



**HAL**  
open science

## Novel missense mutation c.2685G>C (p.Q895H) in gene associated with very low levels of VWF mRNA

Noelia Cabrera, Pilar Casaña, Ana Rosa Cid, Saturnino Haya, Andrés Moret, Jose Antonio Aznar

### ► To cite this version:

Noelia Cabrera, Pilar Casaña, Ana Rosa Cid, Saturnino Haya, Andrés Moret, et al.. Novel missense mutation c.2685G>C (p.Q895H) in gene associated with very low levels of VWF mRNA. *Annals of Hematology*, 2008, 88 (3), pp.245-247. 10.1007/s00277-008-0576-7 . hal-00477970

**HAL Id: hal-00477970**

**<https://hal.science/hal-00477970>**

Submitted on 30 Apr 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Novel missense mutation c.2685G>C (p.Q895H) in *VWF* gene associated with very low levels of VWF mRNA

Noelia Cabrera · Pilar Casaña · Ana Rosa Cid ·  
Saturnino Haya · Andrés Moret · Jose Antonio Aznar

Received: 8 February 2008 / Accepted: 21 July 2008 / Published online: 20 August 2008  
© Springer-Verlag 2008

**Abstract** Homozygous patients for null alleles in *VWF* gene show a severe von Willebrand phenotype, whereas compound heterozygous patients only show the phenotype of the expressed allele. Five members of the same family were studied. The two patients showed borderline VWF levels, a mild factor VIII (FVIII) deficiency and a decrease of the binding of VWF to exogenous FVIII. The genetic analyses of the *VWF* gene confirmed that the patients were compound heterozygous for c.2561G>A (R854Q) and c.2685G>C (p.Q895H) mutations. The latter, is located in the 3' extreme of exon 20, and it has not been previously described. Studies of the cDNA from platelet mRNA were performed to investigate the expression of p.H895 allele. The loss of heterozygosity at the cDNA level suggests a lack of expression of the p.H895 allele. The overall studies can explain the type 2N phenotype of the two patients, since the allele carrying the new missense mutation p.Q895H has shown a low expression of *VWF* gene.

**Keywords** *VWF* gene · von Willebrand disease · Missense mutation · Low expression · Type 2N · Factor VIII binding capacity

### Introduction

Von Willebrand disease (VWD) is the most common inherited bleeding disorder in humans, is heterogeneous, and results from quantitative and/or qualitative defects in the von Willebrand factor (VWF). VWF is a large multimeric glycoprotein synthesized in the endothelial cells and in the megakaryocytes. It is required for platelet adhesion to the injured subendothelium and stabilizes coagulation factor VIII (FVIII) in the plasma [1]. The *VWF* gene is localized on chromosome 12, spans 178 kb, contains 52 exons, and is transcribed into an 8.8-kb mRNA [2]. In recent years, many molecular defects of the *VWF* gene, associated with different VWD types, have been identified. (Sheffield University VWF <http://www.vwf.group.shef.ac.uk/>).

VWD type 3 is the most severe variant and is characterized by extremely low or undetectable levels of VWF and by a recessive inheritance. Different mutation types have been detected in severe VWD patients, such as frameshift, nonsense, splice site, and missense mutations. Large gene deletions are not very frequent and are usually associated with inhibitor development. Loss of heterozygosity in the cDNA VWF from the platelets indicates the presence of a null allele [3], although usually the specific molecular defect was unknown. Homozygotes for null alleles produce a total reduction of VWF levels in plasma, heterozygotes, generally, are asymptomatic, and compound heterozygotes for a null allele and other genetic defect show the phenotype of the expressed allele. Furthermore, the presence of null alleles could explain the variable penetrance among some families [4].

We now report one missense mutation c.2685G>C (p.Q895H), which produces a very low level of VWF mRNA. This mutation, combined with the common

N. Cabrera · P. Casaña (✉) · A. R. Cid · S. Haya · A. Moret ·  
J. A. Aznar  
Unidad de Coagulopatías Congénitas.  
Hospital Universitario La Fe.,  
Av. Campanar 21,  
46009 Valencia, Spain  
e-mail: casanya\_pil@gva.es

**Table 1** Phenotype and genotype data of the family

Subject	FVIII:C (IU/dL)	VWF:Ag (IU/dL)	VWF:RCo (IU/dL)	VWF:FVIII B/VWF:Ag	Mutation
I:1	103±49	61±28	74±20	0.98	[p.Q895H]
I:2	121	74	121	n.a.	[p.R854Q]
II:1	15±3	31±6	32±2	0.1	[p.R854Q] + [p.Q895H]
II:2	19±2	32±2	38±5	0.1	[p.R854Q] + [p.Q895H]
II:3	84	70	114	1.05	none
Normal	60–140	48–140	50–170	~1	

The mean and SD are shown when three or more determinations were performed.  
n.a. Not available

p.R854Q mutation, which in homozygosity causes type 2N VWD, allows the clinical expression of just the p.Q854 allele.

### Materials and methods

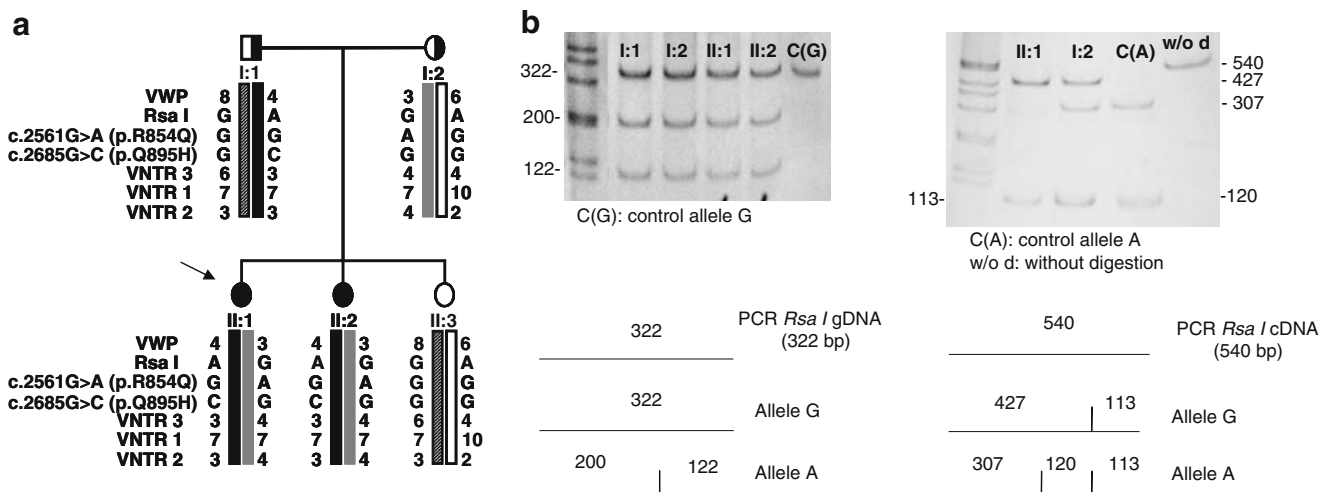
Five members belonging to the same family were studied. They gave their consent after they were informed of the kind of study they were to take part in.

For diagnosis, determination of FVIII procoagulant activity (FVIII:C), VWF antigen (VWF:Ag), ristocetin cofactor (VWF:RCo), VWF collagen-binding capacity (VWF:CB), and analysis of the multimeric structure were performed as previously described [5]. The VWF-binding assay (VWF:FVIII B) was carried out with minor changes to the method previously reported [6].

The segregation polymorphism analyses were performed as previously described [5]. The screening of the 52 exons

and their boundary exon/intron of the *VWF* gene was carried out in the index case by single strand conformation polymorphism analyses (SSCPs), conformation-sensitive gel electrophoresis (CSGE), and sequencing of abnormal patterns.

To analyze the expression of *VWF* gene alleles, platelet-rich plasma (PRP) was obtained by centrifugation at 150×g for 10 min at 20°C. Platelet pellets were immediately prepared to extract platelet RNA or frozen at –80°C. Platelet RNA was prepared from these pellets with *RNeasy Total RNA kit*. Platelet mRNA was reverse transcribed using the *StrataScript First Strand cDNA Synthesis Kit*. The cDNA was subsequently amplified by polymerase chain reaction to obtain a 540-bp fragment spanning from exon 17 to 21 of *VWF* gene. Restriction endonuclease assays with *MspI*, *HpyCH4IV*, and *RsaI*, were performed as recommended by the manufacturer and were analyzed by electrophoresis on 10% acrylamide/bis (29:1) gels and silver stain.



**Fig. 1** **a** Pedigree from family with the segregation of polymorphisms and the p.R854Q and p.Q895H mutations in *VWF* gene. *Solid symbols* represent affected members, and *half-filled symbols* represent the heterozygous state. The *arrow* marks the index case. **b** *RsaI* restriction analysis at gDNA and cDNA level. The resulting 322-bp

fragment in gDNA and the 540-bp fragment in cDNA are digested by *RsaI*, and the predictive size of the bands is given for the allele A and G, corresponding to the presence or the absence of the polymorphic *RsaI* site

## Results and discussion

The proband (II:1) and one of her sisters (II:2), both blood group O, showed mild FVIII deficiency ( $15 \pm 3$  and  $19 \pm 2$  IU/dL respectively), below borderline VWF:Ag, VWF:RCo, and VWF:CB levels, and normal multimeric structure. The two patients were classified as VWD 2N based on marked decrease of the capacity of plasma VWF to bind exogenous FVIII. In addition, plasma VWF levels from parents were normal, and the father showed normal VWF:FVIII/VWF:Ag ratio (Table 1). Family segregation analyses of *VWF* gene markers showed that two of the sisters shared the same haplotypes (Fig. 1a). SSCP analyses showed an abnormal pattern in the exon 20 fragment. The DNA sequence analysis of this fragment revealed two mutations: c.2561G>A (p.R854Q) and c.2685G>C (p.Q895H). We did not find any other candidate mutations in the rest of the sequences of the *VWF* gene analysed. Family study of the p.R854Q mutation was performed by restriction enzyme digestion with *MspI*. To corroborate the new nucleotide substitution c.2685G>C (p.Q895H), we digested the 301-bp fragment corresponding to exon 20 with the restriction enzyme *HpyCH4IV*, since it generates a new restriction site. The analyses performed confirmed that the father carried the c.2685G>C (p.Q895H) change, the mother carried the c.2561G>A (p.R854Q) mutation, whereas the two sisters were compound heterozygous for the two mutations.

The *HpyCh4IV* restriction analysis was performed in at least 100 normal chromosomes in order to rule out the possibility of this new change being a polymorphism.

The two sisters with both mutations, [p.R854Q] + [p.Q895H], showed a marked decrease in the ability to bind exogenously added FVIII. Therefore, it might be thought that the new p.Q895H mutation on the second VWF allele may explain the observed type 2N VWD phenotype (Table 1). However, taking into account that the guanine 2685 is the last nucleotide in the 3' extreme of exon 20, a splice site prediction program was used in order to evaluate if this new mutation affects this donor splice site ([http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)). In the normal sequence, the donor site was predicted with a score of 0.86. For the changed c.2685G>C (p.Q895H), this score was reduced to 0.46. All this suggests that, in the p.H895 allele, an incorrect intron splicing could occur and that may affect the mRNA stability. To evaluate the expression of *VWF* gene in both alleles, we studied the platelet mRNA.

We performed restriction analyses with *RsaI* enzyme, since all the family members were heterozygotes for the polymorphism 15/292 A/G (p.T/A789). The two sisters (II:1, II:2) and their father (I:1) showed, at cDNA level, a loss of heterozygosity of the allele A which is associated with the changed c.2685G>C (p.Q895H) (Fig. 1b). This loss of heterozygosity suggests that the p.H895 allele is silent at mRNA level. The mother showed a heterozygous pattern both at gDNA as at cDNA level, which indicates that both alleles are expressed.

In summary, our patients are compound heterozygous for both missense mutations, p.Q895H and p.R854Q, as only the latter is expressed; their phenotype is characteristic of the recessive type 2N variant. Heterozygous family members for the p.Q895H mutation, as well as for p.R854Q mutation, result in asymptomatic phenotypes.

Furthermore, future in vitro expression studies with recombinant VWF could confirm whether the p.Q895H mutation is really the cause of low transcription of the *VWF* gene.

**Acknowledgments** This work was partly supported in part by CSL Behring, and by FIS grant no. PI020612 (Spain). We wish to thank all the staff of the “Unidad de Coagulopatías Congénitas de la Comunidad Valenciana” for their technical and clinical assistance.

## References

1. Ruggeri ZM, Zimmerman TS (1987) von Willebrand factor and von Willebrand disease. *Blood* 70:895–904
2. Mancuso DJ, Tuley EA, Westfield LA, Worrall NK, Shelton-Inloes BB, Sorace JM, Alevy YG, Sadler JE (1989) Structure of the gene for human von Willebrand factor. *J Biol Chem* 264: 19514–19527
3. Nichols WC, Lyons SE, Harrison JS, Cody RL, Ginsburg D (1991) Severe von Willebrand disease due to a defect at the level of von Willebrand factor mRNA expression: detection by exonic PCR-restriction fragment length polymorphism analysis. *Proc Natl Acad Sci USA* 88:3857–3861
4. Eikenboom JC, Reitsma PH, Peerlinck KM, Briet E (1993) Recessive inheritance of von Willebrand's disease type I. *Lancet* 341:982–986
5. Casaña P, Martínez F, Haya S, Espinos C, Aznar JA (2001) Significant linkage and non-linkage of type 1 von Willebrand disease to the von Willebrand factor gene. *Br J Haematol* 115:692–700
6. Casonato A, Pontara E, Zerbinati P, Zucchetto A, Girolami A (1998) The evaluation of factor VIII binding activity of von Willebrand factor by means of an ELISA method. *Am J Clin Pathol* 109:347–352