Factor XIII Val 34 Leu
A Novel Association With Primary Intracerebral Hemorrhage

Andrew J Catto, BSc, MRCP; Hans P Kohler, MD; Sally Bannan, BSc; Max Stickland, AIBMS; Angela Carter, BSc; Peter J Grant, MD, FRCP

Background and Purpose—A common G-to-T point mutation (Val 34 Leu) in exon 2 of the α-subunit of the factor XIII is strongly negatively associated with the development of myocardial infarction. This result suggests that factor XIII Val 34 Leu is interfering with the formation of cross-linked fibrin. The role of factor XIII Val 34 Leu in the pathogenesis of cerebral infarction and primary intracerebral hemorrhage is unknown.

Methods—Six hundred twelve patients with acute stroke, defined by World Health Organization criteria and cranial CT, and 436 age-matched control subjects free of cerebrovascular disease were genotyped for the factor XIII Val 34 Leu mutation. Venous blood was drawn for the determination of hemostatic variables and lipids. Factor XIII genotype was determined through a single-stranded conformational polymorphism technique and plasminogen activator inhibitor (PAI)-1 4G/5G promoter genotype by allele-specific polymerase chain reaction.

Results—The mutation was more frequent in patients with primary intracerebral hemorrhage (n=62) (54.8%; P=.05) than in control subjects (41.7%) or in patients with cerebral infarction (n=529) (46.5%; P=.22). There was no relationship between PAI-1 levels and the PAI-1 4G/5G genotype.

Conclusions—There was a slightly higher incidence of factor XIII Val 34 Leu in patients with PICH. This may be related to impaired cross-linking of fibrin and/or coagulation proteins. (Stroke. 1998;29:813-816.)

Key Words: factor XIII ■ fibrin ■ intracerebral hemorrhage ■ mutation ■ risk factors

The importance of the hemostatic system in the pathogenesis of atherothrombotic disorders has been demonstrated in prospective studies. These have identified increased concentrations of fibrinogen, factor VII, and suppressed fibrinolysis resulting from elevated PAI-1 in relation to MI. Elevated levels of tissue plasminogen activator and fibrinogen are risk factors for CI. In MI, abnormal fibrin structures have been reported, and factor XIII may have a role in this process.

Factor XIII is activated by thrombin, cleaving the α-subunit between Arg37 and Gly38 releasing the N-terminal 37 residues (activation peptides). The α-subunit selectively cross-links fibrin monomers and other substrates, including α-2 antiplasmin. Cross-linked fibrin shows increased resistance to fibrinolysis and enhanced mechanical strength.

We have studied a G-to-T point mutation in codon 34 of exon 2 that codes for a valine-leucine (factor XIII Val 34 Leu) change in the α-subunit, 3 amino acids from the thrombin activation site, in 398 subjects examined for investigation of coronary artery disease determined by angiography. The prevalence of the mutation was lower in subjects with MI, indicating that factor XIII Val 34 Leu is protective against atherothrombotic disease. However, in subjects with factor XIII Val 34 Leu and MI, higher PAI-1 levels and an increased frequency of the PAI-1 promoter 4G/4G genotype suggest that inhibition of fibrinolysis negates the protective effects of factor XIII Val 34 Leu, which supports a functional role for this mutation.

In view of the apparently protective role of factor XIII Val 34 Leu in the pathogenesis of MI, we hypothesized that possession of the mutation might either be protective against CI or predispose to the development of PICH through the formation of weaker fibrin structures. The aims of this study were therefore to investigate (1) the association of the factor XIII Val 34 Leu genotype and the phenotypes CI and PICH and (2) the relationships between PAI-1 levels, the PAI-1 4G/5G genotype, and the factor XIII Val 34 Leu mutation in the pathogenesis of these discrete phenotypes.

Subjects and Methods

Study Population
We recruited 612 consecutive inpatients with acute stroke defined by World Health Organization Criteria (excluding subarachnoid hemorrhage) and 435 healthy control subjects closely matched for age, sex, and domicile and free of clinically detectable cerebrovascular disease, as described previously. All subjects gave informed consent according to a protocol approved by the Research Ethics Committees.

Identification of Stroke Phenotype
To determine the stroke phenotype (CI or PICH), cranial CT was performed within 10 days of stroke. Cases with CI were classified...
Selected Abbreviations and Acronyms
CI = confidence interval
IC = intracerebral hemorrhage
MI = myocardial infarction
PAI = plasminogen activator inhibitor
PCR = polymerase chain reaction
PICH = primary intracerebral hemorrhage

Analysis of Circulating PAI-1 Activity
Nonfasting venous blood samples were taken from both cases (within 10 days of stroke) and controls between 8 and 11 AM and placed in 0.1 mol/L sodium citrate (9:1, vol/vol) in ice water for assay of PAI-1 activity, then centrifuged at 2500 g at 4°C for 30 minutes. Samples were stored at −80°C. PAI-1 activity was measured by a chromogenic assay (Spectrolyse, Biopool). The inter- and intra-assay coefficients of variation for the assay were 5.7% and 3.1%, respectively. Cholesterol and total triglyceride levels were measured with the Hitachi 747 automated analyzer (Boehringer Mannheim).

Extraction of DNA and Genotype Determinations
Genomic DNA was extracted from 10 mL venous blood and anticoagulated with 1.6 mg/mL EDTA, with use of a detergent/salt exchange method described previously.13

Identification of the Factor XIII Val 34 Leu Genotype
A 183-bp fragment of exon 2/intron B of the factor XIII gene was amplified by PCR, with 5'-GACCTTGTAAGTCAAAAATGTC as 5' primer and 5'-GACCTTGTAAGTCAAAAATGTC as the 3' primer. PCR reaction conditions were as described previously.8 To detect the substitution of guanine to thymine (Val 34 Leu), we used a random selection of samples using dye-labeled terminators (ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer) on an ABI 373A sequencer. There were no discrepancies between genotypes determined by the two methods.

Identification of the PAI-1 4G/5G Promoter Genotype
The PAI-1 4G/5G promoter polymorphism was determined for each subject by PCR amplification of genomic DNA with use of allele-specific primers and PCR conditions, as previously described. Samples were classified into one of three possible genotypes (4G/4G, 5G/5G, or heterozygous 4G/5G) and validated using known genotypes verified by direct sequencing.

Statistical Methods
Body mass index, cholesterol, triglycerides and PAI-1 activity were log transformed. PAI-1 levels in cases and controls were compared using unpaired t tests. Levels were expressed as geometric means and antilogged, with 95% CIs. χ2 analysis was used to compare the genotype distributions. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs. The determinants of PICH were studied in a logistic regression model, with the covariates age, sex, current smoking, and antilogged, with 95% CIs.

Results
Characteristics of the Study Population
The median age of the case patients (n = 612) and control subjects (n = 436) was 73.0 years. Conventional vascular risk factors, namely, ischemic heart disease, MI, diabetes mellitus, hypertension, and current smoking, were more common in the case patients (P < .01). Cholesterol levels were lower in cases than controls (5.1 mmol/L [95% CI, 3.4 to 7.3] and 5.8 mmol/L [CI, 5.6 to 5.9], respectively; P = .003). Factor XIII Val 34 Leu genotypes were available in all cases and controls; PAI-1 4G/5G genotypes were available in 558 cases and 172 controls. PAI-1 activity was measured in the first 312 cases and in 213 controls. In cases, the median PAI-1 level was 9.8 U/mL (95% CI, 8.8 to 10.9); in controls, 8.8 U/mL (CI, 7.8 to 10.1) (P < .0001).

Genotype Distribution in All Cases and Controls
The distribution of the factor XIII Val 34 Leu genotype in cases was G/G, 326 (53.3%); G/T, 240 (39.2%); and T/T, 46 (7.5%); in controls it was G/G, 254 (58.3%); G/T, 157 (36.0%); and T/T, 25 (5.7%). Distribution of the PAI-1 4G/5G genotypes in cases was 4/4, 148 (26.9%); 4/5, 270 (49.1%); and 5/5, 132 (24.0%); in controls it was 4/4, 60 (33.9%); 4/5, 84 (47.5%); and 5/5, 33 (18.6%). There was no difference in the distribution of the factor XIII Val 34 Leu (or PAI-1 4G/5G) genotypes between cases and controls (P = .21 and P = .13, respectively). There was no age relation with genotype (case patients < 60 years (n = 103) compared with age-matched control subjects (n = 80); χ2 = 0.16, P = .68), nor was there any relation in controls when analyzed only by age subdivided by decades (χ2 [test for trend] = 0.19, P = .20).

Genotype Frequencies in PICH
Table 1 demonstrates an excess of the mutation in patients with PICH (n = 62) compared with control subjects (P = .05). In the logistic regression model, significant independent variables for PICH were a past history of ischemic heart disease (P = .006) and diabetes mellitus (P = .04) but not factor XIII Val 34 Leu genotype (P = .22). In this model there was no interaction between ischemic heart disease and factor XIII Val 34 Leu genotype (P > .05).

There was no difference in distribution of the PAI-1 4G/5G genotype and factor XIII Val 34 Leu genotypes between...
cases of both PICH and CI and no significant relationships between factor XIII Val 34 Leu and other stroke risk factors.

Discussion

The results from this study indicate an association between possession of a G-to-T point mutation coding for factor XIII Val 34 Leu and PICH. We hypothesized that possession of factor XIII Val 34 Leu mutation might favor the formation of weaker fibrin structures, thereby protecting against CI or predisposing to PICH. This study supports our previous findings that factor XIII Val 34 Leu may play a significant role in the pathogenesis of vascular disorders. The lack of association of the mutation with ischemic stroke is interesting when contrasted with MI. However, this may be accounted for by the heterogeneous nature of ischemic stroke compared with that of MI.

The precise role for fibrin in the pathogenesis of cerebrovascular disease has not been established. However, the formation of cross-linked fibrin from fibrin monomer is pivotal for the development of a stable thrombus, and abnormalities of fibrin structure and architecture are associated with premature MI in men. This process involves the action of thrombin on fibrinogen to produce soluble fibrin and the activation of factor XIII, which cross-links fibrin, thereby rendering it more resistant to fibrinolysis. Lysis of fibrin is dependent on the binding of tissue plasminogen activator and plasminogen to fibrin with local plasmin production in an environment protected from the circulating PAI-1.

In our population, levels of PAI-1 were elevated in PICH (median, 11.4 U/mL) as well as in CI (12.1 U/mL). Unlike in MI, we did not demonstrate a relationship between the factor XIII Val 34 Leu mutation and levels of PAI-1 or the 4G/5G genotype, although there was a trend to higher levels of PAI-1 in subjects with PICH and the factor XIII Val 34 Leu mutation than in those with PICH and the wild types (11.8 U/mL and 7.8 U/mL, respectively) (Table 2).

The results from this study indicate a slightly higher incidence of the factor XIII Val 34 Leu mutation and risk of PICH, although the present study does not provide direct evidence that factor XIII Val 34 Leu results in direct functional alteration in the protein. The findings of this study have to be viewed in the light of the drawbacks associated with a case-control study and the relatively small numbers of PICH subjects available for study in such a cohort. However, while this finding was not an independent relationship in this population and the findings could not be regarded as causal, these observations are consistent with previous findings in relation to MI, which indicates that this mutation justifies further study. Attention should be directed to the heterogeneous nature of PICH, because the factor XIII Val 34 Leu mutation may be more strongly related to the different pathological variants of PICH, such as lipohyalinosis or amyloid angiopathy. Furthermore, the influence of very early mortality from PICH may be acting to dilute the strength of the association, a possibility that should be considered in future studies of the factor XIII Val 34 Leu mutation and PICH. This might be addressed through use of archival DNA methods in subjects who have died from PICH.

Acknowledgments

This study was supported by the Stroke Association. HPK is funded by the Royal Society, London, United Kingdom.

References


Factor XIII Val 34 Leu: A Novel Association With Primary Intracerebral Hemorrhage
Andrew J Catto, Hans P Kohler, Sally Bannan, Max Stickland, Angela Carter and Peter J Grant

Stroke. 1998;29:813-816
doi: 10.1161/01.STR.29.4.813

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/29/4/813

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/