Journal of Health Sciences

SHORT COMMUNICATION Open Access

A genotyping assay for missense mutation in *WISP3* gene associated with childhood onset pseudorheumatoid arthropathy

Lejla Pojskic[1]*, Ismet Gavrankapetanovic[2], Naida Lojo-Kadric[1], Rifat Hadziselimovic[1,3], Kasim Bajrovic[1]

[1]Institute for Genetic Engineering and Biotechnology, University of Sarajevo, Bosnia and Herzegovina, [2]Clinic for Orthopedics and Traumatology, University Clinical Center of Sarajevo, Bosnia and Herzegovina, [3]Faculty of Science, University of Sarajevo, Bosnia and Herzegovina

ABSTRACT

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 (*WISP3*) located on chromosome position 6q22. Up to date, there is only a handful of *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 *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 *WISP3* genotyping for confirmation of suspected diagnosis of PPD.

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

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.

Conclusion: Due to its nature, the identified mutation C223G in exon 4 in 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 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.

Keywords: Progressive pseudorheumatoid dysplasia (PPD); genetic diagnosis; WISP3 mutation; familial mutation

*Corresponding Author: Lejla Pojskic, Laboratory for Human Genetics, Institute for Genetic Engineering and Biotechnology, University of Sarajevo, Zmaja od Bosne 8-Kampus, Bosnia and Herzegovina. Phone: +38733220926, E-mail: lejla.kapurpojskic@ingeb.unsa.ba

Submitted: 08 June 2015 / Accepted: 28 August 2015

DOI: http://dx.doi.org/10.17532/jhsci.2015.241

INTRODUCTION

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

UNIVERSITY OF SARAJEVO
FACULTY OF HEALTH STUDIES

© 2015 Pojskic, *et al.;* licensee University of Sarajevo - Faculty of Health Studies. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 act 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 homoeostasis. 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.

TABLE 1. PCR conditions for detection of mutation in exon 4

Compound	Volume (1X)
10X PCR Buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.4)	1.25
MgCl2	0.625 mM
dNTPs	0.5 mM
Forward and reverse primer for exon 4 (F 5'-CATGGCTTCTTTGGCAATTT-3' R 5'-TCATACCTGTCTGAGGCAAAG-3')	0.25 mM
DNA templates	20.5 ml
Taq polymerase	2 unit

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 1 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

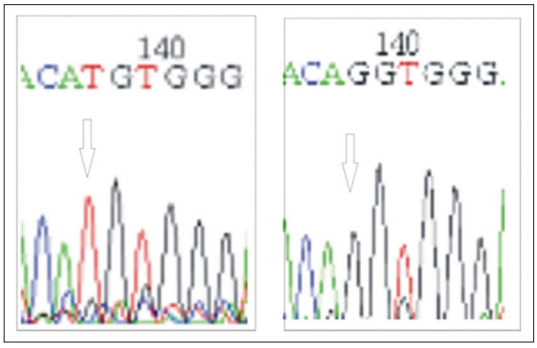

FIGURE 1. Mutation detection in exon 4 of a proband (right) in comparison to wildtype homozygote (left). Thymine on the 667 place of coding sequence is altered to Guanine, which corresponds to TGT to GGT code (p.Cys223Gly)

TABLE 2. Mutations observed previously in various population data sets and their effect on polypeptide chain

Mutations and polymorphisms on WISP3 gene				Reference
Position	DNA change	Protein change	Origin of the patient	
Ex. 1	c. 43delGC	A15fsX	USA	Hurvitz et al. (1999)
Intr. 1	g.IVS1+2insT	Splicing defect	Jordan	
Ex. 1	c. 136C>T	Q46X	China	Yue et al. (2009); Ye et al. (2012); Yu et al. (2015)
Ex. 2	c. 156C>A	C52X	Italy, France, Lebanon, Syria, Turkey, India	Hurvitz et al. (1999); Delague et al. (2005); Temiz et al. (2011); Dalal et al. (2012)
Ex. 2	c. 232T>C	C78R	France	Hurvitz et al. (1999)
Ex. 2	c. 233G>A	C78Y	India	Dalal et al. (2012)
Ex. 2	c. 246delA	P82fsX104	Saudi Arabia, Jordan	Hurvitz et al. (1999)
Ex. 2	c. 248G>A	G83E	Lebanon, Syria, India	Delague et al. (2005); Dalal et al. (2012)
Ex. 2	c. 340C>T	C114R	India	Dalal et al. (2012)
Ex. 2	c. 341G>A	C114Y	China	Yue et al. (2009);
Ex. 2	c. 342T>G	C114W	China	Ye et al. (2012); Yu et al. (2015); Sun et al. (2012)
Ex. 3	c. 348C>A	Y116*	India	Dalal et al. (2012)
Ex. 3	c. 433T>C	C145R	India	Dalal et al. (2012)
Ex. 3	c. 434G>A	C145Y	Italy	Hurvitz et al. (1999)
Ex. 3	c. 535_536delTG	C179fsX	Syria	Delague et al. (2005)
Ex. 3	c. 589G>C	A197fsX201	Syria	Delague et al. (2005)
Ex. 4	c. 624_625insA	C209fsX229	China	Ye et al. (2012)
Ex. 4	c. 667T>G	C223G	China	Ye et al. (2012); Yu et al. (2015); Luo et al. (2015)
Ex. 4	c. 682T>C	S228P	India	Dalal et al. (2012)
Ex. 4	c. 716_722delAAATGAG	Q239fs*16	China	Sun et al. (2012)
Ex. 4	c. 729_735delGAGAAAA		China	Ye et al. (2012)
Ex. 4	c. 739_740delTG	C247fsX277	Caucasian, India	Dalal et al. (2012)
Ex. 4	c. 756C>A	C252*	China	Luo et al. (2015)
Ex. 5	c. 802T>G	C268G	India	Dalal et al. (2012)
Ex. 5	c. 840delT	F280fsX312	China	Liao et al. (2004); Zhou et al. (2007); Yang et al. (2013)
Ex. 5	c. 857C>G	S286*	China	Yu et al. (2015)
Ex. 5	c. 863insAC	T288fsX313	USA	Hurvitz et al. (1999)
Ex. 5	c. 866_867insA	Q289fsX302	China	Ye et al. (2012); Yu et al. (2015); Sun et al. (2012)
Ex. 5	c. 866delAG	Q289fsX301	Iran	Hurvitz et al. (1999)
Ex. 5	c. 947_951delAATTT	Q316fsX320	India	Dalal et al. (2012)
Ex. 5	c. 993G>A	W3331X	Italy	Hurvitz et al. (1999)
Ex. 5	c. 1000T>C	S334P	China	Zou et al. (2007); Liao et al. (2004); Sun et al. (2012)
Ex. 5	c. 1010G>A	C337Y	India	Dalal et al. (2012)

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.

REFERENCES

1. Hartwell LH. Genetics: From Genes to Genomes, Third Edition. McGraw-Hill Companies; 2004. p.30-36.
2. The American Society of Human Genetics Board of Directors and The American College of Medical Genetics Board of Directors. Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents. Bethesda, Md: ASHG;1995.
3. Thompson MW, McInnes RR, Willard HF. Genetics in Medicine, Fifth edition. Thompson & Thompson W.B. Saunders Company;1991.
4. Bass HN, Beavers J. Perinatal Screening for Congenital Malformations and Genetic Disorders: Current Status and Future Directions. The Permanente Journal. 2002;6(1):15-19.
5. Lyon E. When is a mutation associated with the disease? Genetic applications in Practice. NCHPEG. 2006;1:p 4-6.
6. Nakamura Y, Weidinger G, Liang GO, Aquilina-Beck A, Tamai K, Moon, RT. et al. The CCN family member Wisp3, mutant in progressive pseudorheumatoid dysplasia, modulates BMP and Wnt signaling. The Journal of Clinical Investigation. 2007;117:3075-3086. http://dx.doi.org/10.1172/JCI32001.
7. Yue H, Zhang ZL, He JW. Identification of novel mutations in *WISP3* gene in two unrelated Chinese families with progressive pseudorheumatoid dysplasia. Elsevier (Bone) 2009;44:547-554. http://dx.doi.org/10.1016/j.bone.2008.11.005.
8. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1988;16:1215. http://dx.doi.org/10.1093/nar/16.3.1215.
9. Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ. 2000.;pp.365-386.
10. Hurvitz JR, Suwairi WM, Hul WM, El-Shanti H, Superti-Furga A, Roudier J. et al. Mutations in the CCN gene family member WISP3 cause progressive pseudorheumatoid dysplasia. Nature Genetics. 1999;23:94-98. http://dx.doi.org/10.1038/12699.
11. Ye J, Zhang HW, Qiu WJ, Han LS, Zhang YF, Gong ZW, et al. Patients with progressive pseudorheumatoid dysplasia: from clinical diagnosis to molecular studies. Mol Med Rep. 2012;5(1):190-5.
12. Yu Y, Hu M, Xing X, Li F, Song Y, Luo Y, et al. Identification of a mutation in the WISP3 gene in three unrelated families with progressive pseudorheumatoid dysplasia. Mol Med Rep. 2015;12(1):419-25. http://dx.doi.org/10.3892/mmr.2015.3430.
13. Delague V, Chouery E, Corbani S, Ghanem I, Aamar S, Fischer J et al. 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. 2005;138A(2):118-26 http://dx.doi.org/10.1002/ajmg.a.30906.
14. Temiz F, Ozbek MN, Kotan D, Sangun O, Mungan NO, Yuksel B, et al. A homozygous recurring mutation in WISP3 causing progressive pseudorheumatoid arthropathy. J Pediatr Endocrinol Metab. 2011;24(1-2):105-8. http://dx.doi.org/10.1515/jpem.2011.117.
15. Dalal A, Bhavani GSL, Togarrati PP, Bierhals T, Nandineni MR, Danda S, et al. Analysis of the WISP3 gene in Indian families with progressive pseudorheumatoid dysplasia. Am J Med Genet A. 2012;158A(11):2820-8. http://dx.doi.org/10.1002/ajmg.a.35620. Bosnia and Herzegovina.
16. Sun J, Xia W, He S, Zhao Z, Nie M, Li M, et al. Novel and recurrent mutations of WISP3 in two Chinese families with progressive pseudorheumatoid

dysplasia. PLoS One. 2012;7(6):e38643. http://dx.doi.org/10.1371/journal.pone.0038643.

17. Luo H, Shi C, Mao C, Jiang C, Bao D, Guo J, et al. A novel compound WISP3 mutation in a Chinese family with progressive pseudorheumatoid dysplasia. Gene. 2015;564(1):35-8. http://dx.doi.org/10.1016/j.gene.2015.03.029.
18. Ehl S, Uhl M, Berner R, Bonafé L, Superti-Furga A, Kirchhoff A. Clinical, radiographic, and genetic diagnosis of progressive pseudorheumatoid dysplasia in a patient with severe polyarthropathy. Rheumatol Int. 2004;24(1):53-6. http://dx.doi.org/10.1007/s00296-003-0341-0.
19. Liao EY, Peng YQ, Zhou HD, Mackie EJ, Li J, Hu PA, et al. Gene symbol: WISP3. Disease: spondyloepihyseal dysplasia tarda with progressive arthropathy. Hum Genet. 2004;115(2):174.
20. Yang X, Song Y, Kong Q. Diagnosis and surgical treatment of progressive pseudorheumatoid dysplasia in an adult with severe spinal disorders and polyarthropathy. Joint Bone Spine. 2013;80(6):650-2. http://dx.doi.org/10.1016/j.jbspin.2013.03.006.
21. Zhou HD, Bu YH, Peng YQ, Xie H, Wang M, Yuan LQ, Jiang Y, Li D, Wei QY, He YL, Xiao T, Ni JD, Liao EY. Cellular and molecular responses in progressive pseudorheumatoid dysplasia articular cartilage associated with compound heterozygous WISP3 gene mutation. J Mol Med. 2007; 85:985-96 http://dx.doi.org/10.1007/s00109-007-0193-2.
22. Zhang Y, Pan Q, Zhong H, Merajver S, Kleer C. Inhibition of CCN6 (WISP3) expression promotes neoplastic progression and enhances the effects of insulin-like growth factor-1 on breast epithelial cells. Breast Cancer Research. 2005;7:1080-1089. http://dx.doi.org/10.1038/sj.onc.1205462.
23. Huang W, Zhang Y. Inhibition of CCN6 (Wnt-1-Induced Signaling Protein3) Down-Regulates E-Cadherin in the Breast Epithelium through Induction of Snail and ZEB1. The American Journal of Pathology. 2008;172:893- 904. http://dx.doi.org/10.2353/ajpath.2008.070899.
24. Zhang Y, Pan Q, Zhong H, Merajver S, Kleer C, Zhi-Fen W, Livant D. WISP3 is a novel tumor suppressor gene of inflammatory breast cancer. Oncogene. 2002;21:3172-3180. http://dx.doi.org/10.1038/sj.onc.1205462.