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TNFα gene G-308A polymorphism and the risk of ischemic stroke

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ABSTRACT

TNFα, a significant immune mediator, may contribute to the initiation and progression of the ischemic stroke. Genetics of TNFα molecule may have an important role in the risk of ischemic stroke. The most interesting aspects of the G-308A polymorphism remain unexplained; there are many discrepancies between the results. Differences in the ethnicity of the studied cohorts may be taken as one of the possibility. Our study material consisted of 101 patients with ischemic stroke, including 30% classified as lacunar stroke. The diagnosis was based on the presence of rapidly developing neurological signs lasting longer then 24 h and confirmed by neuroimaging matter. All patients were of Polish Caucasian origin. Randomly selected 100 individuals without any sign of the vascular disease of central nervous system were taken as the control material. The frequency of polymorphism G-308A in TNFα gene was determined as described by Rubattu et al. [11]. The genotype distribution in our material was similar and statistically insignificant between patients and controls. The heterozygotic G/A genotype was detected in 9% of patients and in 15% of control materials, homozygotic A/A was found in 5% of patients and only in one of control and G/G in 87% of patients and in 84% of control individuals. Our results are negative with respect to the impact of 308 TNFα polymorphism on the risk of ischemic stroke in Caucasians living in Poland.

Słowa kluczowe:
polimorfizm
TNFα
udar niedokrwienny.

STRESZCZENIE

Polimorfizm promotora genu G-308A TNFα a ryzyko udaru niedokrwiennego. Czynnik martwicy nowotworu alfa (TNFα) jest ważnym mediatorem immunologicznym i może współdziałać w zapoczątkowaniu i postępie udaru niedokrwiennego. Genetyka cząsteczki TNFα może mieć ważną rolę w ryzyku udaru. Najbardziej interesujący aspekt polimorfizmu G-308 A pozostaje niewyjaśniony, z powodu wielu różnic w wynikach badań. Różnice

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etniczne w badanych kohortach mogą stanowić jedną z przyczyn wystąpienia udaru niedokrwiennego. Nasz badany materiał dotyczył 101 chorych z udarem niedokrwiennym, w tym 30% zdiagnozowano jako udar lakunarny. Rozpoznanie było oparte na nagłym występowaniu objawów neurologicznych, trwających dłużej niż 24 godziny i potwierdzonych przez metody neuroobrazowania. Wszyscy badani byli Polakami rasy kaukaskiej. 100 przypadkowo wybranych osób bez objawów choroby naczyniowej ośrodkowego układu nerwowego stanowiło materiał kontrolny. Częstość występowania polimorfizmu G-308A genu TNFα była określana jak opisano przez Rubattu i wsp. /2005/ [11]. Rozdział genotypów w naszym materiale był podobny i nieistotny statystycznie pomiędzy chorymi i grupą kontrolną. Heterozygoty G/A były stwierdzone u 9% chorych i w 15% przypadków grupy kontrolnej, homozygoty A/A były stwierdzone u 5% chorych i tylko u 1 osoby kontrolnej, a G/G u 87% chorych i u 84% osobników kontrolnych. Nasze wyniki są więc negatywne odnośnie możliwego znaczenia polimorfizmu G-308A TNFα w ryzyku udaru niedokrwiennego u osób rasy kaukaskiej, żyjących w Polsce.

1. Introduction

The ischemic stroke is one of the most frequent causes of mortality and morbidity in the general population. There are now increasing number of evidences that proinflammatory cytokines contribute to the pathomechanism of stroke.

The tumor necrosis factor α (TNFα) seems to be one of the most important cytokines. There are many studies concerning the genetics of TNFα in connection with cerebro-vascular diseases in various world regions. However, there are still several controversies related to that subject.

Um et al. [1] reported that the TNFα GG genotype in Koreans happened to increase the relative risk for cerebral infarction and concluded that the TNF-alpha 308 locus plays an important role in the pathogenesis of cerebral infarction. Lee et al. [2] presented evidence that TNF-alpha gene polymorphism is associated with the increased susceptibility to ischemic stroke in Korean population. Trifunovic Cvetkovic et al. [3] claimed that in Swedish patients the A1A1 genotype of TNFα is associated with risk of stroke. According to Lalouschek et al. [4] carriers of the TNF-alpha (308) A allele face an increased risk of stroke only in association with a febrile episode.

Harcom et al. [5] presented evidence demonstrating that patients with ischemic stroke in Hungarian population had a significantly lower frequency of the 308A allele compared to healthy subjects. Of great interest are the conclusions of Tong et al. [6] that TNF-alpha 308 GA heterozygotes in the Chinese and Uyghur population may be protected against ischemic stroke. Results presented by Kim et al. [7] suggested that TNF-α 308 G→A and 238 G→A polymorphisms are responsible for susceptibility to ischemic stroke in Korean population.

Pereira et al. [8] basing on their metaanalyses concluded that the G-308A polymorphism is unlikely to be associated with an increased risk of ischemic stroke in Caucasians whereas in Asians such polymorphism might represent a protective factor.

Recent publications of metaanalyses by Cui et al. [9] express an opinion that despite some controversies it may be assumed that TNF-α is involved in the pathogenesis of stroke.

The significant differences between the European and Asian populations concerning the impact of the TNF-α gene in the pathogenesis of stroke certify that further respective studies in Caucasians are necessary.

2. Material and methods

The study material consisted of 101 patients (69 males and 32 females), aged 48–85 years, with ischemic stroke. The diagnosis was based on the presence of rapidly developing neurological signs lasting longer than 24 h. Using the TOAST criteria proposed by Goldstein et al. [10] the studied patients have been classified to the following subgroups: 30 cases – lacunar stroke, 18 patients – cardioembolic, 16 cases – atherosclerotic group and 16 as undetermined etiology – cryptogenic. The diagnosis was confirmed by neuroimaging methods (CT scan or/and MRI). All individuals were of Polish Caucasian origin. Patients with hemorrhagic stroke were excluded. Randomly selected 100 unrelated individuals without any signs of the vascular disease of central nervous system, matched for gender and age, were taken as control material. The control material consisted of 60 males and 40 females, aged 46–78 years with diagnose of lumbar pain (65 cases) and neurotic symptoms (35 patients). Both subgroups were without any inflammatory symptoms.

Methods: The frequency of polymorphism G-308A in tumor necrosis factor-alpha (TNF-α) gene was determined as described by Rubattu et al. [11]. The genomic DNA was extracted from a whole blood, collected on EDTA, using the isolation kit of A&A Biotechnology. In the next step, PCR was used to amplify a 519 bp fragment of the TNF promoter region using selected primers: 5′-CAAACACAGGCCTCAGGACTC-3′ and 5′-AGGGAGCGTCTGCTGGCTG-3′.

Then, 519 bp product was submitted to NESTED PCR using specific primers designed with the last nucleotide

Table 1 – Genotype and allele frequencies for the tumor necrosis factor (TNFα) in cases of ischemic stroke and controls (number of individuals and percentage of the given genotype).

TNFα	Ischemic stroke	Controls
Genotype		
GG	87 (87%)	84 (84%)
GA	9 (9%)	15 (15%)
AA	5 (5%)	1 (1%)
Allele		
G	91 (91%)	91 (91%)
A	9 (9%)	9 (9%)
G vs A	$p = 0.862$	
GG vs GA, AA	OR = 0.845 ($p = 0.697$) 95% CI = 0.388–1.838	
GG, GA vs AA	OR = 5.156 ($p = 0.212$) 95% CI = 0.591–44.95	

OR, odd ratio; CI, confidence interval.

complementary to the allelic variant, according to G and T at the end of primer.

The allelic type was determined by electrophoresis in 1.5% agarose gel, according to the presence of specific length NESTED PCR products.

3. Results

The genotype and allelic distributions were compared between controls and patients with stroke. In the control group the heterozygotic G/A genotype of TNF was detected in 15% of individuals. Homozygotic A/A was found in only one control and the G/G in 84% control individuals. Allelic distribution in the control group accorded to 9% A and 91% G individuals. The genotype distribution in patients was similar and statistically insignificant. The heterozygotic G/A genotype was detected in 9 individuals (9%), homozygotic A/A was found in 5 individuals (5%) and G/G in 87 individuals (87%), with allelic distribution of A = 9% and G = 91% (Table 1).

4. Discussion

A significant role of an inflammatory mechanism in stroke is evident. Clinical implications of brain–immune interactions and ischemic stroke were presented in a recent comprehensive review paper of Kamel and Iadecola [12]. The authors indicate that the immune activation may cause secondary tissue injury, but it is not clear whether modulating the acute immune response can produce clinical benefits in the treatment of stroke patients. The inflammatory reaction can be influenced by functional polymorphism in compounds of the immune system. An increased expression of proinflammatory cytokines, including tumor necrosis factor (TNFα) has been found in the plasma and cerebro-spinal fluid of patients with acute ischemic stroke. TNFα, a significant immune mediator, may contribute to the initiation and progression of the ischemic stroke. Tuttolomondo et al. [13] showed significantly higher TNFα plasma levels in stroke patients of cardioembolic subtype, whereas in lacunar subtype TNFα plasma level was significantly lower. In our material the lacunar stroke subtype was as high as 30% and cardioembolic subtype as 18%. Therefore it seems clear that the group of ischemic stroke patients taken as a whole compared with the control material showed no differences in the genotype and allelic distribution. Genetics of TNFα molecule, or variability in its gene, may have an important role in the risk of ischemic stroke. The most studied and interesting aspects of the G-308A polymorphism remain unexplained: there are many discrepancies between the results. However, the cause of this is not clear. Differences in the ethnicity of the studied cohorts may be taken as one of the possibilities. In Caucasians living in European countries, such as Hungary and Austria, as well as in pediatric stroke patients in Turkey (Karahan et al. [14]), TNFα 308 G/A polymorphism did not associate with the risk of ischemic stroke. Our results obtained in the cohort of Caucasians patients living in Poland are also negative with respect to the impact of G-308A TNFα polymorphism on the risk of ischemic stroke. In contrast are the findings of Rubattu et al. [11], who established a role of TNFα gene variants on juvenile ischemic stroke in Italy. Also Trifunovic Cvetkovic et al. [3] indicated an association between allele A1 of the TNFα polymorphism and stroke in hypertensive male Swedish cases.

The interesting point raised by Gupta et al. [15] should be added that G-308A polymorphism of the TNFα gene may be associated with hypertension and hypercholesterolemia. This indirectly leads to ischemic stroke. Such a relationship must be taken into account.

The conclusion has to be issued that further studies are required in various groups of patients in different countries and other parts of Poland, as long as this very serious problem is not validly resolved. However it seems that studies of 101 patients living in one region of Poland are conclusive for that geographical region.

5. Conclusion

G-308A TNFα polymorphism is not related to the risk of ischemic stroke in Caucasians living in western Poland.

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 involv-

ing humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

REFERENCES

[1] Um JY, An NH, Kim HM. TNF-alpha and TNF-beta gene polymorphisms in cerebral infarction. J Mol Neurosci 2003;21:167–71.

[2] Lee BC, Ahn SY, Doo HK, Yim SV, Lee HJ, Jin SY, et al. Susceptibility for ischemic stroke in Korean population is associated with polymorphisms of the interleukin-1 receptor antagonist and tumor necrosis factor-α genes, but not the interleukin-1β gene. Neurosci Lett 2004;357:33–6.

[3] Trifunovic Cvetkovic J, Wiklund PG, Ahmed E, Weinehall L, Hallmans G, Lefvert AK. Polymorphisms of IL-1β, IL-1Ra, and TNF-α genes: a nested case–control study of their association with risk for stroke. J Stroke Cerebrovasc Dis 2005;14:29–35.

[4] Lalouschek W, Schillinger M, Hsieh K, Endler G, Greisenegger S, Marculescu R, et al. Polymorphisms of the inflammatory system and risk of ischemic cerebrovascular events. Clin Chem Lab Med 2006;44:918–23.

[5] Harcom P, Laki J, Kiszel P, Széplaki Z, Szolnoki Z, Kovács M, et al. Decreased frequency of the TNF2 allele of TNF-alpha-308 promoter polymorphism is associated with lacunar infarction. Cytokine 2006;21:100–5.

[6] Tong Y, Geng Y, Xu J, Wang Z, Zhang Y, Lin L, et al. The role of functional polymorphisms of the TNF-alpha gene promoter in the risk of ischemic stroke in Chinese Han and Uyghur populations: two case–control studies. Clin Chim Acta 2010;411:1291–5.

[7] Kim OJ, Lee JH, Choi JK, Oh SH, Hong SH, Oh D, et al. Association between tumor necrosis factor-alpha (−308G→A and −238G→A) polymorphisms and homocysteine levels in patients with ischemic strokes and silent brain infarctions. Cerebrovasc Dis 2010;30:483–90.

[8] Pereira TV, Rudnicki M, Franco RF, Pereira AC, Krieger JE. Effect of the G-308A polymorphism of the tumor necrosis factor alpha gene on the risk of ischemic heart disease and ischemic stroke: a meta-analysis. Am Heart J 2007;153:821–30.

[9] Cui G, Wang H, Li R, Zhang L, Li Z, Wang Y, et al. Polymorphism of tumor necrosis factor alpha (TNF-alpha) gene promotor, circulating TNF-alpha level, and cardiovascular risk factor for ischemic stroke. J Neuroinflammation 2012;10:235.

[10] Goldstein L, Jones M, Matchar D, Edwards LJ, Hoff J, Chilukuri V, et al. Improving the reliability of stroke subgroup classification using the trial of ORG 10172 in acute stroke treatment (TOAST) criteria. Stroke 2001;32:1091–7.

[11] Rubattu S, Speranza R, Ferrari M, Evangelista A, Beccia M, Stanzione R, et al. A role of TNF-alpha gene variant on juvenile ischemic stroke: a case–control study. Eur J Neurol 2005;12:989–93.

[12] Kamel H, Iadecola C. Brain–immune interactions and ischemic stroke. Neurol Rev 2012;69:576–81.

[13] Tuttolomondo A, Di Raimondo D, Pecoraro R, Arnao V, Pinto A, Licata G. Inflammation in ischemic stroke subtypes. Curr Pharm Des 2012;18:4289–310.

[14] Karahan ZC, Deda G, Sipahi T, Elhan AH, Akar N. TNF-α-308 G/A and IL-6-174 G/C polymorphisms in the Turkish pediatric stroke patients. Thromb Res 2005;115:393–8.

[15] Gupta V, Gupta A, Jafar T, Gupta V, Agrawal S, Srivastava N, et al. Association of TNF-α promoter gene G-308A polymorphism with metabolic syndrome, insulin resistance, serum TNF-α and leptin levels in Indian adult women. Cytokine 2012;57:32–6.