The Egyptian Journal of Medical Human Genetics 18 (2017) 299–303

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Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

Case Report

Progressive pseudorheumatoid dysplasia in North and West Africa: Clinical description in ten patients with mutations of *WISP3*

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ARTICLE INFO

Article history:
Received 31 October 2016
Accepted 27 November 2016
Available online 5 January 2017

Keywords:
Bone
Dysplasia
Autosomal recessive
Mutation
WISP3
Consanguinity

ABSTRACT

Progressive pseudorheumatoid dysplasia is a rare autosomal recessive spondyloepiphyseal dysplasia characterized by predominant involvement of articular cartilage with progressive joint stiffness and enlargement in the absence of inflammation. Short stature, joint contractures, gait disturbance, and scoliosis and/or kyphosis, resulting in abnormal posture and significant morbidity are generally seen over time. This condition is caused by mutations of *WISP3*, a gene located on chromosome 6q21.

Ten patients pertaining to 3 families originated from Tunisia, Morocco and Senegal, with progressive pseudorheumatoid dysplasia are described. Three exhibited marked muscle weakness resulting in delayed diagnosis. Genetic studies of *WISP3* in these three consanguineous kindred identified two mutations, each at the homozygous state: c.624_625insA (p.C209Mfs*21), also named c.624dupA, in exon 4, in the Tunisian and Senegalese patients, and c.48+2dupT, a splice donor site mutation in intron 1, in the Moroccan patients.

The clinical features and the molecular studies of the *WISP3* gene are discussed.

1. Introduction

Progressive pseudorheumatoid dysplasia (PPRD), also referred to as spondyloepiphyseal dysplasia tarda with progressive arthropathy or Progressive pseudorheumatoid arthropathy of childhood is a rare autosomal recessive skeletal disorder (OMIM 208230). It is characterized by an abnormal cartilage homeostasis responsible for an axial and peripheral skeletal dysplasia, associated with pain, stiffness, and swelling of multiple joints mainly wrists, fingers, hips, and knees. Walking difficulties often appear, due to muscle weakness and flexion contractures and stiffness of the hip and knee joints. The age of onset is in early childhood, with most affected children presenting with disease features by the age of 8-years. Adults with PPRD have usually short stature. The diagnosis is comforted radiologically: vertebral bodies are flattened with anterior ossification defects; the acetabular portion of the pelvis is irregular, the capital femoral epiphyses enlarged, the joint spaces narrowed, and the ends of the proximal and middle phalanges are expanded. The articular space may be narrow, but destructive bone changes and the inflammatory markers characteristic of juvenile rheumatoid arthritis are always lacking. The disorder is caused by loss of function mutations of *WISP3* (Wnt1-inducible signaling pathway protein 3), a gene located on chromosome 6q21 (MIM 603400) [1,2].

WISP3 encodes a 354-amino-acid protein, a member of the CCN (Connective tissue growth factor, Cysteine-rich 61, Nephroblastoma over-expressed) family of connective tissue growth factors, known to be mostly extracellular-matrix associated proteins. WISP3 is essential for maintaining cartilage integrity mainly by regulating the expression of the type II collagen and aggrecan in chondrocytes. WISP3 also is involved in regulation of cell migration and adhesion, cell proliferation, differentiation, and survival in connective tissues [1–3].

Peer review under responsibility of Ain Shams University.
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http://dx.doi.org/10.1016/j.ejmhg.2016.11.004
1110-8630/

We hereby report the clinical features, the radiographic data of bones, and the molecular studies of the *WISP3* gene in consanguineous patients from Tunisia, Morocco and Senegal.

2. Patients and methods

2.1. Ethical statement

This study was carried out with protocols approved by the Ethics Committees of the Monastir-Sousse Hospitals and Universities (Tunisia), the Assistance-Publique Hôpitaux de Paris, France and the Saint-Joseph University of Beirut (Lebanon). Informed consent was obtained from the patients and/or their parents before blood sampling and DNA analyses.

2.2. Family 1

Patients of family 1 consist of 2 Tunisian consanguineous sibs (Fig. 1A), originating from the Centre of Tunisia, now aged 28 and 21, respectively. Early developmental milestones were normal for the affected siblings. Walking difficulties appeared between 13 and 18 years, due to flexion contractures of their hip and knee joints, followed simultaneously, or, a few months later by painful swelling of the interphalangeal joints with limited flexion, increasing stiffness of the limb joints and spine with limited flexion and extension of the knees, wrists, and elbows. Marked muscle weakness, general wasting, and short stature (height below the 3rd percentile) were also noted.

The skeletal survey revealed broad ends of the short tubular bones, especially in proximal phalanges; narrow joint spaces between the carpal bones and the interphalangeal joints, flattened vertebral bodies with rounded end plates, irregular upper and lower plates, short pedicles, wide epiphyses of the knees, short and wide femoral neck, large capital femoral epiphyses, flat and irregular articular surfaces of the knees, and narrow joint spaces (Fig. 2). Hips replacement was performed for both sibs when they were 21 and 22 years.

2.3. Family 2

Family 2 (Fig. 1B), is originated from Marakech Morocco. Parents are second-degree cousins and are clinically healthy. The elder affected sister developed difficulties in walking associated with lower limb deformities at the age of 3. An initial diagnosis of rickets was made but without any improvement after prolonged vitamin D supplementation. Subsequently, joint contractures appeared gradually in the hips, knees and fingers. Later on, marked generalized muscle weakness appeared. Although CK levels were normal, a neuro-muscular disorder was suspected and a biopsy of the deltoid muscle performed. Results of the latter were unconclusive. Motor performance kept declining, and she became wheel-chair-bound at age 13. Her younger sister, now aged 12, had a similar clinical history but presented with additional, most probably fortuitous, mild intellectual disability.

Both patients were only able to sit unsupported, had short stature (135 cm and 130 cm; largely below the 3rd percentile) and presented with multiple joint deformities, particularly in the fingers. Interestingly, neither of them ever complained of any specific pain in any joints. Radiological examination revealed enlarged epiphyses in the fingers, elbows, and knees; platyspondyly, scoliosis, irregularities and destruction of the acetabula, and deformed femoral heads.

2.4. Family 3

Six consanguineous siblings, ranging from 6 to 21 years of age, originating from Senegal, West Africa, were investigated (Fig. 1C). The eldest affected child achieved normal motor milestones and started complaining of difficulties in walking together with painful ankles, hips and shoulders when he was 10 years old. In parallel, he gradually developed mild muscle wasting in the distal part of the lower limbs. Muscle weakness extended in the upper limbs over the subsequent three years. Waddling gait and mild scapular winging were noted on examination. Electrophysiological studies performed at age 15 revealed minor changes consistent with a chronic neurogenic process. A muscle and nerve biopsy done shortly afterwards turned out to be inconclusive.

On examination, the decline in motor functional abilities contrasted with the preservation of muscle strength and the absence of sensory impairment. The patient continued complaining of occasional pains in hips and knees, but never exhibited general symptoms such as fever or skin rashes. He was primarily considered to have an axonal form of Charcot-Marie-Tooth disease. A closer clinical re-evaluation at age 16 showed the presence of stiff joints in the shoulders, elbows, hips and wrists whereas fingers and knees appeared normal. No growth retardation was noted. Height was at the 35th percentile. In contrast, the radiological evaluation was very suggestive of a severe spondyloepiphyseal dysplasia with marked diffuse osteoporosis, platyspondyly, and moderate enlargement of epiphyses associated with mild narrowing of the articular space in both hips. The course of the disease has been slowly deteriorating since then, with enlargement of finger joints, marked waddling gait and decline in motor abilities at age 19. The other siblings had similar symptoms with some degree of intra-familial variability whereas both parents were totally healthy.

2.5. Molecular studies

Given that clinical presentation and radiological abnormalities were suggestive of PPRD, molecular studies of the *WISP3* gene were undertaken. EDTA blood samples were collected for genetic studies and DNA was extracted from lymphocytes by standard methods [4].

The coding sequence of *WISP3* (GenBank Accession Numbers: NM003880 and NM198239) gene was sequenced in all families members, after their DNA was amplified by PCR (GeneAmp PCR system 9700 from ThermoFisher). Primers sequences were designed as previously described in Delague et al. [5], and checked for specificity using BLAST (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi), DNA sequences were obtained from UCSC or Genbank databases. PCR reactions were performed using Taq DNA polymerase (Invitrogen Life technologies, Carlsbad, CA, USA). The amplification conditions for each PCR were 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, the specific annealing temperature for 30 s, and 72 °C for 30 s, with a final extension of 10 min at 72 °C.

PCR products from genomic DNA were purified using the GenElute PCR clean-up kit (Sigma-Aldrich, USA), and both strands of the resultant products were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) under standard conditions. The labelled products were subjected to electrophoresis on an ABI 3500 Genetic Analyzer sequencing system Kit (Applied Biosystems, Foster City, CA, USA). Electropherograms were analysed using Sequence Analysis Software version 5.2 (Applied Biosystems, Foster City, CA) and compared to reference sequences using ChromasPro version 1.7.5.4 (Technelysium, Queensland, Australia).

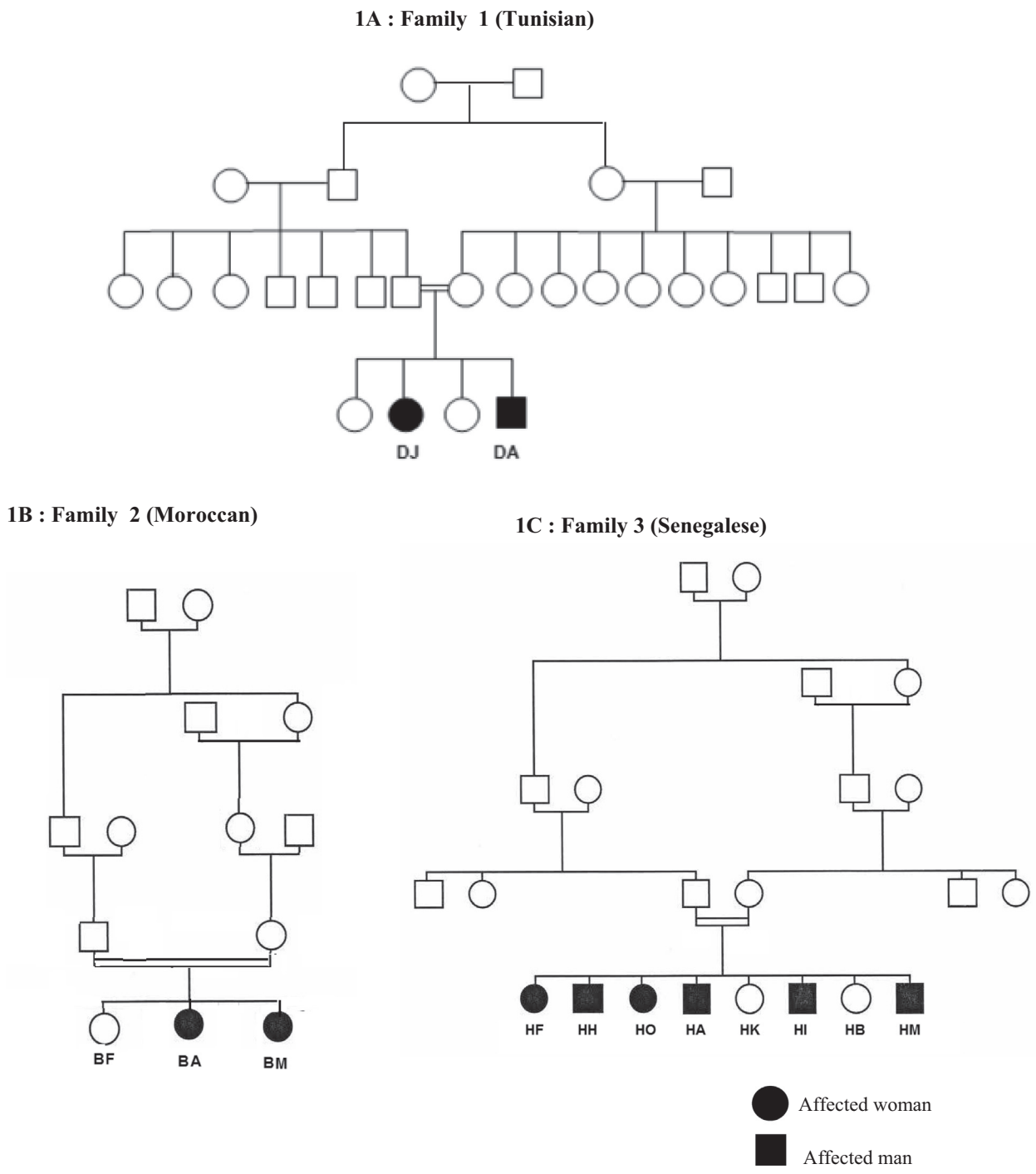

Figure 1. Pedigree of the 3 families.

2.6. Molecular findings

The exploration of the entire coding sequence of *WISP3* allowed the identification of the causative mutations in the patients. Patients of family 1 (Tunisian family), were found homozygous for an insert mutation c.624_625insA (p.C209Mfs*21), also named c.624dupA, in exon 4 of *WISP3*. This frameshift mutation leads to the substitution of the Cytosine at position 209 in a Methionine creating a new reading frame which introduces a premature stop codon 21 residues downstream. The Senegalese siblings (Family 3) were homozygous for the same mutation. The consanguineous Moroccan siblings (Family 2) were homozygous for the splicing mutation c.48+2dupT in intron 1.

3. Discussion

Here, we report 3 families that fulfilled the clinical and radiological criteria of PPRD. The latter has been estimated to occur in approximately 1 per million people in European countries but seems much more common in Turkey and the Middle East, although its prevalence in these regions is unknown (5). The condition in all regions is likely to be underdiagnosed because it is often misdiagnosed as juvenile rheumatoid arthritis (JRA). PPRD

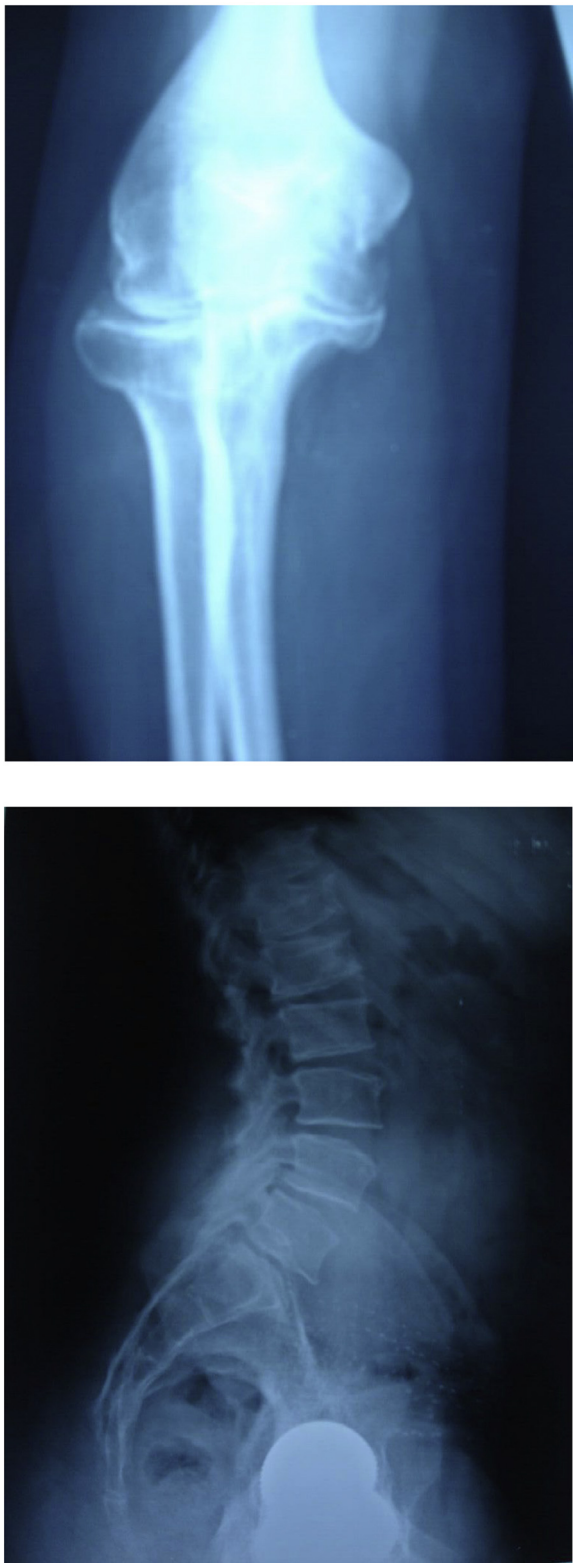

Figure 2. X-ray showing (A) wide epiphyses of the knee with flat and irregular articular surfaces, and narrow joint spaces, and (B) the flattened vertebral bodies with rounded end plates, irregular upper and lower plates, and short pedicles.

is clearly differentiated from JRA by absence of inflammation, extra-skeletal manifestations and articular bone erosion, and different radiological features. In PPRD there is no improvement with steroids and immune-suppressive drugs.

Often noted in some PPRD patients and in all the present cases, is the muscle weakness, precipitating loss of ambulation, and leading to diagnostic mistakes. The muscular wasting might be explained by accumulation of reactive oxygen species. Indeed, it has been showed that WISP3 functions as a ligand and has a potential role in regulating the superoxide dismutase gene expression [6] and the levels of reactive oxygen species (ROS) [7]. It is considered that disturbed expression of ROS contributes, in part, to the pathology of many muscular dystrophies [8]. Thus, in PPRD, the muscle weakness, due to abnormal levels of ROS, would be a direct consequence of the mutations of *WISP3*.

The molecular diagnosis and the search of the mutations of *WISP3* allow to avoid clearly diagnosis mistakes and to confirm without ambiguity a PPRD. The c.48+2dupT splicing mutation found at the homozygous state in the Moroccan family has already been reported in Jordanian patients [1,9,10], raising the question of a possible founder effect owing to the Moslem conquests from the Arabian Peninsula to the North Africa from the seventh century.

The mutation c.624_625insA (p.C209Mfs*21), found in the Tunisian and Senegalese patients has been described in only four patients at the compound heterozygous state: two were Chinese [11], one from the USA [12] and the fourth from Poland [12]. The premature stop codon, carried by our patients at the homozygous state, generates a short truncated and not functional WISP3 protein, 230 amino-acids long, instead of 354. The parents inhabit neighbouring villages and the historical data on both the families support the hypothesis that the mutation would have segregated in the two families from a common ancestor who lived in the late fourteenth century in the region of Moknine and Sayada (Centre of Tunisia, near the Mediterranean sea) [13].

In conclusions, we have reported the clinical and molecular study of patients with PPRD from Tunisia, Morocco and, for the first time, Senegal. The identification of the mutations in *WISP3* gene, as well as of a possible founder effect, presents a major interest in these consanguineous families in order to put in place usefully the genetic counselling and, if feasible, the prenatal diagnosis.

Acknowledgements

We are thankful to Dr. Christine Thémar-Noel (CHU Robert-Debré, Paris) and Dr. Louis Viollet (Salt Lake City, USA) for having kindly referred the families from Morocco and Senegal.

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