ORIGINAL ARTICLE

Molecular characterization of exon 28 of von Willebrand's factor gene in Nigerian population

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Abstract

Background: Polymorphisms in von Willebrand factor (VWF) gene are an important contributor to the expression of VWF gene and differences in ethnic distribution of these single nucleotide polymorphisms (SNPs) exists.
Aims: Our objective was to molecularly characterize the exon 28 of the VWF gene in the three major ethnic groups of Nigeria.
Subjects and Methods: We recruited 90 subjects, 45 had a history of bleeding. Questions included those used in the Zimmerman Program for the Molecular and Clinical Biology of von Willebrand disease (VWD), and the bleeding scores were calculated using the Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD scoring system. Full blood count, coagulation profile, VWF:antigen level and VWF:collagen-binding activities were carried out. Data were analyzed using GraphPad Prism (5.03). GraphPad Software, Inc USA. The BigDye terminator chemistry was used to determine the nucleotide sequences of VWF gene (exon 28).
Results: Eight SNPs were identified, rs 216310 (T1547), rs 1800385 (V1565L), rs1800384 (A1515), rs1800383 (D1472H), rs 1800386 (Y1584C), rs 216311 (T1381A), rs 216312 (intronic) and rs 1800381 (P1337).
Conclusion: The SNPs rs 216311, rs 1800383 and rs 1800386 associated significantly with bleeding in study subjects. rs1800386 occurred in all with bleeding history, no ethnic variations were noted.

Key words: Exon 28, polymorphism, single nucleotide, von Willebrand factor

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Introduction

The von Willebrand factor (VWF) gene is a multimeric, multifunctional, and a highly polymorphic glycoprotein. Several genetic variations including mutations, transcript variants, and single nucleotide polymorphisms (SNPs) which alter function and or quantity of VWF have been described. The dysfunctions of the VWF gene may result in von Willebrand's disease a common bleeding disorder.

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Of more than fifty countries in Africa, information on von Willebrand disease (VWD) has been reported only in about five countries. The first family with the condition in Africa was reported in 1969[1] in South African Bantu. In the Western Cape of South Africa, the prevalence of the various subtypes of VWD was reported.[2] Ten cases reported in Kenya were without details. The cases reported in Arab were mostly among individuals with Arab ancestry.[3] In Zimbabwe, a retrospective study of hospital records revealed two probable cases among 95 patients with hemophilia A and 11 with hemophilia B between 1980 and 1986, but full investigation and family studies were not performed. In Nigeria, we have been unable to find documented cases of

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von Willebrand's disease. VWD results from quantitative and or qualitative defects in VWF gene. VWD is inherited as an autosomal dominant or recessive disorder with different clinical expressions depending on the type.[4] Common bleeding symptoms are epistaxis, menorrhagia (in women), easy bruising, oral cavity bleeding, bleeding after dental extraction, and postoperative bleeding.

Subjects and Methods

Patient population

The study involved three major ethnic groups in Nigeria: Hausa, Igbo, and Yoruba. Subjects with various bleeding disorders (index cases) were included in the study questions for probable VWD included those used in the Zimmerman Program for the Molecular and Clinical Biology-VWD and the bleeding scores calculated using the Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD scoring system. The subjects were recruited at three different locations within the country. Site A: Ondo Specialist Hospital Akure, Ondo State. Site B: 103 Battalion, Nigerian Army Enugu, Enugu State. Site C: University of Nigeria, Enugu, Enugu State and University of Nigeria Teaching Hospital Ituku Ozalla, Enugu State. Subjects were excluded from the study if they have malignant disease, metabolic disorders, or if their parent and grandparents were not from the same ethnic group as the subject. The study was among the adult population aged 20–60 years. Out of the ninety subjects enrolled in the study, 45 subjects reported a bleeding history.

Sample collection

Five milliliters of blood was collected into an ethylenediaminetetraacetic acid (EDTA) nonvacuum tube, for full blood counts and ABO blood grouping. Cells from the EDTA bottle were used to prepare DNA for sequencing. For coagulation profile and VWF functional assays: 4.5 ml of the blood was added into 500 µl of 3.2% trisodium citrate containing 5% N (2-hydroxyethyl)-piparazine-*N*-2-ethanesulfonic acid buffer.

Preparation of platelet-poor plasma

The 4.5 ml of blood in 3.2% trisodium citrate was centrifuged 1 h after collection at 1500 g for 15 min; supernatant plasma was re-spurn at 1500 g for 15 min until a platelet count of <10,000/µL was obtained.

The University of Nigeria Research Ethics Committee approved the study NHREC/05/01/2008B the study lasted from November 2010 to 2012. All participants gave their consent before sample collection.

The full blood count and platelet count were done using a hematology auto analyzer BC 5300 (Mindray, China).

von Willebrand factor:antigen and von Willebrand factor:collagen-binding activity testing

VWF:antigen (Ag) level and VWF:collagen binding (CB) were assayed using commercial ELISA kits from Technoclone, Austria (lot no. RA42B00.01 and RB49B00.01, respectively). FVIII activity was measured using FVIII immuno depleted human plasma from Diagen, UK.

DNA sequence analysis

gDNA was isolated using Tri Reagent (Sigma-Aldrich® - United States). Polymerase chain reaction amplification and sequencing of the exon 28 of VWF gene was carried out at ACGT, USA; primers were designed to specifically amplify exon 28 of the VWF gene. Bidirectional gene sequencing was carried out at the exon 28 of the VWF gene, using BigDye Terminator version 3.1 Applied Biosystems, UK-cycle sequencing chemistry software. The exon 28 is the largest exon of the VWF gene and is about 1.8 kb. Of the 484 classified mutations in the VWF gene, 252 (52%) occur in exon 28 alone while the remaining 232 about 48% occur in the rest of the 51 exons of the VWF gene.[5] Sequence data were analyzed using Sequencher 5.1-bulid (Gene Codes Corporation) and compared with the reference sequence NC-000012.11.

Statistical analysis

This was calculated using GraphPad Prism (5.03). GraphPad Software, Inc USA. For the comparison of two groups, Mann–Whitney U-test was performed, $P < 0.05$ was considered statistically significant. GraphPad Statmate 2 GraphPad Software, Inc USA was used to calculate the power of the study with 45 subjects in each group the study has a 95% power to detect a difference between means of 0.82 with a significance level (alpha) of 0.05 (two-tailed).

Reference range

The reference range for VWF:Ag level were mean ± 2 standard deviation 1.359 ± 0.1646 U/ml in blood group A, 1.338 ± 0.2224 U/ml in blood group B, 1.326 ± 0.4437 U/ml in AB blood group, and 1.233 ± 0.27301 U/ml in blood group O, based on testing 200 normal individuals.

Results

The hematological variables, PT, APTT, and FVIII: C activity showed no significant difference between the index cases and control groups while VWF:Ag and VWF:CB showed significant differences $P < 0.05$ [Tables 1 and 2].

Eight SNPs were detected in this study population rs216310 (T1547), rs1800385 (V1565 L), rs1800384 (A1515), rs1800383 (D1472H), rs1800386 (Y1584C), rs216311 (T1381A), rs216312 (intronic), and rs1800381 (P1337), [Table 3].

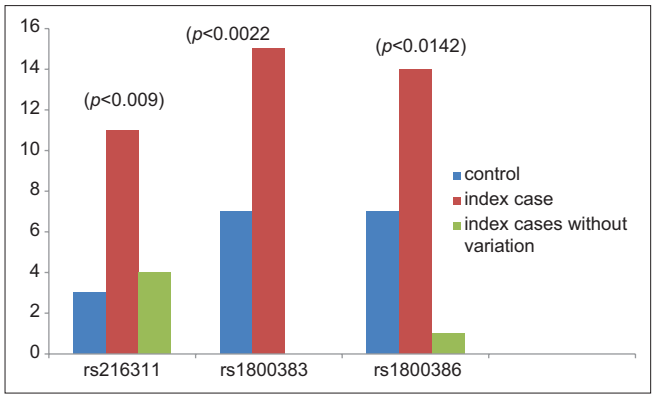

Figure 1: Association of rs216311, rs1800383, and rs18003386 with bleeding, $P < 0.05$ for the three single nucleotide polymorphisms

Table 1: Comparison of mean±2 standard deviation of hematological variables, prothrombin time, activated partial thromboplastin time, von Willebrand factor: antigen, von Willebrand factor:collagen binding, and Factor VIII:C for studied subjects

Variables	Mean±2SD		*P*
	Index cases	Control groups	
Plat×10^9/L	222.4±61.88	224.7±64.39	0.5859
PWD	15.97±0.403	16.0±0.421	0.9193
PCT (L)	2.124±0.537	2.097±0.488	0.8118
MPV (fl)	9.587±1.044	9.653±1.094	0.8811
Hemoglobin (g/dl)	13.04±1.60	13.45±1.32	0.2687
WBC×10^9/L	5.534±1.846	5.168±0.158	0.4851
PT (s)	22.09±7.795	21.06±4.348	1.000
APTT (s)	47.55±9.576	43.73±8.754	0.0864
FVIII:C (IU/dl)	80.78±11.57	79.11±6.085	0.4302
VWF:Ag (U/ml)	0.602±0.094	1.286±0.101	0.001
VWF:CB (U/ml)	0.464±0.328	0.879±0.309	0.0001

VWF=von Willebrand factor; CB=Collagen binding; Ag=Antigen; FVIII=Factor VIII; APTT=Activated partial thromboplastin time; WBC=White blood cell; PT=Prothrombin time; MPV=Mean platelet volume; PCT=Plateletcrit; PWD=Platelet distribution width

Three of the SNPs: rs216311, rs1800383, and rs1800386 associated significantly with bleeding [Figure 1]. The CDS located SNPs (rs1800384, 1800385), rs216310, and rs1800381 did not associate significantly with bleeding, $P > 0.05$. The SNPs rs216311, rs1800383, and 1800386 associated significantly with bleeding in this study. dbSNP: rs1800386 identified in our index cases have been reported previously[6,7] and in association with type 1 VWD in a Canadian population.

Discussion

The phenotypes of the study subjects showed a significant difference in the VWF:Ag and VWF:CB activity [Table 1] between index and control groups. Low VWF:Ag has been suggested as either a risk factor or a disease in VWD.[8] The ability of VWF to bind collagen (VWF: CB) is dependent on the presence of high-molecular-weight multimers. Decrease in VWF:CB relative to VWF:Ag (<0.7) is associated with qualitative VWD.

Of the eight SNPs detected, T1547 has been reported in association with type 3 VWD in a German population,[9] whereas in this study, T1547 was not associated significantly with bleeding [Figure 1]. However, Y1584C reported as not relevant in some studies or in association with type 1 VWD[6,10] was found to be associated significantly with bleeding in this study $P = 0.014$. T1381A and D1472H associated significantly with bleeding $P = 0.009$ and $P = 0.002$, respectively [Figure 1], but were reported with unknown pathogenicity[11,12] for North American, British, and Japanese populations. rs1800383 (D1472H), rs1800386 (Y1584C), and rs216311 (T1381A), despite being significantly linked with bleeding, were also detected in some of the control subjects. This agrees with the fact that the expression

Table 2: Clinical data, phenotypes, and detected single nucleotide polymorphisms for some of the index cases

Subject ID	Sex	Ethnic group	ABO	Reason for inclusion	Bleeding score	SNP detected	VWF:Ag (U/ml)	VWF:CB (U/ml)	FVIII:C (IU/dl)	CB/Ag
E92	Male	Igbo	O	GI bleeding, epistaxis	>8	D1472H, Y1584C, T1381A, rs216312, P1337	0.25	0.15	90	0.60
E69	Female	Igbo	O	Severe menorrhagia	>8	D1472H, T1547, V1565L, Y1584C, T1381A	0.26	0.25	80.4	0.96
E59A	Female	Igbo	O	Postpartum hemorrage	>8	D1472H, V1565L, Y1584C, T1381A, P1337	0.22	0.16	98.5	0.73
E19	Female	Yoruba	B	Postpartum hemorrage	>8	D1472H, T1547, V1565L, Y1584C, T1381A, rs216312	0.47	0.39	79.8	0.83
E10	Male	Yoruba	O	Epistaxis severe	<4	D1472H, T1547, Y1584C, T1381A, rs216312, P1337	0.37	0.25	96.4	0.68
E35	Female	Yoruba	B	Epistaxis/bleeding gum	<4	D1472H, V1565L, A1555, Y1584C, T1381A, P1337	0.48	0.33	80.4	0.69
E61	Male	Hausa	O	Epistaxis	<4	D1472H, T1547, V1565L, Y1584C, T1381A	0.42	0.56	76.4	1.33

SNP=Single nucleotide polymorphism; VWF=von Willebrand factor; CB=Collagen binding; Ag: Antigen; FVIII=Factor VIII

Table 3: Sequence variations identified in exon 28 of von Willebrand factor gene in the three major ethnic groups of Nigeria

SNP ID	Protein position/ amino acid change	Cases	Total	Hausa	Igbo	Yoruba
rs216310	T1547T	Control	10	3	4	3
		Index	9	4	1	4
rs1800385	V1565L	Control	10	3	4	3
		Index	9	3	2	4
rs1800384	A1555A	Control	13	3	5	5
		Index	2	0	0	2
rs1800383	D1472H	Control	7	2	3	2
		Index	15	5	5	5
rs1800386	Y1584C	Control	7	1	4	2
		Index	14	5	4	5
rs216311	T1381A	Control	3	1	1	1
		Index	11	3	4	4
rs216312	Intronic	Control	10	4	3	3
		Index	8	3	2	3
rs1800381	P1337P	Control	12	4	4	4
		Index	7	2	2	3

SNP=Single nucleotide polymorphism

of bleeding phenotype is highly variable.[4] Levels of plasma VWF are largely determined by genetic factors, with estimates of heritability in humans ranging from 25% to 32%, by pedigree analysis[13] to 66–75% in twin studies.[14] ABO blood group accounts for one-third of the genetic variations in VWF plasma levels. Loci responsible for the remaining two-thirds are being elucidated.[15] Studies from the European and Canadian joint studies on type 1 VWD have shown that disease diagnosis does not segregate with VWF genotype in approximately 50% of families, supporting the existence of additional genetic factors. Study on genetic modifiers of type 2 and type 3 VWD may help to explain the relationship of VWF genotype and the clinical phenotype.

Conclusion

The eight SNPs detected in this study have been reported in other populations, but the degree of their association with a bleeding phenotype varies for different populations. The three SNPs that associated significantly with bleeding in this study were also detected in the control subjects, thus explaining the phenotypic variability.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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