodermatoses or hearing loss, nor in the additional 100 Northern European controls published previously (Smith et al, 2002), excluding the possibility that
V37E represents a non-consequential sequence polymorphism. No sequence aberrations were found in four other connexin genes expressed in stratifying epithelia of ectodermal origin, including GJB3, GJB4, GJB5, and GJA1.

Discussion

Connexins are a large family of small integral membrane proteins utilized by most vertebrate tissues to establish direct cell–cell communication through gap junction channels. Similar to certain other adhesion molecules, such as claudins, occludin, or PMP22, they contain four hydrophobic domains transversing the plasma membrane (M1–M4, Fig 2). These α-helices separate 2 highly conserved hydrophilic loops extending into the extracellular space from 3 relatively variable cytoplasmic domains. Six connexin molecules oligomerize into homomeric or heteromeric connexin hemichannels, which are integrated into the plasma membrane where they may accumulate to gap junction plaques and associate with their counterparts in adjacent cells to complete intercellular channels. These channels permit the diffusion of small molecules (up to ~1 kDa) between cells and mediate the exchange of signals and nutrients, thereby coordinating cellular activities and response to stimuli. In the skin, up to 10 different connexin molecules have been detected, which are expressed in spatial and temporal overlapping patterns, and are partially shared by other stratified epithelia (Richard, 2000a; Di et al, 2001). Despite the apparent redundancy of the gap junction system, each connexin appears to have specific properties, which is illustrated by the fact that inherited defects in several connexin genes result in related yet distinct skin disorders, including PPK associated with SNHL, Vohwinkel syndrome and KID syndrome (GJB2), Clouston syndrome (GJB6), and erythrokeratodermia variabilis (GJB3 and GJB4) (Richard, 2003a).

We report a patient with an unusual clinical presentation resulting in diagnostic difficulties. He presented with a combination of clinical features characteristic of KID syndrome, including congenital bilateral SNHL, keratitis, and mild erythroderma. In addition, he had congenital absence of hair, PPK, and nail dystrophy, which are also seen in Clouston syndrome, and generalized spiny hyperkeratotic papules as described in HID syndrome (Hystrix-like Ichthyosis Deafness syndrome) (Traupe, 1989). In contrast to all other KID and HID syndrome, patients harboring pathogenic mutations in GJB2 (Richard et al, 2002; van Geel et al, 2002; van Steensel et al, 2002), our molecular studies revealed a heterozygous mutation in GJB6. The identified mutation V37E in GJB6 was recently observed in a patient with Clouston syndrome without evidence for abnormal sweating, hearing, photophobia, and keratitis (Smith et al, 2002). These findings illustrate a perplexing phenotypic variability of the GJB6 mutation V37E and underscore the profound influence of genetic and epigenetic factors that modify the clinical phenotype. In fact, other GJB6 mutations originally described in patients...
with Clouston syndrome, such as G11R and A88V, may also result in forme fruste with hypertrophic nail dystrophy closely resembling pachyonychia congenita, alone or in combination with hypotrichosis (Van Steensel et al, 2003). In contrast, mutation T5M has been associated with SNHL without any other associated features (Grifa et al, 1999). It is tempting to speculate that the unique disorder of the patient studied here might be related, at least in part, to the presence of a homozygous sequence polymorphism in GJB2 replacing valine27 with isoleucine. Despite detailed functional analyses of numerous recessive and dominant Cx26 mutations, the functional properties of this particular polymorphic variant of Cx26 remain unknown. Other Cx26 mutations that were originally considered non-consequential polymorphisms, such as M34T and V37I (orthologous to the GJB6 mutation reported here), were subsequently found to represent recessive alleles with reduced or absent channel permeability (Bruzzone et al, 2003; Oshima et al, 2003). Hence, it will be interesting to learn if V271-Cx26 has consequences on Cx26 function and, perhaps, on assembly or channel properties of mixed channels formed with wild-type Cx30 or mutant V37E-Cx30, which could aggravate the pathological processes.

Our findings demonstrate for the first time that KID syndrome is genetically heterogeneous and caused by mutations in either GJB2 or GJB6. Therefore, KID syndrome is another example of a connexin disorder attributable to mutations in more than one connexin gene, as are EKV (GJB3, GJB4), non-syndromic SNHL (GJB2, GJB6, GJB3), or cataract (GJA3, GJA8). Although dominant Cx26 mutations in KID syndrome either affect a hypermutable CpG dinucleotide in the first extracellular domain or cluster in the N-terminus, the mutation identified here, V37E, lies in the first transmembrane helix of Cx30. Based on a 3-dimensional map of truncated Cx43 (Unger et al, 1999) as well as SCAM (scanning cysteine accessibility mutagenesis) analysis of Cx32 (Zhou et al, 1997), this domain has been shown to line the cytoplasmic end of the channel pore and is part of a voltage sensing and transduction mechanism. Consequently, ion selectivity and other channel properties critically depend on the sequence, charge, and side chain conservation of this domain. Replacing this hydrophobic valine in
Cx30 with a charged glutamate acid residue can be expected to disrupt the alpha-helical structure of this transmembrane domain and ultimately alter the assembly and function of Cx30 in our patient. This prediction is supported by an in vitro expression study of V37E-Cx30 in HeLa and NEB1 keratinocytes, in which the mutant protein was synthesized but failed to be transported to the cell membranes and therefore could not produce gap junction plaques (Common et al, 2002). These results are similar to those obtained for Cx26 mutations causing PPK/SNHL (Marziano et al, 2003) and KID syndrome (Yi, 2003) as well as mutations in other connexin genes with a cutaneous phenotype (Di et al, 2002; Rouan et al, 2003). Whereas the assembly and trafficking defect observed for several Cx26 mutants could be overcome by co-expression with wtCx30 or wtCx30, the presence of mutant Cx30 subunits in gap junction plaques was shown to inhibit dominantly the function of homotypic or mixed gap junction channels, thus disturbing normal intercellular communication (Marziano et al, 2003). These data have important implications for understanding the pathomechanisms of KID syndrome and provide strong experimental evidence for the existence of mixed (heterotypic/heteromeric) Cx26/Cx30 gap junctions in the non-sensory epithelia of the inner ear, where loss of either protein results in SNHL (Cohen-Salmon et al, 2002; Kudo et al, 2003; Teubner et al, 2003). Both Cx26 and Cx30 are closely related proteins with 76% amino acid identity and are co-expressed in human corneal epithelium as well as in sweat glands, sweat ducts, and hair follicles (Richard et al, 2002; Coutinho et al, 2003). In contrast to Cx30, which is prevalent in the upper differentiated layers of interfollicular epidermis, Cx26 is only expressed at low levels in palmpoplantar epidermis but is strongly induced in response to wounding or other trauma (Lucke et al, 1999; Coutinho et al, 2003). Together with our molecular findings, this overlapping but not identical tissue expression of Cx26 and Cx30 implies some common functions and perhaps direct interaction of both proteins in many ectodermal epithelia, and may form the basis for the observed pleiotropism of GJB2 and GJB6 mutations, which can result in SNHL, skin, hair, nail, or corneal pathology alone or in combination (Richard, 2003a). The prominent hair phenotype of GJB6 mutations points to a hitherto unexplored role of Cx30 in keratinization of hair follicles.

In summary, our data illustrate the increasing complexity and overlap between cutaneous connexin disorders. The same Cx30 gene mutation may account for Clouston syndrome or KID syndrome, which makes it difficult to draw clinical and prognostic predictions, such as ocular or audiologic involvement, based on the underlying mutation alone. Moreover, KID syndrome results from pleiotropic mutations in 2 different yet closely related connexin genes that impair the function of the inner ear, cornea, skin, and appendages with significant variability of the resulting phenotype. The shared tissue expression and function of cutaneous connexins may serve as a basis for the broad clinical overlap observed among the genetic disorders resulting from alterations in these gap junction proteins. Based on our results, we suggest considering connexin gene mutations in any patient with a congenital disorder of cornification with or without erythema associated with SNHL or involvement of other ectodermal tissues, whereby mutation analysis should include all genes with potential cutaneous phenotype, including Cx26, Cx30, Cx30.3, and Cx31.

Materials and Methods

Patients and biological materials A 6-7-day-old boy and his unaffected mother were ascertained and gave written informed consent to participate in genetic research studies approved by the institutional review board of Thomas Jefferson University, Philadelphia, PA. DNA samples of both individuals were obtained from buccal mucosa cells (Richards et al, 1993). A paraffin-embedded 3-mm punch biopsy of lesional skin from the left arm of the 6.5-month-old proband was analyzed by light microscopy (H/E).

DNA amplification and mutation analysis DNA samples were amplified by PCR for direct DNA sequence analysis of the coding regions of GJB6, GJB2, GJB3, and GJB4. Primer sequences were derived from the genomic gene sequences (GenBank accession numbers: AJ005585; NT_009799 [GJB6]; U43932; M86849; NT_009799 [GJB2]; AF097370; AL121988 [GJB3]; AL121988 [GJB4]; AF097971 [GJB5]; NT_033944.2 [GJA1]) and are listed as follows: [GJB6]: (sense) 5’-AGAATGCTTTCAGGGTGAGG-3’ (nucleotides 196 to 178 from ATG translation start site) and (antisense) 5’-GATCTGAGGCAAGCTCGCTGGA-3’ (nucleotides 413 to 432) as well as (sense) 5’-GGCTTACACAGGGACGAAA-3’ (nucleotides 85 to 104) and (antisense) 5’-GTTTGGTATGCCTTCTTGGAG-3’ (nucleotides 835 to 854); [GJB2]: (sense) 5’-GTTCTGTTCACTGATCGGC-3’ (nucleotides 172 to 157) and (antisense) 5’-GTTTGGTATGGCTTCTTGGGG-3’ (nucleotides 835 to 853); and [GJB3]: (sense) 5’-TGGCCTTTTACCTGTTCTCCTTACCAA-3’ (nucleotides 177 to 153) and (antisense) 5’-ATGGCTTTGCTCCCACCCTCTCT-3’ (nucleotides 896 to 917). PCR reactions were performed using 200 ng genomic DNA, 2.5 IU Taq DNA polymerase, 10% Q-solution (Qiagen, Valencia, CA), and standard PCR conditions for 60 μL total volume. PCR cycling conditions were 94 °C for 2 min; 36 cycles of 94 °C for 30 s; 57 °C [GJB6/56 °C [GJB2; GJB5] for 45 s; 72 °C for 60 s; and finally 72 °C for 7 min. Amplicons were gel purified (QiAquick gel extraction kit, Qiagen) and directly sequenced using the BigDye terminator sequencing system on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA). Primers and PCR conditions for amplification and sequencing of GJB3, GJB4, and GJA1 were used as previously published (Richard et al, 2000b; Paznekas et al, 2002; Richard et al, 2003b). Sequence variants were confirmed by bi-directional DNA sequencing and in case of mutation 110T → A by denaturing high performance liquid chromatography (dHPLC). This method was also used to exclude the mutation from 100 population-matched control chromosomes. For dHPLC analysis, a 321 bp fragment of GJB6 was PCR-amplified from genomic DNA with primers (sense) 5’-GACGCTGCACACTTTCATC-3’ and (antisense) 5’-ATGGCTTTGCTCCCACCCTCTCT-3’. Each sample was denatured at 94 °C for 10 min, cooled to 65 °C for 10 min to allow heteroduplex formation, and maintained on ice until loading. Ten micro liter of each sample was separately injected and analyzed on a WAVE™ instrument (Transgenomic). Heteroduplex formation, and maintained on ice until loading. Ten micro liter of each sample was separately injected and analyzed on a WAVE™ instrument (Transgenomic).


Appendix: Electronic database information