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Case Report

Phenotype and HOXD13 gene mutation in a big synpolydactyly (SPD) pedigree

Xiao-Jie Chen[1*], Shu-Min Ren[2*], Xiang-Dong Kong[2], Chun-Guang Qiu[1]

[1]Department of Cardiovascular, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, P. R. China; [2]Center of Prenatal Diagnosis, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, P. R. China. [*]Equal contributors.

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Abstract: Objective: To study the phenotype and HOXD13 gene mutation of a big synpolydactyly (SPD) Pedigree in Henan Province of China. Methods: The SPD pedigree was taken as research subjects; 13 surviving patients were examined clinically; PCR was used to amplify HOXD13 gene first exon and PCR products were sequenced to detect mutations. Results: In the SPD pedigree, the proportion of sick men and women was the same; delivery was continuous, and there was no cross-hereditary, showing autosomal dominant inheritance; patients showed difference degrees of SPD, but there was a big difference in expressivity among patients; there were nine additional trinucleotide repeats in polyalanine coding sequence in the first exon of HOXD13 gene of patients (totally 27 bp); this repetition makes the alanine residues in poly-alanine chain expand from 15 to 24. Conclusion: The SPD pedigree showed autosomal dominant inheritance; patient phenotypes were complex, with obvious differences in expressivity, but explicit rate was 100%; HOXD13 gene was the causative gene for congenital SPD pedigree.

Keywords: Congenital synpolydactyly, HOXD13, mutation analysis

Introduction

Congenital syndactyly (SD) is a rare genetic disease with developmental abnormalities in the hands and feet as the main symptoms, mostly showing autosomal dominant inheritance and partially showing chromosome syndrome. Autosomal dominant inherited SD can be divided into five subtypes in clinical: I, II, III, IV, V types [1]. Congenital synpolydactyly (SPD) belongs to type II SD, usually exhibiting 3, 4 finger and 4, 5 toe involvement, two-finger (toe) connection by webs, which cannot be separated [1]. 1996, Muragaki et al [2] found that HOXD13 SPD gene first exon-encoding polyalanine expansion (PAE) mutation existed in three American SPD families, so HOXD13 was determined as its virulence gene, meanwhile the study also raised that PAE as a mutation mechanism may play an important role in the occurrence of hereditary diseases. The author deeply studied the clinical phenotypes and genetic of a big SPD pedigree in Henan Province of China, analyzed and summarized clinical phenotypes and chromosome abnormalities and the role of HOXD13 gene mutations in the SPD pedigree, in order to find a simple and accurate method for the prenatal diagnosis of SPD pedigrees and provide a theoretical basis for exploring the role of HOXD13 first exon gene-encoding PAE in SPD pedigree carcinogenesis.

Subjects and methods

Subjects

Research objects were from the genetic counseling clinic of prenatal diagnosis center in the First Affiliated Hospital of Zhengzhou University. The family has five generations and a total of 48 members, of which 16 were patients, each 8 cases of men and women; in the group three elderly patients had died, without atavistic phenomenon, consistent with autosomal dominant inheritance characteristic (**Figure 1**). Pedigree patients had unilateral or bilateral 3, 4 and 4, 5 finger (toe) syndactyly malformation, while there were significant differences in expressivity. The hands of severe patients manifested as 3, 4 web space and bone fusion companied with partial or complete polydactyly in web space, the uneven development of 3rd and 4th fingers, resulting in the inclination of fusion finger to one side; feet displayed as 4, 5 syndactyly accompanied with complete polydactyly in web

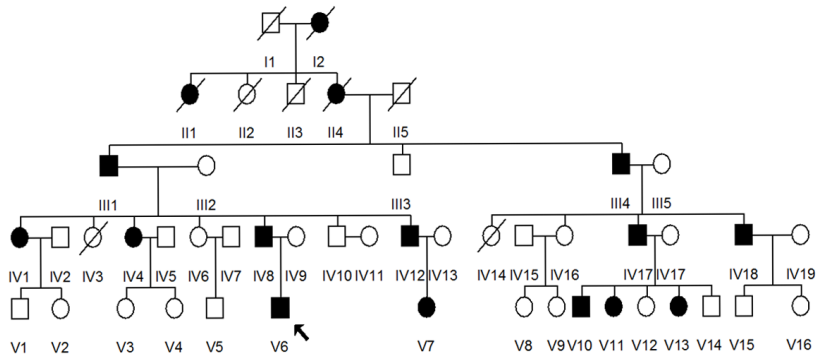

Figure 1. Pedigrees of finger (toe) syndactyly with polydactyly. Note: The hollow circular represents normal female; Hollow Square represents the normal male; black circles represent female patient; Black Square represents the male patient; crossed graph represents death, graph indicated by the arrow represents the proband.

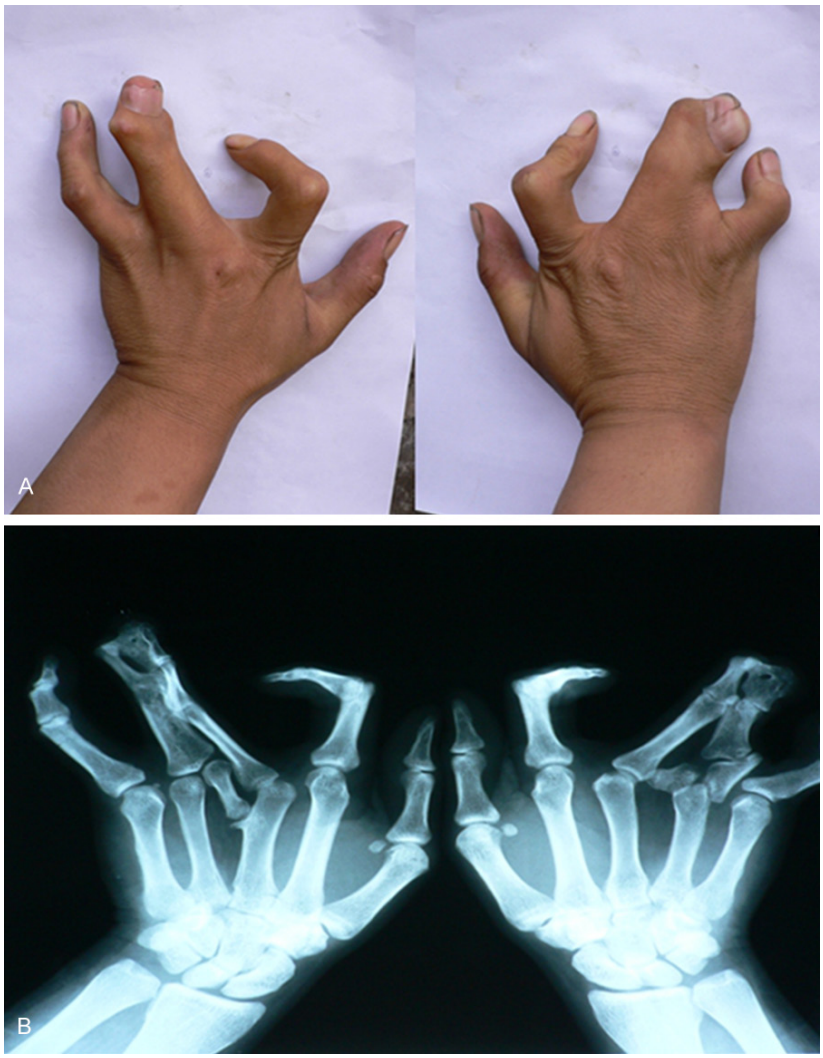

Figure 2. Hands photos and X-ray of proband V6. A: Hands photos of proband V6; B: X-ray of proband V6 hands.

space, and fusion toe mostly inclined to one side. Mild patients showed only web space fusion, finger (toe) infusion, and unilateral involvement. Proband V6, male, 8 years old when visited. The hands of the proband (V6) and his father (IV8) showed 3, 4 web space infusion as well as the nails; bone fusion in web space, inseparably, uneven development; the fourth knuckle outward tilted; fused fingers had poor activity; the development and activities of rest three fingers were normal; the proband (V6) was also accompanied by small phalanx deformity (**Figures 2A**, **3A**). Proband showed 4, 5 toe syndactyly with polydactyly (**Figure 4A**). X-rays showed that three out of five (proband V6, proband father IV8 and uncle IVI2) showed that: the proximal phalanx base branch of the 4th finger was connected to the third finger palm and joints of the 3rd finger, as a "bridge" like structure; the third finger and the fourth finger showed bony fusion; the 4th finger proximal phalanx appeared broadening or widening bifurcation (**Figures 2B**, **3B**). Proband X-rays showed bilateral bony toe syndactyly with polydactyly; the fifth toe of right foot bent inward because of dysplasia (**Figure 4B**). Examination: heart, lungs, abdomen showed no abnormalities; clear sanity, normal intelligence, no limb and spinal deformity.

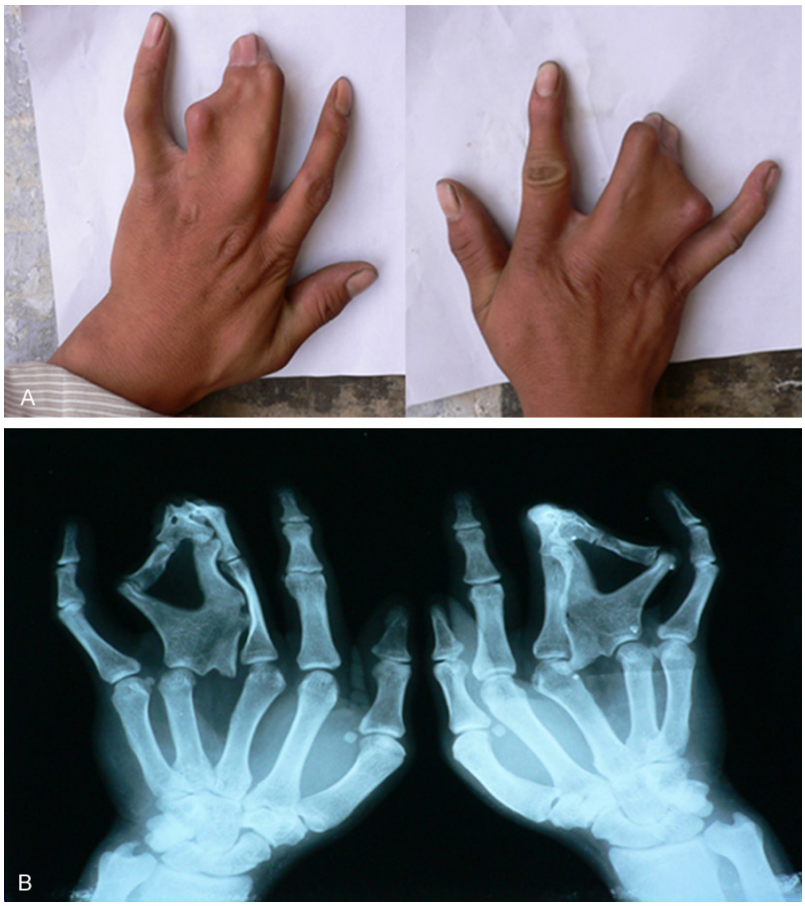

Figure 3. Hands photos and X-ray of proband father's V6. A: Hands photos of proband father's V6; B: X-ray of proband father's V6 hands.

Methods

Extraction of genomic DNA: In eligible for informed consent, 1 mL venous blood was drawn from patients and their healthy immediate relatives (a total of 24 individuals) and anticoagulated by EDTA. 10 cases of unrelated healthy donors were taken as control, including five cases of men and women respectively. TIANGEN Genomic DNA extraction kit was used to extract genomic DNA (Tiangen Biochemical Technology Co., Ltd., Beijing), and DNA was frozen at -20°C for spare.

HOXD13 gene mutational analysis: Primers for HOXD13 first exon were designed [3]; the amplification of repeated GCN sequence coding polyalanine and the sequence of both sides was performed; the upstream primer: 5'-CTTTCTCTCCGCGCCTGTGTTCG-3', downstream primer: 5'-CTACAACGGCAGAAGAGGACGACG-3'; primers were synthesized by Shanghai Sangon Biological Engineering Co., Ltd. The amplified fragment was 161 bp. 25 μL reaction system contains: GC Buffer II 12.5 μL, dNTP 4 μL, primer 2 μL, DNA template 2 μL, LA Taq enzyme 1.5 μL, add sterile double distilled water to 25 μL; the reagents were added on the ice box and double-distilled water was used as blank control. PCR reaction condition: 96°C denaturation for 5 min; 96°C 30 s, 60°C 30 s, 72°C 30 s, 30 cycles, final extension at 72°C for 10 min. PCR products were detected by 2% agarose gel electrophoresis and the fragment of normal products was collected for sequencing. Positive PCR products in agarose electrophoresis were separated by 5% polyacrylamide gel electrophoresis; abnormal bands were subjected to recycling and re-amplification (25 cycles) to collect PCR product for sequencing. And then purified normal and abnormal fragments were sequenced by Shanghai Sangon Biological Engineering Co., Ltd in both directions; the mutant fragment sequencing results and the DNA sequences of HOXD13 first exon in GenBank were compared.

Results

PCR amplification

All the normal hand-foot appeared 161 bp single band. All the patients, except 161 bp, have a 188 bp long fragment (**Figure 5**).

HOXD13 polyalanine chain gene mutation analysis

Sequence analysis showed that there were nine additional trinucleotide repeat sequences (a total of 27 bp) in poly-alanine chain coding sequence of the first exon of HOXD13 gene in patients of this SPD pedigree; this insertion makes the alanine residues in polyalanine

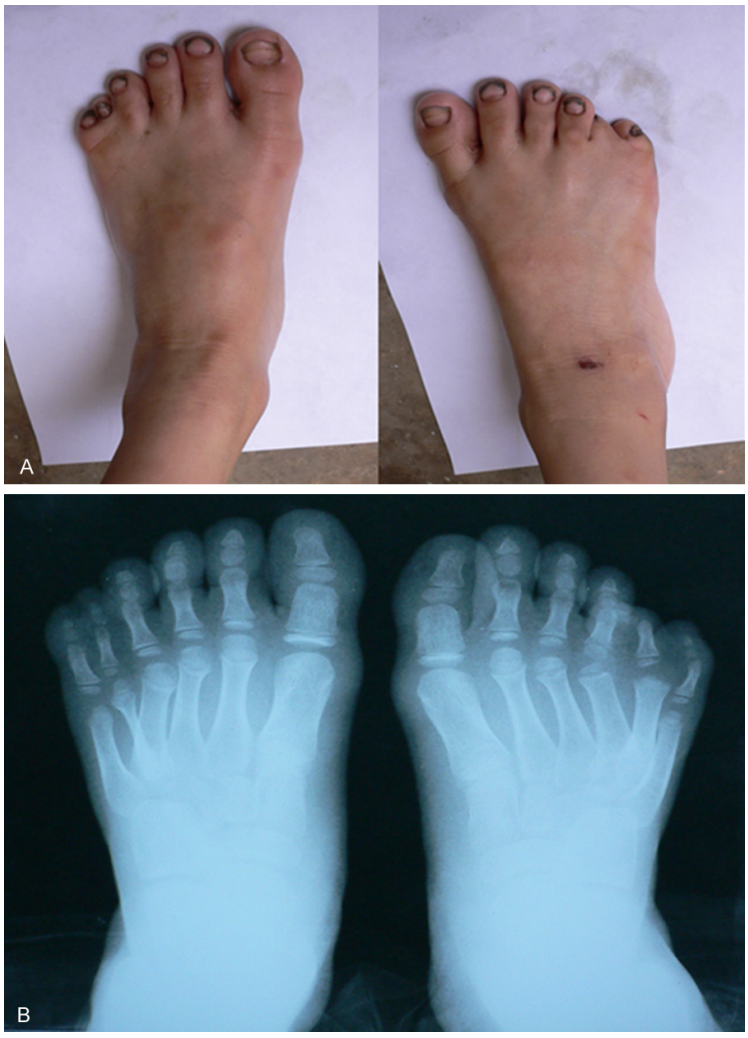

Figure 4. Photographs and X-rays of proband V6 feet. A: Photographs of proband V6 feet; B: X-rays of proband V6 feet.

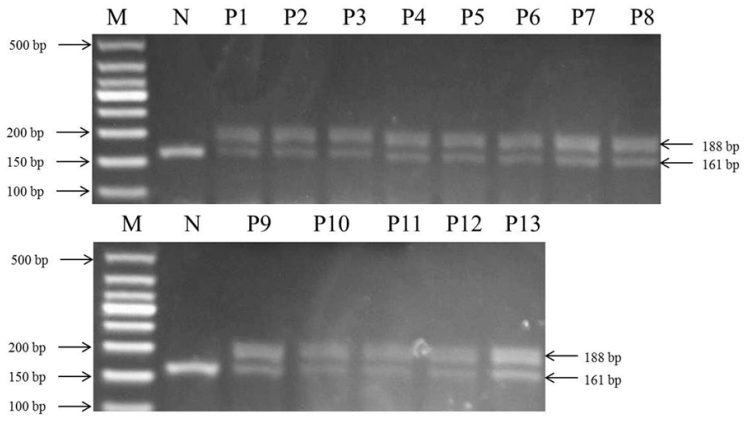

Figure 5. Agarose gel electrophoresis results of HOXD13 PAE gene sequence. Note: M is the standard molecular weight, from the top to bottom were 500, 400, 350, 300, 250, 200, 150, 100 bp; N is normal control; P1~P13 are pedigrees patients.

chain extend from the normal 15 to 24. Inserted fragment was the 5th to 13th sites in normally-encoding poly-alanine chain, shown in **Figure 6**.

Discussion

HOXD gene is located on chromosome 2q31; the 5'end is HOXD13 gene; gene full-length is 1365 bp, and coding region is 1008 bp. The gene contains two exons; the first exon in the 5'end contains poly-alanine chain incomplete trinucleotide (GCN) repeat sequence; the second exon in 3'end encodes a highly conserved homologous box domain, and introns contain a CA repeat sequence [4].

Since Muragaki et al [2] found that the HOXD13 gene-encoding PAE mutation was closely associated with SPD incidence, a number of studies have detected this new extend mutation in several non-associated SPD pedigrees at home and abroad, and HOXD13 gene-encoding PAE mutation is the common feature of these causative mutations. The poly-alanine residue in HOXD13 gene first exon encoding the N-terminal of protein extends from the normal 15 to 22, 23, 24, 25, or 29, respectively, and this polyalanine chain extending has genetic stability [5-13]. SPD pedigree in this study has typical phenotypes; pathogenic mechanism is that alanine residues of polyalanine chain extend from the normal 15 to 24; additional nine alanine residues are inserted (5th~13th). All patients in the pedigree carried heterozygous mutation; phenotypes and genetic mutations exhibited genetic stability in the transmission of generations; the individual with this genotype

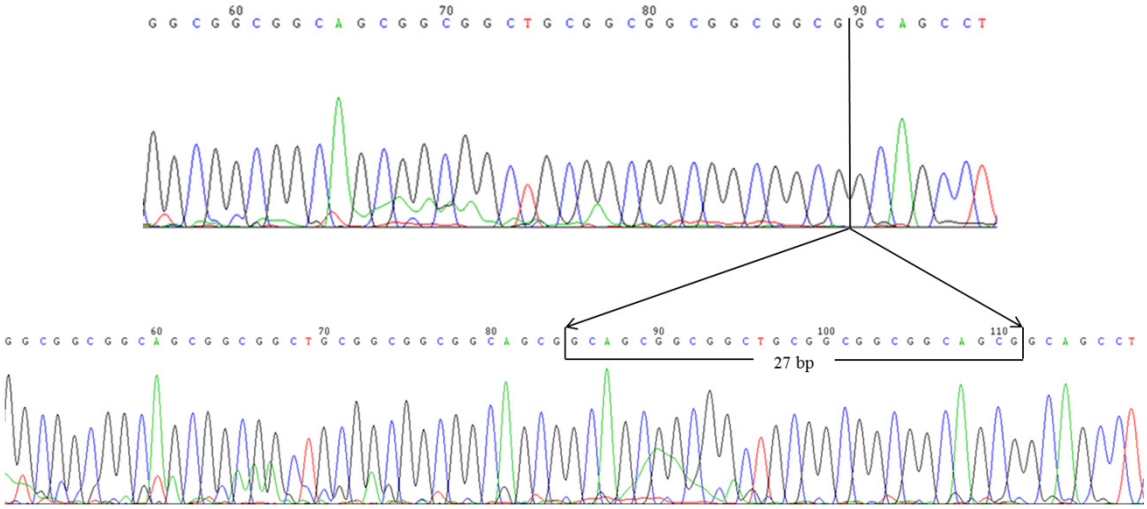

Figure 6. Nucleic acid sequence analysis of polyalanine chain-coding region in HOXD13 gene.

showed a certain degree of clinical symptoms, suggesting that the mutation in this pedigree is indeed caused by HOXD13 gene mutation.

There is a big difference in the penetrance and expressivity of SPD between the different families and different patients within the same family. Studies have shown that the severity of SPD penetrance and phenotypes significantly increased with increasing length of the poly-alanine chain [14]; the number of inserted alanine residue associated with the SPD is mostly 7, 9 or 10, and it has been reported that in pedigrees with the alanine chain extended ≤6 residues or reduced two or four residues, no SPD-associated symptoms were observed [8, 15]. The mechanism of PAE leading to the occurrence of genetic disease is still unclear. Albrecht et al [16] believe that when the length of the poly-alanine chain exceeds a certain threshold value, the mutant protein gathers in the cytoplasm to form inclusion bodies, and longer PAE-caused polyalanine chain is likely to gather in the cytoplasm, thereby impeding the transcription factor enter the nucleus, affecting its normal regulation. Chromosome t (12; 22) can cause atypical SPD symptoms; since FBLN1 gene is expressed in limb development-associated extracellular matrix, t (12; 22)-caused FBLN1 gene disruption may be its pathogenesis mechanism [17]. This study detected chromosomes of proband and his father in the pedigree and found no abnormalities, suggesting that chromosome number and structural abnormalities were not the pathogenic mechanism of the family.

Albrecht et al found that the aggregation degree of mutant protein in cytoplasm was positively correlated with the increased number of alanine residues after extending, which well explains the correlation of PAE mutation with penetrance and expressivity [16]. Studies have shown that homozygous phenotype of HOXD13 mutation is more severe than that of heterozygotes. Heterozygotes of HOXD13 polyalanine chain extending usually manifest as 3, 4 finger syndactyly, 4, 5 (toe) syndactyly, polydactyly and other typical SPD phenotypes. The homozygotes also appear shortened phalanges, multi-directional SPD, Carpal fusion and other complex expression [7, 18]. However, recent studies have found that, individuals with reduced four or seven alanine residues in HOXD13 gene also have SPD related phenotypes; the pathogenic mechanism may be that decreased alanine residue has a significant inhibitory effect on HOX gene-related functions [19, 20]. In this study, nine alanine residue sequences were inserted in the HOXD13 gene of patients of SPD pedigree; from the dose effect of mutation, the severity of SPD clinical symptoms and penetrance were in line with its genotype. This provides a foundation for the study of prenatal gene diagnosis of SPD pedigrees and provide a theoretical basis for exploring the role of HOXD13 gene first exon-encoding PAE in SPD family.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chun-Guang Qiu, Department of Cardiovascular, The First Affiliated Hospital of Zhengzhou University, Jianshe Donglu 1#, Zhengzhou 450052, P. R. China. Tel: +86-0371-66862729; Fax: +86-15037133788; E-mail: qx-guangd@163.com

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