



# 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 1.3/10,000 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 ( $\mu\text{g}$ ) 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  $> 100\times$ , 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': CACTGGCGTACTGACTGC; 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 ( $-4.13\text{SDS}$ ) 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 ( $-5.09\text{SDS}$ ) and her weight was 38.0 kg ( $< 3\text{rd}$  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).

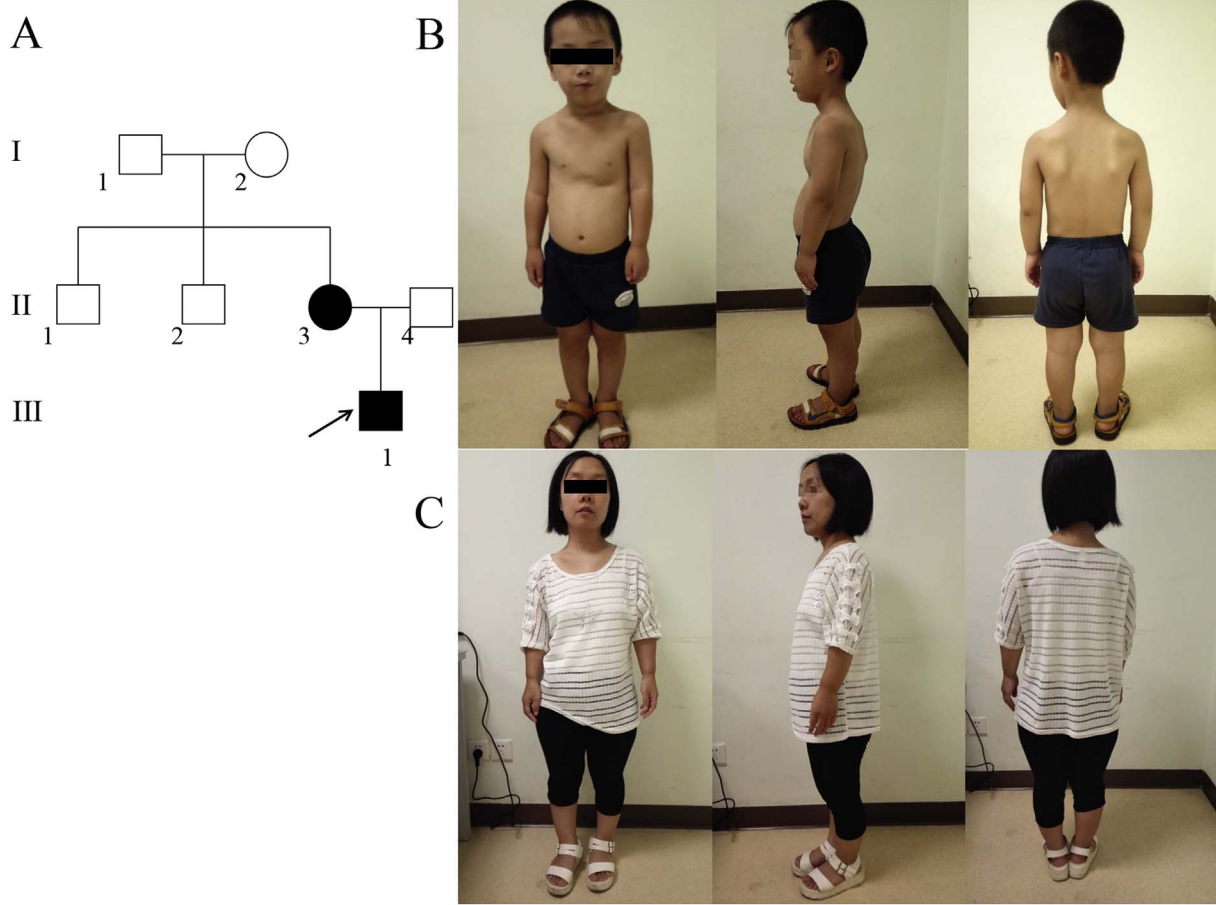
Radiographic examinations were performed on the two patients. Radiological studies of the patient III-1 revealed short extremities, unchanged interpedicular distances from L1 – L5, broadening the metaphysis of proximal tibial, and 'fish mouth-shaped' sciatic notch (Fig. 2A). For patient II-3, the skeletal 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,



**Fig. 1.** The Chinese family with SD in our study. (A) Pedigree drawing of the family. Square symbol = male, round symbol = woman, filled symbol = affected, unfilled symbol = unaffected, arrows = probands. (B) Pictures of patient III-1. (C) Pictures of patient II-3.

and valgus deformity (Ornitz and Legeai-Mallet, 2017). Compared with ACH, the skeletal abnormalities of HCH are usually milder. The average heights of HCH affected adults are 146.1 cm in males and 137.6 cm in females (Korkmaz et al., 2012), whereas patients with ACH is 125 cm in males and 124 cm in females, respectively (Trotter and Hall, 2005). The most common radiologic features of HCH include short stubby extremities, broadening metaphyses, gradually narrowing or unchanged interpedicular distances from L1 – L5, and short iliac bones with ‘fish mouth-shaped’ sciatic notch (Song et al., 2012). The clinical and radiographic findings of HCH is atypical, diverse, and milder than those of ACH, which makes HCH difficult to diagnose early (Brook and de Vries, 1998; Matsui et al., 1998), with most diagnoses being made in the late childhood.

Song et al. tested 58 clinically diagnosed HCH patients for mutations in *FGFR3* and the short stature homeobox (*SHOX*) gene. They found that 32.8% (19/58) had *FGFR3* mutations and 10.3% (6/58) had *SHOX* mutations, while 56.9% (33/58) were negative for mutations in *FGFR3* and *SHOX*, suggesting that other undiscovered gene mutations are associated with this disease (Song et al., 2012). According to the Human Gene Mutation Database (HGMD, <http://www.hgmd.org/>), 66

mutations in *FGFR3* have been reported (updated on January 2017), approximately one third of which (23/66) were reported to be associated with HCH. Approximately 70% of the affected patients of HCH are caused by heterozygous mutations in *FGFR3*. The most common mutations of HCH are in exon 10 of gene *FGFR3*: c.1620C > A and c.1620C > G transition, causing the substitution of asparagine by lysine at codon 540 (p.N540K). The two *FGFR3* missense mutations occurred in 70% of the cases of HCH (Prinos et al., 1995). Other rare *FGFR3* mutations associated with HCH accounted for < 2% of *FGFR3* mutations (Bellus et al., 1995; Bellus et al., 1996; Deutz-Terlouw et al., 1998; Grigelioniene et al., 1998; Bellus et al., 2000; Mortier et al., 2000; Winterpacht et al., 2000). Biochemical analysis have shown that the *FGFR3* mutations could increase the efficiency of receptor phosphorylation in the absence of ligand, leading to the defect of cartilage differentiation long bone growth plate (He et al., 2012). In this study, we employed TES to detect mutations in a Chinese family with SD. A c.1145G > A variant (p.G382D) in *FGFR3* was found in the patients III-1, II-3, and her fetus, which was predicted to lead to damaging consequences analyzed by SIFT, PolyPhen2, and Mutation Taster. This variation was verified by Sanger sequencing. The mutation was not

**Table 1**  
Summarization of anthropometric measurements of the affected family members.

Patient	Age <sup>a</sup>	Gender	Height (cm)	Ht-SDS	U:L	Arm span (cm)	Sitting height, ratio to the total height	Head circumference (cm)
III-1	8 y 7 m	M	109.6	- 4.05	1.35	103.7	0.62	52.6
II-3	32 y	F	135.1	- 4.81	1.41	125.9	0.63	56.6

<sup>a</sup> y: year, m: month.



**Fig. 2.** Radiographic findings of the two patients. (A) Images of Patient III-1. (a-b) Radiographic findings of the spine of patient III-1. The patient displayed unchanged interpedicular distances from L1-L5. (c) Radiographic findings of the pelvis of patient III-1. Narrow pelvis, 'fish mouth-shaped' sciatic notch was observed. (d) Radiographic findings of the long bones of patient III-1. Short stubby extremities and broadening of the metaphysis of proximal tibial, were observed. (B) Images of Patient II-3. (a-b) Radiographic findings of the spine of patient II-3. The patient displayed unchanged interpedicular distances from L1-L5. (c) Radiographic findings of the pelvis of patient II-3. Narrow pelvis, 'fish mouth-shaped' sciatic notch and short femoral necks, were observed. (d) Radiographic findings of the long bones of patient II-3. Short stubby extremities, were observed.

present in otherwise healthy family members or the 100 ethnicity-matched controls. Co-segregation of the mutation and disease suggested that this was a pathogenic mutation. Analysis with Clustal X v.2 (<http://clustal.org/clustal2/>) revealed that the glycine at position 382 is highly conserved among species, suggesting that it is structurally and functionally important. These findings indicated that *FGFR3* mutation was probably harmful and pathogenic in the patients presented here.

*FGFR3* gene encodes fibroblast growth factor receptor 3, which is a member of the fibroblast growth factor receptor (*FGFR*) family. These family members combine with acidic and basic fibroblast growth hormone and play an important part in the development and maintenance of bone structure (Peters et al., 1993). The *FGFR3* structure is mainly composed of three parts, including an extracellular domain, a transmembrane domain, and an intracellular domain. A novel mutation was identified in the transmembrane domain of *FGFR3* at position 382, which is strongly conserved among species. Previous studies have shown that various pathogenic missense mutations located in the transmembrane domain of *FGFR3* are associated with ACH or HCH (Shiang et al., 1994; Bonaventure et al., 1996; Heuertz et al., 2006). Among them, G380R is a hot spot mutation, leading to gain-of-function via ligand-independent stabilization of *FGFR3* dimers. This stabilization occurs through the formation of hydrogen bonds between the side-chains of the two arginine residues, demonstrating the importance of this functional domain. Mutation G382D is adjacent to two mutations, the V381E HCH mutation and the G380R ACH mutation, and we compare the clinical and radiographic features of the mutations listed in Table 2. From the table, we can see that a gradient of severity exists

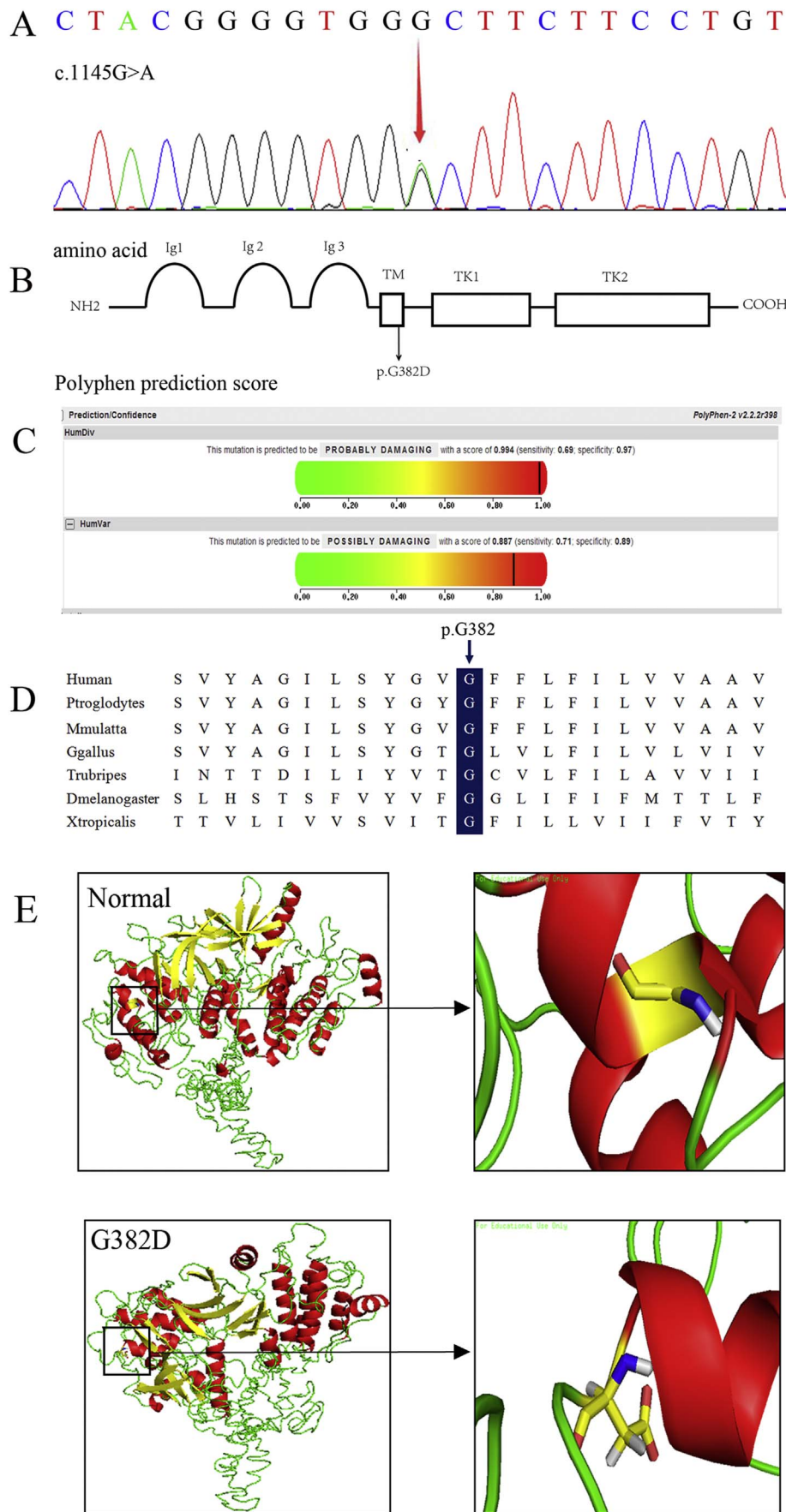
for these mutations, as follows: G380R homozygote > G380R compound heterozygote > V381E heterozygote > our case. > 90% of the ACH cases are caused by G380R mutation in exon 10 of *FGFR3* gene (Satioglu-Tufan et al., 2006; Placone and Hristova, 2012). Heuertz S et al. reported the c.T1142A (V381E) mutation in a sporadic case of HCH (Heuertz et al., 2006). The major skeletal abnormalities described by Heuertz S et al. included dysplasia of the femoral necks, mild metaphyseal cupping in the metacarpals, and narrowing interpedicular distances in the 8-year-old patient. In the present study, the interpedicular distances from L1 – L5 in the patients were not gradually narrowing, but aequilate. Compared with adjacent ACH mutations, the severity of the clinical phenotype was reduced, which suggests that residues 381 and 382 are less critical for receptor activation than residue 380, or that the hydrogen bonding strength is lower. The milder phenotypes of our patients may also be caused by the conservation of the amino acid substitution.

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*. However, the correlations between the phenotype and genotype remain unclear and studies on more patients will be needed to provide more comprehensive clinical and genetic data.

#### Competing interests

The authors declare that they have no competing interests.





**Fig. 3.** Sequencing results and bioinformatic analysis of the gene mutation in our study. (A) Partial sequence diagram of *FGFR3* exon 9 in our case. A heterozygous mutation c.1145G > A transition, causing the substitution of glycine by aspartic acid at codon 382, is shown by an arrow. (GenBank accession number: NM\_000142.4) (B) The structure domains of *FGFR3*. Mutations at the protein level are indicated below the domains. (C) Score of the novel damaging mutation c.1145G > A (p.G382D) in Polyphen v.2. (D) Cross-species conservation of *FGFR3* around p.Gly382 is displayed. (E) Protein structure prediction of wild-type and mutant *FGFR3*.

**Table 2**  
Comparison of G380R, V381E substitution in *FGFR3* and our case.

	G380R homozygote	G380R compound heterozygote	G380R heterozygote	V381E heterozygote	Our case
<b>Clinical features</b>					
Frontal bossing/depressed nasal bridge/midface hypoplasia/macrocephaly	Severe	Moderate/Severe	Moderate/Severe	+	+
Disproportionate short-limb stature	Severe	+	+	+	+
Brachydactyly and trident hand	Severe	+	+	?	–
Bowlegs	Severe	+	+	?	+(mild)
<b>Developmental delay</b>					
Gross motor	Severe	Moderate/severe	+	–	–
Mental retardation	Severe	Mild to Severe	–	–	–
Hypotonia	Severe	Moderate/severe	+	–	–
<b>Radiological findings</b>					
Shortening of long bones with metaphyseal flare	Similar to TD	Moderate/severe	Moderate/severe	+	+
Narrowing of the inferior lumbar interpedicular distances	Severe	Moderate/severe	Moderate	+	–
Squared shortened ilia/narrowed sacroiliac notches	Similar to TD	Moderate/severe	Moderate	?	+

References: +, present; –, not present; ?, unknown status; Thanatophoric dysplasia: TD.

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**Author contributions**

J.C., G. L., and C.X. designed the study. J.C., J.Y., and S.Z. performed the study and analyzed the data. J.C. wrote the main manuscript. All authors read and approved the final manuscript.

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