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Interleukin-6 gene –174 C/G and apolipoprotein E gene polymorphisms and the risk of Alzheimer disease in a Polish population

Polimorfizmy –174 C/G genu interleukiny 6 oraz genu apolipoproteiny E a ryzyko rozwoju choroby Alzheimera w populacji polskiej

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Abstract

Background and purpose: Inflammation plays a prominent role in Alzheimer disease (AD) pathogenesis. Interleukin-6 (IL-6), a pro-inflammatory cytokine, and some genetic variations in the IL-6 gene have been reported to be associated with a risk of AD. However, the results of the conducted studies are equivocal.
Material and methods: We genotyped IL-6 (–174 C/G) and apolipoprotein E gene (APOE) common polymorphisms in a large case-controlled study in a Polish population. We included 361 patients aged ≥ 65 years with AD (mean age 75.8 ± 5.3 years, 232 females [64.3%]) and 200 controls (75.3 ± 7.4 years; 119 females [59.5%]), without any neurological deficit, cognitive complaints or history of neurological diseases. The IL-6 polymorphism was genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA).
Results: The distribution of the IL-6 (–174 C/G) genotypes was similar to that in the controls (AD: C/C = 15.79%, C/G = 51.25%, G/G = 32.96% vs. controls: C/C = 21.50%, C/G = 45.50%, G/G = 33.0%, $p > 0.05$). Our study confirms previous reports that APOE 4 is strongly related to the risk of AD (OR = 6.17; 95% CI: 4.01-9.49). APOE status

Streszczenie

Wstęp i cel pracy: Choroba Alzheimera jest najczęstszą chorobą zwyrodnieniową ośrodkowego układu nerwowego. Patogeneza choroby nie została poznana; uważa się, że procesy zapalne odgrywają istotną rolę w jej rozwoju. Interleukinę 6 (IL-6), będącą cytokiną prozapalną, oraz niektóre polimorfizmy genu IL-6 wiąże się ze wzrostem ryzyka zachorowania na chorobę Alzheimera, jednak wyniki badań nad tym zagadnieniem nie są jednoznaczne.
Materiał i metody: Do badania włączono 361 chorych ≥ 65. roku życia [średnia wieku 75,8 ± 5,3 roku, 232 kobiety (64,3%)] oraz 200 osób z grupy kontrolnej [75,3 ± 7,4 roku; 119 kobiet (59,5%)] bez skarg na zaburzenia funkcji poznawczych, chorób neurologicznych w wywiadzie i deficytu neurologicznego w badaniu klinicznym. Osoby chore i zdrowe były rasy kaukaskiej. Oznaczono polimorfizm IL-6 (–174 C/G) oraz apolipoproteiny E (APOE). Polimorfizm pojedynczego nukleotydu –174 C/G genu IL-6 został oznaczony za pomocą sond TaqMan na urządzeniu ABI 7900HT (Applied Biosystems, Foster City, CA).
Wyniki: Rozkład genotypu IL-6 (–174 C/G) u osób z chorobą Alzheimera i w grupie kontrolnej był podobny (choroba Alzheimera: C/C = 15,79%, C/G = 51,25%, G/G = 32,96%;

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did not affect the distribution of the studied IL-6 polymorphism.

Conclusion: IL-6 (–174 C/G) polymorphism is not a risk factor for late onset AD in a Polish population.

Key words: Alzheimer disease, interleukin-6, APOE, polymorphism, risk factor, Polish population.

grupa kontrolna: C/C = 21,50%, C/G = 45,50%, G/G = 33,0%, $p > 0,05$). Badanie potwierdziło, że APOE 4 jest czynnikiem ryzyka choroby Alzheimera (OR = 6,17; 95% CI: 4,01–9,49). Polimorfizm APOE nie miał wpływu na rozkład polimorfizmu IL-6.

Wniosek: Polimorfizm IL-6 (–174 C/G) nie jest czynnikiem ryzyka choroby Alzheimera o późnym początku w populacji polskiej.

Słowa kluczowe: choroba Alzheimera, interleukina 6, APOE, polimorfizm, czynniki ryzyka, populacja polska.

Introduction

Alzheimer disease (AD) is the most common neurodegenerative disease. The main pathological feature of AD is the presence of abnormally accumulated proteins and loss of neurons in specific brain regions [1]. The progressive neurodegenerative process in AD is accompanied by chronic inflammation, including activation of microglia and astrocytes that express pro-inflammatory cytokines [2].

Several lines of evidence point to the involvement of interleukin-6 (IL-6) in pathogenesis of AD. Both experimental and clinical data indicate that brain expression [3-6], plasma [7-11] and cerebrospinal fluid levels [12] of IL-6 may affect plaque formation, cognitive decline or dementia both in cross-sectional and longitudinal follow-up studies.

The IL-6 gene in humans is located on chromosome 7 (7p21). The (–174 C/G) polymorphism in the promoter region of the IL-6 gene was reported to affect the IL-6 gene transcription rates [13] and IL-6 plasma levels in AD patients [13,14], implicating its role in development of AD.

Studies analysing a link between the IL-6 (–174 C/G) polymorphism and risk of AD in different ethnic groups have yielded conflicting results; some found an association [15-19] whereas others did not [14,20-28]. The aim of the present study was to investigate a possible association between the IL-6 (–174 C/G) polymorphism and the risk of AD in a case-control study in a large Polish population sample.

Material and methods

Participants

We studied a total sample of 561 subjects. The AD group consisted of 361 patients who were at least 65 years old at the onset of the disease (mean age, 75.8 ± 5.3 years, 232 females [64.3%]) and had no family history of AD. Onset was defined as age at which memory loss or change in behaviour was first noted. The patients were recruited from the Outpatient Memory Clinic, Neurology Department, University Hospital in Krakow (58.5%) and the Department of Neurodegenerative Disorders, Medical Research Centre, Polish Academy of Sciences in Warsaw. Clinical diagnosis of probable AD was made according to the National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [29]. The evaluation included medical, neurological and neuropsychological examination, interview with a close informant, laboratory testing and computed tomography or magnetic resonance imaging. The details of patients' evaluation are described comprehensively elsewhere [30].

The control group included 200 unrelated caregivers or volunteers (mean age: 75.3 ± 7.4 years; 119 females [59.5%]), consecutively recruited from the population of Krakow. They had no apparent neurological disease or psychiatric syndrome, cerebrovascular disease or severe functional limitations and obtained > 26 points on the Mini-Mental State Examination (MMSE) [31]. Controls and AD patients were Caucasian and of Eastern European descent.

All participants gave informed consent and the study was approved by the Local Ethical Committee.

Genetic analyses

The IL-6 promoter (–174 C/G) and the APOE common polymorphism were studied. DNA was extracted from leucocytes using the standard protocol. The IL-6 polymorphism was genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). The APOE

polymorphisms were genotyped by a PCR-based assay simultaneously utilizing two distinct restriction enzymes (HaeII and AflIII enzymes were applied).

Statistical analyses

The genotype and allele frequencies of the IL-6 polymorphism were compared between cases and controls using the χ^2 test (SPSS for Windows, v.10.0). The Hardy-Weinberg equilibrium was verified for all tested populations. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were estimated by logistic regression, controlling for APOE 4 carrier status, gender, and age. The level of significance was set at $p < 0.05$.

To achieve 80% power in this study, with 361 cases and 200 controls, at 0.05 alpha error the effect of the IL-6 allele would have to be OR > 1.44.

The results of our study were combined with those of the previous studies assessing the significance of the studied polymorphism using a fixed or random effect model with the software MIX version 1.7.

Results

Genotype distributions of the IL-6 (–174 C/G) polymorphism and the APOE gene common polymorphism in both groups were in Hardy-Weinberg equilibrium. The frequencies of the G/G genotype and the G allele were similar in AD patients and controls and the difference did not reach significance ($p = 0.20$ and 0.30, respectively). Table 1 shows the IL-6 genotype and allele distribution in patients and controls.

The genotype with the APOE 4 allele was found in 54.2% of the studied patients and in 16.0% of the controls ($p < 0.001$). The possession of at least one E4 allele was associated with a risk of AD in univariate analysis (OR = 6.16, 95% CI: 4.01-9.49, $p < 0.0001$) and after adjustment for gender and age at disease onset (OR = 6.17, 95% CI: 4.01-9.50, $p < 0.0001$). We examined the distribution of the IL-6 genotype and allele frequencies after stratification of the data by APOE 4 carrier status. We found that the distribution of the studied polymorphism did not differ between the carriers and non-carriers of the APOE 4 allele (Table 1).

After pooling the results of our study with the results of the 14 previous studies in Caucasians available in the AlzGene database (http://www.alzgene.org/meta.asp?geneID=53) we found that the studied polymorphism did not affect the risk of developing AD when the significance of the C vs. T allele was considered (OR = 1.0012; 95% CI: 0.9355-1.0714, z-statistic = 0.0334, $p = 0.97$).

Table 1. IL-6 (–174 C/G) and APOE (ε2/3/4) genotype and allele frequencies for patients with Alzheimer disease (AD) and controls

IL-6 (–174 C/G) genotypes	AD patients (N = 361)	Controls (N = 200)	χ^2 – statistic value	p-value
CC	57 (15.80%)	43 (21.50%)	3.21 (df = 2)	0.20
GC	185 (51.25%)	91 (45.50%)		
GG	119 (32.96%)	66 (33.0%)		
IL-6 (–174 C/G) allele				
C	299 (41.41%)	177 (44.25%)	0.84 (df = 1)	0.30
G	423 (58.59%)	223 (55.75%)		
APOE (ε2/3/4) genotypes				
2/2	0 (0%)	2 (1.0%)	89.25 (df = 5)	< 0.0001*
2/3	19 (5.26%)	24 (12.00%)		
3/3	147 (40.72%)	142 (71.00%)		
2/4	8 (2.22%)	7 (3.50%)		
3/4	162 (44.88%)	25 (12.50%)		
4/4	25 (6.93%)	0 (0%)		
2/2 or 2/3 or 3/3	166 (45.98%)	168 (81.0%)	77.21 (df = 1)	< 0.0001
2/4 or 3/4 or 4/4	195 (54.02%)	32 (19.0%)		

**Cochran-Armitage Trend Test; Z = –7.99*

Discussion

We did not find any evidence of an association between the IL-6 (–174 C/G) polymorphism and late onset AD in the Polish population sample. The distribution of the studied polymorphism was similar to that observed in countries at the same geographic latitude, but different when geographical longitude was considered [14]. Our study confirms previous reports that APOE 4 is strongly related to the risk of AD (OR = 6.17; 95% CI: 4.01-9.49) [32,33]. APOE status did not affect the distribution of the studied IL-6 polymorphism.

Previous studies (12 case-control and 2 prospective studies) assessing the connection between the IL-6 polymorphism and the risk of AD brought equivocal results. Faltraco *et al.* [16] reported risk reducing association of the IL-6 C allele in AD. Pola *et al*. [18] found that the G/G polymorphism was associated with increased risk of AD. In other studies on Italian populations the IL-6 C allele increased the risk of AD [12,14], the C/C genotype increased the risk of AD in women [17], and the G/G genotype was lower in AD than in healthy controls [15]. However, other studies did not find an association between the IL-6 polymorphism and AD [14, 20-28].

Differences in study design and the geographical variations of IL-6 frequency may in part explain the different patterns of association between this polymorphism and AD in various studies. In most studies patients with AD had neuropsychological examination but in controls no test battery was used, apart from MMSE; therefore some cases with incipient AD might have been included. Moreover, all studies published to date analysing the role of the IL-6 (–174 C/G) polymorphism in AD have been underpowered. That is why we performed a meta-analysis assessing the significance of the studied polymorphism on all available data from 3107 AD patients and 10 014 controls. This meta-analysis, however, was not able to show the significance of the IL-6 (–174 C/G) polymorphism for the risk of AD.

Conclusion

IL-6 (–174 C/G) polymorphism is not a risk factor for late onset AD in a Polish population.

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Disclosure

The authors report no conflict of interest.

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