Original article

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Impact of the –174G/C interleukin-6 (*IL-6*) gene polymorphism on the risk of paediatric ischemic stroke, its symptoms and outcome

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Abstract

Objectives: *Ischemic stroke remains one of the top ten causes of death in children. There is evidence for the role of pro-inflammatory cytokines, such as IL-6 and the –174G>C promoter polymorphism of the IL-6 gene, in the occurrence and outcome of stroke in adults. The aim of the present study was to determine a possible association between the –174G>C IL-6 polymorphism and occurrence of paediatric stroke, its symptoms and outcome.*

Material and methods: *The study group consisted of 340 individuals: 80 stroke children, 122 parents of patients and 138 controls. The –174G/C polymorphism was genotyped using the RFLP method. For the analysis of the relationship between genotypes and stroke we used two alternative methods: the case-control model and the transmission test for linkage disequilibrium using data from families.*

Results: *We observed no differences in the transmission of alleles from parents to children. We also did not find any statistical differences in distribution of genotypes and alleles between patients and controls. However, the analysis showed that post-stroke epilepsy was genotype-dependent. All children with epilepsy were G allele carriers and none of them was a CC homozygote whereas about 25% of children without epilepsy had the CC genotype.*

Conclusions: *Our study did not show any associations between the IL-6 –174 G>C polymorphism and the occurrence of stroke but we observed a relation between post-stroke epilepsy and the G allele carrier-state.*

Key words: *stroke, children, interleukin-6, polymorphism.*

Introduction

Stroke in adults is the leading cause of morbidity and mortality in developing countries. Although childhood stroke is not that common it remains one of the top ten causes of death in children. Children surviving stroke show a high risk of recurrence and chronic neurological sequels. Multiple risk factors such as congenital and acquired heart diseases, arteriopathies, sickle cell anaemia, haematological disorders or infec-

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tions are identified in children with stroke. However, the aetiology of stroke in many cases remains unknown and seems to be multifactorial [3,17]. The significance of genetic factors in the aetiology of stroke was confirmed by studies of twins, however, genetic and molecular basis of the mechanism underlying paediatric stroke is still poorly understood [4].

There is evidence for the role of pro-inflammatory cytokines, such as interleukin-6 (IL-6), in the occurrence and outcome of stroke in adults. IL-6 showed a significant increase of serum levels already within the first hours following the onset of the disease. The IL-6 level was significantly correlated with increasing volumes of brain lesion, infarct volume, stroke severity, and poor functional and neurological outcome [6,10,18,22,23]. The –174G>C promoter polymorphism of the *IL-6* gene seems to be a functional polymorphism associated with a different level of secreted protein according to the genotype. However, different studies have reported conflicting results. In some populations of patients [5], higher IL-6 levels are associated with the GG genotype while in other populations – with the CC genotype [16]. Some studies in adult patients also showed an association between the –174G>C *IL-6* polymorphism and stroke occurrence, an increased risk of carotid plaque formation, and severity of disability after the event [7,8,13].

The aim of the present study was to determine a possible association between the –174G>C *IL-6* polymorphism and occurrence of paediatric stroke, its symptoms and outcome. To avoid some of the problems often encountered when applying the case-control design, e.g. the proper control group selection, we also used an alternative method, the transmission disequilibrium test (TDT) using data from families.

Material and methods

The study group consisted of 340 individuals, including 80 patients (35 girls and 45 boys), 122 parents of stroke children, and 138 control children. Patients were recruited from the Department of Neuropaediatrics at the Medical University of Silesia in Katowice (42 children), the Department of Developmental Neurology at the Medical University of Gdansk (17 patients), the Department of Paediatric and Adolescent Neurology at the Jagiellonian University Medical College in Krakow (12 children) and the Department of Neurology at the Polish Mother's Memorial Hospital – Research Institute in Lodz (9 patients).

The age of patients ranged from 6 months to 18 years (mean age 8.75 years) at the time of diagnosis, and 2 to 25 years (mean age 14.33 years) at the time of the follow-up.

The control group was age- and sex-matched, and consisted of children with no history of ischemic stroke or other vascular diseases. The age of control children ranged from 3 months up to 18 years (mean age 7.5). There were 73 boys and 61 girls. The study protocol was approved by the Ethics Committee of the Medical University of Silesia in Katowice and written consents were submitted by the patients' parents.

Genetic analysis

Genomic DNA was extracted from peripheral leukocytes using the MasterPure™ genomic DNA purification kit (Epicentre Technologies). The *IL-6* – 174G/C polymorphism was genotyped using the restriction fragment length polymorphism method described by Elghannam *et al.* [9] with own modifications. The amplification parameters were: initial 1 min denaturation at 94°C, followed by 35 cycles of 94°C for 0.5 min, 64°C for 0.5 min, and 72°C for 0.5 min. The product of amplification was digested by the Hsp92II restriction enzyme (Promega) generating fragments: 230 and 75bp for G allele and 121, 109 and 75bp for C allele. The fragments were separated on 8% polyacrylamide gel and visualized by the $AgNO_3$ staining.

Statistical analysis

The allele frequencies were assessed on the basis of the genotype distribution. Hardy-Weinberg equilibrium in all groups was tested by the χ^2 test. The transmission/disequilibrium test was used for the analysis of a possible relationship between the *IL-6* –174G/C polymorphism and stroke [19]. The test was based on the analysis of transmission of specific alleles from the heterozygous parents to their affected children. Transmission of a particular allele to a child is expected to be 50% if there is no association between the allele and the disease. An excess transmission to the offspring is expected if the allele is associated with an increased risk of the disease. The frequencies of transmitted alleles observed in the study were compared with the expected frequencies using the χ^2 test. Alternatively, the case-control method was applied. The genotype and allele frequencies were compared between patients and children without stroke using the χ^2 test. An analysis of clinical symptoms and con-

sequences of stroke according to the genotype (carrier-state of G or C allele) was also performed using the χ^2 test. We analyzed stroke symptoms such as hemiparesis, hemiplegia, facial nerve paresis, impaired consciousness, aphasia, headache, and seizures. During the follow-up examination, hemiparesis, worsening of intellectual function, post-stroke epilepsy, and aphasia were analyzed. The *EpiInfo 6* (WHO) software was used in all statistical analyses.

Results

General characteristics

At the time of diagnosis, the predominant clinical symptoms were hemiparesis (60/80 patients) and central facial nerve paresis (61 children). Impaired consciousness was observed in 45 patients, aphasia occurred in 41, headaches in 40 and seizures in 14 children with stroke. The rarest symptom was hemiplegia. During the follow-up examination most commonly reported symptoms were hemiparesis, usually middle- or low-grade (47 patients), worsening of the intellectual function in 22 patients, post-stroke epilepsy in 12 children, and aphasia in 7 children. Other symptoms were observed in 16 patients. The features of the extrapyramidal syndrome were most common. Using the classification of ischemic stroke types, most patients presented partial anterior circulation infarct (PACI), lacunar infarct (LACI) or total anterior circulation infarct (TACI), only 11 children had posterior circulation infarction (POCI) [2,20].

Genotype analysis

The genotype frequencies were in consistence with the Hardy-Weinberg equilibrium in patients and the control group.

There were 61 full families (both parents and a child) analyzed in the transmission/disequilibrium test. Forty six of them were informative trios ("informative trio" is a family with at least one heterozygous parent). Only in such informative families, it was possible to deduce which allele had been transmitted from the parent to a child. Although the C allele was observed to be more often transmitted, the difference was not statistically significant (Table I).

In the case-control model, we also did not observe any statistical differences in distribution of genotypes and alleles. There was only a tendency to a higher prevalence of C allele and CC homozygotes in the children with stroke (Table II). The frequency of genotypes and alleles did not differ between girls and boys. The analysis of clinical symptoms and outcome of stroke showed that only post-stroke epilepsy was genotype-dependent. All the children with epilepsy were G allele carriers and none of them was a CC homozygote, while about 25% of the children without epilepsy had the CC genotype ($\chi^2 = 4.01$, $P = 0.045$) (Table III).

Discussion

In the present study we did not observe any association between the –174G>C polymorphism and stroke occurrence in children although the frequency of CC

Table I. The results of transmission/disequilibrium test

Allele	Transmitted	Not transmitted	χ^2; P
G	27	36	0.75; 0.37
C	36	27	0.75; 0.37

Table II. Distribution of genotypes and alleles of the *IL-6* gene

Genotype/allele	Stroke children		Control children	
	n	%	*n*	%
GG	21	26.2	40	29
GC	43	53.8	76	55.1
CC	16	20.0	22	15.9
G	85	53.1	156	56.5
C	75	46.9	120	43.5
GG/GC + CC*	$\chi^2 = 0.19$, $P = 0.66$			
GG + GC/CC*	$\chi^2 = 0.58$, $P = 0.45$			
G/C*	$\chi^2 = 0.42$, $P = 0.49$			

**Comparison of stroke children with the control group*

Table III. The analysis of association between genotype and epilepsy

Epilepsy**	GG + GC	CC
0	46	15
1	13*	0

**$\chi^2 = 4.01$, $P = 0.045$*

***Data available for 74 patients*

homozygotes was slightly higher in patients than in control children. The transmission of C alleles from parents to the affected children was also slightly more frequent than G alleles, but similarly the difference was not statistically significant. Applying both methods allowed to obtain more reliable and informative results and to avoid some problems often encountered when applying the case-control design, e.g. the proper control group selection. The research of Turkish children with stroke gave similar findings although the authors conducted only a case-control study [15]. The comparison of both studies showed that the allele frequencies significantly differ between the populations among both patients (46.9% vs. 20.5% for C allele) and control subjects (43.5% vs. 23.3% for C allele). Such ethnic differentiation of allele frequencies seems to make reasonable association studies even if the relationship between the specific polymorphism and the disease was not confirmed in one of the populations.

Acute inflammation plays a key role in secondary brain injury induced by ischaemia [13]. Therefore, we have also analyzed a possible association between the IL-6 –174G>C polymorphism and some clinical symptoms and outcome of stroke in our patients. The analysis showed that only post-stroke epilepsy was genotype-dependent. All children with epilepsy were G allele carriers and none of them was a CC homozygote. The animal studies showed that the IL-6 level may be related to epilepsy. Pro-convulsive effect of recombinant human interleukin-6 was observed in rats. Transgenic mice, whose astrocytes produced higher amounts of IL-6, were characterized by a high susceptibility to seizures induced by even small doses of kainic acid [1]. Accumulating clinical evidence also strongly supports the relevance of inflammation in the pathophysiology of human epilepsy. The central nervous system (CNS) injuries such as stroke are considered common risk factors for developing epilepsy, and long-lasting CNS inflammation develops rapidly after such events. Moreover, astrocytic overexpression of cytokines such as TNF-α or IL-6 results in age-dependent development of neurological dysfunctions, including seizure susceptibility and spontaneous seizures. An antiepileptic activity of selected anti-inflammatory drugs also suggests a role of inflammation in the pathophysiology of epilepsy [21].

A possible role of the *IL-6* polymorphism in the development of post-stroke epilepsy may result from its influence on the IL-6 plasma level. The first report of Fishman *et al*. [11] revealed that IL-6 level was nearly twice as high in subjects with the GG genotype as in those with the CC genotype. However, subsequent studies reported conflicting results [23]. Some authors suggest that the genetic control of the IL-6 production is age and gender dependent. Kilpinen *et al*. [16] showed that neonates with the CC genotype secreted significantly more IL-6 than carriers of the G allele, while the production of IL-6 in adult controls was not associated with the *IL-6* -174 G>C polymorphism.

Concluding, our study did not show any associations between the *IL-6* -174 G>C polymorphism and an occurrence of stroke, however, we observed a relation of post-stroke epilepsy with the G allele carrier-state. Yet the finding should be confirmed in a larger group of patients.

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