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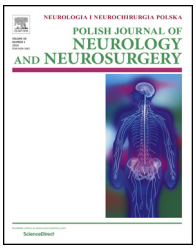

Original research article

Risk factors of stroke and −717A > G (rs2794521) CRP gene polymorphism among stroke patients in West Pomerania province of Poland

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ABSTRACT

Background and purpose: Some of the risk factors of ischaemic stroke influence the development of atherosclerosis, which is a significant cause of vascular incidents. An inflammatory component plays a role in pathogenesis of both atherosclerosis and atrial fibrillation, the most important risk factor of embolic strokes. C-reactive protein (CRP) concentration in blood reflects the inflammatory process. Concentration of this protein depends on the *CRP* gene polymorphism. The aim of the study was to assess the relationship between selected risk factors of stroke and variant of −717A > G (rs2794521) *CRP* gene polymorphism in population of West Pomerania Province of Poland.

Materials and methods: There were 125 consecutive patients with ischaemic stroke analysed, who met the inclusion and exclusion criteria. In all patients, −717A > G *CRP* gene polymorphism was genotyped and analysed in relation to selected stroke risk factors.

Results: Prevalence of type 2 diabetes was lower in patients with AA genotype of −717A > G *CRP* gene polymorphism than in patients with other alleles ($p = 0.017$). Subjects with GG genotype had significantly higher concentration of CRP comparing to AG genotype ($p = 0.023$). No correlation was found between −717A > G *CRP* gene polymorphism and the lipid profile and other selected risk factors of stroke.

Conclusions: In patients with ischaemic stroke in West Pomerania Province, the GG genotype of −717A > G *CRP* gene polymorphism is associated with significantly higher CRP concentration in relation to AG genotype. Patients with AA genotype may be characterised by lower prevalence of type 2 diabetes.

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1. Introduction

Stroke is the third cause of death and first cause of permanent disability, especially among people more than 60 years of age [1]. Between 2004 and 2006, the incidence of ischaemic stroke in European population amounted to 114.7 in men and 74.9 in women, while in Polish population it reached 147.2 and 125.9 per 100 000, respectively [2]. In a study covering relatively small population in Poland between 2005 and 2006, much higher incidence of ischaemic stroke and intracerebral haemorrhage was noted: 211/100 000 for men and 130/100 000 for women (mean 167/100 000) [3].

Pathogenesis of particular types of ischaemic stroke is associated with risk factors. Understanding of these factors enables proper stroke prevention. Inflammatory factors play a substantial role in the process that leads to atherosclerosis development. It is reflected by increased level of C-reactive protein (CRP) in patients with advanced atherosclerosis [4,5]. The blood CRP level is applicable in the assessment of risk and prognosis in stroke patients. Nevertheless, blood level is liable to be modified by environmental (inflammatory disorders, statins) and genetic (gene polymorphism) factors.

CRP is produced as the response on inflammatory stimuli largely under control of inflammatory cytokines including interleukin-6 (Il-6) and tumour necrosis factor-α (TNF-α) [5,6]. Development of atherosclerotic lesions observed in carotid arteries correlates with increased CRP [7]. C-reactive protein level correlates with the increased risk of ischaemic stroke in general population and asymptomatic stenosis of internal carotid artery [8,9]. It was shown that inflammatory factors take part in the atrial fibrillation (AF) pathogenesis that is associated with embolic strokes [10]. Positive correlation between AF incidence and CRP level was found [5,11–14].

The expression of CRP is genetically conditioned. The *CRP* gene is localised on chromosome 1 (1q21–1q23) and is characterised by genetic variability. There has been about 30 *CRP* gene single nucleotide polymorphisms (SNP) described so far [15]. The relationship between particular SNP allele (variants) and diverse CRP expression was shown, but these results indicate both increase and decrease of CRP [7,16–20]. Depending on SNP variant of *CRP* gene, there have been revealed some differences in death risk in stroke patients, risk of recurrent stroke in patients with symptomatic internal carotid artery stenosis, cardiogenic stroke, risk of intracranial atherosclerosis and intima-media complex thickness (IMT) development [15,21–24]. Results of studies investigating relationship between CRP and −717A > G (rs2794521) *CRP* gene polymorphism are contradictory [16,17,25–28]. Individuals carrying the −717A allele had a 6.84-fold higher risk of developing coronary heart disease when compared with those not carrying this allele [28]. The risk of myocardial infarct and ischaemic stroke is decreased depending on −717A > G genotype [15,29]. *In vitro* studies suggested that differences in transcriptional activity depending on −717A > G *CRP* gene SNP – the G to A substitution at the site of rs2794521 resulted in increased transcriptional activity of the promoter of *CRP* gene [30].

As the examples given above, despite intensive studies in the field of *CRP* gene polymorphism role in the stroke pathogenesis, obtained results are contradictory. It is also in regard to the association between −717A > G *CRP* gene SNP, CRP level and incidence risk of vascular episodes. CRP is the independent risk factor of stroke and additionally exert indirectly an effect throughout the pathogenetic link with atherosclerosis, AF and type 2 diabetes [31,32]. Investigation of mutual interactions between *CRP* gene genotype and presence of stroke risk factors may be thus helpful in a detection of novel connections on immune-inflammatory basis.

2. Materials and methods

There were 125 consecutive subjects enrolled in the study, who were hospitalized in the Neurology Clinic of the First Independent Public Clinical Hospital of Pomeranian Medical University (SPSK 1 PUM) in Szczecin between January 2008 and June 2010, of age above 18, that were inhabitants of West Pomerania Province. Main inclusion criterion was diagnosis of ischaemic stroke on the first day after the symptoms onset, confirmed by clinical examination and brain neuroimaging tests (computed tomography or nuclear magnetic resonance) [33]. Patients with both atherothrombotic and embolic stroke were enrolled in the study.

Written consent to participate in the study was obtained from a patient or caregiver. Local Bioethical Committee approval was also obtained (resolution No. BN-001/118/07 KBP, Szczecin, 27th of November 2007).

We excluded patients with body temperature of more than 37.4 °C, clinical or biochemical features of infection, chronic inflammatory disorders, cancers and those treated with any of 3-Hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors (statins) within last year.

Presence of risk factors was established upon history and medical records. Hypertension and diabetes were diagnosed upon history and during hospitalisation. The body mass index (BMI) was calculated by body mass in kilograms divided by squared-height, in metres. Assessment of common, internal carotid arteries and vertebral arteries was made with the use of GE LOGIQ Book XP camera, linear head 7.5 MHz. Transcranial measurement of systolic and diastolic pressure in middle cerebral arteries was made with the use of Multi-Dop P System ultrasonography, head 2 MHz (DWL Elektronische Systeme GmbH). The complex of intima-media was described as the distance between lines demarcating the artery lumen and inner wall (intima) to media and adventitia. IMT was measured on the distal wall of common carotid arteries in the distal part [34,35].

Atherosclerotic plaques within carotid arteries were identified according to guidelines from Manheim consensus [36]. The stenosis grade of carotid arteries was considered for the bifurcation of common carotid artery or extracranial part of internal carotid artery.

Biochemical laboratory tests were made in the Central Laboratory of SPSK 1 PUM. Blood CRP level was measured using immunoturbimetric method enhanced by latex, with the lowest detection level of 1 mg/L.

DNA was isolated from the venous blood that was collected into EDTA anticoagulant tubes.

The −717A > G *CRP* gene SNP (rs2794521:C > T) was detected in the promoter region of *CRP* gene with the use of

Table 1 – Characteristics of the subjects (n = 125)[a].

Variable	Mean (SD)	Median (range)
BMI (kg/m^2)	27.7 (4.2)	27.1 (19.5–43.4)
SBP on admisssion (mm Hg)	160.3 (30.9)	158 (102–280)
DBP on admission (mm Hg)	92.4 (18.6)	90 (60–180)
Glycaemia on admission (mg/dL)	133.3 (56.9)	114 (50–382.9)
CRP (mg/L)	5.77 (7.93)	3.1 (0.5–69.6)
Total cholesterol (mg/dL)	224.8 (44.6)	219 (65–396)
HDL (mg/dL)	49.7 (12.2)	50 (25–88)
LDL (mg/dL)	146.4 (36.2)	141 (55–255)
Triglycerides (mg/dL)	154 (93.6)	135 (53–704)

SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; HDL, high density lipoproteins; LDL, low density lipoproteins.
[a] Body mass index (BMI) was assessed in 86 subjects.

PCR-RFLP method (*Polymerase Chain Reaction – Restriction Fragment Length Polymorphism*) with Bsh1236I enzyme, in the Department of Experimantal and Clinical Pharmacology of Pomeranian Medical University [28].

3. Statistical analysis

Investigated parameters were evaluated by the sample size, arithmetic mean, median, standard deviation, minimum and maximum value (continuous variables). CRP level, because of the right-oblique distribution, was logarithmically transformed. Arithmetic means were compared between studied polymorphisms with the use of one-way analysis of variance (ANOVA) and *least significant difference* (LSD) as *post hoc* test.

The frequency values of discrete variables, characterised by counts and percentages were analysed in fourfold table; Pearson χ^2 test was applied. Statistical significance of all analyses was set as $p < 0.05$.

4. Results

There were 79 (63%) men enrolled in the study; mean age of subjects was 64.3 years (standard deviation [SD] = 11.2, range: 39–89 years). Prehospital hypertension treatment was applied in only 46% subjects. Previous diabetes treatment was used in 12%. The most frequent heart rhythm disturbances were chronic (9.6%) and paroxysmal (7.2%) AF. Alcohol abuse was revealed in 12%. In the period preceding hospitalisation, 20% of patients had been receiving antiplatelets, and only 3% – oral anticoagulants. During hospitalisation, antiplatelet treatment was used in 98%, oral anticoagulants in 13%, alteplase in 5%. Diabetic drugs were used in 18%. (Tables 1 and 2).

Table 2 – Risk factors of ischaemic stroke.

Variable	n	%
Hypertension	100	80
Coronary heart disease	43	34.7
Previous acute coronary syndrome	12	9.6
Type 2 diabetes	27	21.6
Present cigarette smoking	64	51.6
Previous stroke/transient ischaemic attack	10	8
Heart rhythm disturbances	24	19.2
Occlusion of carotid arteries	18	14.6
Stenosis of carotid arteries ≥50%	37	26.1
Atherosclerotic plaques in carotid arteries	95	77.3

In the group of our patients, AA genotype of −717A > G *CRP* gene polymorhism was the most common (n = 67, 53.6%), AG genotype was present in 50 cases (40%) and GG genotype in 8 subjects (6.4%). The genotype distribution was consistent with awaited, appointed upon Hardy–Weinberg rule [37].

CRP level was significantly higher in GG genotype patients when compared to AG genotype (p = 0.23, LSD *post hoc* test). Borderline correlation was observed between CRP level measured on the first day of hospitalisation and −717A > G *CRP* gene SNP in all subjects (p = 0.069, ANOVA test).

As presented in Table 3, there was an association between type 2 diabetes and −717A > G *CRP* gene polymorphism (p = 0.05). When AA genotype was compared to AG + GG genotypes, the AA patients were characterised by significantly less frequent incidence of diabetes in relation to other patients (p = 0.017). No relationship was noted between −717A > G *CRP* gene polymorphism and either selected parameters mentioned in Table 3 or lipid profile (total cholesterol, LDL, HDL, triglycerides). No significant relations were found between −717A > G *CRP* gene polymorphism, systolic and diastolic blood pressures and in relation to left and right IMT.

Table 3 – Comparison of incidence of selected risk factors of stroke and genotype of the −717A > G *CRP* gene.

Parameter	AA genotype (n)	AG genotype (n)	GG genotype (n)	p-Value[a]
Stenosis of carotid arteries ≥50%	16	18	3	NS
Hypertension	55	40	5	NS
Coronary heart disease	22	20	1	NS
Previous acute coronary syndrome	7	5	0	NS
Type 2 diabetes	9	15	3	0.05; 0.017[b]
Previous stroke/transient ischaemic attack	4	6	0	NS
Heart rhythm disturbances	9	13	2	NS
Atherosclerotic plaques in carotid arteries	47	41	6	NS

NS, not significant.
[a] Pearson χ^2 test.
[b] For comparison of: AA *vs.* AG + GG.

5. Discussion

The distribution of −717A > G *CRP* gene polymorphism in our patients was as follows: AA (53.6%), AG (40%), and GG (6.4%). In a study conducted in a group of healthy Korean men, the distribution was different: AA – 2%, AG – 22%, GG – 76% [26]. This difference may result from race dissimilarities that are observed in inflammatory cytokines polymorphisms [38]. On the other hand, the AA genotype may be a stroke risk factor. In another study, a distribution consistent with our results was found [25]. Further studies are necessary to answer the question if −717A > G *CRP* gene polymorphism may be a stroke risk factor.

We were mainly interested in plausible association between −717A > G *CRP* gene polymorphism and selected stroke risk factors. In the first step, the analysed genotype was related to CRP level. It was found in the LSD *post hoc* test that CRP was significantly higher in GG genotype patients when compared to AG ($p = 0.023$), while in the ANOVA test there was no statistical significance, but only a tendency ($p = 0.069$). Till now, only in one study it was shown that stroke patients with AG and GG genotype had higher CRP levels than AA genotype patients [26]. In other studies, there was no relationship found between any of the −717A > G *CRP* gene polymorphisms and CRP level [16,17,25,27,28].

The effect of CRP and other inflammatory cytokines on the risk of type 2 diabetes raises no doubts. The relationship between inflammatory molecules' genetic variants *e.g.* fibrinogen with certain disorders is not clear and fully known [31,32,39]. In the analysed group it was shown that AA genotype compared to other patients was associated with less frequent incidence of type 2 diabetes ($p = 0.017$). There are no previous studies on the association between −717A > G *CRP* gene polymorphism and diabetes, but other *CRP* gene polymorphisms were investigated. No relationship was found in relation to rs12093699 *CRP* gene polymorphism, but significant association was found in rs3093059 (OR 7.01; 95% CI: 1.16–42.22; $p = 0.03$) and rs133552 *CRP* gene polymorphisms [31,40,41].

In our study, no relationship between −717A > G *CRP* gene polymorphism and other, selected stroke risk factors was found. Further search for such relationships seems to be reasonable, especially in relation to risk factors connected with inflammation, such as AF. In the genome-wide association study (GWAS) the relationship between T allele of rs2200733 polymorphism on 4q25 chromosome and cardioembolic stroke, especially with the AF background was found [42].

Observations made in our study are useful in terms of further studies and efficacy improvement of prevention and treatment of ischaemic stroke patients. We are aware of methodological limitations related to the small sample size that additionally were in the acute phase of stroke which is accompanied by the complex immune and inflammatory processes.

6. Conclusions

1. In stroke patients recruited in the West Pomerania province of Poland, the GG genotype of −717A > G *CRP* gene polymorphism is associated with higher CRP level compared to AG genotype.
2. AA genotype patients may have lower risk of type 2 diabetes.

Conflict of interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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