OBJECTIVE: To determine whether heritable thrombophilia and hypofibrinolysis were risk factors for retinal vein occlusion.

DESIGN: Measures of thrombophilia (increased likelihood of thrombus formation) included anticardiolipin antibodies (IgG and IgM), the lupus anticoagulant (including dilute Russell viper venom clotting time), antigenic proteins C and S, and homocysteine. Polymerase chain reaction assays were performed for 3 thrombophilic gene mutations (factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin gene). Measures of hypofibrinolysis (reduced ability to lyse thrombi) included lipoprotein Lp(a), plasminogen activator inhibitor activity, and polymerase chain reaction analysis of the hypofibrinolytic 4G/5G polymorphism of the PAI1 gene. These coagulation measures were performed in 17 patients with retinal vein occlusions with comparison with serologic coagulation measures and polymerase chain reaction assays in 40 and 234 healthy normal volunteers as controls, respectively.

RESULTS: Of 14 patients with retinal vein occlusion with measures of dilute Russell viper venom clotting time, a thrombophilic antiphospholipid antibody, 6 (43%) had abnormal results (>38.8 seconds) compared with 1 (3%) of 30 controls (P = .002). Of 17 patients with vein occlusion, 3 (18%) were heterozygous for the thrombophilic factor V Leiden G1691A mutation compared with 7 (3%) of 233 controls (P = .02). Of 17 patients with vein occlusion, 2 (12%) had normal alleles (5G/5G) for the plasminogen activator inhibitor gene promoter; the other 15 (88%) were heterozygous or homozygous for the 4G polymorphism, which is associated with hypofibrinolysis. Of 234 controls, 85 (36.3%) had the 5G/5G allele; 149 (63.7%) were heterozygous or homozygous for the 4G polymorphism (P = .03). Patients with vein occlusion were more likely to have high levels of the major determinant of hypofibrinolysis, plasminogen activator inhibitor activity. These levels were high (>22 U/L) in 6 (38%) of 16 patients with vein occlusion compared with 1 (2%) of 40 controls (χ² = 12.8; P = .001). Patients with vein occlusion were more likely (8/16 [50%]) to have high levels of hypofibrinolitic Lp(a) (>35 mg/dL) than controls (5/40 [13%]; χ² = 9; P = .003). The median Lp(a) level in patients with vein occlusion who had the 4G/4G genotype was 62 mg/dL compared with 5.3 mg/dL in controls with the 4G/4G genotype (P = .05).

CONCLUSION: Thrombophilia and hypofibrinolysis are possible causes of retinal vein occlusion.


From the Cholesterol Center (Drs Glueck, Vadlamani, and Wang) and the Ophthalmology Division (Dr Bell), Jewish Hospital; the Molecular Diagnostics and Research Center (Drs Gupta and Fontaine) and Hematology/Oncology Division (Mr Stroop and Dr Gruppo), Children’s Hospital, Cincinnati, Ohio.
PATIENTS AND METHODS

STUDY PROTOCOL

The study was approved by the Institutional Review Board of the Jewish Hospital, Cincinnati, Ohio, and was carried out with informed consent.

Blood specimens for measures of thrombophilia, hypofibrinolysis, and lipid profiles were obtained between 8 and 10 AM after an overnight fast from seated patients with vein occlusion. Retinal vein occlusion was diagnosed by the referring ophthalmologists based on either or both the results of fluorescein angiography and characteristic fundus features.

cDNA PCR ASSAYS

A full range of cDNA PCR assays14-21 and measures of thrombophilia and hypofibrinolysis14,20,22-26 were systematically performed in patients with vein occlusion and in the healthy control groups. The PCR analyses of 3 thrombophilic gene mutations (factor V Leiden G1691A mutation,16 the MTHFR C677T mutation,18 and the 20210A allele of the prothrombin gene15) and the hypofibrinolytic 4G/5G polymorphism of the PAI1 gene promoter17,25 were performed at the Coagulation Research Laboratory, Children's Hospital Medical Center, Cincinnati.

Genomic DNA for each PCR assay was obtained by a salting-out procedure. A PCR assay was performed for factor V Leiden to determine the presence or absence of a single point mutation at nucleotide 1691 leading to a glutamine substitution for an arginine residue at amino acid 506 in the factor V molecule. The PCR product was digested with the restriction enzyme MnlI. A PCR assay was performed for the enzyme MTHFR to determine the presence of a point mutation at nucleotide 677 leading to a valine substitution for an alanine residue. The PCR product was digested with the restriction enzyme HinfI. A PCR assay for the prothrombin gene was performed to determine the presence or absence of a point mutation at nucleotide 20210 leading to a G-to-A transition. The PCR product was digested with the restriction enzyme HindIII. A PCR assay was performed for the polymorphism of the PAI1 gene region of the promoter gene 675 base pairs upstream from the transcriptional starting site, resulting in 2 alleles containing either 4 or 5 guanosines.17,25

MEASURES OF THROMBOPHILIA

Measures of thrombophilia included anticardiolipin antibodies (IgG and IgM), antinuclear antibodies (S), anticoagulant proteins (C and S), antithrombin activity, and the lupus anticoagulant (inducing the dilute Russell viper venom clotting time), and homocysteine (Alliance Hospital Laboratories, Cincinnati, Ohio). Established, previously published methods were used.14,19,22-26

After the discovery that resistance to activated protein C (mediated by the thrombophilic mutant factor V Leiden gene) is a major risk factor for venous thrombosis, Williamson, Larson, and Scat and their colleagues reported a higher prevalence of resistance to activated protein C in patients with retinal vein occlusion than in controls. A 1996 study by Graham et al11 however, found no significant association between the factor V Leiden G1691A mutation and the presence of retinal vein occlusion. Furthermore, a recent study by Gottlieb et al12 in patients younger than 50 years with central retinal vein occlusion reported protein C resistance in patients with retinal vein occlusion.
by plasminogen activator inhibitor 1 (PAI-1). The 4G/4G polymorphism of the PAI1 gene is hypofibrinolytic, associated with high levels of plasminogen activator inhibitor activity (PAI-Fx). Low tissue plasminogen activator levels, high PAI-1 levels, or both, major causes of hypofibrinolysis, have been associated with osteonecrosis and are also risk factors for arterial thrombosis. Lp(a), an atherogenic, hypofibrinolytic, cholesterol-carrying lipoprotein, may inhibit the conversion of plasminogen to plasmin. A high Lp(a) level is a risk factor for osteonecrosis and is an independent risk factor for coronary artery thrombosis. Not depicted in Figure 1 because it has multiple postulated interactions with the coagulation cascade is the common mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, associated with increased levels of the thrombophilic amino acid, homocysteine.

Further confirmation of pathogenic relationships between hypofibrinolysis and thrombophilia and retinal vein occlusion, with better understanding of the molecular genetic cause of venous thrombosis, should promote further studies of the surveillance, prevention, and treatment of central retinal vein thrombosis.

Our specific aim was to systematically assess measures of thrombophilia and hypofibrinolysis in patients with retinal vein occlusion, including newly developed complementary DNA (cDNA) polymerase chain reaction (PCR) assays for mutant genes that affect coagulation.

**RESULTS**

**PATIENTS WITH RETINAL VEIN OCCLUSION**

Retinal vein occlusion was bilateral in 3 patients. There were 9 women and 8 men, 1 of whom was African American, and the rest were white. The age of patients was 52 ± 10 years (median, 51 years; range, 33-69 years) and of controls was 37 ± 7 years (median, 37 years; range, 24-54 years). Of the 40 adult controls, 23 were female; 36 were white, 2 African American, and 2 “other.” Six patients (35%) had hypertension that was well controlled with antihypertensive agents, 3 (18%) ingested 7 or more alcoholic beverages per week, 2 (12%) had type 2 (mature-onset) diabetes mellitus, and 1 (6%) smoked 1 pack or more of cigarettes per day.

Three (18%) of the patients had a history of osteonecrosis, 4 patients (24%) had other thrombotic events, primarily deep venous thrombosis not including osteonecrosis. No patients had been given high-dose corticosteroid therapy (prednisone, >20 mg/d, for ≥ week) before the development of retinal vein thrombosis. One patient received high-dose corticosteroid treatment of his retinal vein thrombosis for 2 months; this antedated and contributed to his subsequent development of osteonecrosis of the hips, requiring bilateral hip replacement.

Exclusion of the 3 patients who had both osteonecrosis and retinal vein thrombosis from the patient-control comparisons did not alter any of the statistical comparisons. One of these 3 patients was heterozygous
for the mutant factor V Leiden gene, and 1 was homozygous for the 4G/4G polymorphism of the PAI1 gene.

THROMBOPHILIC AND HYPOFIBRINOLYTIC GENE MUTATIONS (PATIENTS VS CONTROLS)

Of the 17 patients with vein occlusion, 3 (18%) were heterozygous for the thrombophilic factor V Leiden G1691A mutation compared with 7 (3%) of 233 controls (P = .02, Fisher exact test) (Figure 2). Of the 3 patients heterozygous for the factor V Leiden G1691A mutation, 2 were also homozygous for the 4G/4G polymorphism of the PAI1 gene, and 1 of these 2 patients also had high Lp(a) levels (70 mg/dL). One patient was heterozygous for the mutant PAI1 gene and also had high Lp(a) levels (40 mg/dL).

The distribution of the MTHFR C677T mutation did not differ between patients and controls (P = .41) (Figure 2).

All 17 patients with measurement of the 20210*A allele of the prothrombin gene had the normal "wild-type" gene, which was not different (P > .41) from 9 (3.8%) of 234 controls (Figure 2).

The distribution of the factor V Leiden G1691A mutation, the 4G/5G polymorphism of the PAI1 gene promoter, the 20210*A allele of the prothrombin gene, and the MTHFR C677T mutation in 17 patients with retinal vein occlusion and in 234 controls.

THROMBOPHILIC FACTORS

Measurements of levels of antigenic proteins C and S, anticardiolipin antibodies IgG and IgM, and homocysteine did not differ between patients and controls (P > .10; data not shown). The major component of the lupus anticoagulant, however, dilute Russell viper venom clotting time, differed between patients and controls. Of 14 patients with dilute Russell viper venom clotting times, 6 (43%) had prolonged (abnormal) times (>38.8 seconds) compared with 1 (3%) of 30 controls (P = .002, Fisher exact test) (Figure 4). The frequency of the 4G allele was 21 (0.62) of 34 in patients vs 196 (0.42) of 468 in controls (χ² = 5.1; P = .02).

DIFFERENCES BETWEEN PATIENTS AND CONTROLS IN MAJOR COAGULATION MEASURES

Thrombophilic Factors

Patients with vein occlusion were more likely to have high levels of hypofibrinolytic Lp(a) (>35 mg/dL)²²,²³ (8/16 [50%]) than controls (5/40 [13%]) (χ² = 9; P = .003) (Figure 4). Patients were more likely to have high levels of the major determinant of fibrinolysis, PAI-Fx. Levels of PAI-Fx were high (>22 U/L)²² in 6 (38%) of 16 patients.
compared with 1 (2%) of 40 controls ($P = .001$, Fisher exact test) (Figure 4).

Hypofibrinolytic disorders often occurred in clusters that were present with and without inclusion of the 3 patients who also had osteonecrosis. Of the 6 patients with high PAI-Fx levels, 4 (67%) also had high Lp(a) levels, and 2 (33%) also had prolonged dilute Russell viper venom clotting times. Of the 8 patients with high Lp(a) levels, 4 (50%) had high PAI-Fx values.

Of the 6 patients homozygous for the 4G/4G polymorphism in the promoter sequence of the PAI1 gene, 5 had measures of Lp(a). Of these 5 patients, 4 (80%) also had high Lp(a) levels; of 9 patients heterozygous for the 4G allele, 4 (44%) also had high Lp(a) levels, whereas the 2 patients with the 5G/5G genotype had normal Lp(a) levels.

When classifying the patients and controls by the PAI1 gene polymorphism, patients homozygous for the 4G allele had much higher median Lp(a) levels than controls (62 vs 5.3 mg/dL; $P = .05$) (Figure 5). Patients heterozygous for the 4G/5G trait also had higher median Lp(a) levels than controls heterozygous for the 4G/5G allele (16 vs 3.3 mg/dL; $P = .06$) (Figure 5). Patients with the 5G/5G genotype had higher median Lp(a) levels than controls with the same genotype (17.5 vs 6.7 mg/dL), but this difference was not significant ($P = .62$) (Figure 5).

When the relationships between the PAI1 gene and Lp(a) levels were compared in patients vs controls, the slopes of the 2 regression lines differed ($P = .04$) (Figure 6). In patients, but not in controls, increased 4G alleles for the PAI1 gene were associated with increased Lp(a) levels (Figure 6).

**Lipids and Lipoprotein-Cholesterol Levels**

Of 16 patients with vein occlusion having lipid profiles in the fasting state, 10 (63%) had high total cholesterol levels ($>5.17$ mmol/L [$>200$ mg/dL]), 2 (13%) had high triglyceride levels ($>2.82$ mmol/L [$>250$ mg/dL]), 1 (6%) had low high-density-lipoprotein–cholesterol levels ($<0.90$ mmol/L [$<35$ mg/dL]), and 8 (50%) had high low-density-lipoprotein–cholesterol levels ($>3.36$ mmol/L [$>130$ mg/dL]).

In our study, patients with retinal vein occlusion were more likely than healthy normal controls to have the heritable thrombophilic factor V Leiden G1691A mutation and the thrombophilic antiphospholipid lupus anticoagulant. They were also more likely to have homozygosity or heterozygosity for the heritable 4G PAI1 allele, and accompanying this, were much more likely to have high levels of the hypofibrinolytic PAI-Fx. Patients with retinal vein occlusion who were homozygous for the 4G PAI1 allele were more likely than controls to have high Lp(a) levels, a double dose of hypofibrinolytic factors that, we postulate, contribute to retinal vein occlusion. Patients were also more likely than controls to have high levels of the heritable hypofibrinolytic Lp(a). The lipoprotein Lp(a) is postulated to be hypofibrinolytic by virtue of its sequence homology with plasminogen, thus competing with plasminogen for fibrin binding and hindering fibrin digestion. It also can interact with cellular plasminogen receptors with a plasminogenlike affinity. Most relevant to the present study, where 4G/4G homozygosity of the PAI1 gene was associated with high Lp(a) levels, Lp(a) enhanced PAI1 transcription and expression on cultured endothelial cells. As noted by Rosendaal, venous thrombosis is a multicausal disease; usually more than 1 coagulation abnormality needs to be present before thrombosis occurs. “The younger an individual, the more risk factors are required to precipitate thrombosis.” This is relevant to retinal vein occlusion because systemic workup for the cause of the disease is usually limited to younger
patients. The common concurrence of other venous thrombosis and central retinal vein occlusion should promote studies of the pathogenesis of this type of conjoint thrombosis.

Whether synergism between the patients’ high low-density-lipoprotein–cholesterol and high Lp(a) levels, known to cause arterial occlusive disease,26 could have played any role in their ophthalmic disease is unknown. However, 2 earlier studies1,2 implicated hyperlipidemia as a risk factor for retinal vein occlusion, consistent with the findings of the present study.

In agreement with previous studies of retinal vein occlusion, we found a high prevalence of the hypofibrinolytic Lp(a),7 the thrombophilic lupus antibody,2 and thrombophilic resistance to activated protein C.8-10 A new finding in the present study was that patients with vein occlusion were more likely to be heterozygous or homozygous for the 4G PAI1 allele than controls (P = .03) and were more likely than controls to have high levels of hypofibrinolytic PAI-Fx (P = .001). These findings have not, to our knowledge, been reported previously in retinal vein occlusion.

A second new finding in the present study was that patients with vein occlusion with the 4G/4G and 4G/5G genotypes were more likely to have high Lp(a) levels than normal controls with the same genotypes. Furthermore, the slopes of the regression lines for the relationship between the PAI1 gene and Lp(a) differed between patients and controls, suggesting that in patients with vein occlusion, but not in healthy controls, increased mutant alleles for the PAI1 gene were associated with Lp(a).34

In aggregate, patients with retinal vein occlusion were likely to have heritable thrombophilia, hypofibrinolysis, or both. As might be expected from this mixed increased prevalence of thrombosis and hypofibrinolysis, 7 (42%) of the patients had thrombi in other vascular beds; 3 (18%) had sustained osteonecrosis (thought to be caused by venous thrombosis in bone),14,19,22,23,26 and 4 (24%) had a history of deep venous thrombosis, known to be caused by thrombophilia and hypofibrinolysis.14,19,22,23

Before the discovery of resistance to activated protein C, mediated by the mutant factor V Leiden gene, although diabetes mellitus, hyperlipidemia, hypofibrinolysis, and thrombophilia had been identified as causes of retinal vein occlusion,1,7,13 other retrospective studies35-38 of the causes of central retinal vein occlusion in young patients were inconclusive. The newly discovered, most common heritable thrombophilia, however, resistance to activated protein C, appears to be 4 to 5 times more common than other known inherited thrombophilias or hypofibrinolys in young patients with central retinal vein occlusion.8-10 The PCR assay for polymorphism of the PAI1 gene has only recently become available17,25 and, to our knowledge, has not been used previously in the evaluation of patients with retinal vein occlusion. Major recent advances have been made in coagulation measurements that allow the diagnosis of the most common coagulation disorders, with the recognition of resistance to activated protein C10 and with cDNA PCR measurements of the mutant factor V Leiden gene12,16,19 and the 4G/4G polymorphism of the PAI1 gene.17,25 By enabling the diagnosis of common, heritable coagulation disorders, these cDNA PCR methodological advances8-10,16,19,25 provide a higher level of certainty, unaffected by age,19,16,25 that thrombophilia and hypofibrinolysis are causes of retinal vein occlusion. Furthermore, the diagnosis of heritable thrombophilia, hypofibrinolysis, or both, as putative causes of retinal vein occlusion provides important prospective diagnostic insight into the high likelihood of other thrombotic disorders9 in patients with central retinal vein occlusion. Thus, 18% of our patients had a history of osteonecrosis,19,10,22 and 24% had other thrombotic events exclusive of osteonecrosis, primarily deep venous thrombosis. The recognition of resistance to activated protein C in patients with retinal vein occlusion also has important ramifications for their first-degree relatives.39 In 177 subjects from 34 kindreds in which 1 family member had resistance to activated protein C, Svensson and Dahlback39 reported that 27% had a history of thrombosis. At age 45 years, the likelihood that a subject with resistance to activated protein C would be free of thrombosis was only 59% compared with 97% in subjects without resistance to activated protein C.

The recognition of frequent hypofibrinolysis and thrombophilia as possible causes of retinal vein occlusion1,7,13 calls for further prospective analyses and studies. Patients with retinal vein occlusion, particularly those with a family history of thrombosis,8 should have tests designed to diagnose heritable thrombophilias and hypofibrinolysis, including assessment of the factor V Leiden G1691A mutation. These analyses should include studies of the 4G/5G polymorphism of the PAI1 gene promoter, the prothrombin gene, the MTHFR C677T mutation, PAI-Fx, Lp(a), the lupus anticoagulant antibody, and anticardiolipin antibodies. Patients with these predominantly heritable abnormalities are at increased risk of both venous and arterial thrombi.

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Corresponding author: Charles J. Glueck, MD, Cholesterol Center, Jewish Hospital, 3200 Burnet Ave, Cincinnati, OH 45229 (e-mail: glueckch@healthall.com).

ARCH OPHTHALMOL/VOL 117, JAN 1999

48

Figure 6. Relationships between 4G/5G polymorphisms of the PAI1 gene promoter and lipoprotein Lp(a) in 16 patients with retinal vein occlusion and 40 controls. The slopes differ (P = .04).


A look at the past . . .

Dr Edward L. Holmes has resigned the Professorship of Ophthalmology and Otolaryngology in the Rush Medical College of Chicago, which he has held since 1860. He will be succeeded by Dr Harper of the Chicago University, with which the Rush Medical College will be united. Dr Holmes will be seventy years of age on February 11, 1898. He was the founder of the Illinois Eye and Ear Infirmary, one of the few State institutions of its kind in America. His valuable contributions to the science and practice of ophthalmology are well known. His genuine kindness and sterling character are highly appreciated by all who have had the privilege of being acquainted with him.