Plasma Clearance Receptors of FVIII and VWF

Subjects: Biochemistry & Molecular Biology

Contributor: Andrey G. Sarafanov

Congenital deficiency in blood coagulation factor (F) VIII (FVIII) results in excessive bleeding. The disease (hemophilia A) is treated with infusions of therapeutic FVIII concentrates, either plasma-derived (pdFVIII) or produced with recombinant DNA technology (rFVIII). Due to the relatively short FVIII plasma half-life (~12 h), such treatment requires frequent FVIII infusions (3–4 per week in prophylaxis) that calls for developing more efficient longer-acting FVIII products, in particular with an extended plasma lifetime, commonly termed half-life (EHL). Relevant protein modifications are performed via genetic and/or chemical modification of rFVIII, and efficient designs require understanding the molecular mechanisms of FVIII plasma clearance.

Keywords: hemophilia A ; factor VIII ; von Willebrand factor ; therapeutic factor VIII ; extended half-life factor VIII ; plasma clearance

1. Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1)

LRP1 was the first found FVIII clearance receptor, described independently by research groups of Saenko and Lenting in 1999 [1][2]. LRP1 is the major clearance receptor for both FVIII and VWF, based on many in vitro, animal, and genetic studies [3]. In circulation, LRP1 is expressed on hepatic cells where it internalizes ~15 ligands, such as alpha-2 macroglobulin, proteases, coagulation factors, and lipoproteins, as well as a large variety of ligands in other tissues [4][5]. LRP1, similar to other related receptors (see below), interacts with its ligands via a flexible string of adjacent complement-type repeat domains (CRs), and lysine residues on ligands are the major determinants for the interactions [6].

On FVIII, such lysine residues are located on the Light Chain (LCh) throughout its A3, C1, and C2 domains [2][7][8]. The FVIII-LRP1 interaction (K_D ~20 nM) occurs via formation of multiple alternative binding combinations in a dynamic mode where a real-time interactive site involves a string with three or more CR domains of the receptor and multiple lysines of FVIII, as found by us recently [8].

Upon FVIII activation, another LRP1-binding site is exposed on the A2 domain. The functional relevance of that may reflect a participation of LRP1 in plasma clearance of inactivated FVIIIa remnants (A2 domain and A1/A3′-C1-C2 heterodimer). Indeed, each of these fragments is cleared from mouse plasma with the involvement of LRP1 [9][10].

On VWF, the LRP1-binding site is located within its A1 domain [11]; thus, all VWF monomers carry such sites. In contrast to VWF sites for binding FVIII, its LRP1-binding sites are not exposed under static fluid conditions in vitro due to folded state of molecule and become exposed only when it unfolds under fluid shear force in blood vessels [12].

2. Low-Density Lipoprotein Receptor (LDLR)

LDLR was identified as the second FVIII plasma clearance receptor in 2005 [13]. LDLR belongs to a large family of endocytic receptors named after LDLR itself and involving seven members in mammals, including LRP1 [14]. The family members are expressed in many tissues where they interact with numerous ligands and regulate many processes, including those with medical importance [4][5][15][16].

Expressed in the liver, LDLR catabolizes a smaller number of ligands, mainly low-density lipoprotein (LDL), relevant to cholesterol metabolism [17]. The role of LDLR in FVIII clearance was supported in a mouse model, where LDLR deficiency resulted in ~1.5-fold prolongation of FVIII half-life, similarly to LRP1. In turn, combined deficiency in both LRP1 and LDLR resulted in ~5-fold prolongation of FVIII plasma half-life with an increase in its plasma level. The authors concluded that LRP1 and LDLR cooperate in FVIII plasma clearance [13], which was supported in further genetic studies [18][19].

Compared to LRP1, the affinity of LDLR for FVIII is lower (K_D 30–60 nM), which may explain its apparently smaller contribution to FVIII plasma clearance. As it was found previously, the organization of FVIII binding sites for both receptors

is similar as they are located on the FVIII LCh with the same significance of the C1 domain for LDLR binding [20] and due to presence of the binding site also for LDLR in the A2 domain in FVIIIa [21][22]. Reanalysis of these data [20], considering the FVIII-LRP1 interaction mode [8], indicates that the mechanism of FVIII-LDLR interaction is also based on formation of alternative binding combinations.

In addition to LDLR and LRP1, other members of the LDLR family exposed to circulation are very low-density lipoprotein receptor (vLDLR) and megalin, expressed on endothelial and kidney cells, respectively. Both can interact with FVIII and FVIIIa in vitro similarly to LRP1 and LDLR, however, with unknown functional relevance [21][22]. In particular, the role of vLDLR in FVIII clearance was not supported in the mouse model [23].

3. Cell-Surface Heparan-Sulfate Proteoglycans (HSPGs)

HSPGs have covalent attachment of several negatively charged sulfated polysaccharide chains and are localized on cell membranes. There is a large variety of HSPGs which interact with numerous protein ligands via their basic residues [24] [25]. HSPGs serve as receptors or coreceptors, in particular of LRP1, facilitating its ligand recognition. In plasma, such ligands involve lipoprotein lipase [26], apo E-containing lipoproteins [27][28], thrombospondin [29], thrombin-protease nexin 1 complex [30], tissue factor pathway inhibitor [31], thrombin, antithrombin III, and FX [25], and FVIII [32].

In these processes, HSPGs preconcentrate the ligands to facilitate their further interactions with other receptors. In particular, FVIII has extremely low plasma concentration (≤1 nM) [33], insufficient for its effective interaction with LRP1 (K_D ~20 nM). The involvement of HSPGs, abundantly expressed on cells [32], altogether with the cell membrane interaction of FVIII [34], is believed to increase its local concentration and facilitate LRP1 binding to LRP1. Similarly, HSPGs may facilitate FVIII interactions with other clearance receptors.

4. Asialoglycoprotein Receptor (ASGPR)

Other clearance receptors of FVIII and VWF (lectins) recognize their carbohydrates. Activated form of FVIII contains four N-linked glycans, whereas the dissociated B-domain contains ~14 N- and ~7 O-linked glycans [35][36]. VWF contains 23 glycans (N- or O-linked) per monomer [3][37], with an estimated number of >1000 on the molecule. In both proteins, the N-glycans contain bi-, tri-, and tetra-antennary structures with high mannose portions, ABO(H)-determinants; and the O-glycans contain mainly T-antigen. These glycans (as well as in other proteins) are terminally capped by sialic acid sugars with ≥80% occupancy [36][37][38][39][40][41]. Notably, the N-glycosylation of rFVIII differs from pdFVIII mainly in sialic acids, ABO(H) blood group, etc. The greatest difference was found in rFVIII expressed in human embryonic kidney (HEK) cells, whereas the use of baby hamster kidney (BHK) and Chinese hamster ovary (CHO) cells also resulted in such a difference [36]. The half-life of VWF is dependent on its ABO(H) group glycans, where the O-type glycan-bearing VWF variants have significantly shorter plasma half-life, associated with FVIII level as noted above [42].

ASGPR (Ashwell receptor), a member of the C-type family of lectins recognizing terminal asialic sugars [43], was found to be a clearance receptor for both FVIII and VWF. ASGPR binds with high affinity to FVIII asialylated N-glycans (K_D ~2 nM), where the major binding determinant is the abundantly glycosylated B-domain. However, removal of the B-domain does not significantly affect FVIII plasma half-time in various species including humans. Therefore, the authors concluded that ASGPR likely plays a role only in quality control of FVIII biosynthesis by eliminating incompletely glycosylated protein [44]. Other causes of proteins desialylation are proteins “aging” [45] and infection with pathogens [46][47]; both processes likely engage ASGPR in the clearance of such proteins.

Regarding VWF, a deficiency in ASGPR resulted in ~1.5-fold prolongation of its plasma half-life in a mouse model; in parallel, FVIII level was increased [46]. Other plasma ligands of ASGPR include chylomicron remnants, fibronectin, lactoferrin, immunoglobulin A, members of the prolactin/growth hormone family, lipoprotein(a), urokinase-type plasminogen activator [44], and platelets [46].

5. Sialic Acid Binding Immuno Globulin-like Lectin Member 5 (SIGLEC5)

In humans, there are 14 members of SIGLECs. These receptors are expressed on cells of hematopoietic origin, whereas each member is found on specific cell type(s). SIGLEC5 recognizes the two most common sialic acid sugars, N-acetylneuraminic acid and N-glycolylneuraminic acid, and is expressed on macrophages. SIGLEC5 binds to both FVIII and VWF with high affinity (K_Ds ~8 and ~14 nM, respectively). In cell culture, SIGLEC5 mediated internalization of both proteins; and in mice, overexpression of SIGLEC5 resulted in significant decrease in FVIII and VWF levels [48].

Within the FVIII/VWF complex, FVIII does not interact with SIGLEC5. In contrast to VWF, desialylation of FVIII does not affect its binding to SIGLEC5, indicating that FVIII interacts with receptors via protein moiety. Indeed, each of full-length (FL) FVIII and a B-domain deleted (BDD) FVIII, lacking ~85% of FL-FVIII glycans, had similar binding to SIGLEC5. The authors concluded that SIGLEC5 may regulate plasma level of the FVIII/VWF complex [48].

6. C-Type Lectin Domain Family 4 Member M (CLEC4M)

CLEC4M is a mannose-specific endocytic receptor expressed on endothelium of liver, lymph nodes, and placenta. CLEC4M interacts with both FVIII and VWF via their N-glycans and internalizes both proteins in cell culture; in the case of FVIII, the interaction occurred in either a VWF-dependent or independent manner. Overexpression of CLEC4M in mice resulted in decreased plasma levels of both FVIII and VWF. The authors suggested that CLEC4M is a clearance receptor of both FVIII and VWF [49][50].

This role of CLEC4M was further supported by finding association of polymorphism in the receptor gene with FVIII and VWF plasma levels (see below). Other known ligands of CLEC4M include pathogens, such as *Mycobacterium tuberculosis*, HIV-1, influenza A, SARS-CoV, Ebola, hepatitis C, and West Nile viruses [51], whereas no endogenous glycoprotein ligands besides FVIII and VWF were reported.

7. Stabilin-2 (STAB2)

STAB2 is a class H endocytic receptor, recognizing glycosaminoglycans (mucopolysaccharides), and is expressed on endothelial cells in liver, spleen, and lymph nodes. Studies demonstrated association between STAB2 genetic polymorphism and plasma levels of both FVIII and VWF [52][53][54][55]. STAB2 binds and internalizes VWF, and binds FVIII in a VWF-independent mode. In STAB2-deficient mice, the half-lives of VWF and FVIII were prolonged ~1.5-fold upon injection of VWF, whereas FVIII itself interacted weakly with STAB2. The authors concluded that STAB2 is a clearance receptor of the VWF and FVIII/VWF complex. Other ligands of STAB2 include hyaluronic acid, heparins, chondroitin sulfates, collagen, and advanced glycation end products [56].

8. Scavenger Receptor Type A Member 5 (SCARA5)

SCARA5 (SR-A5) is expressed on endothelial cells in the spleen and kidneys, where it recognizes lipopolysaccharides, ferritin, polyanions, and bacteria [57], indicating that recognized determinants are carbohydrates. SCARA5 binds VWF with very high affinity (K_D ~0.5 nM), whereas it does not bind FVIII. SCARA5-expressing human cells bound and internalized VWF, but not FVIII. Under the condition of SCARA5 deficiency in mice, the half-life of injected VWF was prolonged by ≤1.3 times. The authors concluded that SCARA5 is an endocytic receptor for VWF [57]. This was supported by the finding of association of SCARA5 gene polymorphism with VWF levels [53].

9. Scavenger Receptor Type A Member 1 (SCARA1)

SCARA1 (SR-A1) is localized on macrophages where it recognizes polysaccharides, lipopolysaccharides, spectrin (a marker of apoptotic cells), bacteria, extracellular matrix, proteoglycans, etc. [58], most likely via carbohydrates. SCARA1 binds to VWF with high affinity (K_D ~14 nM) via multiple sites suggested to be on the D'D3, A1, and D4 domains via both protein and glycan determinants. VWF bound to SCARA1-expressing cells and had slower plasma clearance in SCARA1-defcient mice. Two VWF mutants, known for increased plasma clearance in humans, had significantly higher binding to SCARA5 in a purified system and on macrophages, and slower plasma clearance in SCARA1-defcient mice. The authors suggested that SCARA1 contributes to increased plasma clearance of certain VWF variants [59].

10. Macrophage Galactose-Type Lectin (MGL)

MGL is a C-type lectin, expressed on macrophages and recognizing terminal N-acetyl galactosamine or galactose sugars. FVIII binds to MGL via asialic O-linked glycans on the B-domain. In MGL-deficient mice, FVIII level was ~1.3-fold elevated, consistent with a role of MGL in clearance of both FVIII and VWF. In VWF-deficient mice, FVIII half-life was reduced ~8-fold, but prolonged 3–4-fold upon (i) inhibition of MGL with antibodies and (ii) depletion of macrophages by a specific reagent (clodronate). The authors concluded that MGL plays an important role in macrophage-mediated plasma clearance of both FVIII and the FVIII/VWF complex [60].

In an earlier study, dose-dependent binding of VWF to MGL was observed, as well as an enhanced clearance of hypo sialylated VWF in VWF/ASGPR-deficient mice, inhibited by coadministration of an MGL antibody. In MGL-deficient mice,

VWF levels were significantly elevated [61]. Altogether, these studies demonstrated a role of MGL in plasma clearance of FVIII via both VWF-dependent and independent pathways.

11. Other Factors Interacting with FVIII and VWF, or Affecting Their Plasma Levels

In addition to receptors mentioned above, other factors were also indicated to participate in FVIII plasma clearance based on less-in-depth studies. Two lectins, Galectin-1 and -3 (Gal-1, Gal-3), expressed on endothelial cells and recognizing *β*-galactoside residues, bound FVIII with high affinity (K_Ds 0.1–0.5 nM) via N- and O-linked glycans, mostly located on the B-domain. Notably, a BHK-cell-derived rFVIII had enhanced affinity to both receptors compared to the CHO-cell-derived rFVIII [62], consistent with their difference in N-glycans mentioned above [36]. VWF also bound both galectins, mostly via N-glycans but with lower affinity (K_Ds 20–80 nM) [63]. The authors concluded that Gal-1, Gal-3 may influence FVIII procoagulant activity and VWF-mediated thrombus formation [62][63].

In several studies involving more than 50,000 subjects total, genetic polymorphism (a single nucleotide or amino acid change) in many genes was associated with FVIII and VWF plasma levels. The FVIII-linked genes were *STAB2*, *VWF*, *F8*, *ABO*, *SCARA5*, *STXBP5*, *LBH*, *FAM46A*, *VAV2*, *ACCN1*, *KATNB1*, *LDLR*, and *LRP1*; and the VWF-linked genes were *STXBP5*, *SCARA5*, *ABO*, *STAB2*, *VWF*, *STX2*, *TC2N*, *CLEC4M*, *VPS8, EBP41L4A*, *KRT18P24*, and *SAFB2* [18][19][52][53][64]. A later study of Sabater-Lleal et al. identified additional 7 FVIII- and 11 VWF-linked genes/gene groups, supported by functional validation of results using cell culture where silencing of respective genes resulted in change of FVIII and VWF levels [65].

Recently, Swystun and Lillicrap described particular variants of *LDLR*, *ASGPR*, *CLEC4M*, *TC2N*, and *ABO(H)* affecting the plasma half-lives of FVIII and VWF in support of development of personalized treatment plans for patients with hemophilia A [42]. It should also be noted that elevated FVIII levels are associated with increased risk of thrombosis [66]. Overall, the genetic studies supported the role of FVIII and VWF clearance receptors reviewed above, whereas the roles of other identified genes remain to be explored.

12. Summary—FVIII Determinants to Be Modified to Extend the Plasma Half-Life

Knowledge derived from understanding FVIII clearance mechanisms indicates that major FVIII determinants to clearance receptors are located on the LCh. This portion of FVIII carries extended binding sites for LRP1, LDLR, and VWF, for which interactions with FVIII significantly affect its plasma half-life. Therefore, blocking these sites on FVIII may potentially decrease or abolish these interactions and extend FVIII plasma half-life.

References

1. Saenko, E.L.; Yakhyaev, A.V.; Mikhailenko, I.; Strickland, D.K.; Sarafanov, A.G. Role of the low density lipoprotein-related protein receptor in mediation of factor VIII catabolism. J. Biol. Chem. 1999, 274, 37685–37692.
2. Lenting, P.J.; Neels, J.G.; van den Berg, B.M.; Clijsters, P.P.; Meijerman, D.W.; Pannekoek, H.; van Mourik, J.A.; Mertens, K.; van Zonneveld, A.J. The light chain of factor VIII comprises a binding site for low density lipoprotein receptor-related protein. J. Biol. Chem. 1999, 274, 23734–23739.
3. Lenting, P.J.; Christophe, O.D.; Denis, C.V. von Willebrand factor biosynthesis, secretion, and clearance: Connecting the far ends. Blood 2015, 125, 2019–2028.
4. Au, D.T.; Strickland, D.K.; Muratoglu, S.C. The LDL Receptor-Related Protein 1: At the Crossroads of Lipoprotein Metabolism and Insulin Signaling. J. Diabetes Res. 2017, 2017, 8356537.
5. Cooper, J.M.; Lathuiliere, A.; Migliorini, M.; Arai, A.L.; Wani, M.M.; Dujardin, S.; Muratoglu, S.C.; Hyman, B.T.; Strickland, D.K. Regulation of tau internalization, degradation, and seeding by LRP1 reveals multiple pathways for tau catabolism. J. Biol. Chem. 2021, 296, 100715.
6. Fisher, C.; Beglova, N.; Blacklow, S.C. Structure of an LDLR-RAP complex reveals a general mode for ligand recognition by lipoprotein receptors. Mol. Cell. 2006, 22, 277–283.
7. van den Biggelaar, M.; Madsen, J.J.; Faber, J.H.; Zuurveld, M.G.; van der Zwaan, C.; Olsen, O.H.; Stennicke, H.R.; Mertens, K.; Meijer, A.B. Factor VIII Interacts with the Endocytic Receptor Low-density Lipoprotein Receptor-related Protein 1 via an Extended Surface Comprising “Hot-Spot” Lysine Residues. J. Biol. Chem. 2015, 290, 16463–16476.

8. Chun, H.; Kurasawa, J.H.; Olivares, P.; Marakasova, E.S.; Shestopal, S.A.; Hassink, G.U.; Karnaukhova, E.; Migliorini, M.; Obi, J.O.; Smith, A.K.; et al. Characterization of interaction between blood coagulation factor VIII and LRP1 suggests dynamic binding by alternating complex contacts. J. Thromb. Haemost. 2022, 20, 2255–2269.
9. Kurasawa, J.H.; Shestopal, S.A.; Woodle, S.A.; Ovanesov, M.V.; Lee, T.K.; Sarafanov, A.G. Cluster III of low-density lipoprotein receptor-related protein 1 binds activated blood coagulation factor VIII. Biochemistry 2015, 54, 481–489.
10. Sarafanov, A.G.; Makogonenko, E.M.; Pechik, I.V.; Radtke, K.P.; Khrenov, A.V.; Ananyeva, N.M.; Strickland, D.K.; Saenko, E.L. Identification of coagulation factor VIII A2 domain residues forming the binding epitope for low-density lipoprotein receptor-related protein. Biochemistry 2006, 45, 1829–1840.
11. Wohner, N.; Legendre, P.; Casari, C.; Christophe, O.D.; Lenting, P.J.; Denis, C.V. Shear stress-independent binding of von Willebrand factor-type 2B mutants p.R1306Q & p.V1316M to LRP1 explains their increased clearance. J. Thromb. Haemost. 2015, 13, 815–820.
12. Castro-Nunez, L.; Dienava-Verdoold, I.; Herczenik, E.; Mertens, K.; Meijer, A.B. Shear stress is required for the endocytic uptake of the factor VIII-von Willebrand factor complex by macrophages. J. Thromb. Haemost. 2012, 10, 1929–1937.
13. Bovenschen, N.; Mertens, K.; Hu, L.; Havekes, L.M.; van Vlijmen, B.J. LDL receptor cooperates with LDL receptor-related protein in regulating plasma levels of coagulation factor VIII in vivo. Blood 2005, 106, 906–912.
14. Dieckmann, M.; Dietrich, M.F.; Herz, J. Lipoprotein receptors—An evolutionarily ancient multifunctional receptor family. Biol. Chem. 2010, 391, 1341–1363.
15. Go, G.W.; Mani, A. Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis. Yale J. Biol. Med. 2012, 85, 19–28.
16. Lane-Donovan, C.; Philips, G.T.; Herz, J. More than cholesterol transporters: Lipoprotein receptors in CNS function and neurodegeneration. Neuron 2014, 83, 771–787.
17. Fass, D.; Blacklow, S.; Kim, P.S.; Berger, J.M. Molecular basis of familial hypercholesterolaemia from structure of LDL receptor module. Nature 1997, 388, 691–693.
18. Martinelli, N.; Girelli, D.; Lunghi, B.; Pinotti, M.; Marchetti, G.; Malerba, G.; Pignatti, P.F.; Corrocher, R.; Olivieri, O.; Bernardi, F. Polymorphisms at LDLR locus may be associated with coronary artery disease through modulation of coagulation factor VIII activity and independently from lipid profile. Blood 2010, 116, 5688–5697.
19. Vormittag, R.; Bencur, P.; Ay, C.; Tengler, T.; Vukovich, T.; Quehenberger, P.; Mannhalter, C.; Pabinger, I. Low-density lipoprotein receptor-related protein 1 polymorphism 663 C > T affects clotting factor VIII activity and increases the risk of venous thromboembolism. J. Thromb. Haemost. 2007, 5, 497–502.
20. Kurasawa, J.H.; Shestopal, S.A.; Karnaukhova, E.; Struble, E.B.; Lee, T.K.; Sarafanov, A.G. Mapping the binding region on the low density lipoprotein receptor for blood coagulation factor VIII. J. Biol. Chem. 2013, 288, 22033–22041.
21. Ananyeva, N.M.; Makogonenko, Y.M.; Kouiavskaia, D.V.; Ruiz, J.; Limburg, V.; Meijer, A.B.; Khrenov, A.V.; Shima, M.; Strickland, D.K.; Saenko, E.L. The binding sites for the very low density lipoprotein receptor and low-density lipoprotein receptor-related protein are shared within coagulation factor VIII. Blood Coagul. Fibrin 2008, 19, 166–177.
22. Ananyeva, N.M.; Makogonenko, Y.M.; Sarafanov, A.G.; Pechik, I.V.; Gorlatova, N.; Radtke, K.P.; Shima, M.; Saenko, E.L. Interaction of coagulation factor VIII with members of the low-density lipoprotein receptor family follows common mechanism and involves consensus residues within the A2 binding site 484–509. Blood Coagul. Fibrin 2008, 19, 543–555.
23. Bovenschen, N.; van Dijk, K.W.; Havekes, L.M.; Mertens, K.; van Vlijmen, B.J. Clearance of coagulation factor VIII in very low-density lipoprotein receptor knockout mice. Br. J. Haematol. 2004, 126, 722–725.
24. Park, P.W.; Reizes, O.; Bernfield, M. Cell surface heparan sulfate proteoglycans: Selective regulators of ligand-receptor encounters. J. Biol. Chem. 2000, 275, 29923–29926.
25. Bernfield, M.; Gotte, M.; Park, P.W.; Reizes, O.; Fitzgerald, M.L.; Lincecum, J.; Zako, M. Functions of cell surface heparan sulfate proteoglycans. Annu. Rev. Biochem. 1999, 68, 729–777.
26. Chappell, D.A.; Fry, G.L.; Waknitz, M.A.; Muhonen, L.E.; Pladet, M.W.; Iverius, P.H.; Strickland, D.K. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. J. Biol. Chem. 1993, 268, 14168–14175.
27. Ji, Z.S.; Fazio, S.; Lee, Y.L.; Mahley, R.W. Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. J. Biol. Chem. 1994, 269, 2764–2772.

28. Mahley, R.W.; Ji, Z.S. Remnant lipoprotein metabolism: Key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J. Lipid Res. 1999, 40, 1–16.

29. Mikhailenko, I.; Kounnas, M.Z.; Strickland, D.K. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates the cellular internalization and degradation of thrombospondin. A process facilitated by cell-surface proteoglycans. J. Biol. Chem. 1995, 270, 9543–9549.

30. Knauer, M.F.; Crisp, R.J.; Kridel, S.J.; Knauer, D.J. Analysis of a structural determinant in thrombin-protease nexin 1 complexes that mediates clearance by the low density lipoprotein receptor-related protein. J. Biol. Chem. 1999, 274, 275–281.

31. Warshawsky, I.; Broze, G.J., Jr.; Schwartz, A.L. The low density lipoprotein receptor-related protein mediates the cellular degradation of tissue factor pathway inhibitor. Proc. Natl. Acad. Sci. USA 1994, 91, 6664–6668.

32. Sarafanov, A.G.; Ananyeva, N.M.; Shima, M.; Saenko, E.L. Cell surface heparan sulfate proteoglycans participate in factor VIII catabolism mediated by low density lipoprotein receptor-related protein. J. Biol. Chem. 2001, 276, 11970–11979.

33. Butenas, S.; Parhami-Seren, B.; Undas, A.; Fass, D.N.; Mann, K.G. The “normal” factor VIII concentration in plasma. Thromb. Res. 2010, 126, 119–123.

34. Castro-Nunez, L.; Koornneef, J.M.; Rondaij, M.G.; Bloem, E.; van der Zwaan, C.; Mertens, K.; Meijer, A.B.; Meems, H. Cellular uptake of coagulation factor VIII: Elusive role of the membrane-binding spikes in the C1 domain. Int. J. Biochem. Cell. Biol. 2017, 89, 34–41.

35. Fay, P.J. Factor VIII structure and function. Int. J. Hematol. 2006, 83, 103–108.

36. Canis, K.; Anzengruber, J.; Garenaux, E.; Feichtinger, M.; Benamara, K.; Scheiflinger, F.; Savoy, L.A.; Reipert, B.M.; Malisauskas, M. In-depth comparison of N-glycosylation of human plasma-derived factor VIII and different recombinant products: From structure to clinical implications. J. Thromb. Haemost. 2018, 16, 1592–1603.

37. Canis, K.; McKinnon, T.A.; Nowak, A.; Haslam, S.M.; Panico, M.; Morris, H.R.; Laffan, M.A.; Dell, A. Mapping the N-glycome of human von Willebrand factor. Biochem. J. 2012, 447, 217–228.

38. Canis, K.; McKinnon, T.A.; Nowak, A.; Panico, M.; Morris, H.R.; Laffan, M.; Dell, A. The plasma von Willebrand factor O-glycome comprises a surprising variety of structures including ABH antigens and disialosyl motifs. J. Thromb. Haemost. 2010, 8, 137–145.

39. Hironaka, T.; Furukawa, K.; Esmon, P.C.; Fournel, M.A.; Sawada, S.; Kato, M.; Minaga, T.; Kobata, A. Comparative study of the sugar chains of factor VIII purified from human plasma and from the culture media of recombinant baby hamster kidney cells. J. Biol. Chem. 1992, 267, 8012–8020.

40. Lenting, P.J.; Pegon, J.N.; Christophe, O.D.; Denis, C.V. Factor VIII and von Willebrand factor--too sweet for their own good. Haemophilia 2010, 16 (Suppl. S5), 194–199.

41. Matsui, T.; Titani, K.; Mizuochi, T. Structures of the asparagine-linked oligosaccharide chains of human von Willebrand factor. Occurrence of blood group A, B, and H(O) structures. J. Biol. Chem. 1992, 267, 8723–8731.

42. Swystun, L.L.; Lillicrap, D. Current Understanding of Inherited Modifiers of FVIII Pharmacokinetic Variation. Pharmgenom. Pers. Med. 2023, 16, 239–252.

43. Grewal, P.K. The Ashwell-Morell receptor. Methods Enzymol. 2010, 479, 223–241.

44. Bovenschen, N.; Rijken, D.C.; Havekes, L.M.; van Vlijmen, B.J.; Mertens, K. The B domain of coagulation factor VIII interacts with the asialoglycoprotein receptor. J. Thromb. Haemost. 2005, 3, 1257–1265.

45. Yang, W.H.; Aziz, P.V.; Heithoff, D.M.; Mahan, M.J.; Smith, J.W.; Marth, J.D. An intrinsic mechanism of secreted protein aging and turnover. Proc. Natl. Acad. Sci. USA 2015, 112, 13657–13662.

46. Grewal, P.K.; Uchiyama, S.; Ditto, D.; Varki, N.; Le, D.T.; Nizet, V.; Marth, J.D. The Ashwell receptor mitigates the lethal coagulopathy of sepsis. Nat. Med. 2008, 14, 648–655.

47. Ward, S.; O’Sullivan, J.M.; O’Donnell, J.S. von Willebrand factor sialylation-A critical regulator of biological function. J. Thromb. Haemost. 2019, 17, 1018–1029.

48. Pegon, J.N.; Kurdi, M.; Casari, C.; Odouard, S.; Denis, C.V.; Christophe, O.D.; Lenting, P.J. Factor VIII and von Willebrand factor are ligands for the carbohydrate-receptor Siglec-5. Haematologica 2012, 97, 1855–1863.

49. Rydz, N.; Swystun, L.L.; Notley, C.; Paterson, A.D.; Riches, J.J.; Sponagle, K.; Boonyawat, B.; Montgomery, R.R.; James, P.D.; Lillicrap, D. The C-type lectin receptor CLEC4M binds, internalizes, and clears von Willebrand factor and contributes to the variation in plasma von Willebrand factor levels. Blood 2013, 121, 5228–5237.

50. Swystun, L.L.; Notley, C.; Georgescu, I.; Lai, J.D.; Nesbitt, K.; James, P.D.; Lillicrap, D. The endothelial lectin clearance receptor CLEC4M binds and internalizes factor VIII in a VWF-dependent and independent manner. J. Thromb.

Haemost. 2019, 17, 681–694.

51. CLEC4M_Human. Available online: https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=10332 (accessed on 10 April 2023).
52. Huffman, J.E.; de Vries, P.S.; Morrison, A.C.; Sabater-Lleal, M.; Kacprowski, T.; Auer, P.L.; Brody, J.A.; Chasman, D.I.; Chen, M.H.; Guo, X.; et al. Rare and low-frequency variants and their association with plasma levels of fibrinogen, FVII, FVIII, and vWF. Blood 2015, 126, e19–e29.
53. Smith, N.L.; Chen, M.H.; Dehghan, A.; Strachan, D.P.; Basu, S.; Soranzo, N.; Hayward, C.; Rudan, I.; Sabater-Lleal, M.; Bis, J.C.; et al. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. Circulation 2010, 121, 1382–1392.
54. van Loon, J.; Dehghan, A.; Weihong, T.; Trompet, S.; McArdle, W.L.; Asselbergs, F.W.; Chen, M.H.; Lopez, L.M.; Huffman, J.E.; Leebeek, F.W.; et al. Genome-wide association studies identify genetic loci for low von Willebrand factor levels. Eur. J. Hum. Genet. 2016, 24, 1035–1040.
55. Desch, K.C.; Ozel, A.B.; Halvorsen, M.; Jacobi, P.M.; Golden, K.; Underwood, M.; Germain, M.; Tregouet, D.A.; Reitsma, P.H.; Kearon, C.; et al. Whole-exome sequencing identifies rare variants in STAB2 associated with venous thromboembolic disease. Blood 2020, 136, 533–541.
56. Kayashima, Y.; Clanton, C.A.; Lewis, A.M.; Sun, X.; Hiller, S.; Huynh, P.; Wilder, J.; Hagaman, J.; Li, F.; Maeda-Smithies, N.; et al. Reduction of Stabilin-2 Contributes to a Protection Against Atherosclerosis. Front. Cardiovasc. Med. 2022, 9, 818662.
57. Swystun, L.L.; Ogiwara, K.; Lai, J.D.; Ojala, J.R.M.; Rawley, O.; Lassalle, F.; Notley, C.; Rengby, O.; Michels, A.; Nesbitt, K.; et al. The scavenger receptor SCARA5 is an endocytic receptor for von Willebrand factor expressed by littoral cells in the human spleen. J. Thromb. Haemost. 2019, 17, 1384–1396.
58. Cheng, C.; Hu, Z.; Cao, L.; Peng, C.; He, Y. The scavenger receptor SCARA1 (CD204) recognizes dead cells through spectrin. J. Biol. Chem. 2019, 294, 18881–18897.
59. Wohner, N.; Muczynski, V.; Mohamadi, A.; Legendre, P.; Proulle, V.; Ayme, G.; Christophe, O.D.; Lenting, P.J.; Denis, C.V.; Casari, C. Macrophage scavenger receptor SR-AI contributes to the clearance of von Willebrand factor. Haematologica 2018, 103, 728–737.
60. Ward, S.E.; Guest, T.; Byrne, C.; Lopes, P.; O'Sullivan, J.M.; Doherty, D.; O'Connell, D.; Gutierrez Llaneza, S.; Chion, A.; Fazavana, J.; et al. Macrophage Galactose Lectin Contributes to the Regulation of FVIII (Factor VIII) Clearance in Mice-Brief Report. Arterioscler. Thromb. Vasc. Biol. 2023, 43, 540–546.
61. Ward, S.E.; O'Sullivan, J.M.; Drakeford, C.; Aguila, S.; Jondle, C.N.; Sharma, J.; Fallon, P.G.; Brophy, T.M.; Preston, R.J.S.; Smyth, P.; et al. A novel role for the macrophage galactose-type lectin receptor in mediating von Willebrand factor clearance. Blood 2018, 131, 911–916.
62. O'Sullivan, J.M.; Jenkins, P.V.; Rawley, O.; Gegenbauer, K.; Chion, A.; Lavin, M.; Byrne, B.; O'Kennedy, R.; Preston, R.J.; Brophy, T.M.; et al. Galectin-1 and Galectin-3 Constitute Novel-Binding Partners for Factor VIII. Arterioscler. Thromb. Vasc. Biol. 2016, 36, 855–863.
63. Saint-Lu, N.; Oortwijn, B.D.; Pegon, J.N.; Odouard, S.; Christophe, O.D.; de Groot, P.G.; Denis, C.V.; Lenting, P.J. Identification of galectin-1 and galectin-3 as novel partners for von Willebrand factor. Arterioscler. Thromb. Vasc. Biol. 2012, 32, 894–901.
64. Antoni, G.; Oudot-Mellakh, T.; Dimitromanolakis, A.; Germain, M.; Cohen, W.; Wells, P.; Lathrop, M.; Gagnon, F.; Morange, P.E.; Tregouet, D.A. Combined analysis of three genome-wide association studies on vWF and FVIII plasma levels. BMC Med. Genet. 2011, 12, 102.
65. Sabater-Lleal, M.; Huffman, J.E.; de Vries, P.S.; Marten, J.; Mastrangelo, M.A.; Song, C.; Pankratz, N.; Ward-Caviness, C.K.; Yanek, L.R.; Trompet, S.; et al. Genome-Wide Association Transethnic Meta-Analyses Identifies Novel Associations Regulating Coagulation Factor VIII and von Willebrand Factor Plasma Levels. Circulation 2019, 139, 620–635.
66. Bank, I.; Libourel, E.J.; Middeldorp, S.; Hamulyak, K.; van Pampus, E.C.; Koopman, M.M.; Prins, M.H.; van der Meer, J.; Buller, H.R. Elevated levels of FVIII:C within families are associated with an increased risk for venous and arterial thrombosis. J. Thromb. Haemost. 2005, 3, 79–84.