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Association of *IL8* and *IL10* gene allelic variants with ischemic stroke risk and prognosis

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Aim. *Evaluating a role of IL8 gene –781 C/T, and IL10 gene –592C/A polymorphisms as genetic markers of ischemic stroke risk.* ***Methods***. *A case group consisted of 183 patients with ischemic stroke, which were treated in the Brain Vascular Pathology unit of SI «Institute of Gerontology of NAMS of Ukraine». A control group included 88 healthy individuals older than 65 years without any history of ischemic stroke. Genotyping was performed using PCR followed by restriction fragment length polymorphism analysis.* ***Results***. *Significantly ($P < 0,05$) higher frequency of IL8 –781T allele carriers in the case group (81,6 %) comparing to the control (70,1%) was revealed. –781T allele carriers have nearly 2-fold increased ischemic stroke develop- ment risk (OR = 1.886; 95 % CI: 1.041–3.417). Significantly ($P < 0,05$) higher frequency of IL10 gene –592C allele carriers was observed in the patients with ischemic stroke (98,2%) comparing to the control (90,7 %). The ischemic stroke development risk in such individuals is 5-fold increased (OR = 5.71; 95 % CI: 1.48–22.11). It was revealed that –592C allele homozygotes with ischemic stroke have more than 2-fold higher improvement (according to the Rankin scale) chances during the first fortnight of treatment (OR = 2,76; 95 % CI: 1,26–6,07).* ***Conclusions***. *On the basis of the obtained significant differences, IL8 gene –781T and IL10 gene –592C variants may be considered the factors of ischemic stroke hereditary susceptibility. Besides, IL10 gene –592CC genotype is a genetic marker of the patients state positive dynamics during first two weeks of treatment.*

Keywords: interleukin, ischemic stroke, polymorphism.

Introduction. Inflammation is a key process in organism protection, which is activated in response to different traumas and injuries [1]. Cerebral ischemia induces quick inflammatory reaction involving several cell types [1]. The whole range of modern studies is focused on ischemia-related inflammatory signaling proving its involvement in all stages of the ischemic cascade [2]. Cytokine environment (a network of interacting cytokines and their receptors) is a crucial contributor to the inflammatory response and thus is closely connected to the pathophysiology of ischemia-induced brain injury, especially ischemic stroke. The balance between pro- and anti-inflammatory cytokines is significantly altered by changes in respective gene expression mostly due to polymorphisms in their promoter and intron regions [3, 4]. In order to investigate certain interleukin role in the stroke pathogenesis two genes were chosen: pro-inflammatory interleukin 8 (*IL8*) and anti-inflammatory interleukin 10 (*IL10*). Interleukin 8 is a cytokine of chemoattraction, which also functions as growth and angiogenesis factor. It induces immune cell infiltration in ischemia zone and may participate in reperfusion [5]. *IL8* gene is located on chromosome 4 in position 4q13–q21 and consists of 4 exons and 3 introns [6]. The total amount

of 235 SNPs is reported for this gene [7]. A common C to T transition in position –781 of first intron in *IL8* gene creates a recognition site for the transcription factor C/EBP β that affects the expression and leads to a higher production of the respective protein [8]. Therefore, it was selected as a possible genetic marker of the ischemic stroke risk. Interleukin 10 is an anti-inflammatory cytokine associated with the tissue repair and cytoprotective effects [9]. *IL10* gene is located on chromosome 1 in position 1q31–q32. It consists of 5 exons and 4 introns [10]. The total amount of 187 SNPs is reported for this gene [7]. A promoter variant –592C/A is located in the Sp1 transcription factor recognition site leading to an altered affinity of this factor to DNA sequence and reduced level of the respective cytokine [11]. A functional role of this polymorphic variant makes it a possible genetic marker of the ischemic stroke risk.

The aim of this study was to evaluate the role of the *IL8* gene –781C/T, and *IL10* gene –592C/A polymorphic variants as genetic markers of the ischemic stroke risk.

Matherials and methods. *Study groups*. The case group consisted of 183 patients with ischemic stroke (men – 95, women – 88, average age – 64.6 ± 9.1). All the patients have undergone examination and treatment in the Brain Vascular Pathology unit of «Institute of Gerontology of NAMS of Ukraine». The patients were documented according to a standard protocol comprising the following information: medical history (including general diseases and previous medication with particular reference to the vascular diseases); vascular risk factors (including hypertension, diabetes, cigarette smoking, body mass index); results of clinical and instrumental investigations (including carotid and vertebral ultrasound, electroencephalography, echocardiography, MRI and CT); stroke cause and stroke severity as measured by validated scales.

The control group included healthy individuals older than 65 years (n = 88, men – 35, women – 53, average age – 73.9 ± 6.4) without the history of ischemic stroke. The individuals comprising this group were subjected to the standard interview including their medical history and vascular risk factors.

All the participants were non-related and represented the general population of Ukraine without selection on ethnical background. An informed consent was obtained from each participant prior to blood collection and DNA extraction. This study was approved by the Ethical Committee of Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine.

Genotyping. The DNA was extracted from peripheral blood leukocytes according to standard procedures. Genotyping for the *IL8* gene –781C/T, and *IL10* gene –592C/A polymorphisms was performed by the PCR with following restriction fragment length polymorphism (RFLP) analysis as described elsewhere [8, 12].

Statistical analysis. The $\chi 2$ test was used to detect deviations from Hardy–Weinberg equilibrium in genotype distribution. Fisher's exact test (Mid–P method) was used to estimate the difference in genotype and allelic distribution. In order to assess the association of certain genotype with ischemic stroke development OR index was calculated. A P-value of less than 0.05 was regarded as significant. Statistical analysis has been performed using GenePop and OpenEpi statistical packages [13, 14].

Results and discussion. The observed genotype distributions for two studied polymorphic variants did not deviate from the ones expected according to the Hardy–Weinberg equilibrium in all investigated groups (Table 1).

The analysis for the *IL8* gene –781 C/T polymorphic variant revealed a significantly higher frequency of –781T allele carriers in the case group (81.6 %) comparing to the control group (70.1 %). Further statistical analysis showed that the carriers of *IL8* –781T allele have nearly 2-fold increased risk of ischemic stroke development (OR = 1.89; CI 95 %: 1.04–3.42). The obtained data may be explained as follows: the ischemic injury is a result of the cellular and molecular events cascade, caused by a lack of blood flow with further hypoxia [2, 9]. The hypoxic damage leads to the «danger molecules» production by injured and dying cells – a crucial trigger of post-ischemic immune system activation and ischemic area enlargement [9]. Pro-inflammatory interleukin 8 surplus that is characteristic of –781T allele carriers may promote the expansion of ischemic injury area and its transformation to an infarction zone.

The significantly higher frequency of the *IL10* gene –592C allele carriers was observed in the patients with ischemic stroke (98.2 %) comparing to the control

Table 1
Genotype and allele frequency for studied polymorphic variants with results for Hardy–Weinberg probability test and association tests

Polymorphism	Genotype	Case group		Control group		Odds Ratio		
		n	%	*n*	%	P	OR	95 % CI
–781C/T IL8 gene	*CC*	33	18.4	26	29.9		0.53	0.29–0.96
	CT	102	57.0	45	51.7	0.04	1.89	1.04–3.42
	TT	44	24.6	16	18.4			
	Total	179	100	87	100	–	–	–
	Allele					–	–	–
	C	168	46.9		55.7	–	–	–
	T	190	53.1		44.3	–	–	–
Hardy–Weinberg probability test; P-value*		0.0694		0.8298		–	–	–
–592C/A IL10 gene	Genotype							
	CC	116	68.2	49	57.0		5.71	1.47–22.10
	CA	51	30.0	29	33.7	0.01	0.18	0.05–0.68
	AA	3	1.8	8	9.3			
	Total	170	100	86	100	–	–	–
	Allele					–	–	–
	C	283	83.2	127	73.8	–	–	–
	A	57	16.8	45	26.2	–	–	–
Hardy–Weinberg probability test; P-value*		0.4204		0.2625		–	–	–

N o t e. *Estimation of exact P- values conducted by the Markov chain method.

group (90.7 %). The carriers of this allele have almost 6-fold increased risk of the ischemic stroke development (OR = 5.71; 95 % CI: 1.47–22.11). The individuals carrying –592C allele may be assumed to have an impaired primary inflammatory response to the cerebral ischemia because of the anti-inflammatory interleukin 10 increased level. The cerebral tissues, presumably, react slower to hypoxia under such conditions that leads to the reperfusion delay and cell necrosis promotion [2, 9].

In order to evaluate the role of individual's genotype in the process of post-stroke improvement the genotype distributions for two studied polymorphic variants have been analyzed in the group of patients with decreased stroke severity (assessed using Rankin scale on the 3rd and the 14th days of treatment) and no changes in a state. The obtained results are presented in Table 2.

No association has been found between the *IL8* gene –781C/T polymorphic variant genotype and the stroke severity dynamics. Interestingly, the individuals homozygous for *IL10* gene –592C allele have more than 2-fold higher chances of improvement during the first two weeks of treatment (OR = 2.78; 95 % CI: 1.26–6.12). The obtained results about the association of –592C allele with the increased risk of ischemic stroke and at the same time with the positive post-stroke improvement prognosis association may seem controversial at the first glance. However, in fact these data ref-

Table 2
Genotype and allele frequency for studied polymorphic variants and results of association tests in patients with improved state by the 14th day of treatment and patients with no changes in stroke severity

Polemorphism	Genotype	Case group		Control group		Odds Ratio		
		n	%	*n*	%	P	OR	95 % CI
–781C/T IL8 gene	*CC*	6	11.0	26	22.6		0.43	0.16–1.11
	CT	34	63.0	60	52.2	0.08	2.34	0.90–6.07
	TT	14	25.9	29	25.2			
	Total	54	100	115	100	–	–	–
	Allele					–	–	–
	C	46	42.6	112	48.7	–	–	–
	T	62	57.4	118	51.3	–	–	–
	Genotype							
–592C/A IL10 gene	*CC*	43	81.1	65	60.7		2.78	1.26–6.12
	CA	10	18.9	39	36.5	0.01	0.36	0.16–0.79
	AA	0	0.0	3	2.8			
	Total	53	100	107	100	–	–	–
	Allele					–	–	–
	C	96	90.6	169	79.0	–	–	–
	A	10	9.4	45	21.0	–	–	–

lect the contradictory roles of various inflammatory responses in cerebral ischemia. The inflammatory response during the acute phase of cerebral ischemia evokes neuroprotective mechanisms through preconditioning, which leads to the ischemic tolerance [1, 9]. The suppression of pro-inflammatory pathways by high levels of *IL-10* during early stages of ischemic process may have a detrimental effect. On the other hand, during later stages of cerebral ischemia the beneficial aspects of inflammation are outbalanced by its contribution to the ischemic lesion progression. The pro-inflammatory cytokine (interleukin 6, 8, 1 β) production induces the inflammatory molecules expression and circulating lymphocyte infiltration in the area of cerebral infarction, which results in the area expansion. Anti-inflammatory interleukin 10 negatively regulates the pro-inflammatory cytokine production and thus may prevent injured area enlargement [1, 2, 9].

Conclusions. On the basis of revealed significant differences it was established that the *IL8* gene –781T and *IL10* gene –592C variants may be considered the genetic markers of the ischemic stroke development risk. On the other hand, the *IL10* gene –592CC genotype is associated with the positive post-stroke improvement prognosis. Though, it is important to mention that the final conclusion about the involvement of studied markers in the cerebral ischemia pathogenesis would be possible to make only after the verification of the obtained results by independent study.

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Асоціація алельних варіантів генів *IL8* і *IL10* з ризиком розвитку і прогнозом ішемічного інсульту

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Резюме

Мета. *Оцінити роль поліморфних варіантів –781C/T гена IL8 і –592C/A гена IL10 як генетичних маркерів ризику розвитку*

*ішемічного інсульту. **Методи**. До групи дослідження ввійшли 183 пацієнти з ішемічним інсультом, які перебували на стаціонарному лікуванні у відділенні судинної патології головного мозку ДУ «Інститут геронтології НАМН України»; до контрольної – 88 здорових людей старше 65 років без історії ішемічного інсульту. Генотипування проводили методом ПЛР з наступним аналізом поліморфізму довжини рестрикційних фрагментів. **Результати**. Виявлено статистично достовірно (P < 0,05) вищу частоту носіїв алеля IL8 –781T у групі пацієнтів з інсультом (81,6 %) порівняно з контрольною групою (70,1 %). Носії алеля IL8 –781T мають майже вдвічі вищий ризик розвитку ішемічного інсульту (OR = 1,886; ДІ 95 %: 1,041–3,417). Статистично достовірно (P < 0,05) вища частота носіїв алеля –592C гена IL10 спостерігалась у пацієнтів з ішемічним інсультом (98,2 %) порівняно з контрольною групою (90,7 %). Ризик розвитку ішемічного інсульту (OR = 5,71; ДІ 95 %: 1,48–22,11) у носіїв цього алеля у 5 разів вищий. Встановлено, що в осіб, гомозиготних за алелем –592C гена IL10, у яких розвинувся ішемічний інсульт, шанси на покращення стану (за шкалою Ренкіна) протягом перших двох тижнів майже втричі більші (OR = 2,76; ДІ 95 %: 1,26– 6,07). **Висновки**. На підставі отриманих статистичних відмін- ностей встановлено, що алелі –781T гена IL8 і –592C гена IL10 є факторами спадкової схильності до розвитку ішемічного інсульту. Крім того, генотип –592CC гена IL10 є генетичним маркером позитивної динаміки стану пацієнта у перші два тижні лікування.*

Ключові слова: інтерлейкін, ішемічний інсульт, поліморфізм.

Ассоциация аллельных вариантов генов *IL8* и *IL10* с риском развития и прогнозом ишемического инсульта

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Резюме

***Цель**. Оценить роль полиморфных вариантов –781C/T гена IL8 и –592C/A гена IL10 в качестве генетических маркеров риска развития ишемического инсульта. **Методы**. В исследуемую группу вошли 183 пациента с ишемическим инсультом, находившихся на стационарном лечении в отделении сосудистой патологии головного мозга ГУ «Институт геронтологии НАМН Украины»; в контрольную – 88 здоровых людей старше 65 лет без истории ишемического инсульта. Генотипирование проводили методом ПЦР с последующим анализом полиморфизма длины рестрикционных фрагментов. **Результаты**. Выявлено статистически достоверно (P < 0,05) более высокую частоту носителей аллеля IL8 –781T в группе пациентов с инсультом (81,6 %) по сравнению с контрольной группой (70,1 %). Риск развития ишемического инсульта у носителей аллеля IL8 –781T почти вдвое выше (OR = 1,886; ДИ 95 %: 1,041–3,417). Статистически достоверно (P < 0,05) более высокая частота носителей аллеля –592C гена IL10 наблюдалась у пациентов с ишемическим инсультом (98,2 %) по сравнению с контрольной группой (90,7 %). Риск развития ишемического инсульта у носителей этого аллеля в 5 раз выше (OR = 5,71; ДИ 95 %: 1,48–22,11). Установлено, что у лиц, гомозиготных по аллелю –592C гена IL10, у которых развился ишемический инсульт, шансы на улучшение состояния (по шкале Рэнкина) в течение первых двух недель почти втрое больше (OR = 2,76, ДИ 95 %: 1,26–6,07). **Выводы**. На основании полученных статистических различий установлено, что аллели –781T гена IL8 и –592C гена IL10 являются факторами наследственной предрасположенности к развитию ишемического инсульта. Кроме того, генотип –592CC гена IL10 является генетическим маркером положительной динамики состояния пациента в первые две недели лечения.*

Ключевые слова: интерлейкин, ишемический инсульт, полиморфизм.

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