Q1311X: a novel nonsense mutation of putative ancient origin in the von Willebrand factor gene

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Summary

Type 3 von Willebrand disease, a recessive autosomally inherited bleeding disorder, refers to complete deficiency of von Willebrand factor (VWF). The novel Q1311X mutation was detected in the homozygous state in four Spanish patients from two apparently unrelated families of gypsy origin. The lack of specific amplification of platelet VWF cDNA from two of the patients indicates reduced levels of mutated gene expression. The similar haplotype linked to mutated alleles suggests a common origin. On the basis of the two instabilities observed and the estimated mutation rate of the microsatellites of intron 40 of the VWF gene, we can estimate that this mutation could have arisen about 2300 years ago.

Keywords: von Willebrand disease, type 3 VWD, nonsense mutation, tetranucleotide repeats, microsatellite instability.

INTRODUCTION

Type 3 von Willebrand disease (VWD) is a recessive hereditary severe bleeding disorder caused by a total quantitative deficiency of von Willebrand factor (VWF). VWF is a large multimeric glycoprotein that is synthesized in the megakaryocytes and endothelial cells from an mRNA of 8.8 kb. The VWF gene spans 178 kb in length and contains 52 exons (Mancuso et al, 1989). A pseudogene, spanning exons 23±34 of the gene, shows about 97% homology (Mancuso et al, 1991). Currently, there are nearly 200 mutations registered in the VWF database (http://mmg2.im.med.umich.edu/vWF/), but most of them are associated with qualitative defects as the different phenotypes are confined to specific regions of the VWF. By contrast, the mutations in quantitative variants may lie on the whole VWF gene, and characterizing them is a time consuming and difficult process. Only five different nonsense mutations have been recorded in type 3 VWD. In this study, we report on the characterization of a new nonsense mutation in the A1 domain of VWF, associated with reduced levels of platelet mRNA, that was found in two apparently unrelated families of gypsy origin.

MATERIAL AND METHODS

Patients. The laboratory and clinical data on the four patients with type 3 VWD are summarized in Table I, together with those of 10 relatives. In family 2, the probands' parents are cousins. No multimers were detected by luminography (detection limit , <0.1%) in the four patients, while the multimeric structure was normal for their relatives.

Genetic analysis. The search for mutations was carried out by single-stranded conformation polymorphism (SSCP) analysis in a VWF gene-specific fragment (exon

28), as previously described (Casaña et al, 1998). The mutation was identified by automatic sequencing and was confirmed by MaeI restriction analysis (see legend to Fig 1). The total RNA was purified with the RNeasy kit (Qiagen) from the platelets in a 20-ml sample of citrated blood. Reverse transcription was carried out using the reverse transcription polymerase chain reaction (RT-PCR) kit (Stratagene), and the specific cDNA amplification was performed with Amplitaq Gold (Perkin Elmer). To analyse the VWF cDNA (exon 27±28), a 461-bp fragment was amplified (nucleotides 3645±4105 of cDNA). In addition, a 274-bp fragment of factor (F)VIII Cdna (exons 11±13) was analysed as a control (Pieneman et al, 1995).

RESULTS

Single-stranded conformation polymorphism analysis of the 487-bp DdeI-digested fragment showed an abnormal electrophoretic pattern in the four patients. Automatic sequencing revealed the 3931C>T transition that gives rise to a TAG stop codon at codon 1311 of the VWF instead of the normal glutamine (Q1311X). This transition, not previously described, was confirmed and detected in other family members by *Mae*I digestion (Fig 1B and D). It was not detected in 130 unrelated chromosomes.

The fact that the four patients showed the same haplotype except for the VNTR3 polymorphism (Fig 1A and C) suggests a common ancestral origin for the three unrelated chromosomes bearing the mutation. Once the mutation rates (μ) of the linked microsatellites are known, it may be possible to estimate the age of the mutation, assuming a constant rate neutral mutation process. The number of microsatellite variations that would have accumulated in the individuals after t generations is Poisson

distributed with a mean $\lambda = \mu t$ (Kaplan et al, 1994). To our knowledge, only six instabilities of any of the three microsatellites in intron 40 have been reported elsewhere (Eikenboom et al, 1993; Haddad & Sparrow, 1997; Brinkmann et al, 1998). In our laboratory, we detected one instability in VNTR2 (Casaña et al, 1995) and another one in VNTR3 in a total of 358 meiotic events analysed for the three microsatellites. On the basis of these data, and combining the mutability rate of these three microsatellites, i.e. eight instabilities detected in a total of 4201 allele transmissions, we estimated an averaged mutation rate of $\mu = 0.0019$ [95% confidence interval (CI) = 0.00099-0.00381]. Because of their low mutability rates, the remaining polymorphisms were negligible. The nine tetranucleotide alleles present in the three unrelated chromosomes carrying the Q1311X mutation have undergone at least two instabilities, which correspond to a mean of $\lambda = 0.2222$ (2/9). This gives a total time ($t = \lambda/\mu$) of 117 generations, which, assuming a generation time of 20 years, would imply 2340 years as a likely antiquity of the common ancestor.

Studies by RT-PCR yielded the expected 461-bp fragment of VWF in the control and in the two heterozygous females analysed, but this fragment was undetected in the two homozygous patients studied. The mutation was detected in the cDNA of one heterozygote, whereas in the other one only the normal allele was present (data not shown).

DISCUSSION

The 3931C>T transition that gave rise to a truncated protein in the A1 domain of VWF has not been reported previously. Three of the five different nonsense mutations

summarized in the database are recurrent and have been found in patients of Finnish, Swedish, Dutch, German,

Turkish, Japanese and Italian origin. The 3931C>T transition does not affect CG dinucleotides. This, together with the fact that the patients have similar haplotypes, is highly suggestive of their common origin. The estimate of about 2300 years can only be taken as tentative. Nevertheless, the study of more chromosomes carrying Q1311X would enable a more accurate estimation of the mutation age. Our data suggest that this mutation, detected so far in only four Spanish patients of gypsy origin, could be previous to the first dispersion of the original gypsy population from north-west India. The oldest record of this event dates from the year 1025, and by the middle of the fifteenth century the gypsies were settled in Europe from Denmark to Spain. Consequently, given that the Q1311X mutation could be ancient and widely distributed, the direct detection of this might help to improve diagnosis of type 3 VWD patients, permit earlier identification in heterozygotes and allow more accurate genetic counselling.

A very low level of mutant VWF mRNA was reported earlier for a heterozygote type 3 VWD patient for the mutation R2535X (Eikenboom et al, 1992). Nonsense mutations for a variety of different genes have been associated with dramatic reductions in their mRNA levels (reviewed by Cooper & Krawczak, 1993). However, another mutation located upstream (2680delC, in exon 18) showed significant levels of mRNA (Mohlke et al, 1996). Our cDNA studies are consistent with reduced levels of the VWF mRNA in platelets from homozygous patients. The VWF cDNA studies in the two heterozygotes showed variable results. These discrepancies might represent different degrees of degradation/processing of mRNA in the cell, and different stages of cell differentiation could have practical implications for the detection of silent alleles.

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