Role of Factor XIII Val34Leu Polymorphism in Retinal Artery Occlusion

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Background and Purpose—Factor XIII (FXIII) Val34Leu, a common polymorphism in the gene for factor XIII, has been associated with a lower risk of stroke, myocardial infarction, and deep vein thrombosis. Ineffective fibrin cross-linking has been suggested to be causative. The aim of the present case-control study was to investigate the role of FXIII Val34Leu polymorphism in patients with retinal artery occlusion.

Methods—A total of 108 patients with retinal artery occlusion and 313 age- and sex-matched controls were genotyped for the FXIII Val34Leu polymorphism. Factor XIII Val34Leu genotypes were determined by use of allele-specific polymerase chain reaction.

Results—Homozygous Leu genotype was found significantly more often in control subjects than in patients with retinal artery occlusion (P=0.018), with an odds ratio of 0.22 (95% confidence interval 0.07 to 0.74). Distribution of the Val/Val and Val/Leu genotypes did not differ significantly between groups.

Conclusions—Because prevalence of homozygous Leu genotype was significantly higher in controls, we conclude that the Leu/Leu genotype is associated with a protective effect against retinal artery occlusion. (Stroke. 2001;32:2759-2761.)

Key Words: coagulation ■ factor XIII ■ genetics ■ retinal artery occlusion

Factor XIII (FXIII) is a transglutaminase that plays a decisive role in the coagulation cascade and in fibrinolysis. In plasma, FXIII circulates as a heterotrimer consisting of 2 A-subunits (FXIIIA) that possess catalytic activity, and 2 carrier B-subunits (FXIIIB). Thrombin leads to activation of FXIII by cleaving the peptide bond at Arg37 to Gly38 of FXIIIA subunit. Activated FXIII catalyzes the formation of (γ-Glu)-Lys bonds between fibrin monomers, which causes increased rigidity of the thrombus.

Furthermore, FXIII increases resistance of the fibrin clot to plasmin degradation by cross-linking of α2-antiplasmin to fibrin. Consequently, deficiency of FXIII has been associated with severe bleeding diathesis and a higher risk of miscarriage in female patients.

Recently, a common polymorphism of the FXIIIA gene, which is characterized by a Val→Leu exchange at amino acid position 34 (FXIIIVal34Leu) 3 amino acids from the thrombin activation site, has been identified. The Leu allele has been associated with decreased risk of stroke, myocardial infarction, and deep vein thrombosis and increased risk of intracerebral hemorrhage.

Nevertheless, several other studies found no such effect, which has resulted in an ongoing debate on the role of this polymorphism for arterial and venous thrombosis.

Retinal artery occlusion (RAO) is a common vision-threatening disease that primarily affects patients >60 years of age. Occlusion of the central retinal artery or 1 of its branches leads to ischemic infarction of the retina and severe visual loss. Among others, embolization, intraluminal thrombosis, and hemorrhage under an atherosclerotic plaque have been reported as mechanisms responsible for RAO, and several risk factors, including arterial hypertension, diabetes mellitus, hyperhomocysteinemia, and elevated plasma lipoprotein(a) level, have been identified.

However, not all cases can be explained fully by the known risk factors alone and the precise pathomechanism of RAO is still unknown. Therefore, the aim of our present study was to investigate a possible role of FXIII Val34Leu in patients with RAO.

Subjects and Methods

The present study was designed to be a retrospective case-control study to analyze the role of biochemical and genetic risk factors for RAO. Patients and controls were selected from the registry of subjects who had been admitted to our department between September 1996 and June 2000. Participating subjects were invited to our department and were seen between January 2000 and June 2000. The local ethics committee approved the study, and all subjects gave written, informed consent before being enrolled.

Diagnosis of RAO was made on clinical grounds by ophthalmoscopic fundus examination that revealed 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 RAO, whereas involvement of 1 of its branches was classified as branch RAO. Confirmation by fluorescein angiography was performed only if an element of doubt existed as to classification.

Exclusion criteria for patients with RAO comprised malignancy and vasculitis. Of the 117 patients who had been diagnosed with RAO, 9 were not included in the present study (4 patients refused...}

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study participation, 3 suffered from malignancy, and 2 had arteritis temporalis). Thus, the patient group consisted of 108 subjects.

The control group consisted of 313 sex- and age-matched patients referred to our department for reasons other than retinal vascular occlusion, anterior ischemic optic neuropathy, or vasculitis. Subjects with a history of deep vein thrombosis, myocardial infarction, malignancy, or stroke were not eligible to be controls.

Arterial hypertension was defined as having a history of arterial hypertension or the intake of antihypertensive drugs. Subjects were classified as diabetics if they were being treated for insulin-dependent or non-insulin dependent diabetes mellitus. According to their smoking status, subjects were defined as nonsmokers, exsmokers (exsmokers had to have stopped smoking for at least 1 year), or current smokers. Myocardial infarction was defined as a history of myocardial infarction and ECG findings that confirmed myocardial infarction. Subjects were classified as having had a stroke if they had a history of stroke, neurological deficits that lasted >24 hours, and infarction as confirmed by CT scans. Hypercholesterolemia was defined as intake of lipid-lowering drugs or a fasting plasma cholesterol level >220 mg/dL.

Genotype Determination
Venous blood was collected in 5-mL EDTA tubes and stored at −20°C. After recruitment of study participants was completed, genomic DNA was isolated from each subject by standard methods and stored at 4°C. Genotyping for the FXIII Val34Leu polymorphism was done by allele-specific polymerase chain reaction as described previously.23

Statistics
Descriptive statistics were used to calculate frequencies and percentages of categorical variables. Continuous data are given as mean ± SD. We performed the Kolmogorov-Smirnov test to assess normal distribution and Levine’s test for homogeneity of variances. Means were compared by use of independent-sample t test, whereas proportions were compared by use of χ² test statistics or, when appropriate, Fisher’s Exact Test. Odds ratios and 95% confidence intervals (CIs) were calculated by use of multiple logistic regression analysis. All probability values are 2-tailed, and all CIs were exact. Odds ratios and 95% confidence intervals were calculated by use of multiple logistic regression analysis. All probability values are 2-tailed, and all CIs were calculated by use of multiple logistic regression analysis. All probability values are 2-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, IL).

Results
The study group consisted of 108 patients (48 females and 60 males) with RAO, whereas the control group included 313 patients (131 females and 172 males). Among patients with RAO, 59 (54.6%) and 49 (45.4%) were classified as having central RAO and branch RAO, respectively. Mean age of patients was 69.0 ± 12.6 years (range 41 to 89) and of controls was 68.6 ± 10.6 years (range 40 to 90). Table 1 shows baseline parameters and clinical characteristics of both groups. Besides myocardial infarction and stroke, which were exclusion criteria for control subjects, arterial hypertension, and exsmoking status were significantly more frequent in patients than in control subjects, whereas nonsmoking status was found significantly more often in the control group.

Table 2 shows distribution of genotypes of the Val34Leu polymorphism in patients and control subjects and also shows Leu allele frequency. Homozygous Leu genotype was significantly more frequent in patients than in controls (P=0.018). Odds ratio of the homozygous FXIII Leu/Leu genotype was 0.22 (95% CI 0.07 to 0.74). Adjustment for arterial hypertension and smoking status did not change the effect of the polymorphism significantly.

Exclusion of patients with a history of stroke (n=17) and myocardial infarction (n=12) from the study group yielded an odds ratio of 0.30 (95% CI 0.09 to 1.01). Comparison between genotype distribution among patients with RAO did not reveal any significant difference (branch RAO versus central RAO, Val/Val 33 [55.9%] versus 27 [55.1%], Val/Leu 26 [44.1%] versus 19 [38.8%], and Leu/Leu 0 [0%] versus 3 [6.1%]). Allelic frequencies for controls were similar to those previously reported by other investigators.8–13

Discussion
Our present study shows an inverse association between homozgyosity for FXIII Val34Leu polymorphism and presence of RAO. These findings are consistent with the hypothesis that the homozygous Leu genotype is associated with a protective effect against arterial thrombosis.

Several studies have observed a protective effect of the FXIII Val34Leu polymorphism against stroke, myocardial infarction, and deep vein thrombosis. In earlier studies, enzyme activity has been shown to be higher in Leu/Leu homozygotes than in subjects with the more frequent Val/Val genotype, whereas intermediate levels have been determined in heterozygotes.24,25 Recently, activation of the FXIII by thrombin has been reported to occur faster and to require less thrombin in subjects with FXIII 34Leu versus FXIII 34Val, whereas FXIII activity after full activation was independent of the genotype.18,26,27 However, increased rates of fibrin cross-linking and protection of FXIII Val34Leu against thrombosis seem to be paradoxical; the exact mechanism by which the polymorphism exerts its protective effect is still unknown. Possible explanations for this effect include ineffective cross-linking of fibrin monomers or, alternatively.

| TABLE 1. Baseline Characteristics of Patients and Controls |
|---------------------------------|-----------------|
|                                | Patients With RAO | Controls |
|                                | (n=108)          | (n=313)  |
| Men                             | 60 (55.6)        | 172 (55.0) |
| Women                           | 48 (44.4)        | 141 (45.0) |
| Mean ± SD age, y                | 69.0 ± 12.6      | 68.6 ± 10.6 |
| Arterial hypertension           | 66 (61.1)        | 130 (41.5)† |
| Diabetes mellitus               | 21 (19.4)        | 60 (19.2)  |
| Hypercholesterolemia            | 52 (48.1)        | 166 (53.0) |
| Myocardial infarction           | 12 (11.1)        | ...       |
| Stroke                          | 17 (15.7)        | ...       |
| Smoker                          |                   |           |
| Current                         | 19 (17.6)        | 45 (14.4)  |
| Non                             | 50 (46.3)        | 220 (70.3)† |
| Ex                              | 39 (36.1)        | 48 (15.3)† |

Values for genotypes are n (%); data were compared by χ² test. *P<0.05; †P<0.001.

| TABLE 2. Distribution of FXIII Val34Leu Genotypes |
|---------------------------------|-----------------|
|                                | Patients With RAO | Controls |
|                                | (n=108)          | (n=313)  |
| Val/Val                        | 60 (55.5)        | 156 (49.8) |
| Val/Leu                        | 45 (41.7)        | 123 (39.3) |
| Leu/Leu                        | 3 (2.8)          | 34 (10.9) |
| Leucine allele frequency       | 0.236            | 0.305     |

Values for genotypes are n (%); data were compared by χ² test.
deficient cross-linking of such other natural substrates for FXIII as α2-antiplasmin and von Willebrand factor.28

In our present study, homozygous Leu genotype was found more frequently in the control group than in patients with RAO. Therefore, we assume a protective effect exists of the homozygous Leu genotype against RAO. After exclusion of patients with a history of stroke and myocardial infarction, the odds ratio did not substantially differ. Nevertheless, 95% CI included 1.00, which is most probably due to the large number of patients excluded (26.9% of all patients with RAO). Thus, inclusion of more patients with no history of myocardial infarction and stroke will be necessary to determine the effect of the polymorphism in patients free of those cardiovascular diseases.

In the present study, protection against RAO was restricted to the homozygous Leu genotype, although a protective effect of the heterozygous Leu genotype against stroke and myocardial infarction has been demonstrated in recent studies.11,13

A subanalysis comparing genotype distribution between patients with central and branch RAO revealed no significant difference. Thus, we assume that FXIII Val34Leu polymorphism is a risk factor for both types of RAO. However, this conclusion has to be considered with caution, given that the number of patients included in this subanalysis was small.

The present study was retrospective; therefore, it shares the general limitations of retrospective studies. Because an individual’s genotype does not change during his or her lifetime, we do not regard this as a major problem for our present study. Furthermore, because RAO is a rare disease, prospective studies would require tens of thousands of probands followed for several years to reach an adequate number of cases, which would exceed the financial and staffing abilities of most ophthalmological departments.

In conclusion, our present study suggests an inverse association between Val34Leu polymorphism and RAO. However, large prospective studies are needed to confirm our findings and to define the effect of this genetic risk factor for the development of RAO.

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