Identification of a Novel Missense FBN2 Mutation in a Chinese Family with Congenital Contractural Arachnodactyly Using Exome Sequencing

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Abstract

Congenital contractural arachnodactyly (CCA, OMIM 121050), also known as Beals-Hecht syndrome, is an autosomal dominant disorder of connective tissue. CCA is characterized by arachnodactyly, narrow body habitus, elongated limbs, chest wall deformities, kyphoscoliosis, congenital contractures and a crumpled appearance of the helix of the ear. The aim of this study is to identify the genetic cause of a 4-generation Chinese family of Tujia ethnicity with congenital contractural arachnodactyly by exome sequencing. The clinical features of patients in this family are consistent with CCA. A novel missense mutation, c.3769T>C (p.C1257R), in the fibrillin 2 gene (FBN2) was identified responsible for the genetic cause of our family with CCA. The p.C1257R mutation occurs in the 19th calcium-binding epidermal growth factor-like (cbEGF) domain. The amino acid residue cysteine in this domain is conserved among different species. Our findings suggest that exome sequencing is a powerful tool to discover mutation(s) in CCA. Our results may also provide new insights into the cause and diagnosis of CCA, and may have implications for genetic counseling and clinical management.

Introduction

Congenital contractural arachnodactyly (CCA, OMIM 121050), also known as Beals-Hecht syndrome, is an autosomal dominant disorder of connective tissue. CCA is phenotypically related to but genetically distinct from Marfan syndrome (MFS) [1]. It was first introduced by Rodney Beals and Frederick Hechet in 1971. It is characterized by contractures, arachnodactyly, narrow body habitus, elongated limbs, chest wall deformities, scoliosis, muscular hypoplasia and crumpled ears [2,3]. It can be divided into classical CCA and severe/lethal CCA. Patients with severe/lethal CCA show cardiac abnormalities (mitral valve prolapse, atrial septal defect, ventricular septal defect and aortic hypoplasia) and gastrointestinal anomalies.
(duodenal atresia, intestinal malrotation), in addition to anomalies presented in classical CCA [4]. The estimated incidence of CCA is not clear but seems to be less frequent than that of MFS with the incidence of 1:10,000 [5]. CCA showed no geographic or ethnic predilection. Although it has been described mainly in Caucasian families of European origin, CCA has also been reported in Japanese, people of Indian descent, African Americans, African Blacks [6], and a Chinese family [7]. The FBN2 gene, discovered during the cloning of the FBN1 gene, was mapped to chromosome 5q23-q31 and was linked to CCA [8].

The main purpose of this study was to identify the gene responsible for a 4-generation Chinese family with CCA that is characterized by arachnodactyly, camptodactyly, kyphoscoliosis and large joint contracture. We found a heterozygous c.3769T>C transition (p.C1257R), which co-segregates with patients in this family and are absent in normal controls, in the FBN2 gene. The missense mutation disrupts the overall integrity of the FBN2 protein. Our data indicate that it is a pathogenic mutation.

Materials and Methods

Participants and clinical evaluation

A 4-generation, 15-member Chinese family of Tujia ethnicity with familial CCA was recruited from the Third Xiangya Hospital, Central South University (Fig 1). Blood samples were collected from 14 members of the family, including 7 affected individuals (I:2, II:2, II:6, III:1, III:3, III:4 and IV:2,) and 7 unaffected members (II:1, II:3, II:4, II:5, II:7, III:2, and IV:1) (Fig 1). Blood samples were also collected from 100 unrelated normal controls (male/female: 50/50, age 45.7±6.5 years). Written informed consent was obtained from all subjects, and this study was approval from the Ethics Committee of the Third Xiangya Hospital, Central South University, China.
Exome capture and sequencing

Genomic DNA was extracted from blood samples using standard phenol-chloroform extraction method [9]. Exome capture was performed in the proband (III:4), by BGI-Shenzhen using NimbleGen SeqCap EZ Human Exome Library v2.0 (Roche NimbleGen, Inc., Madison, WI, USA), and sequencing was performed using a HiSeq 2000 platform (Illumina, San Diego, CA, USA). All steps were performed according to the manufacturer’s instructions [10]. The enriched library targeting the exome was sequenced on the HiSeq 2000 platform to get paired-end reads with a read length of 90-bp [11]. Exome sequencing depth of 68.01× were obtained to provide sufficient depth to accurately call variants at 99.17% of each targeted exome.

Read mapping and variant analysis

The clean reads of each individual were aligned to the human reference genome (UCSC Build 37.1, hg19) using Burrows-Wheeler transform (BWA; Cambridge, UK) and made variant calls with Genome Analysis Tool Kit (version 2.1), and completed functional annotation of the variants with BGI in-house script [12,13]. Alignment of the sequences from one affected individual of the family was performed using SOAPaligner (soap2.21) after the duplicated reads were deleted, and SNPs were called using SOAPsnp (1.05) set with the default parameters [14]. Insertions or deletions (indels) affecting coding sequence or splicing sites were identified [15]. The thresholds for calling SNPs and short insertions or deletions included the number of unique mapped reads supporting a SNP ≥4 and the consensus quality score ≥20. The quality score is a Phred score, generated by the program SOAPsnp1.05, quality score 20 represents 99% accuracy of a base call. All candidate mutations in the subject were filtered against the single nucleotide polymorphism databases (dbSNP build 137, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), ethnic Han Chinese individuals from Beijing available in the 1000 Genomes Project (1000genomes release_20100804, http://www.1000genomes.org/), HapMap (2010–08_phaseII+III, http://hapmap.ncbi.nlm.nih.gov/) and YanHuang (http://yh.genomics.org.cn/) project [16].

Mutation validation

Sanger sequencing was performed to confirm the presence and identity of potential disease-causing variants with ABI3500 sequencer (Applied Biosystems Inc., Foster City, CA, USA). PCR amplification was conducted as described previously [17], and the primer sequences were as follows: 5′-ATACCTGCACACGATCTCCC-3′ and 5′-AAGCAGACCTGACAATGTGG-3′.

Bioinformatics analysis of the mutation

The Basic Local Alignment Search Tool (http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) was used to perform multiple sequence alignment. Online tools, including MutationTaster (http://www.mutationtaster.org/), Polymorphism Phenotyping version 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/, scores less than 0.05 are deleterious), were used to evaluate the possible effects of amino acid substitution on protein structure and function in terms of chemical change, sequence conservation, and likelihood of pathogenicity [18,19,20].

Results

Clinical findings

Clinical features of the seven patients in this family who participated in this study are shown in Table 1. The proband (III:4), a 11 year old girl, was noted at birth to have contractures of the
fingers without any prenatal complication, and had some improvement of her contractures
with age. She had normal mental and motor development, and the results of blood and urine
examinations were normal. On physical examination, all seven patients (I:2, II:2, II:6, III:1,
III:3, III:4 and IV:2) showed arachnodactyly and camptodactyly (Fig 2), two patients (II:6,
III:3) showed kyphoscoliosis and large joint contracture, and one patient (III:6) had cardiovas-
cular abnormalities. There was no evidence of tall stature, crumpled ears or ocular complica-
tions. Compare to female patients, male patients experienced more severe phenotypes, such as
kyphoscoliosis and cardiovascular complications, which have not been reported before.

**Mutation screening**

We performed exome sequencing of the proband (III:4, Fig 1) in the Chinese family with CCA,
and 6.74 billion bases of 90-bp paired-end read sequence were generated. Among the 6.74 bil-
lion bases, 6.40 billion (94.85%) passed the quality assessment, 6.22 billion (92.22%) were
aligned to the human reference sequence, and 4.27 billion bases (63.35%) were mapped to the
targeted bases with a mean coverage of 68.01-fold. A total of 111,167 genetic variants, including
14,779 non-synonymous variants, were identified in either the coding regions or the splice
sites. We excluded known variants identified in dbSNP137, 1000 human genomes project,
HapMap, and YanHuang. After this, we reduced the number of candidate genes by more than
93.17%. The sequencing data of our study was deposited in NCBI Sequence Read Archive
(SRA) database (study accession number: SRP071315).

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**Table 1. Clinical and genetic data of 7 patients with FBN2 c.3769T>C (p.C1257R) mutation.**

| Subject | I:2 | II:2 | II:6 | III:1 | III:3 | III:4 | IV:2 |
|---------|-----|------|------|-------|-------|-------|------|
| Gender  | Female | Female | Male | Female | Male | Female | Female |
| Age (years) | 85 | 58 | 44 | 39 | 30 | 11 | 10 |
| Genotype | Heterozygote | Heterozygote | Heterozygote | Heterozygote | Heterozygote | Heterozygote | Heterozygote |
| Tall stature | - | - | - | - | - | - | - |
| Crumpled ear | - | - | - | - | - | - | - |
| Arachnodactyly | + | + | + | + | + | + | + |
| Camptodactyly | + | + | + | + | + | + | + |
| Large joint contracture | - | - | + | - | + | - | - |
| Kyphoscoliosis | - | - | + | - | + | - | - |
| Muscle hypoplasia | - | - | - | - | - | - | - |
| Cardiovascular complication | - | - | - | - | - | - | - |
| Ocular complication | - | - | - | - | - | - | - |

+, present; -, absent

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**Fig 2. (A) The phenotype and (B) X-ray images of hands from an affected member (IV:2) of the family.**

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A prioritization scheme was applied to identify the pathogenic mutation in the proband. After validation by Sanger sequencing, a c.3769T>C (p.C1257R) mutation in the FBN2 gene was identified in the proband (Fig 3). Two male patients (II:6, III:3) and five female patients (I:2, II:2, III:1, III:4, IV:2) in the family were subsequently found to carry the same heterozygote mutation. The variant co-segregated with disease phenotype in this family, and was absent in unaffected individuals in this family and in 100 ethnicity-matched unrelated controls.

Bioinformatics analysis of the mutation

The cysteine at position 1257 is phylogenetically conserved among various species (Fig 4). MutationTaster predicted that the mutation was disease-causing with a probability value close to 1. PolyPhen-2 analysis produced a score of 0.991 on the HumVar database (sensitivity, 0.50; specificity, 0.95), which is predicted to be probably damaging. The SIFT prediction revealed a score of 0.00, indicating that the substitution is predicted to affect protein function. Our data indicated that the variant c.3769T>C (p.C1257R) in the FBN2 gene was likely deleterious and was the disease-causing mutation for CCA in our family.

Discussion

CCA is a well-characterized autosomal dominant disorder with variability in the clinical expression and intragenic heterogeneity [21]. CCA is phenotypically related to MFS, including tall stature, marfanoid habitus, arachnodactyly, camptodactyly, kyphoscoliosis and pectus excavatum [22]. However, CCA patients have crumpled appearance of ear helix and multiple joint contractures (especially elbow, knee and finger joints), and usually do not produce the ocular and life-threatening cardiovascular complications observed in MFS [23,24]. CCA can be distinguished from MFS genetically because CCA is caused by the mutation of the FBN2 gene, whereas MFS is caused by the FBN1 gene defects. Fibrillin 1 and fibrillin 2 are major structural components of the extracellular microfibrils with an average diameter of 10 nm, and they have virtually super imposable structures, with almost every domain encoded by a separate exon [25]. Fibrillin is a component of the extracellular matrix microfibrils, which play an essential...
Fibrillin 1 plays a role in the formation of elastic fibers and the deposition of tropoelastin, and performs anchoring functions in some tissues. Fibrillin 1 is involved in tissue rigidity while fibrillin 2 is associated with tissue elasticity [26,27].

The FBN2 gene (OMIM 612570) located at chromosome 5q23-31, consists of 65 exons and encodes a 2,912-amino acid protein [28]. It is characterized by long introns taking up the first half of the gene and the largest intron is 54,341 bps (between coding exons 5 and 6), and densely packed exons in the second half. The evolutionary conservation of intron length in the FBN2 gene across mammals indicates that the introns may have some function in regulating gene expression. The large introns may contain promoters and/or enhancer elements for non-coding RNAs, or influence the rate of production of mature mRNA [29]. At least 48 mutations in the FBN2 gene have been found to be responsible for CCA. These mutations vary from point mutations to gross deletions. Twenty-nine missense mutations, 13 splice-site mutations, a nonsense mutation, a small insertion, a small deletion, a gross insertion and 2 gross deletions are described in Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php).

Fibrillin 2 mutations associated with CCA occur in a rather limited region in exons 23–35, a cbEGF rich region of fibrillin 2, similar to where severe MFS cluster in FBN1, between exons 23 and 34, the so-called "neonatal region", while only two are recorded near the N terminus, in exons 8 and 17 [24,30].

| Species              | p.C1257R |
|----------------------|----------|
| Homo sapiens         | IMNGGCDTQCTNSEGSYEC |
| Pan troglodytes      | IMNGGCDTQCTNSEGSYEC |
| Callithrix jacchus   | IMNGGCDTQCTNSEGSYEC |
| Sus scrofa           | IMNGGCDTQCTNSEGSYEC |
| Bos taurus           | IMNGGCDTQCTNAEGSYEC |
| Equus przewalskii   | IMNGGCDTQCTNSEGSYEC |
| Ovis aries           | IMNGGCDTQCTNSEGSYEC |
| Felis catus          | IMNGGCDTRCTNSEGSYEC |
| Oryctolagus cuniculus| IMNGGCDTRCTNSEGSYEC |
| Mus musculus         | IMNGGCDTQCTNSEGSYEC |
| Rattus norvegicus    | IMNGGCDTQCTNSEGSYEC |
| Gallus gallus        | IMNGGCDTHCTNSEGSYEC |

Fig 4. Conservation analysis of FBN2 p.C1257 amino acid residue.
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Exome sequencing revealed a T>C substitution at nucleotide 3769 (p.C1257R) in the FBN2 gene of the proband. The mutation is located in exon 29, which encodes a calcium-binding epidermal growth factor-like (cbEGF) domain. The mutation alters the amino acid 1257 from cysteine to arginine, and is predicted to disrupt the secondary structure of the domain. This position is highly conserved among many different species, suggesting it is important for the stability and function of the protein (Fig 4) [31]. The abnormal fibrillin 2 may perturb the assembly of fibrillin into multimeric beaded microfibrils, normal function or stability of the microfibrils, and disrupt elastic fibrillogenesis [32,33]. Patients in this family have classical phenotype of CCA, without any evidence of tall stature and crumpled ears. One unique feature of our CCA family is that male patients present with more severe phenotype, such as large joint contracture, kyphoscoliosis and cardiovascular complications, which does not showed in five female patients. Such difference between genders may be related to genetic background, epigenetic and environmental factors, including the difference in the secretion of sex hormone and growth hormone, and a combination of subtle anatomical and physiological variations between males and females, etc. Further studies to use appropriate genetic-deficient animal models, detect the sex and growth hormone level in CCA patients, and analyze large sample of patients with CCA may facilitate a more thorough understanding of these differences in this disease.

Mouse models with spontaneous mutations, chemical mutagenesis, radiation-induced mutations and knockout mutant in the Fbn2 gene have been described in the literature. Current mouse models recapitulate some, but not all aspects of the phenotype in human. The typical phenotype in homozygous mutation mouse models is syndactyly, and other manifestations, including transitory neonatal contractures, deafness and muscle weakness. No external ear deformities, arachnodactyly or spine anomalies have been reported, suggesting species-specific differences in development [34].

In summary, our data show that the novel missense mutation p.C1257R in the FBN2 gene is the genetic cause of a Chinese Tujia family with CCA. Exome sequencing provides a highly efficient and cost-effective approach for identifying disease-causing gene of CCA while exclude gene(s) responsible for phenotype-similar disorders, such as MFS. Our findings may provide new insights into the cause of CCA and may have implication in genetic counseling for families with CCA.

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Author Contributions
Conceived and designed the experiments: HD Q. Lu ZS. Performed the experiments: HD Q. Lu HX XD ZY Q. Lin JX LG. Analyzed the data: HD LY YG Q. Lin JX LG. Contributed reagents/materials/analysis tools: HD Q. Lu HX XD LY ZY YG ZS. Wrote the paper: HD Q. Lu.

References
1. Maslen C, Babcock D, Raghunath M, Steinmann B. A rare branch-point mutation is associated with missplicing of fibrillin-2 in a large family with congenital contractual arachnodactyly. Am J Hum Genet. 1997; 60: 1389–1398. PMID: 9199560
2. Langenskiold A. Congenital contractural arachnodactyly. Report of a case and of an operation for knee contracture. J Bone Joint Surg Br. 1985; 67: 44–46. PMID: 3988142
3. Jurko AJ, Krsiakova J, Minarik M, Tonhajzerova I. Congenital contractural arachnodactyly (Beals-Hecht syndrome): a rare connective tissue disorder. Wien Klin Wochenschr. 2013; 125: 288–290. doi: 10.1007/s00508-013-0358-7 PMID: 23999522
4. Babcock D, Gasner C, Francke U, Maslen C. A single mutation that results in an Asp to His substitution and partial exon skipping in a family with congenital contractual arachnodactyly. Hum Genet. 1998; 103: 22–28. PMID: 9737771
5. Putnam EA, Park ES, Aalfs CM, Hennekam RC, Milewicz DM. Parental somatic and germ-line mosaicism for a FBN2 mutation and analysis of FBN2 transcript levels in dermal fibroblasts. Am J Hum Genet. 1997; 60: 818–827. PMID: 9106527
6. Vlijmen E. Congenital contractual arachnodactyly (Beals syndrome). J Med Genet. 1994; 31: 640–643. PMID: 7815423
7. Chen Y, Lei YP, Zheng HX, Wang W, Cheng HB, Zhang J, et al. A novel mutation (C1425Y) in the FBN2 gene in a father and son with congenital contractual arachnodactyly. Genet Test Mol Biomarkers. 2009; 13: 295–300. doi: 10.1089/gtm.2008.0132 PMID: 19473076
8. Wang M, Clericuzio CL, Godfrey M. Familial occurrence of typical and severe lethal congenital contractual arachnodactyly caused by missplicing of exon 34 of fibrillin-2. Am J Hum Genet. 1996; 59: 1027–1034. PMID: 8900230
9. Liang H, Zheng W, Xu H, Lei J, Song Z, Jiang X, et al. No evidence of association between the LINGO4 gene and essential tremor in Chinese Han patients. Parkinsonism Relat Disord. 2012; 18: 303–305. doi: 10.1016/j.parkreldis.2011.10.017 PMID: 22104011
10. Guo Y, Yuan J, Liang H, Xiao J, Xu H, Yuan L, et al. Identification of a novel COL4A5 mutation in a Chinese family with X-linked Alport syndrome using exome sequencing. Mol Biol Rep. 2014; 41: 3631–3635. doi: 10.1007/s11033-014-3227-1 PMID: 24522658
11. Zhang J, Barbaro P, Guo Y, Alaola A, Li J, et al. Utility of next-generation sequencing technologies for the efficient genetic resolution of haematological disorders. Clin Genet. 2016; 89: 163–172. doi: 10.1111/cge.12573 PMID: 25703294
12. Liu H, Wu S, Duan L, Zhu W, Zhang S, Hu X, et al. Identification of a novel EXT1 mutation in patients with hereditary multiple exostosis by exome sequencing. Oncol Rep. 2015; 33: 547–552. doi: 10.3892/or.2014.3610 PMID: 25421355
13. Shi Y, Li Y, Zhang D, Zhang H, Li Y, Lu F, et al. Exome sequencing identifies ZNF644 mutations in high myopia. PLoS Genet. 2011; 7: e1002084. doi: 10.1371/journal.pgen.1002084 PMID: 21695231
14. Gilissen C, Arts HH, Hoischen A, Spruijt L, Mans DA, Arts P, et al. Exome sequencing identifies WDR35 variants involved in Sensenbrenner syndrome. Am J Hum Genet. 2010; 87: 418–423. doi: 10.1016/j.ajhg.2010.08.004 PMID: 20817137
15. Guo Y, Yuan L, Yi J, Xiao J, Yuan J, Xiong W, et al. Identification of a novel GJA3 mutation in congenital nuclear cataract. Orphanet J Rare Dis. 2015; 92: 337–342. doi: 10.1097/OPX.0000000000000518 PMID: 25635993
16. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003; 31: 3812–3814. PMID: 12824425
17. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7: 248–249. doi: 10.1038/nmeth0410-248 PMID: 20354512
18. Scola RH, Werneck LC, Iwamoto FM, Ribas LC, Raskin S, Correa Neto Y. Congenital contractual arachnodactyly with neurogenic muscular atrophy: case report. Arq Neuropsiquiatr. 2006; 64: 259–262. PMID: 16740166
19. Jones JL, Lane JE, Logan JJ, Vanegas ME. Beals-Hecht syndrome. South Med J. 2002; 95: 753–755. PMID: 12144083
20. Matsumoto T, Watanabe A, Migitu M, Gocho Y, Hayakawa J, Ogawa S, et al. Transient cardiomyopathy in a patient with congenital contractual arachnodactyly (Beals syndrome). J Nippon Med Sch. 2006; 73: 285–288. PMID: 17106180
21. Ramirez F, Pereira L. The fibrillins. Int J Biochem Cell Biol. 1999; 31: 255–259. PMID: 10216958
26. Takaesu-Miyagi S, Sakai H, Shiroma T, Hayakawa K, Funakoshi Y, Sawaguchi S. Ocular findings of Beals syndrome. Jpn J Ophthalmol. 2004; 48: 470–474. PMID: 15486770
27. McClure SD, Van de Velde S, Fillman R, Yandro S. New finding of protrusio acetabuli in two families with congenital contractural arachnodactyly. A report of seven cases. J Bone Joint Surg Am. 2007; 89: 849–854. PMID: 17403810
28. Frédéric MY, Monino C, Marschall C, Harroun D, Faire L, Jondeau G, et al. The FBN2 gene: new mutations, locus-specific database (Universal Mutation Database FBN2), and genotype-phenotype correlations. Hum Mutat. 2009; 30: 181–190. doi: 10.1002/humu.20794 PMID: 18767143
29. Davis MR, Summers KM. Structure and function of the mammalian fibrillin gene family: implications for human connective tissue diseases. Mol Genet Metab. 2012; 107: 635–647. doi: 10.1016/j.ymgme.2012.07.023 PMID: 22921888
30. Belleh S, Zhou G, Wang M, Der Kaloustian VM, Pagon RA, Godfrey M. Two novel fibrillin-2 mutations in congenital contractural arachnodactyly. Am J Med Genet. 2000; 92: 7–12. PMID: 10797416
31. Putnam EA, Zhang H, Ramirez F, Milewicz DM. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nat Genet. 1995; 11: 456–458. PMID: 7493032
32. Chaudhry SS, Gazzar J, Baldock C, Dixon J, Rock MJ, Skinner GC, et al. Mutation of the gene encoding fibrillin-2 results in syndactyly in mice. Hum Mol Genet. 2001; 10: 835–843. PMID: 11285249
33. Boregowda R, Paul E, White J, Ritty TM. Bone and soft connective tissue alterations result from loss of fibrillin-2 expression. Matrix Biol. 2008; 27: 661–666. doi: 10.1016/j.matbio.2008.09.079 PMID: 18838118
34. Miller G, Neilan M, Chia R, Gheryani N, Holt N, Charbit A, et al. ENU mutagenesis reveals a novel phenotype of reduced limb strength in mice lacking fibrillin 2. PLoS One. 2010; 5: e9137. doi: 10.1371/journal.pone.0009137 PMID: 20161761