DATA REPORT

Novel *WISP3* mutations causing progressive pseudorheumatoid dysplasia in two Chinese families

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Progressive pseudorheumatoid dysplasia (PPD) is a rare disease caused by mutations in the gene for Wnt1-inducible signaling pathway protein 3 (*WISP3*). Here, we report the clinical and radiographic manifestations of two Chinese PPD patients. We performed whole-exome sequencing for one patient and sequenced the *WISP3* for the other. Three *WISP3* mutations (c.396T > G, c.721T > G and c.679dup) were identified; the two missense mutations were novel. Our study expanded the *WISP3* mutation spectrum.

Human Genome Variation (2016) **3**, 16041; doi:10.1038/hgv.2016.41; published online 8 December 2016

Progressive pseudorheumatoid dysplasia (PPD; MIM 208230) is an autosomal recessive skeletal dysplasia characterized by the predominant involvement of articular cartilage with progressive stiffness and enlargement of joints in the absence of inflammation. PPD shares similar skeletal manifestations with mucopolysaccharidosis, rheumatoid arthritis and ankylosing spondylitis.[1,2] Early development of PPD in patients is normal; however, joint contractures develop in the hands in early childhood and spread to the knees, hips, other large joints, and the spine. Laboratory assessments for rheumatoid factor and inflammation are always negative. A lack of response to anti-rheumatic drugs is characteristic.[3] Adult patients are generally severely handicapped due to sustained cartilage loss and pernicious skeletal changes.

PPD is caused by Wnt1-inducible signaling protein 3 (*WISP3*) mutations. *WISP3*, located on chromosome 6q22, encodes a connective tissue growth factor involved in cell growth and differentiation.[4–6] The expression of *WISP3* is vital for human cartilage homeostasis and bone growth, and participates in controlling aggrecan and the cartilage-specific protein collagen II in chondrocytes.[7,8] To date, 55 *WISP3* mutations have been reported globally, 12 of which were from Chinese patients. In this report, we present the clinical manifestations and radiographic features of two unrelated Chinese PPD patients in whom we found two novel and one recurrent *WISP3* mutations.

All participants provided informed consent. The study was approved by the Ethics Committee, Drum Tower Hospital, Nanjing, China and was in accordance with the principles of the Helsinki Declaration.

Patient 1 was a 27-year-old female who presented with multiple joint swelling and hip pain. Her birth history was unremarkable. There was no family history of PPD or other skeletal diseases. The joint swelling began at the age of 12 in the proximal interphalangeal joints and then developed in the elbows and knees without distinct pain or limited motion in the joints. By the age of 14, she noticed limb paresis with an unstable gait. She had difficulty walking, occasional joint pain, and increasing stiffness and swelling of the limb joints and spine. She underwent bilateral total hip joint replacement at our center because she lost her mobility completely after a fall at age 20. On physical examination, she had normal intelligence and face. Her height was 150 cm (−2.8 s.d.). A short trunk without kyphosis, flexural elbows and a limited range of movement in the wrists were noted. She suffered from progressive joint pain, especially in the back. Laboratory examinations, including assessments of inflammation markers and rheumatoid factor, were within the normal range.

Her X-ray imaging revealed defective ossification of the anterior portions of the upper and lower end plates and slightly flattened thoracic vertebrae (Figure 1a,b). There was a mild S-shaped deformity of the tibiae without other abnormalities. Narrow joint spaces, irregular densities and cyst-like structures in the femoral head and neck indicated marked precocious osteoarthrosis (Figure 1c). The articular surfaces of the knee were flat and irregular, and the joint space had disappeared (Figure 1d). The ends of the short tubular bones were broad, which was most pronounced in the distal ends of the proximal phalanges. The joint spaces of the carpal bones were narrow (Figure 1e). The interphalangeal joints appeared swollen (Supplementary Figure S1a).

Patient 2 was a 17-year-old male referred to us because of short stature. His parents were cousins and were healthy with no evidence of arthritis. His limb muscle weakness and hip pain after walking for long distances began at the age of 12. Anti-inflammatory treatment was proposed, which resulted in no improvement, and systemic inflammation was excluded. Physical examination at 17 showed a height of 150 cm (−3.8 s.d.). Multiple joint contracture, short trunk, thoracic kyphosis and swollen interphalangeal joints were noted (Supplementary Figure S1b–d). Laboratory examinations were within the normal range.

Radiological findings indicated decreased heights of the vertebrae (Figure 1f), lessened joint spaces in the hips with heavy

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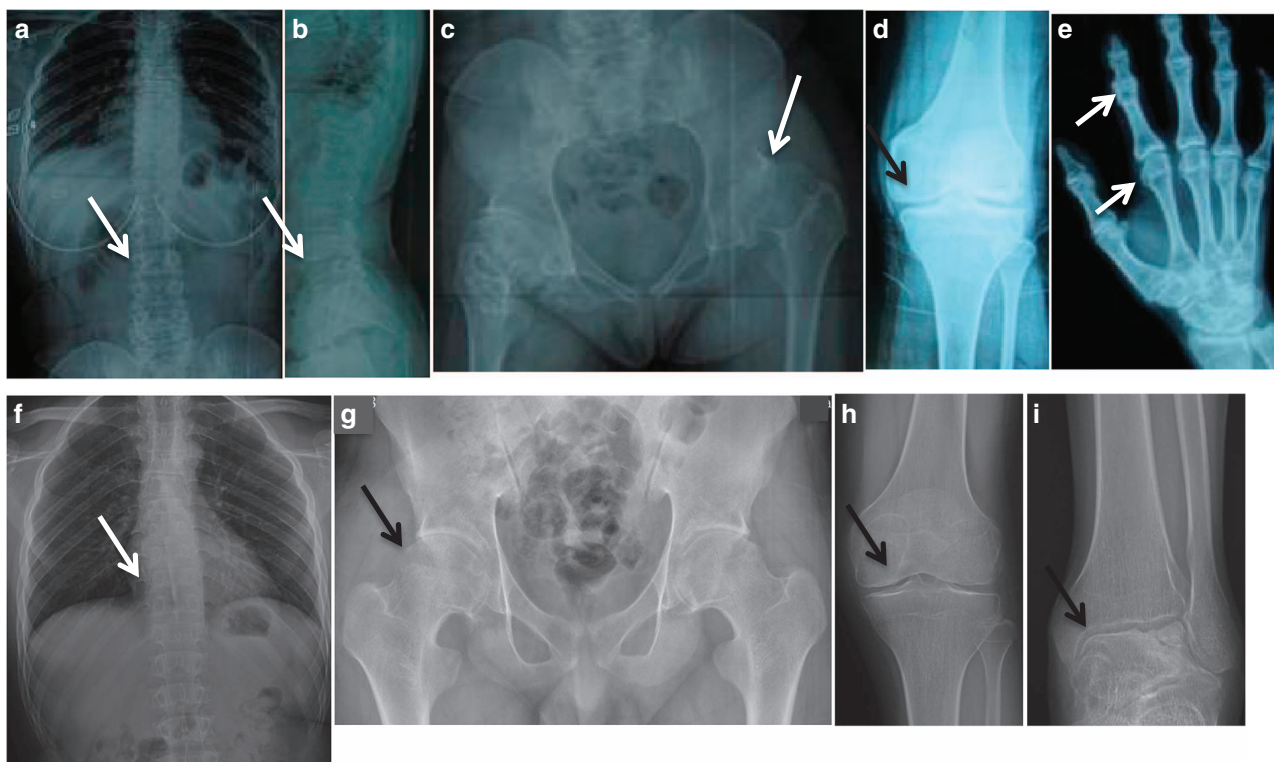
Figure 1. Radiographic features of the two patients with progressive pseudorheumatoid dysplasia. (**a–e**) Patient 1 at age 15. (**f–i**) Patient 2 at age 17. (**a**, **b**) Thoracolumbar spine. Platyspondyly with anterior breaking of the vertebral bodies. (**c**) Pelvis. Narrow joint spaces and subluxation of the hip joints and irregular densities and cyst-like structures in the femoral head and neck. (**d**) Knee. The articular surface was flat and irregular, and the joint space was narrow. (**e**) Hand. Enlargement of the interphalangeal and metacarpal epiphyses. (**f**) Chest. Decreased heights of the vertebrae. (**g–i**) Large joints of the lower extremities showed narrow joint spaces.

cartilage loss, large femoral epiphyses and enlargement of the metaphysis of the interphalangeal joints (Figure 1g–i).

In retrospect, the radiographic findings of the two patients were quite consistent with previous reports of PPD;[9–11] however, because of the closure of the growth plates and secondary changes related to aging, a definitive diagnosis was difficult. Therefore, we performed molecular analysis of the patients. We extracted genomic DNA from the peripheral blood using a QIAGEN DNA minikit (Qiagen, Hilden, Germany).

Due to the difficulty in diagnosis, we conducted whole-exome sequencing in Patient 1. In brief, genomic DNA was divided into smaller fragments of 200–250 bp with an ultrasonic instrument (Covaris LE220, Woburn, MA, USA). Ampure Beads (Beckman Coulter, Brea, CA, USA) purification was used to add poly A through a joint reaction at the end of the purified DNA fragments. Agene-trapping chip (Roche NimbleGen, Madison, WI, USA) was used to hybridize and capture the DNA fragments. After hybridization, the captured DNA was sequenced on Illumina HiSeq2500 Analyzers (Illumina, SanDiego, CA, USA) and read on quality analysis pipeline (PIQA).[12] We used BWA v0.59[13] to align the sequence reads to the human genome reference (build 37) and removed duplicated reads from the subsequent analyses. Sequences variants were identified by comparison with the NCBI reference sequence (NM 198239.1) and annotated by ANNOVAR (http://www.openbioinformatics.org/annovar). The $20 \times$ coverage for the RefSeq coding region was 98.13% (Supplementary Figure S2).

Because we noticed the same symptoms in patient 1, we examined patient 2 by direct sequencing for *WISP3* instead of whole-exome sequencing. The entire coding region of *WISP3* was sequenced using five pairs of primers (the sequences are available on request). Sanger sequencing was then performed in the proband's family members.

Patient 1 had two novel missense mutations, NM_198239.1 (WISP3_v001):c.396T > G [p.(Cys132Trp)] and NM_198239.1 (WISP3_v001): c.721T > G [p.(Cys241Gly)] (Figure 2a). Both mutations had not been previously reported and were not found in sequencing data projects (Exome Aggregation Consortium Exome; http://exac.broadinstitute.org/). Patient 2 had ahomozygous frameshift mutation, NM_198239.1 (WISP3_v001): c.679dup [p.(Cys227Leufs*21)] (Figure 2b), which has previously been described in two PPD individuals.[14] One adenine-ribonucleotide duplication altered the reading frame downstream of codon 227 and caused a premature stop signal (base sequence T-G-A) at codon 247(C227fs*21). SIFT and Polyphen-2[15] both predicted these mutations to cause severe damage to the *WISP3* protein function.

We performed Sanger sequencing for the two families and confirmed the mutations in the patients and their siblings (Figure 2a,b). In patient 1, the maternal mutation c.396T > G [p.(C132W)] and the paternal mutation c.721T > G [p.(C241G)] were highly conserved among diverse species (Figure 2c).

Thus, we have found two novel *WISP3* mutations in cases of PPD. Our study performed the whole-exome sequencing, which allowed us to obtain molecular results faster, especially when traditional specific diagnosis takes a longer time due to sequencing a large gene. The exome allows for the possibility of analyzing the first candidate gene and checking the presence of mutations in other genes at the same time.[16,17] Our study has further expanded the *WISP3* mutation spectrum and thus contributed to the earlier detection of this rare disease, providing significant benefits to patients' families and greater awareness of the growing amount of PPD patients for future clinical diagnosis.
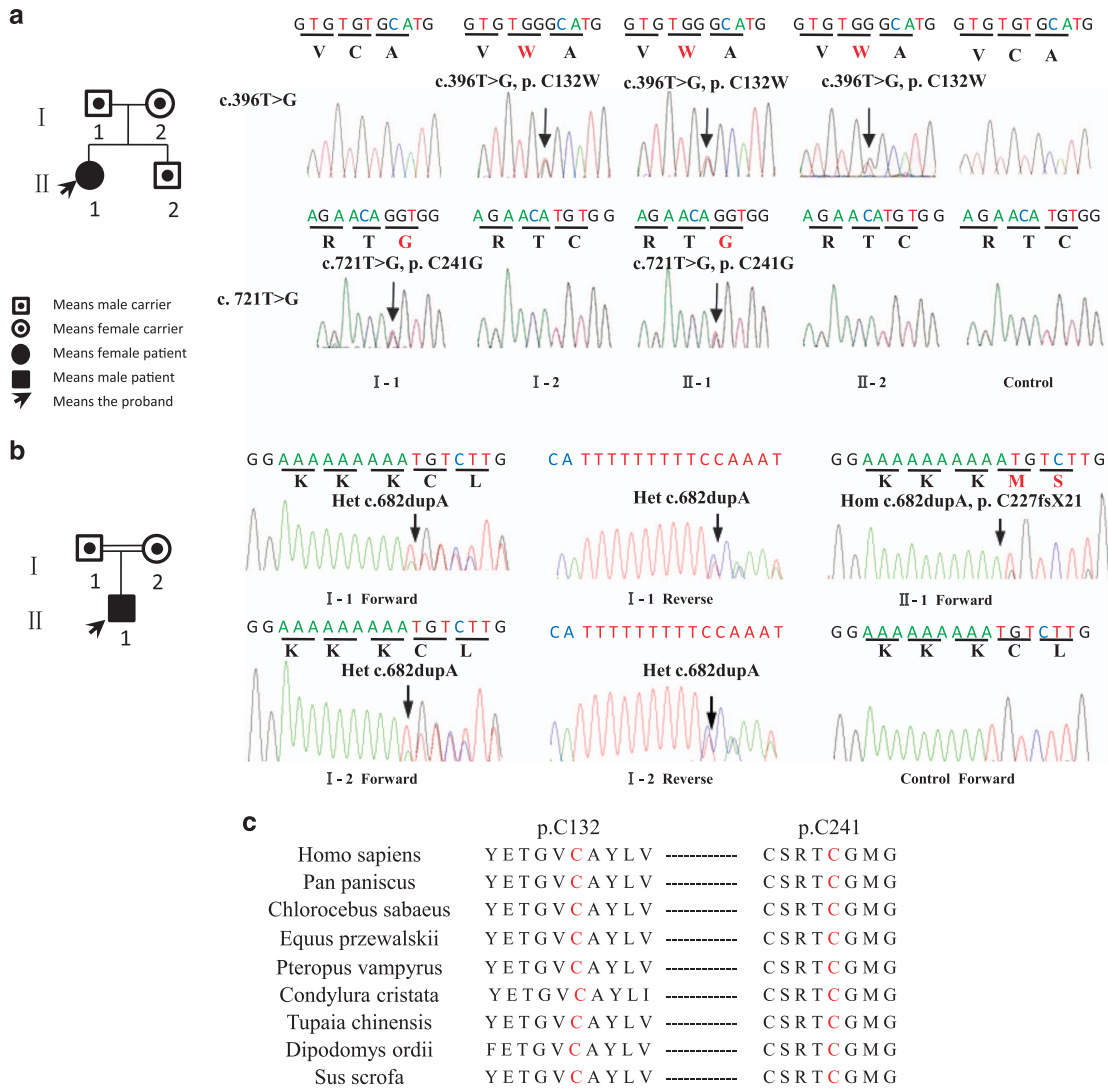
Figure 2. *WISP3* mutations in the patients with progressive pseudorheumatoid dysplasia. (**a**) Family of patient 1. Patient 1 was a compound heterozygote of the mutationsc.396T > G [p.(C132W)]and c.721T > G [p.(C241G)]. Mother of patient 1 was confirmed to have one mutation (c.396T > G of *WISP*), whereas father and brother of patient 1 were confirmed to have the other mutation (c.721T > G of *WISP*). This suggested that the two mutations in patient 1 were compound. Neither of the two mutations was present in the 50 controls. (**b**) Family of patient 2. Patient 2 was a homozygote of the mutation c.679dup [p.(C227Lfs*21)]. The frameshift mutation of the carrier parents needs to be read in theelectropherogram on both the forward and reverse sides. (**c**) Amino acid alignments in different species around the missense mutation. p.C132and p.C241 are highly evolutionarily conserved.

COMPETING INTERESTS

The authors declare no conflict of interest.

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Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv)