Original Article

A novel variant in fibrillin-1 is responsible for early-onset familial thoracic aortic aneurysms in Marfan patients

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Background: Marfan syndrome (MFS) is an inherited connective tissue disorder that affects the skeletal, ocular, and cardiovascular system. The disease's severity and clinical manifestations vary greatly due to pathogenic variants which, combined with a lack of research on the correlation between MFS's genotype and phenotype, make MFS a challenging disease to diagnose. This study aims to further the understanding of MFS by shedding light on the clinical manifestation of a novel variant in fibrillin-1 (*FBN1*)—the protein responsible for the genetic defects that lead to MFS.

Methods: A patient was diagnosed with MFS by combining a clinical examination (based on the 2010 revision to Ghent nosology criteria) with a targeted next-generation sequence analysis. The functional analysis of the causal mutation and the clinical details of the affected patient were then analyzed.

Results: The *FBN1* heterozygous variant c.5081_5082insT, which is known to delete large fragments from amino acids 1702 to 2871, was found in the proband patient and her son. The two also displayed the skeletal and cardiovascular manifestations of MFS. In addition, the 14-year-old son was identified as having a dilated aortic bulb at the same rupture site of the proband's dissection, and the proband's mother also died at age 32 due to aortic dissection.

Conclusions: The *FBN1* variant c.5081_5082insT (p.Leu1694fs*9) is a pathogenic mutation that can cause MFS patients to experience early-onset familial thoracic aortic aneurysms (TAA). We hope that this discovery can provide further insight into the treatment of MFS patients with truncating variants in exons 42-65.

Keywords: Thoracic aortic aneurysm/dissection; frameshift mutation; fibrillin-1 (*FBN1*); Marfan syndrome (MFS)

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Introduction

Marfan syndrome (MFS) is a connective tissue disorder with pleiotropic manifestations involving the cardiovascular, ocular, skeletal, skin, lung, and central nervous systems. The disease's diagnosis is based primarily on the clinical interaction of these systems as outlined in the Ghent nosology criteria. Mortality due to the disease is mainly associated with the development of cardiovascular events, such as heart failure, aortic aneurysm, and subsequent aortic dissection (1).

More than 90% of MFS cases are caused by pathogenic

heterozygous variants which are encoded in the extracellular matrix glycoprotein fibrillin-1 (FBN1). Previous studies have been explored the relationship between the location and type of *FBN1* mutations and the clinical outcomes of MFS patients (2). However, these efforts have been proven to be difficult by the known inter-familial and intra-familial clinical variability in MFS patients (3). Furthermore, mutations in *FBN1* lead to different clinical disorders, such as non-syndromic familial thoracic aortic aneurysms, familial kyphoscoliosis, adolescent idiopathic scoliosis, and Weill-Marchesani syndrome *et al.* (4). *FBN1* perform important biological functions as a calcium-binding microfibrillar structural molecule and a regulator of transforming growth factor β (TGF-β) signaling (5), beyond serving as scaffolds for elastin deposition. In this manner, the tissue-specific fibrillin microfibril network structure cooperated with biological functions to force influence on tissue growth and homeostasis (4). This indicates that more studies on genotype-phenotype correlation are warranted.

In this study we have sought to identify and determine how a novel variant in *FBN1* might be responsible for early-onset familial thoracic aortic aneurysms (TAA) in MFS patients. We believe such research can contribute to improving the diagnosis and treatment of MFS patients with truncating variants in exons 42-65.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3104).

Methods

Ethical compliance

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee at the First Affiliated Hospital of Gannan Medical University (No. 201713104150) and informed consent was taken from all the patients.

Subjects and DNA preparation

The proband patient first presented to the hospital complaining of acute chest pain and was subsequently diagnosed with a thoracic aortic dissection according to a computed tomography angiogram (CTA). She was then diagnosed with MFS according to the 2010 revised Ghent nosology criteria (6). The clinical phenotype of her first-degree relatives was also investigated. After the relevant consent forms were signed, peripheral blood samples were collected from these relatives, and genomic DNA samples were isolated.

Next-generation DNA sequencing and variant calling

In light of the characteristic clinical features of MFS, the blood samples of the patient's relatives were screened for the 13 genes associated with hereditary thoracic aortic disease (*ACTA2*, *COL3A1*, *FBN1*, *FBN2*, *MYH11*, *MYLK*, *PLOD1*, *SLC2A10*, *SMAD3*, *TGFBR1*, *TGFBR2*, *EFEMP2*, and *ELN*). This gene panel screening was carried out by using a next-generation sequencing (NGS) tool (Realomics Inc., Shenzhen, China). The average sequencing depth was 150 x, and qualified sequence reads were arrayed to the human reference genome (NCBI GRCh37) using the Burrows-Wheeler Aligner (version 0.5.17; http://bio-bwa.sourceforge.net/) (7). SAMtools (version 0.1.18, http://samtools.sourceforge.net/). Picard (http://picard.sourceforge.net/) and GATK (http://www.broadinstitute.org/gsa/wiki/index.php/Home_Page) were used for removing duplicate reads, alignments, and recalibrations. Potential single-nucleotide variants (SNVs) and small insertions and deletions (indel) were called and filtered using GATK3.7. High-confidence SNV and indel variants were then noted using snpEff (Version 4.2; http://snpeff.sourceforge.net/), and all variants were annotated according to the control population of the 1000 Genomes Project (2014 October release, http://www.1000genomes.org), ExAC (http://exac.broadinstitute.org), EVS (http://evs.gs.washington.edu/EVS), the disease databases of ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and Online Mendelian Inheritance in Man (OMIM; http://www.omim.org).

Statistical analysis

Considering MFS is a rare Mendelian disorder, we calculated the frequency of each variant in gene targets in the proband by using a large control dataset from 2504 individuals in the 1000 Genomes Project phase 5 dataset, which contains data from individuals from five "superpopulations" (European, African, East Asian, South Asian, and ad-mixed American). We repeated the analysis for variants filtered according to minor allele frequency (MAF) thresholds <0.05%. We then filtered rare variants

according to two scenarios protein-altering variants and loss-of- function (LOF) variants. The interpretation of the pathogenicity of all genetic variants was undertaken following the guidelines of the American College of Medical Genetics and Genomics (8). In the genomic DNA of the patient, we classified a frameshift variant of the *FBN1* gene as "pathogenic/likely pathogenic" due to multiple lines of pathogenic evidence. According to the above guidelines, this result details when a variant has a greater than 90% chance of being disease-causing. Mutationtaster2 was used (9) to further determine whether the putative functions of the mutations were disease causing.

Sanger sequencing

The frameshift variant of *FBN1* (NM_000138.4) was confirmed by Sanger sequencing. The targeted fragment was amplified by polymerase chain reaction (PCR) using a primer pair (GCATCACCAACCCTCCAATC; TGCTGAGTCTACAAGTCTGGT). PCR products were purified and sequenced by Tianyi Huiyuan Inc. (Guangzhou, China).

Results

Family pedigree and patient information

The proband (II-1), a 39-year-old female, was admitted to the hospital due to acute chest pain and was diagnosed with Standford B aortic dissection according to CTA (*Figure 1A*). She was then treated with an endovascular stent graft that spanned from the aortic arch to the descending aorta. In addition to the patient's aortic dissection, other clinical features of MFS were identified. These included the dilation of her carotid and pulmonary artery, as well as multiple skeletal disorders as described in *Table 1*. Her mother resembled the proband in looks and died at age 32 due to acute chest pain caused by aortic dissection. In accordance with the Ghent nosology criteria, the proband (II-1) was diagnosed as MFS because his features met the criteria that is a systemic score >7 and family history. The patient's 14-year-old son also displayed a dilated aortic bulb at the descending aorta next to the aortic arch, the same rupture site as that of the patient's dissection (*Figure 1B*), consequently was diagnosed MFS syndrome based on his family history and clinical phenotype.

Sequencing analysis

Given the high-mortality risk of the *FBN1* variant, we performed genetic counseling and next-generation gene panel sequencing. Bioinformatic analysis revealed a novel *FBN1* frameshift variant c.5081_5082insT (p.Leu1694Phefs*9) in exon 42 (chr15:48755421), which was further confirmed by Sanger sequencing (*Figure 1C*,*1D*). The heterozygous peak of Sanger sequencing for the 2 patients demonstrated that the indel mutation was not yet present in the unaffected relative (*Figure 1C*). The insertion variant was further confirmed using clone-based sequencing (*Figure 1D*). We also found that the mutation c.5081_5082insT did not exist in known databases (UMD-FBN1 (http://www.umd.be/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), UCSC common SNP (http://genome.ucsc.edu/), dbSNP (http://www.bioinfo.org.cn/relative/dbSNP%20Home%20Page.htm), and the 1000 Genomes Project) or in published articles. These results reveal that the proband (II-1) passed this *FBN1* variant to her son (III-1) and that the genotype is segregated by phenotype in this family.

Further analysis indicated that this insertion caused a change in amino acids 1694 to 1701 and a deletion of large fragments in amino acids 1702 to 2871 (*Figure 1D*). Compared to the wild-type gene, this truncated protein lacks 23 exons in the C-terminus, corresponding to 2 TGF-β domains, 18 cbEGF-like domains, and 1 fibulin-like domain (*Figure 1E*). We further determined that this gene variation likely affects protein function and may subsequently result in disease (as predicted by the MutationTaster2). In accordance with standards and guidelines for the interpretation of sequence variants developed by the American College of Medical Genetics and Genomics, c.5081_5082insT can be considered as a pathogenic variant of this MFS family accompanied with early-onset familial thoracic aortic aneurysms (8). Considering that MFS's hereditary fashion, we also genotyped her little son at the pathogenic locus using Sanger sequencing and consequently excluded him as an MFS patient.

Discussion

The genotype-phenotype correlation was recognized as an important factor not only in diagnosis but also in the

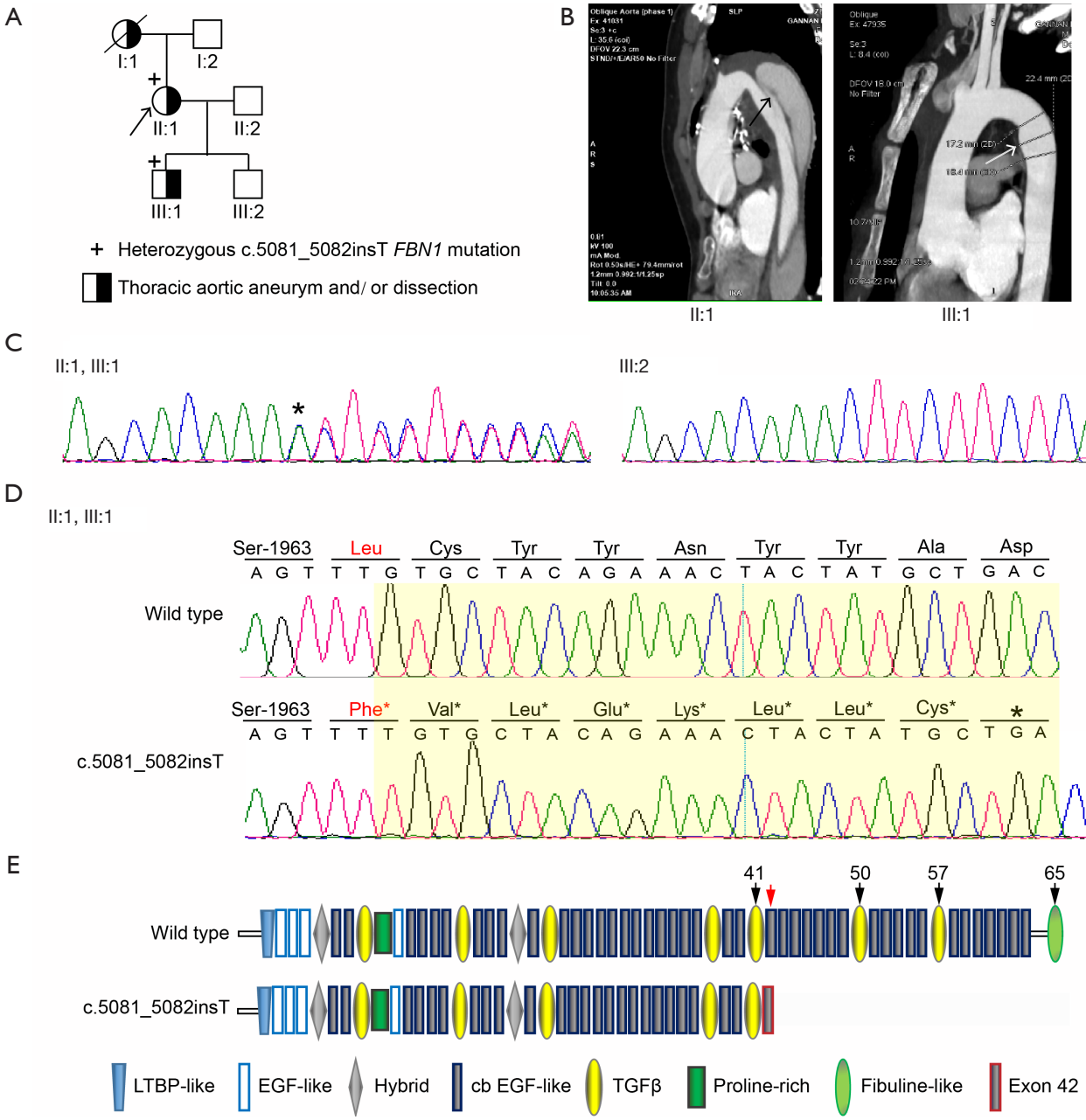

Figure 1 Family pedigree and molecular characterization of the variant c.5081_5082insT in *FBN1*. (A) Family pedigree. The individuals affected (I:1, II:1, III:1) are indicated by black squares and circles, and the proband is indicated by an arrow. (B) Thoracic aortic features of the proband (II:1) and her son (III:1). (C) The heterozygous variant c.5081_5082insT in exon 42 of *FBN1*. Sanger sequencing showed that the insertion was carried by II:1 and III:1 (indicated by the asterisk) and the corresponding normal sequence in unaffected family members. (D) Sequencing analysis of DNA clones from 2 affected individuals who had the heterozygous c.5081_5082insT variant. The upper tracer is the wild-type clone sequence, the lower tracer is the mutant clone sequence with the variant c.5081_5082insT. (E) The mutation identified in *FBN1*. Exon encoding and the different domains are numbered in bold, and the insertion is in red. The upper tracer is the integral FBN1, and the lower tracer is the truncated protein. cb-EGF, calcium-binding epidermal growth factor; EGF, epidermal growth factor; *FBN1*, gene encoding fibrillin-1; LTBP, latent TGF-β binding protein; TGF-β, transforming growth factor β.

Table 1 The clinical features of the family as detailed in the Ghent nosology criteria

Clinical features	II:1	III:1
Cardiovascular features		
Increased aortic z score at the sinuses of Valsalva	Present	Present
Dilatation or dissection of descending aorta or abdominal aorta	Present	Present
Dilatation of the pulmonary artery	Present	Present
Dilatation of the carotid artery	Present	Unknown
Mitral valve prolapses	Absent	Absent
Ocular features		
Ectopia lentis	Absent	Absent
Myopia (–3 diopters)	Absent	Present
Skeletal features		
Wrist and thumb sign	Present	Present
Pectus carinatum	Present	Absent
Pectus excavatum or chest asymmetry	Present	Absent
Pneumothorax	Absent	Absent
Protrusio acetabulae	Present	Unknown
Hindfoot deformity	Absent	Absent
Plain flat foot	Absent	Absent
Reduced upper segment/lower segment and increased arm span/height without severe scoliosis	Absent	Absent
Scoliosis >20 degrees or thoracolumbar kyphosis	Present	Absent
Reduced elbow extension	Absent	Absent
Facial features (dolichocephaly, malar hypoplasia, enophthalmos, retrognathia, down-slanting palpebral fissures)	Absent	Absent
Dura		
Lumbosacral dural ectasis	Present	Unknown
Skin system		
Skin striae	Absent	Absent

risk stratification and clinical management, of patients suspected to suffer from MFS (10). As MFS based purely on the mutation type is challenging, establishing a better genotype-phenotype correlation may prove to be an important step in the future treatment of MFS. In this case study of a Chinese family, we discovered that the novel insertion mutation c.5081_5082insT (p. Leu1694Phefs*9) is associated with MFS characterized by early-onset familial TAA and accompanying multiple skeletal defects. The presence of the premature termination codon truncates FBN1 protein, causes an haploinsufficiency (HI)-inducing effect, and consequently reduces the amount of functional FBN1.

The mutations that take place in *FBN1* have been classified into different categories including missense, which has a so-called dominant negative (DN) effect, and frameshift, nonsense and splice, all of which have a HI effect (11). A few studies have observed a trend in the association between genotype and clinical outcomes. For example, in a multicenter clinical trial, Faivre

et al. evaluated the effect of mutation type and location, concluding that MFS patients with truncating variants in FBN1 have a more severe skeletal and skin phenotype compared with MFS patients with missense mutations (2). Moreover, several single-center prospective cohort studies have analyzed the correlation between aortic events and mutation classifications in MFS patients, suggesting that those diagnosed with truncating variants in the *FBN1* gene have a higher chance of experiencing an aortic event (10,12). Recently, Arnaud *et al.* further found that those truncating mutants not only experienced the high risk of aortic events but also consequently had a shorter life expectancy, compared with MFS patients with in-frame variants (13). Otherwise, Baudhin *et al.* observed that 48% of MFS patients with a truncating, or splicing variant did not report an aortic event (12). Unlike truncating mutations, the number of missense mutations were enough to establish the correlation between MFS severity and mutation location. The missense mutations in exons 24-32 had been proven consistently by several studies that these variants resulted in early onset aortic risk and even severe forms of MFS through a dominant negative effect (2). However, in this region, truncating variants were not associated with aortic risk (13). These findings suggest that further research into the location of the pathogenic truncating variants of individuals with MFS may have significant implications for understanding the severity of the disease.

In this present study, the *FBN1* variant, c.5081_5082insT, was noted to cause a truncated C-terminus, resulting in a lack of 1169 amino acids translated by exons 42-65. The 2 patients diagnosed with the variant also fulfilled the criteria for skeletal and cardiovascular manifestation, but notably showed no ocular or skin anomalies. They both also had a positive wrist and thumb sign, with only the proband experiencing pectus carinatum, chest asymmetry, and scoliosis (Figure S1). What is also important to note is that the proband presented thoracic aortic dissection at 39 years of age, her mother died from aortic dissection at age 32, and her 14-year-old son has been identified as having an aortic sinus dilatation and a dilated aortic bulb at the descending aorta next to the aortic arch. This indicates that the c.5081_5082insT mutation caused by MFS has resulted in the patient's son having a high-mortality risk, as well as a high risk of experiencing some form of aortic complication, which in the future may require surgery. Our study also identified that the patient's son displayed some features of MFS which have not yet been defined as MFS in the Ghent nosology criteria, indicating the clinical values of etiological diagnosis and genetic counseling.

Ultimately, this research sheds further light on the important influence exons 42-65 have on aortic development. A previous report has described those patients with a mutation located in the 3' region (exon 59-63) of *FBN1* were associated with a mild phenotype characterized by a lack of significant aortic pathology (14). The fragment from exons 44-49 have also been proven to regulate the bioavailability of TGF-β and consequently contribute to Marfan pathogenesis (15). This has allowed us to recognize the important role TGF-β signaling plays in aortic aneurysm progression (16). Losartan, a TGF-β inhibitor, has also been noted to be particularly effective at reducing aortic root dilatation in MFS patients with HI *FBN1* mutations as opposed to those with from DN mutations (17). Xu *et al.* reported that there was a significantly higher frequency of aortic dissection than aortic aneurysm in Marfan syndrome patients carried *FBN1* frameshift and nonsense mutations, otherwise the in-frame mutants experienced the opposite tendency (18). Besides, the truncating mutations of *FBN1* also contribute to non-syndromic sporadic or familial aortic aneurysms and dissections (19). Considering the severity of aortic involvement in truncating mutants, the location of truncating mutations in *FBN1* associated with aortic events might enrich our knowledge on clinical management.

Conclusions

Through this study we have identified and confirmed a novel truncating mutation, c.5081_5082insT (p. Leu1694fs*9) in the *FBN1* gene. This MFS mutation is characterized by early-onset familial TAA and is accompanied by variable skeletal manifestations. These findings make a significant contribution to research on the treatment and management of MFS patients with truncating variants in exons 42 to 65.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-3104). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee at the First Affiliated Hospital of Gannan Medical University (No. 201713104150) and informed consent was taken from all the patients.

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Supplementary

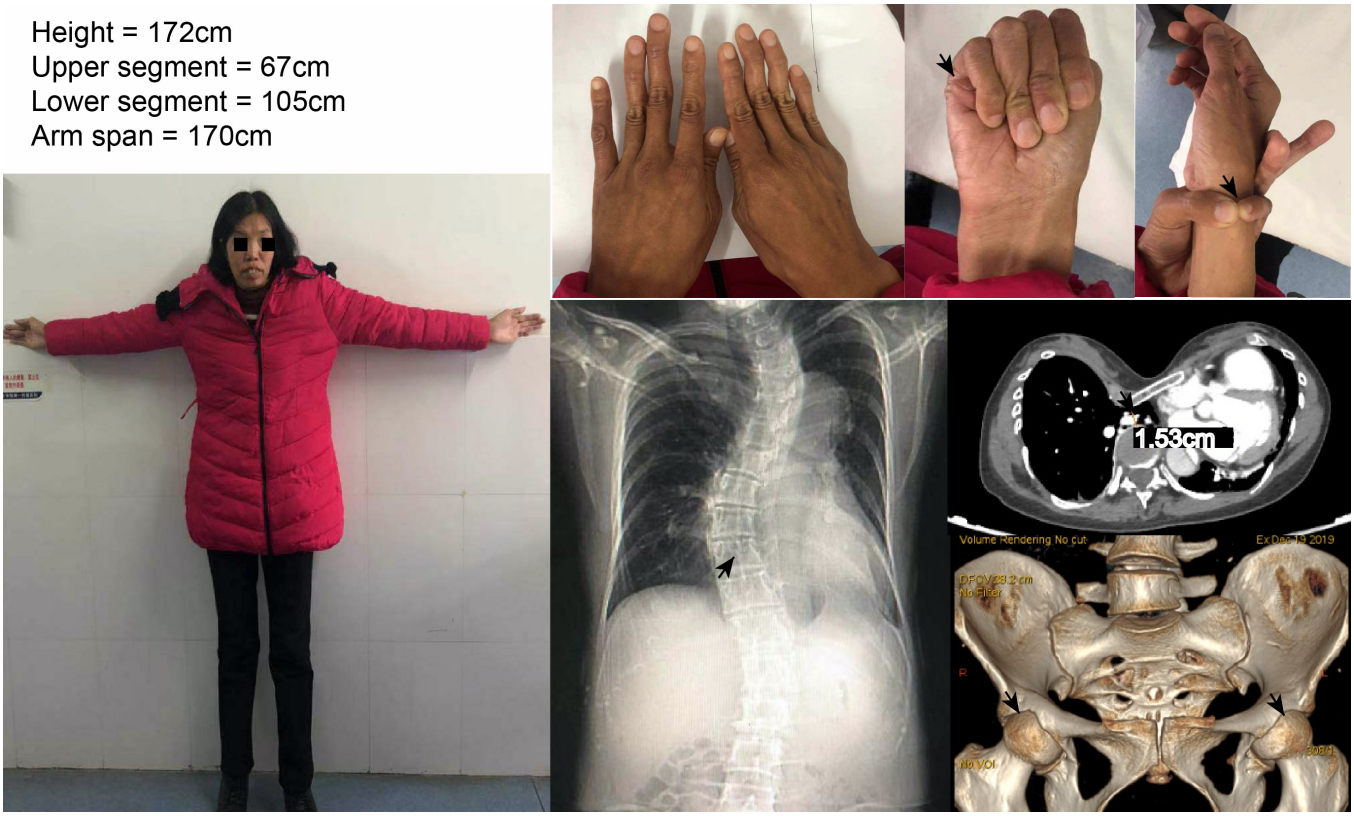

Figure S1 Skeletal features of the proband: note the wrist and thumb sign, pectus carinatum, and mild scoliosis.

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