How much do we really know about von Willebrand disease?

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Purpose of review
In the last nine decades, large advances have been made toward the characterization of the pathogenic basis and clinical management of von Willebrand disease (VWD), the most prevalent inherited bleeding disorder. Pathological variations at the von Willebrand factor (VWF) locus present as a range of both quantitative and qualitative abnormalities that make up the complex clinical spectrum of VWD. This review describes the current understanding of the pathobiological basis of VWD.

Recent findings
The molecular basis of type 2 (qualitative abnormalities) and type 3 VWD (total quantitative deficiency) have been well characterized in recent decades. However, knowledge of type 1 VWD (partial quantitative deficiency) remains incomplete because of the allelic and locus heterogeneity of this trait, and is complicated by genetic variability at the *VWF* gene, interactions between the *VWF* gene and the environment, and the involvement of external modifying loci. Recent genome wide association studies and linkage analyses have sought to identify additional genes that modify the type 1 VWD phenotype.

Summary
Understanding the pathogenic basis of VWD will facilitate the development of novel treatment regimens for this disorder, and improve the ability to provide complementary molecular diagnostics for type 1 VWD.

Keywords
bleeding disorder, von Willebrand disease, von Willebrand factor

INTRODUCTION

Von Willebrand disease (VWD), the most prevalent inherited bleeding disorder, was first described in 1926 by the Finnish physician, Eric von Willebrand, in a large family living in the Åland archipelago in the Baltic Sea [1]. The female index case had a history of early mucus membrane and ankle bleeds, and expired at the age of 14 from her fourth menstrual bleed. The recognition of von Willebrand factor (VWF) as the deficient or dysfunctional protein in VWD occurred in 1971 with the development of an immunological test to differentiate VWF from its binding partner, factor VIII (FVIII), and thus distinguish VWD from hemophilia A [2]. Shortly thereafter, forms of VWF with variant functional properties were identified, leading to the classification of qualitative and quantitative VWF deficiencies and detailed phenotypic testing [3]. In 1985, the cloning of the *VWF* cDNA [4–7] initiated advancements in molecular diagnostics for VWD and characterization of the functional aspects of this molecule that have continued to date (Fig. 1).

VWF is a large multimeric glycoprotein that regulates hemostasis by mediating platelet–platelet binding and adhesion to the endothelium, and acts as a carrier for coagulation FVIII (Fig. 2a). VWF is synthesized in endothelial cells and megakaryocytes in a complex biosynthetic process that involves C-terminal dimerization, N-terminal multimerization, cleavage of an N-terminal propeptide, and posttranslational modification involving N- and O-linked glycosylation and sulfation. This yields a mature VWF molecule that circulates as a series of multimers ranging in size between 0.5 and 20 mDa. VWF is either constitutively released from endothelial cells or retained in endothelial intracellular

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KEY POINTS

- The genetic and mechanistic basis of most type 2 and 3 VWD cases are well characterized.
- Type 1 VWD involves impaired VWF synthesis, secretion, or accelerated clearance.
- Overall, 35% of type 1 VWD cases do not have a candidate variant in the *VWF* cDNA.
- Recent studies have identified novel loci that may modulate the type 1 VWD phenotype.

storage vesicles termed Weibel–Palade bodies (WPB) or platelet α-granules for release upon hemostatic challenge.

VWD is the most prevalent hereditary bleeding disorder affecting between 0.1 and 1% of individuals [8–10]. Patients with VWD typically present with excessive bleeding from mucocutaneous membranes of the nasopharynx, genitourinary, and gastrointestinal tracts and can present with complications associated with surgery, trauma, menorrhagia, and childbirth. VWD is classified into three subtypes with type 1 (~65–80% of cases) and type 3 (~1/1 000 000 cases) involving partial and severe quantitative deficiencies, respectively. Type 2 VWD (~20–35% cases) involves qualitative defects in VWF, which impair its platelet- and/or collagen-binding (subclass 2A, 2B, 2M), or FVIII-binding (2N) properties (Table 1).

TYPE 3 VON WILLEBRAND DISEASE

Type 3 VWD (VWF:Ag <0.01–0.05 IU/ml) is inherited in a recessive or codominant fashion with index cases being either homozygous or compound heterozygous for two *VWF* null alleles [11]. Recent studies have reported that in approximately 50% of type 3 families, *VWF* null alleles demonstrate a codominant phenotype where obligate carriers present with type 1 VWD [12,13]. Type 3 VWD variants can be found throughout the *VWF* gene and the mutational spectrum encompasses nonsense and missense variants, insertions, and small and large gene deletions. Mechanistically, type 3 VWD is predominantly associated with impaired VWF biosynthesis and/or disruption of the protein conformation that inhibits secretion. Studies of endothelial progenitor cells derived from type 3 VWD patients demonstrate heterogeneity of phenotypes, including null VWF production, and both quantitative and qualitative defects in WPB formation [14].

TYPE 2A VON WILLEBRAND DISEASE

Type 2A VWD is classified by the loss of the platelet-binding function of VWF associated with reduced high-molecular-weight multimers (HMWMs). This can be the result of impaired multimerization of VWF during biosynthesis [15], or increased proteolysis of HMWMs by the VWF protease ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) [16], although some type 2A variants are not easily classified and variants may influence various overlapping aspects of VWF synthesis and storage, secretion, and proteolysis [17]. Type 2A VWD is inherited in an autosomal dominant manner with over 80 unique variants reported in the literature. The majority of type 2A variants (~80%) can be found in the A2 domain of VWF (exon 28) near the site of ADAMTS13 cleavage (Fig. 2a). Additional 2A substitutions are found in the VWF propeptide (VWFpp)

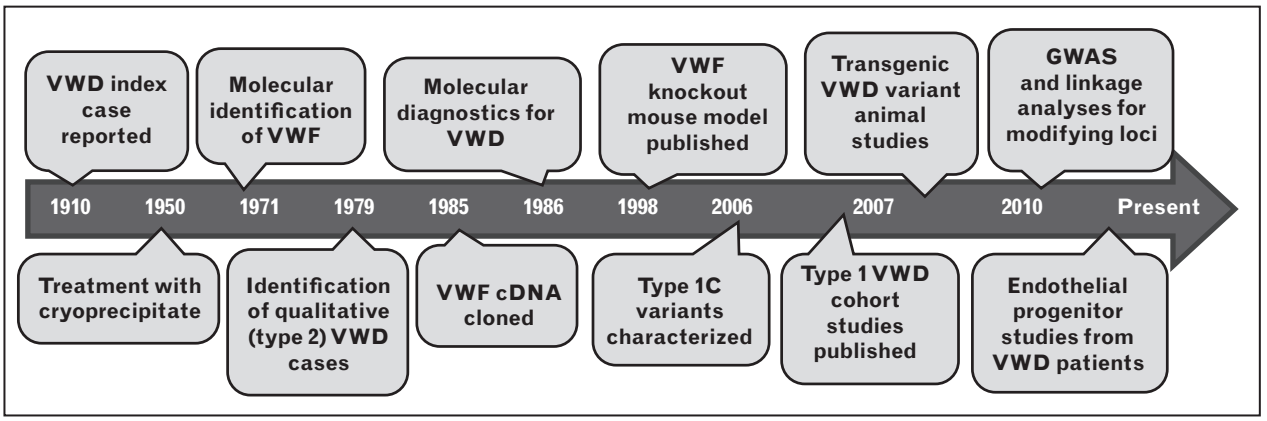

FIGURE 1. A history of the molecular characterization of von Willebrand disease. GWAS, genome wide association studies; VWD, von Willebrand disease; VWF, von Willebrand factor.

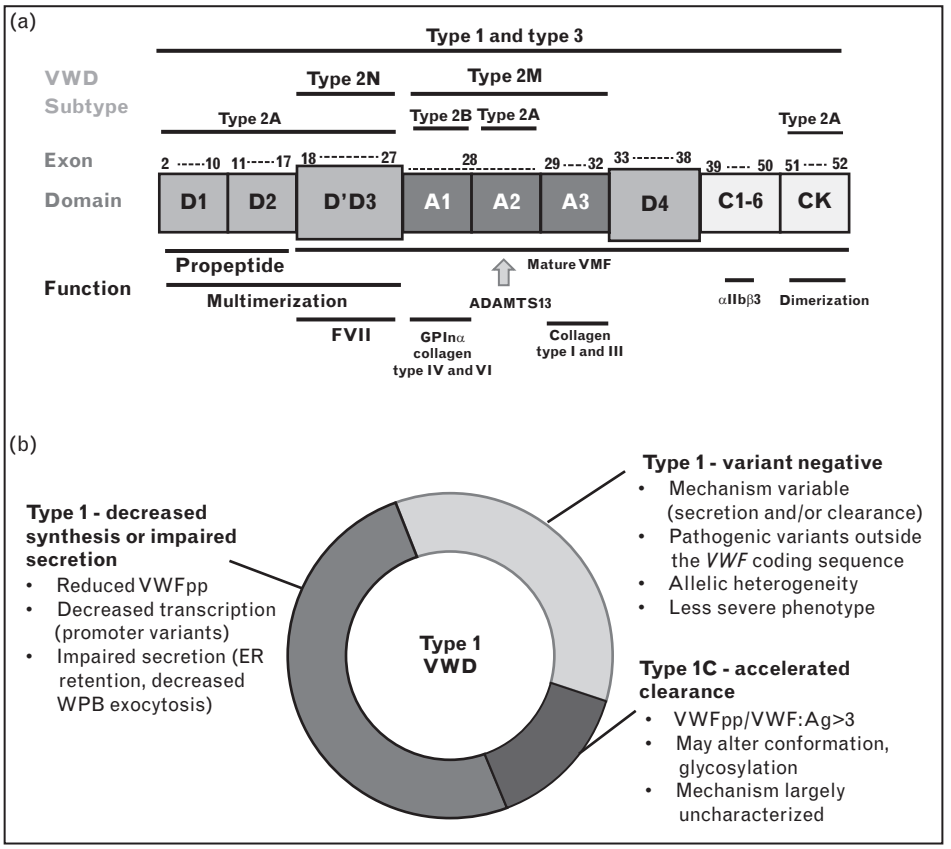

FIGURE 2. (a) Structure and function of von Willebrand factor in relation to von Willebrand disease. (b) Mechanisms of quantitative von Willebrand factor deficiency in type 1 von Willebrand disease. ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CK, cystine knot; ER, endoplasmic reticulum; FVIII, factor VIII; GP, glycoprotein; GWAS, genome wide association studies; VWD, von Willebrand disease; VWF, von Willebrand factor, VWFpp, von Willebrand factor propeptide; WBP, Weibel–Palade bodies.

and D'D3 domains, which are involved in VWF multimerization (10%), and the cystine knot domain which regulates VWF dimerization (10%).

TYPE 2B VON WILLEBRAND DISEASE

Type 2B VWD is characterized by gain-of-function VWF variants that have enhanced binding to the platelet glycoprotein Ibα (GPIb) receptor [18]. This disorder is phenotypically characterized by loss of HMWMs and chronic thrombocytopenia because of accelerated platelet clearance [19]. Bleeding in type 2B VWD can be aggravated by conditions that increase VWF plasma levels, including inflammation, trauma, or pregnancy. Type 2B variants are observed exclusively in the VWF A1 domain (exon 28), and to date at least 30 unique missense substitutions have been identified (Fig. 2a). They are thought to induce a conformational change in the orientation of the A1 domain that results in the exposure of the cryptic GPIb-binding site. Molecular diagnostics for type 2B VWD are important to distinguish this disorder from its genocopy, platelet type VWD, which involves gain-of-VWF-binding function variants in the gene for GPIbα (*GPIBA*) [20].

TYPE 2M VON WILLEBRAND DISEASE

Type 2M VWD is characterized by qualitative defects in VWF adhesive activity associated with normal multimer distribution. Type 2M VWD typically involves impaired binding of VWF to the platelet GPIb receptor, or to various forms of collagen [21]. Recent studies of type 2M VWD collagen-binding variants by Flood *et al.* [22▪▪,23,24] have highlighted the utility of assessing VWF binding to collagen types I, III, IV, and VI as part of a routine diagnostic workup for VWD. Variants that impair binding of VWF to GPIb occur in the A1 domain, whereas collagen-binding variants occur in the A1 and A3 domains of VWF (Fig. 2a). Type 2M VWD typically displays a dominant inheritance pattern, and is generally associated with a milder bleeding phenotype than the other type 2 subtypes [25].

Table 1. von Willebrand disease types and subtypes

VWD Subtype	Frequency of diagnosis	Mechanism	Phenotype	Variant location(s)	Inheritance pattern	Mutation type
Type 3	1/1 000 000	Total quantitative deficiency	VWF:Ag <0.05 IU/ml VWF:RCo <0.05 IU/ml FVIII:C <0.15 IU/ml	Whole VWF gene	Autosomal recessive, codominant	Nonsense, missense, insertions, deletions
Type 2A	10–20%	Impaired multimerization	Reduced HMWMs VWF:RCo 0.05–0.5 IU/ml RCo/Ag ratio <0.7	Propeptide, D'D3, A2, CK	Autosomal dominant	Missense
Type 2B	5–10%	Gain-of-function GPIb binding	Low platelet count VWF:RCo 0.05–0.5 IU/ml Reduced HMWMs RCo/Ag ratio <0.7	A1	Autosomal dominant	Missense
Type 2M	3–5%	Impaired platelet or collagen binding	VWF:RCo 0.05–0.5 IU/ml RCo:Ag ratio <0.70 Normal multimers	A1, A3	Autosomal dominant	Missense
Type 2N	2–5%	Decreased FVIII binding	FVIII:C <0.5 U/ml FVIII < VWF:Ag	D'D3	Autosomal recessive	Missense
Type 1	65–80%	Partial quantitative deficiency	Low VWF:Ag (<0.5 IU/ml) Low VWF:RCo (<0.5 /ml)	Whole VWF gene	Autosomal dominant, occasional recessive, or codominant	Nonsense, missense, insertions, deletions
Type 1C	~15% of type 1	Partial quantitative deficiency Accelerated clearance	Accelerated clearance (desmopressin trial) VWFpp/VWF:Ag >3	D'D3, D4	Autosomal dominant	Missense

CK, cystine knot; FVIII, GP, glycoprotein; factor VIII; HMWM, high-molecular-weight multimer; VWD, von Willebrand disease; VWF, von Willebrand factor, VWFpp, von Willebrand factor propeptide.

TYPE 2N VON WILLEBRAND DISEASE

Type 2N VWD involves impaired binding of VWF to FVIII, resulting in accelerated proteolysis and clearance of FVIII. Type 2N VWD presents clinically with normal VWF antigen and activity levels, but low FVIII:C (0.05–0.4 IU/ml) and is therefore, frequently misdiagnosed as mild hemophilia A. Type 2N is inherited in an autosomal recessive manner with patients either homozygous or compound heterozygous for two different 2N alleles or one 2N and one null allele [26]. Type 2N VWD variants cluster in the D'D3 region of the VWF molecule, with the majority of severe 2N variants localized to a flexible loop in the D' region (Fig. 2a) [27▪▪]. Type 2N variants typically result in either a loss of cysteine residues or positive charge, although the R763 and R760 variants, which impair propeptide cleavage, can also result in type 2N VWD due presumably to steric hindrance of the FVIII-binding site on VWF by the retained propeptide [28].

TYPE 1 VON WILLEBRAND DISEASE

Type 1 VWD is the most prevalent form of this disorder, accounting for up to 80% of all cases. There continues to be a lack of international consensus concerning the precise phenotypic definition of type 1 VWD, but it is generally characterized by a mild-to-moderate quantitative deficiency of VWF with plasma VWF levels between 0.05 and 0.5 IU/ml, although some would place the upper limit of VWF as low as 0.3 IU/l. Thus, in some classifications, patients with a bleeding phenotype and VWF levels between 0.3 and 0.5 IU/ml are referred to as 'low VWF level' patients. Type 1 VWD is predominantly inherited in an autosomal dominant manner, although recessive cases have been reported [29▪]. Pathogenic variants can be found throughout the entire full-length molecule, and to date more than 100 type 1 VWD variants have been described, with missense variants accounting for the majority of pathogenic candidates [30–33].

Type 1 VWD is also complicated by a genotype–phenotype relationship that is characterized by variability in both phenotypic penetrance and expressivity. For example, there is increasing evidence of an ethnic predisposition to VWD, and studies of some variants associated with type 1 and 2 VWD in Caucasian populations are present in normal populations of African individuals, which may in part be related to coinheritance of these variants with *VWF* and other genetic polymorphisms that can independently modify VWF:Ag levels [34–36]. Variability in type 1 VWD expressivity may be in part associated with the expression of variant monomers within the multimeric structure, which has recently been described as a potential pathogenic basis for the variable expressivity of a type 2 substitution [37▪▪]. Additionally, although the heritability of VWF plasma levels is estimated at approximately 65% [38–42], the interaction between genotype and the environment may also modify the type 1 phenotype. For example, VWF:Ag levels are known to increase with age at a rate of approximately 0.8% per year, and recent studies have reported the normalization of VWF:Ag levels in type 1 patients over time [43▪,44▪].

TYPE 1: IMPAIRED SYNTHESIS OR SECRETION

The cause of type 1 VWD involves variants that influence the rate of VWF synthesis, secretion from the endothelial cell, and clearance from the plasma (Fig. 2b). Type 1 variants with impaired secretion are distinguished from those with an accelerated clearance profile based on surrogate assessments or responsiveness to desmopressin. As we now understand that VWF and FVIII are expressed by endothelial cells and that the two proteins circulate in the plasma as a noncovalent complex, an increased ratio (FVIII:C/VWF:Ag) is indicative of decreased VWF:Ag secretion [45]. Additionally, these individuals have reduced levels of VWF propeptide, which is released with full-length VWF in equimolar ratios. Type 1 secretion variants tend to localize in the VWF D1, D2, and D3 domains [46].

The mechanistic basis for type 1 VWD secretion defects are relatively well characterized and can encompass decreased synthesis related to variants in the promoter [47], although the majority of type 1 variants are associated with intracellular retention in the endoplasmic reticulum, abnormal WPB formation, and/or impaired secretion of VWF as observed in heterologous expression systems and in patient-derived endothelial progenitor cells [48–50]. Often, the characterization of type 1 variants is not entirely clear cut, with the display of both quantitative and more subtle qualitative defects. The most frequently occurring type 1 variant is Y1584C, which has been described in up to 20% of index cases [51]. It is associated with plasma VWF:Ag levels of ~0.4 IU/ml and a variable bleeding score. Heterologous in-vitro systems and animal models demonstrate that Y1584C is associated with increased intracellular retention and decreased secretion relative to wild-type VWF, a mild increase in susceptibility to ADAMTS13 cleavage, decreased HMWMs, and impaired thrombus formation *in vivo* [52].

TYPE 1C: ACCELERATED CLEARANCE

Type 1 accelerated clearance variants (termed type 1C), which make up approximately 15–20% of all type 1 cases, are characterized by measuring VWF half-life in response to desmopressin (DDAVP) or by the VWFpp/VWF:Ag ratio surrogate assessment (where a ratio >3 is indicative of accelerated clearance) [53]. Although the type 1C VWD subtype is associated with a more severe reduction in plasma levels of VWF:Ag (usually ~0.10–15 IU/ml), as compared with the rest of the type 1 population, there are no reported differences in bleeding scores between these two patient populations presumably because of the normal release of platelet-derived VWF [46]. Variants associated with type 1C are located predominantly in the VWF D3, A1, and D4 domains.

The half-life of VWF is regulated by a number of different mechanisms, including posttranslational modification of the VWF protein (e.g., glycosylation) [54], exposure to shear which alters the three-dimensional conformation of the molecule [55], and the interaction between VWF and clearance receptors that are expressed on the sinusoidal endothelial cells and macrophages in the liver and/or spleen [56,57]. The mechanistic basis for many of the type 1C variants is currently under investigation, but may involve altered glycosylation and/or the exposure of a cryptic-binding site on the VWF molecule that changes the affinity of VWF for one or more clearance receptors. The R1205H 'Vicenza' variant is the prototypical type 1C substitution [58]. Patients with the R1205H variant typically have a VWFpp/VWF:Ag more than 10 and plasma levels of VWF:Ag between 0.10 and 0.15 IU/ml [53]. Recent studies suggest that accelerated clearance of the R1205H variant can occur through a macrophage-dependent mechanism [59▪▪] that may involve low-density lipoprotein receptor-related protein 1.

TYPE 1: VARIANT NEGATIVE

Although genetic testing is routinely used as a complementary diagnostic methodology for types

2 and 3 VWD, molecular diagnostics for the type 1 VWD heterogeneous trait is an ongoing challenge. Four large multicenter studies have consistently reported a molecular diagnosis for approximately 65% of cases of type 1 VWD [30–33], which contrasts to the more than 90% rate of genetic diagnosis for the type 2 and 3 populations. As such, type 1 VWD is distinct in that it displays both locus and allelic heterogeneity. Interestingly, the 35% of type 1 VWD cases that are variant negative tend to be associated with a milder bleeding phenotype (Fig. 2b). It is thought that for some variant negative cases, pathogenic variants may be found in deep intronic sequences, or at distal regulatory regions of the *VWF* gene. Although more detailed characterization of the whole *VWF* gene is required to identify these variants, it is becoming increasingly evident that loci external to the *VWF* gene also contribute to the type 1 VWD phenotype. The classical example of locus heterogeneity that influences plasma VWF levels in type 1 VWD is the ABO blood group locus, which has been recognized for several decades to contribute to approximately 30% of the genetic influence on plasma VWF:Ag levels. ABO (H) antigens are elaborated on the N-linked glycans of VWF, and individuals with group O VWF have approximately 25% lower VWF levels than non-O VWF individuals [60]. Thus it follows that individuals with type O VWF are more frequently diagnosed with type 1 VWD than non-O individuals. The specific mechanism by which type O VWF levels are reduced is not precisely characterized, although it appears to be regulated through a clearance-dependent process [61].

Recently published genome wide association studies (GWAS) and linkage analyses have sought to identify additional loci that can modify plasma levels of VWF [42,62]. Of particular note is the Cohorts for Heart and Aging Research in Genome Epidemiology GWAS meta-analysis, which identified polymorphisms at novel loci that associated with plasma levels of VWF [62]. In addition to previously recognized associations with the ABO blood group locus and the *VWF* gene, six novel loci were identified that may contribute to the regulation of plasma VWF levels through modulating VWF secretion and clearance. Recent studies have begun to confirm the role of these proteins in regulating VWF plasma levels, and the association of polymorphisms at these loci with plasma levels of VWF in VWD. For example, syntaxin-binding protein 5 (STXBP5) is a member of the soluble NSF attachment protein receptor (SNARE) family of proteins, which have functional roles in regulating intracellular transport, and functions as a negative regulator of WPB exocytosis [63▪▪]. Additionally, C-type lectin domain family 4, member M (CLEC4M) [64] and stabilin-2 [57] are both endocytic receptors expressed on the sinusoidal endothelial cells of the liver, and regulate plasma levels of VWF via clearance-related mechanisms. Further studies are required to fully characterize the contribution of GWAS-identified novel loci to the type 1 VWD phenotype with the long-term goal of incorporating these variants into a diagnostic algorithm for this trait [64,65▪,66▪] (Table 2).

CONCLUSION

The last 90 years of VWD research have yielded major advances in both molecular and phenotypic characterization of the disorder along with prophylactic and on-demand treatment regimens (Fig. 1). However, there is still much to understand regarding the underlying pathogenic mechanisms of type 1 VWD. Modern genomic technologies, including whole (gene)ome sequencing, and epigenetic

Table 2. Loci that associate with plasma von Willebrand factor levels in normal individuals

Gene (protein)	Protein function	Mechanism	Refs
ABO blood group locus	Glycosyltransferase	Glycosylation (clearance)	[60,62]
CLEC4M	Endocytic lectin receptor	Clearance	[62,64]
SCARA5	Endocytic scavenger receptor	Clearance	[62]
STAB2	Endocytic pattern recognition receptor	Clearance	[57,62]
STX2	SNARE protein regulating exocytosis	Secretion	[62]
STXBP5	SNARE protein regulating exocytosis	Secretion	[62,63▪▪]
TC2N	Unknown	Undetermined	[62]
Chromosome 2 – 2q12–2p13	Multiple loci	Undetermined	[42]
UFM1	Protein conjugation	Undetermined	[66▪]

CLEC4M, C-type lectin domain family 4, member M; *SNARE*, soluble NSF attachment protein receptor; *SCARA5*, scavenger receptor class A, member 5; *SNARE*, soluble NSF attachment protein receptor; *STAB2*, stabilin-2; *STX2*, syntaxin 2; *STXBP5*, syntaxin-binding protein 5; *TC2N*, tandem C2 domain nuclear; *UFM*, ubiquitin-fold modifier 1.

studies will hopefully further our understanding of this complex inherited trait [67,68].

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Conflicts of interest

There are no conflicts of interest.

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