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The β-fibrinogen –455G/A gene polymorphism and the risk of ischaemic stroke in a Polish population

Polimorfizm –455G/A genu β-fibrynogenu a ryzyko udaru niedokrwiennego w populacji polskiej

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Abstract

Background and purpose: Ischaemic stroke is considered to be multifactorial and interactions between environmental and genetic factors play an important role. Although vascular risk factors are well known, the genetic ones are still undiscovered. In the present study, we assessed the significance of the β-fibrinogen –455G/A gene polymorphism and the risk of ischaemic stroke in a Polish population.

Material and methods: 426 ischaemic stroke patients classified according to stroke aetiologies (small vessel disease, large vessel disease or cardioembolic stroke) and 234 controls were included in the study. The association of the β-fibrinogen genotypes with ischaemic stroke was tested using logistic regression analysis under dominant, recessive or additive models of inheritance.

Results: The allele and genotype distributions of the β-fibrinogen –455G/A gene polymorphism did not differ significantly between patients and controls (patients: G – 75%, GG – 56.6%, GA – 36.8%, AA – 6.6%; controls: G – 73.7%, GG – 57.3%, GA – 32.9%, AA – 9.8%; $p > 0.05$, χ^2). In addition, logistic regression analysis adjusted for the known risk factors, i.e. hypertension, ischaemic heart disease, myocardial infarction, hypercholesterolaemia, diabetes mellitus and smoking, did not show a role of the studied polymorphism in ischaemic stroke.

Streszczenie

Wstęp i cel pracy: Etiologia udaru niedokrwiennego mózgu jest wieloczynnikowa. Istotną rolę odgrywają w niej interakcje pomiędzy czynnikami środowiskowymi i genetycznymi. Naczyniowe czynniki ryzyka udaru mózgu są dość dobrze poznane, natomiast rola czynników genetycznych pozostaje wciąż niejasna. W prezentowanym badaniu oceniano znaczenie polimorfizmu –455G/A genu β-fibrynogenu w kontekście ryzyka wystąpienia udaru niedokrwiennego mózgu w populacji polskiej.

Materiał i metody: Do badania włączono 426 chorych na udar niedokrwienny mózgu sklasyfikowanych zgodnie z etiologią udaru (choroba małych naczyń, choroba dużych naczyń lub udar sercowozatorowy) oraz 234 osoby z grupy kontrolnej. Związek pomiędzy badanym polimorfizmem a udarem niedokrwiennym mózgu został zbadany przy użyciu regresji logistycznej w dominującym, recesywnym i addytywnym modelu dziedziczenia.

Wyniki: Nie stwierdzono istotnej różnicy w rozkładzie alleli i genotypów polimorfizmu –455G/A genu β-fibrynogenu pomiędzy pacjentami a osobami z grupy kontrolnej (pacjenci: G – 75%, GG – 56,6%, GA – 36,8%, AA – 6,6%; grupa kontrolna: G – 73,7%, GG – 57,3%, GA – 32,9%, AA – 9,8 %; $p > 0{,}05$, test χ^2). Ponadto w modelu regresji logistycznej uwzględniającym wpływ znanych czynników ryzyka, takich

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Conclusions: The β-fibrinogen –455G/A gene polymorphism is not a risk factor for ischaemic stroke in a Polish population.

Key words: ischaemic stroke, fibrinogen, single nucleotide polymorphism.

jak: nadciśnienie tętnicze, choroba niedokrwienna serca, zawał mięśnia sercowego, hipercholesterolemia, cukrzyca i palenie tytoniu, nie wykazano roli badanego polimorfizmu w udarze niedokrwiennym mózgu.

Wnioski: Polimorfizm –455G/A genu β-fibrynogenu nie jest czynnikiem ryzyka udaru niedokrwiennego mózgu w populacji polskiej.

Słowa kluczowe: udar niedokrwienny mózgu, fibrynogen, polimorfizm.

Introduction

Ischaemic stroke is one of the leading causes of disability and death in the developed countries [1]. Apart from the known risk factors, such as older age, hypertension, atrial fibrillation, diabetes mellitus, smoking or positive family history, there are data showing that genetic factors may play a role in the risk of ischaemic stroke, but they are not thoroughly investigated [2]. It is well known that plasma fibrinogen levels are regarded as an independent risk factor for overall ischaemic stroke, as well as for all main etiological subtypes [3]. Moreover, it is proved that fibrinogen levels are significantly higher in large vessel and cardioembolic strokes as compared to small vessel or cryptogenic strokes [3]. There are several factors that could affect plasma fibrinogen concentrations [4,5]. Among them, the A allele of the β-fibrinogen –455G/A gene single nucleotide polymorphism (SNP) (rs 1800790) is mentioned [4]. It was shown that the A allele of this SNP is associated with high plasma fibrinogen levels [4].

In the present study we investigated the relationship between the –455G/A SNP in the β-fibrinogen gene and ischaemic stroke risk in a Polish population.

Material and methods

Consecutive patients with ischaemic stroke, admitted to the Stroke Unit, Department of Neurology, University Hospital in Krakow, Poland, between 2008 and 2010, participated in the study. During hospitalization, patients underwent neuroimaging, ECG, carotid ultrasound and echocardiography. A standardized questionnaire on demographics, medical history and vascular risk factors, i.e. hypertension, ischaemic heart disease, myocardial infarction, hypercholesterolaemia, diabetes mellitus and smoking, were taken from all participants. Stroke aetiology was established according to the TOAST criteria [6]. Only patients with known stroke aetiology were included in the study. Control individuals were recruited from relatives of the hospital staff, consecutive spouses of the patients of the Department of Neurology, or from the community. They had no medical history of neurological disorders. All subjects were Caucasian and gave informed consent. The study was approved by the local ethics committee.

Genomic DNA was extracted from peripheral blood using a commercially available kit (Boehringer Mannheim, Germany). The β-fibrinogen –455G/A gene SNP was detected by polymerase chain reaction and restriction enzyme digestion, as described previously [7].

Comparisons between the groups were studied using χ^2 test or Student's t-test. The Hardy-Weinberg equilibrium was tested by χ^2 test. The association of the β-fibrinogen genotypes with ischaemic stroke was tested using logistic regression analysis under dominant (AA + GA vs. GG), recessive (AA vs. GA + GG) or additive (AA vs. GA vs. GG) models of inheritance. A p-value less than 0.05 was considered significant.

Results

Altogether 426 patients with ischaemic stroke out of all 554 ischaemic stroke victims and 234 controls were included in the study. Stroke aetiology was not established in 128 patients. There was no deviation from the Hardy-Weinberg equilibrium regarding the β-fibrinogen –455G/A gene SNP in the studied groups ($p > 0.05$). The demographic characteristics and stroke risk factor profile are summarized in Table 1. Out of the 426 patients, 28.4% ($n = 121$) had small vessel disease, 23% ($n = 98$) had large vessel disease and 48.6% ($n = 207$) had cardioembolic stroke.

The allele and genotype distributions were similar between patients and controls (patients: G – 75%, GG – 56.6%, GA – 36.8%, AA – 6.6%; controls: G – 73.7%, GG – 57.3%, GA – 32.9%, AA – 9.8 %; $p > 0.05$, χ^2 test). Moreover, the frequency of alleles and geno-

Table 1. Demographic characteristics and risk factor profile of the study subjects

	Patients (n = 426)	Controls (n = 234)	Odds ratio*	95% confidence interval*	*P*-value
Age [years], mean (SD)	67.2 (13.3)	67.61 (12.17)	1.00	0.99-1.01	0.87
Male, %	49.3	44.0	0.81	0.59-1.12	0.21
Hypertension, %	81.7	56.8	3.64	2.52-5.26	< 0.001
Ischaemic heart disease, %	54.7	40.7	1.98	1.39-2.80	< 0.001
Myocardial infarction, %	15.0	6.4	2.51	1.39-4.54	0.002
Hypercholesterolaemia, %	55.4	35.8	2.40	1.66-3.47	< 0.001
Diabetes mellitus, %	21.6	14.2	1.71	1.10-2.66	0.017
Smokers, %	33.8	18.0	2.31	1.51-3.54	< 0.001

**Logistic regression analysis adjusted for age and gender*
SD – standard deviation

Table 2. Genotype and allele distributions in controls and patients classified according to stroke aetiologies

	Small vessel disease (n = 121)	Large vessel disease (n = 98)	Cardioembolic stroke (n = 207)	Controls (n = 234)
Genotypes, *n* (%)				
GG	73 (60.3)*	56 (57.1)*	112 (54.1)*	134 (57.3)
GA	40 (33.1)*	38 (38.8)*	79 (38.2)*	77 (32.9)
AA	8 (6.6)*	4 (4.1)*	16 (7.7)*	23 (9.8)
Alleles, *n* (%)				
G	186 (76.9)*	150 (76.5)*	303 (73.2)*	345 (73.7)
A	56 (23.1)*	46 (23.5)*	111 (26.8)*	123 (26.3)

**Stroke subgroups vs. controls, χ^2 test, $p > 0.05$*

types between patients with small vessel disease, large vessel disease or cardioembolic stroke and controls did not differ significantly (Table 2). In addition, logistic regression analysis both crude and adjusted (for hypertension, ischaemic heart disease, cardiac infarction, hypercholesterolaemia, diabetes mellitus and smoking) did not show the significance of the β-fibrinogen –455G/A gene SNP as a risk factor for ischaemic stroke, irrespectively of its aetiologies under dominant, recessive or additive models of inheritance (Table 3).

Discussion

We failed to find any relationship between the β-fibrinogen –455G/A gene SNP and ischaemic stroke in a Polish population. Moreover, logistic regression analysis adjusted for the known risk factors did not show the significance of the studied polymorphism as a risk factor for ischaemic stroke.

In the first positive study, 227 patients with cerebrovascular diseases (185 patients had ischaemic stroke and 42 had transient ischaemic attack or reversible ischaemic neurological deficit) and 225 controls were included. This study showed an association between the AA genotype and large vessel disease stroke ($\chi^2 = 4.0$, $p = 0.045$) [8]. There were a few more reports showing a positive association between the genotype with the A allele and specific ischaemic stroke subgroups, for example in males compared to elderly male controls (OR = 3.84, 95% CI: 1.64-9.02; $p < 0.02$) [9] or in single lacunar stroke as compared to recurrent lacunar strokes (three or more) (OR = 2.72, 95% CI: 1.18-6.27; $p = 0.02$) [7]. Additionally, hypertensive patients carrying the genotype with the A allele had more than four-

Table 3. Relative risk of ischaemic stroke in carriers of the -455G/A single nucleotide polymorphism

	Crude			Adjusted*		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
All strokes (n = 426)						
dominant model	0.99	0.74-1.31	0.92	1.00	0.69-1.45	0.99
recessive model	1.20	0.69-2.08	0.51	1.42	0.69-2.95	0.34
additive model	0.94	0.72-1.21	0.61	0.95	0.70-1.27	0.71
Small vessel disease (n = 121)						
dominant model	1.14	0.73-1.77	0.58	1.30	0.76-2.21	0.33
recessive model	1.54	0.67-3.55	0.31	1.34	0.50-3.56	0.56
additive model	0.86	0.61-1.21	0.39	0.81	0.54-1.23	0.32
Large vessel disease (n = 98)						
dominant model	1.00	0.62-1.60	0.98	0.99	0.56-1.77	0.98
recessive model	2.56	0.86-7.61	0.08	2.48	0.67-9.17	0.17
additive model	0.87	0.60-1.27	0.47	0.88	0.55-1.41	0.60
Cardioembolic stroke (n = 207)						
dominant model	0.88	0.60-1.28	0.51	0.84	0.47-1.48	0.54
recessive model	1.30	0.67-2.54	0.44	0.98	0.35-2.72	0.97
additive model	1.03	0.77-1.37	0.87	1.10	0.72-1.73	0.62

**Adjusted for hypertension, ischaemic heart disease, myocardial infarction, hypercholesterolaemia, diabetes mellitus and smoking*
OR – odds ratio, 95% CI – 95% confidence interval

fold risk for recurrent lacunar strokes compared with patients carrying the GG genotype without hypertension (OR = 4.24, 95% CI: 1.29-13.99; p = 0.02) [7]. On the other hand, the role of the A allele of the β-fibrinogen –455G/A gene SNP in ischaemic stroke was not confirmed in Hungarian [10], Dutch [12], or Korean populations [11].

It is difficult to compare the present results with previously published studies, since two of the six quoted studies had no control groups [7,11], only three research groups took into consideration different stroke aetiologies [7,8,11] and, in addition, the number of subjects included in all studies was relatively small [7-12]; the most numerous group consisted of 299 stroke cases [7]. Finally, the ethnicity of subjects who participated in two out of the six studies were other than Caucasian [9,11].

Briefly, only a meta-analysis of all published results would increase the power of the study and could find a true association between the β-fibrinogen –455G/A gene SNP and ischaemic stroke. However, in relation to the differences in homogeneity between the studied controls (only females or patients with recurrent strokes) [7,12] it would be difficult to conduct it. Thus, the genetic case-control association studies should be designed and performed using similar patterns accepted by all researchers.

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Disclosure

Authors report no conflict of interest.

References

1. Roger V.L., Go A.S., Lloyd-Jones D.M., et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics – 2011 update: a report from the American Heart Association. *Circulation* 2011; 123: e18-e209.

2. Markus H.S. Stroke genetics. *Hum Mol Genet* 2011; 20: R124-R131.
3. Jood K., Danielson J., Ladenvall C., et al. Fibrinogen gene variation and ischemic stroke. *J Thromb Haemost* 2008; 6: 897-904.
4. Tybjaerg-Hansen A., Agerholm-Larsen B., Humphries S.E., et al. A common mutation (G-455—>A) in the beta-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. *J Clin Invest* 1997; 99: 3034-3039.
5. Iacoviello L., Vischetti M., Zito F., et al. Genes encoding fibrinogen and cardiovascular risk. *Hypertension* 2001; 38: 1199-1203.
6. Adams H.P. Jr, Bendixen B.H., Kappelle L.J., et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; 24: 35-41.
7. Martiskainen M., Pohjasvaara T., Mikkelsson J., et al. Fibrinogen gene promoter –455 A allele as a risk factor for lacunar stroke. *Stroke* 2003; 34: 886-891.
8. Kessler C., Spitzer C., Stauske D., et al. The apolipoprotein E and beta-fibrinogen G/A-455 gene polymorphisms are associated with ischemic stroke involving large-vessel disease. *Arterioscler Thromb Vasc Biol* 1997; 17: 2880-2884.
9. Liu Y., Pan J., Wang S., et al. Beta-fibrinogen gene –455A/G polymorphism and plasma fibrinogen level in Chinese stroke patients. *Chin Med J* (*Engl*) 2002; 115: 214-216.
10. Pongrácz E., Andrikovics H., Csornai M., et al. Contribution of the –455G/A polymorphism at beta-fibrinogen gene and of the Leiden mutation to hemorheological parameters in ischemic stroke patients. *Clin Hemorheol Microcirc* 2006; 35: 75-82.
11. Lee S.H., Kim M.K., Park M.S., et al. Beta-fibrinogen gene –455 G/A polymorphism in Korean ischemic stroke patients. *J Clin Neurol* 2008; 4: 17-22.
12. Siegerink B., Rosendaal F.R., Algra A. Genetic variation in fibrinogen; its relationship to fibrinogen levels and the risk of myocardial infarction and ischemic stroke. *J Thromb Haemost* 2009; 7: 385-390.