A genotyping assay for missense mutation in \textit{WISP3} gene associated with childhood onset pseudorheumatoid arthropathy

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\textbf{ABSTRACT}

\textbf{Introduction:} Progressive pseudorheumatoid dysplasia (PPD) is an autosomal recessive genetic disorder reported to be caused by gene alterations of the Wnt1-inducible signaling pathway protein 3 corresponding gene (\textit{WISP3}) located on chromosome position 6q22. Up to date, there is only a handful of \textit{WISP3} mutations identified in Europe, whereas most mutations are identified in Asia and Middle East. According to our knowledge, this is the first report of genetic dissection of \textit{WISP3} associated with spondyloepiphyseal dysplasia tarda from Bosnia and Herzegovina. Based on clinical examination findings (general manifestations, physical examination, characteristics of their bones on X-ray and laboratory results), an index patient was directed to \textit{WISP3} genotyping for confirmation of suspected diagnosis of PPD.

\textbf{Methods:} DNA was extracted from peripheral blood leukocytes. All 5 exons and their exon-intron boundaries of the \textit{WISP3} gene were amplified by polymerase chain reaction (PCR) and sequenced by Sanger method. Segregation analysis was done to confirm the familial carrier status.

\textbf{Results:} A missense mutation (C223G) - homozygous T to G transition at c.667 in exon 4 was identified in index patient. This mutation changed codon CAG to TAG and resulted in a subsequent change of the cysteine to glycine codon. Same mutation was observed in both parents in heterozygous form confirming the familial segregation.

\textbf{Conclusion:} Due to its nature, the identified mutation C223G in exon 4 in \textit{WISP3} gene is the most probably causative for PPD in described patient. Here we describe the PCR based method for genotyping of specific mutation in \textit{WISP3} gene. The identification of this mutation might be a valuable addition to a regional databases on rare genetic variant although a functional analysis should be performed to explain its pathological effect.

\textbf{Keywords:} Progressive pseudorheumatoid dysplasia (PPD); genetic diagnosis; \textit{WISP3} mutation; familial mutation

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\textbf{INTRODUCTION}

\textit{WISP3} gene is a member of the Wnt1 inducible signaling pathway (WISP) protein subfamily, which belongs to the connective tissue growth factor (CTGF) family that is included in various developmental
processes and oncogenesis. WISP3 gene is approximately 18 Mbs proximal to CTGF and 23 Mbs proximal to the human cellular oncogene MYB. This gene spans over 5 exons and encodes a polypeptide consisted of 354 amino acids with molecular weight of approximately 39kD. WISP3 protein has two potential N-linked glycosylation sites and 36 conserved cysteine residues. Normally, WISP3 is predominantly expressed in fetal and adult kidney and testis. Its weaker expression is found in placenta, ovary, prostate and small intestine (1). Besides, it was shown by RT-PCR that WISP3 is expressed in skeletal derived cells such as human synoviocytes, articular cartilage chondrocytes and bone marrow-derived mesenchymal progenitor (2). It is involved in biological processes such as cell to cell signaling, regulation of cell growth, signal transduction. WISP3 has an essential role in normal postnatal skeletal growth and cartilage homeostasis. Mutations on WISP3 gene usually cause skeletal disease known as progressive pseudorheumatoid dysplasia (PPD) or progressive pseudorheumatoid arthropathy of childhood (PPAC) (3,4). It has been observed previously that reduction or loss of function of WISP3 increase the amount of cellular reactive oxygen species (ROS) in human chondrocyte cell line (5). So far it is known that WISP3 promotes superoxide dismutase (SOD) expression and activity in chondrocytes (6). SOD acts as an antioxidant that control the accumulation of ROS thus preventing a destructive impact of ROS to proteins, DNA, lipids, and carbohydrates in cell under stress conditions.

The PPD as an autosomal recessive skeletal disorder itself is associated with mutations in WISP3 gene. It is a rare disorder with estimated incidence of one per million in United Kingdom and relatively high incidence in the Middle East and Gulf States (7). PPD is usually under recognized in early childhood since first clinical symptoms onset between three to eight years of age. Clinical and radiographic examinations show that PPD patients have continuous cartilage loss and changes in bone structure. These destructive changes are seen after skeletal development is completed. Unlike some of the prenatal loss of cartilage growth, PPD is post natal destruction of cartilage and homeoeostasis. Firstly, it was hard to distinguish PPD from juvenile rheumatoid arthritis (JRA). It differs from JRA by the absence of arthritic and other inflammatory changes. Also, pharmacological treatment typical for rheumatic disease did not cure PPD.

The main clinical characteristics of PPD can be listed as: Osseous swelling and stiffness of the joints, progressive corruption of joint mobility, muscle weakness, easy fatigability, and deformation of knee (8). First affected parts are hands often followed by changes in knees, hips, spine and other large joints in the progress of disease. Skeletal radiographs can be done in the early stage of the disease but will not capture any destruction (2). As symptoms progress, changes can be observed radiographically. Scoliosis, kyphosis and lordosis may develop (OMIM 208230). Diagnosis of PPD is based on clinical, radiographic examining and genetic testing. Causal treatment is not available. However, physical therapy can prevent demineralization due to lack of mobility (8).

Alterations in WISP3 gene structure as reported up to date result in frameshift, nonsense or non-synonymous mutations and they are usually changes with pathological significance.

The aim of this case study was to develop a reliable method for detection of missense C223G mutation in WISP3 gene.

METHODS

In this report we describe a method for detection of missense mutation in WISP3 gene based on a familial case of mutation with suspected diagnosis of Progressive pseudorheumatoid dysplasia (PPD). The study was approved by Scientific committee of the Institute for genetic engineering and biotechnology, and it was conducted in accordance with Helsinki declaration. An eight years old girl had been conditionally diagnosed because she suffered motor weakness, joint contractures, stiffness, swelling of joints, knee weakening and cartilage loss that was confirmed by X-ray. Blood samples were taken from the patient and her parents as DNA source. Genomic DNA was extracted using salting out procedure described elsewhere (8) followed by a quantitative and qualitative analysis of DNA extracts using UV spectroscopy at 260nm prior to PCR amplification. Specific PCR primers were designed using Primer3 (Table 1) (9).
PCR was performed for initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation (94°C, 30 s), annealing (56°C, 30 s), and extension (72°C, 45 s) with 5 units of Taq polymerase. This was followed by a final extension step at 72°C for 7 min. The products were analyzed on 1% agarose gels stained with ethidium bromide to check expected size and detected with ultraviolet illumination. Subsequently, PCR amplification of all exons was performed. PCR amplification reaction products were checked for efficiency using gel electrophoresis and ethidium bromide staining. and sequenced by Sanger sequencing. A standard sequencing reaction using same forward primer as used for primary PCR reaction (Macrogen Inc, Korea). The electropherograms were visually inspected for correct nucleotide calls and sequences were aligned using BioEdit software.

RESULTS

In order to design specific and sensitive test to detect for familial mutation in WISP3 causing PPAC, we sequenced the WISP3 locus of the index case with suspected PPD (age 8). After PCR amplification quality check up on an agarose gel electrophoresis, Sanger sequence analysis was performed. Electropherograms were visually inspected for correctness of automatically analyzed by Sequencher software (Gene Codes Corp). All exon sequences from a proband have been aligned with a reference sequence (NM_003880.3) taken from the NCBI database. One discrepancy from reference sequence was found that correspond to c.667T or T>G nucleotide substitution in homozygous form. Mutation affects first nucleotide of TGT cysteine codon that encodes for glycine instead of cysteine on position 223 of corresponding amino acid sequence (Figure 1). We also sequenced exon 4 of WISP3 gene in parents of the proband and found that parents are both heterozygous for the missense c.667T>G mutation suggesting the familial character of the described mutation.

DISCUSSION

WISP3 is an essential gene for the long-term integrity of human cartilage. Individuals with mutation in the functional domain of this gene develop severe osteoarthropathy in the context of a mild skeletal dysplasia. WISP3 gene spans over 5 exons and encodes several functional domains each corresponding to one of the exons. Exon 1 encodes a peptide sequence that plays role in Wisp secretion (7,10-12); exon 2 codes for insulin-like growth factor binding proteins (IGFBPs) that contains twelve cysteine residues (7,10,12-15); exon 3 encodes a cysteine rich, von Willebrand factor type C repeat domain (10,11,13,15); exon 4 contains information for a thrombospondin type I domain biosynthesis (with six cysteine residues) that may bind to sulfated glycosaminoglycan’s either at cell surfaces or in extracellular matrix (11,12,15-18); and exon 5 that encodes a cysteine knot domain comprised of ten cysteine residues possibly involved in dimerization and receptor binding (10-12,14-17,19-24). Until now, a number of mutations and polymorphisms were found throughout the WISP3 sequence with geographically localized origin (Table 2). Registered
WISP3 mutations are of frameshift, nonsense or non-synonymous type mutations located in different domains in most cases classified as disease associated mutations (10,21).

It is not definitely shown how the mutations are able to trigger mechanism on molecular level so that it results in deformation of cartilage. It was suggested that WISP3 regulates the expression of type II
collagen and aggrecan. They are essential cartilage specific molecules which take part in cartilage integrity (22). Various authors investigated the effect of Cys78Arg mutation in IGFBP domain in experiments in vitro (23). Mutant WISP3 protein is not capable of raising expression level of type II collagen and aggrecan compared to wild type. It suggests that Cys78Arg mutant WISP3 is unable to pass conformational changes which are essential in regulation of expression type II collagen and aggrecan (22-24). Another group (6) showed that the loss of function and gain of function approaches deepens insight about biological activity of WISP3 in vivo experiments by using zebrafish as a model organism. It was found that overexpression of WISP3 inhibited Bone Morphogenetic Protein (BMP) and WNT signaling in both zebrafish and human by binding directly to BMPs and to the WNT co-receptors LRP6 and frizzled. BMP and WNT signaling are required during skeletal differentiation, growth, and homeostasis and it has been shown that WISP3 modulate both pathways. It was reported that PPD disease-causing mutations in IGFBP, VWC and CT domains (C78R, C145Y, Q338L) have significant effect on WNT signaling function (21). It implies that protein’s WNT inhibitory function requires proper folding of multiple domains. In case of C78R mutation that corresponds to IGFBP domain, mutant WISP3 is still able to inhibit signaling (7,21).

According to available literature mutation c.667T>G is the second documented mutation in exon 4 that leads to missense amino acid substitution exchange. The exon 4 encoded thrombospondin type 1 domain is rich in cysteine suggesting its importance in physiological role of the WISP3.

CONCLUSION

We developed a familial mutation genotyping assay for WISP3 and characterized segregation mutation of WISP3 at exon 4 c.667T>G that causes the autosomal recessive inherited skeletal disorder PPD or PPAC. Although the nature of amino acid substitution cysteine to glycine at exon 4 that encodes domain rich in cysteine residues suggest pathogenic change, additional functional analysis should be performed to confirm the pathophysiological role of the c.667T>G mutation in PPAC.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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