Original Article
Novel *HOXD13* frameshift mutation causes synpolydactyly and clinodactyly

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Abstract: *HOXD13* is a member of homeobox transcription factor genes essential for the vertebrate body plan. Mutations of this gene have been associated with different types of human limb deformities, including synpolydactyly, clinodactyly, and brachydactyly, but the gene-phenotype relationships are not fully understood. This study reports a novel heterozygous frameshift mutation of *HOXD13* (c.610delC, p. P204fs61x) in a Chinese family with synpolydactyly and clinodactyly. This mutation was detected in the proband by whole-exome sequencing and confirmed by Sanger sequencing. This mutation co-segregated among family members with the phenotype. Furthermore, this study systemically reviewed previous studies and updated the gene-phenotype relationship for human *HOXD13* mutations.

Introduction

HOX genes encode a large family of homeobox transcription factors essential for embryonic patterning [1]. There are four *HOX* gene clusters in human genome *HOXA-D*, each containing 9-11 genes. For each cluster, genes at the 3' end are expressed early, in anterior and proximal regions of the embryo. Genes at the 5' end are expressed later, in posterior and distal regions [2]. The 5' members of *HOX* genes (*HOXA9-13* and *HOXD9-13*) are important for vertebrate limb development. During anterior-posterior asymmetry formation of the limb, Sonic Hedgehog (*Shh*) is locally expressed at the posterior margin, as well as other patterning genes, including *dHand*, *Twist1*, and most 5' members of the *Hoxd* gene cluster. At the anterior side, expression of the transcriptional repressor form of Gli3 (Gli3R) inhibits expression of these genes. The opposing gradient of Shh and Gli3R, as well as their target genes, specifies digit number and identity [3].

Germline mutations of *HOXD13* have been associated with various human congenital limb malformations [4]. This gene is comprised of two exons encoding a protein of 343 amino acids. The N-terminal region of *HOXD13* protein contains several poly-serine and poly-alanine residues, whereas the C-terminal region contains the DNA-binding homeobox domain [5]. Patients with *HOXD13* mutations display various phenotypes, including synpolydactyly 1 (SPD1) [6-9], brachydactyly D (BDD) [10], syndactyly 5 (SDTY5) [11], brachydactyly-syndactyly syndrome (BDSD) [11], brachydactyly E1 (BDE1) [7, 10], VACTERL association (VACTERL) [12], and brachydactyly-syndactyly-oligodactyly syndrome (BDSDO) [13]. However, the gene-phenotype relationship is not fully understood.

The present study identified a novel frameshift mutation of *HOXD13* (c.610delC, p. P204fs61x) in a family with synpolydactyly and clinodactyly. Phenotypes varied between affected family members, exhibiting either synpolydactyly, clinodactyly, or both. Finally, this study systemically reviewed the literature and updated the gene-phenotype relationship of this gene.

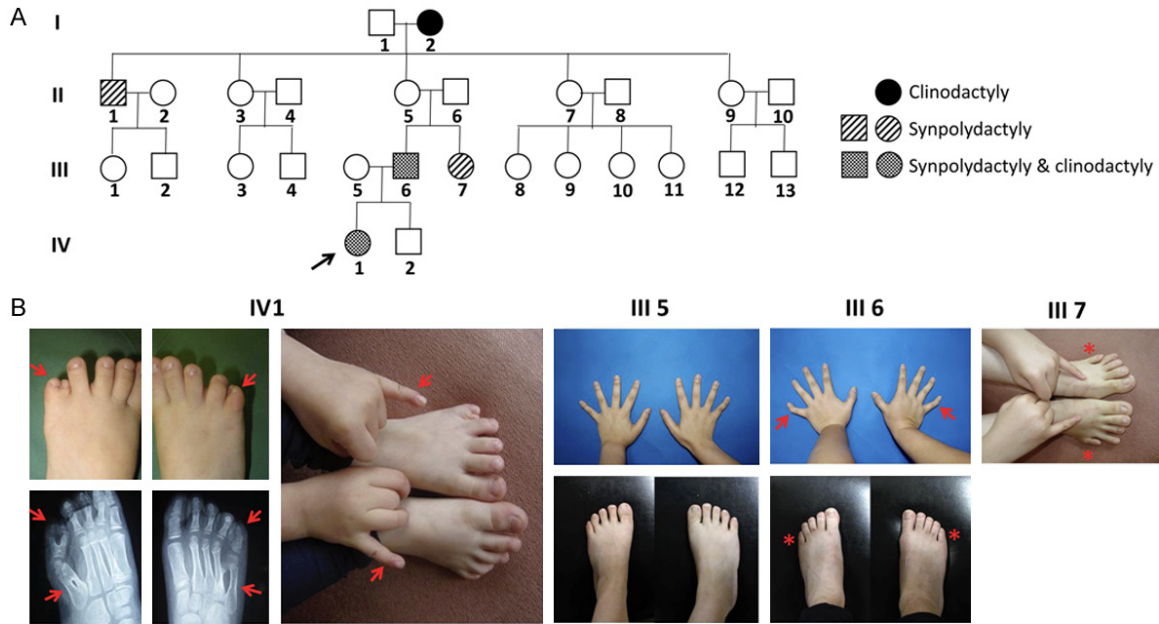# A novel HOXD13 frameshift mutation causes synpolydactyly and clinodactyly

Figure 1. A. Pedigree of a four-generation Chinese family with synpolydactyly and/or clinodactyly. Squares and circles denote males and females, respectively. Unaffected family members are presented as blank squares and circles. Phenotypes of affected family members are as indicated. The proband is denoted by an arrow. B. Clinical features of the proband (IV-1) and other family members (III5, III6 and III7). Arrows indicate limb malformations. Asterisks indicate affected limbs after previous surgery.

Methods

Pedigree recruitment

Members of a four-generation Chinese family with synpolydactyly and clinodactyly were recruited with written consent. Phenotypes of all family members were obtained based on either orthopedist diagnoses or description from the proband's parents. Participant hands and feet were radiographed. A thorough physical examination was performed to exclude other congenital malformations.

Genomic DNA extraction

Peripheral blood of the proband (IV1), her affected father (III6), and unaffected mother (III5) was collected (**Figure 1A**). Genomic DNA was extracted from 200 μl peripheral blood using a QIAGEN DNeasy Blood & Tissue Kit (Cat. No. 69504), following manufacturer instructions. Quantity and quality of extracted DNA was assessed by NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and agarose electrophoresis.

Whole exome sequencing

Genomic DNA of the proband was subjected to whole exome sequencing. Exome capture was performed by using SureSelectXT Human All Exon V5 kit (Agilent Technologies, USA). DNA library was sequenced on an Illumina HiSeq 2000 sequencing system (Illumina, CA, USA) with a read length of 100 base pairs.

Sequencing data analysis

Raw data was converted to Fastq format and the base quality was assessed by FastQC program. Reads were aligned to reference human genome hg19 using BWA (0.7.12) program. Variations of target regions were identified by HaplotypeCaller tool of GATK program (version 3.5). They were then annotated by ANNOVAR program.

Sanger sequencing

To validate this mutation, this study amplified the DNA fragment flanking this position by polymerase chain reaction (PCR) and performed Sanger sequencing. Primer sequences were 5'-TCGTCCTCTTCTGCCGTTG-3' (forward) and 5'-GAGAAAAACGTGTCTGCGCT-3' (reverse).

Results

Clinical report

The great-grandmother (I2) of the proband had bilateral clinodactyly in the fifth fingers. The
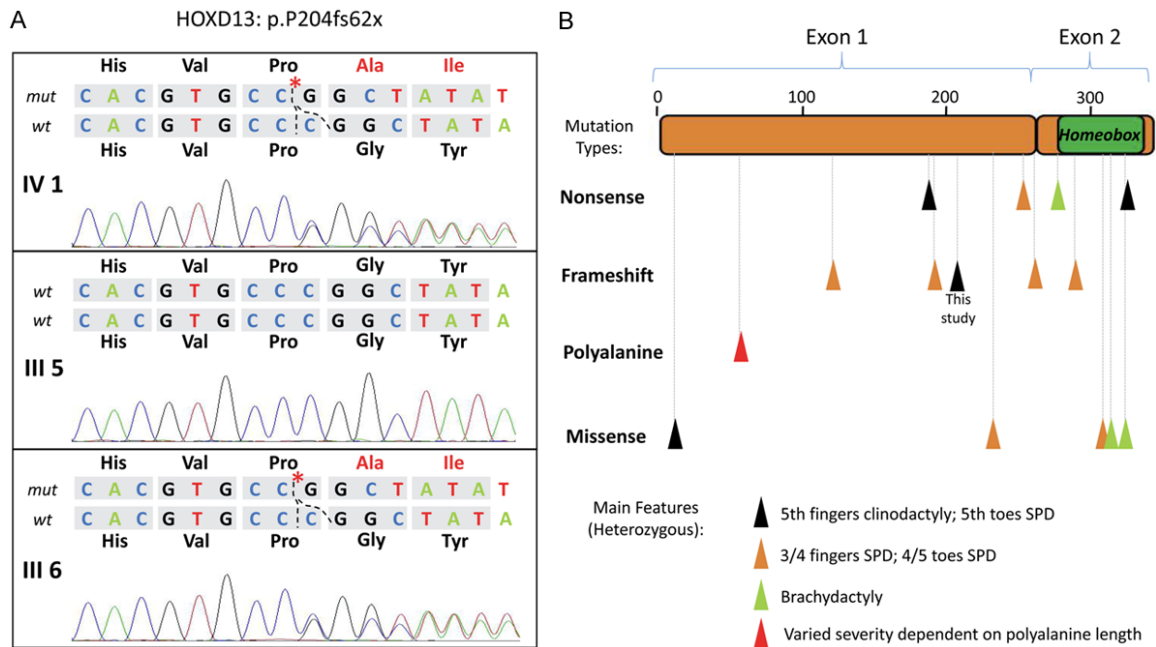
Figure 2. A. Novel single-nucleotide deletion in *HOXD13* of the proband and her father, but not her mother, examined by Sanger sequencing. B. The simplified gene-phenotype relationship of *HOXD13*. Described phenotypes are from individuals with heterozygous mutations; SPD, synpolydactyly. References of this schematic are shown in the discussion section.

proband (IV1) and her father (III6) exhibited bilateral synpolydactyly in the fifth toes, in addition to bilateral clinodactyly in the fifth fingers (**Figure 1B**). However, the other two affected family members (II1 and III7) only had bilateral synpolydactyly in the fifth toes.

Whole exome sequencing data

The unique mapped ratio was 76%. Exome coverage was above 99% and the mean read depth was above 80x. A total of 38,685 single nucleotide variations and 4,759 small insertion and deletions were identified.

Identification of pathogenic mutations

Of identified single-nucleotide variations and insertion/deletions, this study found a heterozygous single nucleotide deletion, c.610delC, in the key limb development gene *HOXD13*. This deletion resulted in a frameshift in the open reading frame of *HOXD13* gene, causing a pre-mature termination of the protein (p. P204fs61x). This mutation was not found in 1000 Genomes [14], Exome Aggregation Consortium [15], and Kaviar [16] databases. According to ACMG guidelines [17], this mutation can be classified as PVS1 (pathogenicity

is very strong) because loss of function of *HOXD13* is a known mechanism for causing synpolydactyly. To validate this mutation, this study amplified the DNA fragment flanking this position by polymerase chain reaction (PCR) and performed Sanger sequencing. Results clearly showed a heterozygous single cytosine deletion in the proband (**Figure 2A**). This mutation was also detected in the proband's affected father, but not in the unaffected mother (**Figure 2A**), further supporting the pathogenicity of this mutation.

Discussion

Germline mutations of *HOXD13* have been frequently detected in human congenital limb malformations [8]. Using WES analysis, the present study identified a novel frameshift mutation of *HOXD13* in a Chinese family with synpolydactyly and/or clinodactyly, with no other obvious malformations.

Many *HOXD13* mutations have been identified in various limb malformations [4]. Reported mutations are of wide range of types, including point mutation, insertion, and deletion. They are located in both exons and splicing sites of this gene, causing protein truncation, amino

acids substitution, insertion, and deletion. In most cases, *HOXD13* mutations are heterozygous and inherit in a dominant manner. Reviewing all PubMed papers reporting *HOXD13* mutations in humans, the gene-phenotype relationship for this gene was summarized (**Figure 2B**). In general, there is a wide spectrum of phenotypes caused by *HOXD13* mutation, shared by different mutation forms, including synpolydactyly of 3rd and 4th fingers or 4th and 5th toes, postaxial polysyndactyly of 5th toe, and clinodactyly or camptodactyly of 4th or 5th fingers. In addition, phenotypes can considerably vary among family numbers bearing the same mutation. Despite this complexity, there is still a certain gene-phenotype relationship for *HOXD13* mutations. For N-terminal polyalanine tract expansion, penetrance, involved limb number, and severity of limb malformation are positively correlated with expansion length [6, 9, 12, 18-33]. The most severe limb malformation has been found in this form of *HOXD13* mutation, probably due to a strong dominant negative effect [21]. Certain missense mutations within the homeobox domain of *HOXD13* can cause severe or uncommon phenotypes, such as brachydactyly and ectrodactyly, not observed in other mutation forms [7, 8, 10, 11, 13, 19, 34-39]. This could be attributed to the gain of function of the homeobox domain. In contrast, the nonsense, frameshift, and splicing site mutations usually cause mild or typical synpolydactyly phenotypes, probably due to nonsense-mediated mRNA decay-caused haploinsufficiency [19, 40-47]. In this study, the p. P204fs61x mutation only resulted in synpolydactyly in the fifth toes and clinodactyly in the fifth fingers. In a recent study, a nearby p. R186x mutation of *HOXD13* was reported to cause very similar phenotypes, indicating a certain gene-phenotype relationship [48]. Homozygous mutations of *HOXD13* have generally resulted in severe limb malformations, such as complete syndactyly, brachydactyly, ectrodactyly, and hypoplastic metacarpals [6, 9, 22, 25, 27-30, 34, 43, 44]. Hypospadias has also been found in several homozygous individuals [25, 29]. The molecular basis of intrafamilial phenotype variation and gene-phenotype relationship require further exploration.

Currently, WES is the most cost-effective method for screening of pathogenic gene mutations for Mendelian inherited diseases. This study has proven the effectiveness of this approach in identification of pathogenic mutations in synpolydactyly. However, due to the strong gene-phenotype relationship for *HOXD13* mutation, a quick candidate gene test before WES will be more efficient when the patient has typical *HOXD13*-related phenotype, such as syndactyly of 3rd and 4th fingers, clinodactyly of 5th or 4th fingers, and synpolydactyly of 5th toes.

In conclusion, this present study identified a novel heterozygous *HOXD13* single-base deletion (c.610delC), causing a frameshift before the homeobox domain (p. P204fs61x), resulting in bilateral synpolydactyly of 5th and 5' toes and/or clinodactyly of 5th fingers in a Chinese family. These findings should help to further delineate the gene-phenotype relationship for *HOXD13* gene.

Disclosure of conflict of interest

None.

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