Role of factor V Leiden and prothrombin 20210A in patients with retinal artery occlusion

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Abstract

Purpose Retinal artery occlusion is a common vision-threatening disease. Among other risk factors, coagulopathies leading to a hypercoagulable state have been associated with retinal artery occlusion. Numerous studies have shown that two genetic variants, factor V Leiden and prothrombin 20210A, cause a procoagulant state. However, their role in the pathogenesis of retinal artery occlusion is still unclear. The purpose of the present study was therefore to investigate a possible association between factor V Leiden, prothrombin 20210A, and retinal artery occlusion.

Methods In the present retrospective case–control study, we studied 136 patients with retinal artery occlusion and 136 age- and gender-matched control subjects. The presence of factor V Leiden and prothrombin 20210A alleles was determined by polymerase chain reaction.

Results The prevalence of heterozygosity for the prothrombin G20210A variant did not significantly differ between patients and controls (three patients *vs* two controls, $P=0.65$). Distribution of factor V Leiden genotypes revealed no significant difference among the two groups (heterozygosity: eight patients *vs* 11 controls, $P=0.47$). As for other risk factors, arterial hypertension, a history of stroke and myocardial infarction were significantly more frequent in patients than in controls.

Conclusion Our data suggest that factor V Leiden and prothrombin 20210A do not play a major role in patients with retinal artery occlusion.

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Keywords: retinal artery occlusion; factor V Leiden; prothrombin 20210A; risk factor

Introduction

Retinal artery occlusion (RAO) is a frequent vision threatening disease, predominantly affecting patients older than 50 years. Severe visual loss is caused by ischaemic retinal infarction because of impaired blood flow in the retinal arteries. Among others, haemorrhage under an atherosclerotic plaque, embolisation, and intraluminal thrombus formation have been suggested to play an essential role in the pathogenesis of RAO.¹ Previous studies have identified arterial hypertension, hyperhomocysteinaemia, arteriosclerosis, and diabetes mellitus as major risk factors.^{1–4} However, some cases cannot be explained by the known risk factors alone and thus identification of other yet unknown risk factors is of great importance.

Variants of genes encoding for proteins in the coagulation cascade, which lead to a permanent hypercoagulable state are potential risk factors for RAO. Among these, factor V Leiden (FV Leiden) and prothrombin 20210A have been shown to be the most frequent hereditary cause of deep vein thrombosis in Caucasians.^{5,6} FV Leiden is characterized by a G–A substitution at nucleotide 1691 of the factor V gene, leading to a procoagulant state by reduced inactivation of FVa.⁷ In 1996, Poort *et al*⁸ reported a G–A transition at nucleotide 20210 of the factor II gene. Heterozygosity for the prothrombin G20210A variant was found to be associated with increased plasma prothrombin levels, thus also exerting procoagulatory effects.⁸

Studies investigating the impact of these genetic variants on arterial thrombosis yielded

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conflicting results.^{9–16} Several case reports described the presence of FV Leiden in RAO,^{17–20} but a subsequent case–control study in 35 German patients with RAO was unable to confirm this association.²¹ A more recent study investigating the prevalence of FV Leiden and prothrombin 20210A in patients without apparent source of embolism found a higher frequency of FV Leiden among patients without reaching statistical significance.²² Owing to the small number of patients included in these studies, data on the impact of FV Leiden and prothrombin 20210A variant on the pathogenesis of RAO are inconclusive.²³ The aim of the present study was to investigate a possible association between these genetic variants and the presence of RAO in a larger cohort of patients.

Material and methods

The present retrospective case–control study included 136 consecutive patients with RAO who met the inclusion criteria, and 136 controls matched for age and gender. All participants were seen at our department between January 1996 and February 2002 and gave written informed consent before enrolment. The study was approved by the local Ethics Committee.

Diagnosis of RAO was made on clinical grounds by ophthalmoscopic fundus examination revealing superficial retinal whitening in the distribution of the involved retinal artery. Occlusion of the central retinal artery, involving the entire retina was classified as central retinal artery occlusion (CRAO), whereas the involvement of one of its branches was classified as branch retinal artery occlusion (BRAO). If there was any element of doubt, fluorescein angiography was performed to confirm RAO.

Exclusion criteria for patients with RAO and control subjects comprised malignancy and vasculitis. The control group was hospital based and consisted of 136 gender- and age-matched (age of controls $\pm$ 3 years) subjects, who were referred to our department for reasons other than retinal vascular occlusion, anterior ischaemic optic neuropathy, or vasculitis. Each patient with RAO was matched with one control subject.

Arterial hypertension was defined by a systolic blood pressure $\geq$ 160 mmHg, diastolic blood pressure $\geq$ 90 mmHg, and/or the current intake of antihypertensive drugs. Subjects were classified as diabetics, when being treated for insulin- or noninsulin dependent diabetes mellitus. According to their smoking status, subjects were defined as nonsmokers or current smokers. Myocardial infarction was defined by a history of myocardial infarction and electrocardiographic findings confirming myocardial infarction. Subjects were classified to have suffered from stroke by a history of

stroke with focal neurological deficits lasting longer than 24 h. Hypercholesterolaemia was defined by the intake of lipid-lowering drugs and/or a fasting plasma cholesterol level above 200 mg/dl.

For genotype determination, venous blood was collected in 5 ml EDTA tubes and genome DNA was isolated by standard methods and stored at 4°C. Genotyping for the prothrombin 20210A variant and FV Leiden was performed as described previously.^{8,24}

Statistics

Descriptive statistics were used to calculate frequencies and percentage of categorical variables. Continuous data are given as mean $\pm$ SD. We performed the Kolmogorov–Smirnov test to assess normal distribution. Proportions were compared using χ^2 -test statistics. Odds ratios and 95% confidence interval (CI) were calculated by multiple logistic regression analysis. All *P*-values are two-tailed and all CIs were calculated at the 95% level. A *P*-value < 0.05 was considered to be significant. Statistical analysis was performed with the SPSS statistical package (SPSS, version 10.0, 1999, Chicago).

Results

The study group consisted of 136 patients (58 females and 78 males) with RAO and 136 control subjects matched for age and gender. Among patients with RAO, 73 (53.6%) and 63 (46.4%) were classified as suffering from CRAO and BRAO, respectively. The mean age of patients was 69.8 ± 10.1 years (range 41.3–89.3 years) and 70.0 ± 10.3 years (range 39.9–89.0 years) in controls. Baseline parameters and clinical characteristics of both groups are shown in Table 1. Arterial hypertension, a history of stroke and myocardial infarction, were found significantly more often in patients with RAO.

Table 1 Baseline characteristics of patients with RAO and controls.

	RAO (n=136)	Controls (n=136)
Female	58 (42.6)	58 (42.6)
Mean age (years $\pm$ SD)	69.8 ± 10.1	70.0 ± 10.3
Range (years)	41.3–89.3	39.9–89.0
Risk factors		
Arterial hypertension	92 (67.6)**	58 (42.6)
Diabetes mellitus	23 (16.9)	25 (18.4)
Hypercholesterolaemia	97 (71.3)	96 (70.6)
Stroke	22 (16.2)**	5 (3.7)
Myocardial infarction	18 (13.2)*	7 (5.1)
Current smoker	26 (19.1)	19 (13.9)

Numbers are given as *n* (%); **P* < 0.05; ***P* < 0.001.

The prevalences of heterozygotes for FV Leiden and the prothrombin G20210A variant were not significantly different among patients and controls (Table 2). Homozygous carriers of FV Leiden or prothrombin 20210A were not found in either patients or controls. Heterozygosity for prothrombin G20210A was associated with an odds ratio of 1.38 (95% CI 0.21–9.19) for RAO, whereas heterozygosity for FV Leiden yielded an odds ratio of 1.01 (95% CI 0.36–2.81). Comparison between genotype distribution among patients with CRAO and BRAO did not reveal any significant difference (CRAO vs BRAO: prothrombin 20210A: 2 (2.7%) vs 1 (1.5%), $P=0.65$; FV Leiden: 5 (6.8%) vs 3 (4.7%), $P=0.61$). In a subgroup analysis including only smokers, FV Leiden was not associated with RAO either ($P=0.38$). No smoker was found among carriers of prothrombin 20210A.

In a logistic regression analysis, case–control status was predicted by arterial hypertension, current smoking status, and a history of stroke, but not by FV Leiden or prothrombin G20210A genotypes (Table 3).

Discussion

In the present study, we investigated a hypothesized association of FV Leiden, prothrombin 20210A, and the presence of RAO in 136 patients. A hypercoagulable state is known to play an important role in venous

thromboembolism and has been suggested as a risk factor for arterial disease in some studies.^{9–12,15} In our study, the prevalence of FV Leiden and the prothrombin 20210A variant did not significantly differ between patients with RAO and control subjects, indicating that both genetic variants are not major risk factors for RAO. Thus, our data confirm the findings of Greiner *et al*,²¹ who also demonstrated a lack of association between FV Leiden and RAO in 35 patients. Analysing genotype distribution of FV Leiden and prothrombin G20210A in patients with CRAO and BRAO separately did not show a significant difference. Therefore, an essential role for both genetic variants in the pathogenesis of either CRAO or BRAO is not suggested. Synergistic and/or additive effects of smoking and FV Leiden and prothrombin 20210A variant for arterial disease have been suggested.¹¹ However, such an interaction could not be observed in the present study.

As for other established risk factors, arterial hypertension was significantly more frequent among patients with RAO and was associated with an odds ratio of 2.88 for RAO. This finding confirms the role of arterial hypertension as a known risk factor for RAO.^{1,2} In contrast to some other investigations, no significant difference in the prevalence of diabetes mellitus and hypercholesterolaemia between patients and controls was observed.^{1,25} A possible explanation for this conflicting result might be that most of our control subjects were recruited among patients undergoing cataract surgery. Both diseases have been associated with increased cataract formation, possibly leading to the high prevalence of diabetes mellitus and hypercholesterolaemia in our control group.^{26,27}

A potential limitation of this study is its retrospective design. Although genotypes do not change during lifetime, we cannot exclude a survival-associated selection bias. Secondly, only two patients younger than 45 years were included in the present study. Therefore, no conclusion can be drawn on FV Leiden and prothrombin 20210A as a risk factor for RAO in young patients. Since thrombophilia-associated vascular events are known to occur frequently in patients younger than

Table 2 Genotype distribution among patients with RAO and controls

	RAO (n=136)	Controls (n=136)
<i>Factor V Leiden</i>		
GG	128 (94.1%)	125 (91.9%)
GA	8 (5.9%)	11 (8.1%)
AA	—	—
<i>Prothrombin G20210A</i>		
GG	133 (97.8%)	134 (98.5%)
GA	3 (2.2%)	2 (1.5%)
AA	—	—

Table 3 Logistic regression analysis of 1:1 matched case–control study

	Odds ratio	95% Confidence interval	P-value
Arterial hypertension (yes/no)	2.88	1.68–4.94	0.001
Diabetes mellitus (yes/no)	0.84	0.43–1.63	0.603
Hypercholesterolaemia (yes/no)	1.19	0.67–2.12	0.555
Myocardial infarction (yes/no)	2.52	0.95–6.67	0.063
Stroke (yes/no)	4.63	1.62–13.23	0.004
Current smoker (yes/no)	2.18	1.07–4.47	0.032
Prothrombin 20210A (yes/no)	1.38	0.21–9.19	0.740
FV Leiden (yes/no)	1.01	0.36–2.81	0.978

45 years, additional studies investigating a possible association between these genetic markers and occurrence of RAO in this age group are warranted.⁶

In conclusion, our data suggest that neither FV Leiden nor prothrombin 20210A are major risk factors for RAO in patients older than 50 years. Thus, routine screening for these genetic variants in unselected patients older than 50 years cannot be recommended.

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