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β Fibrinogen gene -455 G/A polymorphisms as Determinants of Ischemic Stroke Outcome Severity in Response to Aspirin Treatment

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Abstract : Stroke is a leading cause of disability and death. For secondary treatment, aspirin is the agent of choice. Polymorphisms in the promoter region of β fibrinogen gene -455 G/A are associated with increased plasma fibrinogen levels. We evaluated the correlation between β fibrinogen gene-455 G/A promoter polymorphisms and ischemic stroke (IS) outcome on modified Rankin Scale (mRS) in patients treated with aspirin by age groups. A Cohort study design was adopted comprising 136 patients of younger (<55) and older ages (>55). Patients used aspirin for 3 months after IS and completed a detailed stroke-outcome questionnaire. Overall, genotype distribution was 66.2% for GG, 27.2% for GA and 6.6% for AA. The young-age group showed genotypes GG, GA and AA as 30.1%, 15.4% and 6.6%, respectively. After aspirin administration, plasma fibrinogen levels dropped to 248.65±100.71 mg/dl and 235.75±82.01 mg/dl in young and old age groups, respectively. Low and high plasma fibrinogen levels were determined on mRS with a cut-off value of 268.05 mg/dl. In a logistic regression model, the -455 G/A locus genotype showed a significant correlation with age and plasma fibrinogen levels on mRS at day 0 (P<0.25). Poisson Confidence Intervals (PCI) were calculated. Age relative risk was 0.67 (PCI: 0.30-1.49), while fibrinogen-mRS relative risk was 0.78 (PCI: 0.37 – 1.63) at day 0. Aspirin significantly (P<0.05) decreased plasma fibrinogen levels in correlation with promoter polymorphisms in an age-dependent fashion. Significant differences between high and low plasma fibrinogen levels before and after the use of aspirin coincided with substantially reduced mRS scores.
Keywords: β fibrinogen -455 FGB; aspirin; stroke outcome; ischemic stroke risk.

Introduction

Stroke ranks third after ischemic heart disease and cancer as a cause of life-long disability in high-income countries and as a cause of death worldwide.[1] The incidence of stroke varies depending on the country and increases exponentially with age. In Western societies, about 80% of stroke events are due to focal cerebral ischemia secondary to arterial occlusion. The remaining 20% are associated with incident hemorrhage.[2]

Ischemic brain injury is thought to result from a cascade of events starting with energy depletion and ending in cell death. The intermediate factors include an excess of extracellular excitatory amino acids, formation of free radicals, and inflammation.[3,4]

Acute stroke is typically characterized by a sudden onset of focal neurologic deficit, though some patients may have a stepwise or gradual progression into symptomatic stroke. Commonly, this deficit is associated with the following conditions: dysphasia, dysarthria, hemianopia, weakness, ataxia, loss of sensory perception, and neglectfulness. Symptoms and signs are unilateral; and consciousness is generally normal or only slightly impaired, except in some cases involving the formation of infarcts in the posterior circulation.[4]

In two large randomized trials, the use of aspirin (160 or 300 mg per day), initiated within 48 hours after the onset of stroke and continued for 2 weeks or until patient discharge, led to reduced rates of death or dependency upon discharge throughout a follow-up period of 6 months, which was probably due to a reduced risk of recurrent ischemia.[5,6]

Prospective studies with large numbers of samples have suggested that plasma fibrinogen levels may make for an independent risk factor for coronary heart disease and stroke. Fibrinogen, a glycoprotein, is associated with increased risk of arteriosclerosis in humans. G/A variability in the -455 locus of the β fibrinogen promoter region, especially in terms of the carrier status of the A allele, has previously been shown to be associated with elevated plasma fibrinogen levels and an increased risk of cardiovascular disease and ischemic stroke.[7,8]

This study evaluated the correlation between β fibrinogen gene -455 G/A promoter polymorphisms and stroke outcome on the modified Rankin Scale of Ischemic Stroke in patients treated with aspirin by age groups.

2. Material and Methods

2.1. Subjects

All patients with acute ischemic stroke admitted to Adam Malik Hospital after Head CT scans were divided into two groups: those below the age of 55 and those above that age. All subjects received antiplatelet aspirin therapy for 3 months. The study was approved by the human ethics committee of the Medical Faculty of Universitas Sumatera Utara. The study design was fully explained and written study protocols were offered to the patients who satisfied the inclusion criteria. All of the participating subjects signed a written consent form.

2.2. Genetic Analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. The polymerase chain reaction (PCR) primers matched fragments in the promoter region of the β fibrinogen gene with respect to -455 G/A polymorphisms as follows: 5'-GAACATTTTACCTTATGTGAATTAAGG-3' (forward primer) and 5'-GAAGCTCCAAGAAACCATCC-3' (reverse primer). The PCR reaction was performed using Hae III Thermo, whereby 50 µl of the reaction solution were mixed with 50 µg of genomic DNA. 200 ng of each of the appropriate forward and reverse primers were added with 200 µmol/L of each deoxynucleotide triphosphate, and 1U of Dynazyme II DNA Polymerase in a 1x reaction buffer (Finzymes OY). Samples were incubated for 5 minutes at 95^{o} C, which was followed by 34 cycles of 1 minute at 95^{o}C and 1 minute at 72^{o}C. PCR Products (20 micro liters) were digested with 10 U of the HaeIII restriction enzyme (Promega Corp) and resolved in 2% agarose gel to enable the determination of the -455 G/A genotype. The amplification conditions were as follows: an initial denaturing step at 95^{o} C for 7 seconds, followed by 35 amplification cycles of denaturation at 52^{o}C for 45 seconds, 72^{o}C for 30 seconds, 72^{o}C for 7 minutes and 16^{o}C for 7 minutes further.[7] Subsequent digestion with restriction endonuclease HAEIII resulted in fragments of 181 base-pair and 488 base-pair of the more common genotype GG; 488 base-pair and 669 base-pair of genotype GA; and 669 base-pair of genotype AA.

2.3. Stroke Outcome

Prior knowledge of the expected outcome of stroke and its predictors is important for the selection of appropriate instruments of analysis in clinical trials relevant to stroke research. In clinical trials, the modified Rankin Scale (mRS) is widely used. mRS is a global outcome rating scale with values ranging from 0 (no impairment) to 5 (bedridden; incontinent; requiring constant nursing, care and attention) and 6 (a fatal outcome). This study considered mRS scale values 1 and 2 to denote a good outcome, and scale values 3 until 6 to signify a bad outcome.[9]

2.4. Data Analyis

Data analysis was carried out to determine the differences in plasma fibrinogen levels in connection

with the present genotype via the Paired T test, and to determine changes in outcome and plasma fibrinogen levels before and after aspirin use by the Paired T Test and Mc Nemar test. A forward stepwise analysis for the variables of age, genotype and mRS scale value was also performed on day 0 and day 90. For each study, an exact 95% Poisson Confidence Interval was calculated for the respective outcome. Pooled estimates for the event rates and RRs were calculated using the SPSS statistical program. Difference with $P< 0.05$ were considered statistically significant.

3. Results:

The baseline characteristics of plasma fibrinogen levels are shown in fig 1. Fibrinogen levels were observed from the first day following ischemic stroke and throughout 90 days after treatment with aspirin. The mean fibrinogen level value was 305.48 mg/dl on the first day, but decreased to 242.19 mg/dl by day 90.

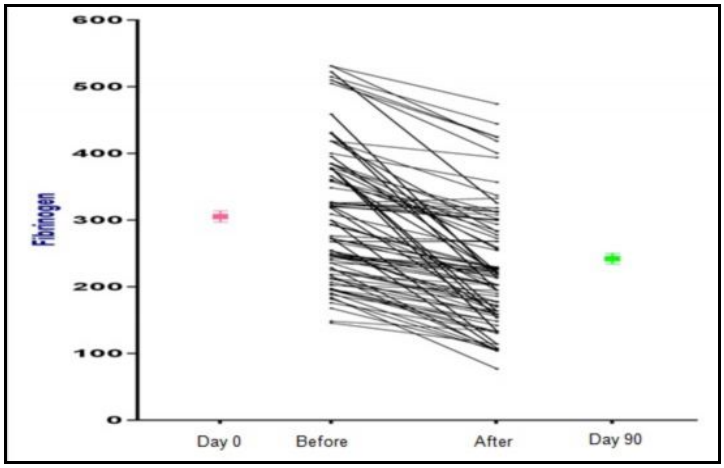

Figure 1. Fibrinogen levels on day 0 vs. day 90

In the 136 samples taken, the genotype distribution of the -455 G/A locus was as follows: 66.2% for GG, 27.2% for G/A and 6.6% for AA. However, at a younger age, the genotypes above were encountered in the following frequencies: GG:60.3%; GA: 30.9%; and AA: 8.8%. Main characteristics of -455 G/A β fibrinogen gene in the patients are shown in figure 2.

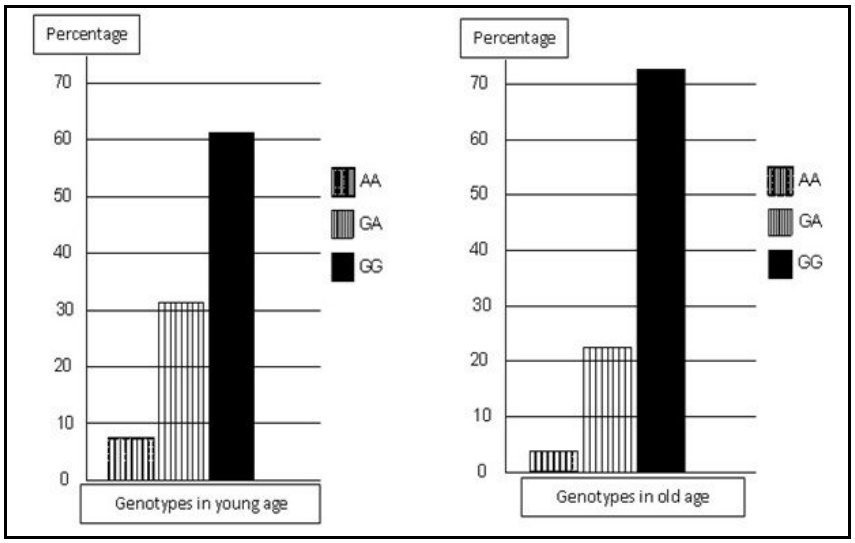

Figure 2. Main β fibrinogen 455 G/A genotype characteristics in ischemic stroke patients

Based on a bivariate test using the paired T test and Mc Nemar test, -455 G/A genotypes promoted significant differences before and after the use of aspirin in terms of genotype GG, GA, G allele, allele A, age and mRS scale (P<0,05). In allele G carriers, plasma fibrinogen levels before the administration of aspirin were shown to be 301.44 ± 96.34 mg/dl, which was reduced to 240.08 ± 90.76 mg/dl after the administration of

aspirin. As for the A allele carries, plasma fibrinogen levels were 362.70 ± 110.95 mg/dl before the administration of aspirin but decreased to 272.04 ± 105.69 mg/dl afterwards.

Differences in plasma fibrinogen levels were observed in each age group. Before aspirin administration, plasma fibrinogen levels were shown to be 310.45 ± 107.29 mg/dl and 300.51 ± 88.51 mg/dl in the younger and the older age groups, respectively. After the administration of aspirin, both the younger group and the older group respectively showed decreased plasma fibrinogen levels of 248.65 ± 100.71 mg/dl and 235.75 ± 82.01 mg/dl. Low and high fibrinogen levels on the mRS scale were recognized using a cut-off value of plasma fibrinogen concentration of 268.05 mg/dl. Plasma fibrinogen levels for day 0 plotted on the mRS scale demonstrated that the low-value fibrinogen levels accounted for as much as 47% of all cases. Conversely, nearly 52% of all subjects showed high-value plasma fibrinogen levels. However, with aspirin use for 90 days, low plasma fibrinogen levels were found in 81% of all patients vs. 19% maintaining the high-value levels. Using Mc Nemar test for statistical analysis, a significant difference was found in plasma fibrinogen levels before and after aspirin use with reference to the mRS scale (P<0.05). Comparisons of patients' characteristics before and after treatment with aspirin are shown in Table 1.

Table 1. Comparisons of characteristics before and after treatment with aspirin

Variable	Before $\bar{x} \pm SD$	After $\bar{x} \pm SD$	p *
Genotype			
GG (66.2%)	298.07 ± 92.52	235.63 ± 85.31	0.0001*
GA (27.2%)	309.59 ± 105.94	250.91 ± 103.28	0.0001*
AA (6.6%)	362.70 ± 110.95	272.04 ± 105.69	0.07*
Allele			
Allele G	301.44 ± 96.34	240.08 ± 90.76	0.001*
Allele A	362.70 ± 110.95	272.04 ± 105.69	0.019*
Young age	310.45 ± 107.29	248.65 ± 100.71	0.001*
Old age	300.51 ± 88.51	235.75 ± 82.01	0.001*
mRS scale			
Low fibrinogen level	43 (47%)	73 (81%)	0.0001**
High fibrinogen level	47 (52%)	17 (19%)	

*Paired T Test, ** Mc Nemar test

Figure 3 below shows the improvement (the left side) and worsening (the right side) of modified Rankin Scale scores after the administration of aspirin relevant to β fibrinogen -455 G/A polymorphisms. The G allele was associated with improved modified Rankin Scale scores over time, whereas the A allele was linked to deteriorating modified Rankin Scale scores over time.

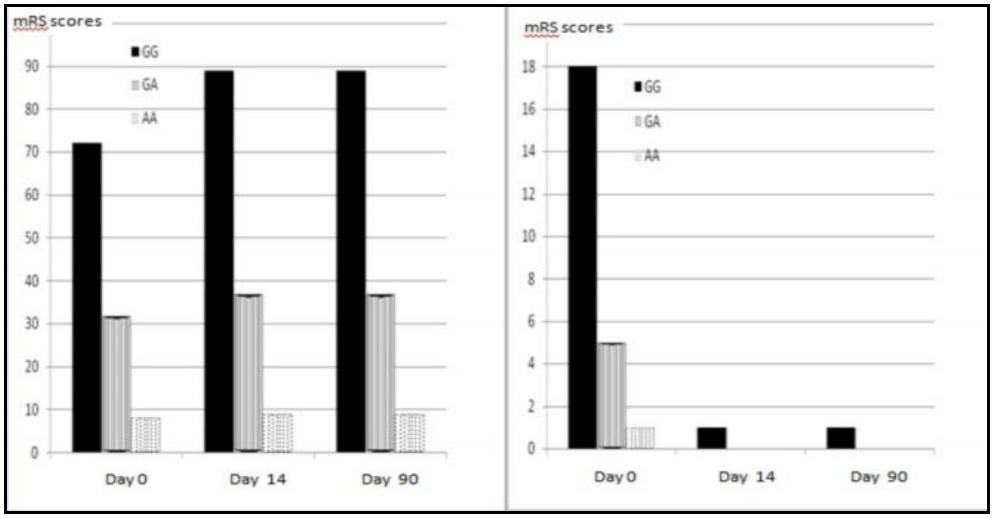

Figure 3. Differences between β fibrinogen -455 G/A gene polymorphisms based on mRS scores

In a forward stepwise logistic regression model, the -455 G/A locus genotypes showed significant interactions with age and plasma fibrinogen levels plotted on the modified Rankin Scale (mRS) for day 0 ($P\leq$ 0,25). For each comparison, a Poisson Confidence Interval was calculated. Comparisons were conducted with age and plasma fibrinogen levels with reference to mRS on day 0. Hence, the identified relative risk of aging was 0.67 (0.30-1.49) and that of fibrinogen-mRS (day 0) was 0.78 (0.37 – 1.63). The Relative Risks (95% CI) which reached statistical significance are shown in Table 2.

Table 2. The influence of genotypes GG, GA and AA on plasma fibrinogen levels and mRS scores on day 0

Variable	B	p Value	Relative Risk	CI 95% Min Max
Age	-0.920	0.166	0.667	0.30 – 1.49
Fibrinogen-mRS day 0	-2.203	0.083	0.778	0.37 – 1.63
Constant	5.949	0.000	0.02	0.00 – 0.03

4. Discussion:

In previous studies, fibrinogen has emerged as a risk factor for stroke, ischemic heart disease, myocardial infarction, venous thrombosis and PAD.[10-12] In this study, impaired plasma fibrinogen levels were observed on the first day following an ischemic stroke in patients treated with aspirin. Observation was continued up to 90 days after treatment. Differences in plasma fibrinogen levels were recorded by age groups: Before aspirin administration, the younger age group showed plasma fibrinogen levels of 310.45 ± 107.29 mg/dl, while the levels in the older age group were 300.51 ± 88.51 mg/dl. After the administration of aspirin, the younger age group showed reduced plasma fibrinogen levels of 248.65 ± 100.71 mg/dl. A similar observation was made in the older age group, with a mean value of 235.75 ± 82.01 mg/dl. Previous findings by Martiskainen et al implied that -455 GA fibrinogen gene polymorphism could contribute to the development and progression of cerebral arteriosclerosis.[7] Nishiuma et al showed a significant correlation (OR 2.05; P=0,05) between fibrinogen genotype and ischemic stroke.[13]

In this cohort study, genotype distribution was as follows: 66.2% for GG, 27.2% for G/A and 6.6% for AA. Those genotypes were encountered in the younger age group with GG accounting for 30.1% of all subjects, GA 15.4% and AA 6.6%. On the other hand, employing the SAM cohort, Martiskainen et al showed the genotypes distributions as 64.9% (GG). 31.8% (GA) and 3.35 (AA).

In 2002, researchers in Korea, Liu et al, reported that the average age of stroke based on β-fibrinogen -455 G/A genotypes were 11.6 years for the GG genotype, 64.39 years for the GA genotype, and 65.09 for the AA genotype (± 10.6 years). They found no significant differences in relation to genotypes GG, GA and AA (P> 0.05).[14] However, in this study, based on a forward stepwise logistic regression model, -455 G/A locus genotypes showed a significant correlation between age and plasma fibrinogen levels using the modified Rankin Scale (mRS) on day 0 ($p\leq$0.25). Identification of stroke risk factors based on the relative risk of aging found the risk of stroke to be 0.67 times higher in advanced ages. On the other hand, higher plasma fibrinogen levels, with reference to the mRS scale on day 0, were shown to increase the risk 0.78 times. In a prospective study, Blake et al reported that the distribution of the C148T β fibrinogen polymorphism was similar among those who subsequently developed cardiovascular events, as compared with those who did not. The adjusted relative risks associated with the presence of the T allele were determined as 0.95 (95% CI 0.75-1.19) for all cases, 1.10 (0,83-1,45) for myocardial infarction, 0.93 (0,65-1,33) for stroke, and 0.71 (0.47-1.1) for venous thromboembolism.[15]

Conclusions

Plasma fibrinogen levels decreased after aspirin administration and in connection with present β fibrinogen polymorphisms in an age-dependent manner (P<0.05). The study encountered significant differences between the high and the low plasma fibrinogen levels before and after the use of aspirin with reference to the mRS scale. Identification of stroke risk factors showed a 0.67-fold increase in relative risk with aging and a 0.78-fold increase with higher plasma fibrinogen levels as demonstrated on the mRS scale on day 0.

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References

1. Hacke,W., Kaste, M, Bogousslavsky, J., Brainin, M, Gugging, M., Chammoro, A., Less, K., Leys, D., Kwiencinki, H., Toni, D. *European Stroke Initiative: Ischemic Stroke Prophylaxis and Treatment*. EUSI. Heidelberg. 2003.
2. Feigin, V.L., Lawes, C.M., Bennett, D.A., Anderson, C.S. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century, *Lancet Neurol* .,Vol.2,pp.43-53, 2003.
3. Worp, H.B., Gijn,J.V. Clinical Practice Acute Ishcemic Stroke. *N. Eng J Med*. Vol.357,pp.572-579, 2007.
4. Ahmed, S.H., Hu, C.J., Paczynski, R., Hsu, C.Y. *Pathophysiology of ischemic injury*. In: Fischer,M. editor. Stroke Therapy. 2 nd ed. Boston Butterworth- Heinemann,2001.pp. 25 – 49.
5. International Stroke Trial Collaborative Group (IST): a randomized trial of aspirin, subcutaneous heparin, both, or neither among 19.345 patients with acute ischemic stroke. *Lancet*., Vol.349,pp.1569-1581,1997.
6. Chinese Acute Stroke Trial (CAST) Collaborative Group: randomized placebo-controlled trial of early aspirin use in 20.000 patients with acute ischemic stroke. *Lancet*., Vol.349, pp.1641-1649,1997.
7. Martiskainen M, Pohjasvaara T, Mikkelsson J, Mantyla R, Kunnas T, Laippala P, Ilveskoski E, Kaste M, Karhunen PJ, Erkinjuntti T. Fibrinogen Gene Promoter -455 A Allele as a risk Factor for Lacunar Stroke. *Stroke*.Vol.34,pp.886-891,2003.
8. Lee, S.H., Kim, M.K., Park, M.S., Choi, S.M., Kim, J.T., Kim, B.C., Cho, K.H. Beta Fibrinogen gene -455 G/A Polymorphism in Korean Ischemic Stroke Patients. *J. Clin. Neurol.,* Vol.4(1),pp.17-22, 2008.
9. Weimar, C., Kurth, T., Kraywinkel, K., Wagner, M., Busse, O, Habert, R.L., Dienner, H.C. Assessment of functioning and disability after ischemic stroke. *Stroke*, Vol.33,pp.2053-2059,2002.
10. Qizilbash N, Jones L, Warlow C, Mann J. Fibrinogen and lipid concentrations as risk factors for transient ischaemic attacks and minor ischaemic strokes. *BMJ*,Vol.303,pp.605-9, 1991.
11. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G.Fibrinogen as a risk factor for stroke and Myocardial Infarction. *N Engl J Med* ., Vol.311,pp.501–505,1984.
12. Kesler, C., Spitzer,C., Stauske,D., Mende, S., Stadlmuller, J., Walther, R. The Apolipoprotein E and β fibrinogen G/A -455 gene plymorphism are associated with ischemic stroke involving large vessel disease. *Arteriosclerosis, Thrombosis, and Vascular Biology.,*Vol.17,pp.2880-2884, 1997.
13. Nishiuma S, Kario K, Yakushijin K, Maeda M, Murai R, Matsuo T, Ikeda U, Shimada K, Matsuo M. Genetic variation in the promoter region of the beta-fibrinogen gene is associated with ischemic stroke in a Japanese population. *Blood Coagul Fibrinolysis*.Vol.9,pp.373-379,1998.
14. Liu, Y., Pan, J., Wang, S., Li X., Huang,Y. Beta Fibrinogen gene -455 A/Gpolymorphism and plasma fibrinogen level in Chinese Stroke patients. Chin. Med. J (Engl)., Vol.115, pp.214-216,2002.
15. Blake, G.J., Schmidtz,C., Lindpaintner, K., Ridker,P.M. Mutation in the promoter region of the β-fibrinogen gene and the risk of future myocardial infarction, stroke and venous thrombosis. *European Heart Journal*., Vol.22, pp.2262-2266, 2001.

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