in the WDR1 gene that encodes for Aip1. The predicted amino acid changes were localized to the conserved regions of C-terminal and N-terminal regions of the β sheet or resulted in missense mutations that are predicted to result in breakdown of the expressed protein. It is likely that all of these mutations significantly reduce the normal activity Aip1 to potentiate the severing activity of ADF/cofilin. The marked increase in neutrophil actin filament content, failure of these cells to form a normal polar structures with lamellipodia at the front, and the marked slowing of cell motility in response to chemoattractants all emphasize the central role of Aip1 in potentiating actin severing activity (see figure panel B). These exciting findings emphasize that the study of genetic diseases, combined with basic cell biology and biochemistry, can provide the fullest understanding of structure–function relationships and promises to provide future targets for controlling inflammation and improving host defense.

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VWD is the most common inherited bleeding disorder in humans. 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 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.

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. 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. In the interpretation of VWF gene sequencing data, one must consider the presence of common, rare, novel, and ethnic-specific DNA variation in populations, the deep catalog of deleterious variants previously reported in patients with VWD, 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 variants 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
structure (mRNA degradation, obscuring 5’ translation initiation), to influencing translation efficiency of the protein (codon bias, mRNA structure, etc). 8

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 allele, resulting in mRNA retention of ~2200 bp of sequence from VWF intron 44 and a predicted abnormal and prematurely truncated VWF protein.

Ablerrant 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. 9 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. 9 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’ 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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Silent disruption: aberrant splicing in VWD

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