Aquaporin 2 gene variations, risk of venous thrombosis and plasma levels of von Willebrand factor and factor VIII

Elevated plasma levels of coagulation factor VIII (FVIII) are a risk factor for venous thrombosis,[1] but determinants of these elevated levels remain largely unknown. We investigated associations of variations in the aquaporin 2 (AQP2) gene with the risk of venous thrombosis and with von Willebrand factor (VWF) and FVIII plasma levels. AQP2 is a water channel that is expressed in epithelial cells of the principal collecting ducts in the kidney.[2] AQP2 transports water from the pre-urine in the collecting ducts into epithelial cells. Subsequently, water is transported back into circulation by AQP3 and AQP4.[2] Expression and localization of AQP2 is regulated by the arginine vasopressin 2 receptor (V2R).[2,3] V2R is expressed on the basolateral membrane of principal collecting duct epithelial cells, but also in vascular endothelial cells, where stimulation of V2R leads to secretion of VWF.

Administration of the synthetic 1-desamino-8-d-arginine vasopressin results in a sharp rise in plasma levels of VWF and FVIII in humans.[3] Decreased activity of the AQP2 water channel could lead to a decrease in blood volume and, therefore, to an increase in activity of the renin angiotensin system (RAS) which regulates blood pressure and vascular tone.[4] RAS stimulates expression of both plasminogen activator inhibitor type-1 (PAI-1) and tissue factor (TF), and inactivates bradykinin which normally stimulates the expression of tissue-type plasminogen activator (t-PA).[5,6]

Finally, via angiotensin II, RAS stimulates synthesis and secretion of vasopressin by the hypothalamus-pituitary axis,[7] which leads to secretion of VWF. It has been shown in rodents that angiotensin II can also directly upregulate the expression of V2R itself.[8] We hypothesized that functional variations in the *AQP2* gene can influence the risk of venous thrombosis and secretion of VWF in the general population.

To test this hypothesis, we studied *AQP2* gene variations in the Leiden Thrombophilia Study (LETS), which consists of 474 consecutive patients and 474 healthy controls, matched for sex and age. All patients were referred for anti-coagulant treatment after a first objectively confirmed episode of deep vein thrombosis. Patients with underlying malignancies were excluded. The design of this study has been previously described in detail.[9] FVIII antigen (FVIII:Ag), VWF antigen (VWF:Ag) and VWF propeptide were measured by ELISA in the plasma of the first 301 patients and 301 controls included in the study.[1,10] Systolic and diastolic blood pressure were measured in all participants on the upper-arm in a resting position. To identify variations in the *AQP2* gene, we resequenced its entire genomic region of 6.6 kb in 25 LETS participants, of which 20 have blood group O, with high VWF:Ag and FVIII:Ag, and who were under 50 years of age. Eighteen single nucleotide polymorphisms (SNPs) were identified in 50 AQP2 alleles (Table 1). Recombination between SNPs was too large to form reliable haplotypes, but they were linked in five haplotype-like clusters. The entire LETS population was successfully genotyped for sixteen of the eighteen SNPs. Two SNPs failed to genotype, but sequencing data place these SNPs, AQP2-16 and AQP2-18, in clusters 5 and 2 respectively. All genotype distributions were in Hardy-Weinberg equilibrium in healthy LETS controls. For all determined AQP2 SNPs, odds ratios (OR) were calculated with 95% confidence intervals (CI95) according to Woolf[11] or Mehta[12] in case of a zero in the equation.

Table 1. ***AQP2*** **gene variations in the LETS.**

Cluster	*SNP*	*rs-number*	*Nucleotide*	*Location*
1	AQP2-1	rs3759125	a > c	upstream
	AQP2-5	rs461872	g > a	intron 1
2	AQP2-2	rs3759126	a > g	upstream
	AQP2NF10	rs3741559	g > a	intron 1
	AQP2-18[a]	rs371777	a > c	intron 3
	AQP2-19	rs403201	c > g	intron 3
3	AQP2-3	rs3782319	g > a	intron 1
	AQP2-10	rs11169225	t > a	intron 1
	AQP2-14	rs12372344	t > c	intron 2
	AQP2NF19	rs7299924	a > g	intron 3
4	AQP2-4	rs3782320	g > a	intron 1
	AQP2-7	rs3782322	g > a	intron 1
	AQP2-8	pending	g > a	intron 1
5	AQP2-6	rs467199	a > g	intron 1
	AQP2-13	rs426496	t > c	exon 2 (silent)
	AQP2-15	rs402813	c > t	intron 3
	AQP2-16*	rs439779	c > t	intron 3
	AQP2-20	rs457487	c > a	3'-UTR

[a]*SNPs that failed to genotype in the LETS.*

Effects on thrombosis risk were observed for SNPs in three of the five clusters, namely clusters 2, 4 and 5 (*Online Supplementary Table 2*). In cluster 2, risk of venous thrombosis was increased 2.2 and 2.5-fold in homozygous carriers of the minor alleles of AQP2-2 and AQP2-19 respectively. In cluster 4, hetero- and homozygous carriers of the minor alleles of all three SNPs showed ORs of approximately 1.5. Finally, in cluster 5, two-fold increases in risk were observed for homozygous carriers of the minor alleles of all four SNPs in this cluster.

Because we hypothesized that *AQP2* gene variations influence thrombosis risk and VWF and FVIII levels via RAS activation, we looked for associations with arterial blood pressure in the control subjects of the LETS. Linear regression analyses showed weak associations of SNPs in clusters 2, 4 and 5 with both systolic and diastolic blood pressure in healthy LETS controls (*Online Supplementary Table 2*).

No associations of plasma levels of VWF propeptide, VWF or FVIII with any of the AQP2 SNPs were observed in healthy LETS controls.

Because the SNPs are in linkage disequilibrium within the clusters, the effects described above may be caused by only one of the SNPs per cluster. Therefore, we used unconditional logistic regression modeling in an attempt to identify the SNPs in each of the clusters that are most likely to be responsible for the observed effects. In cluster 2, effects on risk disappeared for AQP2-2 and AQP2NF10, but appeared to remain for AQP2-19 after adjustment for the other SNPs in the cluster.

The adjusted ORs were 1.5 (0.8 to 2.6) and 2.3 (0.6 to 9.2) for hetero- and homozygous carriers of AQP2-

19 respectively. Associations with blood pressure also remained mainly for AQP2-19. Adjusted regression coefficients were 5.00 (-1.75 to 11.75) and 1.71 (-1.89 to 5.31) for AQP2-19 on systolic and diastolic blood pressure respectively. This indicates that AQP2-19 is probably responsible for the effects on thrombosis risk and blood pressure observed in cluster 2.

In cluster 4, effects on thrombosis risk disappeared for each of the three SNPs after adjustment for the other two SNPs in this cluster. Associations with blood pressure remained for AQP2-4. The adjusted regression coefficients for AQP2-4 were -12.72 (-35.66 to 10.21) and -7.40 (-19.60 to 4.81) for systolic and diastolic blood pressure respectively.

In cluster 5, effects on risk remained strongest for AQP2-20 after adjusting for the other SNPs in the cluster. The adjusted ORs were 1.3 (0.9 to 1.9) and 1.9 (0.9 to 4.1) for hetero- and homozygous carriers of AQP2-20 respectively. Associations with blood pressure remained for SNP AQP2-15, whereas the associations disappeared for AQP2-6, AQP2-13 and AQP2-20. For AQP2-15, the adjusted regression coefficients were 7.21 (-6.09 to 20.51) and 10.46 (3.39 to 17.53) on systolic and diastolic blood pressure respectively. It remains unclear which of the SNPs in cluster 5 is responsible for the observed effects.

In interpreting these results, it should be noted that AQP2-16 and AQP2-18, which failed to genotype, are in clusters 5 and 2 respectively, and that they may be the actual effectors in these clusters.

In conclusion, we observed associations between several AQP2 SNPs and the risk of venous thrombosis. The same SNPs also showed weak associations with arterial blood pressure, but not with plasma levels of VWF propeptide, VWF or FVIII. It is possible that the observed increases in thrombosis risk are mediated through RAS.

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