1 Historical perspective on von Willebrand disease

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Introduction

The history of von Willebrand disease (VWD) and its causative factor, the von Willebrand factor (VWF), spans almost a century and was recently comprehensively reviewed by the late professor Birger Blombäck, who described the first publication by Erik von Willebrand [1], the gene cloning in 1985, and the discovery of the specific metalloprotease, ADAMTS13 [2], that degrades VWF. The purpose of this review is to describe the early history of the understanding of the disease and the first steps in the replacement therapy for its severe forms. Also, we describe in greater detail the findings in a group of different families investigated on the Åland Islands.

The scientist of the disease

Erik Adolf von Willebrand (Figure 1.1) was born in Vasa, Finland, in 1870. He qualified as a medical doctor in 1896 and specialized initially in physical therapy and later in internal medicine at Helsinki. Erik von Willebrand devoted much of his professional life to an interest in blood, especially its coagulation properties. In 1899, he defended a doctoral thesis that dealt with his investigation of the changes that occur in blood following a serious hemorrhage. From 1908 until his retirement in 1935, Erik von Willebrand worked at the Deaconess Institute in Helsinki, where he headed the Department of Internal Medicine between 1922 and 1931. Erik von Willebrand was known for his modesty and integrity, and in his obituary it was said that he "usually preferred to discuss his observations of nature rather than his personal achievements." He died in September 1949, at the age of 89 years.

First description of the disease: the Åland family

In 1926, Erik von Willebrand first described the inherited bleeding disorder in *Finska Läkaresällskapets Handlingar* (in Swedish). He identified features that suggested that this disease was distinct from classic hemophilia and other bleeding disorders known at the time, such as anaphylactoid purpura, thrombocytopenic purpura, and the hereditary thrombasthenia described by Glanzmann. What differentiated this bleeding disorder from classic hemophilia was that it was not frequently associated with muscle and joint bleeding, and it affected both women and men. He stressed that a prolonged bleeding time was its most prominent characteristic. He concluded that the condition was a previously unknown form of hemophilia, and called it "hereditary pseudohemophilia." Erik von Willebrand also discussed the pathogenesis of the condition and felt that the bleeding could best be explained by the combined effect of a functional disorder of the platelets and a systemic lesion of the vessel walls.

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The original observations leading to this new disease were made in several members of a large family (identified as family S) living on the island of Föglö in the Åland archipelago in the Baltic Sea. The index case was a girl aged 5 years, named Hjördis S, who had marked and recurrent bleeding tendencies and was brought to Helsinki for consultation. Both her mother and father were from families with histories of bleeding. The girl was the ninth of 11 children, of whom seven had experienced bleeding symptoms. Four of her sisters had died from uncontrolled bleeding at an early age. Hjördis herself had experienced several severe episodes of bleeding from the nose and lips and following tooth extractions, as well as bleeding in her ankle. At the age of 3 years she bled for 3 days from a deep wound in her upper lip. The bleeding was so severe that she almost lost consciousness and had to be hospitalized for 10 weeks. At the age of 14 years, Hjördis bled to death during her fourth menstrual period.



Figure 1.1 Erik von Willebrand.

Hjördis came from a large family (Figure 1.2). Intrigued by their history, Erik von Willebrand studied the family further with the help of coworkers. He published the pedigree and his clinical and laboratory evaluation in his 1926 paper. He found that 23 of the 66 family members had bleeding problems. The most prominent problem among the affected family members was mucosal bleeding: epistaxis, followed by profuse bleeding from oral lesions, easy bruising, and, in females, excessive bleeding during menstruation and at childbirth. Intestinal bleeding had been the cause of death at early ages in some family members.

In further studies, Erik von Willebrand found two families related to Hjördis S and one unrelated family in whom bleeding symptoms similar to those observed in Hjördis were common [3,4]. In the 1930s, Jürgens, together with von Willebrand [5,6], reinvestigated the patients in Åland and concluded that the disease was due to some impairment of platelet function, including platelet factor 3 deficiency. This observation led to the disease being called von Willebrand–Jürgens thrombopathy, and, although this condition is not officially recognized today, von Willebrand did not dismiss the notion that factors in blood plasma might also be important in the pathogenesis of the disease.

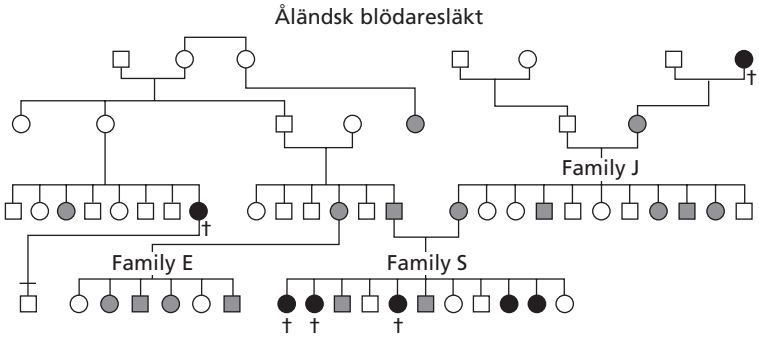


Figure 1.2 The Åland pedigree as originally described in 1926 [1]. The index case, Hjördis, is the ninth sibling in family S (Fam S). □ unaffected male; ○ unaffected female; ■ male with mild bleeding disease; ● female with mild bleeding disease; ● female with severe bleeding disease; † bled to death.

Other early clinical reports

In 1928, Dr. George R. Minot of Boston described five patients from two families with prolonged bleeding times and symptoms similar to the Åland family members. This may have been one of the first descriptions of VWD [7–9]. In the following years, numerous cases similar to those described by von Willebrand were reported, usually under the name of pseudohemophilia. In 1953, Alexander and Goldstein [10] found a dual defect in two patients with hereditary pseudohemophilia. They confirmed the earlier findings of prolonged bleeding time, normal platelet count and function, and abnormal nail bed capillaries. However, they also found a decreased FVIII level (5–10% of normal) and they observed a prolonged coagulation time that was normalized by normal plasma. The prolonged bleeding time, however, was not normalized and this was later explained by the fact that infusion of a restricted volume of plasma does not provide a sufficient amount of VWF [11]. Larrieu and Soulier [12] also found low FVIII activity and a prolonged bleeding time in pseudohemophilia, but otherwise normal clotting factors and platelet parameters. They proposed the name of von Willebrand syndrome for the condition.

The search for a new factor—the bleeding time factor

The first demonstration of the VWF was during the 1950s through a joint effort by Margareta and Birger Blombäck, working in Stockholm with the purification of fibrinogen, and Inga Marie Nilsson, who had established a clinical coagulation unit in Malmö. It was found that fibrinogen purified from Cohn fraction I of human plasma, when specifically obtained in fraction I-0 (AHF-Kabi), was heavily contaminated with an antihemophilic factor, that is plasma factor VIII (FVIII) [13].

At that time, Dr. Nilsson had a 15-year-old female patient named Birgitta who had a severe hemorrhagic diathesis. When she began to menstruate, the condition worsened and she received frequent blood transfusions. However, Birgitta developed serious side-effects from the transfusions and they were stopped. As a consequence, other treatment options had to be considered, and a hysterectomy was planned. Her coagulation evaluation had shown a prolonged bleeding time and a somewhat prolonged coagulation time but normal platelet count and function. FVIII activity was low. Since fraction I-0 had a high concentration of FVIII, it was decided that its effects should be tested in Birgitta. To the surprise of the treating physicians, not only did FVIII activity increase as expected but the bleeding time was also normalized [14]. Subsequently, a hysterectomy was successfully performed under the cover of fraction I-0. According to modern classification, this patient had type 3 VWD. She is now well, and has been on regular prophylaxis with VWF concentrate for many years.

In June 1957, Inga Marie Nilsson, Erik Jorpes, Margareta Blombäck, and Stig-Arne Johansson visited Åland and studied 16 patients who had been examined 25–30 years previously by von Willebrand. No patients who had severe forms of the disease were still living. In their investigation they found FVIII activity to be reduced in 15 of 16 cases [15]. The father of Hjördis had a normal level. The Duke bleeding time varied, with two patients having a definite prolongation and three patients a moderate prolongation. Platelet counts were normal and, in contrast to Jürgens' earlier observation, the platelets themselves were normal with respect to platelet factor 3. One of the patients was given fraction I-0, which normalized the FVIII level and the bleeding time. It could be concluded that the Åland family had the same disease described by several other authors in Europe and the USA [16]. At the same time, Jürgens visited the islands (Erik Jorpes had told him of his team's research plan) and took samples from many of the same patients, and confirmed the decreased FVIII levels [17].

The findings by the Swedish group confirmed what had been documented in a number of Swedish families [18]. The observation was also made that FVIII increased during the first 24 h after infusion of fraction I-0 in patients with VWD, in contrast to what is seen in hemophilia [19]. The results of fraction I-0 infusion in a patient with severe VWD are shown in Figure 1.3. The bleeding time is reduced or normalized; factor VIII clotting activity (VIII:C) increases steadily during the first 24 h whereas the VWF (VIIIR:Ag and VIIIR:RC according to old nomenclature) displays a pharmacokinetic profile as expected and as later shown. Control experiments and further studies [11,20,21] revealed that the bleeding time

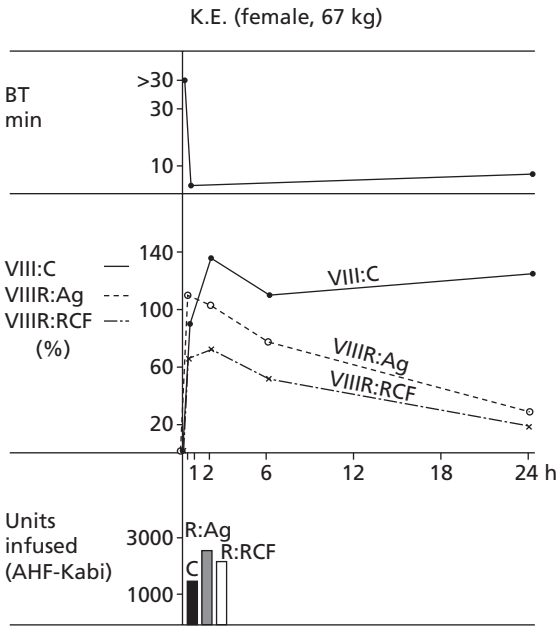


Figure 1.3 VIII:C, VWF:Ag (VIIIR:Ag), VWF:RCo (VIIIR:RCF), and Duke bleeding time (BT) in a patient with severe von Willebrand disease after infusion of human fraction I-0 (AHF-Kabi) [16]. Bleeding time is shortened and VIII:C is successively increased after the initial post-infusion peak during the first 24 h, whereas the von Willebrand factor (VIIIR:Ag and VIIIR:RC) displays a pharmacokinetic profile as expected. Reproduced from Nilson and Holmberg [16].

factor was a plasma factor not earlier described. Fraction I-0 prepared from patients with severe hemophilia A not only corrected the bleeding time in VWD, but also stimulated the production of FVIII activity, whereas fraction I-0 prepared from patients with VWD had no such effect. Purified fibrinogen had no effect on the bleeding time. Still, there was the possibility that the shortening of the bleeding time was due to platelets or platelet factors contaminating fraction I-0. This turned out to be unlikely, since the effect on bleeding time was the same whether the fraction had been prepared from platelet-rich or platelet-poor plasma. Infusion of a platelet suspension from a normal donor to a patient with VWD had no effect on either bleeding time or bleeding tendency, nor did injection of fraction I-0 into a patient with thrombocytopenia. From these findings, it was concluded that the impaired hemostasis in VWD was due to lack of a plasma factor, the bleeding time correcting factor, or the VWF, which occurs not only in normal plasma but also in hemophilia A plasma. This factor not only corrected the prolonged bleeding time, but apparently increased the level of FVIII. Thus, platelets or platelet factors were not identical with the bleeding time factor, which had been proposed by both Rudolf Jürgens and Erik von Willebrand to be responsible, together with a vascular defect, for the bleeding diathesis. These findings have since been widely confirmed. The claim that a previously unknown factor in plasma had been discovered was communicated at the Congress of the International Society of Hematology in Rome in 1958 (see also [20]).

At first, it was not understood how a plasma factor could affect primary hemostasis and shorten the bleeding time. However, Borchgrevink [22] found decreased platelet adhesiveness *in vivo*, and Salzman [23] demonstrated decreased platelet adhesiveness to glass in VWD. Borchgrevink employed the method suggested by Hellem [24], which used a slow flow and could not discriminate between samples from patients with or without VWD. Salzman modified this method and introduced a higher flow, making it more specific for VWD. It was also shown that normal or hemophilic plasma can normalize the reduced platelet adhesiveness as well as the bleeding time in VWD [23,25,26]. In studies using electron microscopy, Jörgensen and Borchgrevink [27] demonstrated a decreased adhesion of platelets to disrupted endothelium in VWD. This observation indicated that the plasma factor lacking in VWD exerted its action in primary hemostasis via the platelets by enhancing their adhesiveness.

During the 1960s, cases of VWD were reported from several countries. The disease was thought to be uniform and was defined as an autosomal dominant inheritable hemorrhagic disease characterized by a prolonged bleeding time, decreased FVIII clotting activity, decreased platelet adhesiveness as measured by the Salzman method, and progressive increase of FVIII activity after infusion of plasma and FVIII concentrate [16].

However, returning to the earlier papers by Erik von Willebrand and Rudolf Jürgens, the findings on the Åland islands showed what appeared to be a discrepancy between the original family S and some of the others investigated; the original von Willebrand family having "pure" VWD while in other families

there were also platelet function defects. Thus, in 1977, Dag Nyman (originally from Åland) and collaborators [28] traveled from Stockholm to Åland to undertake a thorough investigation using new laboratory methods [28]. They found that the families described as having VWD could be divided into four categories: (i) the survivors with a mild disorder from the original family S had the characteristics of type 1 VWD, that is they had similarly decreased levels of VWF:Ag and ristocetin cofactor activity in addition to normal or decreased levels of FVIII, and the platelet aggregation was normal; (ii) one family had a platelet function defect (pure cyclooxygenase defect); (iii) one family had a mixture of VWD and a cyclooxygenase defect; and (iv) one family had a platelet function defect of the aspirin type. These findings, of course, made it easier to investigate the genetic defects of the original VWD (family S).

In the beginning of the 1990s, Zhang and collaborators [29] investigated the DNA sequence from 24 patients with type 3 VWD living in Sweden. They found a cytosine deletion in exon 18 of the *VWF* gene in most of those of Swedish origin and an insertion in exon 28 in those of Finnish origin. Most patients with type 3 VWD were homozygous or double heterozygous for the mutations. Most of the parents had type 1 VWD and were heterozygous. As the Åland population is primarily of Swedish origin, the researchers also investigated family S and found that the surviving members who had type 1 VWD were heterozygous with respect to the mutation in exon 18. There was a small boy with severe VWD whose family was related long ago to another family with VWD from Åland. He was homozygous for the mutation in exon 18 [30].

The end of the beginning

After the publication by Erik von Willebrand in 1926, it took some 30 years until it was clear that a new plasma factor responsible for the hemostatic impairment in VWD had been detected. In that time, a factor concentrate had been produced that was effective in the replacement of VWF: fraction I-0 (or, later, AHF-Kabi). Studies using this concentrate and concentrates purified from different types of bleeding disorders, helped scientists to find and prove the presence of the VWF. This was the end of the beginning.

In 1971, VWF was first detected immunologically and named "FVIII-related antigen" [31]. Since 1985, the VWF has been cloned [32–35], the primary amino-acid sequence has been determined [36], and the complex molecular structure and multiple functions are becoming understood in detail. The metalloprotease ADAMTS13 that cleaves VWF was discovered in 2001 [2]. VWD is no longer a uniform disease [37]. The treatment armamentarium has been developed and includes prophylactic treatment with concentrates in type 3 VWD. It includes desmopressin for most milder cases, new concentrates [38,39], and we are now anticipating development of recombinant VWF for therapeutic use.

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