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A novel FBN2 mutation in a Chinese family with congenital contractural arachnodactyly

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ABSTRACT

Congenital contractural arachnodactyly (CCA, OMIM: 121050) is an autosomal dominant condition that shares skeletal features with Marfan syndrome (MFS, OMIM: 154700), including contractures, arachnodactyly, dolichostenomelia, scoliosis, crumpled ears and pectus deformities but excluding the ocular and cardiovascular complications that characterize MFS. These two similar syndromes result from mutations in two genes belonging to the fibrillin family, FBN1 and FBN2, respectively. We successfully identified a novel FBN2 mutation (C1406R) in a Chinese family with CCA for over five generations. This mutation was detected in the patients of this family but not in the seven unaffected family members or 100 normal individuals. SIFT and PolyPhen analyses suggested that the mutation was pathogenic. We identified a missense mutation in the calcium binding-epidermal growth factor (cbEGF)-like domain. Our study extends the mutation spectrum of CCA and confirms a relationship between mutations in the FBN2 gene and the clinical findings of CCA.

1. Introduction

Congenital contractural arachnodactyly (CCA) is a rare, autosomal dominant connective tissue disorder characterized by contractures, arachnodactyly, dolichostenomelia, scoliosis, crumpled ears and pectus deformities [1,2]. It is caused by mutations in the gene encoding fibrillin-2 (FBN2) on chromosome 5 [1,2]. CCA shares overlapping features with Marfan syndrome (MFS), which is caused by a mutation in the gene encoding fibrillin-1 (FBN1) on 15q15-21.3 [1,2]. These genes encode large, cysteine-rich extracellular matrix glycoproteins, fibrillin-2 and fibrillin-1, which are major components of microfibrils. FBN2 is the only gene known to be associated with CCA. This gene is >28 kb, contains 65 exons, and encodes 2912 amino acids, comprising a multidomain protein with five distinct structural regions. The largest of these structural regions contains 41 calcium binding-epidermal growth factor (cbEGF)-like domains. All of the identified mutations in FBN2 are clustered within a defined region similar to that in which severe MFS mutations cluster in FBN1, specifically between exons 22 and 36 [3].

In many cases, CCA is caused by mutations in the fibrillin2 gene (FBN2), in which 48 mutations have been reported to date, including 31 missense and nonsense mutations, most of which are located in the middle region of the FBN2 gene, from exon 22 to 36 [4–14].

In this study, we identified and characterized a Chinese family with five generations of CCA. One novel mutation (c.4216T>C, p.C1406A) in the FBN2 gene was identified in this family. The presence of the disease-causing mutation is consistent with the clinical diagnosis of CCA, providing information for the genetic counseling of other family members.

2. Materials and methods

2.1. DNA extraction and sequencing

Blood samples were obtained with informed consent from the family members and from 100 normal individuals. Genomic DNA was extracted from the blood samples using a DNA Isolation Kit for Mammalian Blood (Tiangen Biotech, China). Primer sets were used to amplify 65 exons (exon 1–65) of the FBN2 gene (NM_001999) for sequencing. The sequences of the forward and reverse primers of exon 32 were 5′ TACTGGAAAGTGGCTGAC 3′ and 5′ CAAGGCATACTGTTGAAAT 3′, respectively. The PCR conditions for the DNA amplification were as follows: denaturing at 95 °C for 5 min; 30 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s; and a final step for

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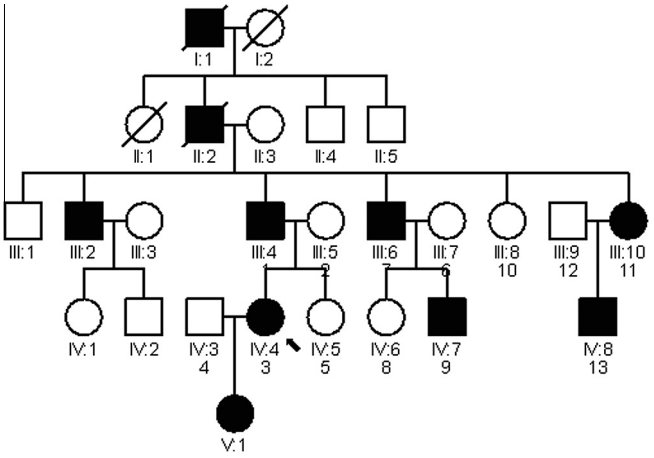

Fig. 1. Pedigree of the family that took part in this study. The patients with congenital contractural arachnodactyly are depicted by darkened symbols. The arrow indicates the proband.

5 min at 72 °C. The variation was further confirmed by digestion with the restriction enzyme MunI. The digested products were separated and analyzed by 8% polyacrylamide gel electrophoresis and silver staining.

2.2. Mutation analysis

To evaluate the mutation, several effective software programs were used. A search was performed for human FBN2 homologs using BLAST on the NCBI web site (http://www.ncbi.nlm.nih.gov). The identified proteins were aligned using ClustalW [15], and a phylogenetic tree was reconstructed with MEGA4 [16] with the neighbor-joining method. The effect of the amino acid substitution on the protein function was evaluated with SIFT [17,18] and PolyPhen.

2.3. Ethics statement

This study was approved by the ethics committee of the Key Laboratory of Reproductive Health of Liaoning Province, and informed consent was obtained from all of the participants.

3. Results and discussion

3.1. Clinical features of patients

We typed the CCA of this family, also known as Distal arthrogryposis type 9, for which ten affected individuals over five generations were evaluated (Fig. 1). All affected members of this family presented with slender, contractural clubbed fingers and toes (Fig. 2A–H), and no neurological or cardiovascular abnormalities were noted. Intrafamilial variation in phenotypic expression was modest. The proband (Patient IV4) was a 36-year-old woman with features typical of CCA. She was tall (163 cm) and thin (56 kg; body mass index = 21.1) with contractural fingers (Fig. 2A) and slender toes (Fig. 2B), scoliosis, crumpled ears, 300° of myopia and exophthalmos. Patient IV7 was 25 years old. He was tall (190 cm) and thin, with contractural fingers (Fig. 2C) and toes (Fig. 2D), scoliosis, a pinched mouth, ptosis and prominent surface moles on the face. Patient III4 was tall (178 cm) and thin (65 kg), with contractural fingers (Fig. 2E), abnormal ears, scoliosis, and ptosis. Patient IV8 was 28 years old. He was tall (183 cm) and had contractural fingers (Fig. 2F), a pinched mouth, ptosis and prominent surface moles on the face. He had undergone surgery to correct his fingers at the age of 3. Patient III6 was tall (187 cm) and presented with contractural fingers (Fig. 2G), abnormal ears, scoliosis, and ptosis. Patient IV10 was 42 years old. She was tall (175 cm) presented with contractural toes (Fig. 2H), abnormal ears and scoliosis.

3.2. Identification of a novel mutation in the FBN2 gene

The sequencing of 65 exons of FBN2 for mutation screening revealed the presence of a heterozygous T → C transversion in the patients at nucleotide position 4216 in exon 32, resulting in a cysteine-to-arginine substitution at amino acid residue 1406 (C1406R) (Fig. 3). This mutation was identified in all affected individuals in the family but not in any of the unaffected family members, five unrelated spouses from other families, or 100 controls. Using the SIFT program, which incorporates sequence homology, the normalized probability of the substitution of C1406 to R was calculated to be 0.00, which was less than the threshold of 0.05. Thus, this mutation was predicted to be deleterious. The PolyPhen program predicted that this mutation was likely to be damaging, with a score of 0.997. Eleven proteins in the FBN2 subfamily were found by a BLAST search that formed a cluster in the phylogenetic tree (Fig. 4). The C1406R mutation occurred in the

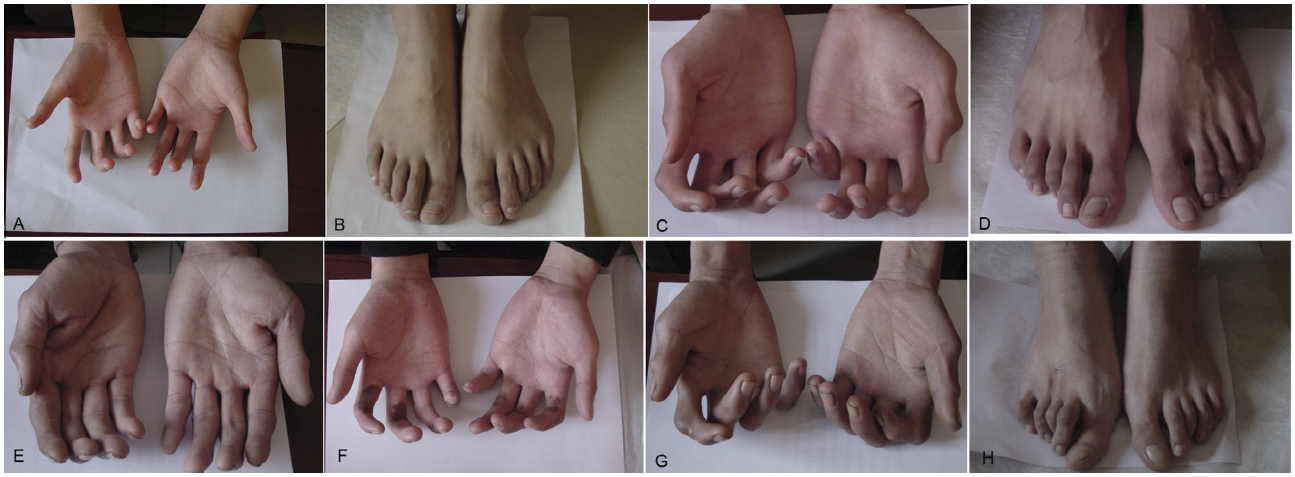

Fig. 2. Phenotype of the family. (A) The proband, hands. (B) The proband, feet. (C) Patient IV-7, hands. (D) Patient IV-7, feet. (E) Patient III-4, hands. (F) Patient IV-8, hands. (G) Patient III-6, hands. (H) Patient III-10, feet.

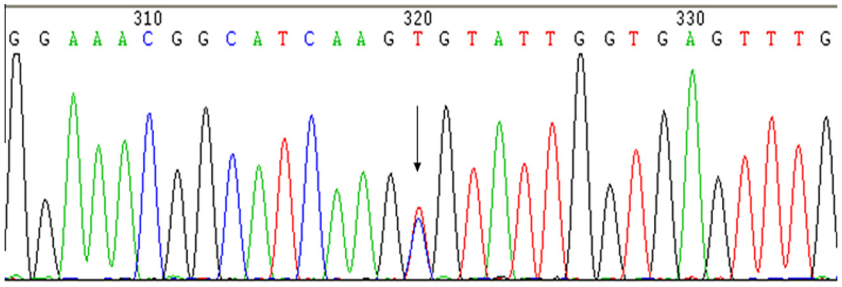

Fig. 3. DNA sequencing results for exon 32 of the FBN2 gene. The arrow indicates a heterozygous T → C transversion at 4216 nt of the cDNA (TGT → CGT, Cys1406Arg).

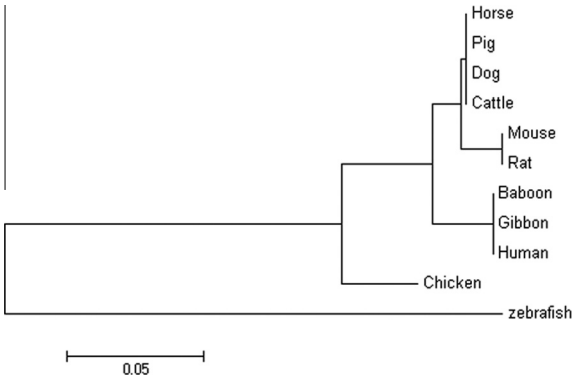

Fig. 4. The phylogenetic tree of FBN2. The bootstrap values are presented on the branches.

22nd cbEGF domain of the FBN2 gene. The alignment of cbEGF22–23 revealed the presence of invariant cysteines in the cbEGF domain that are conserved in a wide range of organisms, ranging from humans to zebrafish (Fig. 5).

3.3. Comparison of DA classifications with the CCA of this family

Distal arthrogryposis (DA) is an inherited limb malformation disorder characterized by congenital contractures of two or more body areas without primary neurological and/or muscular disease. CCA is a unique type of distal arthrogryposis that shares overlapping features with Marfan syndrome and distal arthrogryposis type 1 (DA1), but it is more severe than DA1 and less severe than Marfan syndrome. DA comprises a group of disorders that mainly involve the distal parts of the limbs, characterized by congenital contractures of two or more different body areas. Ten different forms of DA (DA1–DA10) have been reported to be autosomal dominant disorders, and only DA5 shows an autosomal recessive inheritance pattern. In contrast with the majority of families with CCA, in the family evaluated in this study, two affected members presented with prominent surface moles on face, extreme ptosis and scoliosis symptoms were common, and rare instances of myopia and exophthalmos were noted.

Congenital contractural arachnodactyly is an autosomal dominant disorder characterized by dolichostenomelia, contractures, scoliosis, arachnodactyly, crumpled ears and pectus deformities. It is caused by a mutation in the gene encoding fibrillin-2 (FBN2) on chromosome 5q23-q21 [1]. It shares overlapping features with Marfan syndrome (MFS), which is caused by a mutation in the gene encoding fibrillin-1 (FBN1). CCA patients have crumpled ears and congenital contractures and do not typically have the ocular or life-threatening cardiovascular complications observed in MFS [2].

3.4. Prediction of protein functions for the substitutions

Here, we reported a novel mutation involving a T-to-C transition at position 4216 (c.4216T>C) in exon 32 of the FBN2 gene, resulting in a Cys 1406 to Arg change (p.Cys1406Arg). All of the patients in this family were confirmed to have this mutation. Because this mutation has not been previously reported, we selected four software programs to evaluate its pathogenicity and severity. All of the results indicated that this mutation is likely disease-causing because it is located within the region shared by most reported CCA mutations and changes a conserved cysteine to an arginine (C1408R), which is predicted to disrupt microfibril structure.

Acknowledgments

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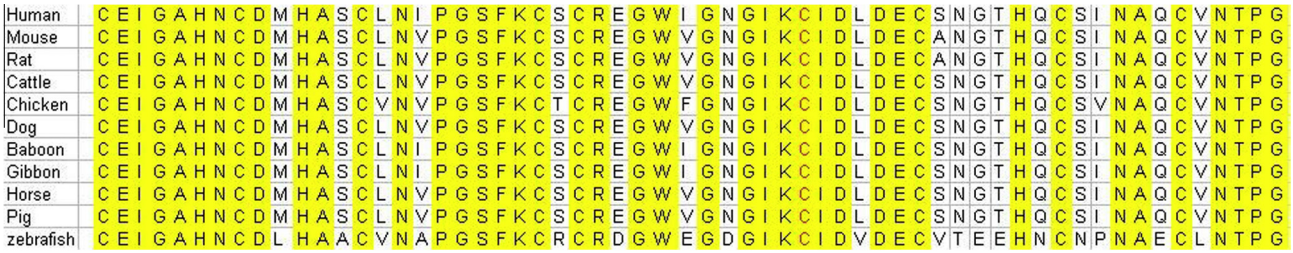

Fig. 5. The alignment of fibrillin cbEGF22–23 of different species. The conserved amino acids are highlighted in yellow, and 1406C is annotated in red.

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