Molecular Biology of von Willebrand Disease (VWD): A Study among Unrelated Pakistani Patients

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Abstract: Von Willebrand Disease (vWD) is an autosomal dominant hereditary blood coagulation disorder that is highly variable in expression. Although the frequency of vWD is low in Pakistan but since the disease has similar manifestations as that of hemophilia A, some of the vWD patients are misdiagnosed. The molecular basis of vWD in Pakistan is also unknown. The main objective of this study is to identify the mutation that produces vWD in Pakistani patients and compare the ethnic differences. For this purpose the blood samples were collected from 11 unrelated vWD patients from southern Pakistan. DNA was extracted by using standard techniques. The DNA for only 15 exons was amplified. The reason for the selection of these exons was that most of mutations in these exons have been reported in South East Asian population. Sequencing results showed two point mutations were present within a same exon 28 in one vWD Sindhi patient, at codon 1229 and 1231 with changes GTT > GGT and AAC > ACC which results in the Asn > Thr and Val > Gly substitution respectively. These mutations have been previously reported in two separate individuals of different ethnic backgrounds. Four known SNPs in eight vWD patients were observed in exon 18, 20, 42. These SNPs are already been documented in other world populations. These results of mutational analysis on vWD patients are significant for the reason that this is the first study that has been carried out in Pakistan and it has provided some insight to molecular pathology of vWD in Pakistan which can be useful in conducting further studies. Also it can be helpful in the molecular diagnosis of vWD.

Key words: Coagulation Disorders • von Willebrand Disease • Molecular Biology • Pakistan

INTRODUCTION

Von Willebrand disease (vWD) is a hereditary autosomal dominant bleeding disorder which results from quantitative or qualitative defects of Von Willebrand factor (vWF) that serves as carrier for factor VIII and adhesive link between platelets and the injured blood vessel wall. It is named after the Finnish physician, Erik Von Willebrand, who first described the condition in 1926 in 24 of 66 members of a family from the Alands islands [1]. The sexes were affected and the bleeding time was prolonged despite normal platelet counts and normal clotting reaction. vWD affects approximately 125 persons per million population, with severe disease affecting approximately 0.5-5 persons per million population [2]. vWF is a large multimeric glycoprotein that circulates in blood plasma at concentrations of approximately 10 mg/mL. In response to numerous stimuli, vWF is released from storage granules in platelets and endothelial cells. It performs 2 major roles in hemostasis. First, it mediates the adhesion of platelets to sites of vascular injury. Second, it binds and stabilizes the procoagulant protein factor VIII (FVIII). vWF exists as a series of multimers, varying in molecular weight between 0.5 (dimer) and 20 million kD (multimer). The building block of multimers is a dimer, held together by disulfide bonds located near the C-terminal end of each subunit [3].

There are 3 main subtypes, classically characterized by excessive mucocutaneous bleeding, a positive family history and abnormal von Willebrand factor (VWF) laboratory studies. Type 1 VWD is a partial deficiency of qualitatively normal VWF, type 2 VWD is caused by functionally abnormal VWF and type 3 VWD represents a virtual absence of the VWF protein [4].

The spectrum of clinical severity is broad, ranging from a severe disorder beginning in early life with
recurring life threatening to a very mild disorder in which abnormal bleeding does not occur except during trauma or surgery. Early bruising is common and pitechia or purpura may be noted. Mucous membrane bleeding including epistaxes, upper GI bleeding and menorrhagia may be prominent. The diagnosis is established by obtaining a appropriate family history on demonstration of a prolonged bleeding time, defective ristocetin-induced platelet aggregation in the presence of normal aggregation to other agent and reduced FVIII R:Ag and FVIIIIC activities. A delayed marked but sustained increase in FVIIIIC activity after infusion of cryoprecipitate confirms the diagnosis and forms the basis of treatment [5].

In Pakistan no molecular study on vWD has been conducted and only clinical data is available for some scattered patients. This study was therefore, planned to:

- Characterize the molecular basis of vWD in Pakistan
- Compare the ethnic differences
- Evaluate the usefulness of the mutational analysis in pre and post-natal diagnosis.

**MATERIALS AND METHOD**

5ml of venous blood samples from 11vWD patients from southern Pakistan were taken for DNA in a EDTA vacutainer tube and the samples were stored at -20 C. The clinical data was collected from the files of the patients and were recorded on a Performa designed for this purpose. 11 patients had family history. Nasal bleeding, bruises, gum bleeding were most common prevalent and common symptoms of the disease. Mostly PT, APTT, BT, CT values were available for nearly all the patients. DNA from blood samples was extracted using Phenol Chloroform Method. The quantity of the extracted DNA in the TE solution was estimated by spectrophotometry yield gel electrophoresis. Out of the 52 Exons, 15 Exons (Exon 3, Exon 5, Exon 6, Exon 7, Exon 9, Exon 10, Exon 16, Exon 18, Exon 19, Exon 20, Exon 27, Exon 28, Exon 42, Exon 43, Exon 45) were selected considering there high mutation rate. In order to design the primers various primer choices were examined for 15 Exons, using the computer program Primer 3. 20 sets of primers were chosen and were synthesized in Primer designing laraotary of CEMB. The primers were mostly 20 nucleotide G+C rich sequence. Sizes of amplified products range from 218pb to 475 bp. Sizes of products to be amplified range from 218 pb to 475 pb. These primers were then utilized to amplify selected portion of DNA using PCR amplification technique of each amplified exon [6]. Amplification was carried out in BIO-RAD I-Cycler and 1-2 ul of genomic DNA was used as template. Quantity and quality of amplification was checked on agarose gel using ethidium bromide staining. All fragments exhibiting aberrant banding pattern were then subjected to cycle sequencing using Big Dye termination technology. Sequencing of the amplified DNA products was done by di-deoxy termination method on ABI 3100 genetic analyzer capillary electrophoresis (Applied Biosystems).

**RESULTS**

Sequencing data was analyzed by using software Chromas2. The sequence was also blasted against normal sequence by using BLAST 2 sequences on NCBI web. BLAST (Basic Local Alignment Search Tool) a search program designed to explore all the available databases against a query protein or DNA sequence. It utilizes the BLAST algorithm for pairwise DNA-DNA or protein-protein sequence comparison. The resulting alignments

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<th>Table 1: Mutations Detected in a vWD Patient</th>
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<th>Table 2: SNPs in vWD Patients</th>
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are presented in graphic form. Any change in the DNA sequence was confirmed by sequencing of the sense and antisense strands for all the patients. Sequencing results showed that out of 11 patients, one patient had 2 point mutations shown in Table 1 and 4 SNPs were detected in 8 samples of patient shown (Table 2).

The mutations were reconfirmed by repeating the sample with the forward and reverse primers.

**DISCUSSION**

Von Willebrand disease (VWD) is an autosomal inherited congenital bleeding disorder which either is due to deficiency or dysfunction of von Willebrand factor (VWF), a large multimeric glycoprotein. VWF has two functions, first it attaches to sub endothelial collagen and to platelets, promoting formation of a platelet plug at the site of injury of small blood vessels and second it binds and transports Factor VIII (FVIII) [7].

Many molecular defects that result in vWD have been described. To date 316 mutations have been reported in VWF gene in different ethnic groups of world population [8]. These defects include point mutations, insertions or deletion of some nucleotides. The majority of abnormalities are missense mutations that are associated with the functionally abnormal vWD types, such as types IIA and type IIB and the variants with defective factor VIII binding. In severe type III vWD gene deletions, non-sense mutations and defective mRNA expression have been described as the cause of quantitative deficiency of VWF.

The size of the samples of this study was small as VWD has very low prevalence in Pakistan and sometimes it is misdiagnosed as Hemophilia A. Blood samples from 11 patients were collected from Karachi, a cosmopolitan city with mix up of individuals of different ethnic backgrounds. 11 vWD patients included in this study are: 3 Pathans, 2 Sindhis, 1 Memon, 1 Brohi, 2 Mahajirs, 1 Sheikh and 1 Behari indicating an ethnic diversity. In this study 15 exons were selected because of high rate of substitution. The second SNP T/C was at codon number 795 with changes TAT -> TAC which resulted into the same amino acid Tyr. 3rd SNP T/C was found in exon 42 at codon number 2413 with changes ACT -> ACC which resulted into the same amino acid Thr. This 4th SNP A/G was detected in 4 patients in exon 20 at codon number 874 with changes AGG -> GGG which resulted into Arg-Gly amino acid substitution. This 4th SNP accounts for 45% of population.
The main objective of this study was to characterize the molecular basis of vWD in Pakistani population as such studies have not been conducted before. The results of this study which are preliminary in nature are significant as these have provided us an insight into the molecular pathology of vWD in Pakistan. Further these results can form the basis of future studies and consequently can be helpful in the molecular diagnosis of the vWD.

REFERENCES

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