ORIGINAL ARTICLE

Association of the 3467C>T mutation (T1156M) in the von Willebrand's factor gene with dominant type 1 von Willebrand's disease

Pilar Casaña[1], Francisco Martínez[2], Saturnino Haya[1], Carmen Espinós[1], José Antonio Aznar[1]

1.- Unidad de Coagulopatías Congénitas de la Comunidad Valenciana. Hospital Universitari La Fe. Avd. de Campanar 21. 46009 Valencia. Spain

2.- Unidad de Genética y Diagnóstico Prenatal. Hospital Universitari La Fe. Valencia. Spain

Corresponsal autor:

Dr. Pilar Casaña

Unidad de Coagulopatías Congénitas de la Comunidad Valenciana.

Hospital Universitari La Fe.

Avd. de Campanar 21. 46009 Valencia. Spain

E-mail: casanya_pil@gva.es

Tel.: +34-96-3868752, Fax: +34-96-3868752 or 718

Abstract

Type 1 is the most frequent form of von Willebrand's disease, which is characterized by a quantitative partial deficiency of von Willebrand's factor. At present, only two mutations located in the D3 domain (C1149R, C1130F) have been reported to cause the classic type 1 variant. The 3467C>T transition that predicts the T1156M amino acid change was detected in seven patients from one family and was not found in 110 normal alleles screened. This is a candidate mutation to cause dominant type 1 variant with complete penetrance. On the other hand, neither of the two mutations mentioned above has been detected in the other 15 families studied with type 1 or possible type 1 patients.

Keywords: Von Willebrand's disease; Type 1 von Willebrand's disease; Mutation detection

Introduction

Von Willebrand's disease (VWD) is the most common hereditary bleeding disorder in humans caused by a qualitative and/or quantitative abnormality in von Willebrand's factor (VWF), which is a large multimeric glycoprotein. Type 1 VWD, a partial quantitative deficiency of VWF, is the most frequent variant of the disease, representing approximately 70% of the cases. The VWF gene, located on the short arm of chromosome 12, spans 178 Kb in length and contains 52 exons [10]. A pseudogene on chromosome 22, spanning exons 23–34 of the VWF gene [11], shows about 97% homology. Currently, there are nearly 200 mutations registered in the VWF database, most of them associated with qualitative defects (http://mmg2.im.med.umich.edu/vWF/). Nevertheless, only two different missense mutations, located in the exon 26 of the VWF gene, have been associated with type 1 VWD and dominant inheritance. In this study, we report on the description of another missense mutation that cosegregates with type 1 VWD in one family.

Material and methods

Patients

Sixteen unrelated Spanish families, comprising 58 patients and 72 relatives, were included in a screening of mutations. The families were informed previously of the investigatory nature of the studies and gave their consent. The patients were diagnosed as type 1 or possible type 1 VWD according to consensus criterion for diagnosis of type 1 recommended by the Subcommittee on VWF of the ISTH [1, 5, 7]. The analytical data on the seven patients with the T1156M mutation are summarized in Table 1. The multimeric structure, detected by luminography, was normal for all of them. Mild-moderate bleeding symptoms such as bleeding after dental extraction, surgery, and

delivery were observed in the patients. They had good response to the vasopressin analogue (DDAVP).

Genetic analysis

The indirect analysis was performed as indicated in the caption to Fig. 1. The bi-point linkage analysis was conducted with the program Mlink from Linkage version 5.1 [9]. A fragment of 301 base pairs (bp), which cover the exon 26, was amplified with primers as previously reported [4]. The polymerase chain reaction (PCR) product was gene specific as checked by *AlwnI*. The search for mutations was carried out by single-strand conformational polymorphism analysis (SSCP) in 10% polyacrylamide (37.5:1) gels, electrophoresed at 5 W for 15 h, and silver stained. The mutation was identified by automatic sequencing and confirmed by *NlaIII* restriction analysis (see legend to Fig. 1). In addition, restriction analysis with *AluI* and *ItaI*, respectively, was used to search for 3445T>C (C1149R) and 3389G>T (C1130F), the other two mutations known to cause dominant type 1 VWD.

Results and discussion

The SSCP analysis showed an anomalous electrophoretic mobility in the samples from seven patients in one family (data not shown). All the other samples analyzed showed the normal pattern. The automatic sequencing of the 301 bp fragment of genomic DNA in one patient revealed the transition 3467C>T that predicts the amino acid change threonine by methionine (ACG>ATG) at codon 1156 (T1156M). This nucleotide change creates a restriction site for the *NlaIII* enzyme. As shown in Fig. 1B, the restriction analysis confirmed that the seven patients carried the mutation in heterozygosis and led us to rule out this change in 110 unrelated normal chromosomes. On the other hand, neither of the C1149R and C1130F mutations was detected in any of

the patients studied. A study on 15 unrelated type 1 VWD patients also reported failure to detect these two mutations [8]. The authors of the present report are currently engaged in the study of the remaining regions of the VWF gene.

In the linkage analysis on the reported family here, a LOD score of 2.70, very close to the significance threshold of 3, was obtained either on the mutated allele or the haplotype obtained by combining the four microsatellite markers.

Diagnosis of von Willebrand's disease may pose problems for clinical practice, particularly in the most frequent form, type 1 [1, 6]. Knowing which mutations cause VWD has obvious advantages regarding the diagnosis and management of VWD. Although direct genetic analysis may currently be an option for the qualitative variants, only seven different mutations have been recorded for type 1 VWD in the database. Only three of them are associated with the classic type 1 dominant form, one of which, the T1156M mutation, is described in this paper. Functional studies have demonstrated retention of the mutated subunits in the endoplasmic reticulum for C1149R mutation [4], which are localized in the D3 domain, a region with many cysteine residues implicated in the multimerization of VWF. The T1156M (3467C>T) mutation does not occur in a cysteine, but is localized between two proximate cysteines. The change of a polar amino acid (T) by another with a nonpolar radical containing a sulfur group (M), which could alter the protein conformation in this region and interfere with the disulfide bond formation, may induce a similar mechanism to that proposed for C1149R and C1130F mutations with dominant negative effect [2, 4].

The CG dinucleotides are mutational hot spots in many genes. The transition described here occurred at a CG dinucleotide. Holmberg recorded the same mutation in a Swedish patient in the database, although the clinical and analytical data from this patient have

not been detailed. Judging from the foregoing account, the T1156M is a candidate mutation as a cause of dominant type 1 VWD with complete penetrance. Direct detection of this mutation, therefore, might help improve diagnosis of type 1 VWD patients.

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Figure Legend

Fig. 1 A Polymorphism segregation analysis. *Squares* represent males, *circles* females, and *filled symbols* affected members; the propositus is indicated by an *arrow*. The following microsatellites were analysed: VNTR3, VNTR1, and VNTR2 (all of them located in intron 40 of the VWF gene) [3], and another in the promoter region (VWP) [12]. **B** Detection of the 3467C>T (T1156M) mutation by restriction analysis in genomic DNA. The digestion with *NlaIII* of the 301 bp fragment generated fragments of 38, 204, and 59 bp from normal alleles, and 38, 149, 55, and 59 bp from mutated alleles. The fragments were analysed in 10% polyacrylamide gels (29:1) and silver stained. Lanes 1 and 17: 1-kb DNA marker. Lanes 3, 4, 5, 9, 13, 14, and 15: heterozygotes for the mutation. Lane 16: nondigested sample

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Table 1 Analytical data from patients with T1156M mutation

	BG	FVW:Ag (U/dl)	FVW:RCo (U/dl)	FVIII:C (U/dl)
II:1		39	27	80
II:3	O^+	37±3	29±2	69±8
III:4		33±5	26±5	75±15
III:6	O^+	28±9	17±4	62±15
III:8	O^+	27±1	17±1	59±3
IV:1	A^-	36±5	31±5	68±11
IV:2	A^+	35	20	70
Normal		45–145	50–150	60–140

BG blood group, *FVW:Ag* antigen of von Willebrand's factor, *FVW:RCo* ristocetin cofactor, *FVIII:C* factor VIII coagulant. The mean and standard deviation are indicated when three or more determinations were carried out