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von Willebrand Disease with G4022A Mutation (vWd Sungnam) : A Case Report

A 10-year-old male patient affected by type 2 von Willebrand disease (vWD) and his family members were investigated by hemostatic and molecular genetic studies. The propositus, who experienced frequent bleeding episodes, was characterized by a normal level of von Willebrand factor (vWF) antigen (54%), reduced vWF ristocetin cofactor activity (5%), decreased factor VIII clotting activity (25%) and absent high molecular weight multimers in the plasma. An exon 28 fragment coding for the A1 and A2 domains was amplified by polymerase chain reaction and sequenced. We found a heterozygous mutation (G4022A), producing an additional *PstI* restriction site, which resulted in the substitution of Arg578Gln. Family studies, including the parents and a brother, were negative for this mutation and vWF abnormalities were not observed. We confirmed that G to A mutation in the region of the platelet glycoprotein Ib binding domain of vWF causes the qualitative type 2 defect in von Willebrand disease.

Key Words : *von Willebrand disease, Hemorrhage, Mutation*

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INTRODUCTION

von Willebrand factor (vWF) is a multimeric plasma glycoprotein that plays a central role in hemostasis (1). von Willebrand disease (vWD) is a syndrome characterized by one or more defects in vWF due to alterations in its genes (2). Over 20 distinct vWD phenotypes have been described, all manifesting as either quantitative (type 1 or 3) or qualitative (type 2) abnormalities in plasma vWF (3). Type 2 vWD is very heterogenous. The defective function of vWF in this type may be attributed to the lack of hemostatically effective large multimers, which are critical to the function of protein in vivo (4). We recently experienced a case of type 2 vWD with a heterozygous mutation (G4022A) in the region of exon 28 of the vWF gene for the platelet glycoprotein Ib binding domain. To the best of our knowledge, this is the first documented case of type 2 vWD with point mutation.

CASE REPORT

Patient

A 10-year-old male patient was admitted for evaluation of mild anemia (Hb: 10.4 g/dL, Hct: 32.4%) and frequent epistaxis. His platelet count (282,000/μL), prothrombin time and activated partial thromboplastin time were normal. His template bleeding time (8 min) was not prolonged. Diagnosis of type 2 vWD was made on the basis of a marked decrease (5%) in ristocetin cofactor activity (vWF:RCo, normal reference range: 43-123%), absence of high molecular weight multimers from plasma (Fig. 1), and normal (54%) vWF antigen (vWF:Ag, normal reference range: 47-134%). Nothing remarkable was found in his family history and laboratory findings for vWF were normal in his parents and a brother (Table 1).

Table 1. Laboratory findings for von Willebrand disease

Lab tests	Patient	Father	Mother	Brother
FVIII	25%	82%	60%	55%
vWF:Ag	54%	118%	91%	122%
vWF:RCo	5%	95%	88%	90%
Multimer	absent HMW	Normal	Normal	Normal
RIPA	normal	ND	ND	ND

HMW: high molecular weight; RIPA; ristocetin induced platelet aggregation; ND; not done

Fig. 1. Plasma vWF multimeric structure analysis. Lane (A): absent high molecular weight multimers in the patient. Lane (B): normal control.

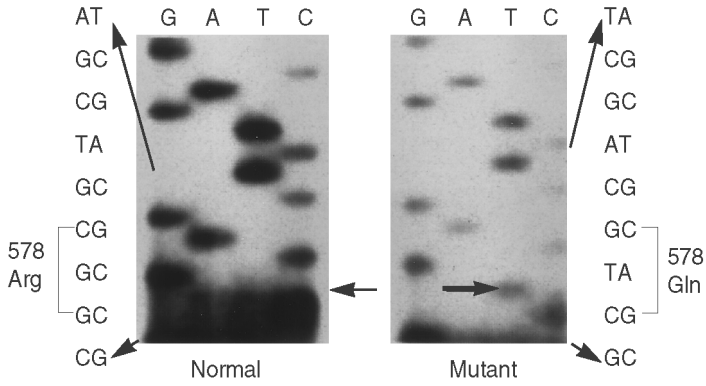

Fig. 2. Nucleotide sequences of vWF exon 28 fragment. A G to A mutation is present at position 4022 resulting in the substitution of arginine (Arg) at codon 578 by glutamine (Gln).

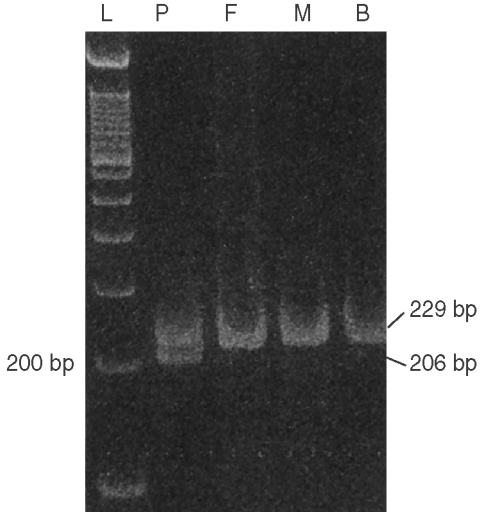

Fig. 3. Detection of Arg578Gln mutation by PstI restriction digestion of 229 bp exon 28 fragment. The new restriction site produces fragments of 206 bp and 23 bp. L: 100 bp ladder, P: patient, F: father, M: mother, B:brother.

Coagulation assays

Plasma samples were assayed for FVIII:C by one stage clotting method, vWF:Ag by enzyme immunoassay (Diagnostica Stago, France), and ristocetin-induced platelet aggregation (RIPA) responses and vWF:RCo by aggregometry (Chronolog, U.S.A.). In the RIPA assay, 0.3-1.2 mg of ristocetin/mL were added to whole blood. No decreased response (19 ohms, normal control 11-23 ohms) to 1 mg/mL, and no enhanced response (5 ohms; normal control 6 ohms) to low dose (0.3 mg/mL) were observed. Multimer analysis was performed by discontinuous 1.2% agarose gel electrophoresis and immunoblotting method (6).

DNA study

Genomic DNA was extracted from peripheral blood leukocytes as previously reported (5). Amplification of genomic DNA was performed by polymerase chain reaction (PCR) and fragments corresponding to the 3' end of exon 28 were amplified with the primer pairs according to the study previously described in detail (7) and directly sequenced with the Sequenase kit (US Biochemical Corp.) (Fig. 2). To confirm the G4022A mutation, amplified exon 28 products (Fig. 3) was digested with PstI (Boehringer Mannheim) and resolved by 1.2% polyacrylamide gel electrophoresis (8).

DISCUSSION

The current classification recognizes three major types of vWD (9). Type 1 and 3 refer to mild and severe quantitative deficiency of vWF, respectively, whereas type 2 refers to qualitative abnormalities. Type 2 is further divided into four subtypes (A, B, M, N) which are thought to reflect distinct functional abnormalities. Type 2A and 2B vWD are characterized by the absence of high molecular weight forms of vWF in the plasma and by the abnormal reactivity of vWF towards platelet glycoprotein Ib (GP Ib), which is decreased in type 2A and increased in type 2B (9). In type 2A, spontaneous mutations in the propeptide region and more commonly, missense mutations in the A2 domain have been detected (10). The patient described here is con-

sistent with type 2A in phenotype characterized by the absence of high molecular weight forms of vWF in the plasma and no increased response to ristocetin. But it is atypical in type 2A genotype because the patient has a well known mutation (G4022A, codon:Arg578Gln) in type 2B (7, 8).

The large majority of patients with type 2B have heterozygous point mutations in a short sequence of exon 28 of vWF gene between codons 540 and 578 (10) and four mutations (Arg543Trp, Arg545Cys, Val553Met and Arg578Gln) account for 90% of type 2B vWD patients studied to date (8). Type 2B is a variant in which the structurally abnormal vWF shows an increased affinity for the platelet vWF receptor, GPIb and this may sometimes give rise to platelet aggregation and thrombocytopenia in vivo (9). Amino acid residues, responsible for the binding of vWF to GP Ib, are located within the A1 domain of vWF between Cys509 and Cys695 (11, 12). The hallmark of type 2B vWD is an enhanced response to ristocetin which is thought to be a consequence of prior absorption of high molecular weight forms of the dysfunctional vWF to platelet receptor GPIb (13).

In the present study, we report the case of a patient with a variant of vWD, presenting a discrepancy between his type 2A phenotype and his type 2B genotype involving the A1 domain.

Type 2M vWD refers to qualitative variants with a decreased platelet dependent function which is not caused by the absence of high molecular weight vWF multimers (14). Type 2N vWD refers to qualitative variants with markedly decreased affinity for factor VIII (15). In addition to the mutation study, absence of high molecular weight vWF multimers and mildly decreased level of factor VIII:C performed on the patient in this study may safely exclude the possibility of type 2M or 2N.

Whereas we cannot provide the real clue for the discrepancy between subtype 2A phenotype and subtype 2B genotype in our patient, it appears that distinct mutations of the same amino acid residues of the vWF subunit can lead to different phenotypes of vWD (16-18). We hypothesized that the possibly misleading phenotype of our patient might be related to the peculiar characteristics of either the platelets or the plasma vWF of our patient, which is unfortunately very difficult to verify. It is apparent from this study that correct diagnosis of type 2 vWD is still difficult to establish because of the phenotypic heterogeneity of the disease. This study thus also illustrate the limitation of phenotype and the importance of the molecular characterization of patients in the correct diagnosis and classification of type 2 vWD.

A family study done to confirm the inheritance of the mutation did not show the same substitution and other family members were phenotypically normal, which suggests a spontaneous mutation in the patient from this family.

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