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A mutation in the connexin 30 gene in Chinese Han patients with hidrotic ectodermal dysplasia

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Summary ***Background:*** hidrotic ectodermal dysplasia (HED) or Clouston syndrome is a rare autosomal dominant disorder affecting the skin and its derivatives. It is characterized by the triad of nail dystrophy, alopecia, and palmoplantar hyperkeratosis. To date, all mutations have been involving in three codons: G11R, A88V and V37E in the connexin 30 (Cx30) gene have been shown to cause this disorder. ***Objective:*** in order to analyze the mutations of the Cx30 gene in Chinese Han patients with HED. ***Methods:*** we collected a large Chinese HED family consisting of a total of 81 individuals including 28 HED patients (14 males and 14 females). The whole coding region of Cx30 was amplified by polymerase chain reaction and products analyzed by direct sequencing, then further confirmed at the mRNA level by RT-PCR. ***Results:*** we detected a transition, 31(G → A), leading to a missense mutation (G11R) in genomic DNAs of 18 patients, and the point mutation was not found in 16 normal individuals in this HED family and in 188 unrelated, population-match control individuals. The transcription of mutated allele was confirmed by RT-PCR of Cx30 mRNA. ***Conclusion:*** our data suggests that a G11R missense mutation in the Cx30 gene can cause HED in Chinese Han population and emphasizes the importance of screening for this as well as other Cx30 gene mutations in the HED.

1. Introduction

Hidrotic ectodermal dysplasia (HED) (Clouston syndrome, MIM #129500) is a rare autosomal dominant epidermal disorder transmitted with high penetrance and variable clinical expressivity. The condition occurs with a frequency of 1:100,000 [1]. It is characterized by the triad of nail dystrophy, alopecia, and palmoplantar hyperkeratosis. Sweat gland function in these patients is normal [2]. The syndrome was first described by Nicolle and Hallipre in 1895 [3], but became well known

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only after the report of a large French–Canadian family by Clouston in 1929 [4]. Genome-wide linkage analysis localized the gene to chromosome 13q11-q12.1 [5]. It wasn't long before colleagues from other parts of the world tested their own patients and confirmed the mapping region [2,6–. Hearing impairment has been reported in few cases of HED [10]. However, Cx30 is expressed in the epidermis [11,12] as well as in brains [13] and inner ear [12], and the possibility that Cx30 could be involved in several different phenotypes was investigated. Mutations in gap junction beta 6 (GJβ6) encoding Cx30 have been shown to underlie hearing loss and HED. A missense mutation (T5M) and a large deletion (342-kb) in Cx30 have been described that causes dominant non-syndromic hearing loss [14,15]. Cx30 gene mutations were identified in 12 Clouston syndrome families of varying ethnicity. There were found clustered to two mutations hotspots, Gly 11 to Arg (G11R) and Ala 88 to Val (A88V) [16]. The different amino acid substitutions have been associated with HED to date: G11R, A88V and V37E [16,17]. Here, we report a family from the Chinese Han population with G11R mutation, which provides further evidence that G11R change in the Cx30 gene is pathogenic in the heterozygous state and that G11R is probably not a 'private' mutation confined to a single geographic region.

2. Report of a case

HED is a rare group of autosomal dominantly inherited genodermatoses, with very few cases reported in the literature. The common diagnostic features among HED patients include (i) dystrophy of the nails which is the key feature of the syndrome and in nearly 30% of those affected there may be no other obvious defects; (ii) hyperkeratosis of the palms and soles, is frequent and may be severe; fissuring is sometimes troublesome; (iii) scalp hair is very sparse, fine, pale and brittle or completely lacking; the eyebrows are thinned or absent, eyelashes are few and small; vellus and pubic and axillary hair are sparse or absent. Ocular abnormalities, hearing loss, hyperpigmentation of the skin, mental deficiency, thickening of the skull, and tufting of the terminal phalanges are found in several HED affected individuals [10].

In an effort to extend the body of literature supporting the role of Cx30 mutation in HED, we studied a pedigree with typical features of HED originating from the Jiangxi Province, China, with 28 affected individuals over five generations, and the mode of inheritance was autosomal dominant with expressivity (Fig. 1). No skipping of generation was observed, indicating that the condition is highly penetrant. The proband (IV2) is a 35-year-old male. The fingers' nails of his are thickened, striated, often discoloured and grow slowly, however his toes' nails are short, thin and brittle. Persistent paronychial infections are frequent and partially destroy the matrix. The skin is thickened beneath the free edges of the nails, over the finger joints and knuckles (Fig. 2a). Hyperkeratosis of the palms and soles, fissuring is sometimes troublesome (Fig. 2b). His scalp hair is severe alopecia in infancy, the eyebrows and eyelashes are completely lacking (Fig. 2c). The pubic and axillary hair or vellus are lost completely. The hair anomaly was severe in childhood with complete alopecia and minimal change with age. Sweating, dentition, hearing, breast development and oral mucosa all appeared normal. The histologic examination of axillary hair skin showed normal epidermis and normal distribution of eccrine glands, remnants of hair follicles with sebaceous glands attached were observed in the lower dermis (Fig. 2d). In this big family (Table 1), the same abnormalities were found in the additional eight patients, whose disease history was identical. Fifteen affected family members were also examined and had very similar abnormalities of the hair, but it is very sparse, fine, and brittle, as well as almost identical disease histories. Only four affected in this big family there are no other obvious defects expect dystrophy of the nails.

3. Material and methods

3.1. Sample collection

We collected a HED family including 18 affected and 16 unaffected individuals and obtained signed informed consents. We used a Kit (QIAGEN) to extract genomic DNA from peripheral blood according to the manufacturer's protocol. In addition, genomic DNAs from 188 normal healthy Chinese Han people were used as controls. mRNA was obtained using axillary biopsy material from the proband, a tissue which constitutively expresses Cx30 gene. Total mRNA of this patient was isolated with TRIZOL reagent (Gibco BRL, American) according to the protocol of the supplier. Reverse transcription (RT) was performed using a corresponding kit (Gibco BRL, American).

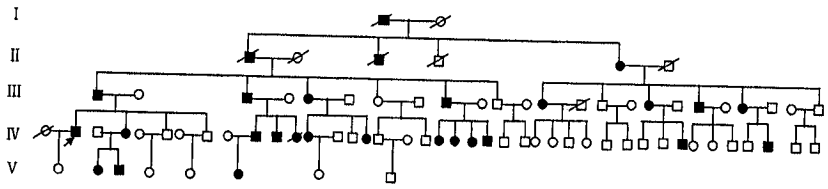

Fig. 1 Pedigree of this family studied.

3.2. Mutation detection and confirmation

A 947-bp fragment spanning the full-length Cx30 gene was amplified with primers (forward, 5′-CAGAAGGAACTTTCAGGTTGG-3′; reverse, 5′-CACCGTGTCACTTTCCCAAG-3′). PCR was performed in 20-μl reaction volume containing 50 ng of genomic DNA, 100 μM dNTPs, 4pmole of each primer, and 0.75 U Hotstar®Taq DNA polymerase in the reaction buffer supplied by the manufacturer (QIAGEN). The PCR conditions were: Hotstar®Taq activation at 95 °C for 15 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 75 s, and then final extension at 72 °C for 7 min. Three microliter aliquots of the PCR products were analyzed on 2% agarose gel electrophoresis. After the amplification, products were purified using a QIAquick PCR Purification Kit (Qiagen), and were sequenced on an Applied Biosystem 3700 automated sequencer using an ABI PRISM fluorescent dye terminator system (Perkin–Elmer, Foster City, CA). Sequence analysis was conducted with the Phred-Phrap-Consed version 12.0 program package.

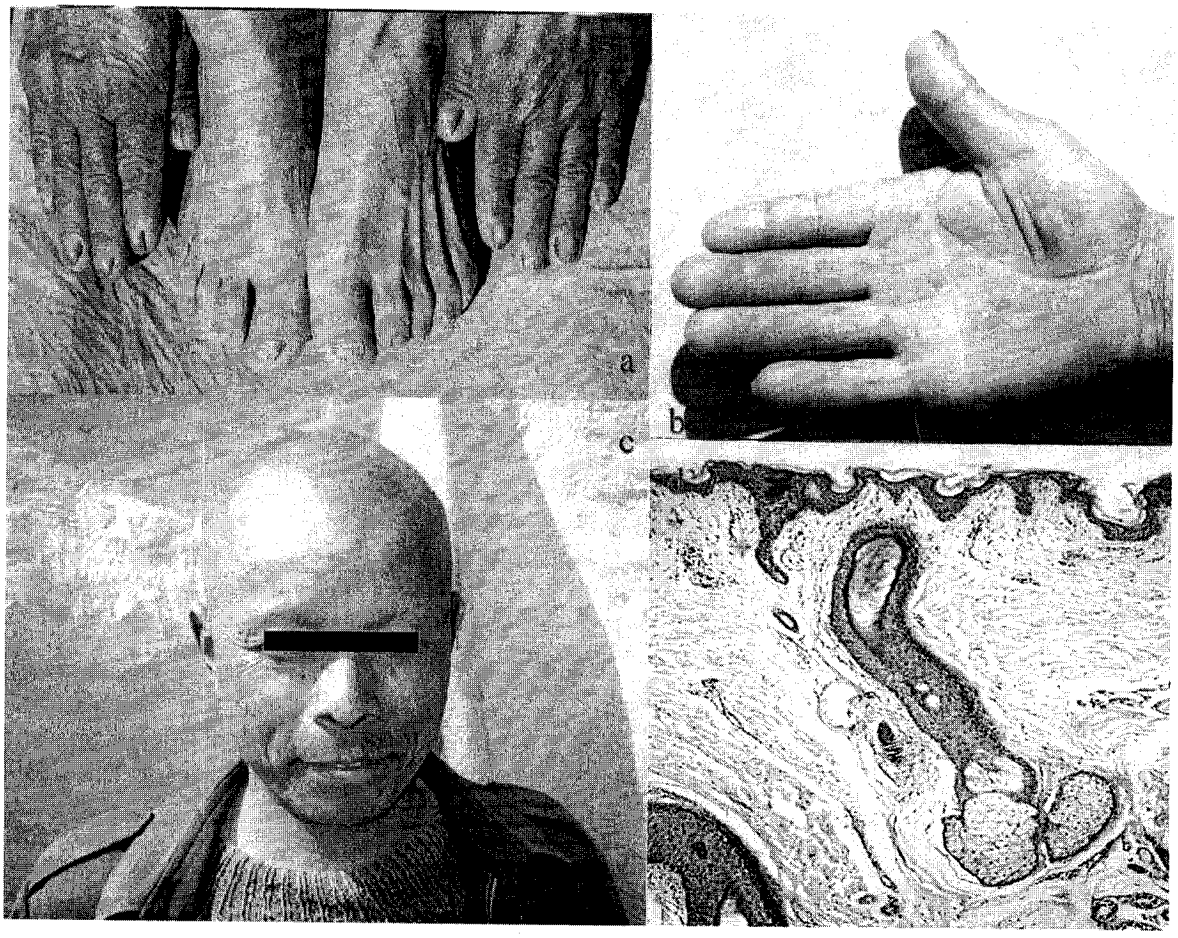

Fig. 2 (a) The fingers' nails of his are thickened, striated, often discoloured and grow slowly, however his toes' nails are short, thin and brittle; (b) shows hyperkeratosis of the palms; (c) shows total alopecia, and virtually absent eyebrows and eyelashes; (d) skin biopsy on the right axillary revealed normal epidermis and a normal distribution of eccrine glands, remnants of hair follicles with sebaceous glands attached were observed in the lower dermis (haematoxylin and eosin; original magnification × 100).

Table 1 Review of clinical findings in affected members of family with hidrotic ectodermal dysplasia

ID no.	Sex	Alopecia	Nails	Soles	Palms	Ages at examination
II5	F	++	+++	+++	++	76
III1	M	+++	+++	+++	+++	60
III3	M	+++	+++	+++	+++	52
III5	F	+++	+++	+++	++	50
III9	M	+++	+++	+++	+++	42
III13	F	−	+	+	−	56
III17	F	+++	+++	+++	++	47
III19	M	++	++	++	++	45
III21	F	+++	+++	+++	++	43
IV2	M	+++	+++	+++	+++	35
IV4	F	+++	+++	+++	+++	34
IV10	M	++	++	++	++	29
IV11	M	++	++	++	++	24
IV13	F	++	+	+	+	30
IV16	F	++	++	+	+	26
IV20	F	++	++	++	++	21
IV21	F	+++	++	++	++	19
IV22	F	+	+	+	+	16
IV23	M	++	+	+	+	13
IV34	M	−	+	+	−	20
IV39	M	−	+	+	−	16
V2	F	+	+	+	+	9
V3	M	−	+	+	−	4
V6	F	+	+	+	+	6

ID no., individual no. in the pedigree; (−), normal; (+), mildly affected; (++), moderately affected; (+++), severely affected.

For mutation detection using as cDNA template, a 783-bp fragment of the Cx30 gene mRNA was amplified from cDNA using sense primer (forward, 5′-GGA CGC TGC ACA CTT TCA TC-3′) and antisense primer (reverse, 5′-GCT TGG GAA ACC TGT GAT TG-3′) in $(NH_4)_2SO_4$ PCR buffer (Bioline) containing 1.5 mM $MgCl_2$ and 4% dimethylsulfoxide. Amplification conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 2 min, and then final extension at 72 °C for 7 min. PCR products were resolved on a 2% agarose gel. The 783-bp fragment was purified and sequenced as above, using primers: ATC CGA ACC TTG TGC TTT TTA, reverse direction sequence.

4. Results

4.1. Cx30 mutation analysis and confirmation

To diagnose the patients at a molecular level, one exon of the Cx30 gene, including flanking splice recognition sequences, was PCR amplified and bi-direction sequenced. We carried out mutation scanning by direct sequencing of DNA from the patients. A heterozygous missense mutation 31G→A in the Cx30 gene was detected by direct sequencing of PCR products derived from genomic DNA from the patients (Fig. 3a). This substitution leads to the predicted amino acid change gly11-to-arg (G11R) among 18 affected individuals. None of the deletions, insertions or missense mutations described here was found in 16 normal individuals of the HED family and 188 unrelated, population-match control individuals, indicating that they do not represent common polymorphisms (Fig. 3b). The mutation was also confirmed in cDNA originating from the patient's skin biopsy (Fig. 3c). The mutation was confirmed in affected individuals from this family. No other changes were noted in any other of these samples.

5. Discussion

Recently, two Cx30 gene mutations (G11R and A88V), in Clouston syndrome were reported in a total of 12 families from different ethic populations [16], while another (V37E) was reported in a sporadic Scottish case [17]. In our patients we found one recurrent mutation (G11R).

Connexins are the major proteins of gap junctions and are important in the key process of

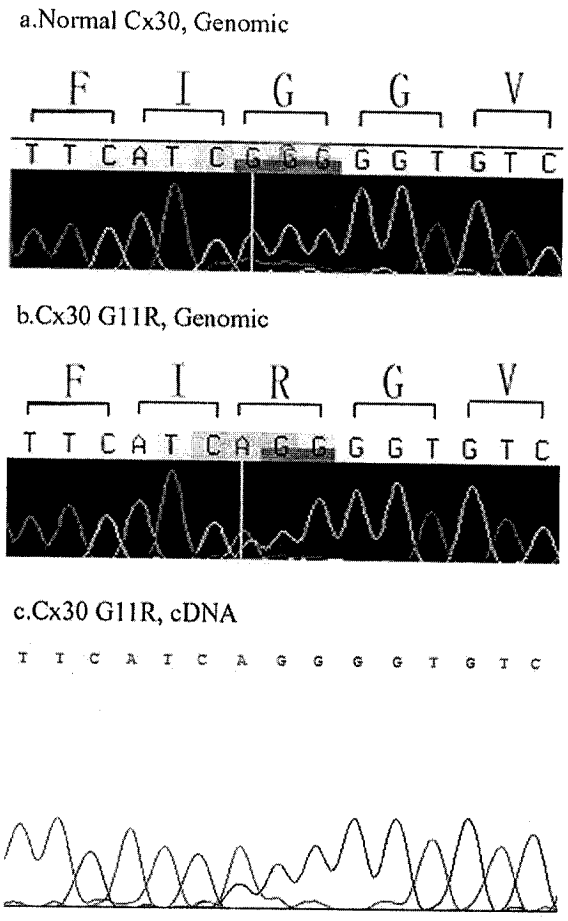

Fig. 3 Cx30 mutation detection and confirmation. (a) Cx30 genomic sequence derived from a normal individual by direct sequencing of PCR products. Coding sequence base numbers 25–39, encompassing codons 9–13 are shown. (b) The equivalent Cx30 sequence as shown in (a), derived from the proband's genomic DNA. The arrow indicates the G31A heterozygous mutation. (c) Equivalent Cx30 sequence as shown in (a), derived from the cDNA prepared from an axillary biopsy from the proband. The arrow indicates the G31A heterozygous mutation. Identification of the mutation in cDNA and genomic DNA in parallel confirms the specificity of the genomic PCR for the Cx30 gene.

intercellular communication. Gap junctions provide direct intercellular communication by linking adjacent cells allowing the direct transfer of small molecules such as metabolites and ions. Cx30 is a member of a family of gap junction proteins forming intercellular channels between adjacent cells.

Connexins have a common structure consisting of four transmembrane domains, two extracellular domains, and three cytoplasmic domains [18,19]. This mutation reported here, G11R, is in the cytoplasmic amino-terminus. The same heterozygous substitution mutations present in the majority of HED patients described to date. Along with glycine 11 and alanine 88, valine 37 is conserved between human and mouse Cx30 and also in connexin 26(Cx26), the most closely related protein to Cx30. HED joins the growing list of inherited skin disorders caused by mutant members of the connexin family of proteins. Two missense mutations, G11R and A88V, affecting conserved amino-acid residues positioned in the cytoplasmatic N-terminal end and in the second transmembrane domains of Cx30, respectively, have been identified in affected unrelated families of many ethnic origins presenting an identical HED phenotype [16]. The importance of the mutated glycine in Cx30 for proper skin gap-junction communication is supported by the fact that an analogous mutation in the neighbouring glycine residue in connexin 31 (Cx31) causes erythrokeratodermia variabilis (EKV). Two missense mutations (G12R and G12D) in the Cx31 gene were observed in EKV [20,21]. The glycine residues at positions 11 and 12 of Cx30 and Cx31, respectively, are conserved residues located in the cytoplasmic amino-termimal domain. G11R, G12R, G12D, three missense mutations affecting residues in the cytoplasmic amino-terminus that are conserved among all -type connexins of different species, and both are predicted to alter charge and structure of this domain. These changes may interfere with conformation and flexibility of the amino-terminus, as well as regulating connexin selectivity and gating polarity [22]. The major variability in the sequence of connexins is found at the intracytoplasmic domains, which could participate in the regulation of the channel activity. The Cx30 gene mutation might also cause HED through haploinsufficiency of gap-junction channels, or by dominant-negative effect on normal Cx30 activity. These mutations appear to affect amino-acid residues that are crucial for the proper assembly or gating polarity of connexons and are supposed to have a dominant inhibitory effect on the function of wild-type connexin channels [21,23]. Therefore, this missense mutation may be a hotspot in this gene, which is known to have a higher mutation rate than other nucleotides.

The involvement of connexins in human disorders has become increasingly evident in recent years. Mutations in connexin genes affect many organs and systems, and the same genes are associated with different disorders. In addition, mutations in different connexins can cause the same or similar disorders. Mutations in either Cx30 or 26 can result in deafness and/or skin pathologies and those in Cx31 can lead to three different phenotypes. The current understanding of the molecular basis of

disorder resulting from mutations in Cx30 allows the use of diagnostic tools for patients and families with this disorder. It remains to be determined why connexin mutations have different effects on the cellular processing of the connexin protein and how tissues are affected differently by particular connexin mutations resulting in skin disease or hearing loss or both disorders.

In conclusion, our report reveals the pathogenic nature of this mutation. Cx30 gene mutations and especially the G11R allele must be considered in families with HED in order to provide the appropriate diagnosis, genetic counseling, and prenatal diagnosis for these families. Together with previous Cx30 gene mutation studies, these results enriched the knowledge about the functional features of connexin protein.

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