Silent disruption: aberrant splicing in VWD

Jill M. Johnsen  BLOODWORKS NW; UNIVERSITY OF WASHINGTON

In this issue of Blood, Yadegari et al.1 report in a patient with type 1 von Willebrand disease (VWD) a novel synonymous von Willebrand factor (VWF) genetic variant and aberrant VWF splicing mechanism leading to VWF mRNA intron retention. The index patient presented with mucosal and provoked bleeding and low VWF level (VWF:Ag) and activity (VWF:GPIb) of 9 and 6 IU/dL, respectively. She also had a normal VWF multimer pattern and had a poor response to desmopressin. This is consistent with qualitatively normal VWF but low VWF due to defective VWF production and/or storage. Neither parent had a history of significant bleeding, but each had borderline-to-low VWF:Ag levels (49 and 44 IU/dL) and similarly low VWF:GPIb levels.

VWD is the most common inherited bleeding disorder in humans.2 Type 1 VWD, the most common form of VWD, is defined by quantitative deficiency of VWF, mucosal bleeding, and autosomal dominant inheritance. However, the diagnosis of VWD can be challenging due to variable penetrance (in which not all relatives with a deleterious VWF gene variant have clinical VWD), expressivity (in which relatives with VWD vary in the severity of bleeding), and overlap of milder VWD symptoms with the bleeding common in normal populations. As often occurs in evaluations of patients for VWD, the patient studied by Yadegari et al did not have all of the criteria for a diagnosis of type 1 VWD because of the absence of a family history. However, she could reasonably still be diagnosed with VWD due to her low VWF laboratory values and personal history of mucosal bleeding.2

Genetic studies of VWF for patients and families with VWD can be useful to inform diagnoses, to provide family and reproductive counseling, and to improve understanding of the molecular mechanisms that cause VWD. The VWF gene harbors all kinds of genetic variation, including single nucleotide variants (SNVs), insertions, deletions, and larger structural variants. In type 1 VWD, deleterious DNA variants have been identified throughout the VWF gene.2 In the performance of VWF gene sequencing, the heterogeneity in the phenotype of VWD and the size of and variation in the VWF gene itself pose significant challenges to detecting and accurately assigning significance to VWF genetic variants. This is particularly true for type 1 VWD, in which the clinical diagnoses can be less clear, and only ~65% of patients have an identifiable causative VWF gene variant.3 In the interpretation of VWF gene sequencing data, one must consider the presence of common, rare, novel, and ethnicspecific DNA variation in populations,4,6 the deep catalog of deleterious variants previously reported in patients with VWD,7 and the predicted functional impact of the variant on the VWF gene. Caution is warranted to avoid both over- and underassigning significance to VWF genetic variants in VWD patients. Additional information from family members and/or additional molecular studies are often needed to support accurate interpretation of the significance of any single VWF gene variant.

Yadegari et al performed VWF gene sequencing found that both the patient and her mother were heterozygous for a novel VWF variant c.7464C>T, p.Gly2488. Importantly, no other candidate DNA variants were detected. This synonymous variant resides well within the boundaries of VWF exon 44. Synonymous SNVs, in which the DNA change does not alter the predicted amino acid, have historically been thought of as “silent” and of little functional significance. However, synonymous variants generally have similar probabilities of being associated with disease as nonsynonymous variants8 and can exert biologically relevant effects through a variety of mechanisms ranging from changing critical regulatory sequences (splice sites, transcription factor binding sites, etc), to altering mRNA

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and other genes, and it has been postulated RNA structure (hash-marked exon) disrupting the consensus splice sites. However, variants mRNA splicing through disruption of genetic variants are thought to alter in VWF. Up to 10% of deleterious allele, resulting in mRNA retention of VWF truncated VWF protein.

Schematic of predicted effect on VWF splicing by the synonymous VWF c.7464C>T variant. (Left) Normally splicing begins with transcription of a VWF pre-mRNA (partial cartoon of VWF exons 44-45 shown on top), followed by docking of U1snRNA (yellow) for the 5’ (donor) splice site during pre-spliceosome formation (middle) and multiple intermediate steps (spliceosome assembly, catalysis, intron lariat formation; not shown); completion of splicing results in spliced mRNA (bottom). (Right) In silico model predicted effect of the synonymous VWF substitution c.7464C>T in exon 44 (dark star) shown transcribed in the pre-mRNA (top); the c.7464C>T variant is predicted to cause a hairpin in the secondary RNA structure (hash-marked exon) disrupting the VWF exon 44 5’s U1snRNA docking site (red Xs); the failure of U1snRNA to dock would result in retention of VWF intron 44 sequence and an abnormal and prematurely truncated VWF protein.

Further VWF protein expression studies in the patient and her mother supported a profound defect in VWF protein intracellular processing and secretion in the patient and VWF protein processing haploinsufficiency in the mother. As in so many other families with VWD, possible explanations include incomplete penetrance in the mother or an undetected deleterious element impacting VWF protein expression inherited from the father or arising de novo. RNA studies detected expression of a normally processed mature mRNA from the nonvariant VWF allele but also aberrant splicing from the c.7464C>T VWF allele, resulting in mRNA retention of ~2200 bp of sequence from VWF intron 44 and a predicted abnormal and prematurely truncated VWF protein.

Aberrant VWF mRNA splicing is known to cause qualitative and quantitative defects in VWF. Up to 10% of deleterious VWF genetic variants are thought to alter VWF mRNA splicing through disruption of consensus splice sites. However, variants more distal to consensus splice sites have been shown to influence splicing in VWF and other genes, and it has been postulated that both intron retention and distal intronic and exonic variants distal could mediate significant aberrant splicing in VWF. The synonymous exon 44 VWF c.7464C>T variant detected by Yadegari et al realizes this prediction, but retention of the VWF intron 44 sequence by c.7464C>T necessarily invokes a noncanonical mechanism as the variant is positioned far (85 bp) from the VWF exon 44 5’s consensus splice site. In silico modeling by Yadegali et al provides insight into the mechanism by predicting that the VWF c.7464C>T variant mediates formation of a pre-mRNA hairpin (see figure), creating a secondary structure which prevents normal recognition of the 5’ docking region for U1snRNA (a critical component of the spliceosome) and therefore disrupting the normal splicing expected at the exon 44–intron 44 boundary.

Thus, Yadegari et al identified a synonymous mid-exonic VWF variant allele and novel VWF aberrant splicing mechanism that account for ~50% of the VWF deficiency in this family. In the process of their investigation, Yadegari et al show the value in pursuing synonymous DNA variants and provide further evidence for aberrant splicing as a common mechanism in the pathogenesis of VWD.

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