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Juvenile Hyaline Fibromatosis: Report of a Case with a Novel ANTXR2 Gene Mutation

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Patient: Female, 2-year-old
Final Diagnosis: Juvenile hyaline fibromatosis
Symptoms: Multiple painless soft tissue masses affecting the ears • forehead • scalp
Medication: —
Clinical Procedure: Excision biopsy • surgery removal
Specialty: Pediatrics and Neonatology • Surgery

Objective: Rare disease
Background: Juvenile hyaline fibromatosis is a rare autosomal recessive disorder with unknown prevalence characterized by abnormal development of hyalinized fibrous tissue usually in the skin, mucosa, bone, and often the internal organs. Here, we report the case of a 7-year-old girl from a family with ANTXR2 mutation confirming JHF.
Case Report: The girl presented with multiple painless soft-tissue swellings affecting the ears, forehead, and scalp. Excisional biopsies of the masses reported positive immunohistochemical staining for collagen type VI in the extracellular matrix area, which indicated collagen VI accumulation. Genetic analysis was performed using whole-exome sequencing. The variants were further validated using Sanger sequencing in trio-based approach. We identified a novel mutation, c.1273_1293delinsTCTTGTGGGTTTGGCT in exon 15 of ANTXR2 gene, leading to a frame-shift of the amino acid from codon 425 to all the rest of the amino acid chain (p.Pro425Serfs). The change of an encoded protein interrupted lysosome-mediated degradation of collagen VI. This finding was compatible with her parents whose genetic tests were both positive for the same heterogenous deletion/insertion mutation. The patient was treated with surgical excision of the tumor masses, which had to be repeated several times due to recurrences.

Conclusions: This novel mutation in exon 15 of the ANTXR2 gene may help improve understanding of genotype-phenotype correlations for this syndrome and provide the basis for diagnostic testing. A multidisciplinary team approach including genetic molecular testing is required for an accurate diagnosis and management of JHF for conducting genetic counseling for affected families as a part of holistic management.

Keywords: ANTXR2 Protein, Human • Collagen Type VI • Hyalinosis, Systemic • Whole Exome Sequencing

Full-text PDF: <https://www.amjcaserep.com/abstract/index/idArt/935921>

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Background

Juvenile hyaline fibromatosis (JHF, OMIM #228600) is a rare autosomal recessive disorder characterized by abnormal accumulation of hyaline material in many connective tissues [1]. Affected individuals typically present with multiple cutaneous nodules, gingival hyperplasia, flexion contractures of the joints, osteolytic lesions, and visceral organs involvement [2,3]. Patients also have thickened skin, hyperpigmentation over bony prominences, and facial papules, which are considered to be the main dermatological manifestations [4]. In JHF, all of these clinical features are generally present within the first few years of life [5].

A mutation of the anthrax toxin receptor-2 (*ANTXR2*; OMIM #608041) gene, also called the capillary morphogenesis protein gene-2 (*CMG2*), is thought to be the cause of JHF [6]. Different types of mutations on *ANTXR2* have been previously found, including missense, frameshift, in-frame, splice-site, and nonsense mutation, with over 70% of the reported mutations being missense or frameshift mutations [2,6]. This gene encodes a type 1 transmembrane protein that contains the von Willebrand Factor A (vWA) domain, which binds to many types of matrix proteins including laminin, fibronectin, and collagens I, IV, and VI. Among these proteins, collagen VI was found to have the highest affinity to Antxr2 vWA domain [7,8]. Therefore, mutations of the *ANTXR2* gene affect the binding of collagen VI to its receptor, consequently interrupting the degradation of collagen VI, which is correlated with the accumulation of collagen VI in patient's connective tissue [5,8]. Although the cause of the disease is known, the exact pathogenesis remains unclear [7]. Herein, we report the case of a 7-year-old girl from a family with the *ANTXR2* mutation as well as her clinical data and genetic findings.

Case Report

A 7-year-old girl was referred to our institution, Songklanagarind Hospital, for further investigation of multiple painless soft-tissue swellings affecting her ears, forehead, and scalp. She was a preterm child of healthy Thai parents who had close blood relation (Figure 1). Her prenatal and perinatal periods were uncomplicated. Her development was totally normal until 2 years of age, at which time she began to develop multiple, slowly-growing, painless, soft-tissue masses at her left ear and forehead. Her parents brought her to the local hospital, where excisional surgery was done to remove the masses. The diagnosis of leiomyoma was made based on pathological examination. She was followed up at that hospital 2-3 times a year to check her mass lesion. Surgery is occasionally needed when there is recurrence. She was then referred to Songklanagarind Hospital at the age of 7 years. At our hospital,

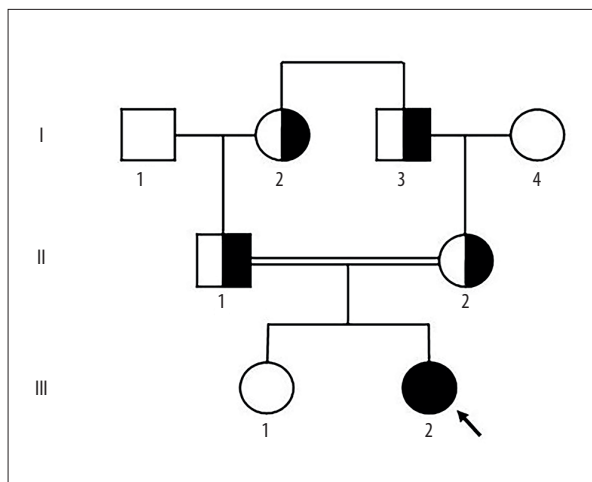


Figure 1. Pedigree chart of the family. The affected patient is indicated by the arrow and filled square. The parents are consanguineous, as shown with a double horizontal line.

her weight was 18.8 kilograms (below the 25th percentile) and her height was 117 cm (below the 40th percentile). A physical examination showed multiple, painless, soft-tissue masses at her forehead, scalp, back, and anterior chest wall. The masses were well-defined, movable, and firm, 1-5 cm in size. No sign of local inflammation was observed. Her heart, lungs, abdomen, and neurological examination were unremarkable. Her cognitive development was appropriate for age. A CT scan of her head showed multiple, well-defined, enhanced, soft-tissue masses at the scalp without intracranial extension (Figure 2).

The child was managed with mass removal. Grossly, the masses were of variable sizes with grey-white cut surfaces. Excisional biopsy of the masses revealed benign fibroblastic cells with an eosinophilic ground substance in the background. Masson staining showed green in the fibrotic areas. Immunohistochemistry was positive for SMA in the spindle cells and collagen type VI in the extracellular matrix area and, conversely, negative for collagen IV, which was previously reported to be potentially associated with the pathogenesis of JHF (Figure 3). All the clinical manifestations and pathological findings were suggestive of juvenile hyaline fibromatosis. However, the patient had recurrence of lesions on the scalp and the right forearm within 1 year after the first surgery.

After obtaining written informed consent from her parents as well as her patient's assent, their peripheral blood samples were collected. Lymphocytes genomic DNA was isolated from those 3 blood samples using a PureLink Genomic DNA kit (Invitrogen, Carlsbad, CA). The patient's gDNA was used for preparing a whole-exome library by using the NextEra rapid capture expanded exome kit (Illumina, Inc., USA) according to manufacturer's protocol. Whole-exome sequencing (WES) was performed using the Illumina HiSeq platform (Macrogen, South

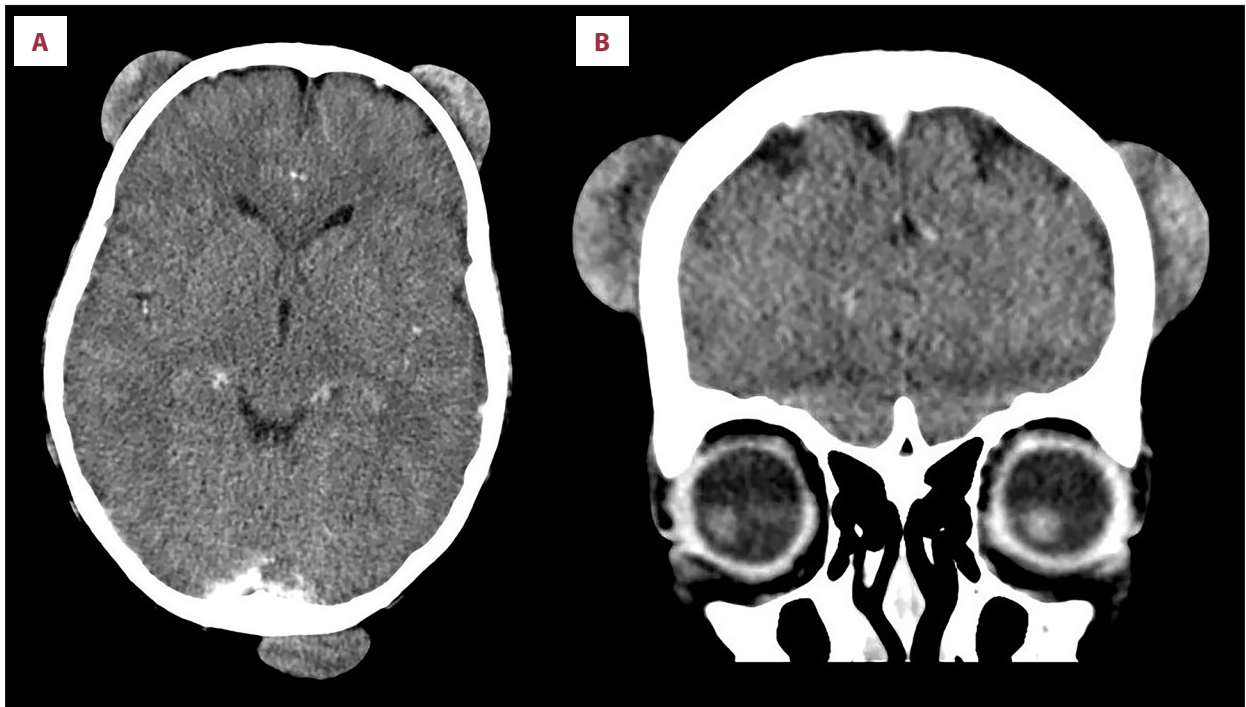


Figure 2. CT of the head of the patient, transverse plane (A) and coronal plane (B), showing multiple well-defined enhanced soft-tissue masses at the scalp, varying in size, with the largest one about 6.3 cm in diameter at the occipital region. The lower skull shows no cortical destruction, periosteal reaction, or sclerotic change.

Korea) with 150 bp paired-end reads. Output data from sequencer, DNA sequence, and quality score were stored in FASTQ file format. The quality of sequence was checked using FastQC software. Sequence reads were trimmed using Trimmomatic v0.38, and then aligned to reference genome GRCh38 by using BWA aligner. Sequence reads were sorted, and duplicates were marked using the Picard program. Variant calling was performed using GATK v4.1.2.0. Finally, variants were annotated using snpEff. Bioinformatic analysis reveal 3 novel frameshift insertion/deletion variants. Two of those are located in exon 13 of different transcript variant of the *ANTXR2* gene including NM_001286780.1: c.1042_1062delinsTCTTGTTGGCT, and NM_001286781.1: c.1042_1062delinsTCTTGTTGGCT. Another one is NM_058172.5: c.1273_1293delinsTCTTGTTGGCT located in exon 15 of another transcript variant.

The variations were validated by Sanger sequencing using the trio-based approach (patient and her parents). We did *ANTXR2* sequencing of patient and her parents' lymphocyte gDNA. Seventeen *ANTXR2* exons were amplified by polymerase chain reaction (PCR) using an annealing temperature of 50°C except for exon 1 and exon 5, for which the PCRs were done at an annealing temperature of 52°C. We used 17 pairs of primers previously designed by Hanks et al [5] in 2003. The PCR products were visualized on 2% agarose gel and purified using a QIAquick PCR Purification kit (Qiagen, Valencia, CA). The products were then sequenced by capillary sequencing method.

We identified an inherited homozygous deletion/insertion variant on exon 15 of the *ANTXR2* gene, which is the same variation discovered from WES. The deletion started from nucleotide 1273 to 1293, and the short nucleotide sequence of 5' TCTTGTTGGCT 3' was inserted in place of the deleted area (NM_058172.5: c.1273_1293delinsTCTTGTTGGCT; **Figure 4**), leading to a frameshift of the amino acid at codon 425 to the rest of the amino acid chain (p.Pro425Serfs). Her parents were subsequently found to be heterozygous for the same deletion/insertion mutation (**Figure 4**). Thus, her parents were both carriers who shared the same mutation. Conversely, no variation on exon 13 was found from Sanger sequencing validation. Moreover, those 2 variants of exon 13 are located at position 79978062-799780077 of the genomic DNA sequence, which is the same position as the variant founded on exon 15. Therefore, those 3 variants discovered from WES are all the same variant, but were located in 3 different transcript variants of the *ANTXR2* gene. This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand.

Discussion

We herein report a mutation of the *ANTXR2* gene in 7-year-old girl with consanguineous parents, resulting in the JHF phenotype. Individuals affected with this disease are classified by the

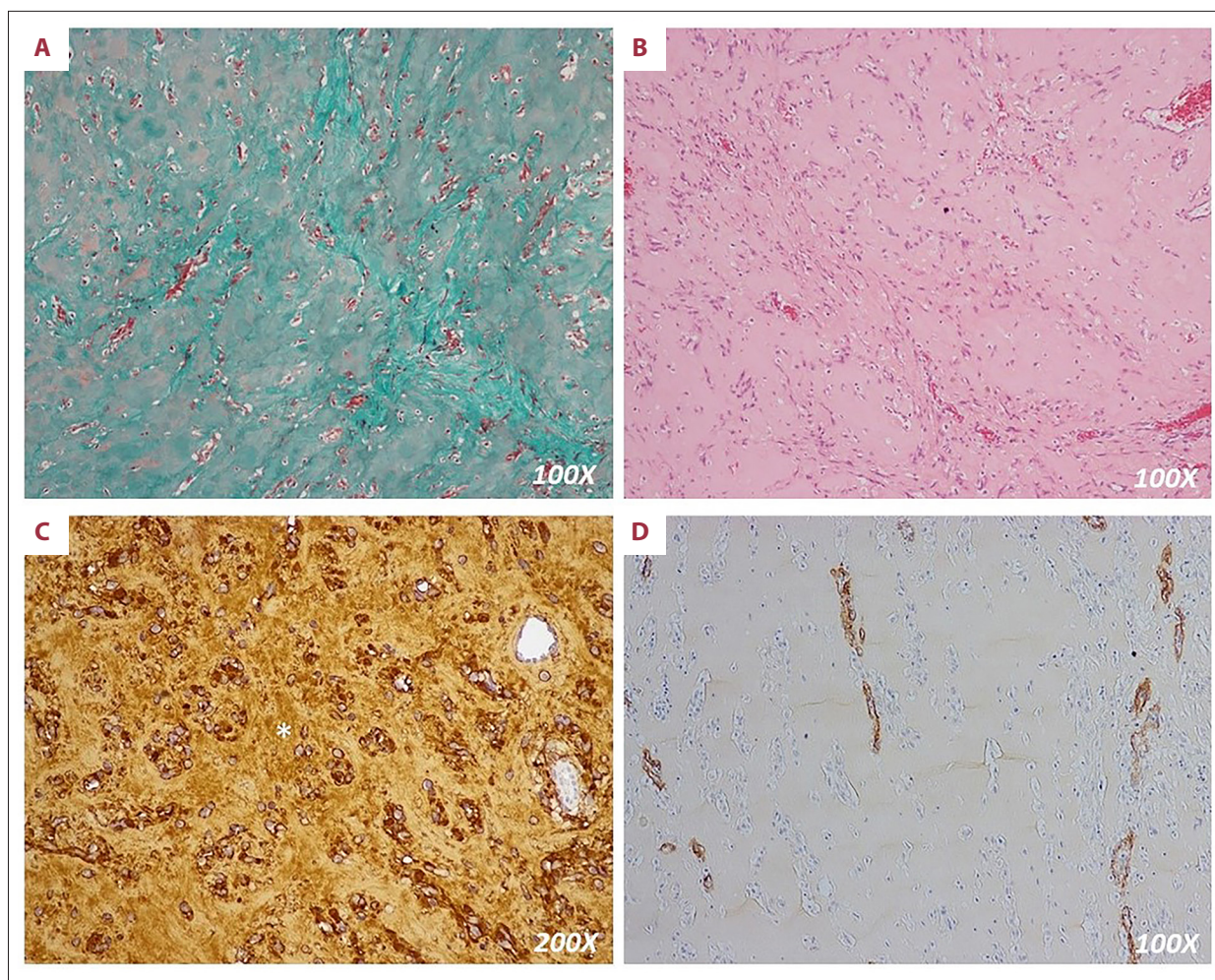


Figure 3. (A) Masson stain showing green in the fibrotic areas, proving the collagen accumulation in ECM. (B) H&E staining shows benign fibroblastic cells with an eosinophilic ground substance in the background. (C) Showing positive immunohistochemical staining for collagen type VI in the extracellular matrix area (*). (D) Showing negative immunohistochemical staining for collagen type IV in patient's biopsy tissue.

disease severity into 4 grades, with grade 1 or mild having only skin or gingival involvement, grade 2 or moderate also having joint or bone involvement, grade 3 having internal organ involvement with or without clinical manifestations, and grade 4 or life-threatening having the highest probability of fatality [2,9]. In our patient, only skin involvement, which is the most common feature of this disease, was found. Interestingly, she did not present with any osteoarticular manifestations, which is the initial presentation in most patients [3]. She did not have persistent diarrhea or recurrent infection, which are the 2 most common presentation in patients with visceral involvement. Moreover, unlike our patient, patients with internal organ involvement generally develop symptoms during the first month of life [3]. Consequently, she was classified as grade 1 JHF.

ANTXR2, a 17-exon gene, is located on chromosome 4q21. It encodes anthrax toxin receptor 2 (Antxr2), a type I transmembrane

protein, which contains 3 domains: a conserved extracellular ectodomain, a single-pass transmembrane domain, and a cytosolic domain. Its ectodomain is composed of a vWA domain which can bound to several matrix proteins, including collagens VI [7,8,10]. The binding of collagen VI to the Antxr2 vWA domain activates intracellular signals such as src-dependent phosphorylation of the Antxr2 cytoplasmic tail and the recruitment of β -arrestin, which triggers endocytosis of collagen VI [8]. The mutation c.1273_1293delinsTCTTGTGGGTTTGGCT causes a translational frameshift of an amino acid chain starting at codon 425. The affected protein region was thought to reside in the Antxr2 cytosolic region, which is a common mutation area for JHF (Figure 5) [9]. This could lead to a protein structure defect that interrupts the lysosome-mediated degradation of collagen VI, which would be compatible with the existence of collagen VI accumulation in JHF patients (Figure 3) [6,8]. The excess collagen can accumulate in the extracellular matrices

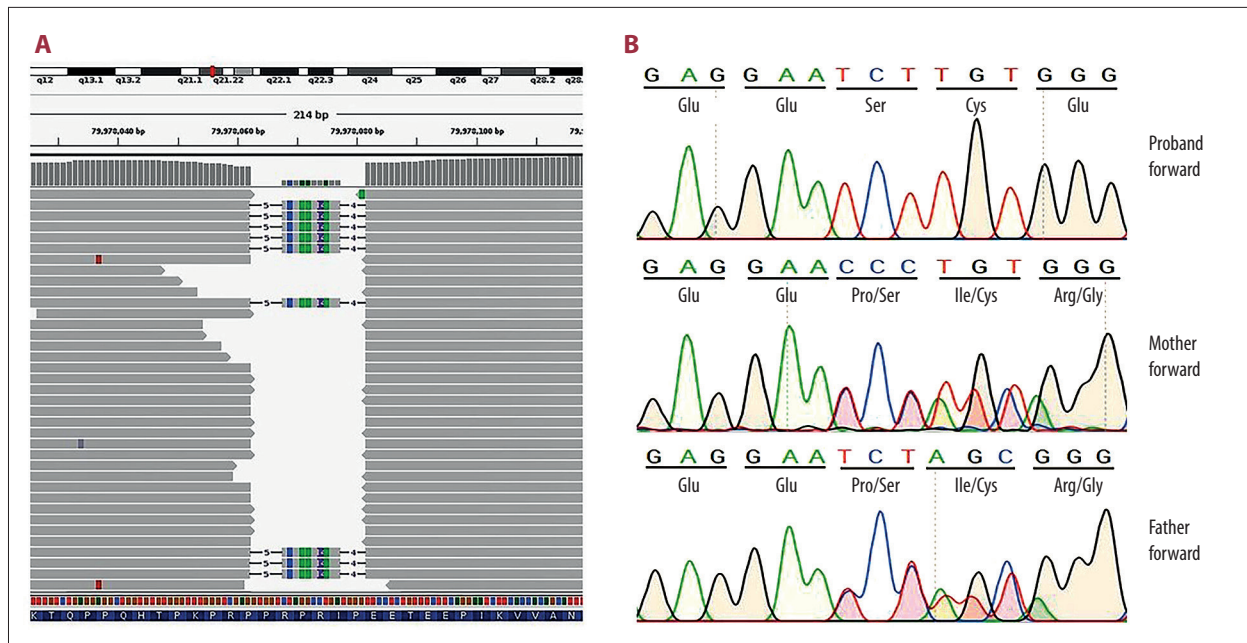


Figure 4. (A) The aligned reads from WES were visualized on Integrative Genomics Viewer (IGV) [14]. The display show area of nucleotide base deletion/insertion (c.1273_1293delinsCTTGTGGGTTTGGCT). (B) The Sanger sequencing of the ANTXR2 gene reveal that proband forward demonstrating a homozygous deletion/insertion mutation on exon 15 (c.1273_1293delinsCTTGTGGGTTTGGCT) leading to a translational frameshift of amino acid chain starting at codon 425. The sequences of the unaffected mother and father show that both are carriers of JHF who share the same mutation.

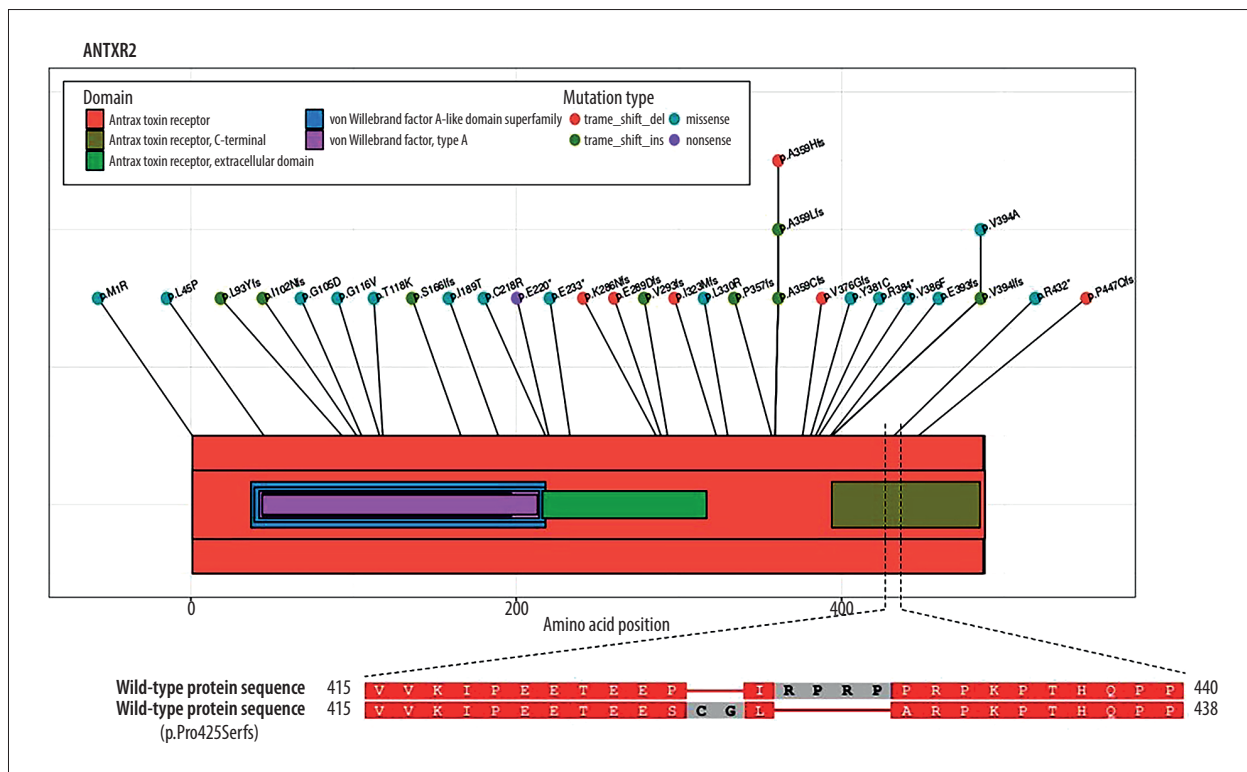


Figure 5. Showing the schematic picture of Antxr2, with protein domains and positions of the previously reported mutations in patients with JHF/IHF and their families. Pairwise alignment between wild-type and mutated protein sequence of our patient was performed using BlastP online software.

(ECM) of connective tissue in several organs of an affected individual, including the skin, joints, gingiva, or even visceral organs. The excess collagen VI accumulation in subcutaneous tissues causes abnormal formation of hyalinized fibrous nodules, which is a significant hallmark of this disease [3]. Burgi and colleagues reported that the uteri of *ANTXR2* homozygous mutant mice displayed progressive fibrosis, which was mainly composed of collagen VI, resulting in destruction of tissue structure and infertility [8]. Concordantly, Van Rijn and colleagues also revealed that the *ANTXR2*-deficient organoids, generated by CRISPR-Cas9 *ANTXR2* knockout human duodenal cell, showed abnormal collagen VI deposition [11]. This finding in animal and organoid models supports the theory that loss of *Antxr2* function leads to collagen VI accumulation and abnormal extracellular matrix composition in human tissue.

In our case, the patient presented with only typical skin manifestations. This finding correlated with the mutation discovered in this patient, since mutations in the nucleotide sequence encoding the *Antxr2* cytosolic domain have been previously found to be clinically less severe than those with mutations in other regions [6]. As only skin manifestations were observed, our patient was managed with surgical excision of the tumor masses, which had to be repeated several times due to recurrences [12].

To date, various therapeutic modalities have been used to treat JHF. Medications, including penicillamine, methotrexate, and steroids, have some benefits for JHF with some success [12,13]. Surgical excision is a good treatment in subcutaneous nodules; however, local recurrence is common [2]. However, there is no curative treatment for this disease, so counseling for families who are known to carry the genetic defect and family planning become imperative. Precise molecular genetic testing not only

allows us to detect diseases earlier but is also important for risk and carrier assessment of family members, which in turn allows proper management and genetic counseling. Moreover, an understanding of the genetic basis of the disease can augment the possibility of future genetic therapies for this disorder. Molecular diagnosis should be offered to every patient with JHF as well as their family members as a part of holistic management, including family planning.

Conclusions

This study reports a novel mutation, c.1273_1293delinsTCTTG TGGGTTGGCT in exon 15 of the *ANTXR2* gene, which caused mild JHF. Information contained in the present report may help increase our understanding of the genotype-phenotype correlations of this syndrome. Due to the nature of the disease, a multidisciplinary team approach is required for accurate diagnosis and management of JHF and for conducting genetic counseling of affected families to prevent disease in future generations.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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