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Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University. KSA

Corresponding author: Fathelrahman Mahdi Hassan. Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University. KSA.E-mail: fathmaga@yahoo.com. ORCID ID: http//www.orcid.org/ 0000-0002-2630-3208.

Phenotypic and Genotypic Characterization of von Willebrand Factor Gene (Exon 18 and 20) in Saudi Healthy Individuals

Faisal M. Alzahrani, Nemat Aldossary, Fathelrahman Mahdi Hassan

ABSTRACT

Introduction: Von Willebrand disease (VWD) is an autosomal congenital bleeding syndrome that was described as being the most widespread genetic condition among men. In Saudi Arabia, the genotyping of the VWF gene is necessary to establish a diagnosis procedure for VWD. **Aim:** The current research, however, attempted to evaluate the phenotypic-genotypic association of the Von Willebrand factor (exon 18 and 20) gene in healthy subjects to establish effective molecular diagnostic strategies. **Methods:** This was a cross-sectional retrospective included 100 healthy people who have been chosen from King Fahad University Hospital. Whole blood samples were collected from all individuals, as well as the laboratory analysis was done using automatic analyzers for; platelet count, ABO blood group and coagulation parameters. DNA Sanger sequencing has been used to sequestrate the full exons 18 and 20. **Results:** In exon 18 of healthy people, three unusual sequence variations (1 missense and 2 synonymous; rs775479826, rs1286572448 and rs369828268) compared to other recorded VWF variations (3 missense and 1 synonymous; c.2365A > G, c.2385T > C, c.2344C > T and c.2340C > G). But in exon 20 only 1 synonymous novel (rs113240752) 1 registered VWF variations in missense (c.2555G > A) were identified. **Conclusion:** The present variants found on those participates could be a realistic approach to detect mutation in the VWF gene to illustrating the relationship of phenotypic and genetic abnormalities variables may lead to determining the functional effect in mutations specific to the Saudi people that can be used to develop a diagnostic tool for VWD in KSA.

Keywords: Phenotypic, Genotypic, von Willebrand Factor, Exon 18, Exon 20 Saudi Healthy.

1. INTRODUCTION

Von Willebrand disease VWD is an autosomal congenital bleeding syndrome that was described as being the most widespread genetic condition among men. The incidence rate hits around 1.2 %. In racial groups, the incidence for VWD appears similar as well as affects both females and males (1-3). All VWF protein defects in patients ' plasma is divided into two (qualitative and quantitative) variables. These changes occur due to differences in the VWF gene, which involve moderate to extreme mucocutaneous bleeding causing VWD with symptoms. VWD lists in three groups according to experimental results and clinical elements: type 1 VWD, type 2 VWD, which would be classified into four subtypes (2A, 2B, 2 M, and 2N) and type 3 VWD, respectively. VWF exons vary considerably, ranging around 41 base pairs (bp) as in the smallest (exon 50) to 1.6 kb as in the highest (exon 28)(4). During most of the genetic study of the VWF gene, several factors need to be considered. The presence of partial VWF, which is located on chromosome 22, was one of these complicated reasons. This VWF pseudo gene is just like the VWF gene and replicates the chromosome 12 sequence throughout exon 23 and exon 34, although (3%) small sequence difference. Therefore, caution must be taken in PCR to ensuring that the amplification is just for the required VWF sequence and the VWF gene was just a significant (4). Besides, the existence of single nucleotide polymorphisms (SNP) within annealing sites of the primary sequence can contribute to a lack of mutation and it could cover genetic defects (5). Due to the difficulty in phenotypically comparing between these patients and healthy people with VWF values, VWD patients could be identified difficultly, especially in mild cases. In Saudi Arabia, the genotyping of the VWF gene is necessary to establish a diagnosis procedure for VWD. This is

due to inadequate knowledge of the genetic basis of the condition, and the minimal findings were seen in KSA on VWF levels among healthy individuals. Also, VWD cases might be diagnosing, especially in mild cases, due to the complexity in phenotypically differentiating between these cases and healthy people with VWF levels (2). A few numbers of studies in Saudi Arabia have been focused on VWF at phenotypic and genotypic which may potentially lead to developing a diagnostic tool in the Kingdome.

2. AIM

The current research, however, attempted to evaluate the phenotypic-genotypic association of the Von Willebrand factor (exon 18 and 20) gene in healthy subjects in the eastern region of Saudi Arabia to establish effective molecular diagnostic strategies, contributing to the avoidance of the false, negative, and false-positive disorder and thus to the reduction of ineffective care.

3. METHODS

This was a cross-sectional retrospective empirical analysis that included 100 healthy people who have been chosen from King Fahad University Hospital's (KFUH) blood bank as donors. Saudi safe citizens ages 18-67, males and females who settle in Saudi Arabia's eastern province. Ethical permission was received from Imam Abdulrahman Bin Institutional Review Board (IRB). After telling them by information of the study and its goals, all volunteers received an oral and written consent form. All participants participating in the study at KFUH blood bank have written and signed informed consent; all people are asked to ask a few questions to assess the bleeding score of each. Whole blood samples have been drawn from all individuals in sodium citrate and EDTA tubes, as well as the laboratory analysis were done using automatic analyzers for; platelet count, ABO blood group and coagulation parameters. For study of VWF: Ag, VWF: RCo, and FVIII: C, plasma was isolated and aliquoted of citrate anticoagulated blood samples. ReliaPrepTM Blood gDNA Miniprep System, which provides pure genomic DNA (Promega, USA), also extracted DNA from entire EDTA blood sample. A number of primers have been used in this experiment to amplify PCR exons 18 as well as 20 VWF, focusing in the most common variants identified (6). After amplification of the DNA use different primers, electrophoresis of the agarose gel has been used to estimate the size of PCR products and to ensure that the DNA amplification was completed (6, 7). DNA products were differentiated by gel according to its size: large DNA fragments migrated slower than that of the smaller DNA fragments which really migrate more rapidly throughout the gel. The MOLEQULE-ON ® PCR/ Gel drug purification package was used before DNA sequencing to purify PCR samples. DNA Sanger sequencing (Biosystem ABI 3730X, Hong Kong) has been used to sequestrate the full exons 18 and 20 and establishes all detected hypothetical mutants. Genotypes were defined using the Sequined MassARRAY MALDI-TOF system for specific SNV markers (95 with lower allele frequencies (MAFs>5%) (Sequenom Inc., San Diego, California, United States). The data sets were evaluated and use both version 21 of SPSS software and the program Excel. Descriptive figures were examined on phenotypic results including platelets with examinations of coagulation. Independent T-test has been assessed for all coagulation features with aged and all coagulation characteristics with one another and $P<0.05$ has been used as the cut-off for statistical significance. Assessment of the Pearson association between age and phenotypic characteristics was also reported among all healthy Saudi participants who enrolled in this study.

4. RESULTS

No one of the 100 participants had bleeding problems as per the demographic survey answers. Gender distributions; around 72% were male but 28% were female with the an average range of 35.92 years, while the minimum age has been 18 years and the maximum age has been 65 years, most individuals (39%) ranged between 18 to 30 years old and most cases without a vWD family history while 4% had reported bleeding signs which is shown in Table 1. The VWF Ag, RiCof, and FVIII activity medians (normal ranges) were 100 (50-160%), 92 (50-200%), and 130.50 (60-150 %), respectively. Average level of VWF: Ag was 113% (range, 3.28), mean level of VWF: RCof was 106.65% (range, 1.66), and means level of FVIII was 140.89% (range, 3.34), as shown in Table 2. In Table 3, as correlated with VWF Ag, VWF activity (RiCof), and FVIII with blood group O and None blood group O; that there would be a clear positive association between the different levels of VWF Ag, FVIII and RiCof between both the different blood groups (p=0.00, 0.04, 0.00 respectively), these variables were reduced to around < 50% in these healthy subjects (1% VWF Ag, 9% RiCof and 2% VWF Ag). The total variations of the sequence found in exon 18; 4 were missense and 3 were synonymous, while in exon 20 they were both missense and synonymous. The consequences of SNPs and MAF of all VWF sequence variations in each of these exons were suggested in the previously recorded

Characteristic	n (%)
Age/ years Mean ± SD; 35.92± 11.96	
18-30	39(39)
31-40	25(25)
41-50	22(22)
> 50	14(14)
Sex	
Male	72(72)
Female	28(28)
Family history of VWD	
Yes	0 (0)
No	100 (100)
Family history of bleeding	
Yes	4 (4)
No	96 (96)

Table 1. Demographic data and characteristics frequency of Saudi healthy individuals

PLT count & Coagulation analysis	Mean	Median	SD	SE	Minimum	Maximum	Range	Arithmetic median, (25th to 75th percentiles)
PLT c/µ	269.48	263	73.32	7.332	59	519	460	263
PT seconds	13.49	13.3	0.72	0.072	11.4	15.8	4.4	13.3 (13-13.8)
APTT seconds	36.09	35.65	4.12	0.412	28.7	57	28.3	35.65 (33.65-37.92)
VWF Ag %	113	100	51	5	48	376	3.28	100 (0.85-1.302)
VWF: RiCof %	106.65	92	50	4.99	35	201	1.66	92 (0.68-1.317)
FVIII %	140.89	130.50	56	5.56	7	341	3.34	130.50 (1.027-1.68)

Table 2. Descriptive statistics of phenotypic data (platelets and coagulation investigation) among Saudi healthy individuals

Laboratory analysis	n (%)		
	Blood group O	Non- Blood group O	P. value*
VWF Ag, %			
< 50	1(1)	0(0)	0.00
50-80	20(20)	1(1)	
81-120	22(22)	24(24)	
121-160	9(9)	11(11)	
>160	2(2)	8(8)	
FVIII, %			
< 60	2(2)	0(0)	0.04
60-100	16(16)	6(6)	
101-150	23(23)	17(17)	
>150	14(14)	22(22)	
RiCof, %			
< 50	9(9)	0(0)	0.00
50-100	25(25)	19(19)	
101-150	13(13)	11(11)	
151-200	6(6)	15(15)	
> 200	1(1)	1(1)	

Table 3. The frequency of VWF Ag, FVIII and RiCof levels associated to Blood group O and Non- Blood group O among Saudi healthy individuals. *Chi-Square, Cramer's V, and Lambda were used to calculate p. value (<0.05 is considered as significant)

differences with their nucleotide and amino acid shifts. In exon 18 of healthy people, three unusual sequence variations (1 missense and 2 synonymous; rs775479826, rs1286572448 and rs369828268) compared to other recorded VWF variations (3 missense and 1 synonymous; c.2365A > G, c.2385T > C, c.2344C > T and c.2340C > G). But in exon 20 only 1 synonymous novel (rs113240752) 1 registered VWF variations in missense (c.2555G > A) were identified as listed in Table 4.

5. DISCUSSION

The VWD has been identified as one of the most severe genetic bleeding disorders worldwide (8). VWD is a common and complicated bleeding problem, including the diagnosis and treatment issues. New diagnostic approaches, including the use of bleeding testing tools and new VWF activity assays, can help to reduce some of those challenges. Even though VWD has a big effect on patient quality of life, it's always important to develop healthcare services. Adding recombinant VWF to existing therapeutic options would enable clinicians to continue adjusting the care to maximize individualized performance. Despite these developments, there was a need for more research to streamline diagnosis and improve care for the patients affected (9). So many studies have also shown that VWF levels interact with ABO blood groups; persons with O blood group have around 25% reduction VWF than those who have non-O blood groups (10, 11). In this experiment, the donor with O blood group had decreased VWF level below the comparison range lower bound (48 IU/dL), while the remaining O-individuals were normal VWF values. The

SNPs	Alleles	Origin Allele	Alternative Allele	Variation type	Exon	Consequence	MAF in ExAC*	Prediction	Nucleotide change	Amino acid change
rs1063856	T>C T>G	T	C/G	SNV	18	Missense	C=0.32321	Likely benign	c.2365A>G	p.Thr789Ala
rs1063857	A>G	A	G	SNV	18	Synonymous	G=0.32312	Likely benign	c.2385T>C	None
rs775479826	G>A	G	A	SNV	18	Missense	A=0.00001	None	None	None
rs61748471	G>A G>T	G	A/T	SNV	18	Missense	A=0.0001	Not provided	c.2344C>T	p.Arg782Trp
rs143904314	G>C	G	C	SNV	18	Missense	C=0.00031	Uncertain significance	c.2340C>G	p.Asn780Lys
rs1286572448	C>T	C	T	SNV	18	Synonymous	T=0.00002	None	None	None
rs369828268	G>C	G	C	SNV	18	Synonymous	C=0.00002	None	None	None
rs113240752	G>A	G	A	SNV	20	Synonymous	A=0.00002	None	None	None
rs216321	T>C	T	C	SNV	20	Missense	T=0.10005	Benign	c.2555G>A	p.Arg852Gln

Table 4. The commonly known and possible variations identified in exons 18 and 20. A According to GRCh37p13. Ex. AC, Exome Aggregation Consortium; MAF, minor allele frequency ;SNP, single nucleotide polymorphism; SNV, single nucleotide variants. *MAF observed in 100 individuals from the healthy Saudi population in the ExAC database. **For missense mutations, the predictions are classified according to damaging (1) or tolerated (0) using Mutation Tester.

new recommendations have established a cutoff value of VWF 30 IU/dL for VWD diagnosis, holding patients with rates above 30 IU/dL and below the lower guideline range limit throughout the low VWF gray zone (12). As found in previous VWF genetic research, patients may be less likely to undergo genetic abnormalities but may have considerable bleeding (13). This enables the low VWF rates, as suggested by Sadler et al., 2006 (14), a risk factor for bleeding. Genetic study is easier than phenotypic analysis due to the current different plasma practice tests necessary for VWD assessment and VWF plasma values induced by stress or related diseases, while VWF genetics were not affected by any of these factors. There may be, furthermore, certain challenges with the genetic testing of VWF; there is a considerable amount of variation in the VWF gene in healthy subjects and several variations previously reported as pathogenic have also been detected in these healthy persons, some of them at reasonably high frequencies, specifically in the African American demographic and possibly other minority groups, such as; p. M740I observed in African American region (15). Novel variations in this analysis should also be approached with caution, as modifications in DNA may not generally indicate improvements in the VWF protein described for the sample according to VWF variant (Scientific and Standardization Committee of the International Society of Thrombosis and Hemostasis at the University of Sheffield (http:/www.vwf.group.shef.ac.uk(14)). The results of this study defined the variants rs1063856 (c.2365A > G; p. T789A) and rs1063857 (c.2385T > C; p. Y795=) found in VWF exon 18, that also encodes part of the D′D3 assembly, functionally crucial for the binding of VWF plasma and FVIII activity levels which are in agreement with previous studies, these references were found in each of these rs1063856 (c.2365A > G; p. T789A) and rs1063857 (c.2385T > C; p. Y795=) (16-20). VWF variants c.2365A > G and c.2385T > C respectively induce VWF biosynthesis clearing and enhance VWF plasma levels, and also the usually inherited VWF variants could significantly impact the protein and could even refer to the hemostatic and thrombotic disease risk / severity according to Ahmad et al., 2018 (21). In this analysis, the variable c.2365A > G in exon 18 appeared associated with a higher levels of vWF: ag and development of FVIII: C, while polymorphism c.2555G > A in exon 20 was associated with a higher levels of FVIII and decreased aggregation of platelets induced by collagen (22). Amino acid replacement p. Arg782Trp (c.2344C > T) was detected in exon 18 in another donor with blood group O with normal laboratory parameters. This variant's MAF became rare < 1% in AAs. It is placed in the VWF D ' domain and has been suspected to affect the behavior or quantity of the released VWF protein and therefore can cause faulty folding of protein (23). A missense type c.2340C > G (p. Asn780Lys) was observed in exon 18 in a donor of 25 years of O blood group with healthy laboratory tests. This variant's minimum allele occurrence is uncommon < 1%. Also, there weren't enough details to justify the much more conclusive classification of this variant according to ClinVar in aggregates of evidence on genomic variation as well as its contribution to people's health.

6. CONCLUSION

This research was able to differentiate many variants; several were new as per the patterns that were missed throughout the previous MCMDM-1VWD report (20, 24) according to weaknesses in the reliability of the methodology being used and prevalence of SNP within PCR specific annealing sites relating to clerical error. The existence of many other genetic abnormalities in VWF in present study may influence the degree of VWF and also the severity of bleeding. The present variants found on those participates could be a realistic approach to detect mutation in the VWF gene to illustrating the relationship of phenotypic and genetic abnormalities variables may lead to determine the functional effect in mutations specific to the Saudi people that can be used to develop a diagnostic tool for VWD in KSA. Further studies are expected to explain the molecular pathophysiology involving bleeding and reduced rates of VWF. It is also suggested to do the same analysis on the full VWF coding area along with the intronic flanking regions, promoters and the 5' and 3' un-translated regions with big error bars. Genotyping of all VWF exons and intron-exon limits in every one of those persons with no coding sequence mutation that set that best range of VWD diagnostic phenotypic results. Precise diagnosis of VWD in exon 28 is most often challenging attributable to its heterogeneous appearance and high analytical variance or identification capacity. Therefore, exon 28 genotyping is indeed extremely beneficial due to the complexity and sequencing analysis of it could be highly recommended in order to possess the first and most common variants in future research.

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