Molecular genetic testing in von Willebrand disease: past, present, and beyond

Omid Seidizadeh[1,2] and David Lillicrap[3]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy and [3]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada

Correspondence: O. Seidizadeh
omid.seidizadeh@unimi.it

D. Lillicrap
david.lillicrap@queensu.ca

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Abstract

The gene for von Willebrand factor (VWF) was among the earliest genomic discoveries in the mid-1980s. It became feasible to use this new knowledge to better understand the genetic mechanisms responsible for von Willebrand disease (VWD) and to utilize the information to generate molecular genetic diagnostic testing strategies. Following initial studies demonstrating large structural variants in type 3 VWD patients, investigations focused on the genetic basis of the various type 2 forms of VWD, demonstrating that the pathogenic variants were localized to regions of the gene encoding functionally distinct domains of the VWF glycoprotein. These observations have resulted in increasing use of molecular genetic diagnosis as either the primary strategy for diagnosing type 2 forms of VWD or, more often, as confirmatory tests to substantiate the results of prior phenotypic analysis. In the meantime, genetic studies of type 3 were demonstrating that a wide range of pathogenic variants located throughout the *VWF* coding sequence were responsible for this severe phenotype. These studies also showed that ~15% of pathogenic variants responsible for type 3 were missense substitutions and that in ~25% of families this severe phenotype, classically thought to be recessive in nature, was transmitted as a semi-dominant trait. Finally, the most prevalent form of VWD, type 1/Low VWF, has proven to be the most challenging for routine molecular genetic testing, with *VWF* coding sequence variants identified in only ~65% of index cases. This review explores various aspects of molecular genetic testing for VWF, commemorating the 100th anniversary of VWD and the 40th anniversary of *VWF* cloning.

Introduction

The history of von Willebrand disease (VWD), has benefited enormously from advances in biomedical science, and no advance has been greater than the characterization of the von Willebrand factor gene (*VWF*) in 1985.[1-4]

While the genetic transmission of VWD had been apparent since the original description of Family S 100 years ago by Erik von Willebrand, the pathophysiology of VWD has been complicated by the fact that the critical protein, von Willebrand factor (VWF) circulates in plasma in a high affinity complex with factor VIII (FVIII),[5] the protein that is deficient or dysfunctional in hemophilia A. Thus, for many years, distinguishing the distinct structural identities and functions of VWF and FVIII was very challenging, and it was not until the 1970s that immunological assays confirmed for the first time that the two proteins were recognizably separate molecules.[6]

With the description of DNA sequencing technology in 1975,[7,8] the world of biomedical science was about to witness a dramatic expansion of possibilities with the cloning of genes that have relevance to medicine. As one early example, characterization of the coagulation genes in the 1980s was greeted by the expectation that this knowledge would advance our understanding of disease mechanisms, provide more precise diagnostic approaches for inherited disorders of hemostasis, and would enable opportunities for novel treatments including recombinant protein production and possibly gene therapy.[9] After what at times may have seemed a slow response to this new knowledge, the subsequent 40 years have seen dramatic enhancements in all three of the promised areas of biomedical molecular science – disease pathogenesis, genetic diagnosis, and genetic therapies.

In this article we focus our attention on the history, current utility, and future potential of molecular genetic diagnosis

of VWD, commemorating the 100th anniversary of the first description of VWD and the 40th anniversary of *VWF* cloning. Molecular genetic strategies were used predominantly for evaluating pathophysiological mechanisms in the years immediately following cloning of *VWF*. Then, as disease mechanisms and genotype-phenotype correlations were more confidently aligned, increasing interest in molecular genetic diagnosis followed. This was first used in the late 1980s and has progressively increased in utility over the past decade.

The von Willebrand factor gene and glycoprotein

The *VWF*, located on the short arm of chromosome 12 (12p13.31), encodes the VWF glycoprotein (Figure 1).[10,11] This gene spans ~178 kb and consists of 52 exons (with a ~8.7 kb cDNA), producing a prepropeptide of 2,813 amino acids; a signal peptide (22 amino acids), a propeptide (741 amino acids), and a mature VWF monomer (2,050 amino acids).[10,11] The VWF glycoprotein contains multiple functional domains that enable its adhesive and carrier roles in blood coagulation, while its multimeric size is precisely regulated by the ADAMTS13 enzyme to avoid the generation of pathological thrombosis. More than 95% of the *VWF* coding sequence is involved in structural domains, many of which are repeated or shared with other proteins.[12] These domains are arranged in the following order: D1–D2 (VWF propeptide)–D'–D3–A1–A2–A3–D4–C1–C2–C3–C4–C5–C6–CK (Figure 1).[13] Of note, a partial, unprocessed VWF pseudogene (*VWFP*) has been mapped to chromosome 22 (22q11.2), spanning approximately 25 kb, from *VWF* exon 23 to exon 34, with 97% homology to the *VWF*.[11] The pseudogene can occasionally participate in pathogenic gene conversion events with the chromosome 12 locus, and in the diagnostic laboratory care must be taken to ensure that the sequences being evaluated are derived from the chromosome 12 gene.[14,15]

History of the discovery of *VWF* and its genetic testing

The early to mid-1980s witnessed a revelation of new knowledge relating to coagulation proteins, with the cloning of the human genes for FVIII, factor IX and VWF all occurring between 1982-1985.[1-4,16,17] During the preceding decade immunological tests had definitively differentiated VWF from its partner protein, FVIII.[6] and the inherent complexity of VWD classification was beginning to become apparent. Cloning of the *VWF* highlighted the large size and complicated organization of this structure with evidence that the *VWF* on chromosome 12 was accompanied by a non-processed partial pseudogene structure on chromosome 22.[11] In addition, a large N terminal domain of VWF, originally referred to as VWF antigen II, was characterized as the 741 amino acid VWF propeptide.[18]

The initial genetic characterization of VWD causing genomic variants was conducted in type 3 VWD patients who had acquired anti-VWF alloantibodies following treatments with VWF concentrates.[19,20] These studies involved combinations of Southern blot and polymerase chain reaction-based sequencing to identify *VWF* deletions of varying sizes, thus confirming a genotype-phenotype correlation that has also been identified in hemophilia A in which FVIII inhibitors are significantly more likely in patients with large multi-domain deletions.[21]

With the arrival of polymerase chain reaction-based genetic analysis in 1985, the potential to generate knowledge on the pathogenesis of the subtypes of VWD was quickly realized. While major genomic deletions and rearrangements were the first to be described in type 3 VWD, single nucleotide substitutions resulting in missense changes were soon being identified in type 2 forms of VWD.[22-24]

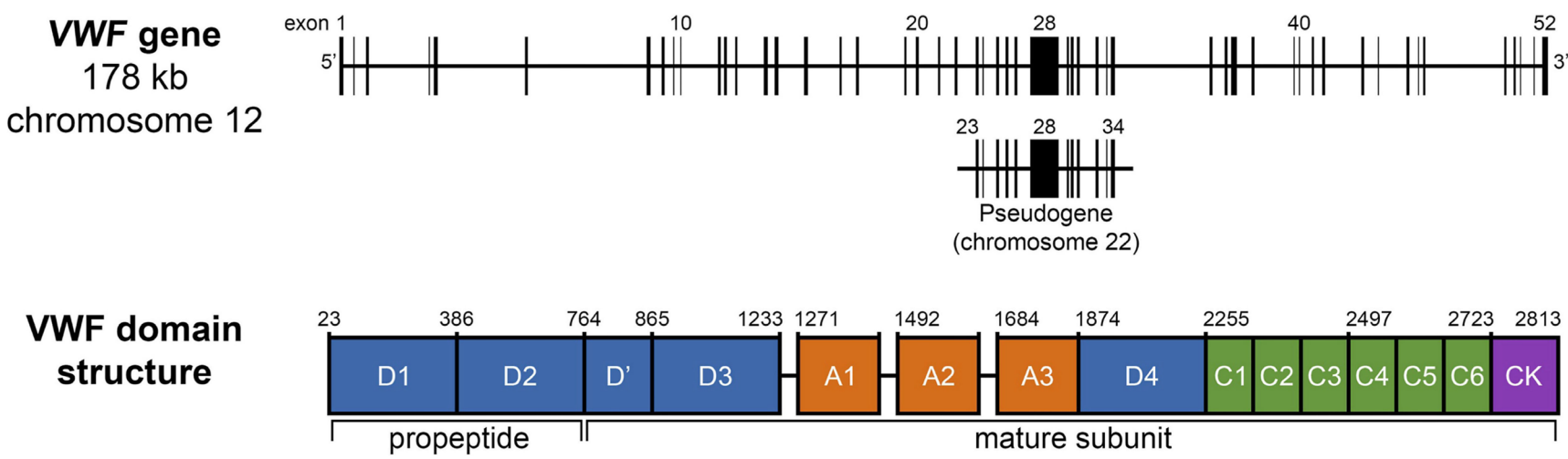

Figure 1. von Willebrand factor gene, pseudogene and protein. The *VWF*, located on chromosome 12 (12p13.31), spans approximately 178 kb. A partial unprocessed von Willebrand factor (VWF) pseudogene, mapped to chromosome 22 (22q11.2), extends about 25 kb and corresponds to exons 23 to 34 of *VWF*, sharing 97% sequence homology with the functional gene. The precursor of VWF is synthesized as a single-chain polypeptide, consisting of a signal peptide, a propeptide (D1–D2 domains), and a segment that forms the mature VWF subunit (D'–CK domains). Figure adapted, with permission, from Seidizadeh *et al.*[15]

Pathogenic variants were soon identified in discrete functional domains of VWF, resulting in types 2A, 2B, and 2N VWD.[25] These observations, made through genetic testing of index cases, were complemented by cell-based and small animal studies to confirm and expand upon the molecular mechanisms responsible for the loss-of-function (types 2A and 2N)[26,27] and gain-of-function (type 2B)[28] phenotypes. Later, with the realization that additional loss-of-function phenotypes can appear with intact multimeric structures that demonstrate reduced binding to glycoprotein Ib or collagen (type 2M), more pathogenic missense variants were identified in regions of the gene encoding the A1 and A3 domains.[29-31]

While significant progress was being made in the understanding of genotype-phenotype associations for types 2 and 3, work started in the 2000s to collect genetic data from large type 1 VWD cohorts.[32-36] These study populations were characterized by low VWF antigen (VWF:Ag) values (either <50 or <30 IU/dL) and had been variably characterized in terms of their clinical bleeding phenotypes, with the routine use of bleeding assessment tools still to come.[37,38] At this time, there was a hope that molecular genetic testing might simplify the diagnosis of type 1 VWD, which was often complicated by the temporal variability of plasma VWF levels.

However, in contrast to what had been hoped, these type 1 genomic studies opened a Pandora's box of complexity that remains only partly resolved some 15 years later. There is now ongoing debate about the benefits of molecular genetic testing for type 1 VWD and these issues will be discussed in more detail later in this review.

Clinical utility of molecular genetic testing in von Willebrand disease

The diagnosis of VWD requires evidence gathered from three sources of information: (i) documentation of a clinical bleeding phenotype; (ii) laboratory test results that are consistent with a deficiency and/or dysfunction of VWF, and (iii) the presence of a family history of this bleeding tendency.[39]

Objective documentation of a bleeding tendency has been markedly improved with the introduction of bleeding assessments tools[40] and there is now a multi-test array of investigations that evaluate the phenotype of plasma VWF.[41-43] These tests quantify the amount of circulating VWF protein, assess the ability of VWF to bind to platelets, collagen, and FVIII, and determine the multimeric profile of the circulating protein.[43] While this comprehensive phenotypic assessment is able to define a VWD diagnosis in most cases, this range of tests is only available in specialist laboratories and, even then, the performance and interpretation of these assays may be problematic.[43,44] The temporal variability of VWF levels and challenging technical performance of tests, such as multimer analysis, ristocetin-induced platelet agglutination, and VWF:FVIII binding, contribute to these phenotype uncertainties.

With the currently available range of phenotypic tests, the diagnosis of VWD will still most often be made without the contribution of molecular genetic analysis. However, there are certain instances in which molecular tests are extremely helpful for arriving at a definitive diagnostic decision (Figure 2).

Type 2 von Willebrand disease

The first and probably most frequently requested molecular diagnostic testing approach relates to the confirmation of type 2 VWD variants.[45] In these disorders, abnormalities of the VWF phenotype should be apparent (e.g., reduced VWF:activity/VWF:Ag ratio, abnormal multimers or lower than expected FVIII:C). However, sometimes these abnormalities are subtle, and their interpretation may not be straightforward.[46] In these cases, the additional information derived from molecular analysis will usually enable a confident dismissal or confirmation of a specific type 2 subtype.

The confirmatory tests for type 2 forms of VWD are aided by the fact that the locations of these pathogenic variants are confined to discrete, functionally related protein domains.[43] As many of the pathogenic variants for several of the different sub-types of VWD are located in exon 28 this is a reasonable place to begin a molecular genetic search. If exon 28 testing proves negative the following sequencing strategy is recommended. For putative type 2A cases targeted sequencing of *VWF* exons 11-17, 22, 25, 26, 27, and 52; for type 2B just exon 28; for type 2M exons 27, 29-32 and for type 2N, the targeted analysis should involve exons 17-21 and 24-27.[47,48] These genomic localization characteristics facilitate a targeted Sanger sequencing approach for most type 2 diagnoses and thus a next-generation sequencing (NGS) strategy is not necessary in many cases. However, with NGS now available in increasing numbers of diagnostic laboratories, the *VWF* is typically sequenced in the context of either whole-exome or whole-genome sequencing analysis.[49]

Types 2A, 2B, and 2M VWD all manifest as dominant traits (with the exception of type 2A subtype IIC) and thus evidence of pathogenic variant heterozygosity is sufficient proof of disease causation.[50] In contrast, type 2N VWD is a recessive trait, and two pathogenic alleles are required to produce the mutant phenotype of a disproportionately reduced plasma FVIII level (Figure 3).[50] As with any recessive condition, this genomic state can be acquired by three mechanisms: (i) homozygosity for a pathogenic variant known to be associated with reduced FVIII binding; (ii) compound heterozygosity for two different pathogenic variants known to cause type 2N disease and (iii) compound heterozygosity for one 2N pathogenic variant and one *VWF* null allele.[51,52] Among these three scenarios, homozygous cases are not particularly rare, as they often result from

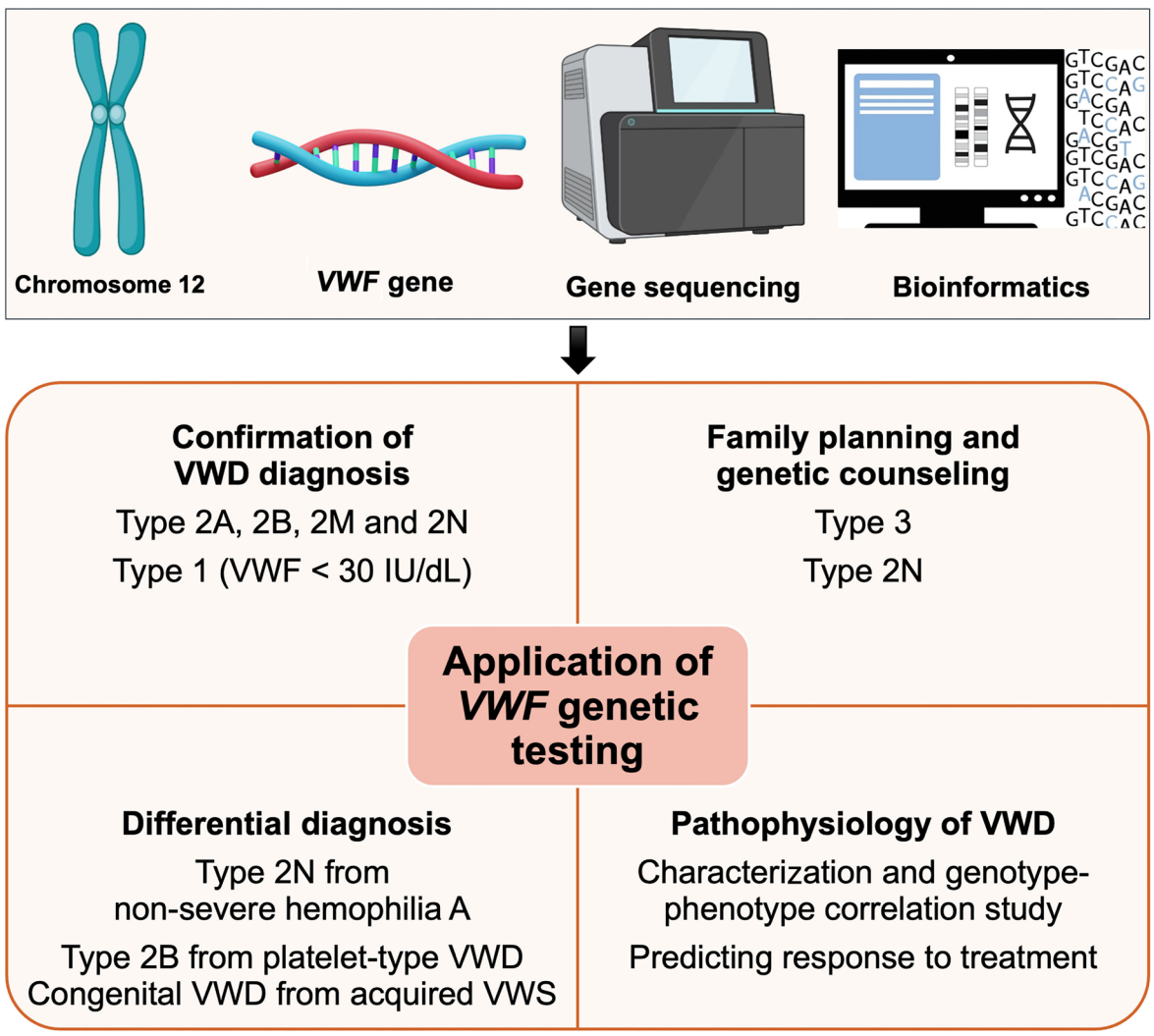

Figure 2. The application of molecular genetic testing of the *VWF* in von Willebrand disease. *VWF* molecular testing is instrumental in confirming certain von Willebrand disease (VWD) diagnoses, facilitating differential diagnoses, and providing insights into the disease's pathophysiology. It also plays a key role in genetic counseling and family planning for certain VWD types. Furthermore, it can enable personalized therapy for specific VWD phenotypes, such as type 2B and type 1C, in which desmopressin is contraindicated or offers limited benefit, respectively. VWS: von Willebrand syndrome.

consanguineous unions. The most common situation is the combination of a type 2N variant with a null allele, whereas compound heterozygosity for two different type 2N variants is rare.

Two type 2 VWD scenarios are especially important to consider. The coagulation phenotypes of type 2B and platelet type-VWD are almost identical and can only be differentiated by rarely used mixing studies with the ristocetin-induced platelet agglutination assay.[53] Importantly, type 2B is always caused by pathogenic variants in exon 28 of *VWF* while platelet type-VWD is the result of gain-of-function variants in the platelet *GPIBA* gene.[54] As treatment choices are different for these two disorders, correct diagnosis is very important and can be most efficiently and precisely made with molecular genetics. The second genocopy dilemma that can be resolved with molecular genetics is the differentiation of type 2N VWD and non-severe hemophilia A. In any patient with a low plasma FVIII level these two disorders should be considered, and while the FVIII binding phenotype of VWF can be used to identify type 2N, in 2025 the most efficient and definitive diagnostic strategy is to use molecular genetics. With the growing use of whole-exome and whole-genome NGS the pertinent information will also be available with the performance of a single test.[55,56] The diagnostic yield from molecular genetic studies of putative type 2 VWD subtypes is ~95% and to date no alternative genetic loci have been identified that result in these phenotypes other than those alluded to in the previous paragraph (*GPIBA* and *F8*). Although rare, concurrent reductions in FVIII and factor V levels can be caused by variants in the *LMAN1* and *MCFD2* genes.[57]

Type 3 von Willebrand disease

The other clear indication for molecular genetic testing in VWD concerns the determination of pathogenic variants responsible for type 3 disease. This analysis requires evaluation of the entire coding sequence of *VWF* as pathogenic variants for this severe phenotype have been documented throughout the gene (Figure 3).[58-60] This analysis has three potential benefits: (i) identification of pathogenic variants to inform future family planning; (ii) identification of type 3 patients with *VWF* genotypes predictive of higher risks for anti-VWF immune responses (e.g., large genomic deletions) and (iii) differentiation of severe acquired von Willebrand syndrome from inherited type 3 VWD. The last benefit is also applicable to other VWD types, as genetic testing can aid in distinguishing acquired from congenital forms of the disease.

Type 1 von Willebrand disease and low von Willebrand factor

The final VWD types to be considered are the most common VWD categories of type 1 or Low VWF. As mentioned previously in this review, several large type 1 cohorts have now been investigated, and all have led to the same conclusion that potential pathogenic variants are only identified in the

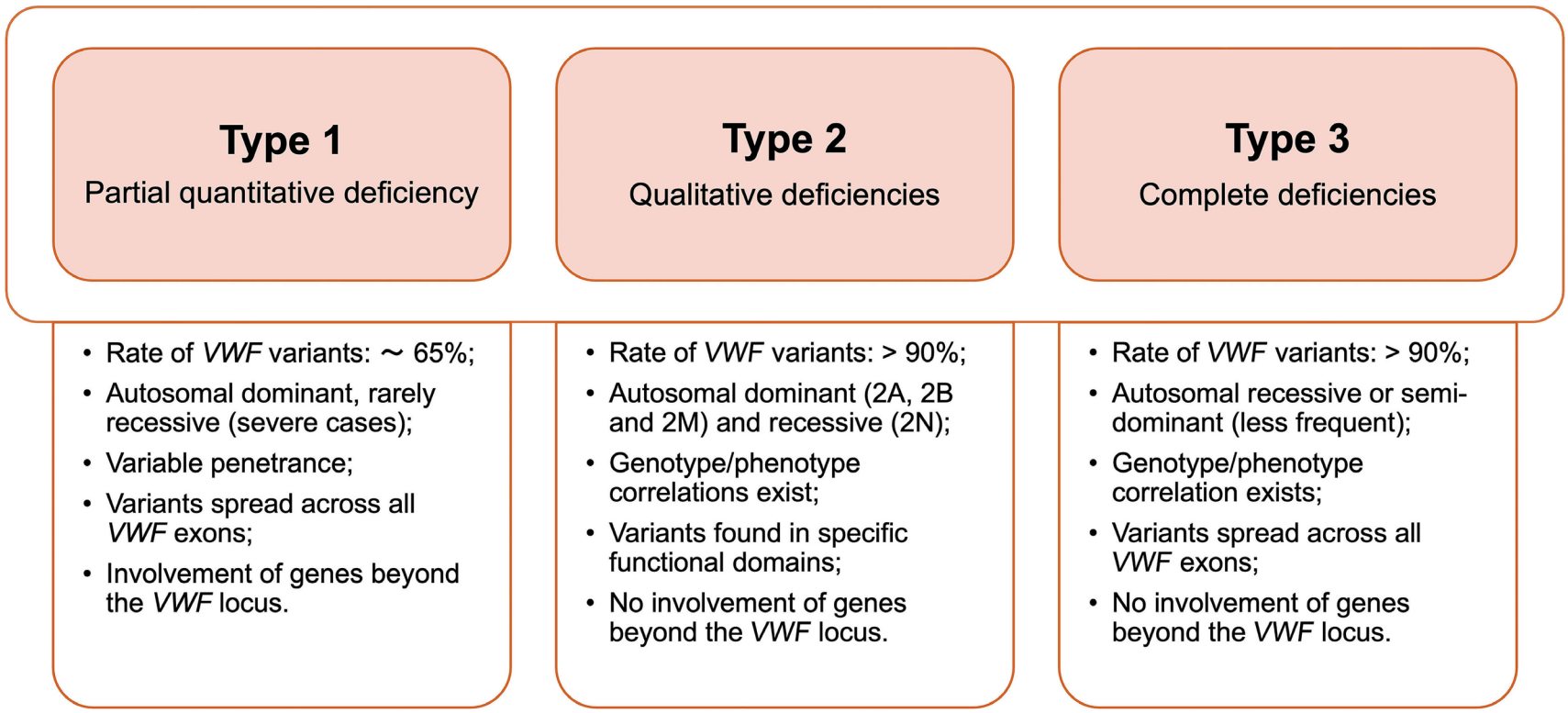

Figure 3. Genetic characteristics of various von Willebrand disease phenotypes.

VWF coding sequence in ~65% of index cases.[33,34,36,60] This number may in fact be an overestimate of the pathogenic variants, as detailed pathogenicity analysis has not been employed in all these studies and some identified variants may be benign. These observations indicate that *VWF* coding region variants will not be found in ~35% of type 1 cases, and even when they are found a strict pathogenicity assessment is recommended. Furthermore, the demonstration of incomplete penetrance or variable expressivity with some genotypes will further complicate the results of genetic analysis. For all these reasons, there has long been a lack of consensus about the utility of genetic testing for type 1.[61,62] One potentially important (although infrequent) justification would be when two individuals with type 1 are planning on having children, with the possibility of giving birth to an infant with severe type 3 VWD. Additionally, since type 1 cases with VWF levels below 30 IU/dL are more likely to possess a pathogenic *VWF* variant, genetic testing can be used to confirm the diagnosis when NGS is available.

The type 1 variant in which genotype data might most clearly benefit clinical management is type 1C disease in which the mutant VWF is cleared significantly faster than the wild-type protein.[63,64] In type 1C, plasma VWF:antigen levels are usually between 10-15 IU/dL and following a trial of desmopressin – the most reliable method for identifying this phenotype – a marked biphasic response is usually evident with an excellent 1 hour VWF recovery followed by a rapid fall in levels at 4 hours. Of note, increased VWF clearance is not exclusive to type 1C and can be seen in several other VWF variants, often coexisting with qualitative or quantitative abnormalities of VWF.[65,66] Type 1C pathogenic variants have been reported across several *VWF* exons, but are usually located in *VWF* exons 26, 27, 28 and 37.[65-67] In a research context, molecular testing can also contribute to enhancing our understanding of the underlying mechanisms leading to reduced VWF levels (Figure 3).[46,68]

Cases of von Willebrand disease without *VWF* genetic variants

Extensive experience over the past 20 years has shown that >90% of types 2A, 2B, 2M, 2N, and type 3 are associated with positive molecular genetic analysis. In most cases these genetic results align with the clinical phenotypes, with types 2A, 2B and 2M being transmitted as fully penetrant dominant traits, and types 2N and 3 as recessive conditions, although a semi-dominant inheritance pattern is present in ~50% of type 3 cases in some studies.[58,60] In marked contrast, as alluded to above, in type 1 VWD/Low VWF, pathogenic variants are missing in ~35% of cases, with a lack of genetic explanation being especially prevalent in patients with VWF levels between 30-50 IU/dL.[35] To date, an explanation for this situation is unclear, but the results of genome-wide association studies conducted over the past 15 years have repeatedly highlighted multi-locus influences on basal plasma VWF levels.[69,70]

The two loci that consistently have the highest ranked associations are ABO and *VWF*. The former association is explained by the more rapid clearance of group “O” VWF due to glycosylation changes,[71] while the VWF association may well concern non-coding pathogenic variants in *VWF* introns and adjacent *cis*-acting regulatory elements. The non-*VWF* loci that have been consistently identified in these genome-wide association studies encode a range of proteins with, in general, clear biologically plausible asso-

ciations with VWF. Examples include proteins involved in VWF biosynthesis and secretion (e.g., *STXBP5* and *STX2*) and clearance receptors contributing to VWF removal from plasma (e.g., *STAB2*, *SCARA5* and *CLEC4M*).[72] In contrast, there are some robust associations for which a biological connection with VWF has yet to be revealed (e.g., *TC2N*).[72] There is no doubt that these genomic studies have added important knowledge related to VWF biology and disease states, but how this information benefits clinical molecular testing and patients' management is not obvious. There may be a future potential for derivation of a polygenic risk score for low VWF states, but this possibility is not close to clinical integration.

Genotype-phenotype correlation

Generally, the severity and frequency of VWD follow opposite trends: type 1 is the most common but has the mildest bleeding symptoms, followed by type 2, which has intermediate severity. Type 3 is the rarest but causes the most severe bleeding (Figure 4A). Understanding the genotype-phenotype correlation in VWD is important in personalized medicine because it can help to predict VWD severity, provide insights into the underlying mechanisms of the disease, and guide treatment decisions. Type 1 Vicenza is a clear example of such correlation, where patients with the p.Arg1205His variant consistently exhibit a significantly shortened VWF half-life, a moderate to severe reduction in VWF plasma levels, typically around 10-15 IU/dL, and ultra-large VWF multimers.[73,74] Over the past four decades, extensive studies on various VWD types have been performed and it has been clear that, generally, a strong genotype-phenotype correlation exists in most VWD types except in type 1/Low VWF cases with VWF levels of 30-50 IU/dL. In these cases, it is not always easy to predict the clinical phenotype based solely on the *VWF* genotype. *VWF* null alleles, typically resulting in loss of function, are classically associated with recessive inheritance and type 3 VWD when biallelic. However, individuals heterozygous for null variants may present with low VWF levels and be diagnosed with type 1 VWD,[60] blurring the line between carrier state and mild disease. We have learnt that, beyond the presence and penetrance of *VWF* pathogenic variant(s), several other factors influence VWD manifestations and may serve as key determinants in its development (e.g,, blood group, age, environmental and physiological factors).[15,72,75] This is especially relevant in cases of mild VWF deficiency. In addition, it has been evident that within the phenotypic classification of VWD, a variety of mutations can be responsible for a particular subtype of disease.

In severe cases of type 1 VWD, as well as in type 2 and type 3 VWD, rare variants of VWF are typically the cause of the disease. However, in milder forms of type 1 VWD (or Low VWF), pathogenic variants often exhibit incomplete penetrance. It is important to recognize that the rarity of a variant does not necessarily indicate causality, and only a

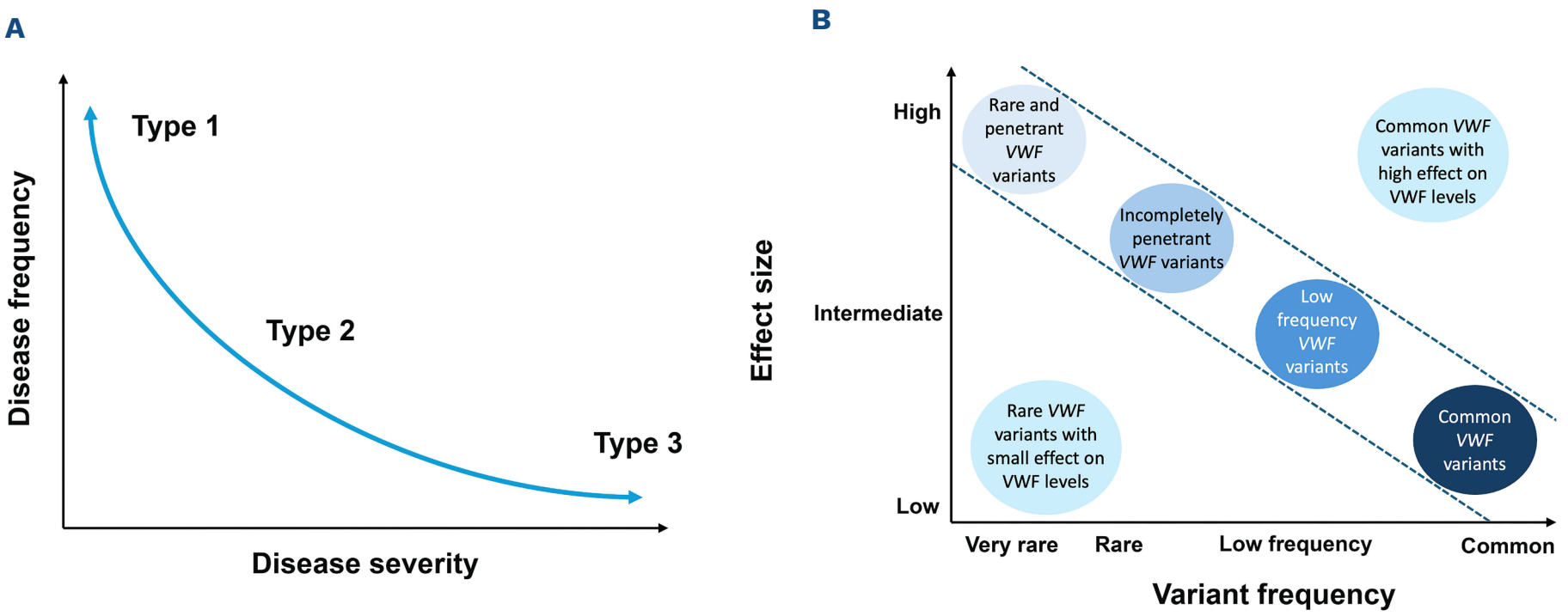

Figure 4. Correlation between disease frequency and severity and association of *VWF* genetic variants with allele frequency and their impact on von Willebrand factor levels. (A) The severity and frequency of von Willebrand disease (VWD) typically exhibit an inverse relationship: type 1 is the most prevalent but presents with the mildest bleeding symptoms, type 2 follows with moderate severity, and type 3, although the rarest, is associated with the most severe bleeding manifestations. (B) In severe cases of type 1 VWD, as well as in type 2 and type 3 VWD, rare *VWF* variants are almost always responsible for the disease. However, in mild forms of type 1 VWD (or Low VWF), variants often exhibit incomplete penetrance. It is important to note that being a rare variant does not necessarily imply causality, and only a few variants with a minor allele frequency up to 1% show a strong association with VWD. Additionally, some common *VWF* variants may contribute to (minor) variations in plasma von Willebrand factor (VWF) levels.

few variants with a minor allele frequency of up to 1% show a strong association with VWD. Additionally, some common *VWF* variants, either alone or in combination, may contribute to minor fluctuations in plasma VWF levels (Figure 4B). It is important to note that the presence of a *VWF* variant does not confirm clinical disease, as some variants may show incomplete penetrance or occur in asymptomatic individuals. Conversely, the absence of a detectable *VWF* variant does not exclude VWD, particularly in cases involving noncoding variants or regulatory defects not captured by standard sequencing approaches.

An additional important consideration is that family studies are critical in the diagnosis of VWD to accurately interpret the pathogenicity of individual *VWF* variants, particularly when multiple variants coexist in the same individual.[33,76] Furthermore, *VWF* mRNA analysis is particularly valuable when genomic sequencing does not fully clarify the pathogenicity of a variant, especially for noncoding, deep intronic, or potential splicing mutations. It can reveal aberrant splicing events, exon skipping, or transcript degradation that may not be predicted from DNA sequencing alone.[77-79] As cells that naturally express VWF are not usually available for mRNA analysis, these studies will almost always require *in vitro* experimental approaches.

Limitations and considerations of genetic testing

While genetic testing for *VWF* offers several advantages in the diagnostic workflow of VWD, there are important limitations to consider. The gene is exceptionally large, which makes full *VWF* sequencing (i.e., coding regions, exon-intron boundaries, and the 5' and 3' untranslated regions) both challenging and time-consuming. Although the availability of NGS has helped address this issue, this technology remains unavailable in many laboratories and is costly. As discussed earlier, another limitation to the application of genetic testing (in type 1 VWD) is variable expressivity and incomplete penetrance of the pathological phenotype. Even with the same mutation, disease severity and clinical presentation can vary greatly between individuals,[35] making it difficult to predict the clinical course of patients based solely on genetic results. Furthermore, due to the large number of genetic variants associated with VWD (>1,500), assessing the pathogenic nature of all these *VWF* variants is challenging and unfeasible. In fact, nearly 50% of variants submitted to ClinVar are classified as variants of uncertain significance, making it difficult to predict how a mutation will manifest clinically. A recent review summarized several other considerations that should be kept in mind when molecular testing is applied for the diagnosis of VWD.[15] These include the difficulty of identifying gene conversions with routine NGS, handling newly identified variants with unclear pathogenicity, overlapping variants among VWD subtypes, and the compounding effects of multiple pathogenic or low-risk variants within the same patient.[15]

Future of *VWF* genetic testing

Since the cloning of the *VWF* in 1985 significant advances have been made in our understanding of the molecular pathology of VWD and this information has resulted in the routine integration of molecular genetic testing for some forms of the disease.

As we look at the future of *VWF* genetic testing, additional clinical management benefits could derive from at least two diagnostic strategies. First, as sequencing costs continue to fall, and bioinformatic pipelines are more readily available, an increasing number of laboratories are using either whole-exome or whole-genome NGS-based analysis to perform their diagnostic testing. The whole-genome sequencing approach provides access not only to the entire *VWF* genomic sequence, enabling detection of potential noncoding pathogenic variants in *VWF* introns and adjacent regulatory sequence, but would also allow for analysis of the growing number of genes that have been associated with the regulation of plasma VWF levels (currently ~25). This latter benefit could facilitate the generation of a polygenic risk score to substantiate the likelihood of a genetic basis for low VWF. Whether this strategy would generate sufficient clinical justification remains to be seen.

In addition to the diagnostic application of molecular testing, there could be a complementary benefit for the subsequent pursuit of various approaches to genomic editing to rescue pathogenic variants (precision medicine). This type of intervention has been successfully developed for the inhibition of specific dominant negative pathogenic *VWF* variants,[80,81] but these studies have all been conducted in cell-based *in vitro* protocols. In contrast, efficiently delivering the machinery to perform this type of treatment *in vivo* is still in an early stage of development.[81,82]

Disclosures

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Contributions

OS and DL contributed equally to this paper.

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