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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
A Novel Connexin 30 Mutation in Clouston Syndrome

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Clouston syndrome (hidrotic ectodermal dysplasia) is an autosomal dominant ectodermal dysplasia characterized by alopecia, palmoplantar hyperkeratosis, and nail dystrophy. Recently, mutations in the GJB6 gene encoding the gap junction protein connexin 30 have been shown to cause this disorder. To date, all mutations have involved two codons: G11R and A88V. Here, we report a novel mutation V37E within the first transmembrane domain of connexin 30 in a spontaneous case of Clouston syndrome. The mutation was detected in genomic DNA, confirmed in reverse transcription polymerase chain reaction products, and was excluded from 100 ethnically matched control individuals by restriction enzyme analysis. Key words: Clouston syndrome/connexin 30/gap junction proteins/GJB6/hidrotic ectodermal dysplasia. J Invest Dermatol 118:530–532, 2002

Hidrotic ectodermal dysplasia (HED, Clouston syndrome, MIM 129500) was first described in 1895 (Nicolle and Hallprén, 1895) and later by Clouston, in families from Quebec (Clouston, 1929, 1939). Although most common in French Canadians, the disorder has been identified in several ethnic groups (McNaughton et al., 1976; Rajagopalan and Tay, 1977; Ando et al., 1988; Patel et al., 1991; Radhakrishna et al., 1997; Kibar et al., 2000). The main features of this autosomal dominant disorder are partial to complete alopecia, palmoplantar hyperkeratosis, and nail dystrophy. Sweat gland function in these patients is normal (Clouston, 1929, 1939).

The molecular basis of clinically similar ectodermal dysplasias such as pachyonychia congenita are now known to be due to mutations in the tissue-specific keratin genes (Irvine and McLean, 1999). Although early studies suggested Clouston syndrome could be due to a keratin defect (Gold and Scriver, 1972; Giraud et al., 1977; Escobar et al., 1983), later genetic linkage studies excluded both keratin gene clusters (Hayflick et al., 1996; Kibar et al., 1996). Genomewide linkage analysis localized the gene to chromosome 13q11-12.1 (Kibar et al., 1996). Linkage was confirmed by several independent mapping studies (Radhakrishna et al., 1997; Taylor et al., 1998; Stevens et al., 1999; Kibar et al., 2000; Lamartine et al., 2000). These analyses demonstrated genetic homogeneity of the disorder and a founder effect in the French Canadian population (Kibar et al., 2000). Two candidate genes within this loci were the gap junction protein connexin 30 (gene name GJB6) (Kelley et al., 1999) and connexin 26 (gene name GJB2) (Lamartine et al., 2000).

As reviewed recently, mutations in connexin 26 are associated with disorders affecting the skin (autosomal dominant Vohwinkel syndrome) or deafness (autosomal recessive DFNB1 or autosomal dominant hearing loss DFNA3) (Kebell et al., 2001). In some cases both skin and hearing ability are affected (Kebell et al., 2001). Connexin 26 was excluded as a candidate for Clouston syndrome (Kebell et al., 1997; Lamartine et al., 2000). Connexin 30 is also associated with some forms of deafness (Gräf et al., 1999) and hearing impairment has been reported in some cases of Clouston syndrome. As connexin 30 is expressed in the epidermis (Dahl et al., 1996; Lamartine et al., 2000), as well as in brain (Nagy et al., 1999) and inner ear (Lautermann et al., 1999), the possibility that connexin 30 could be involved in several different phenotypes was investigated. The human GJB6 gene encoding the 261 amino acid connexin 30 polypeptide was cloned (Gräf et al., 1999) and connexin 30 mutations were identified in 12 Clouston syndrome families of varying ethnicity (Lamartine et al., 2000). These were found clustered to two mutation hotspots, G11R and A88V. Here, we report a novel connexin 30 mutation in a sporadic Scottish case of Clouston syndrome.

MATERIALS AND METHODS

Mutation detection and confirmation Genomic DNA was extracted from whole blood by standard procedures. A 1164 bp fragment spanning the full-length GJB6 gene (connexin 30) was amplified with primers Cx30P1 5'-GGCG AGG GAG TAG TTG TAA-3' and Cx30P2 5'-TGTG TGTGA TGTG TAG-3'. Reactions were performed using polymerase chain reaction (PCR) buffer (67 mM Tris-HCl pH 8.8, 16.6 mM (NH4)2SO4, 0.17 mg/ml bovine serum albumin, and 10 mM 2-mercaptoethanol) containing 1.5 mM MgCl2 and 1 U Taq polymerase (Promega) were amplified as follows: 94°C 5 min × 1; 94°C 30 s, 58°C 1 min, 72°C 2 min × 35; and 72°C 5 min × 1. PCR products were purified using Qiaquick PCR purification kit (Qiagen, Crawley, U.K.) and sequenced on an ABI 3100 (ABI, Foster City, CA) using primers Cx30P1 and Cx30P2 (above).

Dermal fibroblast cells were grown from explants derived from the patient's skin biopsy in Dulbecco's modified Eagle's medium (Sigma, Poole, U.K.) containing 10% fetal bovine serum. miRNA was extracted using the Quickprep micro miRNA purification kit (Amersham Pharmacia Biotech UK); genomic DNA contamination was removed by incubation with RNase-free DNase 1 (Promega) followed by phenol-chloroform extraction and reverse transcription using AMV reverse transcriptase (Promega). cDNA was amplified with Cx30P5 5'-GGA CGC TGC ACA CTT TCA TGA TCC-3' and Cx30P6 5'-GGT GTC ACG ACC TGT GAT TG-3' in (NH4)2SO4 PCR buffer (Bioline) containing 1.5 mM MgCl2 and 4% dimethylsulfoxide. Reactions were denatured at 94°C for 2 min, 1 U Biotaq DNA polymerase (Bioline) was added, and reactions were amplified at 94°C 5 min × 1; 94°C 30 s, 50°C 1 min.
Figure 1. Clinical features of the proband. (a) Total alopecia of the scalp. Eyebrows, eyelashes, and all other body hair was also absent (not shown). (b) Nail dystrophy resembling pachyonychia congenita and keratoderma extending onto fingertips. (c) Papular lesions prominently seen on the forearm.

Figure 2. Molecular genetic analysis. (a) Normal GJB6 sequence corresponding to codons 34–38. (b) The same region of the GJB6 gene as shown in (a), derived from the proband, showing heterozygous missense mutation 110T → A (arrow) predicting the amino acid change V37E. (c) Confirmation of mutation V37E by Xho I digestion. Lane 1, DNA molecular weight markers; lanes 2–6, digested PCR products from normal unrelated controls; lane 7, digested PCR product from affected individual shows an additional band due to a new Xho I site created by the mutation.

RESULTS

Clinical findings The proband, a Scottish Caucasian female, was a sporadic case presenting with total alopecia and absent nails at birth. By the age of 1 year, she had developed 20-nail dystrophy that persisted in adulthood. Papular lesions were noted on the skin, especially on the forearm, as shown in Fig 1. There was also a diffuse, velvet-like keratoderma on the palmar and plantar surfaces that appeared in adolescence. Tooth development, sweat gland function, and hearing were normal. The phenotype of complete alopecia and papular lesions suggested a diagnosis of congenital atrichia (MIM 209500) but the presence of keratoderma was more consistent with Clouston syndrome.

Histology A sample of buttock skin showed normal epidermis and a normal distribution of eccrine glands (data not shown). Remnants of hair follicles with sebaceous glands attached were observed in the lower dermis.

Identification and confirmation of connexin 30 mutation A heterozygous missense mutation 110T → A in the GJB6 gene was detected by direct sequencing of PCR products derived from genomic DNA from the affected individual (Fig 2). This substitution leads to the predicted amino acid change V37E within the first transmembrane domain of the connexin 30 polypeptide. The mutation was also detected in cDNA originating from dermal fibroblasts cultured from the patient’s skin biopsy (data not shown). The base change creates a new Xho I restriction site, which was used to confirm the presence of the mutation in the proband by digestion of PCR products with Xho I restriction enzyme. The mutation was excluded from 100 normal, unrelated individuals using this screening method (Fig 2). During the course of this investigation the hairless (HR) gene was also screened as a candidate for mutations and the patient was found to be heterozygous for R620Q. This amino acid change was originally reported as a mutation in a family with congenital atrichia (Almond et al, 1998) but has since been shown to be a polymorphism (Hillmer et al, 2001).

DISCUSSION

Here we report a novel missense mutation V37E in the GJB6 gene encoding connexin 30 in a sporadic Scottish case of Clouston syndrome. Recently, two GJB6 mutations, G11R and A88V, in Clouston syndrome were reported in a total of 12 families from different ethnic populations (Lamartine et al, 2008).

Connexins have a common structure consisting of four transmembrane domains, two extracellular domains, and three cytoplasmic domains. The mutation reported here, V37E, is in the first of the four transmembrane domains of connexin 30. Along with glycine 11 and alanine 88, valine 37 is conserved between human and mouse connexin 30 and also in connexin 26, the most...
closely related protein to connexin 30). A mutation in the equivalent codon in connexin 26, V37I, has been identified in several families with recessive hearing loss with no skin abnormality (Rabionet et al, 2000). Similarly, mutation V53M in connexin 32 causes Charcot-Marie-Tooth disease (Ikegami et al, 1998). Whereas the latter two mutations involve substitution of one hydrophobic amino acid for another, the V53E mutation reported here changes a hydrophobic residue to a highly charged acidic residue. As this occurs in a transmembrane domain, whose hydrophobic sequence is essential for membrane spanning, this defect is predicted to be highly detrimental to connexin 30 function.

With the recent discovery of mutations in other connexin genes a trend is emerging whereby different mutations in the same gene can cause different diseases. Mutations in either connexin 30 or 26 can result in deafness and/or skin pathologies and those in connexin 31 can lead to three different phenotypes. It remains to be seen how mutations in widely expressed proteins such as connexins can result in diverse phenotypes affecting highly specific epithelial compartments such as the inner ear, hair, nail, and palmo-plantar epidermis.

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