



Original Article

Outcomes of Ischemic Stroke and -455 G/A Beta Fibrinogen Gene Polymorphism

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Objective: Carriage of allele A of the beta-fibrinogen -455 G/A polymorphism is associated with increased plasma fibrinogen level, which induces hypercoagulability and reflects the progression of atherosclerosis. Therefore, it influences outcomes for stroke patients. The effects of beta-fibrinogen gene polymorphism -455 G/A on Barthel Index (BI) and Modified Rankin Scale (mRS) among populations treated with aspirin, based on age group, have not previously been reported. **Methods:** This cohort study was conducted between January and November 2013, the Adam Malik General Hospital, in Medan. Ischemic stroke patients were divided into two groups based on age, i.e. young (< 55 years old) and old (> 55 years old). Each group received a single dose of aspirin (300 mg), followed by 100 mg once daily. Blood plasma fibrinogen levels were measured using the Clauss method. Beta-fibrinogen gene polymorphism -455 G/A was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) using HaeIII enzyme. The outcome of stroke was measured by BI and mRS at days 0, 14 and 90. The study data were analyzed using the paired T test and the unpaired T test, ANOVA, and Chi-square. A p value < 0.05 was considered significant. **Results:** Of the 136 samples analyzed, the frequency of allele A was higher (19.8% in young stroke patients). There was no difference in fibrinogen level according to the polymorphism of gene beta fibrinogen -455 G/A. Polymorphism did not vary by age group. There was no difference in plasma fibrinogen level based on age, either before or after aspirin administration. Plasma fibrinogen levels decreased after aspirin administration and also by polymorphism by age. Age was not associated with the outcomes of ischemic stroke patients treated with aspirin, when assessed by BI and mRS. **Conclusions:** Clinical improvement is greater in genotype GG, GA and AA, which means that genotype GG, GA, AA-containing allele G are better than allele A. There was a significant difference in the lowering of fibrinogen level before and after the aspirin administration.

Keywords: fibrinogen, polymorphism, ischemic stroke, Barthel Index (BI), Modified Rankin Scale (mRS).

1. INTRODUCTION

Increased levels of fibrinogen in the blood are associated with genetic factors, especially the fibrinogen gene, which consists of three types--alpha, beta and gamma. Coordinated interactions between

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genetic variants of these genes may regulate plasma fibrinogen homeostasis, which may predict inter-individual variations in homeostasis. The fibrinogen beta gene is associated with elevated fibrinogen levels.^{1, 2} Elevated fibrinogen levels induce a state of hypercoagulability and reflect the progression of atherosclerosis.³

Increased plasma levels of fibrinogen have been associated with an increased risk of vascular events, including myocardial infarction, stroke, and venous thromboembolism. Synthesis of the beta-polypeptide chain is the rate-limiting stage in fibrinogen formation; it is regulated by a beta fibrinogen promoter in chromosome 4.⁴ Fibrinogen is a glycoprotein associated with an increased risk of atherosclerosis in humans. G/A variability in the -455 locus of the beta fibrinogen promoter region has been shown to be associated with elevating fibrinogen levels, and increased risk of cardiovascular disease and ischemic stroke.⁵

Immobilized fibrinogen on the endothelial cells acts as a substrate in platelets, by binding to alpha₂beta₁ integrins on the adjacent platelet surfaces and adhering to vessel wall/ subendothelial collagen.⁵ Aspirin has a major role in preventing thromboembolic complications due to atherosclerotic disease.⁶ No reports have addressed the effect of beta-fibrinogen gene polymorphism -455 G/A in BI and mRS in population treated with aspirin, by age group. In this study, we analyzed the relationship between beta fibrinogen gene polymorphism -455 G/A and the severity of ischemic stroke outcomes among patients treated with aspirin, by age group.

2. MATERIALS AND METHODS

Patients

All patients with acute ischemic stroke (the term 'acute' was defined as the period of time starting from hospital admission until one week post-admission) who came to

Adam Malik Hospital were divided into two groups: age < 55 years and age > 55 years. They received anti-platelet aspirin for 3 months. The study design was explained to the participants and written information was offered to the patients. The study was approved by the Ethics Committee, Faculty of Medicine, Sumatera Utara University, Medan, Indonesia.

General Clinical Assessment

During the period January-November 2013, a total of 136 patients underwent head CT scans. Using computed tomography scan data, the ischemic stroke cases were examined. Ischemic stroke patients were divided by age group, i.e. young and old and received a single dose of aspirin (300 mg), followed by 100 mg once daily. Blood plasma fibrinogen level was measured using the Clauss method. Beta-fibrinogen gene polymorphism -455 G/A was determined by PCR-RFLP using HaeIII enzyme. Stroke outcomes were measured by BI and mRS, at days 1, 14, and 90.

DNA Procedures

DNA was separated from frozen blood samples using standard procedures. PCR was used in DNA amplification, as previously described.⁵ The PCR reactions were performed with HaeIII Thermo in 50 microliter reaction with 50 microgram of genomic DNA, 200 ng of each appropriate primer (5'-CTCCTCATTGTCGTTGACACCTTGGGAC-3') and 5'-GAATTTGGGAATGCAATCTCT GCTACCT-3', 200 micromol/L of each deoxynucleotide triphosphate, and 1U of Dynazyme II DNA polymerase in 1 x reaction buffer (Finzymes OY). Sample were incubated for 5 minutes at 95° C, followed by 34 cycles of 1 minute at 95°C, 1 minute at 72°C. PCR products (20 microliters) were digested with 10 U of HaeIII restriction enzyme (Promega Corp) and resolved in 2% agarose gel to determine -455 G/A genotype.

Statistical Analysis

Data were expressed as mean \pm standard deviation (\pm SD) of plasma fibrinogen level. Differences between fibrinogen level and polymorphism were analyzed using the paired T test and ANOVA. The paired and unpaired T tests were used to determine differences in plasma fibrinogen level by age. The Chi-square test was used to reveal differences in BI/ mRS values pre- and post-aspirin administration.

3. RESULTS AND DISCUSSION

Fibrinogen -455G/A Polymorphism and Fibrinogen Plasma

Allele distribution was in Hardy-Weinberg equilibrium. Genotype distributions of the -455G/A locus were 66.2%, 27.2%, and 6.6%, for GG, GA, and AA, respectively. The frequency of allele A was 33.8%. These frequencies closely corresponded to the young and old stroke groups. Differences in plasma fibrinogen level were analyzed. Plasma fibrinogen levels were measured on days 0 and 90. The ANOVA test was then run on days 0 and 90 based on the genotype GG, GA, and AA (Table 1).

Table 1: The diagram genotype GG, GA and AA between young and old patients

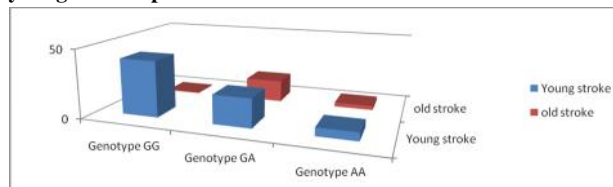


Table 2: Main characteristic of patients' ischemic stroke and their genotype

Genotype	Before Aspirin $\bar{x} \pm SD$	After Aspirin $\bar{x} \pm SD$	P*
GG (n=90)	298.07 \pm 92.52	235.63 \pm 85.31	0.0001
GA (n=37)	309.59 \pm 105.94	250.91 \pm 103.28	0.0001
AA (n=9)	362.70 \pm 110.95	272.04 \pm 105.69	0.07

* Paired T test

The results showed that, among the 136 subjects, fibrinogen levels pre- and post-aspirin administration were 298.07 \pm 92.53 mg/dl, and 235.63 \pm 85.31 mg/dl, respectively, in the GG genotype; 309.59 \pm 105.94 mg/dl and 250.9 \pm 103.28 mg/dl in the GA genotype;

and 362.70 \pm 110.95 mg/dl and 272.04 \pm 105.69 mg/dl in the AA genotype.

Paired T test analysis found a significant difference in decrease of plasma fibrinogen level pre- and post-aspirin administration for genotype GG and GA ($p < 0.05$). However, in Table 2, the p value for the AA genotype was 0.07. There was no significant difference in plasma fibrinogen level between the two age groups, pre- or post-aspirin administration ($p > 0.05$) (Table 2).

The difference of plasma fibrinogen levels by age

The plasma fibrinogen level was 310.45 \pm 107.29 mg/dl among the young patients, versus 300.51 \pm 88.51 mg/dl among the old patients. Post-aspirin administration, the plasma fibrinogen level was 248.65 \pm 100.71 mg/dl versus 235.75 \pm 82.01 mg/dl, respectively. There was a significant difference in decreasing fibrinogen level pre- and post-aspirin administration ($p < 0.05$) (Table 3).

Table 3: Distribution frequency of plasma fibrinogen levels by age

Age	Before Aspirin $\bar{x} \pm SD$	After Aspirin $\bar{x} \pm SD$	$P, *$
Age : < 55 years old	310.45 \pm 107.29	248.65 \pm 100.71	0.001
Age : \geq 55 years old	300.51 \pm 88.51	235.75 \pm 82.01	0.001

* Paired T test

Barthel Index (BI)/modified Rankin Scale (mRS) values pre- and post-aspirin administration, by polymorphism

There was no significant difference in mRS on days 0, 14, and 90 ($p > 0.05$). Genotype frequency did not affect mRS outcome. Better clinical improvement was shown in the GG genotype (65.4 to 52.9 / 52.9 = 25%), GA genotype (27.2 to 23.5 / 23.5 = 15%), and AA genotype (6.6 to 5.9 / 5.9 = 11%) (Table 4).

Table 4: Distribution MRS scale before and after aspirin

Genotype	MRS day 0		MRS day 14		MRS day 90	
	Good n(%)	Bad n(%)	Good n(%)	Bad n(%)	Good n(%)	Bad n(%)
GG	65.4	52.9	52.9	25.0	52.9	25.0
GA	27.2	23.5	23.5	15.0	23.5	15.0
AA	6.6	5.9	5.9	11.0	5.9	11.0

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GG	72(52.9)	18(13.2)	89(65.4)	1(0.7)	89(65.4)	1(0.7)
GA	32(23.5)	5(3.7)	37(27.2)	0(0)	37(27.2)	0(0)
AA	8(5.9)	1(0.7)	9(6.6)	0(0)	9(6.6)	0(0)
<i>p.</i>	0.594		0.773		0.773	

Chi-Square test

Table 5: Distribution Barthel Index score before and after aspirin

Genotype	BI day 0		BI day 14		BI day 90	
	Good	Bad	Good	Bad	Good	Bad
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
GG	39(26.7)	51(37.5)	46(33.8)	44(32.4)	45(33.1)	45(33.1)
GA	18(13.2)	19(14)	15(11)	22(16.2)	24(17.5)	37(27.2)
AA	5(3.7)	4(2.9)	4(2.9)	5(3.7)	5(3.7)	9(6.6)
<i>p.</i>	0.710		0.544		0.310	

Chi-Square test

Table 5 shows no significant difference between BI on days 0, 14, and 90 ($p > 0.05$). Genotype frequency did not affect BI outcome. Clinical improvement was greater in genotypes GG, GA, and AA, meaning the genotype GG-, GA-, AA-containing allele G are better than allele A. Genotype frequency is not affecting the outcome on mRS and BI scale on analysis multivariate (Table 6).

Table 6: Analysis Multivariate about Independent Variable

Variable	B	Sig	Exp(B)	95% CI for exp(B)	
				Min	Max
Age	0.02	0.306	1.021	0.981	1.061
Fibrinogen	-0.002	0.451	0.998	0.993	1.003
Genotype	0.681	0.094	1.976	0.890	4.388
Constant	3.011	0.381	20.6		

In previous studies, fibrinogen has emerged as a risk factor for stroke, ischemic heart disease, myocardial infarction, venous thrombosis, and peripheral artery disease.⁷⁻⁹ Nedeltchev et al., following a prospective study of 203 young ischemic stroke cases, found the mean stroke age was 36 ± 8 years. The common cause of stroke in young age were cardiac embolism, especially cervical artery dissection (54%), followed by atherothrombosis and small-vessel disease (13%).¹⁰ In this study, we report that the AA genotype of the functional fibrinogen promoter -455G/A polymorphism

was associated with fibrinogen plasma and ischemic stroke outcome. Hemorrhage stroke was excluded by computed tomography scan of the head. This study found no significant difference between age and plasma fibrinogen level, pre- or post- aspirin administration. The result of Liu et al. was similar result to the present study ($p > 0.05$).⁹

Kofoed et al. found an association between ischemic stroke caused by fibrinogen level > 3 g/l and < 3 g/l; for ages 20-31 years (RR 5.2, CI 1.1 to 2.6), 52-65 years (RR 2.9, CI 1.6 to 5.4) and > 65 years (RR 1.4 CI 1.0 to 2.0).¹¹ In this study, there was a significant difference in decreased plasma fibrinogen level pre- and post-aspirin administration. Subsequent statistical analysis found no significant difference between age and plasma fibrinogen level.

Nishiuma et al. argued that atherothrombotic and lacunar strokes were more common among in patients with the AA genotype than those in the control group.

¹² In this study, there was no significant difference in mRS score or Barthel Index score. Clinical improvement was greater in genotype GG, GA, and AA, which were genotypes of GG, GA, AA-containing allele G, and were better than allele A.

The following limitations may have influenced the study results. Genetic research should have started during the prenatal period. Assessment and examination of plasma fibrinogen levels was only conducted for a short period.

4. CONCLUSIONS

Clinical improvement is greater in genotypes GG, GA, and AA, which means that the genotype GG-, GA-, AA-containing allele G are better than allele A. There was a significant difference in decreased fibrinogen level pre-and post-aspirin administration.

5. ACKNOWLEDGEMENT

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