Role of Fibrinogen Levels and Factor XIII V34L Polymorphism in Thrombolytic Therapy in Stroke Patients

Rocio González-Conejero, PhD; Israel Fernández-Cadenas; Juan A. Iniesta, MD, PhD; Joan Martí-Fabregas, MD, PhD; Victor Obach, MD; José Alvarez-Sabín, MD, PhD; Vicente Vicente, MD, PhD; Javier Corral, PhD; Joan Montaner, MD, PhD; for the Proyecto Ictus Research Group

Background and Purpose—The identification of genetic and environmental factors that could improve the benefit/risk ratio of thrombolytic therapy in patients with ischemic stroke is crucial.

Methods—We studied the role in the efficacy and side-adverse effects of thrombolytic therapy in stroke of 2 factors involved in the structure and stability of fibrin clot: fibrinogen levels and factor XIII (FXIII) V34L, a common and functional polymorphism. Our study enrolled 200 consecutive patients with stroke who received intravenous recombinant tissue plasminogen activator.

Results—Patients with FXIII V/V genotype and low fibrinogen (<3.6 g/L) displayed the best clinical outcome. In contrast, carriers of the L34 variant and high fibrinogen levels showed almost no clinical response. Moreover, patients with high fibrinogen levels at admission displayed higher mortality than patients with lower fibrinogen levels (22.6% versus 9.7%, P = 0.027; OR = 2.72). The FXIII V34L polymorphism also associated with mortality: 20.0% of L34 carriers but 9.1% of patients with V/V genotype died after thrombolytic therapy (P = 0.034; OR = 2.50). The deleterious effect of this variant seemed to be exacerbated by high levels of fibrinogen, supporting the role of fibrinogen levels in determining the hemostatic consequences of the FXIII polymorphism.

Conclusions—Our study identifies 2 markers involved in fibrin formation associated with the efficacy of thrombolytic therapy and early mortality rates in patients with ischemic stroke. These markers could be useful to identify patients with stroke suitable for a safe thrombolytic therapy. (Stroke. 2006;37:000–000.)

Key Words: genetics ■ risk factors ■ stroke ■ thrombolysis

Thrombolytic therapy is used to achieve the most important objective in arterial occlusion, both in myocardial infarction and ischemic stroke: to quickly restore flow after occurrence of an acute occlusion. Unfortunately, in both disorders there is a significant heterogeneity in the effectiveness and side effects of the treatment. Up to 40% of patients with myocardial infarction treated with thrombolytic drugs do not achieve optimal tissue perfusion. Moreover, there is a considerable mortality associated with thrombolytic therapy in ischemic stroke mainly attributable to bleeding complications. Uncertainty exists about the factors considered as predictors of the safety and efficacy after treatment with recombinant tissue plasminogen activator (r-tPA) in patients with acute ischemic stroke. Very recently, it has been proposed that both elevated plasma levels of cellular fibronectin, and fibrin(ogen) degradation products could predict hemorrhagic transformation after thrombolytic therapy in acute ischemic stroke.

The mechanical strength and resistance to fibrinolysis are enhanced by the formation of covalent γ-glutamyl-γ-lysine bonds between fibrin monomers, reaction that is catalyzed by the activated factor XIII (FXIII). A common polymorphism in the FXIII gene—V34L—is one of the most important functional polymorphisms described so far in the hemostatic system. This polymorphism affects a key valine residue, 3 amino acids upstream to the thrombin cleavage site. It has been clearly demonstrated that the higher rate of proteolytic truncation of L34 variant resulted in earlier activation of FXIII and, consequently, accelerated the cross-linking of fibrin γ- and α-chains and the cross-linking of α2-PI to fibrin. Moreover, this polymorphism also has a significant effect on fibrin clot structure, probably through the alteration of fibrin cross-linking kinetic. Moreover, turbidometric measurements and electron microscopy confirmed the presence of thinner fibrin fibers and decreased porosity in the presence of L34. In spite of contradictory results, paradoxically the FXIII V34L polymorphism might be a relatively weak protective factor in arterial and venous thrombosis.
ingly, the role of this polymorphism on thrombotic disorders could be modulated by certain genetic or environmental factors.12

The relevance of this polymorphism on thrombolytic therapy was recently assessed by our group in acute myocardial infarction. In 2 different groups of patients, we showed clinical evidence that the presence of the L34 variant reduced the efficacy of this therapy in acute myocardial infarction.13,14

Additionally, fibrinogen levels also influence the features of the fibrin clot. High fibrinogen concentrations lead to the formation of a fibrin clot with thin and tightly packed fibers, more resistant to fibrinolytic enzymes.15 Accordingly, fibrinogen levels before the injection of r-tPA could affect the efficiency and side-effects of the thrombolytic treatment.

The aim of our study was to investigate the role, both in efficacy and adverse side effects, of these 2 factors in a wide cohort of consecutive patients with ischemic stroke treated with thrombolytic therapy.

Subjects and Methods

Selection of Patients

We prospectively studied 200 patients who experienced an acute internal carotid artery territory stroke (51% men; mean age 70.2 ± 11.1 years) admitted consecutively into 3 university hospitals between January 2003 and February 2005, who received intravenous r-tPA. Thrombolytic therapy was administered at a dose of 0.9 mg/kg body weight, with an upper dose limit of 90 mg per patient. Ten percent of the total dose was given as a bolus over 1 to 2 minutes, followed by a 60-minute infusion of the remaining dose. Any of the patients had inflammatory, hematological, or infectious diseases, cancer, or severe renal or liver failure. The ethics committee approved the protocol in each center, and informed consent was obtained from patients or their relatives. Medical history recording potential stroke risk factors (age, hypertension, current/former smoker, hypercholesterolemia, type I or II diabetes, atrial fibrillation, and previous ischemic cerebrovascular or coronary heart disease), clinical examination, blood and coagulation tests (including fibrinogen levels, which were measured by Von Claus-thrombin standard method), 12-lead ECG, chest radiography, and noncontrast cranial computed tomography (CT) scan were performed at admission. Stroke subtype was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.16

Stroke severity was assessed by a certified neuroradiologist using the National Institutes of Health Stroke Scale (NIHSS) at admission at 1, 2, 12, 24, 48 hours, and at discharge. Neurological deterioration was defined as death or an increase of >4 points in the NIHSS score between 2 examinations. Finally, modified Rankin scale (mRS) was used to assess clinical outcome at 90 days (mRS 3 mol/L). We defined good outcome as mRS score ≤1.

Serial transcranial Doppler (TCD) assessment was performed by certified sonographers with extensive experience in monitoring recanalization in acute stroke. A standard set of diagnostic criteria was applied to diagnose arterial occlusion, according to the Thrombolysis in Brain Ischemia (TIBI) grading system.17 After the TCD examination performed in the emergency department on admission before r-tPA administration, recanalization was evaluated by the end of r-tPA infusion (1 hour) and again at 24 hours of symptoms onset. Recanalization on TCD was diagnosed as partial when blunted or dampened signals appeared in a previously demonstrated absent or reduced TCD signal.18 Revascularization on TCD was diagnosed as total when Doppler signals reappeared after r-tPA administration. Partial recanalization was considered as being associated with neurological deterioration.

Early CT signs of infarction were evaluated in the first radiological examination, and presence of hemorrhagic transformation was evaluated on a second cranial CT, performed 24 to 36 hours after treatment, or when a neurological deterioration occurred. Hemorrhagic transformation subtype was classified according to the ECASS II criteria.19 We considered symptomatic hemorrhagic transformation as being associated with neurological deterioration.

All CT and TCD examinations were performed by 1 investigator in each center blinded to the clinical and analytical data.

Genetic Analysis

The FXIII V34L polymorphism was determined by genomic polymerase chain reaction–allele specific restriction assay, as previously described.18 Genotyping was performed blinded to the clinical and analytical data.

Statistical Analysis

Continuous variables were tested for normal distribution by Kolmogorov-Smirnov test. The normal distributed continuous variables were described as mean ± SD and those that did not follow normal distribution were described as median (interquartile ranges). Categorical variables were expressed as percentages. Comparison between 2 groups were performed by Mann Whitney U and Student t tests. Univariate statistical analysis was performed by the χ2 test. The strength of the association of major risk factors and the polymorphism with the occurrence of disease was estimated by calculation of the odds ratio (OR) with the EpInfo software. The differences with a 2-tailed P value < 0.05 were considered significant.

Results

Clinical Features of Patients

The prevalence of the FXIII V34L polymorphism was similar to that described by our group in the Spanish population.18 One hundred and twenty-eight patients (64.0%) carried the V/V genotype, 8 (4.0%) were homozygous (L/L), and 64 (32.0%) were heterozygous (V/L). Patients with FXIII V/V genotype displayed similar clinical features than patients carrying the L34 allele (Table 1).

The distribution of fibrinogen values in our sample showed a normal distribution, with a mean value of 3.57 ± 0.79 g/L. Accordingly, we considered patients with high fibrinogen levels those with values ≥ 3.6 g/L. Table 1 also shows the clinical features of patients according to the fibrinogen levels observed at admission.

Efficacy of Thrombolytic Therapy

Recanalization rates increased significantly with time. Thus, the percentage of patients with recanalization 24 hours after thrombolysis was almost twice compared with that observed 1 hour after the treatment (74.5% versus 43.5%; P < 0.001).

The FXIII V34L polymorphism did not significantly modify the rate of recanalization, although carriers of the L34 allele displayed a trend to recanalization resistance at all tested times (Table 2). Fibrinogen levels did not affect the recanalization after r-tPA injection (Table 2).

When exploring the combination of both factors, we observed a lower efficacy of thrombolytic therapy in carriers of the L34 variant who presented high fibrinogen levels, although these differences only achieved statistical significance at 1 hour when comparing with patients V/V and high fibrinogen levels (21.4% versus 57.7%, P = 0.028; Table 2).

This treatment significantly ameliorated the clinical features of patients. Thus, the NIHSS at admission was 16 (11 to 19), but 6 (1 to 15) at discharge (P < 0.001). Interestingly, the clinical evolution was different according to 2 factors in-
TABLE 1. Selected Risk Factors in Patients With Ischemic Stroke and Thrombolytic Treatment According to the FXIII V34L Polymorphism and Fibrinogen Levels

<table>
<thead>
<tr>
<th>FXIII V34L Polymorphism</th>
<th>Fibrinogen Levels, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V/V</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>Male sex, % (n)</td>
<td>50.8 (62/122)</td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
<td>50.4 (59/117)</td>
</tr>
<tr>
<td>Smoker, % (n)</td>
<td>30.0 (33/110)</td>
</tr>
<tr>
<td>DM, % (n)</td>
<td>19.3 (23/119)</td>
</tr>
<tr>
<td>Hypercholesterolemia, % (n)</td>
<td>24.4 (29/119)</td>
</tr>
<tr>
<td>AF, % (n)</td>
<td>40.3 (48/119)</td>
</tr>
<tr>
<td>CHD, % (n)</td>
<td>15.7 (17/108)</td>
</tr>
<tr>
<td>Previous stroke, % (n)</td>
<td>11.2 (13/116)</td>
</tr>
<tr>
<td>Baseline NIHSS, % (n)</td>
<td>16 (11–19)</td>
</tr>
<tr>
<td>Minutes to treatment</td>
<td>156±47</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.56±0.72</td>
</tr>
<tr>
<td>Early CT changes, % (n)</td>
<td>41.6 (42/101)</td>
</tr>
<tr>
<td>Anti-platelet agents, % (n)</td>
<td>18.0 (16/89)</td>
</tr>
<tr>
<td>Basal glucose, mg/dL</td>
<td>117 (103–140)</td>
</tr>
<tr>
<td>(interquartile range)</td>
<td>8 (6/75)</td>
</tr>
</tbody>
</table>

Hypertension was defined as blood pressure exceeding 140 mm Hg systolic or 90 mm Hg diastolic on repeated observations over 3 months or if no blood pressure values were available, when the subject was under treatment with chronic antihypertensive therapy. Current/former smoker, subject smoked >10 cigarettes per day. Minutes to treatment, minutes from symptoms onset until the starting of the treatment. Hypercholesterolemia was defined as a total serum cholesterol level >/=7.2 mmol/L (220 mg/dL).

CHD indicates coronary heart disease; DM, diabetes mellitus; AF, atrial fibrillation.

Low Platelets: <150,000/mL.

volved in the features of the fibrin clot. The clinical response in patients with FXIII V/V genotype was accurate (NIHSS from 16 to 5; mRS 3 mol/L ≤1: 61.7%). In contrast, the NIHSS score displayed minor variations after the thrombolytic therapy and the percentage of patients with mRS 3 mol/L ≤1 was lower in carriers of the L34 variant (Table 2). Fibrinogen levels at admission also influenced the clinical response. Thus, the thrombolytic therapy was clinically excellent in patients with low levels, but not so efficient in patients with high levels (Table 2). Remarkably, the interaction of the 2 parameters significantly correlated with the clinical response. Patients with V/V genotype and low

TABLE 2. Efficacy of Thrombolytic Therapy According to the FXIII V34L Polymorphism and Fibrinogen Concentrations

<table>
<thead>
<tr>
<th>Recanalization</th>
<th>V/V</th>
<th>L</th>
<th>P</th>
<th>&lt;3.6</th>
<th>≥3.6</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour, % (n)</td>
<td>44.9 (40/89)</td>
<td>37.3 (19/51)</td>
<td>0.375</td>
<td>49.5 (45/91)</td>
<td>41.3 (26/63)</td>
<td>0.317</td>
</tr>
<tr>
<td>6 hours, % (n)</td>
<td>60.9 (53/87)</td>
<td>54.0 (27/50)</td>
<td>0.429</td>
<td>67.4 (58/88)</td>
<td>54.0 (27/50)</td>
<td>0.119</td>
</tr>
<tr>
<td>24 hours, % (n)</td>
<td>74.7 (65/87)</td>
<td>68.8 (33/48)</td>
<td>0.457</td>
<td>80.2 (69/86)</td>
<td>74.5 (38/51)</td>
<td>0.434</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurological Symptoms</th>
<th>V/V</th>
<th>L</th>
<th>P</th>
<th>&lt;3.6</th>
<th>≥3.6</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS 1 hour</td>
<td>14 (9–18)</td>
<td>14 (8–18)</td>
<td>0.872</td>
<td>12 (8–17)</td>
<td>15 (9–19)</td>
<td>0.071</td>
</tr>
<tr>
<td>NIHSS 2 hours</td>
<td>10 (5–16)</td>
<td>14 (6–17)</td>
<td>0.115</td>
<td>10 (6–16)</td>
<td>15 (7–19)</td>
<td>0.059</td>
</tr>
<tr>
<td>NIHSS 12 hours</td>
<td>9 (4–16)</td>
<td>13 (6–18)</td>
<td>0.168</td>
<td>9 (4–15)</td>
<td>15 (6–18)</td>
<td>0.006</td>
</tr>
<tr>
<td>NIHSS 24 hours</td>
<td>9 (4–11)</td>
<td>16 (4–17)</td>
<td>0.290</td>
<td>8 (3–14)</td>
<td>14 (5–18)</td>
<td>0.015</td>
</tr>
<tr>
<td>NIHSS 48 hours</td>
<td>7 (1–16)</td>
<td>12 (4–18)</td>
<td>0.037</td>
<td>7 (2–15)</td>
<td>14 (3–18)</td>
<td>0.015</td>
</tr>
<tr>
<td>NIHSS at discharge</td>
<td>5 (1–15)</td>
<td>11 (3–17)</td>
<td>0.112</td>
<td>4 (1–14)</td>
<td>5 (1–19)</td>
<td>0.195</td>
</tr>
</tbody>
</table>

| MRS 3 mol/L ≤1, % (n)  | 33.6 (37/110) | 36.5 (23/63) | 0.703 | 47.5 (38/80) | 20.4 (11/54) | <0.001 |

V/V with fibrinogen levels <3.6g/L and L carriers with ≥3.6g/L fibrinogen at admission.
fibrinogen levels displayed the best clinical outcome. In contrast, carriers of the L variant and high fibrinogen levels showed almost no clinical response (Table 2).

### Hemorrhagic Transformation

Hemorrhagic transformation was observed in 19.4% of patients; 4.8% displayed symptomatic hemorrhagic transformation. Patients carrying the L34 variant presented a higher tendency to hemorrhagic transformation than patients with V/V genotype (23.9% versus 16.8%, respectively) although these differences did not reach statistical significance (P = 0.241; Table 3). However, the incidence of severe hemorrhage (hemorrhagic infarction type 1 and type 2) was significantly higher among carriers of the L34 variant (11.9% versus 3.4%; P = 0.030; Table 3). Interestingly, FXIII polymorphism did associate with the incidence of symptomatic hemorrhagic transformation after thrombolytic therapy. Thus, treatment with r-tPA caused brain bleedings with neurological deterioration in 8.8% of L34 carriers but 2.5% of patients with V/V genotype (P = 0.049; Table 3). In contrast, fibrinogen levels did not determine a major risk for hemorrhagic transformation associated with r-tPA treatment, as shown in Table 3.

Patients with high fibrinogen levels and the FXIII L34 variant reported the highest rates of hemorrhagic transformation (especially severe hemorrhage) and symptomatic hemorrhage after the thrombolytic therapy (Table 3), but these differences did not achieve statistical significance, probably attributable to the small size of the sample (Table 3).

### Mortality Rates

The incidence of mortality within 3 months from the symptoms onset among these patients treated with r-tPA was 12.9%, a similar value to that recently published.4,5 The FXIII V34L polymorphism was significantly associated with the incidence of early mortality associated with thrombolytic therapy in patients with ischemic stroke. Thus, 20.0% of L34 carriers died after thrombolytic therapy, whereas only 9.1% of patients with V/V genotype (P = 0.034; Figure A). Accordingly, carriers of the L34 variant had 2.5-fold risk to die after thrombolytic therapy than patients with V/V genotype. Logistic regression analysis revealed that age (OR = 1.06; 95% CI, 1.00 to 1.13; P = 0.043), atrial fibrillation (OR = 2.78; 95% CI, 1.00 to 7.74; P = 0.05), basal NIHSS (OR = 1.16; 95% CI, 1.02 to 1.31; P = 0.016), and the FXIII V34L polymorphism (OR = 2.95; 95% CI, 1.07 to 8.11; P = 0.035) were independent predictors for mortality after adjusting by risk factors and etiology.

The concentration of fibrinogen also had a significant effect on mortality. Thus, patients with ≥3.6g/L of fibrinogen had 2.7-fold risk to die than patients with fibrinogen levels <3.6g/L (P = 0.027; Figure B). Interestingly, we observed a significant interaction between these 2 factors in the mortality associated with the thrombolytic therapy. The role of the FXIII L34 variant seemed to be specially exacerbated in patients with high fibrinogen levels at admission (35.7% died in contrast to 8.8% of patients with V/V genotype and low fibrinogen levels; P = 0.040; OR = 5.74; Figure C).

### Discussion

Because thrombolytic therapy is the only effective treatment for ischemic stroke, the identification of factors that can improve the benefit/risk ratio of r-tPA administration is of critical importance. In this setting, any element involved in the formation and features of the fibrin clot might play a significant role. Fibrinogen levels just before the injection of r-tPA could be an important factor. Certainly, high fibrinogen levels lead to a less porous and therefore less permeable fibrin clot with thin fibers and have been associated with an increased risk of myocardial infarction and premature coronary artery disease. Here, our data support that fibrinogen levels at admission might play a role in the thrombolytic therapy in stroke, particularly in the efficacy and adverse side-effects of this treatment. Patients with high fibrinogen levels displayed worse clinical response and have 2.7-fold risk to die as a consequence of this therapy.

Additionally, in this study we have identified the first genetic trait that could be associated with r-tPA response among patients with ischemic stroke. A common polymorphism affecting the formation and structure of the fibrin clot, the FXIII V34L, could play a role in the side-effects of this therapy. Our results support that carriers of the FXIII L34 variant have increased symptomatic bleeding rates and, consequently, this polymorphism has a relevant and independent effect on the incidence of early mortality. Thus, carriers of the L34 variant had 2.5-fold risk to die after thrombolytic therapy than patients with V/V genotype. These findings could be
explained by the different structure of the clot associated with the FXIII V34L polymorphism. The thinner fibers of the clot observed in patients with the L34 variant might favor the risk of hemorrhagic transformation observed in these patients. Interestingly, it has been suggested as an opposite role of the FXIII V34L polymorphism in ischemic and hemorrhagic stroke. Thus, although the L34 variant seems to protect against brain infarct (and outweighed the effect of smoking), it significantly increases the risk of hemorrhagic stroke. Additionally, a recent report supports a hemorrhagic role of the L34 variant, because it increases the risk of spontaneous subconjunctival hemorrhage. All these data support our results.

Finally, we observed a remarkable interaction between these 2 factors involved in fibrin formation. As expected, patients who simultaneously had both deleterious factors (FXIII L34 carriers with 3.6 g/L of fibrinogen at admission) showed a much worse clinical response to the fibrinolytic therapy. Moreover, 35.7% of patients with this combination died after the thrombolytic therapy, but only 8.8% of patients with V/V genotype and <3.6 g/L of fibrinogen. Recent in vitro and clinical data support that the hemostatic role of the FXIII V34L polymorphism might depend on the fibrinogen levels, which supports our results. First, the fibrinogen concentration is an important determinant of the effect of FXIII V34L on clot structure. Thus, clots prepared from L/L samples were less permeable at lower fibrinogen concentrations and more permeable at higher fibrinogen levels when compared with V/V samples. At intermediate fibrinogen concentrations, the fibrin fiber diameters were similar across genotypes. Second, the protective effect of the factor XIII V34L polymorphism on the risk of deep venous thrombosis is dependent on the fibrinogen level.

In conclusion, our study found 2 factors involved in the formation and features of the fibrin clot that might play a significant role on the efficacy and mortality associated with thrombolytic therapy in patients with stroke: fibrinogen levels and the FXIII V34L polymorphism. The main limitation of this study is the relative small size of the sample. Further studies including more patients from different popu-
lations are required to confirm these findings. Moreover, it would be relevant to evaluate the association of these factors with others (genetic and environmental) that might influence the efficacy and side-effects of the thrombolytic therapy. For instance, the duration of TCD monitoring to assess r-tPA–induced recanalization was not properly controlled in this study, and an enhancement of ultrasound on r-tPA recanalization has been recently described. Therefore, wider studies controlling these factors and including new polymorphisms are required. These studies might identify those patients that could obtain more benefit of this treatment, and avoid it in those patients with a high probability of severe adverse side-effects.

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Disclosures

None.

References

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