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No association between cytokine gene polymorphism and risk of Alzheimer´s disease in Slovaks

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Clinical and immunopathological evidence support a potential role of inflammatory cytokines in Alzheimer‘s disease (AD). However, studies examining the association between cytokine gene polymorphisms and risk of developing AD yielded conflicting results. The objective of our study was to evaluate the association between the functional polymorphisms in the TNF-α, TGF-β1, IL-10, IL-6 and IFNγ genes, respectively and the risk of AD in Slovak individuals. Fifty sporadic AD patients and 140 non-demented age-matched control subjects were genotyped in our case-control study. The observed allele and genotype frequencies in AD patients and controls did not reveal any statistically significant differences. In conclusion, our data suggest that there is no involvement of cytokine gene genetic variance in the development of AD in the Slovak population.

Key words: Alzheimer´s disease (AD), cytokine genes, single nucleodite polymorphism (SNP)

Alzheimer´s disease (AD) is a progressive neurodegenerative disorder that affects more than 20 million people all over the world. The neuropathological hallmarks of the disease are cortical amyloid plaques formed by aggregation of amyloid β–peptide, neurofibrillary tangles containing phosphorylated tau protein and atrophy. Clinically is AD characterized by deterioration of memory and cognitive function with high prevalence in old age.

Genetic background belongs to major risk factors for developing the disease. Based on the age of onset, AD can be categorized into early-onset (<65 years) and late-onset (≥65 years). Early-onset AD constitutes only about 5% of all AD cases and is mainly caused by mutations in three genes: *APP* coding for β-amyloid precursor protein on chromosome 21, *PSEN1* for presenilin-1 on chromosome 14 and *PSEN2* for presenilin-2 on chromosome 1 showing autosomal dominant mode of inheritance (Chartier-Harlin et al. 1991, Murrell et al. 1991, Levy-Lahad et al. 1995, Tanzi 1999, Rocchi et al. 2003). However, most of AD cases (95%) belong to late-onset and do not follow Mendelian inheritance, despite showing significant heritability (Bergem et al. 1997). Late-onset AD cases lack a clear mode of inheritance like most other psychiatric disorders and have a complex etiology that involves multiple genetic and environmental factors (Bickeboller et al. 1997, Daw et al. 2000, Avramopoulos 2009). The possession of an ApoE ε4 allele on chromosome 19 is the only confirmed genetic factor that has been linked to risk for late-onset Alzheimer's disease so far (Strittmatter et al. 1993). However, 50 percent of Alzheimer's disease cases do not carry the ε4 allele, suggesting that other genetic determinants must exist (Blacker et al. 2003, Bertram et al. 2008, Bertram 2009, Żekanowski and Wojda 2009). As inflammation has been extensively implicated in the pathogenesis of AD, it is logical to assume that some genetic determinats of AD might reside in genes involved in regulation of inflammatory responses, such as the cytokine genes. Genes that code for cytokines are highly polymorphic and many of the polymorphisms occur in regulatory regions influencing the cytokine expression. The most frequent type of mutations is due to a change in a single nucleotide base pair and is called single nucleotide polymorphism (SNP). Numerous genetic association studies investigating the impact of cytokine gene polymorphisms on

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Table I

Frequencies of biallelic polymorphisms in AD patients and controls.

Polymorphism	Allele	AD patients (N=50)		Controls (N=140)		P_c	OR	95 % CI
		n	%	n	%			
TNF-α	A	10	10.00	37	13.21	n.s	0.729	0.348 – 1.529
promoter –308 (A/G)	G	90	90.00	243	86.79		1.370	0.654 – 2.871
TGF-β	C	39	39.00	125	44.96	n.s.	0.782	0.491 – 1.247
codon 10 (C/T)	T	61	61.00	153	55.04		1.278	0.802 – 2.037
TGF-β	C	16	16.00	22	7.91	n.s.	2.219	1.112 – 4.418
codon 25 (C/G)	G	84	84.00	256	92.09		0.451	0.226 – 0.899
IL-10	A	64	64.00	159	56.79	n.s.	1.353	0.844 – 2.169
promoter –1082 (A/G)	G	36	36.00	121	43.21		0.237	0.461 – 1.185
IL-10	C	68	68.00	205	73.21	n.s.	0.777	0.473 – 1.277
promoter –819 (C/T)	T	32	32.00	75	26.79		1.286	0.783 – 2.114
IL-10	A	32	32.00	75	26.79	n.s.	1.286	0.783 – 2.114
promoter –592 (A/C)	C	68	68.00	205	73.21		0.777	0.473 – 1.277
IL-6	C	53	53.00	108	38.57	n.s.	1.795	1.133 – 2.847
promoter –174 (C/G)	G	47	47.00	172	61.43		0.556	0.351 – 0.883
IFN-γ	A	62	62.00	149	53.21	n.s.	1.434	0.899 – 2.289
intron 1 +874 (A/T)	T	38	38.00	131	46.79		0.697	0.440 – 1.112

P_c – corrected *P*-value; n.s. – not significant; OR – odds ratio; CI – confidence interval

susceptibility to AD were published in the recent years reporting conflicting results (Bertram et al. 2007).

The objective of our study was to evaluate the association between 8 functionally important SNP polymorphisms located in five candidate cytokine genes (namely TNF-α, TGF-β1, IL-10, IL-6, and IFNγ) and risk of late-onset AD in Slovak caucasoid individuals.

The investigated group included 50 unrelated patients meeting criteria for probable Alzheimer´s disease with an average age at onset of 77.3 years (median 77 years, M/F ratio 0.47). The diagnosis of AD followed the standard NINCDS-ADRDA Alzheimer‘s Criteria (McKhann et al. 1984, Blacker et al. 1994). Patient samples were collected by the care center for AD patients Centrum Memory in Bratislava, Slovakia. The control group comprised 140 age-matched Slovak volunteers (median 73.1 years, M/F ratio 0.89) without cognitive impairment selected from a larger population sample. Written informed consent for enrolling in the study and for personal data management was obtained from all control subjects as well as the relatives of AD patients.

DNA was extracted from whole blood by a modified salting out procedure (Miller et al. 1988). Cytokine single nucleotide polymorphisms in the TNF-α (promoter –308), TGF-β1 (codon 10, 25), IL-10 (promoter –1087, –819, –529), IL-6 (promoter –174), and IFNγ (intron1 -874) genes were determined by PCR with sequence-specific primers using Cytokine Genotyping Tray CYTGEN from OneLambda, Inc., USA. PCR-amplification was performed as recommended by the manufacturer. Electrophoresis was performed in an 1.5% agarose gel for 20 minutes at 10 V/cm and the gel was UV-photographed.

Table II

Genotype frequencies in AD patients and controls.

Polymorphism	Genotype	AD patients (N=50)		Controls (N=140)		P_c	OR	95 % CI
		n	%	n	%			
TNF-α –308 (A/G)	AA	0	0.00	3	2.14	n.s.	0.389	0.019 – 7.669
	AG	10	20.00	31	22.14	n.s.	0.879	0.395 – 1.956
	GG	40	80.00	106	75.72	n.s.	1.283	0.580 – 2.837
TGF-β codon 10 (C/T)	CC	8	16.00	27	19.42	n.s.	0.790	0.333 – 1.877
	CT	24	48.00	71	51.08	n.s.	0.884	0.462 – 1.688
	TT	18	36.00	41	29.50	n.s.	1.345	0.679 – 2.662
TGF-β codon 25 (C/G)	CC	2	4.00	0	0.00	n.s.	14.381	0.678 – 305.1
	CG	10	20.00	22	15.83	n.s.	1.330	0.580 – 3.047
	GG	38	76.00	117	84.17	n.s.	1.000	0.451 – 2.215
IL-10 –1082 (A/G)	AA	22	44.00	49	35.00	n.s.	1.459	0.756 – 2.817
	AG	20	40.00	61	43.57	n.s.	0.863	0.447 – 1.666
	GG	8	16.00	30	21.43	n.s.	0.698	0.296 – 1.646
IL-10 –819 (C/T)	CC	22	44.00	77	55.00	n.s.	1.459	0.756 – 2.817
	CT	22	44.00	51	36.43	n.s.	1.525	0.794 – 2.930
	TT	6	12.00	12	8.57	n.s.	1.455	0.515 – 4.109
IL-10 –592 (A/C)	AA	6	12.00	12	8.57	n.s.	1.455	0.515 – 4.109
	AC	22	44.00	51	36.43	n.s.	1.525	0.794 – 2.930
	CC	22	44.00	77	55.00	n.s.	1.459	0.756 – 2.817
IL-6 –174 (C/G)	CC	6	12.00	21	15.00	n.s.	0.773	0.293 – 2.041
	CG	21	42.00	66	47.14	n.s.	0.812	0.423 – 1.559
	GG	23	46.00	53	37.86	n.s.	1.189	0.615 – 2.298
IFN-γ +874 (A/T)	AA	16	32.00	41	29.28	n.s.	1.136	0.566 – 2.281
	AT	30	60.00	67	47.86	n.s.	1.634	0.348 – 3.149
	TT	4	8.00	32	22.86	n.s.	0.293	0.098 – 0.877

P_c – corrected *P*-value; n.s. – not significant; OR – odds ratio; CI – confidence interval

Allele and genotype frequencies were evaluated by direct counting, as well as the frequencies of TGF-β and IL-10 haplotypes which can be directly revealed by the used genotyping method. Data were tested for the goodness of fit between the observed and expected genotype frequencies and their fit to Hardy–Weinberg equilibrium. As no significant differences in reported frequencies were observed between men and women both in AD patients and controls, gender was not taken into account further in the presentation of the data. Statistical significance of differences in the allele, genotype and haplotype frequencies in AD patients and controls was evaluated by

Table III

Haplotype frequencies of TGF-β and IL-10 polymorphisms in AD patients and controls.

Polymorphism	Haplotype	AD-patients (N=50)		Controls (N=140)		P_c	OR	95 % CI
		n	%	n	%			
TGF-β codon 10 and 25	TG	56	56.00	155	55.36	n.s.	1.026	0.648 – 1.626
	CG	34	34.00	103	36.78	n.s.	0.885	0.548 – 1.430
	CC	10	10.00	22	7.86	n.s.	1.303	0.594 – 2.857
IL-10 at -1082, –819 and –592	GCC	34	34.00	121	43.21	n.s.	0.678	0.420 – 1.090
	ACC	34	34.00	84	30.00	n.s.	1.202	0.739 – 1.955
	ATA	32	32.00	75	26.79	n.s.	1.286	0.782 – 2.114

P_c – corrected *P*-value; n.s. – not significant; OR – odds ratio; CI – confidence interval

two-sided Fisher´s exact test. As multiple comparisons were made, Bonferroni´s correction was applied. The level of statistical significance was set at 0.05. The odds ratio (OR) and 95% confidence intervals (CI) were calculated as well. All calculations were performed by the Data Analysis and Graphing Software OriginPro, version 8.1.

Different functional polymorphisms within genes modulating inflammation have been suggested to influence the risk of Alzheimer‘s disease (AD) with conflicting results; thus, we have studied eight common functional variations within five cytokine genes, namely the TNF-α (promoter –308), TGF-β1 (codon 10, 25), IL-10 (promoter –1087, –819, –529), IL-6 (promoter –174) and IFNγ (intron1 –874) in 50 AD patients and 140 controls of Slovak population. Allele and genotype frequencies of the investigated biallelic polymorphisms are reported in Table I and II. In Table III haplotype frequencies of TGF-β1 and IL-10 polymorphic variants are given. No statistically significant differences in the allele, genotype and haplotype distribution of the investigated SNPs in AD patients compared to the control group have been observed.

Alzheimer´s disease is a multifactor disease occurring in a sensitive genetic territory (Stozicka et al. 2007). The genetic determinants of Alzheimer‘s disease (AD) remain largely unknown despite early successes in identifying three genes that cause early-onset familial AD and one genetic risk factor for late-onset AD (the ApoE gene). Many SNPs that affect cytokine expression represent disease modifiers and possibly influence the susceptibility to AD. Recent studies have reported a genetic association between SNPs in certain cytokine genes and AD, collectively proposing or negating the involvement of different gene candidates (Bertram and Tanzi 2004, 2008). These controversies are due to known limitations in population-based studies as well as the possible involvement of population-specific factors and unknown pathophysiological mechanisms. Advances in genotyping for large scale genetic studies and haplotype analysis should help reduce the amount of spurious and inconsistent associations and allow incorporating genetic information into the risk assessment process.

In conclusion, in our case-control study no positive associations were found, confirming the greater part of previous reports (Bertram et al. 2008b). Thus, the results obtained in Slovak population do not support the idea that common nucleotide variations in the cytokine genes influence the development of late-onset AD.

In our opinion, the overall chances of an individual developing AD is affected by a “susceptibility profile” reflecting the combined influence of inheriting a certain palette of multiple risk alleles. Obtaining such information could lead to strategies for therapeutic intervention in the early stages of AD.

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