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A. Y. NOSSENT, L. DAI, F. R. ROSENDAAL*, H. L. VOS and J. C. J. EIKENBOOM
*Department of Haematology, Haemostasis and Thrombosis Research Center; *Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands*

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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^{-1} increase the risk of a first venous thrombosis fivefold when compared to levels > 100 IU dL^{-1}. The prevalence of FVIII:C ≥ 150 IU dL^{-1} 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], the persistence of elevated levels over time [3] and familial clustering [4]. The last of these, 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 epinephrine 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^{-1}, FVIII:C levels could be effectively lowered by treatment with propranolol. FVIII:C returned to its initial elevated levels within 2 months after discontinuation of treatment. However, Schönauer *et al.* [7] reported that in patients with venous thromboembolism and FVIII levels > 170 IU dL^{-1}, 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 Thrombophilia Study (LETS). The LETS consists of 474 consecutive patients and 474 controls. All the patients were referred for anticoagulant 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 for 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 enzyme-linked immunosorbent assay 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 SNPs studied 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.

Correspondence: A. Yaël Nossent, Department of Haematology, Haemostasis and Thrombosis Research Center, Leiden University Medical Center, C2-R, PO Box 9600, 2300 RC Leiden, the Netherlands. Tel.: +31 71 5266689/ +31 71 5261648; e-mail: a.y.nossent@lumc.nl

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Table 1 Distribution of beta 2 adrenergic receptor genotypes and haplotypes in patients with venous thrombosis and controls

SNP	Genotype	Case	Control	OR*	CI 95%	Haplotype	Haplotype	Case	Control	OR*	CI 95%
						1					
						16Gly-27Glu-164Thr	HxHx‡	148 (32%)	154 (33%)	1†	
16	Gly/Gly	183 (39%)	180 (38%)	1†			H1Hx	225 (48%)	216 (46%)	1.08	0.81–1.45
	Gly/Arg	219 (47%)	213 (45%)	1.01	0.77–1.34		H1H1	96 (20%)	100 (21%)	1.00	0.70–1.43
	Arg/Arg	66 (14%)	77 (16%)	0.84	0.57–1.24		Total	469	470		
	Total	468	470			2					
						16Gly-27Gln-164Thr	HxHx‡	325 (69%)	340 (72%)	1†	
27	Glu/Glu	96 (20%)	100 (21%)	1†			H2Hx	127 (27%)	120 (26%)	1.11	0.83–1.48
	Glu/Gln	225 (48%)	216 (46%)	1.09	0.78–1.52		H2H2	16 (3%)	10 (2%)	1.67	0.75–3.74
	Gln/Gln	148 (32%)	154 (33%)	1.00	0.70–1.43		Total	468	470		
	Total	469	470			3					
						16Arg-27Gln-164Thr	HxHx‡	183 (39%)	180 (38%)	1†	
164	Thr/Thr	458 (98%)	453 (96%)	1†			H3Hx	219 (47%)	213 (45%)	1.01	0.77–1.34
	Thr/Ile	10 (2%)	17 (4%)	0.58	0.26–1.28		H3H3	66 (14%)	77 (16%)	0.84	0.57–1.24
	Total	468	470				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		

*OR, odds ratio; †Reference Category; ‡'x' indicates any haplotype other than the haplotype studied.

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 analyzed the data stratified by blood group O and non-O in healthy individuals. Again, no effect was observed.

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^{-1} (104.14–117.95), for Gln/Glu: 109.01 IU dL^{-1} (102.18–115.84) and for Glu/Glu: 109.54 IU dL^{-1} (99.50–119.57).

In conclusion, genotypes and haplotypes of the β2AR have no influence on either the occurrence of venous thrombosis or the 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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Congenital afibrinogenemia: report of three cases

M. ANWAR, H. IQBAL, M. GUL, N. SAEED and M. AYYUB
Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi-Pakistan

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Fibrinogen is a 340 kDa major glycoprotein found in plasma that is central to the blood clotting process [1]. It is synthesized primarily in hepatocytes and secreted as a hexamer composed of three pairs of polypeptide chains, Aα, Bβ and γ [2]. Three chains are encoded by different genes (*FGA*, *FGB* and *FGG*, respectively) clustered in a region of approximately 50 kilobases on chromosome 4q28 [3]. During blood coagulation, thrombin converts fibrinogen to fibrin monomers, which associate into staggered overlapping two-stranded fibrils. These then associate laterally to form fibrin bundles [4]. In the final step, these bundles cross-link with each other by formation of covalent intermolecular isopeptide bonds to form a three-dimensional network. This reaction is catalyzed by factor XIIIa, which links the α and γ chains of two adjacent fibrin molecules in the presence of Ca^{2+} [5,6]. Cell-derived tissue transglutaminase can also cross-link fibrin bundles, but it does so by cross-linking two α chains [7].

In addition to end-stage coagulation reactions, fibrinogen also participates in early stages of hemostasis by promoting platelet aggregation [1]. Apart from its role in hemostasis, the fibrin network formed provides a temporary matrix for cell invasion during the subsequent healing process [8,9]. Thus, the clinical manifestations of qualitative or quantitative deficiency of fibrinogen manifest both with hemostasis and healing defects.

Congenital afibrinogenemia (MIM 202400) is a rare autosomal recessive disorder described for the first time in 1920 [10] and is characterized by unmeasurable clottable and extremely low antigen levels in plasma [11]. Diagnosis is often made at birth because of bleeding from the umbilical stump. Some 150 families with about 250 cases of congenital afibrinogenemia have been reported so far [12,13]. We report here three more cases of congenital afibrinogenemia from three different families.

Correspondence: Masood Anwar, Armed Forces Institute of Pathology, Rawalpindi, Pakistan.
Tel.: (51) 561 30077; fax: (51) 9271247; e-mail: afipcomdt@hotmail.com

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One female and two male infants, aged 2, 3 and 5 months, respectively, presented with uncontrolled bleeding from the umbilical stump and hematoma formation at intramuscular (vitamin K) injection sites. They were all products of first-cousin marriages and had been born at full term by normal, vaginal delivery. The number of siblings varied from one to three. There was no family history of bleeding tendency in any of the families.

On examination, no abnormality was detected except for oozing from the umbilical stump and hematomas at injection sites. Blood counts and liver function tests were normal in all infants. However, prothrombin time (PT), partial thromboplastin time with kaolin (PTTK) and thrombin time (TT) were infinitely prolonged in all three patients but were normal in their parents. Fibrin degradation products were not detected in any of the patients. Fibrinogen was undetectable in plasma by the Clauss method. There was no band in the normal fibrinogen position on plasma protein electrophoresis on cellulose acetate membrane. Immunoreactive fibrinogen could not be determined because of a lack of facilities. Details are shown in Table 1. All three patients responded to fibrinogen replacement therapy with cryoprecipitate and were prescribed life-long replacement therapy with fibrinogen concentrates.

Congenital fibrinogen disorders include afibrinogenemia, hypofibrinogenemia and dysfibrinogenemia, which are characterized by the complete absence or extremely low levels of plasma fibrinogen, by reduced amounts of plasma fibrinogen, or by the presence of dysfunctional fibrinogen molecules, respectively [11]. Of these, congenital afibrinogenemia is the rarest and has a high rate in consanguineous families [11], as was the case for the parents of the infants described above. It most commonly presents at birth with abnormal bleeding from the umbilical stump [11], as seen in the three patients described in this report. Other clinical manifestations include bruises, hemarthrosis and gastrointestinal bleeding. The major cause of death is intracranial hemorrhage during infancy or childhood [14]. Only about 250 patients have been reported so far, mostly from Europe and North America [11,15]. From the Indo-Pakistan subcontinent, only three cases have been described previously, two families from Pakistan and one from India [16,17]. We are reporting three more families with one patient in each from Pakistan.