Genetic determinants of clinical variability in type 2 von Willebrand disease: bridging genotype and phenotype

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Abstract

The clinical and genetic features of type 2 von Willebrand disease (VWD) have been described, but genotype-phenotype correlations in large cohorts remain incompletely understood. We investigated the relationship between von Willebrand factor gene (*VWF*) variants and bleeding severity in a large, well-characterized cohort of type 2 VWD patients, aiming to identify genetic determinants underlying clinical variability. Comprehensive laboratory evaluation, *VWF* molecular testing, *in silico* analyses, and bleeding assessment using the ISTH bleeding assessment tool (ISTH-BAT) were performed. Among 371 genetically confirmed cases, ISTH-BAT scores were available for 274 individuals: 83 with type 2A, 69 with 2B, 106 with 2M, and 16 with 2N. The highest bleeding scores were observed in type 2A (median 7), followed by 2B (5), 2M (4), and 2N (4). A total of 67 distinct *VWF* variants were identified. Notably, we observed substantial variability in bleeding severity both across different variants causing the same VWD phenotypes and among individuals carrying the same *VWF* variant. ISTH-BAT scores were significantly higher in females than in males, and in adults compared to children. Among adults, but not children, bleeding scores differed significantly between some subtypes. No significant differences were observed between patients with blood group O and non-O. While certain mucocutaneous bleeding symptoms such as menorrhagia, cutaneous, and epistaxis were commonly observed across all type 2 subtypes, our data highlight important subtype-specific differences in bleeding phenotype profiles. This study provides one of the largest genotype-phenotype datasets in type 2 VWD, revealing marked variability in bleeding severity both across type 2 VWD subtypes and among patients with the same genetic variants.

Introduction

Von Willebrand factor (VWF) is a large multimeric glycoprotein produced primarily by endothelial cells and megakaryocytes; stored in Weibel-Palade bodies and platelet α-granules, respectively.[1] VWF is essential for platelet aggregation at sites of vascular injury by acting as a bridge between platelet glycoprotein Ib (GPIb) and the damaged blood vessel wall (exposed collagen). VWF also binds to coagulation factor VIII (FVIII), protecting it from degradation in the bloodstream and delivering it to the critical sites.[2] A deficiency or dysfunction of VWF can lead to the most common inherited bleeding disorder, von Willebrand disease (VWD).[2] Three types of VWD are distinguished by their unique characteristics, including type 1 with a partial quantitative deficiency of VWF, type 2 with distinct qualitative defects in VWF, and type 3 as the most severe form with a complete or near-complete absence of VWF.[3]

In type 2 VWD, genetic variants in the VWF gene (*VWF*) result in various functional abnormalities of VWF, including reduced VWF-platelet GPIb and collagen binding due to loss of VWF high-molecular-weight multimers (type 2A), Increased affinity for platelet GPIb (type 2B), reduced affinity for GPIb or collagen (type 2M) and for FVIII (type 2N).[4] While types 2A, 2B, and 2M VWD are inherited in an autosomal dominant manner, type 2N is recessively inherited.[5] It is estimated that type 2 VWD affects, per 1,000 individuals globally, 1.3 for type 2A, 1.7 for type 2B, 1.5 for type 2M, and approximately 31 per million for type 2N.[6] Among VWD types, the correlation between genotype and bleeding phenotype in type 1 is complex and not always directly proportional.[7-9] Although some studies have investigated the clinical profile or genetic characterizations of type 2 VWD,[4,10,11] the clinical severity associated with the various genetic variations responsible for different subtypes of type 2 VWD has not been explored in a large cohort.

Understanding how specific *VWF* genetic variants determine or influence the clinical phenotype in different subtypes of type 2 VWD is crucial for accurate diagnosis, personalized treatment, and improved prognosis. Additionally, this knowledge aids genetic counseling and supports advancements in research, potentially leading to new (targeted) therapies and improved patient outcomes.[12]

With this background, the present study aimed to investigate the correlation between genotype and bleeding phenotype in type 2 using one of the largest and well-characterized cohorts of type 2 VWD patients. We further investigated the clinical profile and bleeding severity across the four groups of type 2 patients.

Methods

Study population

We included all genetically confirmed patients diagnosed with type 2 VWD who were referred to the A. Bianchi Bonomi Hemophilia and Thrombosis Center in Milan between January 1, 1995 and April 30, 2025. Each patient underwent comprehensive evaluation for clinical manifestations, biochemical phenotypic tests, and genetic characterization to reach a final diagnosis.[4,13,14] Classification was based on the guidelines set by the International Society on Thrombosis and Hemostasis Scientific and Standardization Committee.[3,15] All participants provided written informed consent for phenotypic and genotypic analyses. The consent process was conducted in accordance with the Declaration of Helsinki.

Laboratory and clinical evaluations

Following blood sample collection, the VWD diagnostic methods, including FVIII activity (FVIII:C), VWF antigen (VWF:Ag), platelet-dependent VWF activity assays (VWF activity), and collagen binding were measured. The details of all VWD diagnostic panel test have been described previously.[4]

The ISTH bleeding assessment tool (ISTH-BAT) was administered to each patient to assess their bleeding history. This questionnaire evaluates the severity, frequency, and treatment requirements for 14 different bleeding symptoms, with each symptom scored on a scale from 0 to 4.

Genetic testing

DNA was extracted from peripheral leukocytes using standard methods. Genetic analysis for patients diagnosed between 1995 and 2017 was performed using polymerase chain reaction and Sanger sequencing.[4] For patients diagnosed from 2018 to 2025, next-generation sequencing (NGS) with a custom target panel was used to sequence the full *VWF* sequencing (i.e., coding regions, exon-intron boundaries, and the 5' and 3' untranslated regions). All variants identified by NGS were confirmed by Sanger sequencing.

Additionally, Sanger sequencing was performed for exons 26 and 28 to avoid missing variants due to low coverage and the limitations of NGS in detecting gene conversions, respectively.

In silico predictions were used to assess variant pathogenicity, by applying CADD (https://cadd.gs.washington.edu/) and REVEL (https://sites.google.com/site/revelgenomics/) scores for missense variants and SpliceAI (https://spliceai-lookup.broadinstitute.org/) for splice-site variants. For variant classification according to American College of Medical Genetics and Genomics (ACMG) guidelines, ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) annotations were used when available; otherwise, classifications were obtained using the Franklin tool (https://franklin.genoox.com/clinical-db/home).

Statistical analysis

Continuous variables were described as median (range), and categorical variables as counts (percentages). Statistical analyses were performed with R statistical software environment (The R Foundation, Vienna, Austria). The Mann-Whitney U test was used to compare medians between two independent groups and a *P* value <0.05 was considered statistically significant.

Results

Patients

In this period (between January 1, 1995 and April 30, 2025), a total of 371 patients with type 2 VWD were genetically confirmed in our center and the ISTH-BAT was available for 274 cases. Therefore, all the analyses were conducted exclusively on this subset of patients, including 83 cases with type 2A, 69 with type 2B, 106 with type 2M, and 16 with type 2N. The sex distribution was 55% females (N=150) and 45% males (N=124) and among patients with available blood group (250/274), 53% had blood group O. Of these 274 cases there were 82 children (<18 years old) and 192 adults. Based on the specific laboratory phenotype plus the location of genetic variants within the VWF glycoprotein domains, the type 2A cohort was subclassified as follows: 2A(IIA) when variants were in the A2 domain, 2A(IIE) when variants were in the D3 domain, and 2A(IIC) when variants were in the D1-D2 domains. Type 2B was further classified as 2B and 2B New York. Type 2M was categorized as 2M, 2M/2A, or 2M with a collagen-binding defect (2MCB).

Laboratory results

Phenotypic laboratory results are summarized in Table 1. The median FVIII:C levels were slightly reduced in type 2A, 2M, and 2N but normal in type 2B. Type 2M exhibited the lowest median VWF:Ag levels, followed by type 2A and 2B, while type 2N showed normal median VWF:Ag levels except for a few cases. Patients with type 2A and 2M had

Table 1. Laboratory results of type 2 von Willebrand disease cohort.

VWD panel tests	Type 2A	Type 2B	Type 2M	Type 2N
Total N=274	83	69	106	16
FVIII:C, IU/dL, median (range)	48 (21-196)	66 (33-108)	49 (23-133)	42 (13-72)
VWF:Ag, IU/dL, median (range)	34 (11-370)	52 (18-170)	29 (12-147)	64 (22-156)
VWF activity, IU/dL, median (range)	13 (3-91)	22 (4-73)	12 (4-101)	51 (18-112)
VWF activity/VWF:Ag ratio, median (range)	0.39 (0.08-1)	0.42 (0.1-1.13)	0.49 (0.12-1.2)	0.73 (0.67-1.4)
*VWF:CB, IU/dL, median (range)	8 (1-59)	23 (3-80)	15 (3-73)	57 (12-134)
*VWF:CB/VWF:Ag ratio, median (range)	0.21 (0.02-1)	0.47 (0.05-1.45)	0.58 (0.08-1.23)	0.87 (0.55-1.47)
**Blood group O, %	45	66	47	72

*Missing number for 2A=7, 2B=3, 2M=4, and 2N=4. VWD: von Willebrand disease; FVIII:C: coagulation factor VIII; von Willebrand factor activity: VWF platelet-dependent activity; VWF:CB: VWF collagen binding. Results are presented as median (range). **Data on blood group were available for 250 cases.

the lowest median VWF activity (13 and 12 IU/dL, respectively), followed by type 2B (22 IU/dL). Type 2A had the lowest median VWF:CB levels (8 IU/dL), followed by type 2M (15 IU/dL) and type 2B (23 IU/dL). The VWF activity/VWF:Ag and VWF:CB/VWF:Ag ratios were low across the subtypes, with values of 0.39 and 0.21 in type 2A, 0.42 and 0.47 in type 2B, and 0.49 and 0.58 in type 2M, respectively. As expected, type 2N patients had normal VWF activity/VWF:Ag and VWF:CB/VWF:Ag ratios.

Bleeding symptoms

Comparison of bleeding severity across type 2 von Willebrand disease
At least one bleeding event was reported in 89% of patients with type 2 VWD. This included 77 of 83 type 2A cases (93%), 60 of 69 type 2B (87%), 92 of 106 type 2M (87%), and 15 of 16 type 2N (94%). The median ISTH-BAT scores varied across type 2 VWD subtypes, with type 2A showing the highest median score of 7 (range, 0-22), followed by type 2B with 5 (range, 0-34). Both type 2M and 2N showed lower median scores of 4, with ranges of 0-24 and 0-23, respectively. When comparing the ISTH-BAT scores across different phenotypes, a significant difference was only found between type 2A and type 2M (P=0.00038). All the other comparisons were not statistically significant (Figure 1A). We further analyzed differences between subtypes: type 2A(IIA) *versus* 2A(IIE), type 2B *versus* 2B NY, and among type 2M, 2M/2A, and 2M(CB). No significant difference was found between 2A(IIA) and 2A(IIE) (P=0.12) or between 2M and 2M/2A (P=0.357). However, significant differences were observed between 2B and 2B NY (P=0.0366), between 2M and 2M(CB) (P=0.0214), and between 2M/2A and 2M(CB) (P=0.0094) (Figure 1B). Females had significantly higher ISTH-BAT scores compared to males (median 6 *vs*. 4; P=0.0038). Adults (N=192) also showed significantly higher ISTH-BAT scores than children (N=82) (median 6 *vs*. 3; P<0.0001). In contrast, no significant difference was observed between patients with blood group O and non-O (median 7 *vs*. 5; P=0.053). Among children, ISTH-BAT scores did not differ significantly across type 2 VWD subtypes. However, in adults, significant differences were found between subtypes 2A and 2M, and between 2M and 2N (*Online Supplementary Figure S1*).
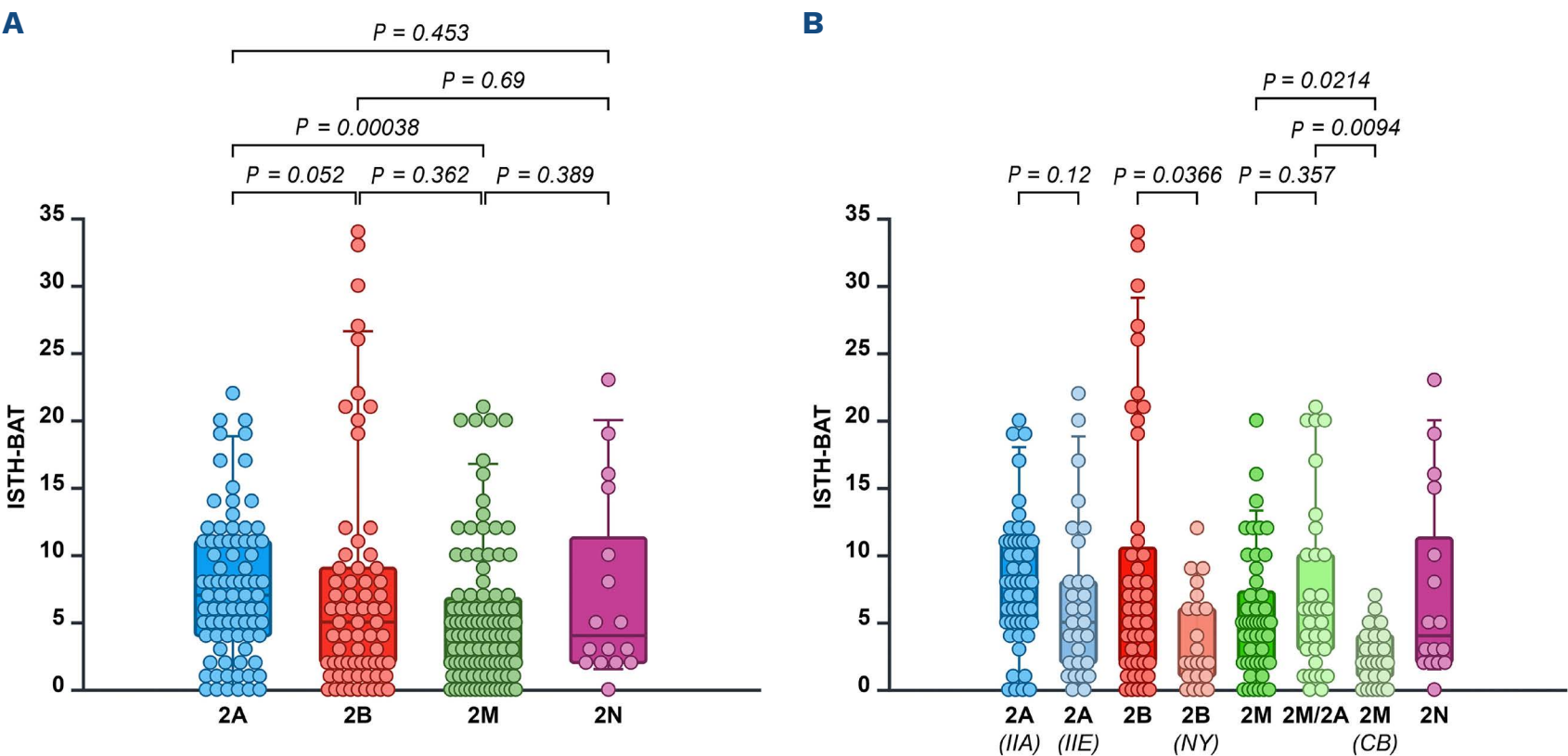
Bleeding symptoms across type 2 von Willebrand disease
The distribution of bleeding symptoms in the cohort (N=274), as assessed by the ISTH-BAT scoring system (Figure 2), showed that the most frequently reported symptoms were menorrhagia (62%), epistaxis (61%), and cutaneous bleeding (59%). Oral cavity bleeding (55%) and postpartum hemorrhage (55%) were also commonly observed. Bleeding from minor wounds and bleeding after tooth extraction occurred in 45% and 37% of cases, respectively, while surgical bleeding was noted in 32%. Other less frequent symptoms included muscle hematomas (15%) and hemarthrosis (11%). Gastrointestinal (GI) bleeding was seen in 15% of the entire cohort, while central nervous system (CNS) bleeding and hematuria were rare, affecting only 1% and 3%, respectively. Bleeding manifestations in type 2 VWD varies across subtypes, as reflected by the ISTH-BAT domain scores. Type 2A was characterized by high rates of cutaneous bleeding (69%), menorrhagia (69%), epistaxis (67%), and postpartum hemorrhage (62%). In addition to other frequent symptoms such as oral cavity bleeding (46%) and bleeding from minor wounds (45%), bleeding after tooth extraction (42%), post-surgical bleeding (40%), and GI bleeding (18%) were also a notable concern (Figure 3A). Type 2B presented with a similar mucocutaneous bleeding pattern but with slightly lower frequencies, including menorrhagia (61%), epistaxis (56%), and postpartum hemorrhage (54%). Other bleeding manifestations in type 2B, whether frequent or rare, are shown in Figure 3B. Type 2M demonstrated a predominantly mucocutaneous bleeding tendency, with epistaxis (61%), menorrhagia (57%), postpartum hemorrhage (53%),

cutaneous (50%), and oral cavity bleeding (41%), being the most common (Figure 3C). However, the overall bleeding severity in type 2M appears lower compared to type 2A and 2B, as seen also from the lower rates of GI bleeding (7% *vs.* 18% and 11%) and surgical bleeding (23% *vs.* 40% and 33%). Type 2N presented a distinct bleeding profile, with hemarthrosis (25%) and muscle hematomas (20%) being notably more frequent than in other type 2 subtypes. This subtype also exhibits lower rates of mucocutaneous bleeding, including cutaneous (56%), and epistaxis (44%), while menorrhagia (58%) and postpartum hemorrhage (37%) are notable concerns (Figure 3D).

Figure 1. Comparison of bleeding severity across type 2 von Willebrand disease subtypes using the ISTH bleeding assessment tool. (A) ISTH bleeding assessment tool (ISTH-BAT) scores in type 2A, 2B, 2M, and 2N von Willebrand disease. (B) ISTH-BAT scores in specific subtypes of type 2 von Willebrand disease, including 2A(IIA), 2A(IIE), 2B, 2B NY, 2M, 2M/2A, and 2M(CB). The line indicates median ISTH-BAT. Created with BioRender.com.
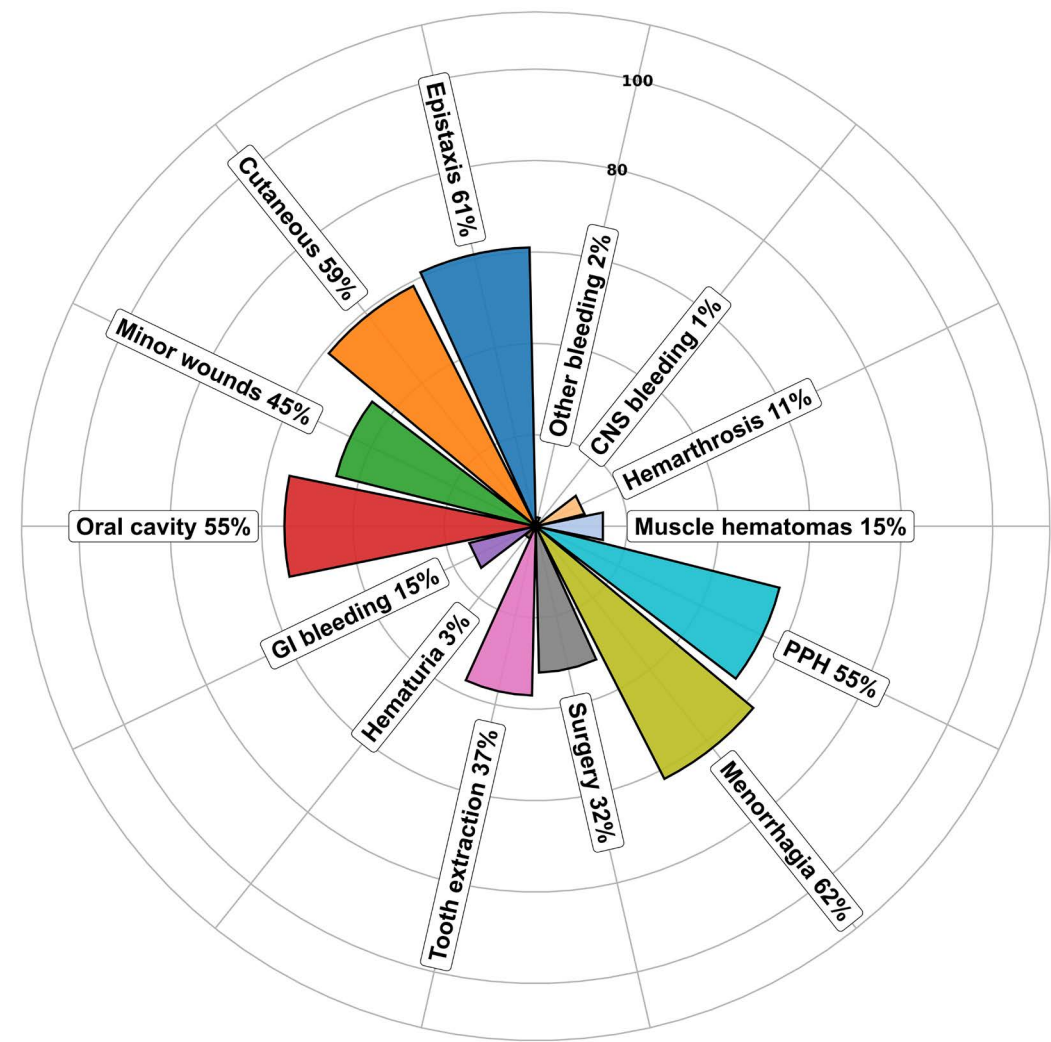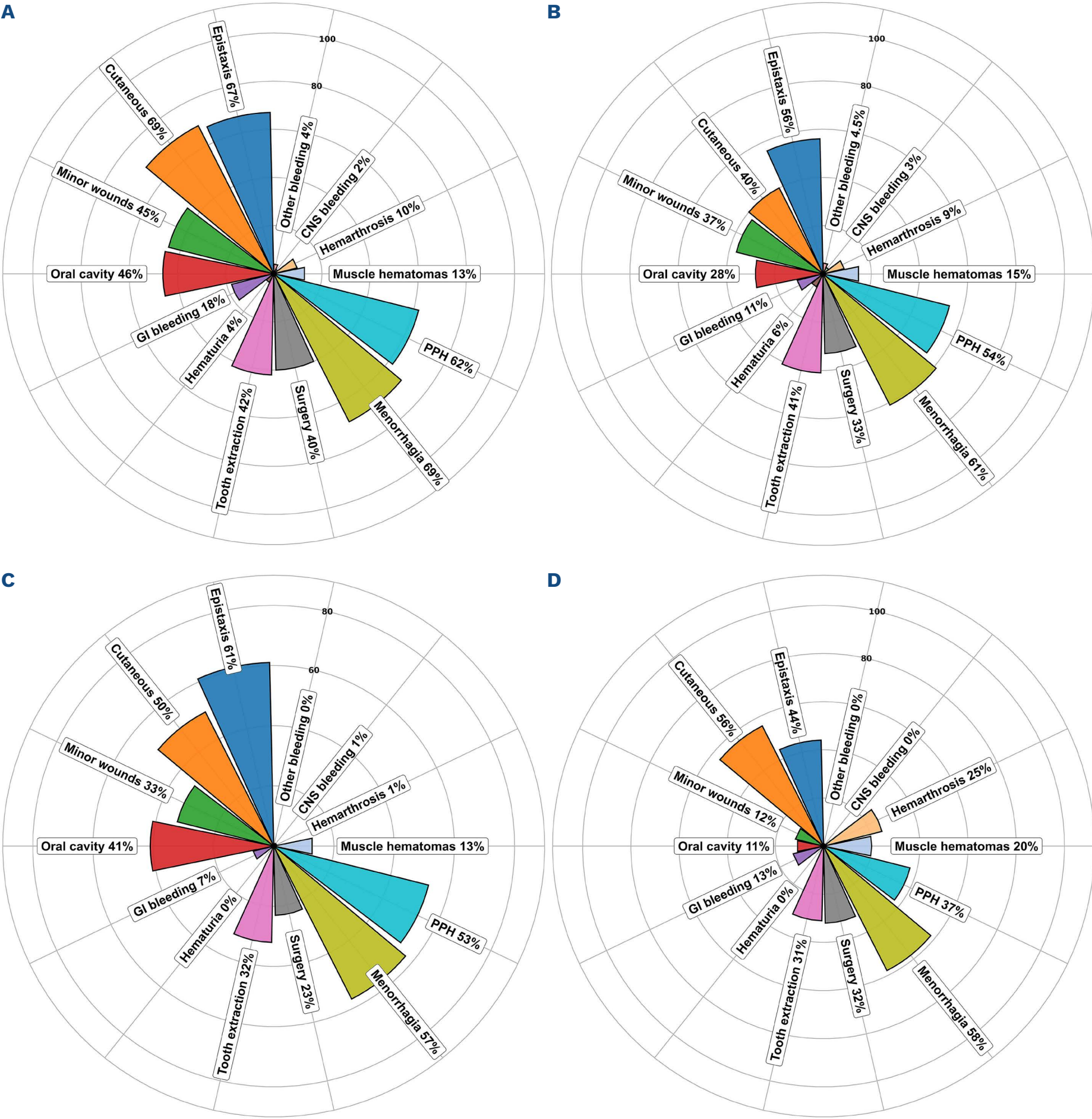

Figure 2. Bleeding profile and frequency of various bleeding symptoms in the overall type 2 von Willebrand disease cohort. Minor wounds: bleeding from minor wounds; GI: gastrointestinal; PPH: postpartum hemorrhage; CNS: central nervous system.

Genetic results

We identified 67 distinct genetic variants; each gene conversion being counted as a single variant. Nearly all variants resulted from single nucleotide changes, except for four deletions. We found more than one genetic variant responsible for type 2 VWD dominant phenotypes in 14 pa-

tients (3 type 2A cases, 4 type 2B, and 7 type 2M). *In silico* predictions and clinical variant classifications according to ACMG guidelines are summarized in *Online Supplementary Table S1*. Most variants had a CADD score >20 (59/67, 88%), and REVEL scores exceeded 0.5 in 87% of evaluated variants (52/60), supporting a likely deleterious effect. Both

Figure 3. Bleeding profile and frequency of various bleeding symptoms across different type 2 von Willebrand disease subtypes.
(A) Type 2A, (B) Type 2B, (C) Type 2M, and (D) Type 2N. Minor wounds: bleeding from minor wounds; GI: gastrointestinal; PPH: postpartum hemorrhage; CNS: central nervous system.

splicing variants were predicted to be pathogenic based on SpliceAI analysis.

## Correlation between genotype and clinical phenotype

To assess the severity associated with each genetic variant of type 2 VWD as stratified by phenotype, a genetic-clinical phenotype correlation analysis was performed (Figures 4-7; *Online Supplementary Figures S2-5*). Overall, the variants exhibited a wide range of bleeding severity, with considerable interindividual variability among cases carrying the same variant. Nevertheless, most type 2 variants were associated with significant bleeding.

Among different variants in type 2A, 17 (12 occurring alone and 5 in combination) were associated with an ISTH-BAT score ≥10 (Figure 4). These included p.Cys1130Cys (c.3390C>T;p.Pro1127_Gly1180delinsArg), p.Leu1281Arg, p.Arg1597Gln, p.Ser1506Leu, p.Ile1628Thr, p.Arg1597Trp, p.Tyr1107Cys, p.Tyr1146Cys, p.Gly1629Arg, p.Gly1631Asp, p.Cys1142Phe, and p.Leu1657Pro. Five variant combinations - p.Asp366Leufs*16/p.Asn528Ser, p.Arg202Trp-Arg1583Gln/p.Cys849Tyr - were also associated with severe bleeding phenotypes (Figure 4). Among them, several variants were

consistently associated with high ISTH-BAT scores; however, others (eg, p.Cys1130Cys, p.Cys1142Phe, p.Gly1629Arg, and p.Gly1631Asp) showed wide variability in bleeding severity among patients with the same genotype. The other type 2A variants with lower ISTH-BATs are shown in Figure 4. Among different variants identified in type 2B, several of them, including p.Arg1306Trp, p.Arg1308Cys, p.Val1316Met, p.Ser1263-Pro1266Leu/p.Cys2557Tyr, p.Arg1308Leu, p.Arg-1308Cys/p.Gly1172Val, and p.Cys275Arg/p.Pro1337Leu were associated with ISTH-BAT scores ≥10 (Figure 5). Overall, type 2B variants exhibited less variability in ISTH-BAT scores than type 2A; however, a few variants (eg, p.Arg1306Trp, p.Arg1308Cys, and p.Val1316Met) demonstrated considerable variation in bleeding severity. Other type 2B variants showed lower ISTH-BAT scores and 2B NY variants were associated with the mildest symptoms.

In type 2M, an ISTH-BAT score ≥10 was observed in genetic variants such as p.Arg1374His, p.Arg1315Cys, p.Arg1315Leu/p.Arg924Gln, p.Tyr1321Cys, p.Asp1283His, and p.Ala1377Val-Arg1379Cys (Figure 6). Additionally, two gene conversions (p.Phe1369Ile-Ser1378Phe-Arg1379Cys and p.Val1360Ala-Phe-1369Ile-Ser1378Phe-Arg1379Cys) were also linked to high
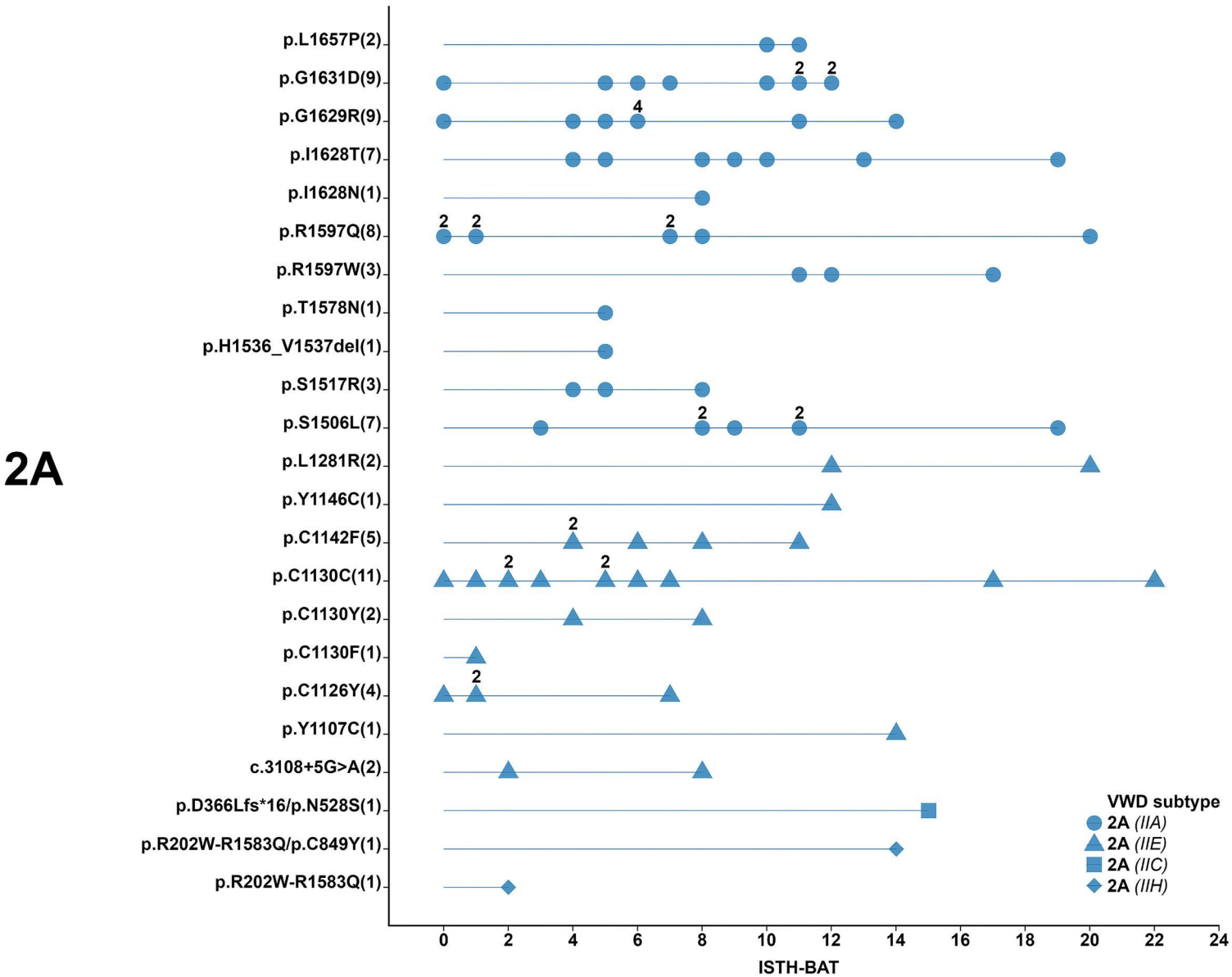
Figure 4. Genotype-phenotype correlation in type 2A von Willebrand disease based on ISTH bleeding assessment tool scores. Variants are ordered by von Willebrand factor domain. Case numbers are shown in parentheses; overlapping cases with identical ISTH bleeding assessment tool (ISTH-BAT) scores are indicated above the data point. VWD: von Willebrand disease.
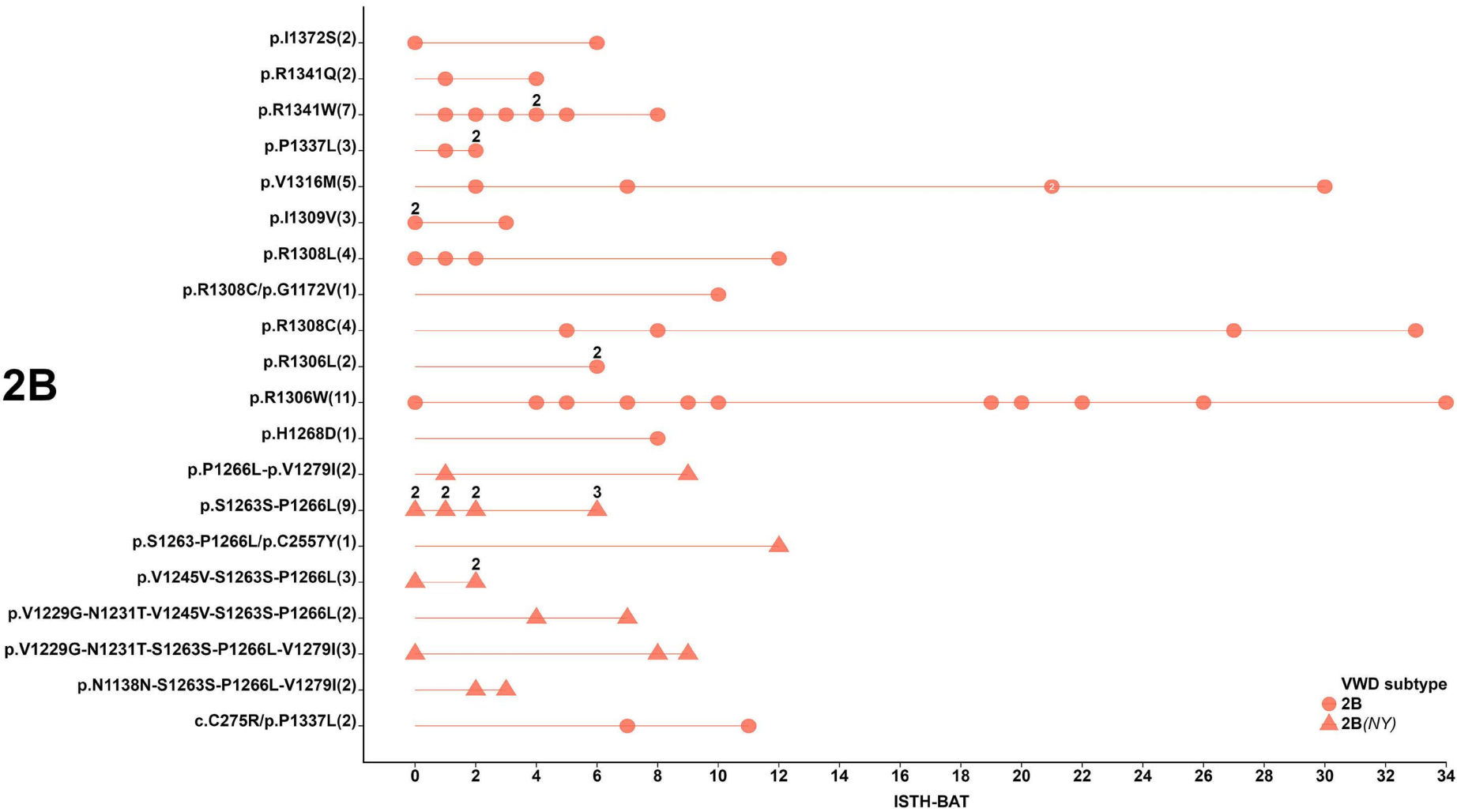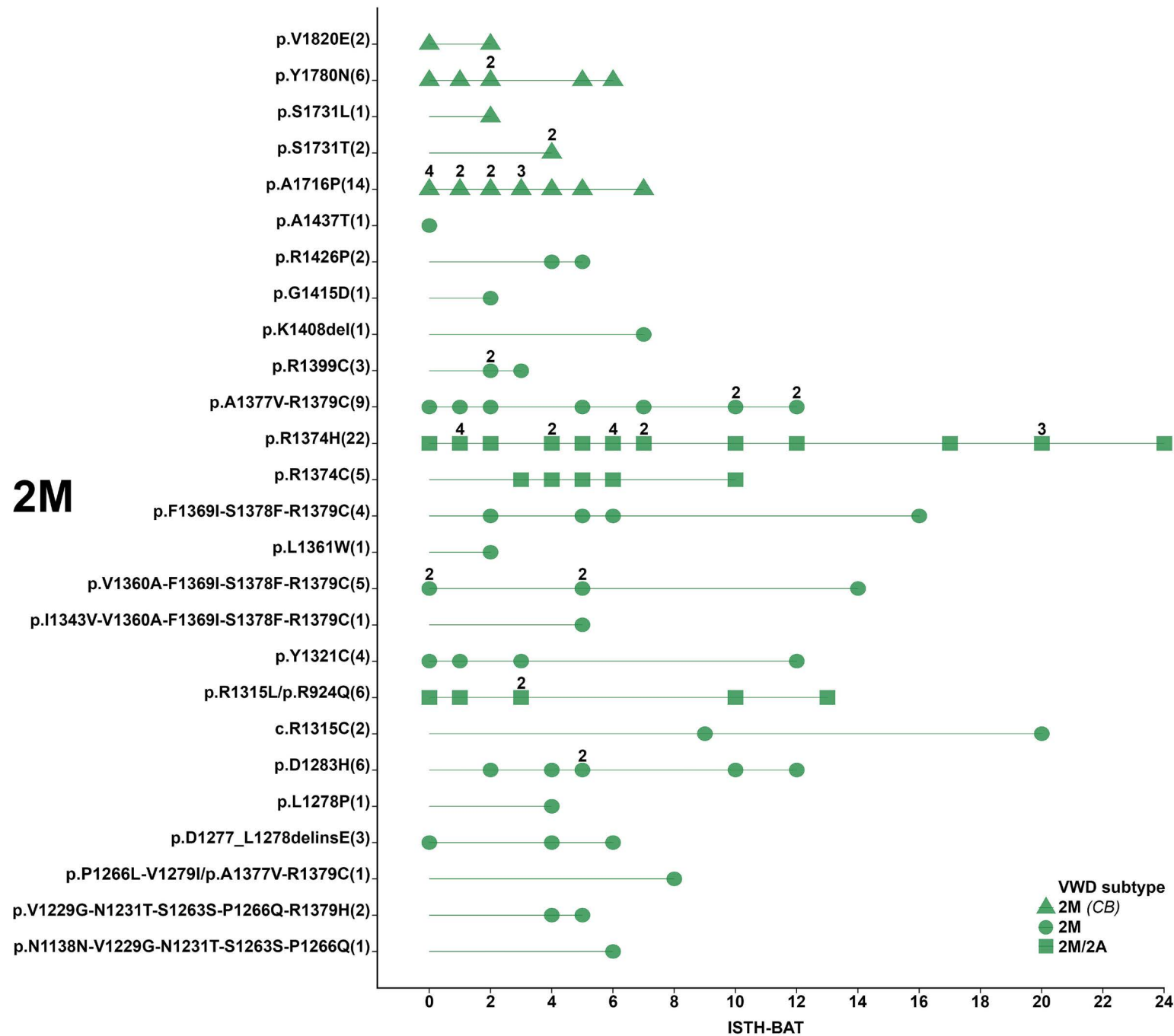
Figure 5. Genotype-phenotype correlation in type 2B von Willebrand disease based on ISTH bleeding assessment tool scores. Variants are ordered by von Willebrand factor domain. Case numbers are shown in parentheses; overlapping cases with identical ISTH bleeding assessment tool (ISTH-BAT) scores are indicated above the data point. VWD: von Willebrand disease.

ISTH-BAT scores. Among cases classified as type 2M(CB), all variants were associated with lower ISTH-BAT scores (Figure 6). Marked variability in BAT scores was observed among patients with the same type 2M variants.

Within the type 2N variants, p.Arg854Gln/p.Arg854Gln, p.Arg854Gln/p.Leu893Arg, and p.Arg854Gln/c.2546+3G>C were found in cases with an ISTH-BAT score ≥10 (Figure 7). Although eight cases were heterozygous for p.Arg854Gln, their FVIII and/or VWF levels suggested the presence of additional, undetected variants. These cases also showed variability in their BAT scores.

Discussion

Few studies have directly explored the genotype and clinical phenotype correlation in type 2 VWD, largely due to the genetic heterogeneity and complexity of the disorder. While significant attention has been devoted to other types of VWD, particularly types 1 and 3,[7-9,16-19] research on type 2 primarily concentrated on identifying genetic variants or examining genotype-laboratory phenotypic correlations.[4,10,11,20,21] However, the relationship between type 2 genetic variants and clinical bleeding manifestations remains poorly understood in the frame of large patient cohorts.

The importance of the bleeding score in VWD has been demonstrated in previous studies, as it helps predict clinical outcomes by quantifying bleeding severity.[22,23] Higher scores have been linked to an increased need for intensive on-demand therapy and may help to identify patients who might benefit from regular prophylaxis.[22]

This study provides a detailed analysis of 274 patients diagnosed with type 2. Following comprehensive clinical, genetic, and phenotypic characterizations of these cases with various type 2 subtypes, the study highlights significant variations in bleeding manifestations and severity across the different subtypes, as well as the impact of genetic variants on clinical outcomes.

A high prevalence of bleeding was observed in this cohort, with 89% of 274 patients reporting at least one bleeding event. Cutaneous bleeding, epistaxis, menorrhagia, oral cavity bleeding, bleeding from minor wounds, and childbirth hemorrhage, were the most frequently observed bleeding symptoms across the entire cohort. These bleeding profiles across the subtypes, as measured by ISTH-BAT scores, were consistent with other reports.[10,24,25] Severe bleeding complications such as hemarthrosis, muscle hematomas, GI bleeding, and CNS bleeding occurred rarely but showed subtype-specific patterns. For example, type 2A was associated with a higher rate of GI bleeding, whereas type 2N
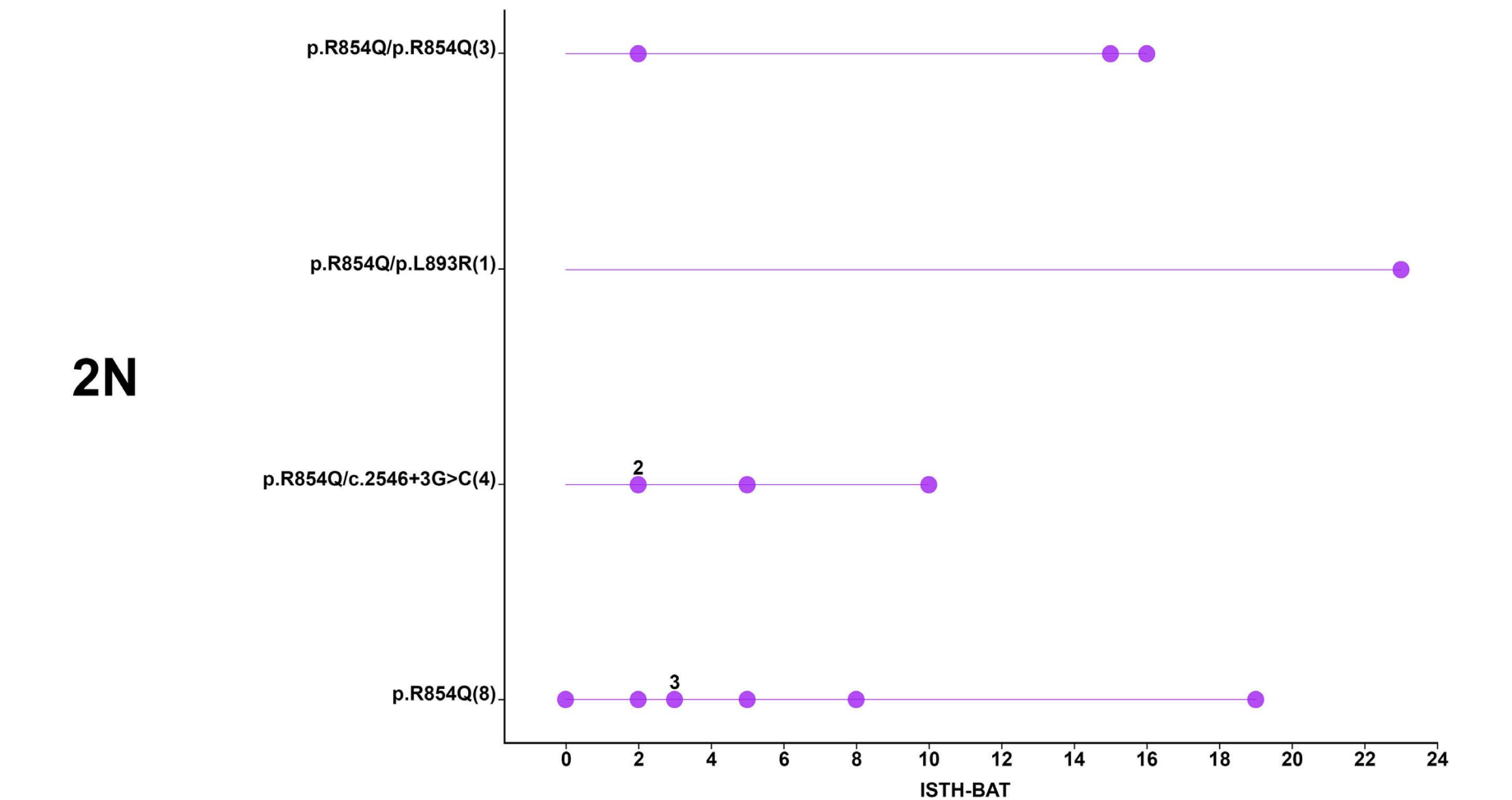
Figure 6. Genotype-phenotype correlation in type 2M von Willebrand disease based on ISTH bleeding assessment tool scores. Variants are ordered by von Willebrand factor domain. Case numbers are shown in parentheses; overlapping cases with identical ISTH bleeding assessment tool (ISTH-BAT) scores are indicated above the data point. VWD: von Willebrand disease.

exhibited a higher incidence of hemarthrosis and muscle hematomas compared to other subtypes. Notably, type 2A exhibited the most severe bleeding phenotype, with particularly high frequencies of epistaxis, menorrhagia, and postpartum hemorrhage, as well as a higher incidence of GI bleeding and surgical bleeding compared to the other subtypes. These findings are in line with previous reports suggesting that type 2A is often associated with a more severe bleeding tendency than other type 2 forms.[24,25] Type 2B patients also presented largely with mucocutaneous bleeding but at slightly lower frequencies. GI and surgical bleeding were less frequent in type 2B compared to type 2A, suggesting that type 2B, while still clinically significant, may be associated with a slightly lower overall bleeding severity, even though this strongly depends on the underlying genetic variants (see below). Type 2M, being characterized by a predominantly mucocutaneous bleeding pattern (epistaxis, oral cavity bleeding, and menorrhagia), exhibited the

lowest rates of severe bleeding complications, such as GI and surgical bleeding, in comparison with both type 2A and 2B. Type 2N, on the other hand, demonstrated a distinct bleeding profile with a higher frequency of hemarthrosis and muscle hematomas than other subtypes, this prevalence of soft tissue bleeding being expected due to the low FVIII. However, other bleeds, although less common, were observed in these patients.

The median ISTH-BAT score for type 2A patients was the highest, followed by type 2B, 2M, and 2N. This aligns with the observed clinical bleeding severity, as type 2A is typically associated with more severe bleeding. A report from the Netherlands found a higher severity in type 2B compared to type 2A, 2M and 2N,[10] but others found a similar bleeding tendency among various type 2.[24,25] In general, our BAT scores were lower than the WiN cohort, probably due to the fact that they included more severe patients plus used a different and self-administered BAT score.[10] An import-

Figure 7. Genotype-phenotype correlation in type 2N von Willebrand disease based on ISTH bleeding assessment tool scores. Variants are ordered by von Willebrand factor domain. Case numbers are shown in parentheses; overlapping cases with identical ISTH bleeding assessment tool (ISTH-BAT) scores are indicated above the data point.

ant finding was the variability in bleeding severity across different subtypes within each type 2 VWD category. While no significant differences were seen between 2A(IIA) and 2A(IIE) or between 2M and 2M/2A, bleeding severity differed significantly between 2B and 2B NY, 2M and 2M(CB), and 2M/2A and 2M(CB), suggesting distinct clinical phenotypes linked to specific subtypes and genetic variants. Although based on smaller sample sizes, previous studies have shown that patients with type 2B NY and type 2M(CB) exhibit milder bleeding severity than those with classical type 2B and type 2M, respectively.[26-28]

Our analysis highlights that bleeding severity, as assessed by ISTH-BAT, is influenced by both sex and age in individuals with type 2 VWD. Females had significantly higher ISTH-BAT scores than males, likely reflecting bleeding challenges related to menstruation and childbirth. Adults also exhibited higher bleeding scores compared to children, which may be attributed to cumulative bleeding events over time and experiencing more clinical challenges. Blood group O was not significantly associated with increased bleeding severity. Notably, while no significant subtype-specific differences were observed in children, adult patients showed distinct bleeding profiles between type 2A and 2M, and between 2M and 2N, suggesting that subtype-related bleeding phenotypes become more apparent with age. These findings underscore the importance of considering age, sex, and VWD subtype in clinical bleeding assessment and management.

The genetic analysis identified a spectrum of 67 distinct variants across the type 2 subtypes. Our findings newly underscore the substantial variability in bleeding severity among different genetic variants and even among individuals carrying the same *VWF* variant, highlighting the complexity of genotype and clinical phenotype correlations in type 2. In type 2A, a wide heterogeneity was observed in ISTH-BAT scores. Seventeen variants such as p.Cys1130Cys, p.Leu1281Arg, p.Arg1597Gln, p.Ser1506Leu, p.Ile1628Thr, and p.Arg1597Trp, were associated with more severe bleeding (ISTH-BAT ≥10). While some variants consistently led to elevated scores, others displayed a broad range of bleeding phenotypes. In contrast, a few were consistently linked to mild symptoms. In type 2B, variants such as p.Arg1306Trp, p.Arg1308Cys, and p.Val1316Met were also associated with more severe bleeding. However, the overall bleeding severity in type 2B was generally lower than in type 2A, as reflected by ISTH-BAT scores <10 for many variants. As with type 2A, some type 2B variants showed notable intravariant variability. Interestingly, gene conversions leading to the 2B NY phenotype were consistently associated with milder bleeding compared to classical 2B variants. The bleeding phenotype in type 2M presented a similarly complex picture. Several variants, including p.Arg1374His and p.Arg1315Cys were associated with higher ISTH-BAT scores, whereas those classified as type 2M(CB) were consistently associated with milder bleeding. Gene conversions also

contributed to higher scores. Again, marked variability was seen among individuals with the same variant, reinforcing the multifactorial nature of bleeding expression. In type 2N, combinations such as p.Arg854Gln/p.Arg854Gln and p.Arg-854Gln/p.Leu893Arg were linked to more severe bleeding. Interestingly, heterozygous carriers of p.Arg854Gln also showed variable phenotypes, suggesting the presence of additional genetic or modifying factors.

Taken together, these findings highlight key insights into type 2 VWD: its inherent genetic and clinical complexity; the variable impact of different variants within the same subtype; and the striking phenotypic variability even among individuals with identical genetic variants. This variability suggests that bleeding severity is influenced not only by the specific VWF variant but also by additional modifiers. Such variability has been previously reported in type 2M cases.[29,30] Over time, it has become evident that several key factors contribute to the variable clinical and laboratory expression of VWD, including age, blood group, type of genetic variants, and environmental exposures.[31,32] These factors may, at least in part, explain the observed such clinical variability.

While this study offers new insights into the genetic basis of various clinical features of type 2 VWD, using one of the largest cohorts to date, several limitations should be acknowledged. First, the retrospective nature of the study may limit the generalizability of the findings to all type 2 patients. Additionally, the sample size for some subtypes, especially type 2N, was limited, warranting larger studies to better characterize bleeding patterns and genetic variability in type 2 VWD. Because data on the total number of surgeries per patient were not available, bleeding after surgery was assessed across the entire cohort based on the presence of any postoperative bleeding events documented in the ISTH-BAT domain. ISTH-BAT scores may be influenced by age, as younger individuals may not have faced certain hemostatic challenges like surgery. Furthermore, sex differences exist since females may score higher due to menstruation or pregnancy, which are not applicable to males.

In conclusion, this study provides a comprehensive overview of the clinical features and genetic characteristics underlying bleeding phenotypes in the four subtypes of type 2 VWD, representing one of the largest genetic and clinical associations of this disorder. Our findings reveal significant variability in bleeding phenotypes across the subtypes: type 2A is associated with the most severe bleeding symptoms, followed by type 2B, while type 2M and type 2N exhibit milder and distinct bleeding tendencies. Notably, type 2B NY variants and type 2M(CB) variants both mitigate the severity of their respective VWD subtypes. Our findings newly underscore the phenotypic heterogeneity of type 2 VWD, demonstrating that even identical *VWF* genetic variants result in widely variable bleeding severity among different individuals.

Data sharing statement

All necessary data are included in the manuscript or the Online Supplementary Appendix. Additional data can be requested from the corresponding author.

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