Determinants of plasma levels of von Willebrand factor and coagulation factor VIII

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Chapter 5

Beta 2 Adrenergic Receptor Polymorphisms: Association with Factor VIII and von Willebrand Factor Levels and the Risk of Venous Thrombosis

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Several studies have shown that elevated plasma levels of coagulation factor VIII (FVIII) are a risk factor of venous thrombosis. This risk remained after correction for the main determinants of FVIII levels, blood group and von Willebrand factor (VWF[1-3]. FVIII activity levels (FVIII:C) ≥ 150 IU/dL increase the risk of a first venous thrombosis fivefold when compared to levels below 100 IU/dL. The prevalence of FVIII:C ≥ 150 IU/dL among thrombosis patients is 25%. Since these levels are found in 10 % of the population, the contribution of elevated FVIII levels to all thrombotic events in the population is considerable[2]. Several lines of evidence support the idea that high FVIII levels are indeed causative to thrombosis and not a consequence of the thrombotic event, such as a dose-dependent relationship with risk[2], persistence of elevated levels over time[3] and familial clustering[4]. The latter, familial clustering, supports the hypothesis that FVIII levels are, at least in part, determined genetically. Because no variations have been found in the FVIII and VWF genes that are associated with thrombosis[1,5], it is likely that genes encoding proteins regulating plasma levels of FVIII and VWF are involved.

A candidate regulator of FVIII levels is the β2 adrenergic receptor (β2AR). It is well known that adrenaline infusion causes a significant rise in FVIII levels. This effect can be blunted by prior administration of a β-blocker. Hoppener *et al.*[6] showed that in patients with venous thromboembolism and FVIII levels > 175 IU/dL, FVIII:C levels could be effectively lowered by treatment with propranolol. FVIII:C returned to its initial elevated levels within two months after discontinuation of treatment. However, Schönauer *et al.*[7] reported that in patients with venous thromboembolism and FVIII levels > 170 IU/dL, propranolol administration could not lower FVIII levels significantly. We approached this issue from a different angle by determining the possible association of single nucleotide polymorphisms (SNP) in the β2AR gene with FVIII and VWF levels and thrombotic risk.

We studied three coding β2AR SNPs, that have previously been implicated in clinically relevant effects[8-10], in a large population based case-control study, the Leiden Trombophilia Study (LETS). The LETS consists of 474 consecutive

patients and 474 controls. All the patients were referred for anti-coagulant treatment after a first objectively confirmed episode of deep vein thrombosis. Patients with underlying malignancies were excluded. The controls were matched for sex and age. DNA samples are available of 469 cases and 470 controls. FVIII:C was measured in the plasma of all these participants by a one-stage clotting assay. VWF antigen and FVIII antigen (FVIII:Ag) were measured by ELISA in the plasma of 301 patients and 301 controls. The design of this study has previously been described in more detail[11]. Using polymerase chain reaction – restriction fragment length polymorphism analyses the three β2AR SNPs, Arg16Gly, Glu27Gln and Thr164Ile, were determined.

The distributions of genotypes for the three studied SNPs were in Hardy-Weinberg equilibrium in the controls. The allele-frequencies in the controls were consistent with those found in previous studies[12,13]. No differences in allelic distributions were observed between the cases and controls (Table 1). There was a protective effect on the occurrence of venous thrombosis for the rare allele of Thr164Ile, however this effect was weak with wide confidence limits around the odds ratio. Based on the frequencies of the SNPs, four different haplotypes were identified in our study-population (Table 1) with the help of special software, Arlequin[14]. These haplotypes corresponded to those reported in previous publications[10,12]. No significant differences were found in the distribution of the haplotypes between cases and controls. The trend we observed for the rare allele of Thr164Ile was recovered in haplotype 4. In addition, homozygotes for haplotype 2 showed an odds ratio of 1.67 (95% confidence interval: 0.75-3.74). Combining this with the results for the individual SNPs, it is unlikely that the gene for the β2AR contains a common polymorphism that is associated with the risk of venous thrombosis.

No association was found between FVIII:C, FVIII:Ag and VWF levels and the different genotypes and haplotypes in the control group. Because ABO blood group strongly influences levels of FVIII and VWF, we also analysed the data stratified by blood group O and non-O in healthy individuals. Again, no effect was observed.

Table 1. Distribution of β2AR genotypes and haplotypes in patients with venous thrombosis and controls.

SNP	Genotype	Case	Control	OR*	CI95
16	Gly/Gly	183 (39%)	180 (38%)	1†	
	Gly/Arg	219 (47%)	213 (45%)	1.01	0.77-1.34
	Arg/Arg	66 (14%)	77 (16%)	0.84	0.57-1.24
	Total	**468**	**470**		
27	Glu/Glu	96 (20%)	100 (21%)	1†	
	Glu/Gln	225 (48%)	216 (46%)	1.09	0.78-1.52
	Gln/Gln	148 (32%)	154 (33%)	1.00	0.70-1.43
	Total	**469**	**470**		
164	Thr/Thr	458 (98%)	453 (96%)	1†	
	Thr/Ile	10 (2%)	17 (4%)	0.58	0.26-1.28
	Total	**468**	**470**		

Haplotype	Haplotype	Case	Control	OR*	CI95
1	HxHx‡	148 (32%)	154 (33%)	1†	
	H1Hx	225 (48%)	216 (46%)	1.08	0.81-1.45
	H1H1	96 (20%)	100 (21%)	1.00	0.70-1.43
	Total	**469**	**470**		
2					
16Gly-27Gln-164Thr	HxHx‡	325 (69%)	340 (72%)	1†	
	H2Hx	127 (27%)	120 (26%)	1.11	0.83-1.48
	H2H2	16 (3%)	10 (2%)	1.67	0.75-3.74
	Total	**468**	**470**		
3					
16Arg-27Gln-164Thr	HxHx‡	183 (39%)	180 (38%)	1†	
	H3Hx	219 (47%)	213 (45%)	1.01	0.77-1.34
	H3H3	66 (14%)	77 (16%)	0.84	0.57-1.24
	Total	**468**	**470**		
4					
16Gly-27Gln-164Ile	HxHx‡	458 (98%)	453 (96%)	1†	
	H4Hx	10 (2%)	17 (4%)	0.58	0.26-1.28
	Total	**468**	**470**		

*Odds Ratio

†Reference Category

"x" indicates any haplotype other than the haplotype studied

O'Donnell *et al* [13] recently reported a significant effect on FVIII:C levels for SNP Glu27Gln in healthy blood group O individuals. They found, that in a group of 59 healthy blood group O individuals, those with genotype Gln/Gln had lower levels of FVIII:C than those with genotype Glu/Glu. However, this effect was very small and only marginally significant. In our study, we could not confirm these results in a group of 201 healthy blood group O individuals. FVIII:C levels within this group were for Gln/Gln: 111.04 IU/dL (104.14-117.95), for Gln/Glu: 109.01 IU/dL (102.18-115.84) and for Glu/Glu: 109.54 IU/dL (99.50-119.57)).

In conclusion, genotypes and haplotypes of the β2AR have no influence on either the occurrence of venous thrombosis or plasma-levels of FVIII and VWF. It remains to be determined what genetic variations are responsible for the familial clustering of elevated levels of FVIII.

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