Premature termination codon mutations in the Von Willebrand factor gene are associated with allele-specific and position-dependent mRNA decay

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Nonsense-mediated mRNA decay (NMD) is an intron-dependent RNA-degradation pathway responsible for depleting transcripts containing premature termination codons (PTCs), presumably to control the synthesis of truncated proteins, potentially deleterious to cells. PTC-bearing (PTC+) mRNAs are unstable only when the PTC is located more than 50-55 nucleotides upstream of the last intron. However, not all genes undergo NMD. Among coagulation genes, NMD was demonstrated for factors V, XI, and XIII, whereas it was shown to be inactive for fibrinogen (FGA, FGG) and factor VIII (FVIII) genes (Online Supplementary Table 1).

Von Willebrand factor (VWF) is a multimeric glycoprotein, synthesized by endothelial cells and megakaryocytes, promoting both platelet adhesion to the subendothelium at sites of vascular injury, and platelet-platelet interactions in high shear-rate conditions. It also binds and stabilizes FVIII. Quantitative VWF deficiency can be classified as partial (VWD1) or complete (VWD3), whereas qualitative defects (VWD2) are subdivided into 4 main types: VWD2A, VWD2B, VWD2M, VWD2N. The aim of this study was to investigate whether PTC-introducing mutations in the VWF gene are associated with NMD. To this purpose, three unrelated Italian probands (P1-P3), heterozygous for at least one truncating mutation, were studied (Figure 1). Their main clinical characteristics are listed in Supplementary Table 2.

P1 has VWD2N and is compound heterozygous for the previously reported R854Q mutation (c.2561G>A, exon 20) and the novel c.2546+3G>C splicing defect (intron 19). P2 has VWD1 caused by compound heterozygosity for 2 novel mutations: C1927R (c.5779T>C, exon 34) and c.8155+6T>C (intron 50). P3 is heterozygous for the VWD3-causing c.6182delT mutation (exon 36).

To evaluate the effect of the novel c.2546+3G>C and c.8155+6T>C splicing mutations on VWF pre-mRNA processing, cDNA regions spanning exons 18-21 and 49-52 were amplified by RT-PCR from platelet- and lymphocyte-derived mRNA of each patient. Sequencing of RT-PCR products demonstrated that c.8155+6T>C causes the skipping of exon 50 (Figure 1B), leading to a PTC in exon 51 (for details on methods, see Supplementary Materials). Concerning c.2546+3G>C, a product lacking exon 19 could be amplified and sequenced only after a second semi-nested PCR (Figure 1A); this mutation would lead to the introduction of a PTC in exon 20. A very low amount of the same skipped transcript could be detected also in the control individual, indicating the existence of a physiologic aberrant splicing event.

To investigate whether the two PTC-introducing splicing defects are associated with mRNA degradation, a fragment containing the relevant missense mutation was PCR amplified from genomic DNA and from platelet and lymphocyte cDNAs of P1 and P2, and sequenced.

Concerning P1, the product obtained from genomic DNA resulted heterozygous for R854Q, whereas platelet- and lymphocyte-derived RT-PCR products were homozygous for this missense substitution (Figure 2A), confirming that the PTC allele was degraded. As for patient P2, the C1927R mutation was detected in the heterozygous state both on genomic DNA and on cDNA (Figure 2A), suggesting that the PTC allele is not subject-
the GeneMapper v4.0 software. On genomic DNA, the wild-type/mutant ratio was equal to ~1, as expected. Conversely, a degradation of 91.2% and 86.1% of the transcripts is PTC-position dependent. Last but not least, we were able to measure the extent of degradation of the transcripts is PTC-position dependent. Moreover, we confirm that NMD susceptibility of VWF might be a modulator of inhibitor development.

Given the effects of VWF inhibitors, i.e. ineffectiveness of replacement therapy and anaphylactic reactions to treatment, it would be important to establish if NMD might be a modulator of inhibitor development. Considering that some PTC-introducing mutations produce truncated VWF proteins are unlikely to be produced as a result of mRNA degradation, a topic on which conflicting data were reported in the literature.4-6 Moreover, we confirm that NMD susceptibility of VWF transcripts is PTC-position dependent. Last but not least, we were able to measure the extent of degradation of the c.6182delT transcript (~90%).

Figure 2. Missense mutations and 1-bp deletion analyses. The central panel shows a schematic representation of part of the VWF gene (introns 18-21 and introns 33-37; exons are represented by boxes, introns by lines, and are not drawn to scale). Panel A: electropherograms of the regions surrounding the missense mutations sequenced on the genomic DNA and on platelet and lymphocyte cDNAs. The position of the mutations is boxed. Panel B, left, shows the electropherograms of the region surrounding the 1-bp deletion obtained by sequencing PCR-amplified fragments from genomic DNA as well as platelet and lymphocyte cDNAs of P3. Panel B, right: GeneMapper windows displaying fluorescence peaks corresponding to wild-type and mutant fragments obtained from genomic DNA as well as platelet and lymphocyte cDNAs. The areas of the fluorescence peaks corresponding to the mutant and wild-type PCR fragments were measured by the GeneMapper v4.0 software. The X-axis represents GeneMapper data points and the Y-axis represents fluorescence units (FUs).

References

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