A Novel Connexin 26 Mutation in a Patient Diagnosed with Keratitis–Ichthyosis–Deafness Syndrome

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Keratitis–ichthyosis–deafness syndrome is a rare disorder characterized by erythrokeratoderma, deafness, and keratitis. Scarring alopecia and squamous cell carcinoma can also occur. Most cases described so far were sporadic. Here we present evidence that keratitis–ichthyosis–deafness syndrome is caused by a mutation in the connexin 26 gene. This finding expands the spectrum of disorders caused by defects in connexin 26 and implies the gene in normal corneal function, hair growth, and carcinogenesis. ***Key words*****: skin cancer/alopecia/gap junction.** ***J Invest Dermatol 118:724–727, 2002***

Keratitis–ichthyosis–deafness (KID) syndrome is a rare autosomal dominant disorder. It is characterized by the occurrence of localized erythematous scaly skin lesions, severe bilateral keratitis, and sensorineural deafness (Rycroft *et al*, 1976; Cram *et al*, 1979; Skinner *et al*, 1981; Singh, 1987; Langer *et al*, 1990; McGrae, 1990; Morris *et al*, 1991; Nurse, 1994; Caceres-Rios *et al*, 1996; Alli and Gungor, 1997; Kone-Paut *et al*, 1998). The term "ichthyosis" is, strictly speaking, not correct, as the skin lesions are more appropriately classified as erythrokeratoderma.

A scarring alopecia can be part of the phenotype. The skin lesions occur predominantly on the face, palms, and soles, and have a typical reticulated pattern that is often called leather-like. Squamous cell carcinoma has been reported in 11% of the patients and may probably be considered as a manifestation of the disease (Grob *et al*, 1987; Madariaga *et al*, 1986; Hazen *et al*, 1989, 1992; Morris *et al*, 1991). Histologic examination usually shows nonspecific changes but may show severe follicular plugging.

The combination of erythrokeratoderma and deafness also occurs in erythrokeratoderma variabilis of Mendes da Costa, an autosomal dominant disorder that has been shown to be caused by mutations in the connexin (CX) genes 30.3 and 31 (Richard *et al*, 1998; Wilgoss *et al*, 1999; Kelsell *et al*, 2000). Although keratitis is not part of erythrokeratoderma variabilis, the skin lesions and sensorineural deafness are similar to those found in KID syndrome. Therefore, we considered the connexin genes that are expressed in skin excellent candidates for KID syndrome.

We ascertained a patient suffering from KID syndrome. She is the only affected person in the family (Cremers *et al*, 1977). The patient, the youngest of nine children, was born at term from consanguineous (third degree) Dutch parents. Pregnancy was uneventful. During the first weeks after birth, thickening and scaling of the skin became apparent, as well as a reddish-brown discoloration of affected skin. The patient reportedly had trouble sweating. At 4 y of age, the parents first noted hearing loss. Psychomotor development was normal. From 11 y of age, the patient developed bilateral keratitis with photophobia. Repeated keratoconjunctivitis with superficial and deep neovascularization of both lenses necessitated the implantation of artificial lenses at age 34. This intervention in turn induced a bullous corneal dystrophy. At 38 y of age, she developed a skin lesion on the right ankle that was initially diagnosed as pseudo-epitheliomatous hyperplasia. Later, the diagnosis was revised as spinocellular carcinoma. The lesion was excised and the patient remains free of disease to date.

Physical examination at age 18 showed red, hyperkeratotic skin on much of the body surface. The nails of hands and feet were thickened. Scalp hair was brittle, eyebrows and eyelashes were sparse, whereas pubic and axillary hair were missing altogether. Mammary gland development was insufficient for age (**Fig 1**). Dentition was abnormal; the teeth were small and abnormally shaped. Ophthalmologic examination showed bilateral bullous corneal dystrophy with neovascularization. Bilateral astigmatism was noted as well. Audiologic examination demonstrated profound bilateral sensorineural hearing loss. No other abnormalities were noted and a karyotype was a normal 46,XX.

Blood was taken from the patient, her mother, and four sibs, and DNA extracted from peripheral blood leukocytes using methods described elsewhere (Miller *et al*, 1988). The father was deceased. We sequenced connexin genes that are known or expected to be involved in skin disorders and sometimes accompanied by deafness. The genes that were analyzed are CX26 (GJB2), 30 (GJB6), 30.3 (GJB3), 31 (GJB5), 31.1 (GJB4), and 37 (GJA4). We did not sequence CX43. It is expressed in skin (Goliger and Paul, 1994), but has been implicated mainly in cardiac morphogenesis and function (Huang *et al*, 1998) and lens function (Gao and Spray, 1998).

Primer sequences were as follows: Cx26F, GCATGCTTGCTTACCCAGACTC; Cx26R, AGGGGAGCAGAGCTCCATTG; Cx30F, AGCAGGGCAGGGAGTTGAAG; Cx30R, TCAGGTTGGTATTGCCTTCTGG; Cx30.3F, CAATCGCACCAGCATTAAGGG; Cx30.3R, TGATCTTATCTGCTGATCTCGCAG; Cx31F, TTCATTCATACGATGGTTTTTCCTC; Cx31R, ACCTCTCCACCTGCCACACC; Cx31.1F, GAACCCAGCTCCTCTAGTGATGG; Cx31.1R, CCATCCAGGCCCAACCTG. The sequences were assembled and analyzed using the Phred-Phrap-Consed software tools (Ewing and Green,

Manuscript received November 20, 2001; revised December 12, 2001; accepted for publication December 20, 2001

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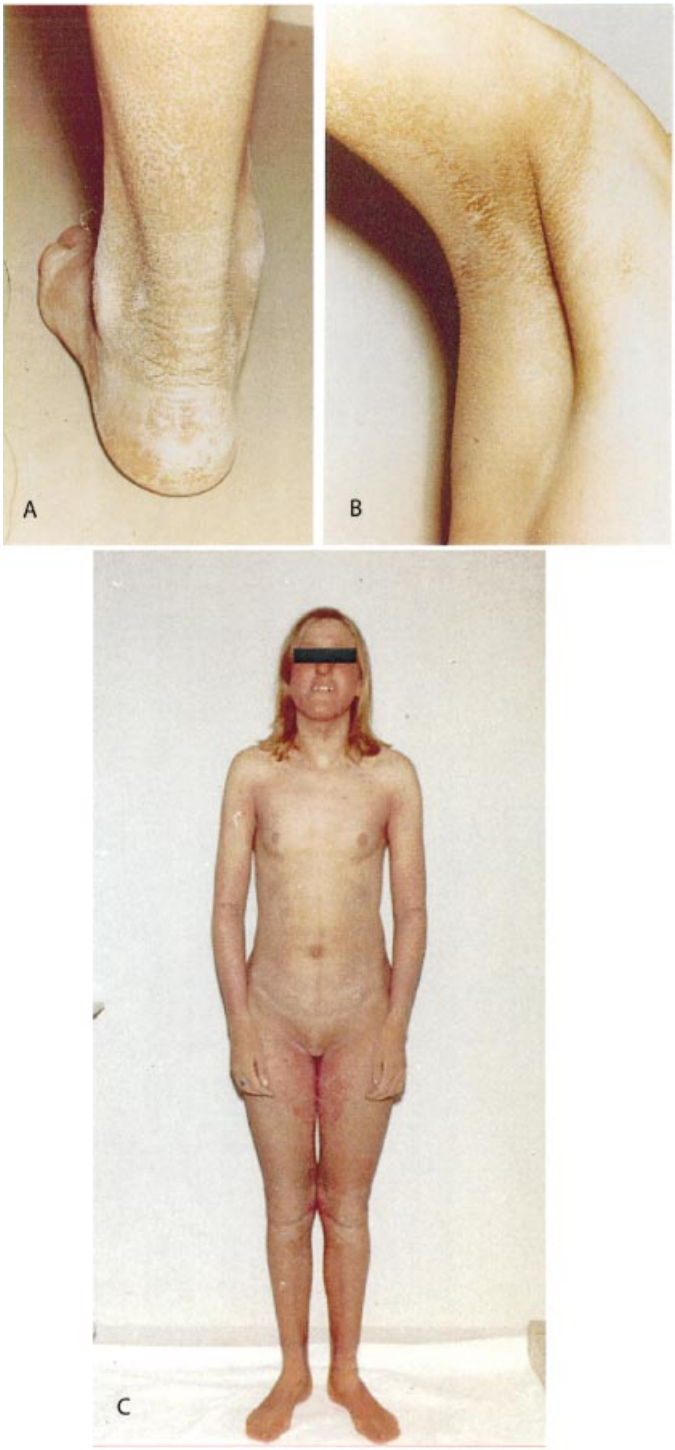

Figure 1. Phenotype of the patient. (*A*) Typical shark-skin-like hyperkeratosis and erythroderma on left calf and ankle. (*B*) Hypotrichosis and hyperkeratosis in right axilla. (*C*) Frontal view of patient. Mask-like erythrokeratoderma of the face, lack of pubic hair, pronounced erythroderma of extremities with sparing of rump. Vestigial mammary glands.

1998; Ewing *et al*, 1998; Gordon *et al*, 1998). No mutations were found in CX30, CX30.3, CX31, and CX31.1. In CX26 the patient had a heterozygous GAC to AAC change in codon 50. This changes a conserved aspartic acid into an asparagine in the first extracellular domain (D50N). Because the G to A change abolishes an *Asp*I (Roche Diagnostics, DE-68305 Mannheim, Germany) restriction site, we examined controls and the family by restriction analysis. The mutation was not present in 164 control alleles and could not be demonstrated in the mother and four sibs either (**Fig 2**). The absence in 164 control alleles and the other family members strongly suggests that it is not a polymorphism; however, recent evidence suggests that some disorders associated with connexin mutations can be digenic. Kelsell *et al* (2000) have demonstrated that a variation in CX26 (M34T) can interact with mutations in CX26 and CX31 to produce a more severe hearing loss than occurs in single CX26/31 mutants. It is possible that a similar phenomenon is at work in KID syndrome, explaining its rarity and the relative lack of instances with autosomal dominant inheritance. We did not find mutations in CX30, CX30.3, CX31, CX31.1, and CX37 or in the other CX26 allele, suggesting that in this particular case a digenic mutation is less likely. The finding of this novel mutation expands the spectrum of disorders in which CX26 is involved. So far, it has been implicated in a variant of Vohwinkel's syndrome (MIM 124500), palmoplantar keratoderma–deafness syndrome and nonsyndromic hearing loss.

The aspartic acid at position 50 is conserved across species and across the connexins suggesting that it is of vital importance for correct functioning of the protein (**Fig 3**). It has been demonstrated that a substitution in CX26 (W77R) impairs transport of small charged molecules across gap junctions (White, 2000). This mutation also leads to inefficient targeting of the protein product to the plasma membrane with subsequent retention in intracellular stores. In addition, the mutated connexin showed limited oligomerization into connexon hemichannels. It is tempting to speculate that the KID syndrome mutation has a similar effect; however, the W77R mutation is recessive, suggesting that the KID mutation must have additional effects.

As the D50N change replaces a charged amino acid by an uncharged one, the substitution can be expected to affect local conformation. It may also influence voltage gating. Recent data suggest that single gene mutations may affect voltage-dependent gating in heterotypic channels such as those formed by CX26 and CX32 (Zhao and Santos-Sacchi, 2000). In addition, the introduction of charge at the start of the first extracellular loop can be expected to disturb local conformation and thus interfere with docking to the partner connexin. It has been demonstrated that local E1 topology is essential for connexon formation (Foote *et al*, 1998). The clustering of skin disease associated CX26 mutations in this domain suggests that this domain is of special importance in skin, either for skin-specific connexon assembly or for interactions with other proteins. In other connexins, the mutations causing erythrokeratoderma variabilis are clustered in the first transmembrane domain, supporting the hypothesis that the CX26 E1 domain has a special function in skin physiology. This issue needs to be addressed in future studies.

Of interest is the role for CX26 in the cornea that is suggested by our findings. The main gap junction protein in the cornea seems to be CX43 (Nishida *et al*, 1996). No CX26 expression has so far been found in corneal epithelium from many animal species (White and Bruzzone, 2000); however, human cornea has to our knowledge not yet been examined for CX26 expression.

Homozygous CX43 knockout mice have lens abnormalities consisting of separation and vacuolization of lens fibrils, interpreted as early signs of cataract (Gao and Spray, 1998). Apparently, this connexin is required for maintenance of osmotic pressure in the lens. It is tempting to speculate that CX26 has a similar role in the human cornea. If corneal keratinocytes were to become separated, infectious agents might be able to establish a presence in between the corneocytes. This would lead to keratitis. Other disorders caused by CX26 mutations are not accompanied by overt corneal disease. Skin symptoms, however, are associated with particular mutations and it is conceivable that the same applies to corneal involvement in which case the communication or osmotic pressure hypotheses would not be tenable as sole explanation. It would be of considerable interest to examine other forms of corneal dystrophy for connexin mutations in order to test this assertion.

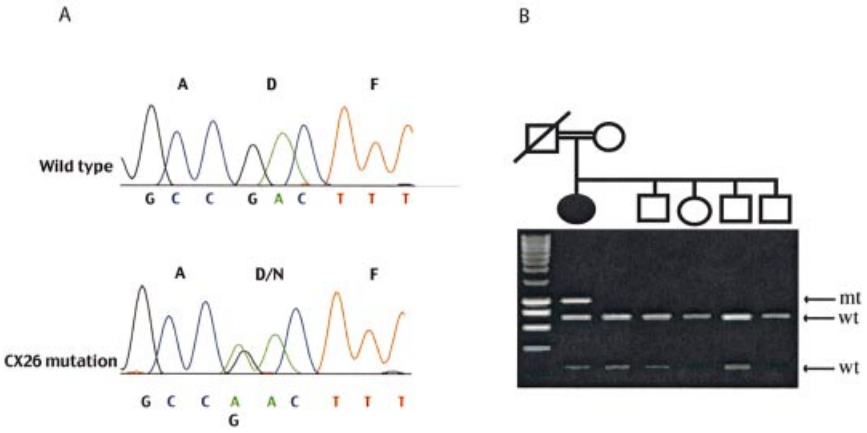

Figure 2. Mutation analysis. (*A*) Sequence traces of wild-type sequence *vs* patient sequence. G→A transversion changing codon 50 from GAC to AAC. (*B*) The mutation abolishes an *Asp*I restriction site. Restriction analysis demonstrates the presence of a mutated allele in the proposita only.

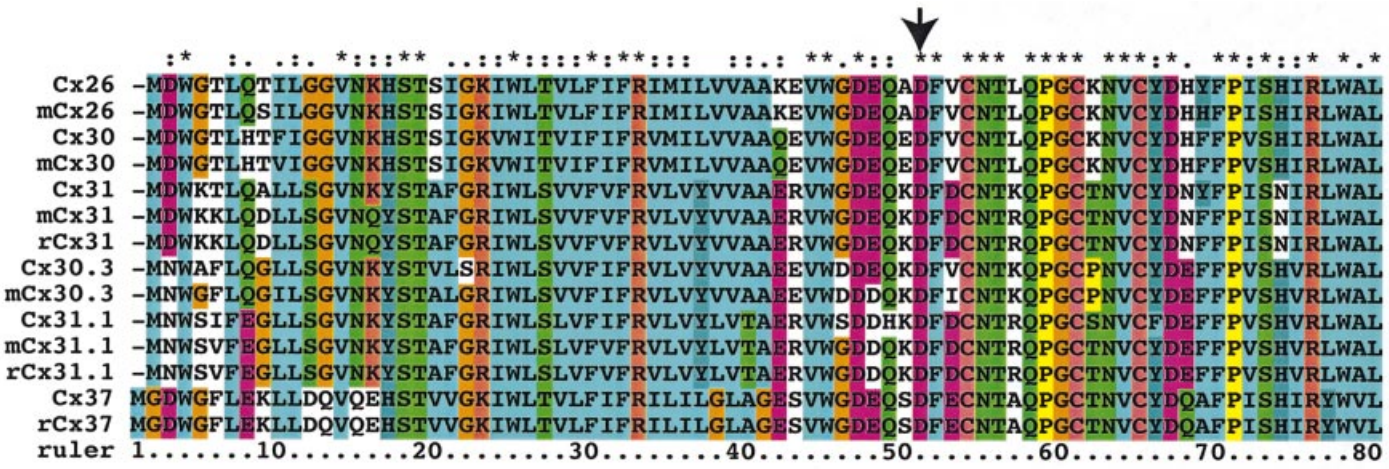

Figure 3. CLUSTALX alignment of connexin proteins from human, mouse, and rat. D50 is conserved in all connexins in the alignment (*arrow*).

The scarring alopecia observed in KID syndrome is probably related to the follicular plugging that is commonly observed. A role for CX26 in hair follicle differentiation is suggested by the hypotrichosis observed elsewhere on the body. Thus far, only CX30 has been implicated in hair growth. This aspect of the phenotype is likely related to the specific mutation we observe here, as other disorders caused by CX26 mutations are not characterized by hypotrichosis. Thus, as in the case of the keratitis, it is not likely that the hypotrichosis is related solely to a disturbance of intercellular communication. The same can be said for the propensity for developing squamous cell carcinomas, which is observed in KID syndrome but not in other disorders caused by connexin mutations. CX26 is known to be reduced or absent in mammary carcinoma cells and is considered a putative tumor suppressor for epithelial tumors (Lee *et al*, 1991, 1992; Tu *et al*, 1998; Singal *et al*, 2000). Other connexins such as CX37 have been shown to be involved in tumorigenesis. Specifically, CX37 mutations have been described in vinyl chloride induced hepatic angiosarcomas (Saito *et al*, 1997) and disturbed gap junction communication has been reported in many other tumor types. CX32 mutant mice are prone to liver cancer (Moennikes *et al*, 2000). No definite connexin mutations have been reported in human cancers or cancer-prone disorders. Our findings are the first to suggest that germline connexin mutations can lead to skin cancer in humans.

CX26 is known to upregulate E-cadherin expression (Stoler *et al*, 1993). As E-cadherin is probably involved in the regulation of hair growth (Van Steensel *et al*, 2000, 2001) and is downregulated in approximately 70% of squamous cell carcinomas examined in one study (Koseki *et al*, 1999) it is likely that alterations of E-cadherin expression are involved in the increased cancer susceptibility and hypotrichosis of KID syndrome.

In conclusion, the finding of a novel CX26 mutation in KID syndrome supports the notion that connexins have functions not directly related to their presence in gap junctions and demonstrates that germ-line connexin mutations can cause cancer in humans. It appears that deafness and erythroderma are symptoms that may be related to disturbed gap junction function *per se*. Other symptoms such as the keratitis and the cancer-proneness seem to be dependent upon mutations in a particular residue suggesting that disturbance of gap junction formation is not sufficient as an explanation and that there may be direct interactions with the cytoskeleton or cell-cycle machinery dependent upon specific amino acid motifs in the connexin protein.

M.v.G. is supported by a grant from Rebirth SA, Luxembourg. M.v.S. is supported by grants from Rebirth SA, Luxembourg and the Dutch Organization for Research NWO grant number 920-03-085.

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