Venous and arterial thrombosis : associations and risk factors

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Risk factors for idiopathic retinal vein occlusion

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Abstract

Risk factors for idiopathic retinal vein occlusion (RVO) are not well defined. We investigated the role of thrombophilia, fibrinolysis and platelet receptors in idiopathic RVO in a case-control study. Cases were 101 patients (55% men) with angiographically confirmed RVO, recruited from four Dutch academic ophthalmic centers. None of them had ocular disease or systemic risk factor predisposing to RVO. Controls (n=94, 52% men) were either brought by cases or selected from hospital contact lens services.

We compared the mean level of endogenous thrombin potential, antithrombin, protein C and S, Factor VIII and homocysteine as well as clot lysis time and thrombin activatable fibrinolysis inhibitor (TAFI) between the two groups. We calculated the odds ratio (OR) of RVO for antithrombin, protein C and S deficiency, hyperhomocysteinemia, factor V Leiden, prothrombin G20210A, lupus anticoagulant, anticardiolipin antibodies, *TAFI* and protein C receptor (*PROCR*) haplotypes (H) and platelet receptor polymorphisms. Increased ORs were observed for *PROCR* H1 (OR: 1.5, 95%CI: 0.8-2.8) and H4 (1.8, 0.6-5.5), elevated TAFI activity (1.5, 0.7-3.3), *TAFI* H1 (1.4, 0.8-2.7) and platelet receptor polymorphisms (rs5918; 1.8, 0.9-3.4 and in a recessive model rs1062535; 1.8, 0.8-3.9 and rs1126643; 1.9, 0.9-4.1) but neither increase reached significance.

None of the established thrombophilia and clot lysis time showed an association with the risk of idiopathic RVO. These results should be interpreted with caution due to limited power to detect small effects.

Introduction

Retinal vein occlusion (RVO) has a prevalence of 5.2 per 1000 individuals in the general population and constitutes the second most common retinal vascular disease after diabetic retinopathy [1]. The four year recurrence rate in the opposite eye is estimated to be 7% [2]. Despite the importance of RVO, the underlying etiology is not fully elucidated.

According to Virchow's triad, a thrombus can be formed following a change in one or more of three elements of vessel wall integrity, hemodynamics or blood coagulability. Risk factors that are correlated with arterial thrombosis, such as hypertension, hyperlipidemia and diabetes, and which are known to affect vessel wall integrity, predispose to RVO [3-5]. This is in line with studies reporting an increased risk of other manifestations of atherosclerosis such as stroke and myocardial infarction in patients with previous RVO [6-8]. The role of thrombophilic defects in the development of RVO is controversial except for elevated levels of homocysteine [9;10], although an association is not established for the C677T dimorphism in the gene of methylentetrahydrofolate reductase (MTHFR) [9;11]. Two meta-analyses showed that the Factor V Leiden (FVL) mutation increases the risk of RVO by about 50 to 60% [9;12], whereas other prothrombotic defects, i.e. prothrombin G20210A and deficiencies of antithrombin, protein C and S are not associated with RVO[9] . The role of lupus anticoagulant and anticardiolipin antibodies in RVO is uncertain [9;10;13]. Elevated TAFI levels are a risk factor for venous thrombosis [14;15] but its role in idiopathic RVO has not been investigated. Likewise, the relation between fibrinolysis and RVO has not received much attention [10;16;17]. Since RVO occurs at arterio-venous crossings where the blood flow is locally turbulent, changes in the platelet reactivity due to polymorphisms in platelet receptors may be important. The few available studies evaluating the role of platelets in RVO are of limited size making this association at best controversial [18-22].FVL (or activated protein C resistance) was found to be more prevalent in younger RVO patients [23;24], which has led to the hypothesis that risk factors other than hypertension and hyperlipidemia are of importance in RVO in young patients. We aimed to investigate the role of thrombophilia, fibrinolysis and platelet function in RVO by comparing their prevalence in RVO patients without known risk factors and in a control group.

Materials and Methods

Study population and data collection

Cases were RVO patients, selected from fluorescein angiography databases of four academic ophthalmic centers in the Netherlands: Academic Medical Center Amsterdam, University Hospital Maastricht, University Hospital Groningen and Eye Hospital in Rotterdam, for whom the diagnosis was established between January 1st 1970 and May 1st 2000. We recruited these patients only if they did not have ocular diseases or risk factors predisposing to RVO, namely open-angle glaucoma, optic nerve head drusen, hypermetropia, ocular inflammation or periphlebitis, hypertension, diabetes, hyperlipidemia, hematological disorders, renal insufficiency, hepatic disease and vasculitis. Controls were brought by the cases but if cases were unable to bring a volunteer, an appropriate healthy control was chosen from the hospital contact lens service that also services the same community. Controls were matched for age (± 3 years) to cases and were not genetically related to each other or to the cases. All participants provided written informed consent and the study was approved by the Medical Ethics Committee of Academic Medical Center Amsterdam, Medical Ethics Committee of University Hospital Maastricht, Medical Ethics Committee of University Hospital Groningen and Medical Ethics Committee of the Rotterdam Eye Hospital.

Patients and controls were subjected to a complete ocular examination including visual acuity measurement, biomicroscopy, intraocular pressure (IOP) measurement and a dilated fundus examination allowing confirmation of the RVO events in the cases, and excluding ocular abnormalities in the controls. Citrated blood was collected and centrifuged within 25 minutes after withdrawal at 4°C (3000 RPM for 10 minutes) and stored at -80°C until assayed.

Endogenous thrombin potential and established thrombophilia

The Endogenous Thrombin Potential (ETP) is derived from a global coagulation assay that measures tissue factor triggered thrombin generation in platelet-poor plasma and provides an estimate of the clotting potential. Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin®, Siemens, Marburg, Germany), 4 μM phospholipids, and 417 μM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf,

Switzerland). The ETP was determined with a calibrated automated thrombograph using a microtiter plate reading fluorometer (Fluoroskan Ascent, ThermoLab systems, Helsinki, Finland) and thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands).

Antithrombin activity was determined with the Berichrom Antithrombin assay (Siemens Healthcare Diagnostics, Marburg, Germany) on a Behring Coagulation System (Siemens Healthcare Diagnostics). Protein C activity (normal values ≥ 70%) was assayed with the Coamatic protein C kit (Chromogenix, Mölndal, Sweden). Total protein S antigen (normal values ≥ 65%) was determined by ELISA (DAKO, Glostrup, Denmark). Free protein S (normal values ≥ 26%) was measured in the supernatant after precipitating the C4b-bound fraction of protein S with polyethylene glycol.

DNA was extracted from peripheral leukocytes. FVL and prothrombin 20210A polymorphisms were detected by routine PCR and restriction enzyme techniques. Factor (F) VIII levels were measured in a one-stage clotting assay with FVIII deficient plasma. The plasma levels were expressed as percentages of pooled normal plasma obtained from more than 150 hospital workers. Total homocysteine levels were measured using a high performance liquid chromatographic assay as reported previously [25]. Normal ranges for fasting and post-loading levels were 6-15 and 18-51 mmol/L for premenopausal women and were 6-19, 25-69, 8-18 and 25-54 mmol/L for postmenopausal women and men, respectively.

Two tests were performed to detect lupus anticoagulant: a diluted prothrombin time (dPT) using 200-fold diluted Innovin (Siemens Healthcare Diagnostics), and a diluted Russel's Viper Venom test (dRVVT, LAC Screen, Gradipore, North Ryde, Australia). dPT and dRVVT were considered prolonged when the ratio of patient plasma to normal plasma was >1.2. In case of a prolonged dRVV screening test, a mixing test with normal plasma (1:1) was performed, and the sample was considered positive when the clotting time of the dRVV confirmation test was more than 20% reduced compared to the dRVV screening test. A patient sample was considered positive for lupus anticoagulant with either a prolonged dRVVT or dPT.

The presence of anticardiolipin antibodies (ACA) IgG and IgM was tested by ELISA (IMTEC, Kordia, Leiden, The Netherlands). In case of anticoagulation therapy (n=2), protein C, S and lupus anticoagulant were not measured.

Using the Genome Variation Server (http://gvs.gs.washington.edu/GVS) common haplotypes were identified in the protein C receptor gene (*PROCR*) in the European Hapmap population. Three tagging single nucleotide polymorphisms (htSNPs; rs867186, rs2069952 and rs2069951) together tag four *PROCR* haplotypes. A htSNP is a polymorphism whose minor allele is specific to one haplotype (H). We used TaqMan SNP genotyping assays to determine the htSNPs. In this assay, fluorescent allele-specific oligonucleotide probes (Applied Biosystems, CA, USA) were used for PCR amplification and fluorescence endpoint reading for allelic discrimination was done on a ABI 7900 HT (Applied Biosystems).

Fibrinolysis parameters and TAFI polymorphisms

Lysis of a tissue-factor induced clot by exogenous tissue-type plasminogen activator was studied by monitoring changes in turbidity during clot formation and the subsequent lysis [26]. The Clot Lysis Time (CLT) was defined as the time from the midpoint of the clear to maximum turbid transition, representing clot formation, to the midpoint of the maximum turbid to clear transition, representing the clot lysis.

Thrombin-Activable Fibrinolysis Inhibitor (TAFI) activity was measured in plasma as described [27], and TAFI antigen was detected in plasma by ELISA as described before [28]. The measurements were done in duplicate. Plasma TAFI levels were expressed as percentage of pooled normal plasma.

We determined four major *TAFI* haplotypes by identifying three htSNPs (rs2146881, rs3742264 and rs1926447) in the European population and subsequently performing TaqMan SNP genotyping assays.

Platelet receptors' polymorphisms

Several SNPs were determined in *glycoprotein (GP) IB* (rs2243039 (Kozak polymorphism) and rs6065), *GPVI* (rs1613662), *GPIa* (rs1126643 and rs1062535), *GPIIB* (rs5911) and *GPIIIA* (rs5918) by TaqMan SNP genotyping assays or PCR and restriction enzyme technique *(*for *GPIa* rs1126643).

Statistical analysis

Student's t-test was used to evaluate the difference in the means of continuous variables between cases and controls. Odds ratios (OR) and 95% confidence

intervals (95%CI) were computed to assess the effect of discrete variables on RVO risk. Odds ratios were calculated to estimate RVO risk for the second and third tertiles of ETP, FVIII, CLT, TAFI activity and antigen level compared with the first tertile. The tertile stratification was done based on the levels in the controls. To investigate the effect of polymorphisms in platelet receptors and different haplotypes of *PROCR* and *TAFI* on the risk of RVO, we calculated odds ratios of RVO in heterozygous and homozygous subjects for the minor allele as compared to homozygous subjects for the common allele (reference group).

Results

Characteristics of the study population

We recruited 101 patients (56 men, 55.4%) with angiographically established RVO as cases and 94 (49 men, 52.1%) healthy subjects as controls. Mean age (±SD) was 48 (±11) and 46 (±10) in cases and controls respectively. The majority of the participants was younger than 60 years (84% of cases and 89% of controls) and of Caucasian origin (83% of cases and 88% of control).

Endogenous thrombin potential and established thrombophilia

Table 1 lists the results of ETP measurements and thrombophilia tests for both study groups. Mean ETP was 106% in both cases and controls and did not show an effect on the risk of RVO.

None of the cases or controls had antithrombin deficiency. Mean antithrombin, protein C activity and total protein S antigen level did not differ between cases and controls, but mean free protein S antigen level was higher in cases than in controls (mean difference: 6%, 95% CI: -10.4 to -2.5). Mildly decreased protein C levels, isolated low levels of free protein S and both total and free low protein S were observed in one case (protein C activity: 68%), two controls (free protein S: 20, 21%) and one control (total protein S antigen: 50% and free protein S: 24%), respectively. These results were based on single measurements. The prevalence of FVL, prothrombin G20210A, hyperhomocysteinemia, lupus anticoagulant and anticardiolipin antibodies as well as the mean FVIII antigen level were not different between cases and controls. Similarly, the risk of RVO was not increased in subjects with higher level of FVIII or homocysteine as compared to those having lower levels. Tertile classification of homocysteine was performed

separately for fasting and post-loading measurements and stratified for sex (data not shown) and showed no association.
In Table 2, the RVO risk associated with each *PROCR* haplotype is shown. Carriers of one or two alleles of H1 or H4 had an increased risk of RVO (OR: 1.5, 95% CI: 0.8 to 2.8 and 1.8, 0.6 to 5.5), although these estimates did not reach statistical significance.

Fibrinolysis parameters and TAFI polymorphisms
Table 3 describes CLT measurements and TAFI antigen and activity levels in cases and controls. Mean CLT was shorter in cases than in controls (mean difference: -7 %, 95% CI: -12.6 to -0.1). Since the distribution of CLT was slightly skewed, we also compared the medians and did not observe a difference between the two groups (71 (range: 40 to 127) in cases and 71 (45 to 150) in controls). No difference in the mean levels of TAFI antigen and activity between cases and controls was detected. However, subjects with TAFI activity in the highest tertile had a 1.5-fold (95% CI: 0.7 to 3.3) increased risk of developing RVO as compared to those in the lowest tertile.
Table 4 lists RVO risks associated with each *TAFI* haplotype. We were not able to assign a haplotype to 10 subjects (eight cases and two controls). Subjects carrying one or two H1 alleles had a slightly higher RVO risk (OR: 1.4, 95% CI: 0.8 to 2.7) than subjects without a H1 allele, although this was not statistically significant. H1 appeared to be associated with increased TAFI activity level.

Platelet receptor polymorphisms
Table 5 presents the association of six SNPs in four platelet receptor genes with the risk of RVO. Carriers of the rs5918 (in *GPIIIa* gene) minor allele appeared to have an increased risk of RVO in a dose-dependent manner (OR in heterozygotes: 1.7, 95% CI: 0.8 to 3.3, OR in homozygotes: 2.8, 0.5 to 15.9). Homozygotes for the minor alleles of the tested SNPs in *GPIa* (i.e. rs1062535 and rs1126643) showed a higher RVO risk (OR: 1.7, 95% CI: 0.7 to 4.1 and 1.8, 0.8 to 4.2 respectively). These SNPs were in strong linkage disequilibrium (r^2=0.82). In a recessive model (i.e. the risk in homozygotes for the minor allele compared with others), the odds ratios were 1.8 (95% CI: 0.8 to 3.9) for rs1062535 and 1.9 (95% CI: 0.9 to 4.1) for rs1126643.

Table 1. Endogenous thrombin potential and various established thrombophilic risk factors in RVO patients and controls

	Patients N= 101	Controls N= 94	Mean difference/ OR	95%CI
ETP, mean levels (%)	106	106	0	-5.9 to 6.3
<99 N (%)	26 (38)	28 (33)	1*	-
99-112 N (%)	18 (26)	29 (34)	0.7	0.3 to 1.5
>112 N (%)	25 (36)	28 (33)	1.0	0.5 to 2.1
Antithrombin, mean levels (%)	103	105	-2	-1.7 to 4.7
Antithrombin deficiency N	0	0	-	-
Protein C, mean levels (%)	109	106	3	-8.9 to 2.1
Protein C deficiency N (%)	1 (1)	0	-	-
Total protein S, mean levels (%)	103	102	1	-6.8 to 3.6
Free protein S, mean levels (%)	50	44	6	-10.4 to -2.5
Protein S deficiency N (%)	0	1 (1)	-	-
FVL N (%)	5 (5)	4 (4)	1.2	0.3 to 4.5
Prothrombin G20210A N (%)	2 (2)	0	-	-
FVIII, mean levels (%)	103	100	3	-9.9 to 5.3
<91 N (%)	27 (31)	32 (34)	1*	-
91-108 N (%)	34 (40)	31 (33)	1.3	0.6 to 2.6
>108 N (%)	25 (30)	31 (33)	0.5	0.5 to 1.9
Hyperhomocysteinemia N (%)	15 (16)	18 (19)	0.8	0.4 to 1.7
LAC N (%)	3 (3)	0	-	-
IgG ACA N (%)	1 (1)	1 (1)	0.9	0.1 to 15.1
IgM ACA N (%)	4 (4)	2 (2)	1.9	0.3 to 10.6
LAC or ACA N (%)	7 (7)	3 (3)	2.3	0.6 to 9.1

Abbreviations: endogenous thrombin potential (ETP), factor V Leiden (FVL), factor VIII (FVIII), lupus anticoagulant (LAC) and anticardiolipin antibody (ACA).
Missing measurements: 31 patients and 9 controls for ETP, 2 patients and 2 controls for FVL, 1 patient for prothrombin G20210A, 15 patients for FVIII, 6 patients for homocysteine, 5 patients and 1 control for protein C, 2 patients and 2 cases for total protein S, 23 patients for free protein S and 7 patients for antithrombin. * Reference category.

Table 2. Common haplotypes of the *PROCR* gene in RVO patients and controls

Haplotype (htSNP)	Patients (%) N= 77	Controls (%) N= 90	OR	95% CI
H1 (rs2069952)				
HxHx	28 (36)	41 (46)	1*	-
H1Hx	41 (53)	40 (44)	1.5	0.8 to 2.9
H1H1	8 (11)	9 (10)	1.3	0.5 to 3.8
H1Hx/ H1H1	49 (64)	49 (54)	1.5	0.8 to 2.8
Frequency H1	37.0	32.2		
H2 (all common)				
HxHx	22 (29)	20 (22)	1*	-
H2Hx	37 (48)	47 (52)	0.7	0.3 to 1.5
H2H2	18 (23)	23 (26)	0.7	0.3 to 1.7
H2Hx/ H2H2	55 (71)	70 (88)	0.7	0.3 to 1.4
Frequency H2	47.4	51.7		
H3 (rs867186)				
HxHx	63 (82)	68 (76)	1*	-
H3Hx	14 (18)	21 (23)	0.7	0.3 to 1.5
H3H3	0 (0)	1 (1)	-	-
H3Hx/ H3H3	14 (18)	22 (24)	0.7	0.3 to 1.5
Frequency H3	9.1	12.8		
H4 (rs2069951)				
HxHx	68 (88)	84 (93)	1*	-
H4Hx	8 (11)	6 (7)	1.6	0.5 to 4.9
H4H4	1 (1)	0 (0)	-	-
H4Hx/ H4H4	9 (12)	6 (7)	1.8	0.6 to 5.5
Frequency H4	6.5	3.3		

* Reference category, Hx: all haplotypes except the one given, htSNP: haplotype tagging SNP, OR: odds ratio, 95% CI: 95% confidence interval

Table 3. Fibrinolysis parameters and TAFI polymorphisms in RVO patients and controls

	Patients N= 101	Controls N= 94	Mean difference/ OR	95%CI
CLT, mean	70	77	-7	-12.6 to -0.1
<93 N (%)	29 (40)	31 (34)	1*	-
93-103 N (%)	23 (32)	31 (34)	0.8	0.4 to 1.6
>103 N (%)	20 (28)	30 (32)	0.7	0.3 to 1.5
TAFI antigen, mean	106	110	6	-0.3 to 8.0
<103 N (%)	35 (51)	33 (36)	1*	-
103-115 N (%)	12 (18)	28 (31)	0.4	0.2 to 0.9
>115 N (%)	21 (31)	30 (33)	0.7	0.3 to 1.7
TAFI activity, mean	102	101	2	-7.4 to 3.8
<93 N (%)	21 (31)	32 (36)	1*	-
93-103 N (%)	19 (28)	28 (32)	1.0	0.5 to 2.3
>103 N (%)	28 (41)	28 (32)	1.5	0.7 to 3.3

Abbreviations: clot lysis time (CLT) and thrombin activatable fibrinolysis inhibitor (TAFI).
Missing measurements: 28 patients and 2 controls for CLT, 32 patients and 3 controls for TAFI antigen, 33 patients and 6 controls for TAFI activity measurements.
* Reference category.

Table 4. Common haplotypes of the *TAFI* gene in RVO patients and controls

Haplotype (htSNP)	Patients (%) N= 69	Control (%) N= 88	OR	95% CI	Mean TAFI (95% CI)**
H1 (rs3742264)					
HxHx	29 (42)	45 (51)	1*	-	97.8 (94.1 to 101.5)
H1Hx	33 (48)	30 (34)	1.5	0.4 to 4.9	100.1 (94.9 to 105.3)
H1H1	7 (10)	13 (15)	0.7	0.1 to 7.3	114.6 (99.7 to 129.5)
H1Hx/ H1H1	40 (58)	43 (49)	1.4	0.8 to 2.7	104.5 (98.5 to 110.5)
Frequency H1	34.1	31.8			
H2 (rs2146881and rs1926447)					
HxHx	39 (57)	51 (58)	1*	-	103.8 (98.3 to 109.4)
H2Hx	27 (39)	33 (38)	0.9	0.3 to 2.8	98.0 (93.4 to 102.6)
H2H2	3 (4)	4 (4)	1.0	0.1 to 13.5	96.5 (89.2 to 103.7)
H2Hx/ H2H2	30 (43)	37 (42)	1.1	0.6 to 2.0	97.8 (93.7 to 102.0)
Frequency H2	23.9	23.3			
H3 (all common)					
HxHx	25 (36)	33 (38)	1*	-	105.1 (98.2 to 112.1)
H3Hx	37 (54)	39 (44)	1.1	0.3 to 4.3	99.4 (94.4 to 104.5)
H3H3	7 (10)	16 (18)	0.6	0.1 to 5.8	96.9 (91.8 to 101.9)
H3Hx/ H3H3	44 (64)	55 (62)	1.1	0.5 to 2.0	98.7 (94.8 to 102.6)
Frequency H3	36.9	40.3			
H4 (rs1926447)					
HxHx	62 (90)	80 (91)	1*	-	101.0 (97.2 to 104.9)
H4Hx	7 (10)	8 (9)	1.1	0.4 to 3.3	104.2 (95.2 to 113.2)
H4H4	0	0	-	-	-
H4Hx/ H4H4	7 (10)	8 (9)	1.1	0.4 to 3.3	104.2 (95.2 to 113.2)
Frequency H4	5.1	4.6			

** Mean TAFI activity level and 95% confidence interval in the controls, * Reference category, Hx: all haplotypes except the one given, htSNP: haplotype tagging SNP, OR: odds ratio, 95% CI: 95% confidence interval

Table 5. RVO risk for different genotypes of polymorphisms in four platelet receptors

Gene SNP	Genotype	Patients N=77(%)	Control N= 90 (%)	OR	95% CI
GPIba					
rs6065	CC	66 (86)	72 (80)	1*	-
	CT	11 (14)	18 (20)	0.7	0.3 to 1.5
	TT	0	0	-	-
	CT/ TT	11 (14)	18 (20)	0.7	0.3 to 1.5
rs2243093	TT	59 (77)	65 (72)	1*	-
(Kozak)	CT	18 (23)	25 (28)	0.8	0.4 to 1.6
	CC	0	0	-	-
	CT/ CC	18 (23)	25 (72)	0.8	0.4 to 1.6
GPIa					
rs1062535	GG	27 (35)	33 (37)	1*	-
	AG	32 (42)	44 (49)	0.9	0.5 to 1.8
	AA	18 (23)	13 (14)	1.7	0.7 to 4.1
	AG/ AA	50 (65)	57 (63)	1.1	0.6 to 2.0
rs1126643	CC	27 (35)	34 (38)	1*	-
	CT	30 (39)	42 (47)	0.9	0.5 to 1.8
	TT	20 (26)	14 (15)	1.8	0.8 to 4.2
	CT/ TT	50 (65)	56 (62)	1.1	0.6 to 2.1
GPIIIa					
rs5911	AA	30 (39)	25 (28)	1*	-
	AC	34 (44)	45 (50)	0.6	0.3 to 1.3
	CC	13 (17)	20 (22)	0.5	0.2 to 1.3
	AC/ CC	47 (61)	65 (72)	0.6	0.3 to 1.2
rs5918	TT	48 (62)	67 (74)	1*	-
	TC	25 (33)	21 (23)	1.7	0.8 to 3.3
	CC	4 (5)	2 (2)	2.8	0.5 to 15.9
	TC/ CC	29 (38)	23 (25)	1.8	0.9 to 3.4
GPVI					
rs1613662	AA	51 (66)	62 (69)	1*	-
	AG	25 (33)	25 (28)	1.2	0.6 to 2.4
	GG	1 (1)	3 (3)	0.4	0.0 to 4.0
	AG/ GG	26 (34)	28 (31)	1.1	0.6 to 2.2

* Reference category, OR: odds ratio, 95% CI: 95% confidence interval

Discussion

In this relatively large study on idiopathic RVO, we were unable to detect a significant association between established thrombophilia factors, fibrinolysis parameters or platelet receptor polymorphisms and idiopathic RVO. However, we observed non-significant risk increases for two haplotypes of *PROCR* (H1 and H4), for higher TAFI activity, for *TAFI* H1 and for several platelet receptor polymorphisms.

The endothelial protein C receptor (EPCR) has a crucial role in the protein C anticoagulant system by reinforcing protein C activation by thrombin [29]. In one study, higher circulating levels of this receptor (soluble EPCR, sEPCR) were associated with the risk of RVO in general [13]. Accordingly, we expected H3 *PROCR* carriers to have higher risk of RVO since H3 *PROCR* carriers are reported to have higher sEPCR levels than other haplotypes [30]. Since our data did not support this hypothesis and also the effect of *PROCR* haplotypes on VTE risk is not clear [30;31], we suggest further investigations before drawing a definite conclusion.

Two small-sized studies did not show an association between TAFI activity and RVO in general [10;17]. We observed that *TAFI* H1, possibly through higher TAFI activity [32], showed a trend toward higher risk of idiopathic RVO. However, CLT did not differ between cases and controls, suggesting that impaired fibrinolysis is not a major contributor in the pathogenesis of idiopathic RVO. Moreover, as higher CLT and ETP, both established risk factors for venous thrombosis [33;34], did not differ between patients and controls, coagulation and fibrinolysis are probably balanced in idiopathic RVO. In contrast to our observation in idiopathic RVO patients, two small studies have reported significant P-values for the association of high Euglobulin Clot Lysis Time and the risk of RVO in general [16;17].

Interestingly, we observed an association between some SNPs in platelet receptor genes and the risk of developing RVO. Functional studies have demonstrated that polymorphisms (rs1062535, rs1126643) in the GPIa component of the GPIa/IIb complex - the receptor for collagen on platelets - lead to a higher density of the complex and subsequently to enhanced adhesiveness to collagen [35]. However, their role in RVO is uncertain; several studies have shown no effect of rs1126643 [19;21;22] while in one study both SNPs were correlated with RVO [20]. Also several

studies have indicated that rs5918 in GPIIIa component of the GPIIb/IIIa complex - the receptor for fibrinogen and von Willebrand factor and the most abundant receptor on platelets - is not associated with RVO [18;19;22].

A limitation of our study is that despite a relative large number of idiopathic RVO patients, there was not sufficient power to detect modest risk increases. For instance the power to detect the observed 1.8 point estimate with our sample size for rs5918 was approximately 50%. For the same reason, we were unable to stratify our analysis for the types of RVO. Also, we did not adjust for multiple testing, which may lead to finding false positive associations.

In conclusion, our data suggest an association between *PROCR* H1 and H4, elevated TAFI activity, *TAFI* H1 and platelet receptor polymorphisms (rs5918, rs1062535 and rs1126643) and idiopathic RVO. We did not observe a role for established thrombophilia factors and CLT, although these results should be interpreted with caution due to the limited power to detect small effects.

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