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Identification of a known GJB6 mutation in an autosomal dominant inherited Chinese family with hidrotic ectodermal dysplasia

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ABSTRACT

Objective: Mutation in the gap junction beta 6 (GJB6) gene has been reported to be associated with an autosomal dominant disorder hidrotic ectodermal dysplasia (HED), characterized by congenital nail clubbing, alopecia and palmoplantar keratoderma. The aim of this study is to investigate relationship between genetic mutation in GJB6 and HED in an affected Chinese family.

Methods: We selected a Chinese HED family consisting of a total of 17 individuals including 8 HED patients (5 males and 3 females). The whole coding region of GJB6 was amplified by polymerase chain reaction and sequenced.

Results: Sequence analysis identified a heterozygous missense mutation c.31G>A (p.G11R) in GJB6 gene of affected individuals, but not in healthy individuals.

Conclusion: A c.31G>A (p.G11R) missense mutation in GJB6 gene is the genotypic characteristic for HED in Chinese population.

KEY WORDS alopecia; congenital nail clubbing; missense mutation; palmoplantar hyperkeratosis

一个中国先天性有汗性外胚层发育不良家系的 GJB6 基因筛查

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[摘要] **目的：**先天性有汗性外胚层发育不良是一种常染色体显性遗传病，GJB6 基因突变与之相关。调查一个来自中国的先天性有汗性外胚层发育不良家系与 GJB6 基因的关系。**方法：**收集一个共有 17 个家系成员（患者 8 人，5 男 3 女）的先天性有汗性外胚层发育不良家系，采集家系成员的外周血，抽提 DNA。然后针对 GJB6 基因的每个外显子设计引物，应用聚合酶链式反应（PCR）方法扩增 GJB6 基因的整个编码区，测序、筛查突变。**结果：**通过对家系患者的 GJB6 基因筛查，发现了一个杂合错义突变 c.31G>A (p.G11R)。**结论：**GJB6 基因错义突变 c.31G>A (p.G11R) 导致该家系先天性有汗性外胚层发育不良。

[关键词] 毛发缺陷；先天性杵状指；错义突变；掌跖角化过度

Dominant and recessive mutations in the gap junction beta 6 (GJB6) gene, encoding connexin30 (Cx30) protein, are associated with a variety of human inherited diseases, primarily affecting the epidermis, hair, nail, and/or the inner ear[1]. The disorder characterized by congenital nail clubbing and alopecia has long been considered as a group of rare, autosomal dominant, and obscure disorders[2]. Clouston[3] first reported an inherited, autosomal dominant skin disorder characterized by nail clubbing, alopecia, and palmoplantar hyperkeratosis in a large French-Canadian family and named as hidrotic ectodermal dysplasia (HED), also called Clouston syndrome (MIM #129500).

Congenital nail clubbing is a distinct rare genodermatosis entity, characterized by shortened of the nail plate, loss of the cuticle and terminal segments of the fingers and toes, resulting from the swelling of the soft tissue of the terminal phalanx of a digit with subsequent loss of the normal angle between nail and nail bed. Also, symmetric, hard, tender, yellow-brown colored keratotic plaques (palmoplantar keratoderma) develops over the entire palms and soles[4]. Congenital alopecia is clinically characterized by shedding of normal scalp hair with failure to re-grow, sparse eyebrow and eyelashes, and lack of secondary axillary, pubic or body hair[5]. In few cases, hearing impairment has also been reported[6]. There are genetic evidences that missense mutation in the GJB6 gene or Cx30 protein is seemed to be a good candidate for HED[7]. In this study, we investigated a Chinese family with multiple affected individuals with congenital nail clubbing and alopecia, and palmoplantar hyperkeratosis showing no evidence of any other abnormality, characterized as HED or Clouston syndrome. As a result, we have identified a heterozygous missense mutation in GJB6 gene in affected individuals with HED from a Chinese family.

1 Materials and methods

1.1 Patients

We selected a Chinese HED family from Hunan Province consisting of a total of 17 individuals including 8 HED patients (5 males and 3 females). The pedigree analysis provided strong evidence of autosomal dominant inheritance, and it accounted for all of the affected persons being heterozygous for a mutant allele (Figure 1). All the affected patients had the similar clinical presentation. At birth, nail clubbing was noticed but alopecia was noticed at 6 years old. Physical examination revealed thickened, striated, and discolored fingers' nails, short, thin and brittle toes' nails, virtually absent eyebrows and eyelashes, lack of body hairs in lower leg portion, hyperkeratosis of the palms (characterized by rough, yellow-brown keratotic, flat-topped papules, resulting in thickening of the stratum corneum, associated with the abnormality of keratin protein), and alopecia (baldness or loss of hair from head or body parts) (Figure 2). Before the study, written informed consent forms were obtained from all the patients and normal individuals.

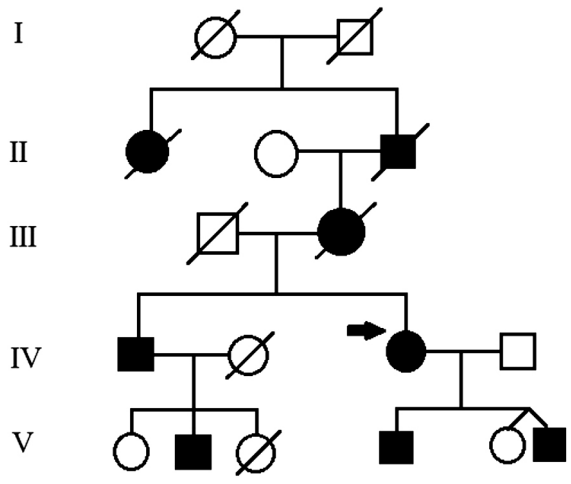

Figure 1 Pedigree analysis of the Chinese family with autosomal dominant nail clubbing and alopecia, taken in this investigation. Normal individuals are shown as clear circles (females) or squares (males), and affected individuals are shown as solid symbols. Deceased individuals are shown with a slash. The arrow indicates the proband.

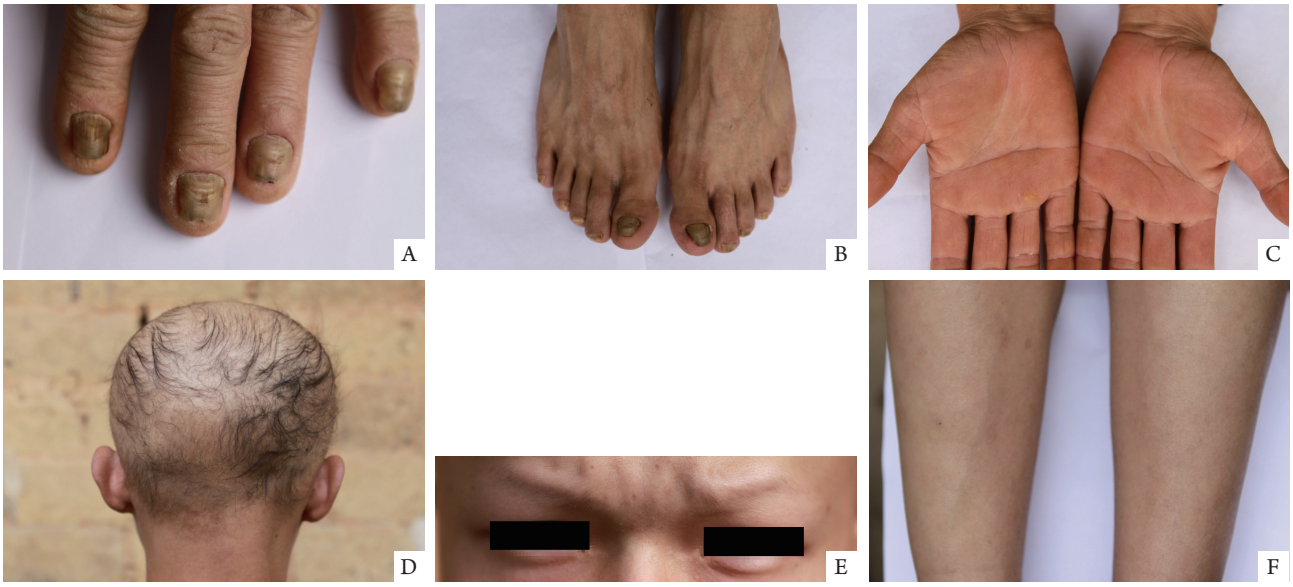

Figure 2 Phenotypes of the studied individuals. A: Thickened, striated, often discolored fingers' nails; B: Short, thin, and brittle toenails; C: Hyperkeratosis of the palms; D: Alopecia; E: Virtually absent eyebrows and eyelashes; F: Lack of body hairs in lower leg portion.

1.2 Methods

1.2.1 Isolation of DNA

Heparinized peripheral blood samples were collected and the genomic DNAs were purified using standard sodium dodecyl sulfate-proteinase K treatment and phenol-chloroform extraction[8]. The purified DNAs were dissolved in tris-ethylene-diamine-tetraacetic acid buffer (TE buffer, pH 7.4) and the concentration of DNA was examined by absorbance at 260 nm and adjusted to 100 ng/mL. The quality of DNA was evaluated by agarose gel electrophoresis.

1.2.2 Polymerase chain reaction

A 1350-bp and 1250-bp fragment spanning the full-length human GJB6 gene was amplified by polymerase chain reaction (PCR) using the following balanced primer pairs, respectively: 1) 5'-AGACTAGCAGGGCAGGGAGT-3' (forward) and 5'- GGAAAAAGATGCTGCTGGTG-3' (reverse); 2) 5'-CCTCCAGCTGATCTTCGTCT-3' (forward) and 5'-GGTTGGTATTGCCTTCTGGA-3' (reverse). Gene-specific PCR primers were derived from the University of California-Santa Cruz (UCSC) human genome database (March 2006, http://genome. ucsc.edu/cgi-bin/hgGateway). Primers to PCR amplified exons and splice junction sites of the genes were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR was performed in 10 μL reaction volume containing 1.0 μL of genomic DNA, 1.0 μL forward primer, 1.0 μL reverse primer, 1 μL 10 × Qiagen HotStar Taq buffer, 2 μL Q-solution, 0.5 μL of 25 mmol/L $MgCl_2$, 0.4 μL dNTPs, 3.05 μL ddH_2O, and 1.0 μL of 5 U/μL HotStar Taq DNA polymerase. Thermal cycling was performed using a thermal cycler (Applied Biosystems). PCR conditions were: 1 cycle of 95 ℃ for 5 min; 8 cycles of 95 ℃ for 30 min, 63 ℃ for 60 min, 72 ℃ for 30 min and decreased 1 ℃ for every cycle; 23 cycles of 95 ℃ for 30 min, 55 ℃ for 60 min and 72 ℃ for 30 min; and 1 cycle of 72 ℃ for 7 min.

1.2.3 Sequencing

After the amplification, PCR products were separated by 1% agarose gel electrophoresis, purified, and then sequenced bidirectionally on an ABI-PRISM3100 automated sequencer (Applied Biosystems). All the sequencing results were assembled, analyzed, and compared to the corresponding wild-type sequences using the SeqMan II program of the Laser gene package (DNA STAR Inc., Madison, WI).

2 Results

To diagnose the patients at a molecular level, one exon of the GJB6 gene, including flanking splice recognition sequences was amplified. PCR resulted in the amplification of GJB6 gene, which was confirmed by 1% agarose gel electrophoresis (Figure 3A), and purified for sequencing. Sequence analysis identified a single nucleotide change c.31G>A in the N-terminal region of GJB6 gene's exon

leading to the substitution of one amino acid on the N-terminal tail of the protein (glycine was substituted by arginine: p.G11R in affected individuals, Figure 3B). None of the deletion, insertion, or missense mutation was found in normal individuals.

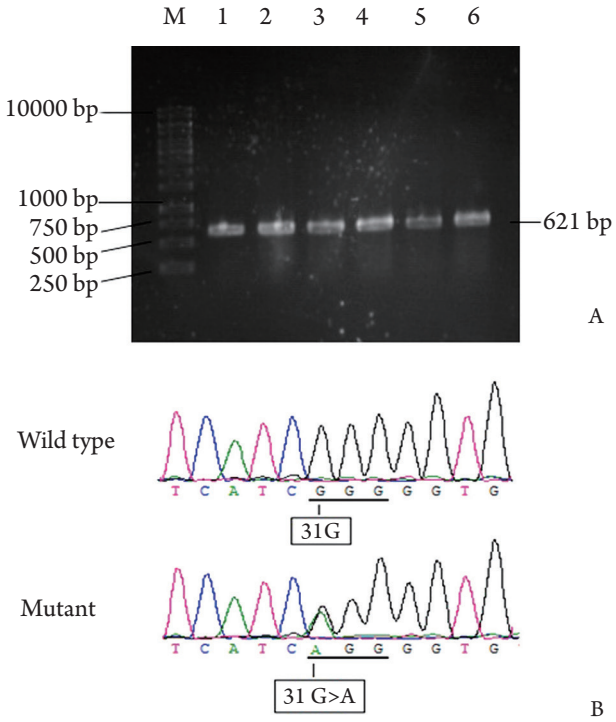

Figure 3 Amplification and sequencing of the exon in GJB6 gene. A: Agarose gel electrophoresis result showing specific band for PCR product (GJB6 gene) of the size 621 bp; M: Marker; 1–6: PCR products from samples of different individuals; B: Partial sequence chromatographs of wild type GJB6 31G (upper panel) and the heterozygous missense mutation 31G>A identified in all patients (lower panel).

3 Discussion

In this study, all the affected patients had similar clinical presentation. No hearing defect, eye abnormalities, dental anomalies, or mental deficiency was found. The life-span for patients is normal. The affected individuals of this Chinese family presented a missense mutation (c.31G>A) in the coding region of the GJB6 gene, which codes for the gap junction protein, connexin 30 (Cx30). Cx30 protein comprises 261 amino acids and is located on the chromosome 13[9]. Cx30 is a member of the connexin protein family which forms channels called gap junctions that permit the transport of nutrients, ions, and signaling molecules between neighboring cells[10], thus coordinating metabolic activities in multi-cellular tissues[11].

Cx30 has 4 trans-membrane domains, 2 extra-cellular domains, and 3 cytoplasmic domains including the amino- and carboxy-terminal regions[10]. Genetic approaches have uncovered a still growing number of mutations in connexins related to human diseases including deafness, skin disease, peripheral and central neuropathies, cataracts, or cardiovascular dysfunctions[12]. The association between connexin mutation and human disorders is being increasingly reported nowadays. Mutation in GJB6 gene changes the building block (amino acid) in Cx30 protein. This change affects the conformation and structural flexibility of N-terminus of Cx30, which regulates the connexin selectivity and gating polarity[2]. This causes abnormal transport activity through the skin gap junctions, which may be associated with the phenotypic characteristics of HED. Three different amino acid substitutions have been identified by Zhang et al[2] in GJB6 gene: p.G11R, p.A88V, and p.V37E. Several other mutation sites have also been reported in GJB6, including p.D50N, responsible for HED; p.G59R, responsible for palmoplantar hyperkeratosis with hearing loss; p.T5M, p.A40V, p.T135K, p.R32Q, p.E101K, p.E147D, p.Y155H, and p.M203V, responsible for deafness[7,9-10,13-19].

We are reporting here the p.G11R mutation, positioned in the cytoplasmatic N-terminal end in GJB6 gene in affected individuals with HED from a Chinese family. Mutation at this position has been reported not only by Zhang et al[2], but also by Lamartine et al[7], Chen et al [5], and also has been indexed by Human Gene Mutation Database (HGMD)[20]. Moreover, Lamartine et al[7] observed no mutation at this position in 118 individuals, who are unrelated to HED. Also, Zhang et al[2] studied 188 normal individuals (HED-unrelated), and reported no mutation at this position. The dbSNP database shows no mutation in this position for the healthy individuals [Reference SNP: rs104894415].

There is no treatment till now for this congenital disorder, and only management is purely supportive. It is hoped that the identification of GJB6 mutations underlying the phenotypes and functional analysis of the mutation will enable a more rapid molecular diagnosis and may reveal the pathological mechanisms, which in future will help to find out treatment of this rare genetic disorder.

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References

1. Common JE, Becker D, Di WL, et al. Functional studies of human skin disease- and deafness-associated connexin 30 mutations [J]. Biochem Biophys Res Commun, 2002, 298(5): 651-656.
2. Zhang XJ, Chen JJ, Yang S, et al. A mutation in the connexin 30 gene in Chinese Han patients with hidrotic ectodermal dysplasia [J]. J Dermatol Sci, 2003, 32(1): 11-17.
3. Clouston HR. A hereditary ectodermal dystrophy [J]. Can Med Assoc J, 1929, 21(1): 18-31.
4. Özdemïr M, Engïn, Baysal İ. Hydrotic ectodermal dysplasia associated with a rib anomaly [J]. Turkiye Klinikleri J Dermatol, 2007, 17(4): 205-209.
5. Chen N, Xu C, Han B, et al. G11R mutation in GJB6 gene causes hidrotic ectodermal dysplasia involving only hair and nails in a Chinese family [J]. J Dermatol, 2010, 37(6): 559-561.
6. Yildirim M, Yorgancilar E, Gun R, et al. Ectodermal dysplasia: otolaryngologic evaluation of 23 cases [J]. Ear Nose Throat J, 2012, 91(2): E28-33.
7. Lamartine J, Munhoz Essenfelder G, Kibar Z, et al. Mutations in GJB6 cause hidrotic ectodermal dysplasia [J]. Nat Genet, 2000, 26(2): 142-144.
8. Davis LG, Dibner MD, Battey JF. Preparation of DNA from eukaryotic cells [M] //Basic methods in molecular biology. New York: Elsevier, 1986: 42-50.
9. Baris HN, Zlotogorski A, Peretz-Amit G, et al. A novel GJB6 missense mutation in hidrotic ectodermal dysplasia 2 (Clouston syndrome) broadens its genotypic basis [J]. Br J Dermatol, 2008, 159(6): 1373-1376.
10. Smith FJ, Morley SM, McLean WH. A novel connexin 30 mutation in Clouston syndrome [J]. J Invest Dermatol, 2002, 118(3): 530-532.
11. Jan AY, Amin S, Ratajczak P, et al. Genetic heterogeneity of KID syndrome: identification of a Cx30 gene (GJB6) mutation in a patient with KID syndrome and congenital atrichia [J]. J Invest Dermatol, 2004, 122(5): 1108-1113.
12. Zoidl G, Dermietzel R. Gap junctions in inherited human disease [J]. Pflugers Arch, 2010, 460(2): 451-466.
13. Grifa A, Wagner CA, D'Ambrosio L, et al. Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus [J]. Nat Genet, 1999, 23(1): 16-18.
14. Gardner P, Oitmaa E, Messner A, et al. Simultaneous multigene mutation detection in patients with sensorineural hearing loss through a novel diagnostic microarray: a new approach for newborn screening follow-up [J]. Pediatrics, 2006, 118(3): 985-994.
15. Yang JJ, Huang SH, Chou KH, et al. Identification of mutations in members of the connexin gene family as a cause of nonsyndromic deafness in Taiwan [J]. Audiol Neurootol, 2007, 12(3): 198-208.
16. YUAN Yongyi, HUANG Deliang, DAI Pu, et al. GJB6 gene mutation analysis in Chinese nonsyndromic deaf population [J]. Journal of Clinical Otorhinolaryngology Head and Neck Surgery, 2007, 21(1): 3-6.
袁永一, 黄德亮, 戴朴, 等. 中国非综合征遗传性聋人群GJB6基因突变分析[J]. 临床耳鼻咽喉头颈外科杂志, 2007, 21(1): 3-6.
17. Nemoto-Hasebe I, Akiyama M, Kudo S, et al. Novel mutation p.Gly59Arg in GJB6 encoding connexin 30 underlies palmoplantar keratoderma with pseudoainhum, knuckle pads and hearing loss [J]. Br J Dermatol, 2009, 161(2): 452-455.
18. Asma A, Ashwaq A, Norzana AG, et al. The association between GJB2 mutation and GJB6 gene in non syndromic hearing loss school children [J]. Med J Malaysia, 2011, 66(2): 124-128.
19. Battelino S, Repič Lampret B, Zargi M, et al. Novel connexin 30 and connexin 26 mutational spectrum in patients with progressive sensorineural hearing loss [J]. J Laryngol Otol, 2012, 126(8): 763-769.
20. Anon. All mutations in GJB6 for Hidrotic ectodermal dysplasia [EB]. Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac.

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