

Retinal vein occlusion and factor V Leiden and prothrombin 20210 G:A mutations

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PURPOSE. *The aim of this study was to establish whether the factor V Leiden mutation and the prothrombin 20210 G:A mutation were risk factors for retinal vein occlusion.*

METHODS. *Blood samples were obtained from 40 patients with retinal vein occlusion and from 50 healthy normal volunteers as controls. Polymerase chain reaction assays were done to detect factor V Leiden and prothrombin 20210 G:A mutations, and the two groups were compared.*

RESULTS. *Two (5%) of 40 patients with retinal vein occlusion and three (6%) of 50 controls were heterozygous for factor V Leiden ($p=0.84$). None of the individuals in either group had the prothrombin 20210 G:A mutation.*

CONCLUSIONS. *There was no significant association between retinal vein occlusion and the factor V Leiden mutation. (Eur J Ophthalmol 2001; 11: 351-5)*

KEY WORDS. *Factor V Leiden mutation, Prothrombin 20210 G:A mutation, Retinal vein occlusion, Central retinal vein occlusion, Branch retinal vein occlusion*

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INTRODUCTION

Retinal vein occlusion (RVO) is a sight-threatening disease that usually causes reduced visual acuity. RVO includes both central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO), and is the second most common retinal vascular disease after diabetic retinopathy (1, 2). Glaucoma, hypertension, hyperlipidemia, cardiovascular disease, hyperviscosity, trauma, hyperopia, and diabetes are risk factors associated with RVO (3-5). However, it is sometimes impossible to pinpoint the causative factor, particularly in younger patients (6), and the pathogenesis of RVO remains unclear.

Resistance to activated protein C (APC) is a risk factor for venous thrombotic disease (7,8), and this resistance is also reportedly associated with RVO (9-13). In the majority of cases, APC resistance is the phenotypic manifestation of a point mutation (G 1691

to A) in the gene for coagulation factor V. The mutant factor V gene is named factor V Leiden (14,15), and has an autosomal dominant inheritance pattern. Factor V Leiden mutation is the most important cause of familial thrombophilia, and is present in approximately 5% of healthy Caucasians (16). Findings are inconsistent regarding the association between factor V Leiden and RVO (17-20).

Another genetic risk factor, a variation in the 3'-untranslated region of the prothrombin gene, has also been linked to increased risk for venous thrombosis (21), and this prothrombin 20210 G:A mutation has been reported in patients with RVO (22, 23).

However, other studies have not confirmed these findings (18, 24).

To further investigate these relationships, we checked for the prevalence of the factor V Leiden mutation and the prothrombin 20210 G:A mutation in RVO patients and in normal controls.

MATERIALS AND METHODS

All patients provided informed consent, and our investigation was conducted according to the Declaration of Helsinki. The study was designed prospectively and included 40 patients who were consecutively diagnosed with RVO by the Baskent University Department of Ophthalmology in two years. In all cases, the diagnosis was made by ophthalmoscopic fundus examination and confirmed by fundus fluorescein angiography.

The control group consisted of 50 consecutive patients who had refractive errors, cataracts, or conjunctivitis, but were otherwise healthy. Controls and patients were age- and sex-matched.

Factor V Leiden assay

DNA was extracted from peripheral blood using the phenol-chloroform extraction and ethanol precipitation method. Screening for the factor V gene 1691 G → A mutation was done with polymerase chain reaction (PCR) amplification of exon 10 using primers 5'TGCCAGTGCTTAACAAGACCA3' and 5'CTTGAA GGAAATGCCCATTA3', followed by Mnl I restriction enzyme digestion and agarose gel electrophoresis. Mnl I digestion of the PCR products produced

various detectable fragments. Mnl I cleaved the normal factor V allele into three fragments of 37, 67, and 116 bp each, whereas the factor V Leiden allele was cut into two fragments of 67 and 153 bp. A 345 bp fragment that included nucleotide 20210 was amplified using a mutagenic primer that introduced a Hind III site, allowing the mutation to be identified. The mutant allele yielded two digestion products of 322 and 23 bp and, since the normal allele lacked the Hind III site, digestion with this enzyme had no effect, yielding a 345 bp fragment.

Prothrombin 20210 G:A assay

Prothrombin gene 20210 G → A transition was also detected by PCR, using the primer 5'TCTAGAACAGTTGCCTGGC3' and a mutagenic primer 5'ATAGCACTGGGAGCATTGAA3'. The amplification products were digested with Hind III restriction enzyme and DNA fragments were resolved using 3% NuSieve agarose gels (21). DNA samples were amplified with the above primers, and a Hind III site was inserted into the mutant allele via the mutagenic primer (21). Mutant alleles were identified by Hind III digestion. In the strategy for direct detection of the 20210 A allele in the prothrombin gene, the Hind III restriction enzyme digestion yields two fragments of 322 bp and 23 bp

TABLE I - PATIENTS' MAIN CHARACTERISTICS

	Age (years) mean ± SD	Age range (years)	Sex (M/F)	<50 years
Patients with RVO (n=40)	59 ± 10	35-77	21/19	10
Control group (n=50)	55 ± 10	34-76	26/24	18

TABLE II - THE GENOTYPIC RATIOS OF FACTOR V LEIDEN MUTATION

	Normal alleles	Homozygous F V Leiden	Heterozygous F V Leiden
Patients with RVO (n=40)	38 (95%)	-	2 (5%)
Control group (n=50)	47 (94%)	1 (2%)	2 (4%)

each. These two bands are found in both homozygous and heterozygous subjects, but only the uncut amplification product (a single band of 345 bp) is seen in normal individuals.

Statistical analysis

The χ^2 test was used for statistical analysis. *p* values < 0.05 were considered significant.

RESULTS

The mean age \pm SD of the patients was 59 \pm 10 years (range 35-77 years), and that of controls was 55 \pm 10 years (range 34-76 years) (Tab. I). Of the 40 RVO patients, 52.5% were male and 47.5% female, and the proportions in the control group were 52% and 48%. Ten RVO patients and 18 controls were younger than 50 years (Tab. I). Of the 40 RVO patients, 19 (47.5%) had CRVO and 21 (52.5%) had BRVO. Five eyes diagnosed as CRVO and four diagnosed as BRVO were classified as ischemic type based on FFA findings. Of the ten patients younger than 50 years, seven had CRVO and three BRVO. With regard to risk factors, ten patients had controlled hypertension, four dyslipidemia, and five diabetes mellitus. In the control group, 25 individuals had refractive errors, 15 had cataracts, and 10 had conjunctivitis, none had systemic problems.

The factor V Leiden mutation was detected in two RVO patients and three (6%) control. Both the RVO patients were heterozygous carriers, while two of the controls were heterozygous and one was a homozygous carrier (Tab. II).

The difference between the RVO group and the controls in terms of frequency of factor V Leiden was not significant ($\chi^2=0.04$, *p*=0.84).

One of the patients with factor V Leiden was a 49-year-old man with non-ischemic type CRVO who had no systemic risk factors, and the other was a 65-year-old woman with BRVO, also non-ischemic, who had hypertension. In the control group, the patients with factor V Leiden were a 47-year-old woman, a 53-year-old man, and a 67-year-old woman. When we compared the frequencies of factor V Leiden in individuals younger than 50 years, again there was no significant difference between RVO patients and con-

trols ($\chi^2=0.19$, *p*=0.66).

We also found no significant differences among the CRVO subgroups based on age (<50 years or \geq 50 years) (*p*=0.36). Again, no significant differences were found between CRVO and BRVO subgroups based on capillary damage (non-ischemic or ischemic) (*p*=0.75 and *p*=0.8 consecutively).

In our analysis of the prothrombin 20210 G \rightarrow A mutation, we detected no heterozygous nucleotide transition (20210 AG) carriers or homozygous nucleotide transition (20210 AA) carriers in any of the RVO patients or controls.

DISCUSSION

We found no association between RVO and the factor V Leiden mutation but found no cases with the prothrombin 20210 G:A mutation. Previous studies have reported that factor V Leiden mutation is a risk factor for RVO (9-11,13,18,19). Larson et al (10) and Williamson et al (11) reported the prevalence of APC resistance in patients with CRVO as 12% and 26%, respectively. However, neither of these studies did genotype testing for factor V Leiden.

Ciardella et al (20) reported that a test for APC resistance with a first generation assay was positive in 45% of patients with RVO, while repeat APC-resistance assay using factor V-deficient plasma gave only 10% positive results. Also, they found no difference between RVO patients and controls with regard to APC resistance.

Factor V Leiden was detected in only 3% of patients with RVO. Those authors concluded that molecular testing for factor V Leiden is the only reliable method of detecting this mutation, and that there is no significant association between factor V Leiden and RVO. We too found no significant difference between RVO patients and controls with regard to the factor V Leiden mutation, with 5% and 6% of each group showing the mutation.

Our study and two others have addressed the role of the Leiden mutation in the etiology of RVO. Linna et al (17) found that out of 46 patients younger than 50 years who had RVO, only 4.3% were heterozygous carriers for factor V Leiden. Graham et al (12) detected only one patient with factor V Leiden in 50 patients with CRVO. Both studies reported no association be-

tween factor V Leiden and RVO. However, Glueck et al (18) found that 18% of 17 patients with RVO were heterozygous for factor V Leiden compared to 3% of controls.

It is difficult to explain these conflicting results. One point may be that Glueck et al only studied a small number of RVO patients. Also, it has been reported that the prevalence of factor V Leiden mutation can vary from 2% to 15%, according to geographical area. This mutation is virtually nonexistent in Chinese, Japanese, and African peoples, and its highest incidence is in Sweden (16, 25, 26). A recent study noted 25% APC resistance in patients with CRVO in Turkey; however, the presence of factor V Leiden was not assessed (27). Previous reports showed that approximately 10% of healthy individuals in the Turkish population are heterozygous for factor V Leiden (28), but 6% of the controls in our study had the Leiden mutation. Another explanation for the different factor V Leiden results may be ethnic differences and gene-environment interactions in patient and control groups. There were also differences in the techniques and study methods.

The prothrombin gene 20210 G:A mutation was re-

ported in case report format in one patient with CRVO (22) and in another with CRVO and central retinal artery occlusion (23). However, a separate study of this mutation by Larsson and Hillarp (24) found that only 3% of 129 patients with CRVO were heterozygous. Subsequently, Glueck et al (18) reported that, of 17 patients with CRVO, none had the prothrombin gene 20210 G:A mutation. We also detected no prothrombin gene 20210 G:A mutations in any of the individuals in our RVO and control groups.

Based on our results, we can conclude that neither the factor V Leiden mutation nor – presumably – the prothrombin gene 20210 G:A mutation is associated with RVO; however, further studies are needed to clearly demonstrate the role of these mutations.

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