

# Current Understanding of Inherited Modifiers of FVIII Pharmacokinetic Variation

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**Abstract:** The inherited bleeding disorder hemophilia A involves the quantitative deficiency of the coagulation cofactor factor VIII (FVIII). Prophylactic treatment of severe hemophilia A patients with FVIII concentrates aims to reduce the frequency of spontaneous joint bleeding and requires personalized tailoring of dosing regimens to account for the substantial inter-individual variability of FVIII pharmacokinetics. The strong reproducibility of FVIII pharmacokinetic (PK) metrics between repeat analyses in the same individual suggests this trait is genetically regulated. While the influence of plasma von Willebrand factor antigen (VWF:Ag) levels, ABO blood group, and patient age on FVIII PK is well established, estimates suggest these factors account for less than 35% of the overall variability in FVIII PK. More recent studies have identified genetic determinants that modify FVIII clearance or half-life including *VWF* gene variants that impair VWF-FVIII binding resulting in the accelerated clearance of VWF-free FVIII. Additionally, variants in receptors that regulate the clearance of FVIII or the VWF-FVIII complex have been associated with FVIII PK. The characterization of genetic modifiers of FVIII PK will provide mechanistic insight into a subject of clinical significance and support the development of personalized treatment plans for patients with hemophilia A.

**Keywords:** factor VIII, hemophilia A, von Willebrand factor, pharmacokinetics, clearance receptor

## Introduction

Hemophilia A (HA) is an X-linked inherited bleeding disorder that involves a quantitative deficiency or qualitative defect of the essential coagulation cofactor factor VIII (FVIII), resulting in impaired thrombin generation and fibrin formation at the site of vascular injury. The severity of a patient's bleeding phenotype is related to the amount of circulating functional FVIII, with mild (5–40%) and moderate (1–5%) HA patients typically displaying prolonged bleeding after injury or trauma.<sup>1</sup> In contrast, severe HA (plasma FVIII levels of <1%), which accounts for approximately 60% of all HA cases, is associated with spontaneous muscle and joint bleeds and can lead to joint arthropathy, a chronic debilitating condition.<sup>2</sup> Hemophilia A affects approximately 1 in 5000 males.<sup>3</sup> While the diagnosis of severe or moderate hemophilia in females is rare, up to 40% of female carriers will have plasma FVIII levels of <40% due to skewed X chromosome inactivation.<sup>4</sup>

Treatment of HA by intravenous infusion of recombinant or plasma-derived FVIII concentrates involves prophylactic or on-demand strategies. Typically, patients with mild or moderate HA with infrequent bleeding events will employ on-demand treatment with factor concentrates. In contrast, patients with severe HA, or mild/moderate cases with a history of frequent bleeding events, will utilize a prophylactic dosing regimen. Prophylactic dosing has been shown to reduce the frequency of joint bleeds and decreases the risk of arthropathy in severe HA patients.<sup>5</sup> These regimens aim to maintain the trough level of FVIII above 1–3% and typically involve thrice weekly infusions of FVIII concentrate with dose calculations based on body mass and in vivo recovery. More recently, proposals to maintain FVIII trough levels above 8–12% have gained support.<sup>6</sup> However, standardized dosing regimens are insufficient to prevent bleeds in all HA patients,<sup>7</sup> as this strategy fails to recognize the substantial inter-individual variability in FVIII pharmacokinetics.<sup>8–12</sup> As a result, the dosage required to maintain FVIII trough levels above the minimum levels is highly variable.<sup>11</sup> There has been widespread interest in developing personalized FVIII dosing regimens to enhance the time spent within an optimal

therapeutic window and to reduce the frequency of joint bleeding.<sup>13</sup> To complement these efforts, studies have now begun to identify the acquired and inherited factors that modify the inter-patient variability of FVIII PK.

## Inherited and Acquired Modifiers of FVIII Pharmacokinetics

The pharmacokinetic profile of infused FVIII concentrates demonstrates low intra-individual variability, with in vivo recovery, half-life, and clearance highly consistent between repeat analyses within the same individual.<sup>14,15</sup> In contrast, the inter-individual variability of FVIII PK is remarkably high, with metrics such as FVIII half-life varying between 6 and 28.8 hours (median ~ 12 hours) in both pediatric and adult populations.<sup>8–12</sup> These findings are independent of the FVIII concentrate used, including recombinant full-length, B-domain deleted, or plasma-derived products. While reports of detailed PK analyses in non-European populations are limited, patient ethnicity has not been reported to significantly modify FVIII PK.<sup>16,17</sup> The intra-individual reproducibility of FVIII PK strongly suggests a genetic basis for this trait. While the heritability, or genetic contribution to the variance of FVIII PK, has not been directly assessed, it is thought to be comparable to the heritability of endogenous FVIII levels in normal individuals, where estimates place the genetic effect between 57% and 85%.<sup>18,19</sup> Indeed, inherited determinants of endogenous FVIII levels in normal individuals have also been associated with modifiers of FVIII PK, including von Willebrand factor (VWF) antigen levels (VWF:Ag), VWF-FVIII binding activity (VWF:FVIIIb), ABO blood group, and VWF-FVIII clearance (Table 1).

In addition to inherited traits, several acquired modifiers of FVIII PK have been identified, including anthropomorphic measurements such as body mass index (BMI) which associates with increased FVIII half-life and delayed clearance.<sup>9,20,21</sup> Age is also a significant modifier of FVIII PK, with FVIII terminal half-life increasing by 0.9 hours for each decade of life.<sup>21,22</sup> In addition, the immunological response to FVIII can modulate FVIII PK. Approximately 30% of severe HA patients will develop neutralizing antibodies (termed inhibitors) to infused FVIII concentrates that inhibit FVIII cofactor activity, and result in rapid clearance of infused FVIII from the plasma.<sup>23</sup> For patients with a positive inhibitor history, FVIII terminal half-life was decreased by 1.3 hours.<sup>22</sup> Additionally, patients with non-neutralizing anti-FVIII IgG antibodies have a shorter reported FVIII half-life as compared to patients with no detectable antibodies.<sup>24</sup>

**Table 1** Influence of Genetic Variants on FVIII PK

Gene	Variant	HGVS	Variant Classification	MAF	Putative Mechanism	Patient Population	Effect on FVIII PK	Ref.
ABO	rs7853989	c.523C>T; p. (Arg175Cys)	Non-synonymous	0.1323	VWF glycosylation	Adolescent/ adults, N=43	Associated with half-life and clearance	[30]
	rs8176719	c.259-1_259insG; p. (Thr87AspfsTer107)	Frame shift	0.3539	VWF glycosylation			
	rs8176743	c.700G>A; p. (Gly234Ser)	Non-synonymous	0.1152	VWF glycosylation			
	rs8176746	c.793C>A; p. (Leu265Met)	Non-synonymous	0.1149	VWF glycosylation			
	rs8176747	c.800G>T; p. (Gly267Ala)	Non-synonymous	0.1149	VWF glycosylation			
ASGR2	rs2289645	c.-95T>C	5'UTR	0.4184	FVIII clearance?	Adolescent/ adults, N=32	FVIII elimination rate constant, alpha half-life, and mean residence time	[74]

(Continued)

Table 1 (Continued).

Gene	Variant	HGVS	Variant Classification	MAF	Putative Mechanism	Patient Population	Effect on FVIII PK	Ref.
CLEC4M	rs868875	c.700+73A>G	3'UTR	0.2551	VWF-FVIII, FVIII clearance	Pediatric, N=43	Associated with clearance	[9]
						Adult, N=44	Associated with clearance	[8]
						Adolescent/adults, N=43	Associated with half-life and clearance	[30]
						Adolescent/adults, N=26	Associated with elimination rate constant, half-life, and Beta rate constant	[65]
	VNTR	N/A	Variable number of tandem repeats	N/A	VWF-FVIII, FVIII clearance	Pediatric, N=43	Associated with clearance, k, and half-life	[9]
						Adult, N=44	Associated with FVIII clearance	[8]
LDLR	rs688	c.1773C>T; (p.Asn591=)	Synonymous	0.3892	FVIII clearance	Adolescent/adults, N=33	Associated with elimination rate constant, alpha distribution half-life, alpha half-life and clearance	[71]
	rs2228671	c.81C>T; p. (Cys27=)	Synonymous	0.0569	FVIII clearance	Adolescent/adults, N=33	Associated with clearance and volume of distribution	
STAB2	rs12229292	c.7248+582G>T	Intronic	0.2337	VWF-FVIII clearance	Pediatric, N=43	Associated with clearance	[9]
						Adult, N=44	No significant association	[8]
						Adolescent/adults, N=43	No significant association	[30]
	rs4981022	c.6987+378G>T	Intronic	0.3067	VWF-FVIII clearance	Pediatric, N=43	Associated with half-life and AUC	[9]
						Adult, N=44	No significant association	[8]
						Adolescent/adults, N=43	No significant association	[30]

(Continued)

**Table I** (Continued).

Gene	Variant	HGVS	Variant Classification	MAF	Putative Mechanism	Patient Population	Effect on FVIII PK	Ref.
TC2N	rs10133762	c.-57+9946A>C	Intronic	0.4672	Unknown	Pediatric, N=43	Associated with clearance	[9]
						Adult, N=44	No significant association	[8]
						Adolescent/ adults, N=43	No significant association	[30]
VWF	rs1063856/ rs1063857	c.2365A>G; p. (T789A)/ c.2385T>C; p.(Y795=)	Non-synonymous/ synonymous	0.3952	Regulates plasma VWF:Ag; VWF clearance	Pediatric, N=43	Associated with clearance	[9]
						Adult, N=44	No significant association	[8]
						Adolescent/ adults, N=43	No significant association	[30]

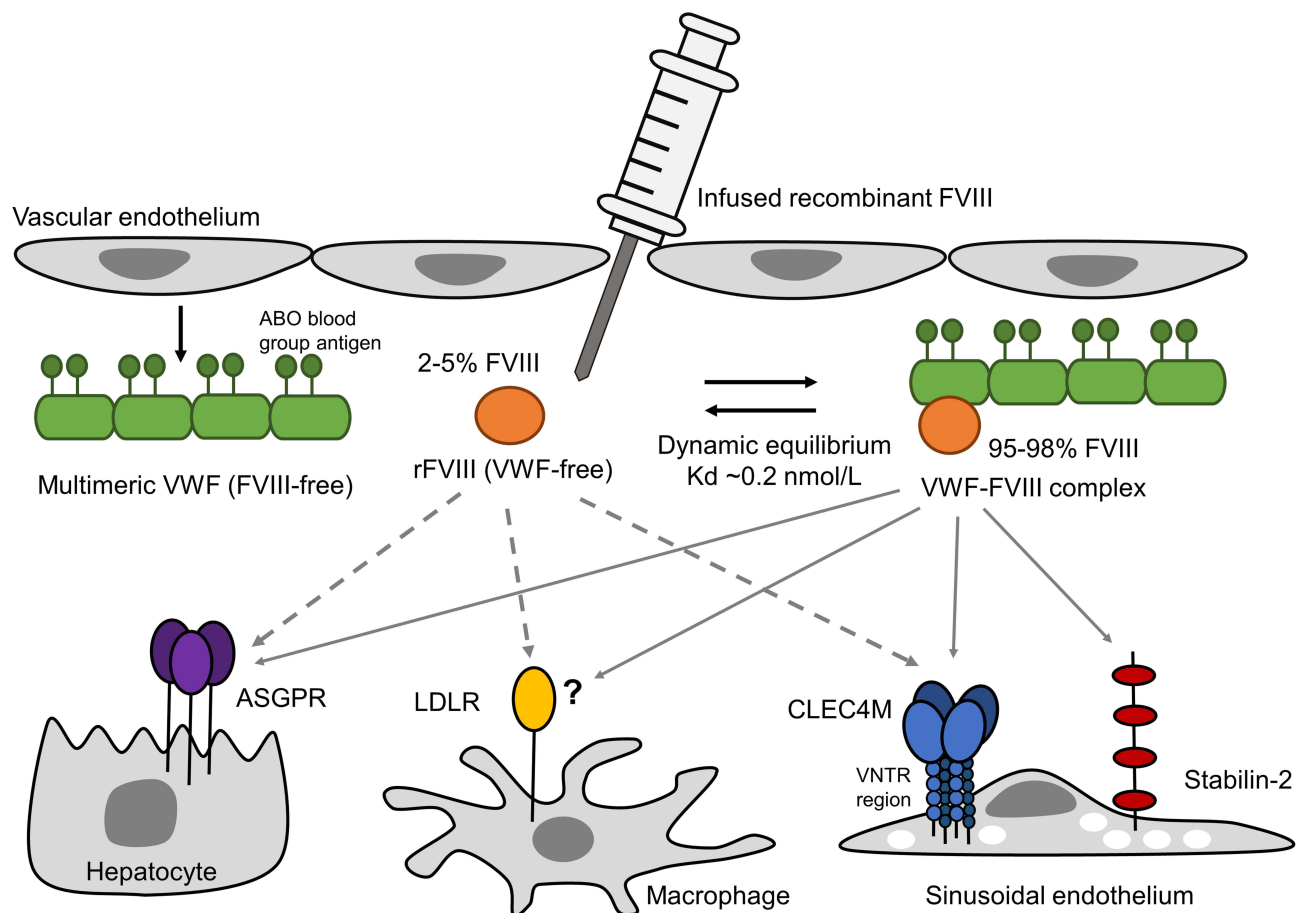
**Notes:** A summary of studies that identify variants that associate with FVIII PK metrics in the ABO blood group locus, ASGR2, CLEC4M, LDLR, STAB2, TC2N, and VWF genes. MAF (Minor allele frequency) data was obtained from gnomAD genomes combined population v3.1.

**Abbreviations:** HGVS, Human Genome Variation Society; N/A, not applicable; UTR, untranslated region; VNTR, variable number of tandem repeats; LD, linkage disequilibrium.

# VWF:Ag Levels and FVIII PK

In the plasma, FVIII circulates as a high-affinity non-covalent complex with its carrier protein VWF (Figure 1).<sup>25</sup> VWF is a large, multimeric glycoprotein that facilitates platelet adhesion and aggregation, and protects FVIII from accelerated proteolysis and clearance by scavenger receptors in the liver and spleen. Plasma levels of VWF strongly associate with endogenous plasma FVIII:C, with estimates suggesting that every 1% change in VWF:Ag will modify plasma FVIII levels by 0.54%.<sup>26</sup> Several studies have now demonstrated that pre-infusion plasma VWF:Ag levels are a major determinant of FVIII PK, with higher VWF:Ag associating with longer FVIII half-life and slower FVIII clearance.<sup>8,9,12,15,27,28</sup> Estimates suggest that each 10% (0.1 IU/mL) increase in plasma VWF:Ag associates with a 16.6 minute increase in the half-life of infused FVIII.<sup>29</sup> Given that plasma levels of VWF:Ag are highly variable (50–200%) in both normal individuals and patients with HA, this may account for up to 25% of the interindividual variability of infused FVIII half-life.<sup>28,30</sup>

Plasma levels of VWF are influenced by pathways that regulate the biosynthesis and secretion of VWF from endothelial cells and platelets, as well as the rate at which VWF is cleared from the plasma.<sup>31</sup> Interestingly, a study that utilized desmopressin to increase VWF secretion into the plasma did not significantly modify FVIII half-life or clearance.<sup>32</sup> The baseline rate of VWF secretion can be estimated through the surrogate measurement of plasma levels of the VWF propeptide (VWFpp), which is synthesized and secreted into the plasma in a 1:1 ratio with mature VWF. Importantly, VWFpp levels do not associate with FVIII PK in pediatric subjects and show a moderate but not statistically significant association in adult subjects.<sup>8,9</sup> The rate of VWF clearance can also be estimated using the surrogate VWFpp/VWF:Ag ratio, which shows a strong correlation with measures of FVIII PK in both pediatric and adult subjects.<sup>8,9,12</sup> Overall, these data suggest that the rate by which VWF is cleared from the plasma is a more significant determinant of FVIII PK than the processes which regulate the synthesis and secretion of endogenous VWF.



**Figure 1** The circulation and clearance of infused FVIII concentrate. In severe hemophilia A, baseline levels of FVIII:C are <1% of normal coagulation factor activity. Prophylactic or on-demand treatment of hemophilia A can involve the intravenous infusion of clotting factor concentrates of either recombinant FVIII or plasma-derived VWF-FVIII products. Once infused into the plasma, 95–98% of FVIII binds to and circulates with the large multimeric coagulation factor von Willebrand Factor (VWF), and the remaining 2–5% of FVIII circulates VWF-free. VWF and FVIII exist in a dynamic equilibrium where the binding is reversible and VWF-FVIII and VWF-free FVIII levels remain at a steady state. VWF is synthesized and secreted by endothelial cells and platelets. Endothelial-cell derived-VWF expresses the A, B, and H blood group antigens. Plasma VWF:Ag levels, ABO blood group, and VWF-FVIII binding activity (VWF:FVIII) have been shown to associate with the variability in FVIII PK metrics. Plasma VWF-FVIII and VWF-free FVIII are cleared by a series of semi-selective receptors expressed by hepatocytes, macrophages, and sinusoidal endothelial cells of the liver and spleen. Clearance of VWF-free FVIII is rapid, with a half-life of approximately 2 hours; the VWF-FVIII complex has a half-life of approximately 12 hours. While it is likely that additional receptors are involved in clearing FVIII concentrates from the circulation, variants in the genes that encode the FVIII or VWF-FVIII clearance receptors asialoglycoprotein receptor (ASGPR) minor subunit (ASGPR2), C-type lectin domain family 4 member M (CLEC4M), low-density lipoprotein receptor (LDLR), and stabilin-2 contribute to the variability in FVIII PK.

## VWF Gene Variants That Modify VWF:Ag

Plasma levels of VWF are regulated by both inherited and acquired factors. Twin and sibling-based studies have estimated the heritability of VWF antigen levels to be approximately 65%.<sup>18,19,33–35</sup> This influence is thought to decline in older populations, as age-related health changes and environmental exposures raise plasma VWF levels. Both variants within the *VWF* gene and variants at other genetic loci including genes that regulate VWF synthesis, secretion, and clearance have a significant influence on VWF plasma levels. The *VWF* gene is highly polymorphic, with an average of 2.5 single nucleotide variants (SNVs) per individual, the majority of which have not been functionally characterized.<sup>36</sup> In populations of normal individuals, common and rare variants in the *VWF* gene have been shown to associate with endogenous plasma levels of VWF:Ag and FVIII:C through biosynthetic and clearance-related mechanisms.<sup>37–39</sup>

The *VWF* gene variants c.2365A>G (rs1063856, p.(T789A)) and c.2385T>C (rs1063857, p.(Y795=)) have a minor allele frequency of approximately 33% in European populations and are inherited in strong linkage disequilibrium ( $r^2=0.98–1.00$ ).<sup>40</sup> Studies involving normal individuals have shown that these variants associate with both plasma VWF:Ag and FVIII:C.<sup>38</sup> Mechanistically, these variants appear to increase VWF protein production and decelerate VWF clearance.<sup>40</sup> In a study of 43 Canadian pediatric patients with severe HA, inheritance of the rs1063856/rs1063857

haplotype significantly associates with the rate of FVIII clearance, suggesting that variants within the VWF gene can modify FVIII pharmacokinetics.<sup>9</sup> This association was not observed in studies of adult/adolescent/adult populations, likely due to the decreased heritability of VWF levels in older age groups.<sup>8,30</sup> To date, other common *VWF* gene variants have not been associated with FVIII PK,<sup>30</sup> and little is known about the potential effect of rare *VWF* gene variants on FVIII PK metrics.

## VWF Gene Variants That Modify VWF-FVIII Binding Activity

The VWF-FVIII complex circulates in the plasma in a dynamic equilibrium, with 95–97% of FVIII bound to VWF, and the remaining circulating as VWF-free FVIII.<sup>41</sup> In the absence of the stabilizing force of VWF, such as in Type 3 von Willebrand disease (VWD) that involves a complete quantitative VWF deficiency, or type 2N VWD where VWF-FVIII binding activity is impaired, the proportion of VWF-free FVIII is increased and the half-life of FVIII is reduced approximately six-fold to 2–3 hours.<sup>42,43</sup> Thus, it follows that the ability of endogenous VWF to bind infused FVIII may impact FVIII PK.

In a study of 43 Canadian pediatric patients with severe HA, VWF-FVIII binding activity significantly correlated with FVIII PK parameters including clearance,  $k$ , half-life, and AUC.<sup>9</sup> When the D'D3 FVIII-binding regions of the *VWF* gene were sequenced, patients with the rare and low-frequency variants p.(Arg826Lys) and p.(Arg852Glu) demonstrated reduced VWF:FVIIIIB but not VWF:Ag when compared to patients without these variants. In a follow up investigation involving 44 adult patients of European descent, VWF:FVIIIIB activity was again significantly associated with VWF clearance,  $k$ , and half-life.<sup>8</sup> Together, these data suggest that the proportion of infused FVIII that circulates VWF-free can contribute to the variability of FVIII PK.

## ABO Blood Group Locus

Preceding its secretion into the plasma, the VWF molecule undergoes significant post-translational modification including signal-peptide and propeptide cleavage, dimerization, multimerization, and glycosylation.<sup>44</sup> Each VWF monomer expresses 13 N- and 10 O-linked glycans which represent up to 20% of its final mass.<sup>45</sup> Endothelial cell-derived VWF also expresses the A, B, and H blood group antigens covalently linked as terminal residues on its N-linked glycans. It is estimated that 25–30% of the variability in plasma VWF:Ag levels can be attributed to ABO blood group status,<sup>33,38</sup> where type O associates with ~25% lower VWF than type non-O (blood type A, B, or AB).<sup>46</sup> This effect is mediated through a clearance-based mechanism, where type O VWF has a significantly shorter half-life when compared with non-O VWF.<sup>47</sup> The ABO system similarly reduces endogenous plasma FVIII levels through the clearance of VWF-bound FVIII.<sup>45,48</sup>

The effect of the ABO blood group system on FVIII PK is well recognized, with slower FVIII clearance and longer half-life observed in non-O as compared to type O subjects.<sup>8,9,12,27,28</sup> The majority of the ABO blood group effect on FVIII PK is mediated through the ABO modification of VWF:Ag. Association analyses performed in pediatric and adult patients have consistently demonstrated that the positive correlation between VWF:Ag and FVIII half-life is stronger in non-O patients as compared with type O patients.<sup>8,9,12,27</sup> The VWFpp/VWF:Ag ratio shows a significant negative correlation with FVIII half-life in non-O but not type O subjects, suggesting that the increase in FVIII half-life for non-O subjects is due to slower clearance of endogenous VWF.<sup>8,9</sup>

The ABO blood group phenotype may also influence a larger proportion of the age-dependent effect on FVIII PK. Studies performed in normal populations have reported that individuals of older age have elevated VWF:Ag, and VWFpp, and decreased VWFpp/VWF:Ag as compared with younger subjects.<sup>49</sup> Interestingly, the effect size is larger for non-O than for type O subjects, suggesting that decreased VWF clearance in older age non-O subjects may drive much of the association between FVIII PK and age; however this effect remains to be directly investigated in a cohort of HA subjects. Overall, the influence of ABO blood group status, VWF:Ag levels, and patient age is estimated to account for up to 34% of the variability in FVIII PK.<sup>27</sup>

Recent evidence has suggested that the ABO genotype, which quantitatively influences A and B antigen loading,<sup>50</sup> may more precisely predict FVIII pharmacokinetics than ABO antigen analysis. A study of 43 adolescent and adult subjects identified a series of 5 *ABO* SNVs routinely used to predict blood type significantly associated with FVIII half-



life and clearance.<sup>30</sup> Each additional copy of the most significant variant, rs7853989, was associated with an increase in FVIII half-life by 1.4 hours, and a decrease in clearance by 0.5 mL/h/kg. In a study of European adults, the ABO blood group genotype associated with FVIII half-life, VWF:Ag, and VWFpp/VWF:Ag.<sup>8</sup> Interestingly, the sole subject with the A/A genotype had the longest FVIII half-life, highest VWF:Ag, and the lowest VWFpp/VWF:Ag in the study, an observation that warrants further investigation in a larger cohort.

## Variants in VWF-FVIII and FVIII Clearance Receptors

Clearance of FVIII involves interactions with a complex series of semi-selective endocytic receptors that bind and internalize either VWF-free FVIII or the VWF-FVIII complex.<sup>25</sup> Given the very low proportion of VWF-free FVIII in the circulation (2–5%) the majority of FVIII is thought to be cleared as part of the VWF-FVIII complex. However, as the half-life of VWF-free FVIII is approximately 6-fold shorter than that of VWF (~2 hours versus ~12 hours), estimates suggest that as much as 24% of FVIII may be cleared independently of VWF.<sup>51,52</sup> The majority of identified VWF-FVIII clearance receptors bind and internalize the VWF-FVIII complex likely through direct interactions with VWF, although a subset of these receptors will also bind directly to FVIII in the absence of VWF.

Murine studies utilizing radiolabeled VWF or FVIII have observed that the spleen has the highest endocytic capacity for VWF and FVIII, followed by the liver.<sup>53,54</sup> However, given the significantly larger size of the liver as compared with the spleen, it is thought that the liver is the predominant site of both VWF and FVIII clearance. Within the liver, macrophage and hepatocyte-expressed clearance receptors for FVIII have been identified through gene candidate studies including LRP1, LDLR, SIGLEC5, SR-A1, MGL, and ASGPR.<sup>55–60</sup> In addition, a series of sinusoidal endothelial cell-expressed VWF-FVIII clearance receptors, CLEC4M, stabilin-2, and SCARA5 were identified by GWAS meta-analyses that investigated associations between endogenous plasma levels of VWF, FVIII, and common single nucleotide variants.<sup>38,39</sup> Subsequent studies confirmed the ability of CLEC4M, stabilin-2, and SCARA5 to bind and endocytose FVIII in a VWF-dependent or -independent manner.<sup>51,61–63</sup> To date, variants in hepatocyte and macrophage-expressed receptors have not been found to associate with plasma VWF or FVIII levels in large-scale association studies involving normal individuals.

The relative contribution of VWF-FVIII clearance receptors to variability in FVIII PK is not currently well understood. However, it is likely that the receptors that contribute to variability in VWF:Ag or endogenous FVIII:C in normal individuals are likely to contribute to the variability of FVIII PK. The relative influence of each receptor on the variability of FVIII PK may be determined by the frequency with which variants at these loci occur, and the degree to which the variants modify receptor function or expression. In addition, the ability of a receptor to interact with both VWF-bound versus VWF-free FVIII, as well as its relative affinity for various FVIII concentrates including plasma-derived, recombinant full-length, or B-domain deleted products, may also play a significant role.

### CLEC4M

CLEC4M (C-type lectin domain family 4 member M) is a calcium-dependent sinusoidal endothelial cell surface-expressed integral membrane protein.<sup>64</sup> The *CLEC4M* single nucleotide variant rs868875 was originally demonstrated to associate with plasma VWF:Ag but not FVIII:C by GWAS.<sup>38</sup> CLEC4M is comprised of a C-terminal carbohydrate recognition domain that facilitates interactions with mannose-containing glycans on both the VWF-FVIII complex, as well as VWF-free recombinant FVIII products.<sup>51,61</sup> The rs868875 SNV located in the 3'UTR of *CLEC4M*, is in linkage disequilibrium with a highly polymorphic variable number of tandem repeat (VNTR) neck region that includes between 3 and 9 repeats of a 23 amino acid sequence.<sup>61</sup> The VNTR region facilitates CLEC4M homo-tetramerization and spatially regulates the position of the carbohydrate recognition domain relative to the cell surface, thereby modifying its ligand binding ability.<sup>64</sup> The VNTR variant has been shown to influence the ability of CLEC4M-expressing cells to bind and internalize the VWF-FVIII complex.<sup>61</sup>

The association between rs868875 and FVIII half-life and clearance has now been described in several studies involving pediatric and adult subjects, and a range of recombinant and plasma-derived FVIII concentrates.<sup>8,9,30,65</sup> For each additional copy of the *CLEC4M* rs868875 allele, FVIII half-life was reduced by 1.1 hours, while clearance was increased by 0.3 mL/h/kg.<sup>30</sup> In pediatric patients, an increased incidence of the rs868875 genotype was observed in

patients with the slowest rate of FVIII clearance.<sup>9</sup> In a study of 26 adolescents and adults that utilized a two-compartment PK model, associations between the rs868875 genotype and elimination rate, half-life, and the Beta rate constant were observed.<sup>65</sup> Interestingly, in a combined analysis, the ABO blood group and the rs868875 *CLEC4M* genotype displayed an interaction, where type-O individuals who were heterozygous or homozygous for the rs868875 minor allele had reduced FVIII half-life, and faster clearance as compared with non-O subjects and subjects homozygous for the reference allele. Currently, a biological interaction between the ABO blood group and *CLEC4M* has not been described.

The association between the *CLEC4M* VNTR genotype and FVIII PK has been characterized in studies involving both pediatric and adult subjects.<sup>8,9</sup> Overall, the VNTR genotype associated with FVIII clearance, elimination rate constant (*k*), and half-life in pediatric subjects. For adult subjects, the association between VNTR genotype was observed for FVIII clearance only. For pediatric and adult subjects, an increased prevalence of VNTR 5 was observed in the quartile of patients with the fastest rate of FVIII clearance, while an increased incidence of the VNTR 9 genotype was observed in the quartile of patients with the longest FVIII half-life in pediatric subjects only. The association between co-inheritance of the VNTR and rs868875 variants was evident in these populations, as all patients homozygous for the rs868875 reference allele had VNTR 6, 7, and 9 combinations, while all patients homozygous for the non-reference allele had VNTR 4 and 5 combinations, suggesting that rs868875 modifies FVIII clearance through linkage with the VNTR variant. Interestingly, no significant association was shown between rs868875 or the VNTR variant and VWF:Ag or VWFpp/VWF:Ag, suggesting that a proportion of this effect may be mediated through clearance of VWF-free FVIII.

## STAB2

Stabilin-2 is a large class H scavenger receptor expressed by liver, spleen, and lymphatic sinusoidal endothelial cells.<sup>66,67</sup> In vitro and in vivo studies have demonstrated that stabilin-2 can bind, internalize, and clear VWF-FVIII from the plasma; however, the interaction between stabilin-2 and FVIII is limited and it likely does not contribute significantly to the clearance of VWF-free FVIII.<sup>63</sup> Association studies performed in normal individuals have observed that both common and rare *STAB2* variants can associate with plasma levels of VWF:Ag and FVIII:C.<sup>37–39</sup> Rare, pathogenic variants in *STAB2* have also been linked with an increased risk for venous thromboembolism (VTE).<sup>68</sup>

In a pediatric study, the *STAB2* deep intronic variant rs4981022 associated with FVIII *k*, half-life, and AUC, while the rs12229292 variant associated with FVIII clearance and AUC.<sup>9</sup> Study subjects in the quartile with the longest FVIII half-life had an increased frequency of the rs12229292 as compared with the quartile of subjects with the shortest FVIII half-life. Importantly, studies conducted in adult subjects, and in mixed pediatric and adult subjects did not detect a significant association between these *STAB2* variants and FVIII PK.<sup>8,30</sup> The difference in these observations may be related to increasing age, and therefore decreased genetic influence of these variants on plasma VWF:Ag in the adult study subjects. Additionally, these studies may not be adequately powered to capture the relatively small effect that these common variants have on FVIII PK in adults.

## LDLR

LDLR (low-density lipoprotein receptor) is a macrophage-expressed scavenger receptor best characterized as a major regulator of plasma cholesterol.<sup>69</sup> In vitro studies have demonstrated that LDLR is capable of binding recombinant FVIII, and LDLR KO mice display prolonged clearance of infused recombinant human FVIII.<sup>60</sup> To date, the ability of LDLR to regulate the clearance of VWF or the VWF-FVIII complex has not been well-characterized. Interestingly, variants in the *LDLR* gene have not been associated with plasma VWF:Ag or FVIII:C in normal individuals by GWAS, although synonymous *LDLR* variants rs2228671 (c.81C>T; p.Cys27=) and rs688 (c.1773C>T; p.Asn591=) have been associated with modestly elevated plasma FVIII levels in coronary artery disease patients.<sup>70</sup>

In a study of 33 adolescent and adult subjects with moderate and severe HA, rs688 was associated with elimination rate constant, alpha distribution half-life, alpha half-life, and intercompartmental clearance, indicating that the rs688 variant may influence the distribution phase of FVIII PK.<sup>71</sup> In contrast, the rs2228671 *LDLR* variant was associated with FVIII clearance and volume of distribution at steady state. The rs688 variant did not associate with plasma VWF:Ag in these patients, suggesting these effects could be mediated through clearance of VWF-free FVIII. Interestingly, variants in the gene that encodes LRP-1



(low-density lipoprotein receptor-related protein-1), a member of the LDLR superfamily of scavenger receptors that has been well characterized as a clearance receptor for VWF and FVIII, have not been associated with FVIII PK metrics.<sup>27,30</sup>

## ASGR2

The ASGPR (asialoglycoprotein receptor) is comprised of two subunits ASGPR1 (asialoglycoprotein receptor 1) and ASGPR2 (asialoglycoprotein receptor 2) and binds to exposed galactose residues on asialoglycoproteins.<sup>72</sup> ASGPR1 and ASGPR2 can combine into dimeric, trimeric, and tetrameric quaternary forms to regulate substrate interactions and endocytosis. The ASGPR binds to plasma-derived FVIII through interactions with the B-domain and FVIII clearance can be attenuated in vivo with asialoorosomucoid (ASOR), an ASGPR ligand.<sup>73</sup> The ASGPR regulates the clearance of desialylated VWF, and mice deficient in *ASGR1* but not *ASGR2* have elevated plasma levels of VWF and FVIII.<sup>58</sup>

The association between a cluster of SNVs in the *ASGR2* untranslated region (UTR) and FVIII PK was investigated in a cohort of 32 adolescent and adult subjects receiving both recombinant full-length, B-domain deleted, and plasma-derived products.<sup>74</sup> Of the 8 variants investigated, rs2289645 (c.-95T>C) significantly associated with FVIII elimination rate constant, alpha half-life, and mean residence time. The other 7 variants tested did not associate with FVIII PK. No significant association was observed between the *ASGR2* genotype and plasma VWF:Ag. This study raises the possibility that ASGPR may interact with desialylated N-linked glycans outside of the FVIII B-domain. Further investigations are warranted to assess the influence of ASGPR on the clearance of endogenous VWF and to resolve the discrepancy between animal models and human studies.

## TC2N

Variants in the *TC2N* gene have been associated with plasma VWF:Ag or FVIII:C in several cohorts of normal individuals.<sup>38,39</sup> The mechanistic relationship between these observations is currently unclear, and a function for TC2N beyond its known role in tumorigenesis is unknown.<sup>75</sup> In a pediatric study of severe HA subjects, the *TC2N* variant rs10133762 was associated with a slower rate of FVIII clearance.<sup>9</sup> However, this association was not statistically significant in adult or mixed adolescent and adult populations.<sup>8,30</sup>

## Genetic Variants That Do Not Associate with FVIII PK

Notably, several FVIII PK investigations have characterized gene variants that did not associate with FVIII PK metrics ([Supplemental Table 1](#)). The *F8* pathogenic genotype, subdivided by mutation class (inversion, deletions, or non-synonymous variants) did not significantly associate with FVIII PK measurements.<sup>27,71</sup> Variants in *STX2* (syntaxin-2) and *STXBP5* (syntaxin-binding protein 5), snare complex proteins which regulate VWF secretion,<sup>76–78</sup> have been associated with endogenous VWF:Ag and FVIII:C levels in normal individuals,<sup>38</sup> but did not significantly modify FVIII PK in a mixed adolescent and adult population.<sup>30</sup> Additionally, variations in the VWF-FVIII clearance receptors LRP-1, SIGLEC5, and SCARA5 did not associate with FVIII PK in pediatric and/or adult subjects.<sup>8,9,27,30</sup> As expected, common variants in genes that may influence the biosynthesis or plasma activity measurement of endogenous FVIII including *F8*, *CIB1*, *CIB4*, *KNG1*, and *TMLHE* did not influence FVIII PK.<sup>30</sup>

## Pharmacokinetics of Extended Half-Life FVIII Products

While recombinant and plasma-derived FVIII concentrates have been the standard of care for hemophilia for many decades, FVIII extended half-life (EHL) products have become widely used in recent years.<sup>79</sup> EHL products, developed to reduce the frequency of intravenous infusion and increase the period spent in the optimal therapeutic window, can achieve an approximate 1.5 – 2-fold increase in FVIII half-life compared with standard half-life (SHL) products. EHL-FVIII typically involves the extension of FVIII plasma half-life either through conjugation of recombinant B-domain deleted (BDD) FVIII to polyethylene glycol (FVIII-PEG) which alters the surface charge of the FVIII molecule and is thought to interfere with clearance receptor interactions, or fusion of BDD FVIII to the Fc region of IgG1 (FVIII-Fc) to increase half-life through the IgG recycling mechanism mediated by the neonatal Fc receptor (FcRn). Importantly, modification of the FVIII molecule by Fc-fusion or PEGylation does not alter its VWF binding capabilities.<sup>80,81</sup>

The pharmacogenomic regulators of EHL-FVIII PK are largely uncharacterized. Similar to SHL-FVIII, the pharmacokinetic profile of EHL-FVIII has higher inter-individual variability than intra-individual variability.<sup>82,83</sup> PK studies in pediatric and adult subjects have demonstrated a terminal half-life range of 6–31.9 hours for FVIII-Fc, and 10.9–23.6 hours for FVIII-PEG, and significant variability for other EHL-FVIII PK metrics.<sup>82,84,85</sup> A report of 38 Finnish adults (74% receiving FVIII-Fc and 26% receiving FVIII-PEG) demonstrated that EHL-FVIII half-life correlates strongly with SHL-FVIII half-life, suggesting that many of the factors that influence PK of SHL products are also important regulators of EHL-FVIII PK.<sup>86</sup>

Several studies of both FVIII-Fc and FVIII-PEG have now observed that pre-infusion VWF:Ag levels positively associate with FVIII terminal half-life, and negatively associated with the rate of FVIII clearance.<sup>82,83,87–90</sup> A large, retrospective multi-center analysis involving over 4800 pediatric and adult patients with severe HA from Canada and the Netherlands reported that the terminal half-life of EHL-FVIII was associated with increasing age at a rate of 1.2 hours/10 years.<sup>22</sup> ABO blood group has also been reported to associate with EHL-FVIII PK in several studies, with EHL-FVIII half-life shorter for subjects with blood type O as compared with non-O subjects.<sup>22,82</sup> To date, little is known regarding the complement of receptors that contribute to the clearance of EHL-FVIII products, or the genetic variants that might influence these pathways. Additional factors that influence the pharmacokinetics of EHL-FVIII products, including VWF:FVIII activity remain to be fully characterized.

More recently, the extension of FVIII half-life to 35–40 hours has been achieved through the generation of a novel FVIII fusion protein, BIVV001.<sup>91,92</sup> Half-life extension is achieved through Fc-fusion, the insertion of two XTEN biodegradable hydrophilic amino acid sequences to impede interaction with clearance receptors, and covalent attachment of the FVIII binding VWF D'D3 domain to prevent interactions with endogenous VWF. Initial clinical trial evidence suggests that this innovation may reduce at least some of the previously documented inter-individual variability of FVIII half-life that is related to its interactions with endogenous VWF.

## Future Directions

Prophylactic treatment regimens have been shown to improve health-related quality of life for patients with severe HA by reducing the frequency of bleeding events and preventing the development of joint arthropathy.<sup>5</sup> To account for the large inter-individual variability of FVIII PK, individual PK analysis has emerged as an essential tool in the optimization of a patient's treatment regimen. This methodology typically involves comparing a patient's post-infusion FVIII levels against population PK data using a Bayesian analysis to obtain more accurate individual PK metrics. Studies demonstrating the improved efficacy of individualized factor replacement therapies over standardized therapeutic regimens are ongoing, and they have the potential to reduce the bleeding frequency and optimize resource use to allow for more equitable access to FVIII replacement therapy.<sup>13</sup>

In recent years, progress has been made toward understanding the pharmacogenomic basis of FVIII PK variability. Despite age-related changes in plasma VWF:Ag levels that are influenced by environmental exposures and patient health, a strong component of FVIII PK is influenced by genetics. Cumulatively, evidence suggests that variants in genes that influence the rate at which VWF is cleared (including VWF-FVIII clearance receptors and the ABO blood group locus) play a more significant role in modifying FVIII PK than determinants that modify VWF synthesis or secretion. Additionally, gene variants that regulate the clearance of VWF-free FVIII, including VWF D'D3 variants that impair VWF-FVIII binding, significantly influence FVIII PK. The genetic influence on FVIII PK appears to be stronger in pediatric subjects, as evidenced by the significant associations between *VWF*, *STAB2*, and *TC2N* variants in pediatric but not adult subjects.<sup>8,9,30</sup> After the influence of ABO, the association between the *CLEC4M* rs868875 variant, which is in linkage disequilibrium with the *CLEC4M* VNTR, appears to be the most robust association in adult and pediatric populations, although *LDLR* and *ASGR2* associations with FVIII PK have been investigated in single cohorts only.

Overall, the majority of FVIII PK-modifying variants are common (minor allele frequency >0.05) and without an apparent pathogenic influence. With the exception of the ABO blood group locus, these variants exert a relatively small effect on FVIII PK. Studies that have investigated associations between genetic variants and FVIII PK metrics have involved relatively small cohorts that test associations with a limited number of gene candidate variants. In addition to the currently identified genetic modifiers of FVIII PK, variants within additional FVIII clearance receptors, genes that

regulate the biosynthesis or glycosylation of VWF, and genes that regulate the immune response to infused FVIII concentrates have the potential to modify FVIII PK. In addition, despite the variability of FVIII concentrates utilized in these studies, no single gene variant has been shown to impact the PK profile of an individual SHL or EHL product to date.

Large-scale, observational, trait-mapping studies involving whole genome or exome sequencing of pediatric and adult subjects are necessary to identify rare variants that modify FVIII PK metrics in an unbiased manner. Such studies may provide valuable confirmation of existing reports and identify the most influential variants in pediatric versus adult populations. They may also identify common variants with a modest effect on FVIII PK, or rare gain- or loss-of-function variants that could exert a large effect on FVIII PK. A better understanding of the genetic modifiers of FVIII PK variability will provide mechanistic insights into pathways that regulate the clearance of FVIII, and support the development of novel strategies to improve FVIII PK. Moreover, these studies will support the continued implementation of personalized treatment plans for patients with hemophilia A.

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