

## Genetic Variations in Exon 3 of VWF Gene in Patients with Von Willebrand Disease (VWD) from South-West Iran

Nasiri M PhD<sup>1</sup>, Galehdari H PhD<sup>2</sup>, Darbouy M PhD<sup>1</sup>, Yavarian M MD<sup>3</sup>, Keikhaee BMD<sup>4</sup>

1-Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran

2- Department of Genetics, University of Shahid Chamran, Ahwaz, Iran.

3- Hematology Research Centre, Shiraz University of Medical Science, Shiraz, Iran

4- Thalassemia and Hemoglobinopathies research center, Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran.

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### Abstract

#### Background

Von Willebrand disease (VWD) is an autosomally inherited bleeding disorder with the prevalence of 1% based on population studies. The disease phenotype is due to quantitative and structural/functional defects in Von Willebrand Factor (VWF) which is a glycoprotein with essential role as a carrier of FVIII in circulation and also it serves the function as hemostasis regulator. VWF is encoded by a large gene located on chromosome 12 which spans 178kb and has 52 exons. Many different mutations are known in VWF gene that can affect the VWD phenotypic features.

#### Materials and Methods

In this study we evaluated genetic variations in exon 45 of VWF gene in Iranian patients suffer from VWD from South-west Iran. Materials and Methods: 36 patients diagnosed with VWD (11 males and 25 females), with different ages, from Khuzestan province are participated in the investigation. Exon 3 with the flanking intronic sequences was amplified by PCR and the amplicons were analyzed by sequencing for any genetic changes (mutations and Single Nucleotide Polymorphism (SNPs)).

#### Results

No mutation was found in our patients in this exon . A novel SNP was recognized in all patients in a homozygous manner, T/C in intron 3 .

#### Conclusion

Although previous molecular investigations of VWD in Iran and some neighboring countries documented several mutations in exon 3, our research showed some contradictory result. The results of our study provided a new insight for further studies, not integrating exon 3 in their analysis .

#### Keywords

von Willebrand Disease, von Willebrand Factor, Polymorphism ; Single Nucleotide

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#### Corresponding Author

Mahbobeh Nasiri, nasiri@iaua.ac.ir ,Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran.

## Introduction

The most common bleeding disorder in human, Von Willebrand disease (VWD), is resulted from defects in central component of Homeostasis, Von Willebrand factor (VWF). The prevalence of VWD is 1% based on population studies (1, 2). VWF is a glycoprotein which mediates adhesion of platelets to vessel injury sites and also carries and stabilizes the FVIII in circulation (3). The clinical manifestations of the disease are mucocutaneous bleeding, post-traumatic and post-surgical bleeding, Menorrhagia and epistaxis (4).

Till now variety of mutations include missense mutations, nonsense mutations, small insertions, and small deletions have been documented for VWD (5). Based on these mutations and their impacts on the pathogenesis of VWD, the disease fall into three categories. Type 1 VWD is due to partial reduction of VWF. It is generally inherited in an autosomal dominant manner and exhibits high heterogeneity, lack of penetrance and variable expressivity (6, 7). Type 2 is related to qualitative abnormalities of VWF and is divided into four subtypes (2A, 2B, 2M and 2N) with determined phenotypic features. The vertical transmission of type 2 VWD suggests the autosomal dominant pattern of inheritance (8). Type 3 is a rare but the most severe form of the disease. It is characterized by not detectable or very low levels of VWF and is inherited in an autosomal recessive fashion (9,11).

The encoding gene for VWF is located on chromosome 12 (12p13.2). It is composed of 52 exons and spans 178 kb of the genome. A partial nonfunctional pseudogene is also located on chromosome 22 with 97% homology to exon 23-34 of main VWF gene sequence (12,13). In addition to different types of mutations that are detected in VWF gene, lots of single nucleotide polymorphisms (SNPs) are also found in this

large gene. SNP markers are just a single base changes in a DNA sequence, with a usual alternative of two possible nucleotides at a given position. SNPs must have prevalence more than 1% in healthy population.

Most SNPs are not responsible for a disease state but others may predispose people to diseases. Thereby, SNPs can be used as biological markers to screen patients for susceptibility to a certain disease by analyzing their DNA for specific SNP profiles (14).

The mature product of the VWF gene is comprised of a series of domains (4, 15, 16). Each domain is encoded by a cluster of exons. Exon 3 contributes to the synthesis of D<sub>1</sub> domain which plays role in VWF secretion and accuracy of protein folding (17). The role of exon 3 in VWF function and previous reports especially in Iran convinced us to choose this exon for mutation screening.

## Materials and Methods

36 patients suffer from VWD (11 males & 25 females) referred to Shafa hospital of Ahwaz from whole parts of Khuzestan province were included to our study. Patients had the age ranging from 6 to 45 year. About 55% (20 out of 36) of patients were born from consanguineous marriage and 50% (18/36) of them were with Arab / Arabian background (the prominent ethnic population in this region of Iran). Except seven families with two affected children, all of other families have had no more than one child suffer from the disease. In this study, PCR- direct sequencing was used to detect mutations in VWD patients.

Whole blood was collected from 36 patients with different types of VWD after being informed of the consent form context. The PCR primers were designed by online version of the primer3 software from the

flanking intronic sequences at both 5' and 3' end of the exon 3 (Table I).

Genomic DNA was extracted using AccuPrep Genomic DNA Extraction Kit (Bioneer Co. South Korea). Based on literature review and the impact of the exon 3 VWF function, we selectively searched for disease causing mutations in this exon as a part of our comprehensive project on VWD. The exon 3 and the flanking introns were amplified by PCR. PCR reaction was done in a total volume of 20µl containing 10 pmol of each primer (TAG Copenhagen A/S, Fruebjergvej3, Denmark) and 50ng gDNA. PCR condition included the initial denaturation at 95°C for 5min followed by 30 cycles at 95°C for 30sec, annealing at 60°C for 30sec and 72°C for 45sec with a final

extension at 72° C for 7 min. PCR products with 377 bp in size were finally separated on 2% agarose gel (Fig.1). Subsequently, the amplicons were sequenced by direct sequencing method on an automated ABI Sequencer (Applied Biosystems, USA) after manufacture's instruction. Sequencing results were analyzed by using software Chromas 2.2 and BLAST of sample sequence with normal sequence on NCBI web.

### Results

No pathogenic mutation was detected in this exon in our sample group. The T to C single nucleotide polymorphism (SNP) in intron 3 was the only genetic variation in this region of the gene. All patients showed homozygous pattern for this polymorphism which is reported for the first time (Fig.2).

Table I: summary of designed primers that have been used in this study for amplification of exon 3.

Primer	Sequence [from 5' to 3']	Product length
VWF X3-F	Gagctgatggtcccagttgtgc	377bp
VWF X3-R	Cgtgtcacagaaaggctgtcc	

The primers were designed with primer 3 free online software.

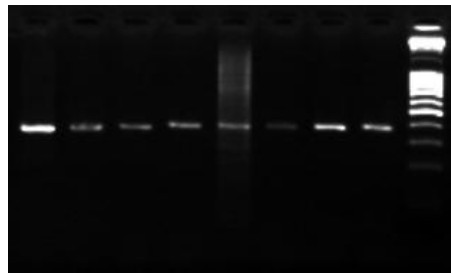


Figure 1: Exon 3 PCR products. The exon 3 and its flanking intronic sequence were amplified with designed primer pairs which produce 377bp amplicons. PCR products were checked on 2% agarose gel. The 100bp ladder was loaded for determining the length of products.

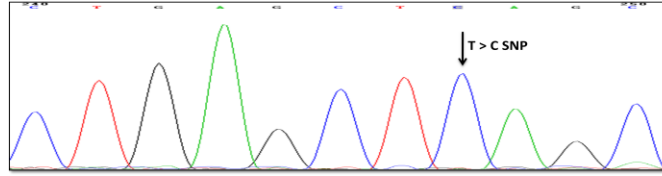


Figure 2: Homozygous pattern for T to C SNP in intron 3 of the VWF gene. The FASTA format of exon 3 sequence of each case was compared with normal sequence in BLAST. the comparison shows the novel sequence variation in intron of exon 3 which result in T to C substitution in homozygous manner.

## Conclusion

Heterogeneous nature of VWD in genotypic base and the severity of the disease make the molecular studying of VWD inevitable (18, 19). The VWF gene is very large in size and lots of mutations and single nucleotide polymorphisms (SNPs) were documented for the disease. Many molecular defects have been described in exon 3 in different ethnic groups. The genetic changes include small deletions, missense mutations and nonsense mutations which are available on HGMD (Human Gene Mutation Database) and ISTH (International Society on Thrombosis and Homeostasis) database (20). two different mutations include a deletion of nucleotide guanine at position 191(G191del) which shift Glysin to terminus (G64AfsX19), (21) and a missense mutation, replace Histidine to Aspartate at codon 47, (22) were described in Iranian type 3 VWD patients by Baronciani et al in the year 2000 and 2003 respectively. Beside these, there are some unpublished mutations in exon 3 of the VWF gene in Turkish VWD patients documented on ISTH. a nonsense mutation which shift serine at codon 71 to terminus (S71X) in heterozygous manner was found among Chinese type 3 VWD patients (23). The other genetic variation in this exon reported in Spain by Corrales et al (24). This nonsense mutation causes the amino acid Argenin at codon 34 change to stop codon. Since Almost all mutations were nonsense mutations or change critical amino acids, cause lack or significant reduction on VWF which was compatible with type 3 VWD.

Existed information makes interest for us to include exon 3 in our study. The results of our work were unpredictable and not compatible with those previous reports. Our study provides new perspective on this part of the gene for other scientists intend to do more work on molecular pathogenesis of VWD. Exon 3 can be excluded from the list of effective exons important for primary mutation screening in Iranian VWD patients and especially in prenatal diagnosis with time limitation.

So it is the first reports of T to C transition in intron 3, more works need to be done for further understanding of the impact of variation on VWF quantity, structure and function. Understanding the details about the roles of this SNP on the pathogenesis of VWD is necessary because maybe achieved by expressing the mutant gene in an expression vector in an appropriate host cell.

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Nasiri Performed the research. Nasiri and Galehdari designed the research study, analysed data and wrote the paper. Yavarian contributed in laboratory equipments. Keikhaee, Pedram and Jalali introduced patients and contributed to data collection. Darbouy edited the manuscript

## Conflict of Interest

The authors have no conflict of interest.

## Reference

- 1-Keeney S, Bowen D, Cumming A, Enayat S, Goodeve A, Hill M; UK Haemophilia Centre Doctors' Organisation (UKHCDO). The molecular analysis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organisation Haemophilia Genetics Laboratory Network. *Haemophilia* 2008;14(5):1099-111.
- 2-Kujovich JL. Von Willebrand disease and pregnancy. *Journal of Thrombosis and Haemostasis* 2005;3:246-253.
- 3-Gupta PK, Kannan M, Saxena R. Carrier detection in severe von Willebrand's disease. *Ann Hematol* 2004;83(10):625-7.
- 4-Goodeve AC. The genetic basis of von Willebrand disease. *Blood Rev* 2010;24(3):123-34.
- 5-Casaña P, Martínez F, Haya S, Tavares A, Aznar JA. New mutations in exon 28 of the von Willebrand factor gene detected in patients with different types of von Willebrand's disease. *Haematologica* 2001; 86(4):414-9.
- 6-Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood* 2003 15;101(6):2089-93.
- 7-Shahbazi S, Mahdian R, Ala FA, Lavergne JM, Denis CV, Christophe OD. Molecular characterization of Iranian patients with type 3 von Willebrand disease. *Haemophilia* 2009;15(5):1058-64.
- 8-Altaf S, Hussain R, Rahman Z, Niazi GA. Molecular biology of von Willebrand Disease (VWD): A study among unrelated Pakistani Patients. *World journal of Medical Science* 2008;3(1):1-4.
- 9-Wood J.A. Treatment of von Willebrand's Disease. *N Engl J Med* 2004;351:683-694
- 10-Gadisseur A, Hermans C, Berneman Z, Schroyens W, Deckmyn H, Michiels JJ. Laboratory diagnosis and molecular classification of von Willebrand disease. *Acta Haematol* 2009;121(2-3):71-84.
- 11-Yawn B, Nichols WL, Rick ME. Diagnosis and management of von Willebrand disease: guidelines for primary care. *Am Fam Physician* 2009;80(11):1261-8.
- 12-Mancuso DJ, Tuley EA, Westfield LA, Lester-Mancuso TL, Le Beau MM, Sorace JM, et al. Human von Willebrand factor gene and pseudogene: structural analysis and differentiation by polymerase chain reaction. *Biochemistry* 1991;30(1):253-69.
- 13-Castaman G, Federici AB, Rodeghiero F, Mannucci PM. Von Willebrand's disease in the year 2003: towards the complete identification of gene defects for correct diagnosis and treatment. *Haematologica* 2003;88(1):94-108.
- 14-Vignal A, Milan D, SanCristobal M, Eggen A. A review on SNP and other types of molecular markers and their use in animal genetics. *Genet Sel Evol* 2002;34(3):275-305.
- 15-Bonthron DT, Handin RI, Kaufman RJ, Wasley LC, Orr EC, Mitsok LM, et al. Structure of pre-pro-von Willebrand factor and its expression in heterologous cells. *Nature* 1986;324(6094):270-3.
- 16-Shelton-Indel BB, Titani K, Sadler JE. cDNA sequences for human Von Willebrand factor reveal five types of repeated domains and five possible protein sequence polymorphisms. *Biochem* 1986;25:3164.
- 17-Mayadas TN, Wagner DD. Vicinal cysteines in the prosequence play a role in von Willebrand factor multimer assembly. *Proc Natl Acad Sci U S A* 1992;89(8):3531-5.
- 18-Rodeghiero F, Castaman G, Tosetto A, Batlle J, Baudo F, Cappelletti A, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. *J Thromb Haemost* 2005;3(12):2619-26.
- 19-Castaman G, Rodeghiero F, Tosetto A, Cappelletti A, Baudo F, Eikenboom JC, et al. Hemorrhagic symptoms and bleeding risk in obligatory carriers of type 3 von Willebrand disease: an international, multicenter study. *J ThrombHaemost* 2006;4(10):2164-9.
- 20-Goodeve AC, Eikenboom JC, Ginsburg D, Hilbert L, Mazurier C, Peake IR, Sadler JE, Rodeghiero F; ISTH SSC Subcommittee on von Willebrand factor. A standard nomenclature for von Willebrand factor gene mutations and polymorphisms. On behalf of the ISTH SSC Subcommittee on von Willebrand factor. *Thromb Haemost* 2001;85(5):929-31.
- 21-Baronciani L, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, et al. Molecular characterization of a multiethnic group of 21 patients with type 3 von Willebrand disease. *Thromb Haemost* 2000;84(4):536-40.
- 22-Baronciani L, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, et al. Molecular defects in type 3 von Willebrand disease: updated results from 40 multiethnic patients. *Blood Cells Mol Dis* 2003;30(3):264-70.
- 23-Wang Y, Li Z, Gu J, Ruan C. von Willebrand disease in China. *Chin Med J (Engl)* 2000;113(8):677-80.
- 24-Corrales I, Ramirez L, Altisent C, Parra R, Vidal F. Rapid molecular diagnosis of von Willebrand disease by direct sequencing. Detection of 12 novel putative mutations in VWF gene. *Thromb Haemost* 2009;101(3):570-6.