Int J Clin Exp Med 2017;10(2):3661-3668
www.ijcem.com /ISSN:1940-5901/IJCEM0041587

Original Article

Association of pro-inflammatory cytokines gene polymorphisms with Parkinson's disease in Chinese population

Weidong Hu[1*], Yun Chen[2*], Heqing Zhao[1]

[1]*Department of Neurology, The Second Affiliated Hospital of Soochow University, Suzhou, China;* [2]*Special Procurement Ward, Department of Geriatrics, The Second Affiliated Hospital of Soochow University, Suzhou, China.* [*]*Equal contributors and co-first authors.*

Received October 10, 2016; Accepted December 8, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: The progressive neurodegenerative process in Parkinson's disease (PD) is accompanied by chronic inflammation, including activation of microglia and astrocytes that express pro-inflammatory cytokines. Up to now, abundant evidence from genetic epidemiology have been assessed the association of pro-inflammation cytokines gene polymorphisms and risk of PD in different ethnicity, but conflicting results were obtained due to the heterogeneity of the genetic background among populations. Here, we recruited 248 PD patients, and 226 matched healthy controls from Jiangsu Province to estimate the influence of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 polymorphism on patients with Parkinson's disease. Single nucleotide polymorphism locus was genotyped using PCR-RFLP. The genotypic and allelic frequency of TNF-α, IL-6 did not show significant difference between PD and normal controls. However, the frequency of wild (AA) and homozygous mutant (CC) genotype IL-10 rs1800896 genotypes in cases and controls was found more in controls (43% and 5.7% respectively), but that of the heterozygous genotype was higher (60.15%) in cases with PD patients. In conclusion, no relationship between IL-6, and TNF-α genotypes or alleles and PD susceptibility was revealed. However, IL-10 rs1800896 GA genotype confers genetically susceptibility to PD in Chinese population.

Keywords: Parkinson's disease, IL-6, IL-10, TNF-α, polymorphism

Introduction

Parkinson's disease (PD) is a degenerative disorder of the central nervous system mainly affecting the motor system [1-3]. Early in the course of the disease, the most obvious symptoms are movement-related; these include shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, thinking and behavioral problems may arise, with dementia commonly occurring in the advanced stages of the disease [4, 5], and depression being the most common psychiatric symptom. Other symptoms include sensory, sleep, and emotional problems. The main motor symptoms are collectively called "parkinsonism", or a "parkinsonian" syndrome [6]. Several genetic and environmental mechanisms are thought to be involved in the pathogenesis of PD [7, 8]. While the contribution of genetic factors to the pathogenesis of the more common sporadic form of PD is still unclear.

Recently findings indicate that that the inflammatory process is an important pathological factor associated with PD [9-14]. In neurodegenerative diseases, activated microglia affect neuronal injury and cell death through the production of glutamate, pro-inflammatory factor, and reactive oxygen species, as well as by mobilizing adaptive immune response and perpetuating neural damage [15]. One hypothesis regarding the cause of the degeneration of nigrostriatal neurons is that PD is triggered by microglial activation because of increased cytokine levels [16, 17]. The genes encoding pro- and anti-inflammatory cytokines may influence cytokine production and thus account for variability in the development of inflammation [18].

The functional promoter polymorphism of nucleotide variations in genes encoding inflammatory molecules, such as IL-6, IL-10 and tumor necrosis factor-α (TNF-α) may influence their biological activities and thus might influ-

ence the risk of PD. For example, Both experimental and clinical data indicate that brain expression, plasma and cerebrospinal fluid levels of IL-6 may affect PD progression [19-24], cognitive decline or dementia both in cross-sectional and longitudinal follow-up studies [25, 26]. The IL-6 gene in humans is located on chromosome 7 (7p21). The (-174 C/G) also known (rs1800795) polymorphism in the promoter region of the IL-6 gene was reported to affect the IL-6 gene transcription rates and IL-6 plasma levels in PD patients implicating its role in development of PD. Meanwhile clinical research has suggested that there is a correlation between the IL-10 SNPs and levels of serum IL-10 with disease severity in PD patients. Many studies have focused mainly on 3' UTR, -1082 G/A (rs1800896) in the IL-10 gene [27].

Meanwhile, several polymorphisms in the promoter region of TNF-α have been associated with different TNF-α expression levels. Of these, the TNF-α -308G/A (also referred to as rs1800629) is the best studied. It involves the substitution of a guanine (G) by a PDenine (A) and is associated with an increase in TNF-α expression levels [28, 29]. Up to now, numberous studies of genetic epidemiology have assessed the association of pro-inflammation cytokines gene polymorphisms and risk of cancer in different populations, but conflicting results were obtained due to the heterogeneity of the genetic background among populations. Furthermore, this support the need for replication studies among all ethnic groups.

Taken together the association of pro-inflammation cytokines and PD, the aim of the present study was to evaluate the influence gene polymorphisms of IL-6, IL-10 and TNF-α on the susceptibility to PD patients in Chinese population.

Patients and methods

Ethics statement

The Medical Ethics Committee of the Second Affiliated Hospital of Soochow University approved this study. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study.

Participants

A total of 248 PD cases, and 226 matched healthy controls fulfilling the criteria for PD were recruited from departments of neurology of the Second Affiliated Hospital of Soochow University from January 2013 to January 2015. All subjects are Han Chinese. All patients underwent a standardized battery of examinations. Subjects with family history of neurodegenerative diseases were excluded. The control group was recruited from the Healthy Examination Center of the Second Affiliated Hospital of Soochow University and confirmed healthy and neurologically normal by medical history, general examinations and laboratory examination.

Genotyping

Genome DNA from whole blood cells of each sample was extracted by using Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instructions. DNA samples were stored at -20°C until analysis. Genotyping for the IL-6 -174 G/C, IL-10 -1188 A/C and TNF-α-308G/A polymorphisms in genomic DNA were performed using the PCR and restriction fragment length polymorphism (RFLP). The genomic region encompassing polymorphism was amplified using the following primers: IL-6 F: 5'-ACT TTT CCC CCT AGTTGTGTCTTTC-3', R: 5'-AGAATGAGCCTCAGACATCTCCAGT-3', IL-10 F: 5'-GGC ATTCTCTTCCAGGTTCTG-3', R: 5'-CCATGGCAACTTGAGAGCTG-3' and TNF-α F: 5'-AGGCAATAGGTTTTGAGGGCCAT-3', R: 5'-TCCCTGCTCCGATTCCG-3'. Polymerase chain reaction products were generated in a 10 μL reaction volume containing 50 ng of genomic DNA, 1× PCR buffer, 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 1 μmol/L of each primer, and 0.25 U of Taq DNA polymerase (Invitrogen Corporation, CarlsbPD, CA). Cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds and a final elongation step at 72°C for 1 minute. Polymerase chain reaction products were digested with 2 U of *NcoI* restriction enzyme at 37°C, according to the manufacturer's instructions (New England BioLabs, Ipswich, MA). IL-6 the amplified fragment of 204 bp was cleaved into two fragments of 24 and 180 bp. The uncut product of 204 bp was identified as CC genotype; the GC genotype was revealed by two fragments of 204 and 180 bp, and the GG by a fragment of 180 bp. And the IL-10 PCR products resulted in either two fragments of 173 and 70 bp (allele C) or a single fragment of 243 bp (allele A). Finally, the -308G allele contains an *NcoI* restriction site not present in the -308A

Table 1. Demographic characteristic of study population

Characteristic	Case	Control	*P*-value
Male [n (%)]	158 (63.71)	142 (62.83)	0.342
Age (years)	79.94 ± 8.120	78.46 ± 6.257	0.765
Age at onset (years)	70.01 ± 7.80		
MMSE	7.56 ± 4.67	28.26 ± 1.08	0.0026

allele; thus, in the presence of the -308G allele, the PCR product (107 bp) is cut into 2 fragments of 80 and 27 bp in length.

Statistic analysis

Data were statistically described in terms of mean ± standard deviation (SD), or frequencies (number of cases) and percentages as required depending on their distribution. The Hardy-Weinberg equilibrium (HWE) was assessed for each variation to identify the deviation. The differences of the genotypes and alleles of detected genes between patients and normal controls were evaluated by using Pearson Chi-square test. Exact test was used inste PD when the expected frequency is less than 5. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Unpaired Student's t test or Mann-Whitney tests were used for two-group comparisons. Statistical analysis of data was performed using the SPSS software package 18.0 (SPSS Inc. USA). *P*-value less than 0.05 was considered statistically significant.

Results

In this study, 248 PD patients (108 males and 140 females) and 226 controls (112 males and 114 females) were screened for IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 polymorphisms using PCR-RFLP methods. No statistically significant differences were observed in age (age at patients compared with age at examination for control subjects) (*P*=0.342) and gender (*P*=0.765) between PD patients and control subjects (**Table 1**). In addition, MMSE scores were significantly lower in PD patients than in control subjects (*P*<001).

Firstly, the frequencies of genotypes and alleles of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 were detected in case and control groups. HWE of rs1800795, rs1800896 and rs1800629 in patients and controls were listed in **Table 2**, and the results showed allelic distribution of detected SNP were not deviated from HWE in both case and control populations. IL-6 rs1800795 in the study population were as follows: 7.4% CC, 33.5% CG and 59.1% GG for the case study group and 4.9% CC, 30.4% GC, and 64.7% GG for the controls, indicating that the genotypes distributions were similar between the cases and the control groups. Also, genomic analysis did not reveal a difference between PD patients and healthy controls in allelic frequency at the -174 position for the IL-6 gene promoter. Similarly, the genotypic and allelic frequency of rs1800629 did not show significant difference between PD and normal controls. Then, Genotype and allele frequency of rs1800629 were detected in PD patients and normal control (**Table 2**). The genotypic and allelic frequency of rs1800629 between cases and health controls did not show significant difference.

Whereas, the frequency of wild (GG) and homozygous mutant (AA) genotype IL-10 rs1800896 genotypes in cases and controls was found more in controls (43% and 5.7% respectively), but that of the heterozygous genotype was higher (60.15%) in cases with PD patients. Significant risk of PD was observed for GA (OR=1.74, 95% CI=1.10-2.41, *P*=0.025) genotype of IL-10. The genomic analysis did not reveal differences in allelic frequencies of the IL-10 (G/A) gene PD patients and healthy controls.

Discussion

The gene single nucleotide polymorphisms (SNP) have been thought to alter expressions or influence certain genes; thus, SNPs could be associated with an altered risk of multiple disease [30-36]. Up to now, the important role of pro-inflammatory cytokines during tumor development and prognosis are increasingly gaining interest. Several lines of evidence point to the involvement of interleukin-6 (IL-6) in pathogenesis of PD. Both experimental and clinical data indicate that brain expression [37], plasma [38-40] and cerebrospinal fluid levels of IL-6 may affect plaque formation, cognitive decline or dementia both in cross-sectional and longitudinal follow-up studies [41-43].

We did not find any evidence of an association between the IL-6 (-174 C/G) polymorphism and PD in the China population sample. The distri-

Table 2. Genotype and allele frequency of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 and Pearson's chi-square test in PD patients and normal controls

Genotype/Allele	Patients (n=248)	Controls (n=226)	*P*-value	OR (95% CI)
IL-6 rs1800795	HWE* *P*=0.25	HWE *P*=0.21		
GG	147	146	0.620	0.905 (0.626-1.320)
GC	83	69	0.077	0.822 (0.681-1.484)
CC	18	11	0.122	4.560 (0.556-37.412)
G	377	361	0.314	0.841 (0.498-1.192)
C	119	91		
IL-10 rs1800896	HWE* *P*=0.28	HWE *P*=0.75		
GG	106	81	0.0521	0.805 (0.526-1.220)
GA	127	140	0.025	1.749 (1.104-2.417)
AA	15	5	0.0519	0.972 (0.653-1.521)
G	339	302	0.275	0.571 (0.328-0.981)
A	157	150		
TNF-α rs1800629	HWE* *P*=0.48	HWE *P*=0.56		
GG	172	169	0.559	0.869 (0.542-1.392)
GA	68	51	0.921	1.258 (0.787-2.010)
AA	8	6	0.350	3.358 (0.347-32.543)
G	412	389	0.356	0.813 (0.524-1.267)
A	84	63		

*Chi-square test for deviation from the Hardy-Weinberg equilibrium (a value of $P<0.001$ was regarded as a deviation from the HWE).

bution of the studied polymorphism was similar to that observed in countries at the same geographic latitude, but different when geographical longitude was considered [44]. Previous studies (12 case-control and 2 prospective studies) assessing the connection between the IL-6 polymorphism and the risk of PD brought equivocal results. Faltraco et al. [45] reported risk reducing association of the IL-6 C allele in PD. Pola et al. [46] found that the G/G polymorphism was associated with increased risk of PD. In other studies on Italian populations the IL-6 C allele increased the risk of PD [47, 48], the C/C genotype increased the risk of PD in women [49], and the G/G genotype was lower in PD than in healthy controls [50]. 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 PD in various studies. In most studies patients with PD neuropsychological examination but in controls no test battery was used, apart from MMSE; therefore some cases with incipient PD might have been included. Moreover, all studies published to date analyzing the role of the IL-6 (-174 C/G) polymorphism in PD have been underpowered. That is why we performed a meta analysis assessing the significance of the studied polymorphism on all available data from 3107 PD 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 PD [39].

IL-10 is an important antitumor cytokine that plays an important role in the development and progress of canc. Variation in the DNA sequence lead to altered IL-10 production, and this can alter individual's susceptibility to cancer. The IL-10 3' UTR G>A polymorphism is a functionally important SNP that alters IL-10 production and it has been a reported potential biomarker for risks of numerous disease, such as hepatitis, psoriasis, Barrett's esophagus, asthma, and arteritis. More importantly, genetic variation in IL-10 was revealed to affect susceptibility to multiple sclerosis, another neurodegenerative disease with evident inflammatory responses. Considering the potential role of IL-10 in PD pathogenesis as well as the involvement of IL-10 polymorphisms in the predisposition to many inflammatory diseases [51-53]. In our study, increased frequency of IL-10 rs1800896 GG and AA homozygous genetype among controls but that of the heterozygous AC genotype

was higher in cases with PD; thus, a significant risk of PD was observed for AC genotype of IL-10 rs1800896, which is consistent with the results of Li [51].

TNF-α gene is located in the class III region of the human major histocompatibility complex (MHC) on chromosome 6p21 [54, 55]. Among the several single nucleotide polymorphisms (SNPs) identified in TNF-α, TNF-α rs1800629 is the most extensively studied. The A allele of this polymorphism can lead to high binding affinity of nuclear factors to the TNF promoter, resulting in a high level of transcription activity and secretion levels of TNF-α. So, it was suggested to have a significant functional effect [56]. A variety of SNPs located in the promoter region of TNF-α gene has been investigated in PD patients by different groups with contrasting results. So, The aim of the present study was to better define the role of TNF-α polymorphisms in PD; In the present study, our results suggested that the genotypes distributions of TNF-α rs1800629 was almost the similar in the cases and the control groups. Previous association studies on the TNF-α gene polymorphism have either shown no association between the -308A allele and PD [57, 58] or a positive association [59] between this allele and early PD onset [57]. Some of these differences may be as a result of the genetic heterogeneity of the population studied [60]. In particular, the TNF gene may exert different effects upon different ethnic groups, depending on its interaction with other unknown susceptibility genes and/or risk factors in another nearby locus. Indeed, the TNF gene is part of a large genomic region on chromosome 6 that contains other genes encoding proteins involved in immune inflammatory responses.

Our results indicate a lack of association between pro-inflammatory cytokines SNP and PD in our China sample, suggesting that genetic, clinical and population heterogeneity are probably responsible for conflicting results in association studies.

In summary, though no any relationship between IL-6, and TNF-α genotypes or alleles and PD susceptibility was revealed, we first identified IL-10 rs1800896 GA genotype confer genetically susceptibility to PD in Chinese population.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Weidong Hu, Department of Neurology, The Second Affiliated Hospital of Soochow University, No. 1055 Sanxiang Road, Suzhou 215004, China. Tel: +86-512-6778-4787; Fax: +86-512-6778-4787; E-mail: huwd2016@163.com

References

[1] Foo JN, Chung SJ, Tan LC, Liany H, Ryu HS, Hong M, Koh TH, Irwan ID, Au WL, Prakash KM, Aung T, Cheng CY, Chong SA, Khor CC, Lee J, Tai ES, Vithana EN, Wong TY, Song K, Liu J and Tan EK. Linking a genome-wide association study signal to a LRRK2 coding variant in Parkinson's disease. Mov Disord 2016; 31: 484-487.

[2] Gazibara T, Kisic-Tepavcevic D, Svetel M, Tomic A, Stankovic I, Kostic VS and Pekmezovic T. Indoor and outdoor falls in persons with Parkinson's disease after 1 year follow-up study: differences and consequences. Neurol Sci 2016; 37: 597-602.

[3] Georgiev D, Dirnberger G, Wilkinson L, Limousin P and Jahanshahi M. In Parkinson's disease on a probabilistic Go/NoGo task deep brain stimulation of the subthalamic nucleus only interferes with withholding of the most prepotent responses. Exp Brain Res 2016; 234: 1133-1143.

[4] Gunay MS, Ozer AY and Chalon S. Drug delivery systems for imaging and therapy of Parkinson's disease. Curr Neuropharmacol 2016; 14: 376-391.

[5] He Q, Koprich JB, Wang Y, Yu WB, Xiao BG, Brotchie JM and Wang J. Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV alpha-Synuclein rat model of Parkinson's disease. Mol Neurobiol 2016; 53: 2258-2268.

[6] Herz DM, Haagensen BN, Nielsen SH, Madsen KH, Lokkegaard A and Siebner HR. Resting-state connectivity predicts levodopa-induced dyskinesias in Parkinson's disease. Mov Disord 2016; 31: 521-529.

[7] Liu S, Zhang Y, Bian H and Li X. Gene expression profiling predicts pathways and genes associated with Parkinson's disease. Neurol Sci 2016; 37: 73-79.

[8] Guo X, Tang P, Li X, Chong L, Zhang X and Li R. Association between two alpha-2-macroglobulin gene polymorphisms and Parkinson's disease: a meta-analysis. Int J Neurosci 2016; 126: 193-198.

[9] Wang J and Tsirka SE. Contribution of extracellular proteolysis and microglia to intracerebral hemorrhage. Neurocrit Care 2005; 3: 77-85.
[10] Block ML and Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 2005; 76: 77-98.
[11] Zhang AM, Ma K, Song Y, Wang B, Feng Y, Liu L and Xia X. Genetic polymorphisms of the IFNlambda genes are associated with biochemical features in Han Chinese with HCV infection from Yunnan province, China. Infect Genet Evol 2014; 21: 161-165.
[12] Zhang AM, Ma K, Song Y, Feng Y, Duan H, Zhao P, Wang B, Xu G, Li Z and Xia X. Mitochondrial DNAs decreased and correlated with clinical features in HCV patients from Yunnan, China. Mitochondrial DNA 2016; 27: 2516-9.
[13] Ma K, Guo L, Xu A, Cui S and Wang JH. Molecular mechanism for stress-induced depression assessed by sequencing miRNA and mRNA in medial prefrontal cortex. PLoS One 2016; 11: e0159093.
[14] Ma K, Zhang H and Baloch Z. Pathogenetic and therapeutic applications of tumor necrosis factor-alpha (TNF-alpha) in major depressive disorder: a systematic review. Int J Mol Sci 2016; 17: E733.
[15] Mateo I, Infante J, Sanchez-Juan P, Garcia-Gorostiaga I, Rodriguez-Rodriguez E, Vazquez-Higuera JL, Berciano J and Combarros O. Serum heme oxygenase-1 levels are increased in Parkinson's disease but not in Alzheimer's disease. Acta Neurol Scand 2010; 121: 136-138.
[16] Wang Y, Cao M, Liu A, Di W, Zhao F, Tian Y and Jia J. Changes of inflammatory cytokines and neurotrophins emphasized their roles in hypoxic-ischemic brain damage. Int J Neurosci 2013; 123: 191-195.
[17] Nagatsu T, Mogi M, Ichinose H and Togari A. Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm Suppl 2000; 60: 277-290.
[18] Pascale E, Passarelli E, Purcaro C, Vestri AR, Fakeri A, Guglielmi R, Passarelli F and Meco G. Lack of association between IL-1beta, TNF-alpha, and IL-10 gene polymorphisms and sporadic Parkinson's disease in an Italian cohort. Acta Neurol Scand 2011; 124: 176-181.
[19] Kaditis AG, Gozal D, Khalyfa A, Kheirandish-Gozal L, Capdevila OS, Gourgoulianis K, Alexopoulos EI, Chaidas K, Bhattacharjee R, Kim J, Rodopoulou P and Zintzaras E. Variants in C-reactive protein and IL-6 genes and susceptibility to obstructive sleep apnea in children: a candidate-gene association study in European American and Southeast European populations. Sleep Med 2014; 15: 228-235.
[20] Cunningham K, Stansfield SH, Patel P, Menon S, Kienzle V, Allan JA and Huston WM. The IL-6 response to chlamydia from primary reproductive epithelial cells is highly variable and may be involved in differential susceptibility to the immunopathological consequences of chlamydial infection. BMC Immunol 2013; 14: 50.
[21] Palhagen S, Qi H, Martensson B, Walinder J, Granerus AK and Svenningsson P. Monoamines, BDNF, IL-6 and corticosterone in CSF in patients with Parkinson's disease and major depression. J Neurol 2010; 257: 524-532.
[22] Shen YQ, Li KS and Neveu PJ. [Kinetic changes of plasma IL-1 and IL-6 levels in MPTP-induced Parkinson's disease model mice and their relationship with brain asymmetry]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2005; 21: 365-368.
[23] Bessler H, Djaldetti R, Salman H, Bergman M and Djaldetti M. IL-1 beta, IL-2, IL-6 and TNF-alpha production by peripheral blood mononuclear cells from patients with Parkinson's disease. Biomed Pharmacother 1999; 53: 141-145.
[24] Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M and Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. Neurosci Lett 1996; 211: 13-16.
[25] Smith KA and Maizels RM. IL-6 controls susceptibility to helminth infection by impeding Th2 responsiveness and altering the Treg phenotype in vivo. Eur J Immunol 2014; 44: 150-161.
[26] Salnikova LE, Smelaya TV, Moroz VV, Golubev AM and Rubanovich AV. Functional polymorphisms in the CYP1A1, ACE, and IL-6 genes contribute to susceptibility to community-acquired and nosocomial pneumonia. Int J Infect Dis 2013; 17: e433-442.
[27] Youssef SS, Abd El Aal AM, Nasr AS, el Zanaty T and Seif SM. Interleukin-12B gene polymorphism frequencies in Egyptians and sex-related susceptibility to hepatitis C infection. J Interferon Cytokine Res 2013; 33: 415-419.
[28] Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R and Belaiche J. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998; 113: 401-406.
[29] Sharma S, Sharma A, Kumar S, Sharma SK and Ghosh B. Association of TNF haplotypes with asthma, serum IgE levels, and correlation with serum TNF-alpha levels. Am J Respir Cell Mol Biol 2006; 35: 488-495.

[30] Zhao Y, Du Y, Zhao S and Guo Z. Single-nucleotide polymorphisms of microRNA processing machinery genes and risk of colorectal cancer. Onco Targets Ther 2015; 8: 421-425.
[31] Gao YW, Xu M, Xu Y, Li D and Zhou S. Effect of three common IL-17 single nucleotide polymorphisms on the risk of developing gastric cancer. Oncol Lett 2015; 9: 1398-1402.
[32] Kakkoura MG, Demetriou CA, Loizidou MA, Loucaides G, Neophytou I, Marcou Y, Hadjisavvas A and Kyriacou K. Single-nucleotide polymorphisms in one-carbon metabolism genes, Mediterranean diet and breast cancer risk: a case-control study in the Greek-Cypriot female population. Genes Nutr 2015; 10: 453.
[33] Ge J, Zhu L, Zhou J, Li G, Li Y, Li S, Wu Z, Rong J, Yuan H, Liu Y, Chi Q, Piao D, Zhao Y and Cui B. Association between co-inhibitory molecule gene tagging single nucleotide polymorphisms and the risk of colorectal cancer in Chinese. J Cancer Res Clin Oncol 2015; 141: 1533-1544.
[34] Geredeli C, Artac M, Yildirim S, Inal A, Dede I, Guler T, Boruban MC, Koral L, Karaagac M, Zamani AG, Altinok T, Aribas O, Bozcuk H and Demirkazik A. Prognostic value of ERCC1, ERCC2, XRCC1, and TP53 single nucleotide polymorphisms in patients with early-stage non-small cell lung cancer. Tumour Biol 2015; 36: 4279-4285.
[35] Zhu S, Shao B, Hao Y, Li Z, Liu H, Li H, Wang M and Wang K. No association of single nucleotide polymorphisms involved in GHRL and GHSR with cancer risk: a meta-analysis. Cancer Biomark 2015; 15: 89-97.
[36] Wu GC and Zhang ZT. Genetic association of single nucleotide polymorphisms in P53 pathway with gastric cancer risk in a Chinese Han population. Med Oncol 2015; 32: 401.
[37] Dursun E, Gezen-Ak D, Hanagasi H, Bilgic B, Lohmann E, Ertan S, Atasoy IL, Alaylioglu M, Araz OS, Onal B, Gunduz A, Apaydin H, Kiziltan G, Ulutin T, Gurvit H and Yilmazer S. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol 2015; 283: 50-57.
[38] Rasmussen L, Delabio R, Horiguchi L, Mizumoto I, Terazaki CR, Mazzotti D, Bertolucci PH, Pinhel MA, Souza D, Krieger H, Kawamata C, Minett T, Smith MC and Payao SL. Association between interleukin 6 gene haplotype and Alzheimer's disease: a Brazilian case-control study. J Alzheimers Dis 2013; 36: 733-738.
[39] Hua Y, Guo X, Huang Q, Kong Y and Lu X. Association between interleukin-6 -174 G/C polymorphism and the risk of Alzheimer's disease: a meta-analysis. Int J Neurosci 2013; 123: 626-635.
[40] Honma T, Hatta K, Hitomi Y, Kambayashi Y, Hibino Y, Konoshita T and Nakamura H. Increased systemic inflammatory interleukin-1ss and interleukin-6 during agitation as predictors of Alzheimer's disease. Int J Geriatr Psychiatry 2013; 28: 233-241.
[41] Uslu S, Akarkarasu ZE, Ozbabalik D, Ozkan S, Colak O, Demirkan ES, Ozkiris A, Demirustu C and Alatas O. Levels of amyloid beta-42, interleukin-6 and tumor necrosis factor-alpha in Alzheimer's disease and vascular dementia. Neurochem Res 2012; 37: 1554-1559.
[42] Chen SY, Chen TF, Lai LC, Chen JH, Sun Y, Wen LL, Yip PK, Chu YM and Chen YC. Sequence variants of interleukin 6 (IL-6) are significantly associated with a decreased risk of late-onset Alzheimer's disease. J Neuroinflammation 2012; 9: 21.
[43] Cojocaru IM, Cojocaru M, Miu G and Sapira V. Study of interleukin-6 production in Alzheimer's disease. Rom J Intern Med 2011; 49: 55-58.
[44] Capurso C. Has dysregulated interleukin-6 gene a role in the development of Alzheimer's disease? Neurosci Lett 2011; 504: 1-3.
[45] Faltraco F, Burger K, Zill P, Teipel SJ, Moller HJ, Hampel H, Bondy B and Ackenheil M. Interleukin-6-174 G/C promoter gene polymorphism C allele reduces Alzheimer's disease risk. J Am Geriatr Soc 2003; 51: 578-579.
[46] Pola R, Flex A, Gaetani E, Lago AD, Gerardino L, Pola P and Bernabei R. The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with Alzheimer's disease in an Italian population [corrected]. Neuroreport 2002; 13: 1645-1647.
[47] Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H and Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 1995; 202: 17-20.
[48] Huell M, Strauss S, Volk B, Berger M and Bauer J. Interleukin-6 is present in early stages of plaque formation and is restricted to the brains of Alzheimer's disease patients. Acta Neuropathol 1995; 89: 544-551.
[49] Licastro F, Grimaldi LM, Bonafe M, Martina C, Olivieri F, Cavallone L, Giovanietti S, Masliah E and Franceschi C. Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. Neurobiol Aging 2003; 24: 921-926.
[50] Arosio B, Trabattoni D, Galimberti L, Bucciarelli P, Fasano F, Calabresi C, Cazzullo CL, Vergani C, Annoni G and Clerici M. Interleukin-10 and interleukin-6 gene polymorphisms as risk fac-

tors for Alzheimer's disease. Neurobiol Aging 2004; 25: 1009-1015.
[51] Li Y, Li Y, Shao Y and Qian Y. IL-10 -1082G/A polymorphism in Alzheimer's disease risk. J Neurol Sci 2011; 309: 154-155.
[52] Zhang Y, Zhang J, Tian C, Xiao Y, Li X, He C, Huang J and Fan H. The -1082G/A polymorphism in IL-10 gene is associated with risk of Alzheimer's disease: a meta-analysis. J Neurol Sci 2011; 303: 133-138.
[53] Combarros O, van Duijn CM, Hammond N, Belbin O, Arias-Vasquez A, Cortina-Borja M, Lehmann MG, Aulchenko YS, Schuur M, Kolsch H, Heun R, Wilcock GK, Brown K, Kehoe PG, Harrison R, Coto E, Alvarez V, Deloukas P, Mateo I, Gwilliam R, Morgan K, Warden DR, Smith AD and Lehmann DJ. Replication by the Epistasis Project of the interaction between the genes for IL-6 and IL-10 in the risk of Alzheimer's disease. J Neuroinflammation 2009; 6: 22.
[54] A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). Nat Genet 1997; 15: 389-392.
[55] Daniels SE, Bhattacharrya S, James A, Leaves NI, Young A, Hill MR, Faux JA, Ryan GF, le Souef PN, Lathrop GM, Musk AW and Cookson WO. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996; 383: 247-250.
[56] Schluter B, Erren M, Schotte H, Junker R, Rust S and Assmann G. The mutagenically separated polymerase chain reaction is a rapid and reliable method for genotyping of the tumour necrosis factor-alpha promoter polymorphism (-308 G/A). Clin Chim Acta 2002; 320: 135-138.
[57] Bialecka M, Klodowska-Duda G, Kurzawski M, Slawek J, Gorzkowska A, Opala G, Bialecki P, Sagan L and Drozdzik M. Interleukin-10 (IL10) and tumor necrosis factor alpha (TNF) gene polymorphisms in Parkinson's disease patients. Parkinsonism Relat Disord 2008; 14: 636-640.
[58] Wu YR, Feng IH, Lyu RK, Chang KH, Lin YY, Chan H, Hu FJ, Lee-Chen GJ and Chen CM. Tumor necrosis factor-alpha promoter polymorphism is associated with the risk of Parkinson's disease. Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 300-304.
[59] Leng A, Mura A, Feldon J and Ferger B. Tumor necrosis factor-alpha receptor ablation in a chronic MPTP mouse model of Parkinson's disease. Neurosci Lett 2005; 375: 107-111.
[60] Meenagh A, Williams F, Ross OA, Patterson C, Gorodezky C, Hammond M, Leheny WA and Middleton D. Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. Hum Immunol 2002; 63: 1055-1061.