Response to desmopressin in patients with mild hemophilia A caused by the *F8* c.1910A>G, p.Asn637Ser mutation

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Therapy for mild hemophilia A consists of desmopressin acetate (1-deamino[8-D-arginine]-vasopressin; desmopressin; DDAVP), which increases factor VIII and von Willebrand factor antigen (VWF:Ag) levels two-fold to six-fold through endogenous release from endothelial cells [1]. The effect of desmopressin varies between patients, and has been suggested to depend on the hemophilia-causing mutation [2–4].

Mild hemophilia is generally caused by *F8* missense mutations. One of these mutations is c.1910A>G, p.Asn637Ser. In our hemophilia treatment center (the Van Creveldkliniek, University Medical Center Utrecht, The Netherlands), a large cohort of patients with this particular mutation is known. To investigate the variability in the increase in FVIII levels after desmopressin infusion in this cohort of patients, a retrospective single-center study was performed.

All male patients with the *F8* c.1910A>G, p.Asn637Ser mutation treated at the Van Creveldkliniek at any time during the past 20 years were included. Baseline characteristics, including residual FVIII level, VWF:Ag level, and blood group, were collected. Age at the time of desmopressin testing and FVIII levels after 30–60 min of intravenous desmopressin infusion were recorded. The dose of desmopressin was 0.3 μg kg^{-1}, which was infused over a period of 20–30 min.

The study was approved by the medical ethics review board of the University Medical Center Utrecht.

For statistical analysis, the mean lowest known FVIII level, baseline FVIII level at the start of desmopressin testing and mean lowest known VWF:Ag level were calculated. For six patients for whom baseline FVIII levels were not measured, the lowest known FVIII levels were imputed. The effects of VWF:Ag levels and blood group on baseline FVIII levels were assessed with linear regression analysis. Determinants with a *P*-value of < 0.15 in the univariate analysis were included in the multivariate analysis.

The mean FVIII level, mean absolute increase in FVIII level and mean proportional increase in FVIII level within 60 min of desmopressin infusion were calculated for our patients. When FVIII levels were measured more than once between 30 and 60 min after the end of desmopressin infusion, the highest level was used for analysis. The effects of age, baseline FVIII level, VWF:Ag level and blood group on the absolute increase in FVIII levels within 60 min after desmopressin infusion were assessed with univariate and multivariate linear regression analysis.

P-values of < 0.05 were considered to be statistically significant. Data were analyzed with IBM SPSS 20 statistical software (IBM Corporation, Armonk, NY, USA).

In 115 patients, mild hemophilia was caused by the *F8* c.1910A>G, p.Asn637Ser mutation. In 77 of these patients, a desmopressin test was performed, and 68 of these could be analyzed. The mean age at desmopressin testing was 21.7 years (range, 3–70 years). The mean lowest FVIII level was 0.19 IU dL^{-1}, but varied greatly, between 6 and 36 IU dL^{-1}. The mean VWF:Ag level was 101 IU dL^{-1} (range, 20–235 IU dL^{-1}). Forty-nine per cent of patients had blood group O, 35% had blood group A, 11% had blood group AB, and 5% had blood group B. We found a strong association between VWF:Ag and FVIII levels (β = 0.06, *P* = 0.003). There was no effect of blood group (O vs. non-O) on FVIII levels (*P* = 0.64). No association was found between age and baseline FVIII level at the time of desmopressin testing (*P* = 0.58).

The mean FVIII level before desmopressin infusion was 20 IU dL^{-1} (range, 13–39 IU dL^{-1}). After desmopressin infusion, the mean absolute increase in FVIII levels was 79 IU dL^{-1} (range, 36–171 IU dL^{-1}; interquartile range [IQR] 57–98), resulting in a mean FVIII peak level of 102 IU dL^{-1} (range, 53–196 IU dL^{-1}; IQR 80–124). The relative FVIII increase after desmopressin infusion varied

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Table 1 Univariate and multivariate linear regression analyses to assess the effects of age, baseline FVIII level, lowest known von Willebrand factor antigen (VWF:Ag) level and blood group on the absolute increase in FVIII levels within 60 min of desmopressin infusion in 68 hemophilic patients with the *F8* c.1910A>G, p.Asn637Ser missense mutation

	Univariate		Multivariate	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value
Age (continuous)	0.41 (0.04–0.77)	0.031	0.43 (0.07–0.79)	0.020
Baseline FVIII level (continuous)	0.94 (− 0.07–1.95)	0.068	1.02 (0.04–2.00)	0.041
Lowest VWF:Ag level (continuous)	0.08 (− 0.12–0.28)	0.41	–	
Blood group (O vs. non-O)	− 10.0 (− 24.4–4.38)	0.17	–	

CI, confidence interval.

from 209% to 892% (IQR 356–556) within 60 min after the end of infusion. A higher baseline FVIII level was independently associated with a higher absolute increase in FVIII level after desmopressin infusion ($P = 0.04$), as was higher age ($P = 0.02$), whereas no effect of the lowest VWF:Ag level ($P = 0.41$) or blood group ($P = 0.17$) on outcome was seen (Table 1).

Our results suggest increases in FVIII levels after desmopressin administration of 10 IU dL^{-1} for every 10 IU dL^{-1} increase in baseline FVIII levels, and of 4 IU dL^{-1} for every 10-year increase in age.

In the literature, it has been shown that desmopressin responses depend on the causative FVIII mutation. In a recent publication, the highest response rates for four known causative FVIII mutations was found for the *F8* c.1910A>G, Asn637Ser mutation [4]. In another study among 62 patients, genetic results predicted desmopressin effects, but the baseline FVIII level did not [5]. In our study, we found that, even within a cohort of patients with similar mutations, response rates differed considerably. One explanation might be variation in VWF:Ag levels, which may have a positive effect on FVIII half-life, resulting in a higher residual FVIII level. In a cohort study of 50 patients, positive associations between FVIII half-life, basal and peak VWF:Ag levels and patient age were found, FVIII half-life varying from 1.3 h to 11.8 h [2]. Unfortunately, in our study, baseline VWF:Ag levels at the time of desmopressin testing and increases in VWF:Ag levels after desmopressin infusion were not routinely measured. Although we found an association between the baseline lowest VWF:Ag level and the baseline FVIII level, no direct effect of the lowest VWF:Ag level on the increase in FVIII level after desmopressin infusion was seen. In this relatively small group of patients, subjects with blood group O had lower absolute increases in FVIII levels after desmopressin infusion. However, this was not significant. In their study, Stoof *et al.* [4] did not find an association between blood group and the effect of desmopressin. Absolute increases in FVIII levels were higher in patients with higher baseline FVIII levels, which suggests that it is not likely that the higher baseline FVIII levels were caused by physical or mental stress [6,7]. If this had been the case, lower increases in FVIII levels after desmopressin infusion would have been seen. In a Canadian study of boys with hemophilia in whom the FVIII response initially failed after desmopressin treatment, a response was seen when they were rechallenged after a mean of 6.3 years [8]. Seary *et al.* [9] found, in their study of 74 boys with 38 different mutations causing moderate or mild hemophilia, that age and FVIII level were strong predictors of response to desmopressin. The stronger effect of desmopressin in older patients in our study may reflect the increase in baseline FVIII levels with age, as previously described by Miesbach *et al.* [10], but our model does not completely explain the variation in the effect of DDAVP. Larger groups of patients are needed to better evaluate the effects of VWF:Ag, blood group and age on baseline FVIII levels and in relation to test results.

However, from our study it may be concluded that the effect of desmopressin varies between patients with the same mutation. Furthermore, linear regression analysis revealed that desmopressin responses improve with age: for every 10-year increase in age, the maximum FVIII response increased by 4 IU dL^{-1}. Especially in patients with initial low/moderate response, it seems reasonable to retest the desmopressin response every 10 years.

Addendum

E. P. Mauser-Bunschoten: designed the study, collected data, and wrote the manuscript. D. E. Fransen van de Putte: designed the study and critically reviewed the paper. J. K. Ploos van Amstel: collected data and critically reviewed the paper. M. Spoor: designed the study, collected data, and critically reviewed the paper. R. E. G. Schutgens: designed the study and critically reviewed the paper.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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The factor V light chain mediates the binding and endocytosis of plasma-derived factor V by megakaryocytes

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Plasma- and platelet-derived factor (F) Va are essential for thrombin generation catalyzed by Prothrombinase assembled on the activated platelet surface [1]. While both the plasma- and platelet-derived molecules can form a functional complex, several studies demonstrate that the two cofactor pools are physically and functionally distinct [2–7]. Despite these differences, the entire platelet-derived pool of the procofactor, FV, is endocytosed from the plasma by megakaryocytes [4,8] via a two-receptor system consisting of a specific, unknown FV receptor and low density lipoprotein (LDL) receptor-related protein-1 (LRP-1) [9], an endocytic receptor belonging to a superfamily of proteins related to the LDL receptor [10]. Flow cytometric analyses and fluorescence microscopy indicate that all megakaryocytes express LRP-1 [9,11]. However, cells that endocytose FV must express both LRP-1 and an unidentified, specific FV receptor. FV binds to the specific FV receptor in a Ca^{2+}-independent manner [9]. This binding interaction facilitates binding of another FV molecule to LRP-1, which mediates endocytosis through Ca^{2+}- [9] and clathrin-dependent [8] mechanisms. Endocytosed FV is modified intracellularly to form the unique platelet-derived cofactor molecule [2–7] and trafficked to α-granules. The platelet-derived cofactor molecule plays the predominant hemostatic role at sites of vascular injury [12]; therefore, elucidation of the mechanisms regulating its endocytosis, modification and storage in α-granules will provide insight into the events leading to formation of a fibrin clot.

The goal of these studies was to identify the specific region(s) of FV involved in its interactions with the membrane receptors that mediate its endocytosis. Plasma FV is converted to FVa via cleavage by thrombin at Arg^{709}, Arg^{1018} and Arg^{1545}, to release the large central B domain and form the active cofactor [1]. FVa consists of a heavy chain (residues 1–709) and light chain (residues 1546–2196) non-covalently linked with Ca^{2+} (Fig. 1A). Endocytosis assays were performed using iodine125-labeled FV (^{125}I-FV), which was prepared and characterized as

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