**WISP3 mutation associated with pseudorheumatoid dysplasia**

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**Abstract**

Progressive pseudorheumatoid dysplasia (PPD) is a skeletal dysplasia characterized by predominant involvement of articular cartilage with progressive joint stiffness. Here we report genetic characterization of a consanguineous family segregating an uncharacterized form of skeletal dysplasia. Whole-exome sequencing of four affected siblings and their parents identified a loss-of-function homozygous mutation in the **WISP3** gene, leading to diagnosis of PPD in the affected individuals. The identified variant (Chr6: 112382301; **WISP3**:c.156C>A p.Cys52*) is rare and predicted to cause premature termination of the **WISP3** protein.

[Supplemental material is available for this article.]

**INTRODUCTION**

Rare genetic conditions involving the skeletal system arise through misregulation in the process of skeletal development (cartilage and bone growth) and remain a diagnostic challenge because of the rarity of the disease and the heterogeneity in the phenotypes (Kornak and Mundlos 2003; Krakow and Rimoin 2010; Chen et al. 2016). Moreover, given the fact that some skeletal phenotypes are driven by several different genes, and that some genes can lead to a variety of different skeletal diseases, achieving a molecular diagnosis can be difficult. Correct diagnosis for progressive pseudorheumatoid dysplasia (PPD) is particularly challenging as it is very rare (~1 in a million) (Wynne-Davies et al. 1982; Garcia Segarra et al. 2012) and has similarities with other disorders (i.e., mucopolysaccharidoses, rheumatoid arthritis, and ankylosing spondylitis) (Spranger et al. 1983; Neerinckx et al. 2015).

**RESULTS**

**Family Description**

Here we report genetic characterization of a consanguineous family segregating PPD. Written informed consent was obtained for all participants. The institutional review boards of the Special Medical Center, Tehran, Iran and Stanford University reviewed the project. The family pedigree is shown in Figure 1A. Affected individuals underwent examination at the Special Medical Center for rare diseases, Tehran, Iran. The patients were asymptomatic at birth, with normal growth, development, and intelligence as well as no facial, joint, and
skeletal system deformity. However, the disease started to manifest at 4–6 years of age in affected individuals and progressively worsened (Table 1). Presenting findings included the enlargement of joints—first in the large joints of the limbs (knees, ankles, and elbow) and then a knobby appearance of the proximal interphalangeal joints of the hands. By the age of 10 yr, a knobby appearance in the metacarpophalangeal and distal interphalangeal joints of the hands was present, as well as the involvement of the spine (mild abnormality). In early adolescence, some affected individuals displayed gait disturbances because of knee deformity and some contracture. In late adolescence and beyond, flexion contracture and stiffness in the large joints had developed (knees, elbows, and hip) and the fingers and toes became short (camptodactylic) (Table 1). Moreover, a skeletal survey showed degenerative changes with generalized osteopenia with the presence of unfused epiphyses in the vertebrae. All together, these clinical data indicate a skeletal dysplasia; however, because of heterogeneity in skeletal abnormalities, it was challenging to precisely make a diagnosis for this abnormality. Therefore, we applied a WES approach to identify the casual gene and came up with a precise diagnosis.

Exome-Sequencing Results

WES to a mean coverage of >80× (Individuals 1.2, 2.1, 2.2, 2.3, and 2.4 of Fig. 1) was performed (Supplemental Table S1). We identified 163,116 variants that are shared in all the
family members and have a genotype quality score of >20 (Table 2). Based on the pedigree, we predicted the disease would follow an autosomal recessive pattern. Thus, we analyzed variants that were homozygous in affected individuals but heterozygous in the healthy parents. Of note, 1064 variants (151 missense variants, 359 variants in 3′UTRs and 5′UTRs, 43 frameshift variants, 34 in-frame deletions and insertions, 43 splicing event–related variants, 276 intergenic variants, 321 intronic variants, 101 synonymous variants, and 12 stop gain, stop lost, and stop retained variants) were identified. We then selected variants with a minor allele frequency (MAF) of <0.01 in public databases: dbSNP Common 144 (Database of Single Nucleotide Polymorphism, NCBI), 1000 Genome project phase 3 (www.1000genomes.org), Exome Aggregation Consortium version 0.3 (ExAC; http://exac.broadinstitute.org/), NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS/). These filtering steps resulted in identifying 286 homozygous variants, of which 26 are exonic and only one variant was predicted to be pathogenic (stop-gain variant).
The identified variant occurs in the exon three of WISP3 gene (WISP3;c.156C>A; p.Cys52∗), is rare (MAF of 0.0008% in ExAC; 0.04% in dbSNP 144; no homozygotes), and is predicted to be deleterious (Table 3). We confirmed the homozygosity of this variant by Sanger sequencing (Fig. 1B) using 5′GGCCTGGAGAAGTGTCAGAT3′ and 5′GTCTCGTA
CCTAGGCCTGTC3′ for PCR amplification and 5′GTCTCGTACCTAGGCCTGTC3′ as a Sanger sequencing primer. We showed that the variant segregates in the family, as all four affected individuals have the homozygous mutation, whereas their parents are heterozygous (Fig. 1B).

**DISCUSSION**

In this study, we employed whole-exome sequencing to identify the underlying genetic variants associated with a rare uncharacterized form of skeletal dysplasia. Abnormalities involving the skeletal system remain a diagnostic challenge because of the heterogeneity of skeletal system diseases. Moreover, given the fact that some skeletal phenotypes are driven by several different genes, and that some genes can lead to a variety of different skeletal diseases, achieving a molecular diagnosis can be quite difficult. To overcome this challenge, we combined the clinical data from a family segregating a rare uncharacterized form of skeletal dysplasia with a comprehensive WES approach. We have sequenced all four affected siblings and their parents to identify the causal mutation associated with this skeletal dysplasia in order to make a better diagnostic. Our study reports a homozygote mutation for rs121908901; WISP3; c.156C>A; p.Cys52*, which introduces a stop codon in the IGFBP

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**Table 2. Variant filtering steps**

| Individual ID | I-2 | I-1 | I-2 | I-3 | I-4 |
|---------------|-----|-----|-----|-----|-----|
| Shared variants | 163,116 |     |     |     |     |
| Homozygote variants in affected but heterozygote variants in parents | 1064 |     |     |     |     |
| 1KG MAF < 0.01 | 373 |     |     |     |     |
| ExAC MAF < 0.01 | 325 |     |     |     |     |
| dbSNP 144 MAF < 0.01 | 322 |     |     |     |     |
| NHLBI MAF < 0.01 | 320 |     |     |     |     |
| UK 10K twins | 286 |     |     |     |     |
| UK 10K ALSPAC | 286 |     |     |     |     |
| Exonic variants | 26 |     |     |     |     |
| Pathogenic (missense or stop gain/loss) | 1 |     |     |     |     |
| Candidate | Chr6:112,382,301; WISP3; c.156C>A; p.Cys52∗ |     |     |     |     |

MAF, minor allele frequency; 1KG, 1000 Genomes project phase 3; ExAC, Exome Aggregation Consortium version 0.3; dbSNP 144, Database of Single Nucleotide Polymorphism, NCBI; NHLBI, Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP).

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**Table 3. Summary of the variant reported in this study**

| Gene | Chr | HGVS DNA reference | HGVS protein reference | Predicted effect | Variant type | dbSNP ID | Genotype | ClinVar accession | ExAC MAF | Inheritance |
|------|-----|-------------------|------------------------|------------------|--------------|----------|----------|------------------|----------|-------------|
| WISP3 | Chr6:112382301 | NM_003880.3: c.156C>A | NP_003871.1: p.Cys52∗ | Stop-gained | rs121908901 Homozygous | SVC000607728 | 6.056e-05 | Homozygous | 05 | recessive |

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domain of the WISP3 protein. Previously, Hurvitz et al. 1999 reported a compound heterozygote involving the same variant in a French family with progressive pseudorheumatoid arthropathy of childhood (Hurvitz et al. 1999).

**WISP3**

The WNT1 Inducible Signaling Pathway Protein 3 (WISP3) gene encodes a member of the connective tissue growth factor (CTGF) family of secreted cysteine-rich, glycosylated proteins that play a multitude of roles in cell growth and differentiation (Bork 1993). The first clinical link between WISP3 and PPD was demonstrated by linkage studies in consanguineous families segregating PPD, which mapped the candidate region to a 3-cM interval between D6S1594 and D6S432 microsatellite markers on Chromosome 6p22 (el-Shanti et al. 1998; Fischer et al. 1998). One year later, Hurvitz et al. (1999) identified mutations in the linkage region, specifically in WISP3, as the strongest candidates to cause this autosomal recessive condition.

**The Spectrum of Mutations in WISP3**

The WISP3 protein contains a signaling peptide (SP) and four conserved cysteine-rich domains that are differentially affected by mutations (Fig. 2). Figure 2 shows a schematic representation of the WISP3 protein domains, and Table 4 represents a comprehensive representation of the mutation spectrum of WISP3 in the literature (Table 4). The insulin-like growth factor–binding domain (IGFBP) has been shown to bind IGF-1 to inhibit signaling, and mutations within have been shown to sensitize articular chondrocytes to IGF-1, causing hypertrophy and diminished production of collagen types II and IX (Liao et al. 2004; Repudi et al. 2013). As shown in Figure 2, this domain contains the highest number of described deleterious mutations (34%), as well as the most affected families. In contrast, the von Willebrand factor type C module (VWC) domain contains only ~7% of described mutations and has the least evidence regarding its possible function in PPD. Although not demonstrated in WISP3, the VWC domain has been shown to interact with BMP and TGF-β family members and to promote oligomerization in other related family members (Holbourn et al. 2008). The thrombospondin domain (TSP) has been identified as a negative regulator of Slug/Notch1 signaling and thus as an anti-angiogenic factor in breast cancer epithelial cells (Huang et al. 2016). Suppression of Notch is likely important in chondrocytes as well, given the observation that Notch signaling promotes ossification and osteoarthritis (Hosaka et al. 2013). This domain contains the second highest number, ~31% of the described deleterious mutations in PPD. Finally, the carboxy-terminal cystine knot–like domain (CTCK) is a commonly identified motif found in proteins that form dimers and bind a variety of ligands (Issacs 1995; Holbourn et al. 2008). For example, in a WISP3-related factor called CCN2
| Variant          | HGMD accession | dbSNP ID     | Protein change         | Citation                                                                 | Population       | No. of families |
|------------------|----------------|--------------|------------------------|---------------------------------------------------------------------------|------------------|-----------------|
| c.43_44delGC     | CD991937       | –            | p.Ala15Thrfs*17        | Hurvitz et al. 1999                                                      | USA              | 1               |
| c.48+2dupT       | CI994276       | rs797044439  | splicing: 2IVS+2       | Hurvitz et al. 1999; Garcia Segarra et al. 2012                         | Jordan, Morocco  | 2               |
| c.49-763G>T      | CS126440       | –            | splicing: IVS2 -763    | Garcia Segarra et al. 2012                                               | Belgium          | 1               |
| c.49-1G>A        | CS159581       | rs781864926  | splicing: IVS2 -1      | Bhavani et al. 2015                                                      | India            | 1               |
| c.105dupT        | CI151711       | –            | p.Gly36fs*10           | Liu et al. 2015                                                          | China            | 1               |
| c.136C>T         | CM091536       | –            | p.Gln46*               | Yue et al. 2009; Ye et al. 2012; Yu et al. 2015                          | China            | 3               |
| c.156C>A         | CM991252       | rs121908901  | p.Cys52*               | Hurvitz et al. 1999; Delague et al. 2005; Garcia Segarra et al. 2012; Rai et al. 2016; Temiz et al. 2011; Bhavani et al. 2015; Madhuri et al. 2016 | Italy, France, Lebanon, Syria, Turkey, Germany, India | 33              |
| c.182G>T         | CM126432       | –            | p.Cys61Phe             | Garcia Segarra et al. 2012                                               | Poland           | 1               |
| c.185delC        | CD126426       | –            | p.Pro62Leufs*4         | Garcia Segarra et al. 2012                                               | Turkey           | 2               |
| c.190G>A         | CM166919       | –            | p.Gly64Arg             | Montane et al. 2016                                                      | Ecuador          | 1               |
| c.197G>A         | CM126433       | rs782172825  | p.Ser66Asn             | Garcia Segarra et al. 2012                                               | USA, Italy, Ecuador | 3           |
| c.232T>C         | CM991253       | rs121908902  | p.Cys78Arg             | Hurvitz et al. 1999                                                      | France           | 1               |
| c.233G>A         | CM129533       | –            | p.Cys78Tyr             | Dalal 2012; Ekbote et al. 2013; Bhavani et al. 2015; Madhuri et al. 2016 | India            | 9               |
| c.236_237CC>AA   | CX126439       | –            | p.Ala79Glu             | Garcia Segarra et al. 2012                                               | Italy            | 1               |
| c.246delA        | CD991938       | rs797044438  | p.Glu84Lysfs*21        | Hurvitz et al. 1999                                                      | Saudi Arabia, Jordan | 3           |
| c.248G>A         | CM129541       | rs147337485  | p.Gly83Glu             | Delague et al. 2005; Dalal et al. 2012; Ekbote et al. 2013; Rai et al. 2016; Temiz et al. 2011 | Lebanon, Syria, India | 10          |
| c.296A>T         | CM159583       | –            | p.Tyr99Phe             | Bhavani et al. 2015                                                      | India            | 1               |
| c.298T>A         | CM159594       | –            | p.Cys100Ser            | Bhavani et al. 2015                                                      | India            | 1               |
| c.327C>A         | CM126423       | –            | p.Tyr109*              | Garcia Segarra et al. 2012                                               | Turkey           | 3               |
| c.340T>C         | CM129534       | –            | p.Cys114Arg            | Dalal et al. 2012                                                        | India            | 1               |
| c.341G>A         | –              | –            | p.Cys114Tyr            | Yue et al. 2009                                                          | China            | 2               |
| c.342_343delTG   | CD126427       | –            | p.Ala115Leufs*16       | Garcia Segarra et al. 2012                                               | Turkey           | 1               |
| c.341G>A         | CM091537       | –            | p.Cys114Tyr            | Yue et al. 2009                                                          | China            | 1               |
| c.342T>G         | CM118811       | –            | p.Cys114Trp            | Sun et al. 2012; Ye et al. 2012; Liu et al. 2015; Yan et al. 2016; Yu et al. 2015 | China            | 5               |
| c.346+1G>T       | –              | –            | p.Tyr109_.,Met195delins9 | Garcia Segarra et al. 2012                                               | Turkey           | 1               |
| c.347-2A>G       | CS159640       | –            | splicing: IVS3 -2      | Bhavani et al. 2015                                                      | India            | 1               |
| c.347_3_347-1delCAG | CD159582 | –            | -                   | Bhavani et al. 2015                                                      | India            | 1               |
| c.346+1G>T       | CS126438       | –            | splicing: IVS3 +1      | Garcia Segarra et al. 2012                                               | Turkey           | 1               |
| c.348C>A         | CM129535       | –            | p.Tyr116*              | Dalal et al. 2012; Madhuri et al. 2016                                  | India            | 2               |
| c.433T>C         | CM129536       | –            | p.Cys145Arg            | Dalal et al. 2012                                                        | India            | 2               |

(Continued on next page.)
Table 4. (Continued)

| Variant         | HGMD accession | dbSNP ID       | Protein change | Citation                                      | Population | No. of families |
|-----------------|----------------|----------------|----------------|-----------------------------------------------|------------|-----------------|
| c.434G>A        | CM991254       | rs121908899    | p.Cys145Tyr    | Hurvitz et al. 1999; Garcia Segarra et al. 2012 | Italy      | 2               |
| c.530C>A        | CM159598       | –              | p.Ser177*      | Bhavani et al. 2015                           | India      | 1               |
| c.536_537delGT  | CD053623       | –              | p.Cys179*      | Delague et al. 2005                           | Syria      | 1               |
| c.589G>C        | CS053500       | –              | Splicing: IVS4 ds -1 | Delague et al. 2005                           | Syria      | 1               |
| c.589+1G>A      | CS1610143      | rs879255273    | Splicing: IVS4 ds +1 | Rai et al. 2016                              | India      | 1               |
| c.589+27C>G     | CS126441       | –              | Splicing: IVS4 ds +27 | Garcia Segarra et al. 2012                    | Italy      | 1               |
| c.594_598delTAGAA | CD1610848   | –              | p.Tyr198*      | Madhuri et al. 2016                           | India      | 1               |
| c.621_622delAAinsT | CX126431 | –              | p.Lys207Asnfs025 | Garcia Segarra et al. 2012                    | USA        | 1               |
| c.624_625insA   | CI105183       | –              | p.Cys209Metfs† | Ye et al. 2010, 2012                           | China      | 3               |
| c.624delA       | CD151709       | –              | p.Lys208fs*24  | Liu et al. 2015                               | China      | 1               |
| c.624dupA       | CI105183       | –              | p.Cys209Metfs† | Ye et al. 2010, 2012                           | China      | 1               |
| c.625dupT       | CI1615597      | –              | p.Cys209Leufs* | Yang et al. 2016                              | China      | 1               |
| c.667T>G        | CM118812       | –              | p.Cys223Gly    | Ye et al. 2012; Luo et al. 2015; Yan et al. 2016; Yu et al. 2015 | China      | 4               |
| c.670G>A        | CM126437       | –              | p.Gly224Arg    | Garcia Segarra et al. 2012                    | Italy      | 1               |
| c.677G>T        | CM126434       | –              | p.Gly226Val    | Garcia Segarra et al. 2012; Madhuri et al. 2016 | UK, India  | 2               |
| c.682T>G        | CM126429       | –              | p.Cys247Leufs* | Ehl et al. 2004; Dalal et al. 2012; Garcia Segarra et al. 2012; Yu et al. 2015 | Caucasian, Germany | 4               |
| c.702C>T        | CM159561       | –              | p.Arg230Leufs* | Bhavani et al. 2015                           | India      | 1               |
| c.708dupC       | CI126430       | –              | p.Asn237Glnfs*3 | Garcia Segarra et al. 2012                    | Turkey     | 2               |
| c.716_722del    | CD124723       | –              | p.Glu239fs*16  | Sun et al. 2012                               | China      | 1               |
| c.719_725delTGAGAAA | CD124723 | –              | –             | Sun et al. 2012                               |            |                 |
| c.725_726delAA  | CD126429       | –              | p.Lys242Argfs*36 | Garcia Segarra et al. 2012                    | Italy      | 2               |
| c.727_731delGAGAA | CD126428 | –              | p.Glu243Lysfs*34 | Garcia Segarra et al. 2012                    | Turkey     | 3               |
| c.729_735delGAGAGA | CD105182 | –              | p.Glu243Aspfs*13 | Ye et al. 2010, 2012                           | China      | 5               |
| c.740_741delGT  | CD044991       | –              | p.Cys247Leufs*31 | Ehl et al. 2004; Dalal et al. 2012; Garcia Segarra et al. 2012; Yu et al. 2015 | Caucasian, Germany | 4               |
| c.756C>A        | CM153375       | –              | p.Cys252*      | Luo et al. 2015                               | China      | 1               |
| c.783+1_783+6delGTAAAG | CD159627 | –              | p.Ile260Asnfs*17 | Bhavani et al. 2015                           | India      | 1               |
| c.802T>G        | CM129538       | –              | p.Cys268Gly    | Dalal et al. 2012                             | India      | 1               |
| c.805delC       | CD159638       | –              | p.Glu269Nfs*44 | Bhavani et al. 2015                           | India      | 1               |
| c.840delT       | HD040019       | rs797044440    | p.Phe280Leufs*33 | Liao et al. 2004; Peng et al. 2004; Yang et al. 2013 | China      | 3               |
| c.850G>T        | CM126424       | –              | p.Gly284*      | Garcia Segarra et al. 2012                    | Turkey     | 1               |
| c.857C>G        | CM126425       | –              | p.Ser286*      | Garcia Segarra et al. 2012; Yu et al. 2015     | Turkey, China | 2               |
| c.862_863dupAC  | CI992094       | rs863223286    | p.Gln289Leufs*25 | Hurvitz et al. 1999                          | USA        | 1               |
| c.866_867insA   | –              | –              | p.Gln289fs*31  | Sun et al. 2012; Ye et al. 2012                | China      | 2               |
| c.866dupA       | CI105184       | –              | p.Ser290Gluufs*13 | Ye et al. 2010, 2012; Sun et al. 2012; Yu et al. 2015 | China, Italy | 9               |

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The cystine knot domain interacts with BMP-2 to activate a signaling program that promotes mature chondrocytes (Maeda et al. 2009). This domain has the third highest number of deleterious mutations at 25%.

In conclusion, our study, in line with previous studies (Hurvitz et al. 1999; Nakamura et al. 2007; Neerinckx et al. 2015; Yu et al. 2015; Rai et al. 2016; Yan et al. 2016), provides further evidence of the essential role of WISP3 in postnatal skeletal growth and cartilage homeostasis in humans.

### METHODS

**Exome Sequencing and Variant Calling**

Exome capture, library preparation, and sequencing, as well as data analysis, were performed as previously described (Reza Sailani et al. 2017). Briefly, exome capture and library preparation were performed using the Agilent SureSelectXT HumanAllExon V5 (product no. 5190–4631). Two micrograms of gDNA was sheared to a peak size of 150–200 bp using the Covaris instrument. Fragmented genomic DNA was purified using Agencourt AMPure XP beads (Beckman Coulter) to remove fragments of <100 bp. Then, according to the manufacturer’s instructions, the purified DNA fragments were then end-repaired, A-tailed, and ligated to indexing-specific paired-end adaptors using the Agilent SureSelect Library Prep Kit, ILM.

The adaptor-ligated libraries were amplified for five cycles with the SureSelect Primer and the SureSelect Indexing Pre-Capture reverse primer. The PCRs were cleaned using the Agencourt AMPure XP beads. To capture exonic regions, 500 ng of each prepared library was hybridized to biotinylated cRNA oligonucleotides for 24 h at 65°C. The captured libraries were pulled down using Dynabeads MyOne Streptavidin T1 (Invitrogen). A post-capture PCR was then performed to amplify the captured libraries and to add the barcode sequences for multiplex sequencing for 14 cycles. Afterward, amplified libraries were purified with AMPure XP Beads. Qubit fluorometer and Bioanalyzer high-sensitivity chips were used to determine the final concentration of each captured library. One library was prepared per sample. Libraries were pooled in three and were paired-end sequenced on a single Illumina HiSeq lane at the Stanford Center for Genomics and Personalized Medicine according to standard protocols.
Bioinformatics Analyses
Raw FASTQ files were aligned to the human genome (hg19 version), and SNPs and indels were called using the BINA pipeline (http://www.bina.com). For variant filtering, Golden Helix VarSeq software (http://goldenhelix.com/products/VarSeq/) was used.

Sanger Sequencing
We used 5’GGCCTGGAGAAGTGTCAGAT3’ and 5GTCTCGTACCTAGGCCTGTC3’ for PCR amplification of the variant sequence. PCR amplification was performed using following reagents: 25 µl REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 1 µl DNA (50 ng/µl), and 22 µl of water per PCR reaction. An initial denaturation step for 3 min at 94° was followed by 35 cycles of 30 sec at 94°, 30 sec at 57°, 30 sec at 72°, and the process completed by a final extension for 7 min at 72°. The PCR amplification resulted in a single DNA band on a standard 1% agarose gel and was purified by Agencourt AMPure XP beads (Beckman Coulter, Inc) before submitting for Sanger sequencing. The reverse primer 5’GTCTCGTACCTAGGCCTGTC3’ was used as sequencing primer. Sanger sequencing was carried out by the Stanford PAN facility using ABI 3130xl Genetic Analyzer.

ADDITIONAL INFORMATION

Data Deposition and Access
The family consented to the genetic study and publication of the genetic and clinical results. The exome-sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra/) under SRA Study SRP106899. The variant was submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number SCV000607728.

Ethics Statement
All participants, or their legal guardian, provided written and informed consent. The institutional review boards of the Special Medical Center, Tehran, Iran and Stanford University reviewed the project. All the affected individuals underwent examination at the Special Medical Center, Tehran, Iran.

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REFERENCES
Bhavani GS, Shah H, Dalal AB, Shukla A, Danda S, Aggarwal S, Phadke SR, Gupta N, Kabra M, Gowrishankar K, et al. 2015. Novel and recurrent mutations in WISP3 and an atypical phenotype. Am J Med Genet A 167A: 2481–2484.
Bork P. 1993. The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett 327: 125–130.

Chen C, Jiang Y, Xu C, Liu X, Hu L, Xiang Y, Chen Q, Chen D, Li H, Xu X, et al. 2016. Skeleton Genetics: a comprehensive database for genes and mutations related to genetic skeletal disorders. Database (Oxford) 2016: baw127.

Dalal A, Bhavani GS, Togarotti PP, Bierhals T, Nandineni MR, Danda S, Danda D, Shah H, Vijayan S, Gowrishankar K, et al. 2017. Analysis of the WISP3 gene in Indian families with progressive pseudorheumatoid dysplasia. Am J Med Genet A 158A: 2820–2828.

Delague V, Chouery E, Corbani S, Ghanem I, Aamr S, Fischer J, Levy-Lahad E, Urtizberea JA, Megarbane A. 2005. Molecular study of WISP3 in nine families originating from the Middle-East and presenting with progressive pseudorheumatoid dysplasia: identification of two novel mutations, and description of a founder effect. Am J Med Genet A 138A: 118–126.

Ehl S, Uhl M, Berner R, Bonaře L, Superti-Furga A, Kirchhoff A. 2004. Clinical, radiographic, and genetic diagnosis of progressive pseudorheumatoid dysplasia in a patient with severe polyarthropathy. RheumatolInt 24: 53–56.

Ekbote AV, Danda D, Kumar S, Danda S, Madhuni V, Gibikote S. 2013. A descriptive analysis of 14 cases of progressive pseudorheumatoid-arthropathy of childhood from south India: review of literature in comparison with juvenile idiopathic arthritis. Semin Arthritis Rheum 42: 582–589.

el-Shanti H, Murray JC, Semina EV, Beutow KH, Scherpbier T, al-Alami J. 1998. Assignment of gene responsible for progressive pseudorheumatoid dysplasia to Chromosome 6 and examination of COL10A1 as candidate gene. Eur J Hum Genet 6: 251–256.

Fischer J, Urtizberea JA, Pavelk S, Vandiedonck C, Brels T, Saker S, Alkatip Y, Prudhommeau D, Scherpbier T, al-Alami J. 1998. Genetic linkage of progressive pseudorheumatoid dysplasia to a 3-cM interval of Chromosome 6. Hum Genet 103: 60–64.

Garcia Segarra N, Mittaz L, Campos-Xavier AB, Bartels CF, Tuysuz B, Alaray E, Cimaz R, Cormier-Daire V, Di Rocco M, Duba HC, et al. 2012. The diagnostic challenge of progressive pseudorheumatoid dysplasia (PPRD): a review of clinical features, radiographic features, and WISP3 mutations in 63 affected individuals. Am J Med Genet C Semin Med Genet 160C: 217–229.

Holbourn KP, Acharya KR, Perbal B. 2008. The CCN family of proteins: structure-function relationships. Trends Biochem Sci 33: 461–473.

Hosaka Y, Saito T, Sugita S, Ikikata T, Kobayashi H, Fukai A, Taniguchi Y, Hirota M, Akiyama H, Chung U, et al. 2013. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. Proc Natl Acad Sci USA 110: 1875–1880.

Huang W, Martin EE, Burman B, Gonzalez ME, Kleer CG. 2016. The matricellular protein CCN6 (WISP3) decreases Notch1 and suppresses breast cancer initiating cells. Oncotarget 7: 25180–25193.

Hurvitz JR, Suwairi WM, Van Hul EV, El-Shanti H, Superti-Furga A, Roudier J, Holderbaum D, Pauli RM, Herd JK, Van Hul EV, et al. 1999. Mutations in the CCN family gene family member WISP3 cause progressive pseudorheumatoid dysplasia. Nat Genet 23: 94–98.

Isaacs NW. 1995. Cystine knots. Curr Opin Struct Biol 5: 391–395.

Kornak U, Mundlos S. 2003. Genetic disorders of the skeleton: a developmental approach. Am J Hum Genet 73: 447–474.

Krakow D, Rimoin DL. 2010. The skeletal dysplasias. Genet Med 12: 327–341.

Liao EY, Peng YQ, Zhou HD, Mackie EJ, Li J, Hu PA, Zhou SH, Won GB, Zhai MX, Luo GB, et al. 2004. Gene symbol: WISP3. Disease: spondyloepiphyseal dysplasia tarda with progressive arthropathy. Hum Genet 115: 169.

Liu L, Li N, Zhao Z, Li W, Xia W. 2015. Novel WISP3 mutations causing spondyloepiphyseal dysplasia tarda with progressive arthropathy in two unrelated Chinese families. Joint Bone Spine 82: 125–128.

Luo H, Shi C, Mao C, Jiang C, Bao D, Guo J, Du P, Wang Y, Liu Y, Liu X, et al. 2015. A novel compound WISP3 mutation in a Chinese family with progressive pseudorheumatoid dysplasia. Gene 564: 35–38.

Madhuni V, Santanathan M, Rajagopal K, Sugumar LR, Kalaji V. 2016. WISP3 mutational analysis in Indian patients diagnosed with progressive pseudorheumatoid dysplasia and report of a novel mutation at pY198. Bone Joint Res 5: 301–306.

Maeda A, Nishida T, Aoyama E, Kubota S, Lyons KM, Kuboki T, Takigawa M. 2009. CCN family 2/connexin-27 and connective tissue growth factor modulates BMP signalling as a signal conductor, which action regulates the proliferation and differentiation of chondrocytes. J Biochem 145: 207–216.

Montane LS, Marin OR, Rivera-Pedroza CI, Vallespin E, Del Pozo A, Heath KE. 2016. Early severe scoliosis in a patient with atypical progressive pseudorheumatoid dysplasia (PPD): identification of two WISP3 mutations, one previously unreported. Am J Med Genet A 170: 1595–1599.

Nakamura Y, Weidinger G, Liang JQ, Aquilina-Beck A, Tamai K, Moon RT, Warman ML. 2007. The CCN family member Wisp3, mutant in progressive pseudorheumatoid dysplasia, modulates BMP and Wnt signaling. J Clin Invest 117: 3075–3086.
Neerinckx B, Thues C, Wouters C, Lechner S, Westhovens R, Van Esch H. 2015. A homozygous deletion of exon 1 in WISP3 causes progressive pseudorheumatoid dysplasia in two siblings. *Hum Genome Var* 2: 15049.

Peng YQ, Liao EY, Gu HM, Wei QY, Zhou HD, Li J, Xie H, Zhai MX, Tan LH, Luo XH, et al. 2004. [Pathology and molecular pathogenesis of spondyloepiphyseal dysplasia tarda with progressive arthropathy caused by compound CCN6 heterogeneous gene mutations]. *Zhonghua Yi Xue Za Zhi* 84: 1796–1803.

Rai E, Mahajan A, Kumar P, Angural A, Dhar MK, Razdan S, Thangaraj K, Wise CA, Ikegawa S, Pandita KK, et al. 2016. Whole exome screening identifies novel and recurrent WISP3 mutations causing progressive pseudorheumatoid dysplasia in Jammu and Kashmir-India. *Sci Rep* 6: 27684.

Repubi SR, Patra M, Sen M. 2013. WISP3–IGF1 interaction regulates chondrocyte hypertrophy. *J Cell Sci* 126: 1650–1658.

Reza Sailani M, Jahanbani F, Nasiri J, Behnam M, Salehi M, Sedghi M, Hoseinzadeh M, Takahashi S, Zia A, Gruber J, et al. 2017. Association of AHSG with alopecia and mental retardation (APMR) syndrome. *Hum Genet* 136: 287–296.

Spranger J, Albert C, Schilling F, Bartsocas C, Stoss H. 1983. Progressive pseudorheumatoid arthritis of childhood (PPAC). A hereditary disorder simulating rheumatoid arthritis. *Eur J Pediatr* 140: 34–40.

Sun J, Xia W, He S, Zhao Z, Nie M, Li M, Jiang Y, Xing X, Wang O, Meng X, et al. 2012. Novel and recurrent mutations of WISP3 in two Chinese families with progressive pseudorheumatoid dysplasia. *PLoS One* 7: e38643.

Temiz F, Ozbek MN, Kotan D, Sangun O, Mungan NO, Yuksel B, Topaloglu AK. 2011. A homozygous recurring mutation in WISP3 causing progressive pseudorheumatoid arthropathy. *J Pediatr Endocrinol Metab* 24: 105–108.

Wynne-Davies R, Hall C, Ansell BM. 1982. Spondylo-epiphysial dysplasia tarda with progressive arthropathy. A “new” disorder of autosomal recessive inheritance. *J Bone Joint Surg Br* 64: 442–445.

Yan W, Dai J, Xu Z, Shi D, Chen D, Xu X, Song K, Yao Y, Li L, Ikegawa S, et al. 2016. Novel WISP3 mutations causing progressive pseudorheumatoid dysplasia in two Chinese families. *Hum Genome Var* 3: 16041.

Yang X, Song Y, Kong Q. 2013. Diagnosis and surgical treatment of progressive pseudorheumatoid dysplasia in an adult with severe spinal disorders and polyarthropathy. *Joint Bone Spine* 80: 650–652.

Ye J, Zhang HW, Wang T, Cao LF, Qiu WJ, Han LS, Zhang YF, Gu XF. 2010. Clinical diagnosis and WISP3 gene mutation analysis for progressive pseudorheumatoid dysplasia. *Zhonghua Er Ke Za Zhi* 48: 194–198.

Ye J, Zhang HW, Qiu WJ, Han LS, Zhang YF, Gong ZW, Gu XF. 2012. Patients with progressive pseudorheumatoid dysplasia: from clinical diagnosis to molecular studies. *Mol Med Rep* 5: 190–195.

Yu Y, Hu M, Xing X, Li F, Song Y, Luo Y, Ma H. 2015. Identification of a mutation in the WISP3 gene in three unrelated families with progressive pseudorheumatoid dysplasia. *Mol Med Rep* 12: 419–425.

Yue H, Zhang ZL, He JW. 2009. Identification of novel mutations in WISP3 gene in two unrelated Chinese families with progressive pseudorheumatoid dysplasia. *Bone* 44: 547–554.