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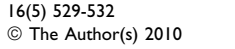

Coinheritance of Severe von Willebrand Disease With Glanzmann Thrombasthenia

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Abstract

A 35-years old male patient presented severe bleeding was diagnosed to have type 3 von Willebrand disease (VWD) and carrier for Glanzmann thrombasthenia (GT). Propositus and family members were studied through basic coagulation tests and genomic DNA analysis. Two offspring of the family were diagnosed to have GT through platelet aggregation along with VWD carrier. The patient with VWD was found positive for homozygous truncating mutation R1659X in *VWF* gene, and all offspring were heterozygous carriers of null allele. Hence, propositus was a carrier of GT with severe type 3 VWD and wife was a carrier of GT. Thus, it is concluded that there is importance of careful studies of patients even from nonconsanguineous families to exclude unusual coinheritance of congenital hemostatic disorders. If single replacement therapy in patient not responding well then probably coexpression of coagulopathies required and multiple replacement therapy should be given according to clinical and laboratory features.

Keywords

von Willebrand Disease, Glanzmann thrombasthenia, bleeding, phenotypes, mutation

Introduction

von Willebrand disease (VWD) is the most common autosomal inherited hemostatic bleeding disorder caused by either quantitative (type 1 and type 3) or qualitative defect (type 2) of von Willebrand factor (VWF). Type 1, types 2A, 2B, and 2M VWD are autosomal dominant, while type 3 and type 2N VWD are autosomal recessive in inheritance.[1] Glanzmann thrombasthenia (GT) is an autosomal recessive–inherited platelet function disorder characterized by a severe reduction in, or absence of, platelet aggregation in response to multiple physiologic agonists due to abnormalities of platelet glycoprotein glycoprotein (GP)IIb and/or GPIIIa.[2,3] Because the mode of inheritance is autosomal, VWD and GT have been observed in both sexes. There are several cases reported for coexpression of VWD with different coagulopathies like hemophilia A and B, factor VIII (FVII) deficiency, FXI deficiency, and FXII deficiency.[4] Here, we are reporting for the first time a patient with severe VWD associated with GT.

Materials and Methods

The study included a 35-years old Indian index patient with VWD and his family members were presenting the phenotypes of VWD as well as GT. Patient had epistaxis, gum bleeding, post-tooth extraction bleeding, and prolonged bleeding after trauma. Patient had with a strong family history of bleeding as female sibling (I-1) died due to bleeding complication (Figure 1). Written informed consent was obtained from the patient and his family members before the start of study. Blood sample was collected from the patient and family members in 3.2% trisodium citrate (1:10) and centrifuged at 1500*g* for 10 minutes. Platelet-poor plasma (PPP) and peripheral blood leukocytes were stored at -70°C.

Coagulation Tests and Aggregation Studies

Bleeding time was done by Ivy method.[5] Activated partial thromboplastin time (APTT) and prothrombin time (PT) tests were performed on automated coagulometer using PPP. Platelet aggregation has been done using platelet-rich plasma (PRP) at a standard count of 300×10^9/L.[6] Mixing aggregation study of patient PRP with normal pool plasma (NPP) was also performed to confirm the diagnosis of VWD, and response was recorded with the use of aggregometer (Chrono-Log, Havertown, Pennsylvania). The factor VIII coagulant (FVIII:C) activity was assayed by manual clotting method using FVIII-deficient plasma (Diagnostica Stago, Chausson, Asnieres,

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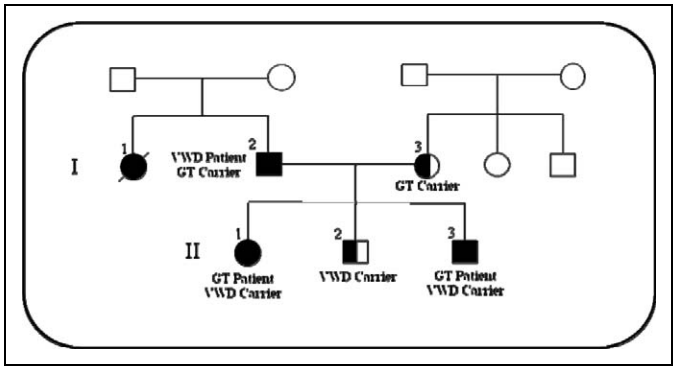

Figure 1. Family pedigree shows the carrier status of the von Willebrand disease (VWD) and Glanzmann thrombasthenia (GT) in members. Female sibling (I-1) died due to bleeding manifestation at the age of 25 years. Patient (I-2) was having type 3 VWD with GT carrier. Two offspring (II-1 and II-3) were presenting phenotypes of GT along with type 1 VWD. Offspring (II-2) was having type 1 VWD.

France) that has been standardized for the routine purpose in our laboratory.

von Willebrand Factor Assays

von Willebrand Factor antigen (VWF: Ag) level was detected in all patients plasma by an enzyme-linked immunosorbent assay kit (REEAD; Corgenix, Westminster, Colorado). Ristocetin cofactor activity (VWF: RCo) was assayed with formalin-fixed platelets.[7]

Genotypic Studies

High-molecular-weight genomic DNA was isolated from leucocytes using phenol–chloroform method[8] and used for polymerase chain reaction (PCR) amplification of *VWF* exons 2-52, as previously described.[9] Polymerase chain reaction was done in a T-gradient Thermoblock device (Dyad; MJ Research, Walthman, Massachusetts) using reagents and Taq polymerase from (Biotools B & M lab, Madrid, Spain), with primers flanking at least 20 base pair (bp) of intronic sequence to also allow detection of splice-site mutations. To avoid amplification of the pseudogene, primers that covered the pseudogenic region from exon 23 through exon 34 were *VWF* gene specific.[10,11] Polymerase chain reaction products were sequenced using an ABI Prism Big Dye Terminator Cycle sequencing ready reaction kit (Applied Biosystems, Weiterstadt, Germany). Mutation that was identified in index patient also screened in family members.

Results and Discussion

Basic coagulation parameters were measured for propositus and family members. Propositus was found normal for platelet counts; PT, APTT, and bleeding time (BT) were found prolonged; and FVIII coagulant activity was found severely low. Bleeding time was found prolonged (>15 minutes) for offspring (II3), and remaining members were not available for this test. Activated partial thromboplastin time, PT, platelet counts, and FVIII coagulant activity were found normal in rest of the family members (Table 1). Family history showed that there was no consanguineous marriage in family.

Platelet aggregation studies for propositus showed reduced aggregation with agonists adenosine diphosphate (ADP) and adrenaline (ADR); aggregation was absent with arachidonic acid (AA) and ristocetin. Mixing aggregation study with NPP and patient's PRP was corrected (Figure 2), which is characteristic of VWD. Offspring (II-1 and II-3) was diagnosed to have GT through platelet aggregation; aggregation was absent with agonists ADP, ADR, and AA and normal with ristocetin. Aggregation in offspring (II-2) was found normal with ADP, ADR, AA, and ristocetin (Table 2).

von Willebrand factor assay showed that propositus was carrying extremely low levels of VWF: RCo and VWF: Ag and characterized for type 3 VWD; all offspring were having borderline of VWF: Ag level and VWF: RCo activity and characterized for type 1 VWD; and wife was having normal level of these assays. Mutation screening in *VWF* gene reveals the presence of homozygous truncating mutation R1659X in exon 28 of propositus. All offspring were carrying heterozygous null allele for this genetic defect.

Phenotypes of the patient and 2 offspring (II-1 and II-3) were severe. Patient was having characteristics of mild VWD as carrying low FVIII: C, VWF: RCo, and VWF: Ag; prolonged bleeding time; and absent aggregation with AA and ristocetin. Being carriers of both diseases has allowed the expression of either disease in some offspring or the simultaneous expression of both in others. Phenotypes in offspring II-1 and II-3 were similar and presented repeated epistaxis, gum bleeding, and prolonged bleeding after trauma. von Willebrand factor analysis values for these offspring reveal the presence of mild VWD, but the bleeding time in offspring (II-3) and aggregation studies in both offspring (II-1 and II-3) reflect that they were carrying compound defect for VWD with GT. Hence, the patient with type 3 VWD was a carrier of GT and all offspring were carriers of *VWF* gene mutation.

Both GT and VWD were characterized by mucocutaneous bleeding. von Willebrand factor is an essential hemostatic multimeric glycoprotein that initiates the platelet plug formation by binding to connective tissue exposed at sites of vascular injury and to receptors GpIbα; it also initiates the binding of platelet to platelet through platelets receptors GpIIb/IIIa. These functions depend on binding sites for many ligands within the repeated multidomain structure of the VWF.[11]

Glanzmann thrombasthenia is an autosomal recessive inheritance with a worldwide distribution. Because the mode of inheritance is autosomal, GT has been observed in both sexes. The carriers for GT are either asymptomatic or having mild clinical features. Glanzmann thrombasthenia occurs in high frequency in certain ethnic populations with an increased incidence of consanguinity such as in Indians, Iranians, Iraqi Jews, Palestinian and Jordanian Arabs, and French Gypsies.[12] Coexpression of VWD and GT is very rare, and only 2 studies

Table 1. Basic Coagulation Parameters in Propositus and Family Members

Family Members	Age	PT (Sec)	APTT (Sec)	BT (Min)	Platelet count (PC) $\times 10^9$/L	FVIII: C (U/dL)	VWF: Ag (U/dL)	VWF: RCo (U/dL)	VWD Type
Propositus	32	14	59.4	>15	248	2.6	1	2	3
Wife	29	13	34	ND	235	114	150	130	N
Daughter	7	13.6	31	ND	211	50	42	38	1
Son (II-2)	4	15	31	ND	205	33	39	40	1
Son (II-3)	11	14.9	32.4	>15	195	54	48	35	1

NOTES: APTT = activated partial thromboplastin time; BT = bleeding time; N = normal; ND = not done; RCo = Ristocetin cofactor activity; VWD = von Willebrand disease; VWF = von Willebrand factor; VWF: Ag = von Willebrand Factor antigen.

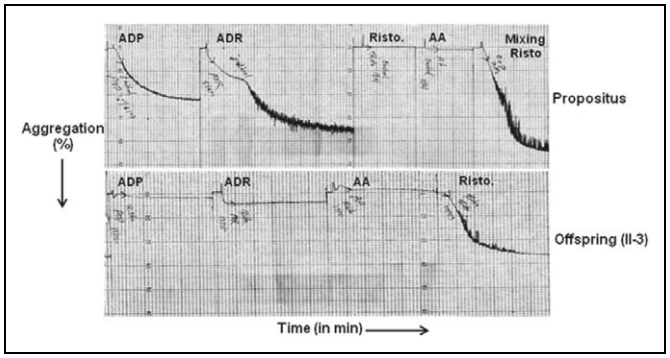

Figure 2. Aggregation with different agonist shows diagnosis of von Willebrand disease (VWD) in propositus as aggregation absent with Ristocetin (1.2 mg/mL) and corrected in mixing study. One of offspring shows pattern of Glanzmann thrombasthenia as aggregation absent with adenosine diphosphate (ADP), adrenaline (ADR), and arachidonic acid (AA) and reduced with ristocetin.

Table 2. Aggregation Studies With Different Agonists

Family Members	ADP (1.25 μmol/L)	ADR (20 μmol/L)	AA (300 μmol/L)	Ristocetin (1.2 mg/mL)
Propositus	Normal	Normal	Absent	Absent
Wife	Normal	Normal	Normal	Normal
Daughter	Absent	Absent	Absent	Normal
Son (II-2)	Normal	Normal	Normal	Normal
Son (II-3)	Absent	Absent	Absent	Normal

NOTES: AA = arachidonic acid; ADP = adenosine diphosphate; ADR = adrenaline.

are reported with mild VWD, one from Arabian consanguineous family[13] and another from a Canadian girl of Southeast Asian ancestry.[14] In this family index, patient was having mild VWD. It is expected where consanguineous marriages are allowed, but the occurrence of coinheritance in the nonconsanguineous family as in current study reflects the relatively wide distribution of these defective alleles. These patients with such compound defects either show high severity in the presence of defective alleles for both GT and VWD or severity is according to single disorder if one allele is normal for any of the defect.

From our experience with this family, we concluded that there is importance of careful studies of patients and their relatives even from nonconsanguineous families, to exclude unusual coinheritance of congenital hemostatic disorders, which might require specially designed approaches to management. These cases are of clinical importance as patient may not be treated only with FVIII concentrate, fresh frozen plasma, and 1,8 deamino-D-arginine vasopressin (DDAVP). Because GT is a platelet function disorder, patients may need platelet concentrates along with other replacements.

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Declaration of Conflicting Interest

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