A Laboratory Phenotype/Genotype Correlation of 1167 French Patients From 670 Families With von Willebrand Disease

A New Epidemiologic Picture

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Abstract: von Willebrand disease (VWD) is a genetic bleeding disease due to a defect of von Willebrand factor (VWF), a glycoprotein crucial for platelet adhesion to the subendothelium after vascular injury. VWD include quantitative defects of VWF, either partial (type 1 with VWF levels <50 IU/dL) or virtually total (type 3 with undetectable VWF levels) and also qualitative defects of VWF (type 2 variants with discrepant antigenic and functional VWF levels). The most bleeding forms of VWD usually do not concern type 1 patients with the mildest VWF defects (VWF levels between 30 and 50 IU/dL).

The French reference center for VWD performed a laboratory phenotypic and genotypic analysis in 1167 VWD patients (670 families) selected by their basic biologic phenotype: type 3, type 2, and type 1 with VWF levels <30 IU/dL. In these patients indeed, to achieve an accurate diagnosis of VWD type and subtype is crucial for the management (treatment and genetic counseling).

A phenotype/genotype correlation was present in 99.3% of cases; 323 distinct VWF sequence variations (58% of novel) were identified (missense 67% versus truncating 33%). The distribution of VWD types was: 25% of type 1, 8% of type 3, 66% of type 2 (2A: 18%, 2B: 17%, 2M: 19%, 2N: 12%), and 1% of undetermined type. Type 1 VWD was related either to a defective synthesis/secretion or to an accelerated clearance of VWF. In type 3 VWD, bi-allelic mutations of VWF were found in almost all patients. In type 2A, the most frequent mechanism was a hyper-proteolysis of VWF. Type 2B showed 85% of patients with deleterious mutations (distinct from type 2B New York). Type 2M was linked to a defective binding of VWF to platelet glycoprotein Ib or to collagen. Type 2N VWD included almost half type 2N/3.

This biologic study emphasizes the complex mechanisms for both quantitative and qualitative VWF defects in VWD. In addition, this study provides a new epidemiologic picture of the most bleeding forms of VWD in which qualitative defects are predominant.

INTRODUCTION

Von Willebrand factor (VWF) is a large multimeric glycoprotein crucial for platelet-dependent primary hemostasis; it is also the carrier protein for coagulation factor VIII (FVIII).1 VWF is synthesized as a 2813-amino-acid (aa) monomeric protein exhibiting repeated domains in the order D1-D2-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK.2 This biologic study emphasizes the complex mechanisms for both quantitative and qualitative VWF defects in VWD. In addition, this study provides a new epidemiologic picture of the most bleeding forms of VWD in which qualitative defects are predominant.
The VWF gene (VWF) is located at the tip of the short arm of chromosome 12 (178 kb of genomic DNA and 52 exons).3,5 Considering its large size and its structural complexity involving many distinct domains and an original multimeric organization, VWF is prone to both quantitative and qualitative defects. In a very large majority of cases, VWF defects are genetically inherited (related to mutations of VWF gene or of other still unknown genes) and lead to von Willebrand disease (VWD).6 In contrast, few VWF defects are acquired by miscellaneous mechanisms, consisting in an acquired von Willebrand syndrome (AVWS).7 VWF defects induce bleeding manifestations, mainly mucosal, which intensity is usually proportional to the severity of the protein deficiency.8 The revised classification of VWD includes 6 types:9 VWD type 1 (OMIM ID#130770) is defined by low levels (<50 IU/dL) of a functionally normal VWF and a dominant inheritance. Within type 1, a ~30 IU/dL threshold (plasma VWF levels lower than 30 IU/dL) is likely to distinguish VWF defects related to VWF gene sequence variation(s) from VWF defects potentially related to other genetic causes/contributors such as gene modifiers.10,11 VWD type 3 defined as severe VWD (OMIM ID#277480) is characterized by undetectable (<1 IU/dL) plasma and platelet VWF levels and a recessive inheritance.12 VWD type 2 (OMIM ID #613554) consisting in qualitative functional VWF defects is very heterogeneous and includes 4 main types:13 type 2A is defined by a decreased binding of VWF to platelet GPIb due to a significant reduction or absence of the high-molecular-weight (HMW) multimers of VWF; type 2M is characterized by a decreased binding of VWF to platelet GPIb or to collagen with normal or subnormal multimeric distribution; type 2B is defined by an increased binding of VWF to platelet GPIb; type 2A, 2B and 2M have in common to affect primary hemostasis and 2B usually does not inherit an impaired binding of VWF to FVIII and a recessive inheritance. A supplementary level of classification is used to distinguish specific mechanisms within type 2A (IIA, IIC, IID, IIE).14 Within type 2B (i.e. “New York” type),15 or within type 1 (i.e. “Vicenza” rapid VWF clearance type).16

The diagnosis of VWD is based on clinical and biological information.17 The laboratory phenotypic investigation for VWD includes many tests ranked in 3 levels.18 Screening assays include classically activated partial thromboplastin time (aPTT), platelet count, and closure time (PFA-100 analyzer). Second-level-specific VWF assays are crucial to diagnose VWF deficiency and they include the measurement of FVIII activity (FVIII:C), VWF antigen (VWF:Ag), and VWF ristocetin cofactor activity (VWF:RCo) allowing the calculation of ratios (VWF:RCo/VWF:Ag and FVIII:C/VWF:Ag) and the measurement of ristocetin-induced platelet aggregation (RIPA). Third-level VWF assays are devoted to a better characterization of VWD types and they include structural assays (VWF multimers analysis, VWF propeptide [VWFpp]) and functional assays (VWF binding to platelet GPIb, to collagen [VWF:CB] and to FVIII [VWF:FVIIIb]).19,20 Some of these assays are available only in some hospital laboratories specialized in the management of inherited bleeding disorders. The genotypic investigation for VWD, also performed in expert laboratories, is considered as a final step to definitely confirm the diagnosis of VWD-specific type.21,22 The diagnosis of VWD type and subtype is crucial for many purposes: to make a differential diagnosis between VWD 2N and hemophilia A or between VWD 2B and a platelet disorder named “pseudo VWD or platelet type VWD,”23 to predict the response to desmopressin24 or the kinetic of VWF during pregnancy, to anticipate the risk of allo-immunization in patients with type 3 VWD,25 and to help for genetic counseling.5

In terms of prevalence, VWD is usually reported to be “the most common human inherited bleeding disorder.” Actually, the 1% prevalence reported to support this observation includes all subjects with either qualitative or any quantitative VWF defects including the mildest quantitative defects (values ranging between 30 and 50 IU/dL), the latter subjects being usually pauci- or nonsymptomatic.26 In contrast, when excluding these milder forms and thus, only considering usually “bleeder” patients (who overlap VWD type 3, type 2, and type 1 with VWF levels usually <30 IU/dL), VWD prevalence is reported to be only ~1/10,000.10,26 This low prevalence, lower than the 1/2000 threshold defining rare diseases, thus allows to consider the most bleeding forms of VWD as a rare disease. This new epidemiologic point of view prompted our group (hemostasis departments from Paris Lariboisière, Lille, Nantes, Bicêtre and Caen university hospitals) to candidate for a national certification for rare diseases provided by the National Plan for Rare Disease (NPRD) on behalf of the French Health Ministry. Our group has been qualified as the national reference center for VWD (CRMW for “Centre de Référence de la Maladie de Willebrand”) since 2006 and we have been collaborating with the main hemostasis departments of France (from about 50 university hospitals) as a national network. The CRMW provides to patients with VWD diagnosed in these latter hemostasis departments, a unique national biologic platform for both the highly specialized phenotype and the genotype of VWD.

The current paper reports the biologic analysis (focus on the phenotypic/genotypic correlation) of 1167 French patients with VWD (from 670 families) enrolled during the first 6 years of functioning of the CRMW. All patients were extensively investigated by the CRMW biologic platform to achieve an accurate diagnosis of VWD type. Thanks to stringent laboratory inclusion criteria required by the definition of rare diseases (limitation to VWD type 3, type 2 and type 1 with VWF levels <30 IU/dL), this cohort brings new insights in the epidemiology of VWD specifically related to mutation(s) of VWF gene.

**PATIENTS, MATERIALS, AND METHODS**

**Patients**

Among the missions of the CRMW, one is to help, on their request, all the physicians of France to make a precise diagnosis of VWD in their patients thanks to a unique biologic platform devoted to an exhaustive phenotypic and genotypic analysis of VWF. On purpose, only patients with the following laboratory inclusion criteria (defined by the CRMW but performed by the enrolling hemostasis departments from at least 2 distinct samples) could beneficiate from further laboratory testing by the CRMW biologic platform: for type 1 phenotype, VWF levels <30IU/dL (together with VWF:RCo/VWF:Ag and FVIII:C/VWF:Ag ratios >0.6); for type 2 phenotype, a decreased or normal VWF levels with a discrepancy between the antigenic and the functional levels (VWF:RCo/VWF:Ag or FVIII:C/VWF:Ag ratios <0 or >0.6); and for type 3 phenotype, VWF plasma levels <5 IU/dL. Only inherited VWF defects were considered: any patient with a clinical context potentially responsible for an AVWS was thus excluded. Affected family
members of index cases (IC) were also eligible. The inclusion period of patients spanned from January 1st, 2007, to December 31, 2012.

Blood Collection, Ethical Issues
Venous blood was collected at the time of enrolment in the study, into 1:10 final volume of 3.2% or 3.8% sodium citrate; platelet-poor plasma was obtained as described. Blood was also collected on EDTA for molecular genetic analysis. An informed consent, specific for the CRMW and explaining that both the phenotypic and genotypic assays were performed for medical diagnosis purposes, was obtained from each patient according to the Declaration of Helsinki. The study was approved by the Ethics Committee of Lille University Hospital. The CRMW database and biobank (plasma and DNA) were declared to the French data protection authority.

Phenotypic Assays
Phenotypic assays performed in VWD patients were as follows. First-level assays performed locally by the enrolling hemostasis departments, consisted in VWF:Ag, VWF:RCO, FVIII:C, platelet count, aPTT, Ristocetin-Induced Platelet Aggregation (RIPA), and PFA-1009 Occlusion Time (ADP and Epinephrine). The second-level VWF assays were performed by the CRMW biologic platform as previously described: VWF multimeric distribution (1.5% SDS-agarose gel electrophoresis); VWF binding to platelet glycoprotein Ib; VWF-CB; VWF:FVIIIb; VWF pp. For each patient, the first-line test panel included VWF multimers and either VWF binding to platelet glycoprotein Ib (phenotype 2A, 2B, 2M) or VWF:FVIIIb (phenotype 2N). Other assays were added as a function of specific phenotypes (VWF:C to document some type 2M or type 1, VWF pp to document VWF clearance in type 1).

Genetic Analysis
The strategy for VWF gene analysis in IC patients was performed as a function of VWD phenotype previously documented by second-level VWF assays (suppl. Material, suppl. Figure 1, http://links.lww.com/MD/A761). Patients genomic DNA was screened for sequence variations by direct sequencing of VWF gene and in some cases, by multiplex ligation-dependent probe amplification (MLPA). HGVS nomenclature (URL: http://www.hgvs.org/mutnomen/) was used for the sequence variations. To analyze conservation across evolution and prediction on structure and functional effect of protein changes, each novel sequence variation was checked on miscellaneous databases (Sheffield mutations database: http://www.aggem.unimelb.edu.au/mutation/ and LOVD VWF database: https://www.ragtime.org/lovdb/VWF/Home.php?select_db=VWF) to determine their deleterious or candidate status. The mutation(s) was (were) qualified to support the phenotype if already reported as deleterious or if candidate but located within a VWF domain likely to explain the phenotype.

RESULTS
Study Population and Demographic Features
According to the inclusion criteria, 1856 subjects were eligible for a phenotypic/genotypic analysis by the CRMW. Six hundred and eighty-nine subjects could not be tested because their blood samples were finally not sent to the CRMW biologic platform. Among the 1167 subjects tested for both phenotype and genotype, 670 were IC (consisting in VWD propositi from 670 unrelated families) whereas 497 were their affected family members. The cohort of 1167 VWD patients exhibited a sex ratio of 1.46F/1M and an age distribution ranging from 6 months to 90 years-old with a 34 y.o. median. The proportion of O blood group was 56%, and in those patients, mean VWF:Ag levels were 191 IU/dL lower than in patients with non-O blood group. The main ethnic groups were White (90% including 15% of people from North Africa) and Afro-Caribbean (10%).

Phenotype/Genotype Correlation
The proportions of the miscellaneous VWD types and subtypes were similar between the 670 IC and the global cohort of 1167 subjects (Figure 1). Among 670 IC, after second-level phenotypic assays and direct sequencing of VWF gene, 648 patients exhibited a genotype supporting their phenotype (1 mutation of VWF gene in 87% of cases, several mutations in 13% of cases) (Figure 2). In contrast, the genotype did not support the phenotype in 22 patients after direct sequencing who thus underwent MLPA analysis (Figure 2). Large deletions, insertions, and duplications were identified in 14 patients (Table 1) but 8 patients remained with a genotype either not explaining or only partially supporting their phenotype (Figure 2).

One hundred and sixty-seven (25%) IC patients were classified as type 1 VWD: normal or subnormal VWF multimeric pattern, presence of 1 heterozygous VWF gene mutation previously described or candidate in type 1 or type 3 VWD (n = 161), or no mutation identified (n = 6) (Figures 2 and 3). Type 1 mutations associated with a decreased synthesis/secretion of VWF were present in 88 patients (Figure 3). Interestingly, 33 patients exhibited mutations inducing an

FIGURE 1. Distribution of von Willebrand disease (VWD) types in the global cohort (n=1167 patients) and in the index cases (n=670 cases). The proportion of each VWD type (1, 2A, 2B, 2M, 2N, and 3) is represented with blue histograms for the global cohort of 1167 patients and with red histograms for the 670 index cases (IC). These proportions are similar for both groups of patients within each VWD type. IC = index cases, VWD = von Willebrand disease.
FIGURE 2. Phenotype/genotype correlation in 670 index cases (IC) patients with von Willebrand disease (VWD). Among 670 IC, after second level phenotypic assays and direct sequencing of VWF gene, 648 patients exhibited a genotype supporting their phenotype: 151 patients had type 1 VWD, 121 patients type 2A, 112 patients type 2B, 127 patients type 2M, 81 patients type 2N, 49 patients type 3, and 7 patients undetermined (U) VWD. In contrast, after direct sequencing, the genotype was not supporting the phenotype in 22 patients (17 patients with no mutation and 5 patients with only 1 mutation although recessive inheritance) who thus underwent Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. MLPA helped identifying large deletions, insertions, and duplications in 14 patients consisting in 10 type 1, 1 type 2A (IIC), and 3 type 3. Finally, 8 patients remained with a genotype either not explaining their phenotype (6 type 1 patients with no mutation found) or only partially supporting their phenotype (2 type 3 patients with a single mutation found). IC = index cases, MLPA = multiplex ligation-dependent probe amplification, VWD = von Willebrand disease.

TABLE 1. von Willebrand Factor Antigen (VWF:Ag) Levels and Genotype of 14 Index Cases Patients With von Willebrand Disease (VWD) in Whom MLPA (multiplex ligation-dependent probe amplification) Was Used to Document the Presence of a Deletion or Duplication

| Patients (VWD Type and Number) | VWF:Ag Range (IU/dL) | VWF Gene–Allele 1 (Direct Sequencing) | VWF Gene–Allele 2 (MLPA) |
|-------------------------------|----------------------|--------------------------------------|--------------------------|
| Type 3 (n = 3)                | <1                   | Non sense mutations                   | Silent allele DupExon38–42 DeleXon1–3 whole deletion (confirmation) |
|                               |                      | p.R324<sup>+</sup>                   |                          |
|                               |                      | p.L414Afs<sup>+</sup> 15 whole deletion (suspicion) |                          |
| Type 1 (n = 10)               | 7–25                 | Normal                               | Silent allele DupExon35–37 DeleXon3–42<sup>1</sup> DeleXon1–3<sup>1</sup> DeleXon6–18 DeleXon19–20 DeleXon32–34 DeleXon33–34 | |
| Type 2A (IIC) (n = 1)         | 18                   | Splice mutation c.1157–1G > A        | Silent allele DupExon 6 |
accelerated VWF clearance with a corresponding phenotype combining plasma VWF levels <15 IU/dL, increased VWF propeptide and sometimes ultralarge VWF multimers. Six patients had mutations localized within the propeptide-cleavage site. Also, 34 patients with type 1 exhibited heterozygous mutations also described in type 3 VWD (Figure 3) and were thus considered as type 3 carriers. In 6 patients with type 1 VWD, no mutation was found.

Four hundred and forty-two IC patients (66%) were diagnosed with type 2 VWD (Figure 2). One hundred and twenty-two patients (18%) exhibited type 2A VWD: 76 patients exhibited type 2A(IIA) VWD consisting in defect of the HMW and intermediate MW VWF multimers, decreased VWF binding to platelet GPIb and previously reported or candidate mutations within VWF A2 domain (n = 72) or VWF A1 domain (n = 4); 42 patients had type 2A(IIE), 3 patients type 2A(IIC), and 1 patient type 2A(IID) (Figure 3). One hundred and twelve patients (17%) exhibited a type 2B VWD including 95 patients with a “classical” type 2B (increased VWF binding to platelet GPIb, variable loss of the high-molecular-weight VWF multimers, moderate or severe thrombocytopenia and previously reported or candidate mutations within VWF A1 domain) and 17 patients with a type 2B “New York” (always normal VWF multimeric pattern, no thrombocytopenia) (Figure 3).

One hundred and twenty-seven patients (19%) were diagnosed with a type 2M VWD: most often smeary VWF multimeric pattern, decreased VWF binding to FVIII and previously reported or candidate mutations within D0- or D3 VWF domains. Among type 2N patients, 37 patients exhibited a type 2N/3 phenotype confirmed by the genetic analysis (1 allele with a 2N VWD mutation and 1 nil allele) (Figure 3).

Fifty-four IC patients (8%) were diagnosed with type 3 VWD: VWF levels <5 IU/dL, no VWF multimer detectable by electrophoresis, presence of 2 VWF gene mutations previously reported or candidate in type 3 VWD (n = 52) or only 1 mutation heterozygous VWF gene mutation identified (n = 2) (Figure 2, Figure 3).
Seven IC patients (1%) remained with a type “undetermined” (U) VWD combining either a type 1 or a type 2 phenotype and new VWF gene mutations (Figure 2, Figure 3 and Table 2).

Focus on VWF Gene Mutations

A total of 323 distinct VWF gene mutations were identified in our VWD cohort including 189 (58%) new mutations. Missense mutations were the most frequent (67%), truncating sequence variations including nonsense mutations, small deletions/duplications and frameshift/splice mutations were 31% of all mutations, whereas large deletions/duplications were 2% of all mutations.

In type 1 VWD, 105 distinct sequence variations were identified (two-third of them being novel): they were spread all over VWF gene and they included a similar proportion of missense (51%) and truncating (49%) mutations (Figure 4).

In type 3 VWD, 61 distinct sequence variations were identified, including two-third of novel mutations (Figure 5). These sequence variations were spread all over VWF gene with

| No. Patients | FVIII:C (IU/dL) | VWF:Ag (IU/dL) | VWF:RCo (IU/dL) | VWF Multimers | VWF Gene Seq variation 1 | VWF Gene Seq variation 2 | VWF Gene Seq variation 3 |
|--------------|----------------|----------------|-----------------|--------------|-------------------------|-------------------------|-------------------------|
| 1            | 41             | 32             | 22              | HMW           | p.T2102H                | –                       | –                       |
| 2            | 22             | 7              | 8               | HMW           | p.G1736Efs*20         | c.5664+5G > A            | p.E2353D                |
| 3            | 83             | 42             | 29              | HMW           | p.E2720Gfs*4          | p.R854Q                 | –                       |
| 4            | 35             | 23             | 18              | Smeary        | p.2814Lext*39         | p.Y1584C                | –                       |
| 5            | 50             | 27             | 28              | HMW           | p.V2517F               | –                       | –                       |
| 6            | 25             | 15             | 13              | Smeary        | p.C2473W               | p.R2575H                | –                       |
| 7            | 70             | 40             | 16              |               | p.A188V                | p.C329S                 | –                       |

\(\_\_\_\) = decreased, FVIII:C = coagulation factor VIII, HMW = high molecular weight, Seq = sequence, VWF:Ag = von Willebrand factor antigen, VWF:RCo = von Willebrand factor ristocetin cofactor activity.

FIGURE 4. Sequences variations of VWF gene in 161 patients with type 1 von Willebrand disease (VWD). In our 161 patients with type 1 VWD, 105 distinct sequence variations spread all over VWF gene were identified. Novel mutations are indicated in blue. Missense mutations (51%) are presented on the top and truncating sequence variations (49%) are indicated on the bottom. In type 1 “IC” (clearance), the most frequent mutations were either the Vicenza mutation p.Arg1205His or others like p.Arg1205Cys, p.Arg1205Leu, p.Cys3115Phe, and dup.exon35–37. Six patients had mutations localized within the propeptide-cleavage site (p.Arg763Gly or p.Arg763Met). Interestingly, almost half of the truncating mutations found at the heterozygous state in our patients with type 1 VWD were also found in association with another mutation in our patients with type 3 VWD. IC = index cases, VWD = von Willebrand disease.
however, a hot spot on the N-terminal part of VWF (D domains); they consisted mainly in truncating mutations leading to silent alleles (82%) whereas missense mutations were rare (18%) (Figure 5).

In type 2 VWD, a total of 118 distinct sequence variations were identified, including one-third of new mutations (Figure 6). These sequence variations were clustered in the A domains of VWF (types 2A, 2B, and 2M) and in the D’-D3 domains of VWF (type 2N); they consisted in missense mutations in a large majority of cases (95%). Interestingly, in type 2N, 22 truncating mutations leading to a silent allele were also found (type 2N/3 patients) including one-third also found in our type 3 VWD patients (Figures 5 and 6).

**DISCUSSION**

The present study was focused on the laboratory analysis of a prospective cohort of 1167 patients with VWD, selected on the basis of a biologic phenotype including type 3, type 2, and type 1 with VWF levels \(<30\) IU/dL. Thanks to the support of the French NPRD from 2007 to 2012, we performed an exhaustive deciphering of both the biologic phenotype and genotype of these patients who constitute one of the largest VWD cohort enrolled and characterized at a national scale. The most recent and very interesting study dedicated to a cohort of VWD patients was published by Battle et al and it was mostly focused on a laboratory phenotype/genotype analysis, mechanisms for VWF deficiency were further elucidated.

On an epidemiologic point of view, theoretically, the expected cohort of French patients matching our inclusion criteria should include \(~6000\) people (1/10,000 prevalence; population in France: 60 million people). During the first 6 years of this study (2007–2012), we prospectively included 1856 eligible patients. Even if we are aware that reaching an exhaustive national enrollment is impossible, our data suggest that the 1/10,000 prevalence estimated for the most hemorrhagic forms of VWD is likely to be close to reality. In addition, our results suggest that the respective proportions of quantitative and qualitative deficiencies of VWF are totally different as a function of the inclusion criteria used to enroll patients with VWF defects. Indeed, in our cohort selected on the basis of a “severe” biologic phenotype mostly associated with a high bleeding risk (i.e., excluding the mildest VWF quantitative defects with VWF levels ranging between 30 and 50 IU/dL), qualitative VWF defects (type 2) are, as it could be expected, the most frequent (66%). In contrast, when including all VWF defects (both the “severe” biologic phenotype and the mildest forms), partial quantitative VWF defects (type 1) are reported to be the most frequent (~75%). For VWD type 3, the proportion of patients of our cohort (8%) is similar to those of the literature. In our cohort, the group of quantitative VWF deficiencies, that is, type 1 and type 3 VWD, showed interesting specific features and also, surprisingly, close genetic relationships. Our 54 IC patients with type 3 VWD exhibited 61 distinct mutations, spread all over VWF gene with a hotspot in the N-terminal part.
Most of them (82%) were truncating mutations predicted to induce a silent allele. These data are in agreement with the /C24100 mutations reported in type 3 VWD in the international literature.11,34,39–43 Our 167 IC patients with type 1 VWD showed 111 distinct mutations, spread all over VWF gene with, however, hotspots within both the N-terminal and the C-terminal parts of VWF. These data are in agreement with the most important studies dedicated to type 1 VWD11,12,24,44–46 altogether including ~300 patients with 85 distinct VWF gene mutations. Interestingly, in our study, half of the sequence variations found in type 1 VWD were truncating mutations which is more important than the 35% to 40% proportion of truncating mutations usually reported in the literature.11,44 In addition, surprisingly, almost half of the truncating mutations identified at a heterozygous state in our patients with type 1 VWD were also found, in association with another mutation, in our patients with type 3 VWD. This genetic overlap between type 1 and type 3 underlines that the penetrance of some type 3 mutations qualified as “recessive” may be variable. In other terms, the genetic basis of some cases of VWD type 1 may not rely on dominant mutations as classically described and these specific patients may be type 3 carriers with a biological type 1 phenotype.47 In our patients with type 1 VWD, missense mutations were present in 50% of cases, which is less than the 55% to 60% frequency usually reported in the literature.5 These missense mutations were mainly responsible for both a defect of synthesis/secretion of VWF or an accelerated clearance of VWF. Interestingly, in terms of mechanisms for VWF quantitative deficiency in our patients with type 1 VWD, 53% of cases were related to a synthesis/secretion defect, 20% of cases to an accelerated clearance (mainly involving the classical “Vicenza” mutation), and 20% presented a type 3 carrier status. Rare mutations in the PP cleaving-site represented 3.5% of cases. The high frequency of the “clearance” type 1 VWD16 has important clinical consequences for patients in terms of response to desmopressin.24 Also, the important proportion of VWF patients with a type 1 phenotype who genetically have a status of type 3 carriers may have crucial implications on genetic counseling.39,47 Finally, no mutation could be found in 6 patients with type 1 VWD and a single mutation was found in 2 patients with type 3 VWD. We can speculate on several hypothesis to explain this absence of mutation. As the 6 patients with type 1 exhibited “borderline” VWF levels of 30 IU/dL, they may have no mutation in the VWF gene.
gene and their decreased VWF levels may be linked to sequence variations of other genetic systems.4 Another hypothesis valuable for both the latter type 1 and type 3 patients is the presence of intronic mutation(s) of VWF gene (potentially leading to a nil allele) undetectable by our gene sequencing methodology. Last but not least, the role of polymorphisms of VWF gene (which combination on the same allele may either be deleterious by themselves or emphasize the deleterious effect of a concomitant mutation) cannot be excluded in these specific cases.

Qualitative VWD molecular variants represented two-third of our cohort (442 IC patients). They exhibit interesting features in terms of both distribution of VWD types (2A, 2B, 2M, and 2N) and mechanisms of VWF deficiency inducing the miscellaneous VWD subtypes. Our current data are in agreement with the majority of missense mutations distributed in various hotspots of our current patients with type 2 VWD, a sequence variation of gene was identified: 118 distinct mutations including a large majority of missense mutations distributed in various hotspots were identified, as usually described in the literature.48 The proportions of types 2A, 2B, 2M, and 2N were almost equivalent. Also, some specific points of our study may be underlined: (i) the important proportion of subtype 2A(III) (34.5%) within type 2A, (ii) the presence of 15% of subtype “New York” within type 2B, (iii) the clear identification of 2 mechanisms for type 2M including both subtype 2M “GPIb” (2M/2A like phenotype) and subtype 2M “collagen,”54 (iv) the important proportion of subtype 2N(3) (46%) within type 2N.55

In conclusion, the current study involving a large series of patients emphasizes the high level of matching between the genotype and the specialized phenotype in VWD.56 This data combined to medico-economic analysis may modify the future combined to medico-economic analysis may modify the future epidemiologic picture of VWD: indeed, if the 30 to 50 IU/dL range of VWF levels <301 IU/dL, VWD will switch from the status of “most common inherited bleeding disorder” to a rare bleeding disorder. Considering this point of view, the 30 to 50 IU/dL range of VWF defects which do not match with a VWF gene abnormality should be renamed “a bleeding risk factor” and not a disease as previously suggested.8 Finally, this work opens the way to further studies dedicated to specific subgroups of our cohort, in order to better investigate the relationships between the bleeding score and both the phenotypic and genotypic profiles.

ACKNOWLEDGMENTS

The authors are grateful to Sophie Capdenat and Sandrine Thoureau-Benghezal (Hospital Lariboisière, Paris), Catherine Mariches and Sylvie Hermoine (Hospital Cardiologique, Lille), Hélène Tout-Mandard (Hospital de Bicêtre, Le Kremlin Bicêtre), Patricia Talarmain and Marie-Annick Gourlaouen (Hospital Hôtel Dieu, Nantes), Elise Vallée (Hospital de la Côte de Nacre, Caen) for expert assistance.

The authors also thank the collaborators of the French Reference Center for von Willebrand disease who enrolled some patients: Claire Barro, CHU de La Tronche; Sophie Bayart, CHU de Rennes; Eric Beltan, CHU de Pointe-à-Pitre, Guadeloupe; Elisabeth Benz-Lemoine, CHU de Poitiers; Claire Berger, CHU of Saint Etienne; Marie-Anne Bertrand, CHU de Bézancourt; Philippe Beurrier, CHU d’Angers; Christine Biron-Andreani, CHU de Montpellier; Florence Blanjouvan, CHU d’Annecy; Jeanne-Yvonne Borg, CHU de Rouen; Tevfik Bouzekedjire, CHU de Bicêtre; Julien Bovet, CHU de Dijon; Catherine Boyer-Neumann, CHU Antoine Béclère Clamart; Marie-Elisabeth Briquel, CHU de Nancy; Sabine Castet, CHU de Bordeaux; Hervé Chambost, CHU de Marseille; Axel Chaminade, CHU Saint Denis de la Réunion; Pierre Chambouni, CHU de Rouen; Erwan Choblet, CHU de Nantes; Nouvelle Calédonie; Sophie Combe, CHU de Bicêtre; Yësin Dargaud, CHU de Lyon; Luc Darnige, CHU Georges Pompidou Paris; Dominique De Prost, CHU Louis Mourier Colombes; 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