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Identification of a novel mutation in the *FGFR3* gene in a Chinese family with Hypochondroplasia

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ABSTRACT

Background: Hypochondroplasia (HCH; OMIM 146000) is a common autosomal dominant skeletal dysplasia characterized by disproportionate short stature, short extremities, relative macrocephaly, and lumbar lordosis. Because of its clinical and genetic heterogeneity, gene mutational analysis is particularly important in diagnosis and the phenotypes may be ameliorated if diagnosed early.

Materials and methods: In this study, we examined a Chinese family with HCH, performed an inductive analysis of their clinical features and radiographic results, and applied targeted exome sequencing (TES) technology to perform a molecular diagnosis.

Results: The proband and his mother all presented disproportionate short stature, short, stubby extremities, unchanged interpedicular distances from L1 − L5, and short iliac bones, with a 'fish mouth-shaped' sciatic notch. The mother received induced abortion recently because an ultrasound showed short femur length of her fetus at 24-week gestation. Eventually, a novel heterozygous mutation (c.1145G > A) in *FGFR3* was identified by TES in the proband, his mother, and her fetus; this causes the substitution of glycine with aspartic acid in codon 382.

Conclusions: In this study, we diagnosed a Chinese pedigree with HCH based on clinical data, radiographic features, and genetic testing results. Our results extend the genetic mutation spectrum of *FGFR3* and demonstrate that TES is an effective method for the diagnosis of skeletal dysplasia in clinical practices.

1. Introduction

Hypochondroplasia (HCH; OMIM 146000) is a common autosomal dominant skeletal dysplasia characterized by disproportionate short stature, short extremities, relative macrocephaly, and lumbar lordosis, with an incidence of approximately 1.3/10,0000 at birth (Andersen and Andersen and Hauge, 1989). However, HCH has similar clinical presentations with three other skeletal dysplasias (SDs): pseudoachondroplasia, achondroplasia (ACH), and thanatophoric dysplasia (TD), which have overlapping clinical phenotypes. Many clinical features of HCH do not appear in early childhood, but develop later in life, which make it difficult to diagnose early based on clinical and radiographic data. Owing to its clinical and genetic heterogeneity, gene mutational analysis is particularly important in diagnosis and may be preventable if diagnosed early (Geister and Camper, 2015).

Mutations in the gene encoding fibroblast growth factor receptor 3 (*FGFR3)* can cause HCH. *FGFR3* is located at 4p16.3, containing 19 exons and 18 introns (Keegan et al., 1991). *FGFR3* is the only known gene associated with HCH. According to GeneReviews (NCBI), a heterozygous mutation of *FGFR3*, N540 K, accounts for 70% of all reported patients (Rousseau et al., 1996; Stenson et al., 2003), whereas mutations at different positions of *FGFR3* only account for fewer cases and some cases that are clinically suspected to be HCH did not have *FGFR3* gene mutation. The latest development of HCH genetic diagnosis raises new questions of possible genetic heterogeneity in HCH.

In this study, we examined a Chinese family with SD, analyzed the patients' clinical and radiographic results in detail, and applied targeted exome sequencing (TES) technology for the probands and their family

Abbreviations: HCH, Hypochondroplasia; SDs, skeletal dysplasias; ACH, achondroplasia; *FGFR3*, fibroblast growth factor receptor 3; TES, targeted exome sequencing

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members. A novel missense mutation in exon 9 of *FGFR3* was identified in the proband. The variant was also found in his mother and her fetus by Sanger sequencing. Our results provide information necessary to improve diagnosis of HCH and will contribute to better genetic counseling in the future.

2. Materials and methods

2.1. Ethical approval

This study was performed in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (IRB) of Xiamen Maternal and Child Health Hospital (Xiamen, China). Written informed consent was obtained from all participants or legal guardians in this study. The methods were carried out in accordance with the approved guidelines.

2.2. Patients

The subject family with SD participated in our study with informed consent in December 2016. Peripheral blood samples were obtained from all individuals, and comprehensive clinical data, such as medical history, pedigree, physical examination, and detection of radiological abnormalities, were collected.

2.3. Candidate gene mutation analysis

Genomic DNA was isolated from peripheral blood leukocytes, using QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Three micrograms (μg) of genomic DNA was employed for exome library construction. Mutations in a short stature custom panel (495 curated genes) were detected by Sinopath Bioinformatics Institute (Beijing, China), utilizing targeted exome sequencing (TES) technology. TES of the genomic DNA was performed on an Illumina Hiseq 4000 platform to obtain paired-end reads with 150 bp read lengths (Wang et al., 2011). The average coverage of the exome was > 100 ×, which permitted examination of the target region with enough depth to exactly match > 99% of the target exome (Guo et al., 2014). Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Applied Biosystems, Foster, CA, USA), and the sequencing was analyzed with an ABI Prism 3130 automated sequencer. The following primers were employed for PCR amplifications: *FGFR3* forward 5′ to 3′: CACTGGCGTTACTGACTGC; and *FGFR3* reverse 5′ to 3′: AGTACCCTAGGCTCTACATGGT.

2.4. Bioinformatics analysis

For bioinformatic analysis, we used three software tools, PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org) and Mutation Taster (http://www.mutationtaster.org/) to predict potential deleterious effects of the missense mutation (Kumar et al., 2009; Adzhubei et al., 2010). To confirm the conservation of amino acid substitutions in the process of species evolution, the typical protein sequences of multiple different species were aligned using Clustal W (UCD, Dublin, Ireland) software to compare mutated positions with conserved domains. Modeling was performed using I-TASSER software, and PyMOL Viewer was used to visualize the effects of altered residues on the protein structures.

3. Results

3.1. Clinical manifestation

The pedigree of the patients is shown in Fig. 1A. The proband (III-1) was the first child in the family with SD. He was born at 39 weeks of gestation by cesarean section. His birth length and weight were reported to be 50 cm (25th–50th percentile) and 3300 g (25th–50th percentile), respectively. He was brought to the Department of Pediatrics at the age of 8 years and 7 months for disproportional short stature. His height was 109.6 cm (− 4.13SDS) and his weight was 21.7 kg (3rd–10th percentile). Other physical examination findings included pectus carinatum, relative macrocephaly (head circumference 52.6 cm), lumbar lordosis, slightly protuberant abdomen and hips, and short extremities. His intelligence was normal (Fig. 1B). The proband's mother (II-3) was 32 year old when she received the physical examination (Fig. 1C). Her height was 133.6 cm (− 5.09SDS) and her weight was 38.0 kg (< 3rd percentile). She received an induced abortion recently because an ultrasound found short femur length (FL) of the fetus. In addition to short stature, other physical examination abnormalities were similar to the proband. She underwent an ultrasound scan examination at 24-week gestation, and the fetus was found to have abnormally short femur and humerus: FL was 2.9 cm, equivalent to 19 w + and humerus length was 2.9 cm, equivalent to 19 w +. The ratio of FL to foot length was 0.73 (normal > 0.9). The ratio of FL to abdominal circumference was 0.15. Biparietal diameter was 6.0 cm and foot length was 4.0 cm, which were normal, equivalent to 24 w. There were no other pathologic results of the fetus. Other body measurements, including arm span and upper-to-lower body-segment ratio (U:L), are presented in the Table 1. All anthropometric measurements were evaluated according to the children's physical development survey data of nine provinces/cities in China (2005).

Radiographic examinations were performed on the two patients. Radiological studies of the patient III-1 revealed short extremities, unchanged interpedicular distances from L1 − L5, broadening the metaphysis of proximal tibial, and 'fish mouth-shaped' sciatic notch (Fig. 2A). For patient II-3, the skeletal defects were similar to those of patient III-1 and all conform to the criteria for HCH (Wynne-Davies et al., 1981) (Fig. 2B). Unfortunately, the mother did not consent to taking radiographic inspections of her aborted fetus.

3.2. Mutation detection

To further confirm the diagnoses, we subsequently applied TES technology for the proband and his family members. Using the Human Gene Mutation Database (HGMD), we identified a novel heterozygous mutation (c.1145G > A) in *FGFR3* in the patients III-1, II-3, and her fetus, causing the substitution of glycine to aspartic acid in codon 382 (Fig. 3A). The substitution was not detected in 100 unaffected individuals, suggesting that it was not a polymorphism. The human *FGFR3* conserved domain consists of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The missense mutation occurred in the transmembrane domain at position 382 (G382D) (Fig. 3B). In addition, it was predicted to be possibly damaging by Polyphen v.2, with a score of 0.887 (Fig. 3C), and SIFT predicted this mutation to be damaging, with a score of 0.01. The gene alteration also presumably influences protein properties, and is pathogenic, as suggested by MutationTaster. Multiple amino acid sequence alignments show that p.Gly382 is conserved across various species by using the Clustal W tool (Fig. 3D). This novel mutation in *FGFR3* is predicted to cause a gain-of-function via ligand-independent activation of *FGFR3*, leading to defects in cartilage differentiation in long bone growth plates (Fig. 3E).

4. Discussion

HCH and ACH are genetic skeletal diseases characterized by short-limb type short stature and have very similar clinical manifestations. Typical clinical manifestations of ACH are as follows: large head with protruding forehead, special facial features (wide between the eyes, flat nose, and prominent jaw), short hands and fingers with a trident appearance, protuberant abdomen and hips, lumbar lordosis, genu varus,

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