

# IL-1 $\beta$ , IL-6, IL-10, and TNF $\alpha$ single nucleotide polymorphisms are associated with cerebrospinal fluid levels of biomarkers of Alzheimer's disease

**Mirjana Babić Leko**

Sveuciliste u Zagrebu Medicinski fakultet

**Matea Nikolac Perković**

Institut Ruder Boskovic

**Nataša Klepac**

Klinicki Bolnicki Centar Zagreb

**Dubravka Švob Štrac**

Institut Ruder Boskovic

**Fran Borovečki**

Sveuciliste u Zagrebu Medicinski fakultet

**Nela Pivac**

Institut Ruder Boskovic

**Patrick R. Hof**

Icahn School of Medicine at Mount Sinai

**Goran Simic** (✉ [goransimic@hotmail.com](mailto:goransimic@hotmail.com))

Sveuciliste u Zagrebu Medicinski fakultet <https://orcid.org/0000-0002-6339-9261>



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## Research

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## Abstract

**Background** Neuroinflammation plays an important role in Alzheimer's disease (AD). During this process, activated microglia release pro-inflammatory cytokines such as interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6 and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) that participate in neuron damage. However, anti-inflammatory cytokines (such as IL-10), which maintain homeostasis of immune response, are also released. Previous studies showed the association of IL-1 $\alpha$ -889C/T (rs1800587), IL-1 $\beta$ -1473G/C (rs1143623), IL-6-174C/G (rs1800795), IL-10-1082G/A (rs1800896) and TNF $\alpha$ -308A/G (rs1800629) polymorphisms with AD.

**Methods** In this study, we assessed whether people carrying certain genotypes in these polymorphisms are more prone to develop AD-related pathology, reflected by pathological levels of cerebrospinal fluid (CSF) AD biomarkers including amyloid  $\beta$ 1–42 ( $A\beta$ 1–42), total tau (t-tau), tau phosphorylated at Thr 181 (p-tau<sub>181</sub>), Ser 199 (p-tau<sub>199</sub>), and Thr 231 (p-tau<sub>231</sub>), and visinin-like protein 1 (VILIP-1). The study included 115 AD patients, 53 patients with mild cognitive impairment (MCI), 11 healthy controls, and 54 patients with other causes of dementia.

**Results** A significant increase in p-tau CSF levels was found in patients with the AA IL-10-1082G/A and GG TNF $\alpha$ -308A/G genotypes, and in carriers of a G allele in IL-1 $\beta$ -1473C/G and IL-6-174C/G polymorphisms. T-tau levels were increased while  $A\beta$ 1–42 levels were decreased in carriers of a G allele in IL-1 $\beta$ -1473C/G polymorphism. An increase in VILIP-1 levels was observed in patients with CG and GG IL-1 $\beta$ -1473C/G, GC IL-6-174C/G and GG TNF $\alpha$ -308A/G genotype.

**Conclusions** These results suggest that persons carrying certain genotypes in IL10 (-1082G/A), IL1 $\beta$  (1473C/G), IL6 (-174C/G) and TNF $\alpha$  (-308A/G) could be more vulnerable to development of neuroinflammation, and consequently of AD.

## Background

Inflammatory processes are enhanced in the brain of Alzheimer's disease (AD) patients [1,2]. Microglial cells become activated and produce high levels of cytokines. In early stages of AD, activated microglia phagocytose amyloid  $\beta$  ( $A\beta$ ) peptide, but when they are activated over extended periods [3], they can no longer clear  $A\beta$ , and the pro-inflammatory cytokines they release participate in propagation of pathological tau proteins and neuron damage [4,5]. The main pro-inflammatory cytokines released from activated microglia are interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6 and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) [6]. During sustained inflammation, anti-inflammatory cytokines (such as IL-10) are also released and maintain homeostasis of the immune response [6]. Single nucleotide polymorphisms (SNPs) in genes for IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10 and TNF $\alpha$  were previously associated with AD [7,8]. It was shown that certain SNPs can influence gene transcription and consequently the amount of the produced cytokines [9–11]. The association of these SNPs with AD has been mostly tested in epidemiological studies by comparison of genotype distribution between AD patients and healthy controls (HC). Only a few studies measured levels of cerebrospinal fluid (CSF) AD biomarkers in patients with *IL-10*-1082G/A, *IL-1 $\beta$* -1473C/G, *IL-1 $\alpha$* -889C/T, *IL-6*-174C/G and *TNF $\alpha$* -308A/G genotypes [12,13]. CSF AD biomarkers such as amyloid  $\beta$ <sub>1–42</sub> ( $A\beta$ <sub>1–42</sub>), total tau (t-tau), tau phosphorylated at amino acids Thr 181 (p-tau<sub>181</sub>), Ser 199 (p-tau<sub>199</sub>), and Thr 231 (p-tau<sub>231</sub>), and visinin-like protein 1 (VILIP-1) serve as endophenotypes of AD, as they reflect AD-related pathology [14]. CSF  $A\beta$ <sub>1–42</sub> [15] and phosphorylated tau proteins [16] are indicators of senile plaques and neurofibrillary tangles in the brain, respectively, while CSF VILIP-1 and t-tau reflect neurodegeneration [17,18]. Here we assessed possible differences in the levels of CSF AD biomarkers ( $A\beta$ <sub>1–42</sub>, t-tau, p-tau<sub>181</sub>, p-tau<sub>199</sub>, p-tau<sub>231</sub> and VILIP-1) among patients with different *IL-10*-1082G/A, *IL-1 $\beta$* -1473C/G, *IL-1 $\alpha$* -889C/T, *IL-6*-174C/G and *TNF $\alpha$* -308A/G genotypes to test whether people carrying certain genotypes are more prone to develop AD-related pathology as reflected by their levels of CSF biomarkers.

## Methods

### Subjects

This study included 115 AD patients and 53 mild cognitive impairment (MCI) patients, 11 HC, and 54 patients with other causes of dementia (14 with vascular dementia [VaD], three with mixed dementia [AD+VaD], 23 with frontotemporal dementia [FTD], 7 with dementia with Lewy bodies [DLB], 3 with nonspecific dementia [ND], 3 with Parkinson's disease [PD], and 1 with corticobasal syndrome [CBS]; **Table 1**). All patients were recruited at the Clinical Hospital Center Zagreb. They gave informed consent for participation in this study and for lumbar puncture. Patients' cognitive status was tested using a battery of neuropsychological tests, including the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog), Montreal Cognitive Assessment (MoCA) and Mini-Mental State Examination (MMSE) [19]. In addition to neuropsychological testing, complete blood tests (levels of folic acid (B9), vitamin B12, thyroid function test, serology for Lyme's disease and syphilis) and a full neurological examination were done. Dementia due to AD was diagnosed by using the National Institutes on Aging – Alzheimer's Association (NIA-AA) criteria of McKhann et al. [20], while for MCI diagnosis the criteria of Albert et al. [21] were used. FTD was diagnosed according to Neary et al. [22], while VaD was diagnosed using the criteria of National Institute for Neurological Disorders and Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINCDS-AIREN) [23], and the Hachinski Ischemic Score (HIS) [24]. All procedures were in accord with the Helsinki Declaration [25] and were approved by the Ethical Committee of the Clinical Hospital Center Zagreb (case no. 02/21 AG, class 8.1-18/82-2 from April 24, 2018) and by the Central Ethical Committee of the University of Zagreb Medical School (case no. 380-59-10106-18-111/126, class 641-01/18-02/01 from June 20, 2018).

### DNA analysis

Plastic syringes with 1 ml of acid citrate dextrose as an anticoagulant were used for collection of venous blood. DNA was isolated from the peripheral blood by the salting-out method [26]. SNPs were determined using TaqMan® SNP Genotyping Assays (Applied Biosystems) by ABI Prism 7300 Real Time PCR System apparatus (Applied Biosystems, Foster City, CA). Following polymorphisms were determined; *IL-1α*-889C/T (rs1800587), *IL-1β*-1473G/C (rs1143623), *IL-6*-174C/G (rs1800795), *IL-10*-1082G/A (rs1800896) and *TNFA*-308A/G (rs1800629).

### Analysis of CSF biomarkers

CSF was collected by lumbar puncture between intervertebral spaces L3/L4 or L4/L5. CSF was centrifuged at 2,000 *g* for 10 min, aliquoted and stored at -80°C in polypropylene tubes. Levels of CSF biomarkers were determined by following enzyme-linked immunosorbent assays (ELISA): VILIP-1 (VILIP-1 Human ELISA, BioVendor, Brno, Czech Republic), t-tau (Innotest hTau Ag, Fujirebio, Gent, Belgium), Aβ<sub>1-42</sub> (Innotest β-amyloid1-42, Fujirebio), p-tau<sub>181</sub> (Innotest Phospho-Tau [181P], Fujirebio), p-tau<sub>199</sub> (TAU [pS199] Phospho-ELISA Kit, Human, Thermo Fisher Scientific Waltham, MA), and p-tau<sub>231</sub> (Tau [pT231] Phospho-ELISA Kit, Human, Thermo Fisher Scientific,).

### Statistical analysis

Data normality was tested using the Kolmogorov–Smirnov test. However, because of the small number of subjects in some groups, non-parametric statistics were used regardless of the results of the test for normality. Levels of CSF biomarkers were compared among groups using the non-parametric Kruskal-Wallis test. Pairwise comparisons were done by *post-hoc* non-parametric test with calculation of the corrected *p* value. All statistical analyses were done in SPSS 19.0.1 (SPSS, Chicago, IL, USA). The level of statistical significance was set at α = 0.05.

## Results

There was no significant deviation from the Hardy–Weinberg distribution in subjects carrying any of analyzed genotype [*IL-1α*-889 ( $\chi^2=0.120$ ; df=1; *p*=0.729), *IL-1β*-1473 ( $\chi^2=0.150$ ; df=1; *p*=0.699), *IL-10*-1082 ( $\chi^2=0.597$ ; df=1; *p*=0.439), *IL-6*-174 ( $\chi^2=0.501$ ; df=1; *p*=0.479), *TNFA*-308 ( $\chi^2=0.009$ ; df=1; *p*=0.921)].

No association between *IL-1α*-889C/T (rs1800587) polymorphism and CSF biomarkers was detected in any of the analyzed groups.

### *IL-10*-1082G/A (rs1800896)

P-tau<sub>181</sub> levels were significantly different between MCI patients with different *IL-10*-1082 genotype (H test=7.183, df=2, *p*=0.028). There was an increase in p-tau<sub>181</sub> levels in MCI patients with AA compared to AG *IL-10*-1082 genotype (Kruskal-Wallis [K-W]) post hoc *p*=0.050) (**Figure 1**). P-tau<sub>181</sub> levels were also increased in patients with AA compared to GG and AG *IL-10*-1082 genotype (MCI patients: U=182, Z=-2.680, *p*=0.007; MCI patients and HC combined: U=309.5, Z=-2.355 *p*=0.019, **Figure 1**).

### *IL-1β*-1473C/G (rs1143623)

T-tau (H test=6.385, df=2, *p*=0.041), p-tau<sub>199</sub> (H test=6.717, df=2, *p*=0.035) and VILIP-1 (H test=6.351, df=2, *p*=0.042) levels were significantly different between AD and MCI patients with different *IL-1β*-1473 genotype (**Figure 2**). T-tau (K-W post hoc *p*=0.035\*) p-tau<sub>199</sub> (K-W post hoc *p*=0.031) and VILIP-1 (K-W post hoc *p*=0.044) levels were significantly increased in AD and MCI patients with CG compared to CC *IL-1β*-1473 genotype (**Figure 2**).

P-tau<sub>199</sub> levels were significantly increased in patients with CG and GG compared to CC *IL-1β*-1473 genotype (MCI patients: U=150.5, Z=-2.177, *p*=0.029; AD and MCI patients: U=2577.5, Z=-2.569, *p*=0.010, group of AD and MCI patients and HC combined: U=3099.5, Z=-2.248, *p*=0.025, **Figure 3**).

P-tau<sub>231</sub> levels were also significantly increased in patients with CG and GG compared to CC *IL-1β*-1473 genotype (AD and MCI patients combined: U=2613, Z=-2.129, *p*=0.033; AD, MCI patients, and HC combined: U=3046, Z=-2.087, *p*=0.037; all patients: U=5214.5, Z=-2.049, *p*=0.040, **Figure 3**).

Aβ<sub>1-42</sub> levels were significantly decreased (*t*=2.117, df=166, *p*=0.036), while t-tau (U=2660, Z=-2.391, *p*=0.017) and VILIP-1 (U=2563.5, Z=-2.208, *p*=0.027) levels were significantly increased in AD and MCI patients with CG and GG genotype compared to patients with CC *IL-1β*-1473 genotype (**Figure 4**).

### *IL-6*-174C/G (rs1800795)

P-tau<sub>199</sub> levels were increased in MCI patients (U=156.5, Z=-2.050, *p*=0.040) and MCI patients and HC combined (U=297, Z=-1.964, *p*=0.049) with GG and GC compared to CC *IL-6*-174 genotype (**Figure 5**). VILIP-1 levels were also significantly different in MCI patients with different *IL-6*-174 genotype (H test=6.695, df=2, *p*=0.035). There was an increase in VILIP-1 levels in MCI patients with GC compared to GG *IL-6*-174 genotype (K-W post hoc *p*=0.039; **Figure 5**).

### ***TNFA*-308A/G (rs1800629)**

As only three AD patients were carriers of AA *TNFA*-308 genotype (**Table 1**), these patients were grouped together with carriers of AG *TNFA*-308 genotype. P-tau<sub>231</sub> (U=805.5, Z=-2.220, p=0.026) and VILIP-1 (U=762.5, Z=-2.517, p=0.012) levels were significantly increased in AD patients with GG compared to AA and AG *TNFA*-308 genotype (**Figure 6**). Additionally, p-tau<sub>231</sub> levels were significantly increased in patients with GG compared to AG *TNFA*-308 genotype (in AD, MCI patients and HC combined, K-W post hoc p=0.038), in AD and MCI patients (K-W post hoc p=0.039), and in AD patients (K-W post hoc p=0.015\*); **Figure 7, Table 2**). VILIP-1 levels were also significantly increased in AD patients with GG compared to AG *TNFA*-308 genotype (K-W post hoc p=0.002; **Figure 7, Table 2**). Levels of t-tau, p-tau<sub>181</sub>, p-tau<sub>199</sub>, p-tau<sub>231</sub> and VILIP-1 were significantly increased in patients with AA compared to AG *TNFA*-308 genotype in all patients (when all subjects were grouped together, in AD, MCI patients and HC combined, in AD and MCI patients combined, and in AD patients), while levels of t-tau and VILIP-1 were increased in patients with AA compared to GG *TNFA*-308 genotype (when all subjects were grouped together and in AD, MCI patients and HC; **Table 2, Figure 8**). The three AD patients carriers of AA *TNFA*-308 genotype, could not be evaluated separately and should be validated in a larger of population.

## **Discussion**

Few studies have investigated whether levels of CSF AD biomarkers differ among patients with different *IL-10*-1082G/A, *IL-1β*-1473C/G, *IL-1α*-889C/T, *IL-6*-174C/G and *TNFA*-308A/G genotypes that were previously associated with AD [12,13]. We compared the levels of six AD CSF biomarkers (Aβ<sub>1-42</sub>, t-tau, p-tau<sub>181</sub>, p-tau<sub>199</sub>, p-tau<sub>231</sub> and VILIP-1) among patients with aforementioned genotypes. This study gave several notable findings. Levels of Aβ<sub>1-42</sub> were decreased, while levels of t-tau were increased in carriers of G allele in *IL-1β*-1473C/G polymorphism. T-tau levels were also significantly increased in patients with CG *IL-1β*-1473C/G genotype. P-tau levels were significantly increased in patients with AA *IL-10*-1082G/A and GG *TNFA*-308A/G genotype, and in carriers of G allele in *IL-1β*-1473C/G and *IL-6*-174C/G polymorphisms. Levels of VILIP-1 were increased in patients with CG and GG *IL-1β*-1473C/G, GC *IL-6*-174C/G and GG *TNFA*-308A/G genotype.

SNPs in genes for *IL-1α*, *IL-1β*, *IL-6*, *IL-10* and *TNFA* can influence transcription and consequently the amount of the produced cytokines [9–11]. Decrease in the amount of anti-inflammatory cytokines and increase in pro-inflammatory cytokines results in increased inflammation, favouring the development of AD [27]. In that way certain genotypes in these SNPs (*IL-10*-1082G/A, *IL-1β*-1473C/G, *IL-1α*-889C/T, *IL-6*-174C/G and *TNFA*-308A/G) can make some people more vulnerable to the development of neuroinflammation and consequently the development of AD. Given that the production of *IL-10* is significantly decreased in carriers of the *IL-10*-1082 A genotype [28,29], a decrease in anti-inflammatory cytokine *IL-10* levels could result in increased inflammation, favouring the development of AD [27]. It was found that the C *IL-6*-174 allele is associated with decrease in *IL-6* plasma levels [10] so this genotype could be protective against AD. *TNFA* being a main pro-inflammatory cytokine, its higher production is associated with increased inflammation and AD progression. *TNFA* inhibitors have been suggested as potential therapeutics for AD [30]. The influence of *TNFA*-308 polymorphism on *TNFA* protein production remains however unclear. Most studies reported that the A *TNFA*-308 allele is associated with increased production of *TNFA* [9,31,32], while some studies did not find differences in *TNFA* protein levels in patients with different *TNFA*-308 genotypes [33]. Regarding polymorphisms in additional pro-inflammatory cytokines *IL-1α* and *IL-1β* that were also tested in this study, it was showed that T allele in the *IL-1α*-889 polymorphism was associated with increased transcriptional activity in *IL-1α* gene and overexpression of *IL-1α* protein [34,35], while G allele in *IL-1β*-1473 polymorphism was associated with weaker promoter activity [36]. Our results support most of these studies, because we observed pathological levels of CSF AD biomarkers in carriers of A allele in *IL-10*-1082 polymorphism, carriers of G allele in *IL-6*-174 polymorphism and carriers of A allele in *TNFA*-308 polymorphism. However, regarding polymorphisms in genes for *IL-1α* and *IL-1β*, our results differed from aforementioned studies. CSF AD biomarkers did not differ between patients with different *IL-1α*-889 genotypes, while levels of CSF AD biomarkers were pathological in carriers of G allele in *IL-1β*-1473 polymorphism.

*IL-10*-1082G/A (rs1800896), *IL-1β*-1473C/G (rs1143623), *IL-1α*-889C/T (rs1800587), *IL-6*-174C/G (rs1800795) and *TNFA*-308A/G (rs1800629) polymorphisms were previously associated with AD in epidemiological studies. Studies on association of *IL-10*-1082G/A polymorphism and AD yielded inconsistent results. Associations between the A allele in *IL-10*-1082 polymorphism and increased risk for AD or the G allele and decreased risk for AD have been reported [11,37–43]. However, other investigators found no association between *IL-10*-1082 polymorphism and AD [44–52] or showed GG *IL-10*-1082 genotype to be significantly increased in AD patients [53] and AA *IL-10*-1082 genotype to decrease the risk for AD [54]. Meta-analyses revealed an association between *IL-10*-1082 AA and AG genotype and increased risk for AD [55], and an association between *IL-10*-1082 GG genotype and reduced risk for AD [56]. However, the meta-analysis of Mun et al. found no association between *IL-10*-1082 polymorphism and AD risk [8]. Our results agree with studies showing association between *IL-10*-1082 A genotype and increased risk for AD [11,37–43].

Cytokine *IL-1β* is likely involved in cognitive decline related to inflammation [57]. As such, polymorphisms in *IL-1β* were studied to assess possible association with AD (for example, *IL-1β*-511, *IL-1β*-31 and *IL-1β*+3953 polymorphisms [8,58–60]). Association of *IL-1β*-1473G/C polymorphism with AD was assessed in only two studies. There was no significant difference in distribution of *IL-1β*-1473 genotypes between AD patients and controls [61,62]. In contrast to these studies, we observed levels of various CSF AD biomarkers to be altered in subjects with different *IL-1β*-1473 genotypes. Our results indicate that *IL-1β*-1473 polymorphism may represent a consistent marker of AD and that the frequency of *IL-1β*-1473 genotypes should be further tested on larger AD and MCI cohorts.

The association of *IL-6*-174C/G polymorphism with AD is ambiguous. Some studies found an association between a C allele in *IL-6*-174 polymorphism and decreased risk for AD [63–70], while others found no association between the *IL-6*-174 polymorphism and AD [47,48,52,54,71–80]. Additionally, some studies found the C allele in the *IL-6*-174 polymorphism to be associated with increased risk for AD [38,41,81–83]. Meta-analyses testing association of *IL-6*-174 polymorphism with AD also returned inconsistent results. Dai et al. [84] and Qi et al. [85] showed the CC *IL-6*-174 genotype to be associated with decreased

risk for AD, while Mun et al. showed that the *IL-6*-174 polymorphism is not associated with AD [8]. Our results support studies showing that the CC *IL-6*-174 genotype is associated with a decreased risk for AD [63–70,84,85].

Studies on association of pro-inflammatory IL-1 $\alpha$  cytokine brain overexpression with AD [86] showed that the presence of a T allele in the *IL-1 $\alpha$* -889 polymorphism is associated with an increased risk for AD [87–96]. Other studies however did not report an association between this polymorphism and AD [48,53,54,97–116]. Yet, meta-analyses demonstrated that an association between the *IL-1 $\alpha$* -889 polymorphism and AD exists [8,117–119]. Our study found no association between this polymorphism and CSF biomarkers in any of the analyzed groups.

Variable results were also obtained from investigations of the association between the *TNFA*-308A/G polymorphism and AD. Several confirmed that presence of the A allele in the *TNFA*-308 polymorphism increases the risk for AD [46,120–122], while others found no association between this polymorphism and AD [12,33,47,70,123–128]. Other authors suggested that the A allele in the *TNFA*-308 polymorphism is protective against AD [13,129,130]. Meta-analyses also gave inconsistent results. Furthermore, Di Bona et al. [131] did not confirm the association between *TNFA*-308 polymorphism and AD. The meta-analysis of Lee et al. [7] showed that the A allele in the *TNFA*-308 polymorphism may be a risk factor for AD in East Asians, but not in Middle Easterners and Europeans. Wang [132] confirmed that the A allele increases risk for AD in Asians but decreases risk in Northern Europeans. Our study included only three AD patients with the AA *TNFA*-308 genotype. These three patients had pathological levels of all examined CSF AD biomarkers, except for A $\beta$ <sub>1-42</sub> (Table 2). This result remains however inconclusive due to the small sample. We also detected pathological levels of CSF AD biomarkers in patients with the GG *TNFA*-308 genotype. The levels of CSF AD biomarkers in patients with different *TNFA*-308 genotypes were also investigated by Sarajärvi et al. [13] and Laws et al. [12]. Although the genetic analysis of Sarajärvi et al. [13] showed that A allele carriers are less susceptible for AD than GG homozygotes, their analysis of biomarkers in patients with different *TNFA*-308 genotypes revealed that levels of A $\beta$ <sub>1-42</sub> were pathological in carriers of an A *TNFA*-308 allele compared to GG homozygotes [13]. This contrasts with our study as we detected pathological CSF levels of p-tau<sub>231</sub> and VILIP-1 in GG homozygotes in comparison to carriers of an A *TNFA*-308 allele, and we found no differences in CSF A $\beta$ <sub>1-42</sub> levels between patients with different *TNFA*-308 genotype. The findings of Laws et al. support our results [12]. Although the results of our previous genetic study [128] showed no significant difference in distribution of *TNFA*-308 genotypes between AD patients and HC, in the present study we detected pathological levels of CSF p-tau<sub>231</sub> and VILIP-1 in AD patients with the GG compared to AG *TNFA*-308 genotypes. Other groups also did not detect a difference in distribution of *TNFA*-308 genotypes between AD patients and HC, but observed difference in distribution of haplotypes (that include the *TNFA*-308 polymorphism) between AD patients and HC [130,133]. Thus, the scope of our next study should be analysis of *TNFA* haplotypes' distribution between AD patients and HC. Our study suggest that heterozygosity in *TNFA*-308 polymorphism could be protective against AD, as pathological levels of CSF AD biomarkers were detected in both AA and GG *TNFA*-308 homozygotes. This deserves further validation.

IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10 and TNF $\alpha$  were also studied as potential biomarkers of AD. However, the results on measurement of these and other inflammatory markers in body fluids were inconsistent [134]. Thus, recently a lot of meta-analyses were conducted with purpose to determine the potential of inflammatory markers as biomarkers of AD. The increase in IL-6 was associated with all-cause dementia, but not AD in meta-analyses of Darewsh et al. [135] and Koyama et al. [136]. Additional meta-analyses observed increase in peripheral IL-6, IL-1 $\beta$  [137–139] and TNF- $\alpha$  [139] in AD patients compared to HC. However, meta-analyses of Saleem et al. [140] and Su et al. [138] observed no significant difference in inflammatory markers between MCI patients and HC. Brosseron et al. [134] divided inflammatory markers measured in body fluids into three groups by involvement in the disease; 1) cytokines unchanged during disease (like IL-1 $\alpha$ ), 2) cytokines that increase slightly but steadily during disease (like IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) and 3) cytokines that have a peak when MCI converses to AD.

## Conclusions

In conclusion, our study reveals altered levels of CSF AD biomarkers in carriers of different genotypes in *IL-10*-1082A/G, *IL-1 $\beta$* -1473C/G, *IL-6*-174C/G and *TNFA*-308A/G polymorphisms, while CSF AD biomarkers did not differ between patients with different *IL-1 $\alpha$* -889C/T genotypes. These polymorphisms as potential genetic biomarkers of AD should be further compared with CSF AD biomarkers on bigger cohort of patients and comparison with neuroimaging AD biomarkers should be also made. Additionally, it should be assessed whether different genotypes in these polymorphisms are the cause of the observed inconsistencies in the levels of these cytokines measured in body fluids, as well as their relationship to inflammasome and microglial activation [5].

## Abbreviations

A $\beta$ , amyloid  $\beta$ ; AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CBS, corticobasal syndrome; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; HC, healthy control; HIS, Hachinski Ischemic Score; IL, interleukin; K-W, Kruskal-Wallis; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ND, nonspecific dementia; NINCDS-AIREN, National Institute for Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences; PD, Parkinson's disease; p-tau<sub>181</sub>, tau phosphorylated at Thr 181; p-tau<sub>199</sub>, tau phosphorylated at Ser 199; p-tau<sub>231</sub>, tau phosphorylated at Thr 231; SNP, single nucleotide polymorphisms; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; t-tau, total tau; VaD, vascular dementia; VILIP-1, visinin-like protein 1.

## Declarations

**Ethics approval and consent to participate**

All procedures were approved by the Ethical Committee of the Clinical Hospital Center Zagreb (case no. 02/21 AG, class 8.1-18/82-2 from April 24, 2018) and by the Central Ethical Committee of the University of Zagreb Medical School (case no. 380-59-10106-18-111/126, class 641-01/18-02/01 from June 20, 2018). All patients gave informed consent for participation in this study and for lumbar puncture.

### Consent for publication

All patients gave consent for publication.

### Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

GŠ conceived and directed the study. NK and FB performed the clinical assessments and lumbar puncture. MNP, DŠŠ, MBL and NP determined IL-1 $\beta$ , IL-6, IL-10, and TNF $\alpha$  genotypes. MBL and GŠ determined levels of CSF biomarkers. MBL and GŠ completed statistical analysis. PRH substantially contributed to the interpretation of data and to manuscript preparation. All authors contributed to revising and editing the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript. All authors met the criteria for authorship, as defined by the International Committee of Medical Journal Editors.

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## References

1. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's Dement Transl Res Clin Interv*. 2018;4:575–90.
2. Šimić G, Španić E, Langer Horvat L, Hof PR. Blood-brain barrier and innate immunity in the pathogenesis of Alzheimer's disease. *Prog Mol Biol Transl Sci*. 2019;168:99–145.
3. Kosciak RL, Betthausen TJ, Jonaitis EM, Allison SL, Clark LR, Hermann BP, et al. Amyloid duration is associated with preclinical cognitive decline and tau PET. *bioRxiv*. 2019; Sept 23, doi:10.1101/778415.
4. Hickman SE, Allison EK, El Khoury J. Microglial dysfunction and defective  $\beta$ -amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci*. 2008;28:8354–60.
5. Španić E, Langer Horvat L, Hof PR, Šimić G. Role of microglial cells in Alzheimer's disease tau propagation. *Front Aging Neurosci*. 2019;11:271.
6. Su F, Bai F, Zhang Z. Inflammatory cytokines and Alzheimer's disease: a review from the perspective of genetic polymorphisms. *Neurosci Bull*. 2016;32:469–80.
7. Lee YH, Choi SJ, Ji JD, Song GG. Association between TNF- $\alpha$  promoter -308 A/G polymorphism and Alzheimer's disease: a meta-analysis. *Neurol Sci*. 2015;36:825–32.
8. Mun M-J, Kim J-H, Choi J-Y, Jang W-C. Genetic polymorphisms of interleukin genes and the risk of Alzheimer's disease: an update meta-analysis. *Meta Gene*. 2016;8:1–10.
9. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor- $\alpha$  promoter polymorphism effects transcription. *Mol Immunol*. 1997;34:391–9.

10. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest.* 1998;102:1369–76.
11. Vargas-Alarcón G, Juárez-Cedillo E, Martínez-Rodríguez N, Fragoso JM, García-Hernández N, Juárez-Cedillo T. Association of interleukin-10 polymorphisms with risk factors of Alzheimer's disease and other dementias (SADEM study). *Immunol Lett.* 2016;177:47–52.
12. Laws SM, Pernecky R, Wagenpfeil S, Müller U, Förstl H, Martins RN, et al. TNF polymorphisms in Alzheimer disease and functional implications on CSF  $\beta$ -amyloid levels. *Hum Mutat.* 2005;26:29–35.
13. Sarajärvi T, Helisalmi S, Antikainen L, Mäkinen P, Koivisto AM, Herukka S-K, et al. An association study of 21 potential Alzheimer's disease risk genes in a Finnish population. *J Alzheimer's Dis.* 2010;21:763–7.
14. Babić Leko M, Willumsen N, Nikolac Perković M, Klepac N, Borovečki F, Hof PR, et al. Association of MAPT haplotype-tagging polymorphisms with cerebrospinal fluid biomarkers of Alzheimer's disease: a preliminary study in a Croatian cohort. *Brain Behav.* 2018;8:e01128.
15. Grimmer T, Riemenschneider M, Förstl H, Henriksen G, Klunk WE, Mathis CA, et al. Beta amyloid in Alzheimer's disease: increased deposition in brain is reflected in reduced concentration in cerebrospinal fluid. *Biol Psychiatry.* 2009;65:927–34.
16. Bürger K, Ewers M, Pirttilä T, Zinkowski R, Alafuzoff I, Teipel SJ, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain.* 2006;129:3035–41.
17. Babić M, Švob Štrac D, Mück-Šeler D, Pivac N, Stanić G, Hof PR, et al. Update on the core and developing cerebrospinal fluid biomarkers for Alzheimer disease. *Croat Med J.* 2014;55:347–65.
18. Babić Leko M, Borovečki F, Dejanović N, Hof PR, Šimić G. Predictive value of Cerebrospinal fluid visinin-like protein-1 levels for Alzheimer's disease early detection and differential diagnosis in patients with mild cognitive impairment. *J Alzheimer's Dis.* 2016;50:765–78.
19. Boban M, Malojčić B, Mimica N, Vuković S, Zrilić I, Hof PR, et al. The reliability and validity of the Mini-mental state examination in the elderly Croatian population. *Dement Geriatr Cogn Disord.* 2012;33:385–92.
20. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* 2011;7:263–9.
21. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* 2011;7:270–9.
22. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology.* 1998;51:1546–54.
23. Román GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology.* 1993;43:250–60.
24. Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, et al. Cerebral blood flow in dementia. *Arch Neurol.* 1975;32:632–7.
25. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA.* 2013;310:2191–4.
26. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16:1215.
27. Magalhães CA, Carvalho M das G, Sousa LP de, Caramelli P, Gomes KB. Alzheimer's disease and cytokine IL-10 gene polymorphisms: is there an association? *Arq Neuropsiquiatr.* 2017;75:649–56.
28. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson I V. An investigation of polymorphism in the interleukin-10 gene promotor. *Eur J Immunogenet.* 1997;24:1–8.
29. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum.* 1999;42:1101–8.
30. Chang R, Yee K-L, Sumbria RK. Tumor necrosis factor  $\alpha$  inhibition for Alzheimer's disease. *J Cent Nerv Syst Dis.* 2017;9:1179573517709278.
31. Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF- $\alpha$  and TNF- $\beta$  by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol.* 1993;23:224–31.
32. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor promoter on transcriptional activation. *Proc Natl Acad Sci U S A.* 1997;94:3195–9.
33. Tarkowski E, Liljeroth AM, Nilsson A, Ricksten A, Davidsson P, Minthon L, et al. TNF gene polymorphism and its relation to intracerebral production of TNF $\alpha$  and TNF $\beta$  in AD. *Neurology.* 2000;54:2077–81.
34. Wei X, Chen X, Fontanilla C, Zhao L, Liang Z, Dodel R, et al. C/T conversion alters interleukin-1A promoter function in a human astrocyte cell line. *Life Sci.* 2007;80:1152–6.
35. Dominici R, Cattaneo M, Malferrari G, Archi D, Mariani C, Grimaldi L, et al. Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 $\alpha$ . *Immunogenetics.* 2002;54:82–6.
36. Lee K-A, Ki C-S, Kim H-J, Sohn K-M, Kim J-W, Kang WK, et al. Novel interleukin 1 $\beta$  polymorphism increased the risk of gastric cancer in a Korean population. *J Gastroenterol.* 2004;39:429–33.
37. Lio D, Licastro F, Scola L, Chiappelli M, Grimaldi LM, Crivello A, et al. Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease. *Genes Immun.* 2003;4:234–8.

38. Arosio B, Trabattoni D, Galimberti L, Bucciarelli P, Fasano F, Calabresi C, et al. Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease. *Neurobiol Aging*. 2004;25:1009–15.
39. Bagnoli S, Cellini E, Tedde A, Nacmias B, Piacentini S, Bessi V, et al. Association of IL10 promoter polymorphism in Italian Alzheimer's disease. *Neurosci Lett*. 2007;418:262–5.
40. Combarros O, Sánchez-Juan P, Riancho JA, Mateo I, Rodríguez-Rodríguez E, Infante J, et al. Aromatase and interleukin-10 genetic variants interactively modulate Alzheimer's disease risk. *J Neural Transm*. 2008;115:863–7.
41. Vural P, Değirmencioğlu S, Parıldar-Karpuzoğlu H, Doğru-Abbassoğlu S, Hanagasi HA, Karadağ B, et al. The combinations of TNF $\alpha$ -308 and IL-6 -174 or IL-10 -1082 genes polymorphisms suggest an association with susceptibility to sporadic late-onset Alzheimer's disease. *Acta Neurol Scand*. 2009;120:396–401.
42. Arosio B, Mastronardi L, Vergani C, Annoni G. Interleukin-10 promoter polymorphism in mild cognitive impairment and in its clinical evolution. *Int J Alzheimers Dis*. 2010;2010:1–5.
43. Fraga VG, Guimarães HC, Teixeira AL, Barbosa MT, Carvalho MG, Caramelli P, et al. Polymorphisms in cytokine genes influence cognitive and functional performance in a population aged 75 years and above. *Int J Geriatr Psychiatry*. 2017;32:1401–10.
44. Scassellati C, Zanardini R, Squitti R, Bocchio-Chiavetto L, Bonvicini C, Binetti G, et al. Promoter haplotypes of interleukin-10 gene and sporadic Alzheimer's disease. *Neurosci Lett*. 2004;356:119–22.
45. Culpán D, Prince JA, Matthews S, Palmer L, Hughes A, Love S, et al. Neither sequence variation in the IL-10 gene promoter nor presence of IL-10 protein in the cerebral cortex is associated with Alzheimer's disease. *Neurosci Lett*. 2006;408:141–5.
46. Ramos EM, Lin M-T, Larson EB, Maezawa I, Tseng L-H, Edwards KL, et al. Tumor necrosis factor  $\alpha$  and interleukin 10 promoter region polymorphisms and risk of late-onset Alzheimer disease. *Arch Neurol*. 2006;63:1165.
47. Shawkatová I, Javor J, Párnická Z, Vrazda L, Novák M, Buc M. No association between cytokine gene polymorphism and risk of Alzheimer's disease in Slovaks. *Acta Neurobiol Exp (Wars)*. 2010;70:303–7.
48. Cousin E, Macé S, Rocher C, Dib C, Muzard G, Hannequin D, et al. No replication of genetic association between candidate polymorphisms and Alzheimer's disease. *Neurobiol Aging*. 2011;32:1443–51.
49. Heun R, Kölsch H, Ibrahim-Verbaas CA, Combarros O, Aulchenko YS, Breteler M, et al. Interactions between PPAR- $\alpha$  and inflammation-related cytokine genes on the development of Alzheimer's disease, observed by the Epistasis Project. *Int J Mol Epidemiol Genet*. 2012;3:39–47.
50. Torres KC, Araújo Pereira P, Lima GS, Bozzi IC, Rezende VB, Bicalho MA, et al. Increased frequency of T cells expressing IL-10 in Alzheimer disease but not in late-onset depression patients. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2013;47:40–5.
51. Kang H-J, Kim J-M, Kim S-W, Shin I-S, Park S-W, Kim Y-H, et al. Associations of cytokine genes with Alzheimer's disease and depression in an elderly Korean population. *J Neurol Neurosurg Psychiatry*. 2015;86:1002–7.
52. Toral-Rios D, Franco-Bocanegra D, Rosas-Carrasco O, Mena-Barranco F, Carvajal-García R, Meraz-Ríos M, et al. Evaluation of inflammation-related genes polymorphisms in Mexican with Alzheimer's disease: a pilot study. *Front Cell Neurosci*. 2015;9:148.
53. Ribizzi G, Fiordoro S, Barocci S, Ferrari E, Megna M. Cytokine polymorphisms and Alzheimer disease: possible associations. *Neurol Sci*. 2010;31:321–5.
54. Moraes CF, Benedet AL, Souza VC, Lins TC, Camargos EF, Naves JOS, et al. Cytokine gene polymorphisms and Alzheimer's disease in Brazil. *Neuroimmunomodulation*. 2013;20:239–46.
55. Zhang Y, Zhang J, Tian C, Xiao Y, Li X, He C, et al. The -1082G/A polymorphism in IL-10 gene is associated with risk of Alzheimer's disease: a meta-analysis. *J Neurol Sci*. 2011;303:133–8.
56. Di Bona D, Rizzo C, Bonaventura G, Candore G, Caruso C. Association between interleukin-10 polymorphisms and Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimer's Dis*. 2012;29:751–9.
57. Benke KS, Carlson MC, Doan BQ, Walston JD, Xue QL, Reiner AP, et al. The association of genetic variants in interleukin-1 genes with cognition: findings from the cardiovascular health study. *Exp Gerontol*. 2011;46:1010–9.
58. Ma SL, Tang NLS, Lam LCW, Chiu HFK. Lack of association of the interleukin-1 $\beta$  gene polymorphism with Alzheimer's disease in a Chinese population. *Dement Geriatr Cogn Disord*. 2003;16:265–8.
59. Wang W-F, Liao Y-C, Wu S-L, Tsai F-J, Lee C-C, Hua C-S. Association of interleukin-1 beta and receptor antagonist gene polymorphisms with late onset Alzheimer's disease in Taiwan Chinese. *Eur J Neurol*. 2005;12:609–13.
60. Payão SLM, Gonçalves GM, de Labio RW, Horiguchi L, Mizumoto I, Rasmussen LT, et al. Association of interleukin 1 $\beta$  polymorphisms and haplotypes with Alzheimer's disease. *J Neuroimmunol*. 2012;247:59–62.
61. Mustapić M, Presečki P, Mimica N, Pivac N, Folnegović Šmalc V, Mück-Šeler D. Dopamine beta-hydroxylase and inflammatory cytokines in Alzheimer's disease. *Period Biol* 112, Suppl 1. 2010;41.
62. Yin Y, Liu Y, Pan X, Chen R, Li P, Wu H-J, et al. Interleukin-1 $\beta$  promoter polymorphism enhances the risk of sleep disturbance in Alzheimer's disease. *PLoS One*. 2016;11:e0149945.
63. Pola R, Flex A, Gaetani E, Lago AD, Gerardino L, Pola P, et al. The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with Alzheimer's disease in an Italian population [corrected]. *Neuroreport*. 2002;13:1645–7.
64. Shibata N, Ohnuma T, Takahashi T, Baba H, Ishizuka T, Ohtsuka M, et al. Effect of IL-6 polymorphism on risk of Alzheimer disease: genotype-phenotype association study in Japanese cases. *Am J Med Genet*. 2002;114:436–9.
65. Faltraco F, Bürger K, Zill P, Teipel SJ, Möller H-J, Hampel H, et al. Interleukin-6-174 G/C promoter gene polymorphism C allele reduces Alzheimer's disease risk. *J Am Geriatr Soc*. 2003;51:578–9.



66. Infante J, Sanz C, Fernández-Luna JL, Llorca J, Berciano J, Combarros O. Gene-gene interaction between interleukin-6 and interleukin-10 reduces AD risk. *Neurology*. 2004;63:1135–6.
67. Combarros O, Infante J, Llorca J, Peña N, Fernández-Viadero C, Berciano J. Interaction between interleukin-6 and intercellular adhesion molecule-1 genes and Alzheimer's disease risk. *J Neurol*. 2005;252:485–7.
68. Koivisto AM, Helisalmi S, Pihlajamäki J, Moilanen L, Kuusisto J, Laakso M, et al. Interleukin-6 promoter polymorphism and late-onset Alzheimer's disease in the Finnish population. *J Neurogenet*. 2005;19:155–61.
69. Fontalba A, Gutiérrez O, Llorca J, Mateo I, Vázquez-Higuera JL, Berciano J, et al. Gene–gene interaction between CARD8 and interleukin-6 reduces Alzheimer's disease risk. *J Neurol*. 2009;256:1184–6.
70. Flex A, Giovannini S, Biscetti F, Liperoti R, Spalletta G, Straface G, et al. Effect of proinflammatory gene polymorphisms on the risk of Alzheimer's disease. *Neurodegener Dis*. 2013;13:230–6.
71. Bagli M, Papassotiropoulos A, Jessen F, Schmitz S, Rao ML, Maier W, et al. Identical distribution of the alpha 2-macroglobulin pentanucleotide deletion in subjects with Alzheimer disease and controls in a German population. *Am J Med Genet*. 2000;96:775–7.
72. Bhojak TJ, DeKosky ST, Ganguli M, Kamboh MI. Genetic polymorphisms in the cathepsin D and interleukin-6 genes and the risk of Alzheimer's disease. *Neurosci Lett*. 2000;288:21–4.
73. Capurso C, Solfrizzi V, D'Introno A, Colacicco AM, Capurso SA, Capurso A, et al. Interleukin 6-174 G/C promoter gene polymorphism and sporadic Alzheimer's disease: geographic allele and genotype variations in Europe. *Exp Gerontol*. 2004;39:1567–73.
74. Depboylu C, Lohmüller F, Gocke P, Du Y, Zimmer R, Gasser T, et al. An Interleukin-6 promoter variant is not associated with an increased risk for Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2004;17:170–3.
75. Zhang Y, Hayes A, Pritchard A, Thaker U, Haque MS, Lemmon H, et al. Interleukin-6 promoter polymorphism: risk and pathology of Alzheimer's disease. *Neurosci Lett*. 2004;362:99–102.
76. Ravaglia G, Paola F, Maioli F, Martelli M, Montesi F, Bastagli L, et al. Interleukin-1 $\beta$  and interleukin-6 gene polymorphisms as risk factors for AD: a prospective study. *Exp Gerontol*. 2006;41:85–92.
77. van Oijen M, Arp PP, Jong FJ de, Hofman A, Koudstaal PJ, Uitterlinden AG, et al. Polymorphisms in the interleukin 6 and transforming growth factor  $\beta$ 1 gene and risk of dementia. *Neurosci Lett*. 2006;402:113–7.
78. Paradowski B, Celczyńska D, Dobosz T, Noga L. Polymorphism 174 G/C of interleukin 6 gene in Alzheimer's disease - preliminary report. *Neurol Neurochir Pol*. 2008;42:312–5.
79. Capurso C, Solfrizzi V, Colacicco AM, D'Introno A, Frisardi V, Imbimbo BP, et al. Interleukin 6–174 G/C promoter and variable number of tandem repeats (VNTR) gene polymorphisms in sporadic Alzheimer's disease. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2010;34:177–82.
80. Klimkowicz-Mrowiec A, Wołkow P, Spisak K, Maruszak A, Styczyńska M, Barcikowska M, et al. Interleukin-6 gene (-174 C/G) and apolipoprotein E gene polymorphisms and the risk of Alzheimer disease in a Polish population. *Neurol Neurochir Pol*. 2010;44:537–41.
81. Licastro F, Grimaldi LME, Bonafè M, Martina C, Olivieri F, Cavallone L, et al. Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. *Neurobiol Aging*. 2003;24:921–6.
82. Mansoori N, Tripathi M, Luthra K, Alam R, Lakshmy R, Sharma S, et al. MTHFR (677 and 1298) and IL-6-174 G/C genes in pathogenesis of Alzheimer's and vascular dementia and their epistatic interaction. *Neurobiol Aging*. 2012;33:1003.e1–1003.e8.
83. Rasmussen L, Delabio R, Horiguchi L, Mizumoto I, Terazaki C-R, Mazzotti D, et al. Association between interleukin 6 gene haplotype and Alzheimer's disease: a Brazilian case-control study. *J Alzheimer's Dis*. 2013;36:733–8.
84. Dai L, Liu D, Guo H, Wang Y, Bai Y. Association between polymorphism in the promoter region of Interleukin 6 (-174 G/C) and risk of Alzheimer's disease: a meta-analysis. *J Neurol*. 2012;259:414–9.
85. Qi H-P, Qu Z-Y, Duan S-R, Wei S-Q, Wen S-R, Bi S. IL-6-174 G/C and -572 C/G Polymorphisms and risk of Alzheimer's disease. *PLoS One*. 2012;7:e37858.
86. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging*. 2000;21:383–421.
87. Du Y, Dodel RC, Eastwood BJ, Bales KR, Gao F, Lohmüller F, et al. Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. *Neurology*. 2000;55:480–3.
88. Grimaldi LM, Casadei VM, Ferri C, Veglia F, Licastro F, Annoni G, et al. Association of early-onset Alzheimer's disease with an interleukin-1 $\alpha$  gene polymorphism. *Ann Neurol*. 2000;47:361–5.
89. Nicoll JA, Mrak RE, Graham DI, Stewart J, Wilcock G, MacGowan S, et al. Association of interleukin-1 gene polymorphisms with Alzheimer's disease. *Ann Neurol*. 2000;47:365–8.
90. Rebeck GW. Confirmation of the genetic association of interleukin-1 $\alpha$  with early onset sporadic Alzheimer's disease. *Neurosci Lett*. 2000;293:75–7.
91. Combarros O, Sánchez-Guerra M, Infante J, Llorca J, Berciano J. Gene dose-dependent association of interleukin-1 $\alpha$  [-889] allele 2 polymorphism with Alzheimer's disease. *J Neurol*. 2002;249:1242–5.
92. Hedley R, Hallmayer J, Groth DM, Brooks WS, Gandy SE, Martins RN. Association of interleukin-1 polymorphisms with Alzheimer's disease in Australia. *Ann Neurol*. 2002;51:795–7.
93. Sciacca FL, Ferri C, Licastro F, Veglia F, Biunno I, Gavazzi A, et al. Interleukin-1 $\beta$  polymorphism is associated with age at onset of Alzheimer's disease. *Neurobiol Aging*. 2003;24:927–31.
94. Hayes A, Green EK, Pritchard A, Harris JM, Zhang Y, Lambert JC, et al. A polymorphic variation in the interleukin 1 $\alpha$  gene increases brain microglial cell activity in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2004;75:1475–7.

95. Seripa D, Matera MG, Forno GD, Gravina C, Masullo C, Daniele A, et al. Genotypes and haplotypes in the IL-1 gene cluster: analysis of two genetically and diagnostically distinct groups of Alzheimer patients. *Neurobiol Aging*. 2005;26:455–64.
96. Zhou Y, Zhang Z, Zhang J, He X, Xu T. [Association between interleukin-1 $\alpha$ -889 C/T polymorphism and Alzheimer's disease in Chinese Han population]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. 2006;28:186–90.
97. Minster RL, DeKosky ST, Ganguli M, Belle S, Kamboh MI. Genetic association studies of interleukin-1 (IL-1 $\alpha$  and IL-1 $\beta$ ) and interleukin-1 receptor antagonist genes and the risk of Alzheimer's disease. *Ann Neurol*. 2000;48:817–9.
98. Ki CS, Na DL, Kim DK, Kim HJ, Kim JW. Lack of association of the interleukin-1 $\alpha$  gene polymorphism with Alzheimer's disease in a Korean population. *Ann Neurol*. 2001;49:817–8.
99. Prince JA, Feuk L, Sawyer SL, Gottfries J, Ricksten A, Nägga K, et al. Lack of replication of association findings in complex disease: an analysis of 15 polymorphisms in prior candidate genes for sporadic Alzheimer's disease. *Eur J Hum Genet*. 2001;9:437–44.
100. Fidani L, Goulas A, Mirtsou V, Petersen RC, Tangalos E, Crook R, et al. Interleukin-1 $\alpha$  polymorphism is not associated with late onset Alzheimer's disease. *Neurosci Lett*. 2002;323:81–3.
101. Green EK, Harris JM, Lemmon H, Lambert JC, Chartier-Harlin MC, St Clair D, et al. Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? *Neurology*. 2002;58:1566–8.
102. Mattila KM, Rinne JO, Lehtimäki T, Röttä M, Ahonen JP, Hurme M. Association of an interleukin 1 $\beta$  gene polymorphism (-511) with Parkinson's disease in Finnish patients. *J Med Genet*. 2002;39:400–2.
103. Pirskanen M, Hiltunen M, Mannermaa A, Iivonen S, Helisalmi S, Lehtovirta M, et al. Interleukin 1 $\alpha$  gene polymorphism as a susceptibility factor in Alzheimer's disease and its influence on the extent of histopathological hallmark lesions of Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2002;14:123–7.
104. Tsai S-J, Liu H-C, Liu T-Y, Wang K-Y, Hong C-J. Lack of association between the interleukin-1 $\alpha$  gene C(-889)T polymorphism and Alzheimer's disease in a Chinese population. *Neurosci Lett*. 2003;343:93–6.
105. Clarimón J, Bertranpetit J, Calafell F, Boada M, Tàrraga L, Comas D. Joint analysis of candidate genes related to Alzheimer's disease in a Spanish population. *Psychiatr Genet*. 2003;13:85–90.
106. Kuo Y-M, Liao P-C, Lin C, Wu C-W, Huang H-M, Lin C-C, et al. Lack of association between interleukin-1 $\alpha$  polymorphism and Alzheimer disease or vascular dementia. *Alzheimer Dis Assoc Disord*. 2003;17:94–7.
107. McCarron MO, Stewart J, McCarron P, Love S, Vinters HV, Ironside JW, et al. Association between interleukin-1 $\alpha$  polymorphism and cerebral amyloid angiopathy-related hemorrhage. *Stroke*. 2003;34:e193–5.
108. Li X-Q, Zhang J-W, Zhang Z-X, Chen D, Qu Q-M. Interleukin-1 gene cluster polymorphisms and risk of Alzheimer's disease in Chinese Han population. *J Neural Transm*. 2004;111:1183–90.
109. Nishimura M, Sakamoto T, Kaji R, Kawakami H. Influence of polymorphisms in the genes for cytokines and glutathione S-transferase omega on sporadic Alzheimer's disease. *Neurosci Lett*. 2004;368:140–3.
110. Wang H-K, Hsu W-C, Fung H-C, Lin J-C, Hsu H-P, Wu Y-R, et al. Interleukin-1 $\alpha$  and -1 $\beta$  promoter polymorphisms in Taiwanese patients with dementia. *Dement Geriatr Cogn Disord*. 2007;24:104–10.
111. Déniz-Naranjo MC, Muñoz-Fernandez C, Alemany-Rodríguez MJ, Pérez-Vieitez MC, Aladro-Benito Y, Irurita-Latasa J, et al. Cytokine IL-1 $\beta$  but not IL-1 $\alpha$  promoter polymorphism is associated with Alzheimer's disease in a population from the Canary Islands, Spain. *Eur J Neurol*. 2008;15:1080–4.
112. Dursun E, Gezen-Ak D, Ertan T, Bilgiç B, Gürvit H, Emre M, et al. Interleukin-1 $\alpha$  -889 C/T polymorphism in Turkish patients with late-onset Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2009;27:82–7.
113. Hu J, Li G, Zhou D, Zou Y, Zhu Z, Xu R, et al. Genetic analysis of interleukin-1 $\alpha$  C(-889)T polymorphism with Alzheimer's disease. *Cell Mol Neurobiol*. 2009;29:81–5.
114. Serretti A, Olgiati P, Politis A, Malitas P, Albani D, Dusi S, et al. Lack of association between Interleukin-1 $\alpha$  rs1800587 polymorphism and Alzheimer's disease in two independent European samples. *J Alzheimers Dis*. 2009;16:181–7.
115. Vendramini AA, Lábio RW de, Rasmussen LT, Reis NM dos, Minett T, Bertolucci PHF, et al. Interleukin-8-251T > A, interleukin-1 $\alpha$  -889C > T and apolipoprotein E polymorphisms in Alzheimer's disease. *Genet Mol Biol*. 2011;34:1–5.
116. Tian M, Deng YY, Hou DR, Li W, Feng XL, Yu ZL. Association of IL-1, IL-18, and IL-33 gene polymorphisms with late-onset Alzheimer's disease in a Hunan Han Chinese population. *Brain Res*. 2015;1596:136–45.
117. Hua Y, Zhao H, Kong Y, Lu X. Meta-analysis of the association between the interleukin-1 $\alpha$  -889C/T polymorphism and Alzheimer's disease. *J Neurosci Res*. 2012;90:1681–92.
118. Qin X, Peng Q, Zeng Z, Chen Z, Lin L, Deng Y, et al. Interleukin-1 $\alpha$  -889C/T polymorphism and risk of Alzheimer's disease: a meta-analysis based on 32 case-control studies. *J Neurol*. 2012;259:1519–29.
119. Li B-H, Zhang L-L, Yin Y-W, Pi Y, Guo L, Yang Q-W, et al. Association between interleukin-1 $\alpha$  C(-889)T polymorphism and Alzheimer's disease: a meta-analysis including 12,817 subjects. *J Neural Transm*. 2013;120:497–506.
120. Wang B, Zhou S, Yang Z, Xie Y, Wang J, Zhang P, et al. Genetic analysis of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) G-308A and Saitohin Q7R polymorphisms with Alzheimer's disease. *J Neurol Sci*. 2008;270:148–51.
121. Yang L, Lu R, Jiang L, Liu Z, Peng Y. Expression and genetic analysis of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) G-308A polymorphism in sporadic Alzheimer's disease in a Southern China population. *Brain Res*. 2009;1247:178–81.

122. Ardebili SMM, Yeghaneh T, Gharesouran J, Rezazadeh M, Farhoudi M, Ayromlou H, et al. Genetic association of TNF- $\alpha$  -308 G/A and -863 C/A polymorphisms with late onset Alzheimer's disease in Azeri Turk population of Iran. *J Res Med Sci*. 2011;16:1006–13.
123. Zhang P, Yang Z, Wan C-L, Zheng W-D, Zhang C-F, Li S, et al. Neither the tumor necrosis factor  $\alpha$  -308 A/G polymorphism nor the  $\alpha$ 2-macroglobulin polymorphism was associated with late-onset Alzheimer's disease in the Chinese population. *Yi Chuan Xue Bao*. 2004;31:1–6.
124. Lio D, Annoni G, Licastro F, Crivello A, Forte GI, Scola L, et al. Tumor necrosis factor- $\alpha$  -308A/G polymorphism is associated with age at onset of Alzheimer's disease. *Mech Ageing Dev*. 2006;127:567–71.
125. Gnjec A, D'Costa KJ, Laws SM, Hedley R, Balakrishnan K, Taddei K, et al. Association of alleles carried at TNF $\alpha$  -850 and BAT1 -22 with Alzheimer's disease. *J Neuroinflammation*. 2008;5:36.
126. Tedde A, Putignano AL, Nacmias B, Bagnoli S, Cellini E, Sorbi S. Lack of association between TNF- $\alpha$  polymorphisms and Alzheimer's disease in an Italian cohort. *Neurosci Lett*. 2008;446:139–42.
127. Manoochehri M, Kamali K, Rahgozar M, Ohadi M, Farrokhi H, Khorshid HRK. Lack of association between Tumor Necrosis Factor- $\alpha$  -308 G/A polymorphism and risk of developing late-onset Alzheimer's disease in an Iranian population. *Avicenna J Med Biotechnol*. 2009;1:193–7.
128. Mustapić M, Popović Hadžija M, Pavlović M, Pavković P, Presečki P, Mrazovac D, et al. Alzheimer's disease and type 2 diabetes: the association study of polymorphisms in tumor necrosis factor- $\alpha$  and apolipoprotein E genes. *Metab Brain Dis*. 2012;27:507–12.
129. Perry RT, Collins JS, Harrell LE, Acton RT, Go RC. Investigation of association of 13 polymorphisms in eight genes in southeastern African American Alzheimer disease patients as compared to age-matched controls. *Am J Med Genet*. 2001;105:332–42.
130. Culpan D, MacGowan SH, Ford JM, Nicoll JAR, Griffin WS, Dewar D, et al. Tumor necrosis factor- $\alpha$  gene polymorphisms and Alzheimer's disease. *Neurosci Lett*. 2003;350:61–5.
131. Di Bona D, Candore G, Franceschi C, Licastro F, Colonna-Romano G, Cammà C, et al. Systematic review by meta-analyses on the possible role of TNF- $\alpha$  polymorphisms in association with Alzheimer's disease. *Brain Res Rev*. 2009;61:60–8.
132. Wang T. TNF- $\alpha$  G308A polymorphism and the susceptibility to Alzheimer's disease: an updated meta-analysis. *Arch Med Res*. 2015;46:24–30.e1.
133. Collins JS, Perry RT, Watson B, Harrell LE, Acton RT, Blacker D, et al. Association of a haplotype for tumor necrosis factor in siblings with late-onset Alzheimer disease: the NIMH Alzheimer Disease Genetics Initiative. *Am J Med Genet*. 2000;96:823–30.
134. Brosseron F, Krauthausen M, Kummer M, Heneka MT. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. *Mol Neurobiol*. 2014;50:534–44.
135. Darweesh SKL, Wolters FJ, Ikram MA, de Wolf F, Bos D, Hofman A. Inflammatory markers and the risk of dementia and Alzheimer's disease: a meta-analysis. *Alzheimer's Dement*. 2018;14:1450–9.
136. Koyama A, O'Brien J, Weuve J, Blacker D, Metti AL, Yaffe K. The role of peripheral inflammatory markers in dementia and Alzheimer's disease: a meta-analysis. *J Gerontol Ser A Biol Sci Med Sci*. 2013;68:433–40.
137. Lai KSP, Liu CS, Rau A, Lanctôt KL, Köhler CA, Pakosh M, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. *J Neurol Neurosurg Psychiatry*. 2017;88:876–82.
138. Su C, Zhao K, Xia H, Xu Y. Peripheral inflammatory biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. *Psychogeriatrics*. 2019;19:300–9.
139. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry*. 2010;68:930–41.
140. Saleem M, Herrmann N, Swardfager W, Eisen R, Lanctôt KL. Inflammatory markers in mild cognitive impairment: a meta-analysis. *J Alzheimers Dis*. 2015;47:669–79.

## Tables

**Table 1. Frequency of *IL-1 $\alpha$*  -889C/T, *IL-1 $\beta$*  -1473C/G, *IL-6* -174C/G, *IL-10* -1082G/A and *TNF $\alpha$*  -308A/G genotypes, and levels of A $\beta$ <sub>1-42</sub>, t-tau, p-tau<sub>181</sub>, p-tau<sub>199</sub>, p-tau<sub>231</sub> and VILIP-1 in AD and MCI patients, HC, and in patients with other causes of dementia.**

	<i>IL-1<math>\alpha</math></i>			<i>IL-1<math>\beta</math></i>			<i>IL-6</i>			<i>IL-10</i>			<i>TNF<math>\alpha</math></i>			MMSE	Age	Gender	A $\beta_{1-42}$ (pg/ml)	T-tau (pg/ml)	p- tau <sub>181</sub> (pg/ml)	p- tau <sub>199</sub> (pg/ml)	p- tau <sub>231</sub> (U/ml)
	AA	GG	AG	CC	GG	CG	CC	GG	GC	GG	AA	AG	AA	GG	AG	Mean ± SD	Median (25–75th percentile)	F/M	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
AD	6	66	43	60	7	48	39	21	55	23	37	55	3	89	23	19.9 ± 4.5	73 (67-77)	62/53	536.9 ± 296.9	520.0 ± 394.4	80.0 ± 47.8	4.4 ± 3.5	3.9 ± 5.5
MCI	5	30	18	40	1	12	13	12	28	9	24	20		43	10	25.1 ± 2.9	70 (59-74)	27/26	723.4 ± 371.9	246.4 ± 158.0	57.6 ± 30.9	3.4 ± 2.4	1.8 ± 3.2
HC	1	9	1	4	2	5	7	1	3	2	4	5		9	2	27.8 ± 1.9	54 (45-61)	7/4	885.5 ± 540.2	255.4 ± 320.9	44.4 ± 25.6	1.4 ± 1.0	1.1 ± 1.9
VaD	2	7	5	9	1	4	2	5	7	2	8	4		9	5	22.2 ± 5.0	71 (63-77)	6/8	502.9 ± 235.9	516.3 ± 325.8	72.7 ± 36.2	5.3 ± 5.5	1.4 ± 0.8
FTD		14	8	14	2	6	4	8	10	4	7	11		21	1	16.5 ± 5.2	61 (56-64)	11/11	413.0 ± 191.1	479.9 ± 409.3	67.1 ± 37.2	5.5 ± 5.6	1.9 ± 2.4
DLB	2	2	3	5		2	1	2	4		4	3		6	1	19.3 ± 3.9	70 (68-75)	2/5	428.3 ± 259.8	126.4 ± 44.9	41.9 ± 28.8	2.5 ± 1.6	0.6 ± 0.2
AD + VaD		1	2	2		1	2	1		2	1				3	19.3 ± 4.0	78	0/3	553.2 ± 37.2	579.7 ± 460.4	74.1 ± 28.6	2.8 ± 1.9	1.9 ± 1.8
PD		1	2	1	1	1	2	1				3		1	2	22.5 ± 10.6	65	1/2	384.7 ± 282.3	132.7 ± 179.6	24.8 ± 13.7	0.8 ± 1.2	0.4 ± 0.3
CBS		1		1			1				1				1	27	51	1/0	917.6	578.3	84.3	1.7	0.8
ND		1	2	3			1	2			1	2		2	1	20.7 ± 5.5	68	2/1	382.6 ± 145.2	195.2 ± 113.9	22.9 ± 7.7	2.2 ± 1.9	0.3 ± 0.3

A $\beta_{1-42}$ , amyloid  $\beta_{1-42}$  protein; AD: Alzheimer's disease; AD + VaD: mixed dementia; CBS: corticobasal syndrome; DLB: dementia with Lewy bodies; FTD: frontotemporal dementia; HC: healthy control; IL: interleukin; MCI: mild cognitive impairment; ND: nonspecific dementia; p-tau<sub>181</sub>, tau protein phosphorylated at threonine 181; p-tau<sub>231</sub>, tau protein phosphorylated at threonine 231; p-tau<sub>199</sub>, tau protein phosphorylated at serine 199; PD: Parkinson's disease; SD: standard deviation; T-tau; total tau; TNF $\alpha$ : tumor necrosis factor  $\alpha$ ; VaD: vascular dementia; VILIP-1, visinin-like protein 1.

**Table 2.** Comparison of A $\beta_{1-42}$ , t-tau, p-tau<sub>181</sub>, p-tau<sub>199</sub>, p-tau<sub>231</sub> and VILIP-1 levels in different groups of patients with *TNF $\alpha$*  -308A/G (rs1800629) genotypes.

<i>TNF<math>\alpha</math></i> -308A/G genotype				
		AA vs GG	AA vs AG	GG vs AG
	All patients (AD, MCI, HC, VaD, FTD, DLB, AD+VaD, CBS, nonspecific dementia, PD)			
<b>A<math>\beta</math><sub>1-42</sub></b>	KW	H test=1.126, df=2, p=0.570		
<b>Total tau</b>	KW	H test=7.538, df=2, p=0.023*		
	PH KW	p=0.029*	p=0.018*	p=1.000
<b>p-tau<sub>181</sub></b>	KW	H test=7.121, df =2, p=0.028*		
	PH KW	p=0.100	p=0.038*	p=0.415
<b>p-tau<sub>199</sub></b>	KW	H test=7.630, df =2, p=0.022*		
	PH KW	p=0.075	p=0.028*	p=0.419
<b>p-tau<sub>231</sub></b>	KW	H test=9.220, df =2, p=0.010*		
	PH KW	p=0.164	p=0.038*	p=0.077
<b>VILIP-1</b>	KW	H test=8.473, df =2, p=0.014*		
	PH KW	p=0.039*	p=0.015*	p=0.516
AD, MCI patients + HC				
<b>A<math>\beta</math><sub>1-42</sub></b>	KW	H test=2.799, df=2, p=0.247		
<b>Total tau</b>	KW	H test=7.714, df=2, p=0.021*		
	PH KW	p=0.031*	p=0.017*	p=1.000
<b>p-tau<sub>181</sub></b>	KW	H test=8.561, df=2, p=0.014*		
	PH KW	p=0.115	p=0.026*	p=0.161
<b>p-tau<sub>199</sub></b>	KW	H test=8.244, df=2, p=0.016*		
	PH KW	p=0.065	p=0.020*	p=0.347
<b>p-tau<sub>231</sub></b>	KW	H test=9.680, df=2, p=0.008*		
	PH KW	p=0.269	p=0.045*	p=0.038*
<b>VILIP-1</b>	KW	H test=10.203, df=2, p=0.006*		
	PH KW	p=0.050*	p=0.010*	p=0.151
AD, MCI patients				
<b>A<math>\beta</math><sub>1-42</sub></b>	KW	H test=1.789, df=2, p=0.409		
<b>Total tau</b>	KW	H test=8.110, df=2, p=0.017*		
	PH KW	p=0.032*	p=0.014*	p=0.821
<b>p-tau<sub>181</sub></b>	KW	H test=8.826, df=2, p=0.012*		
	PH KW	p=0.135	p=0.027*	p=0.120
<b>p-tau<sub>199</sub></b>	KW	H test=8.686, df=2, p=0.013*		
	PH KW	p=0.076	p=0.019*	p=0.234
<b>p-tau<sub>231</sub></b>	KW	H test=9.485, df=2, p=0.009*		
	PH KW	p=0.296	p=0.048*	p=0.039*
<b>VILIP-1</b>	KW	H test=11.854, df=2, p=0.003*		
	PH KW	p=0.058	p=0.007*	p=0.054
AD patients				
<b>A<math>\beta</math><sub>1-42</sub></b>	KW	H test=1.559, df=2, p=0.459		
<b>Total tau</b>	KW	H test=8.735, df=2, p=0.013*		
	PH KW	p=0.062	p=0.014*	p=0.301
<b>p-tau<sub>181</sub></b>	KW	H test=9.392, df=2, p=0.009*		
	PH KW	p=0.229	p=0.029*	p=0.057
<b>p-tau<sub>199</sub></b>	KW	H test=9.671, df=2, p=0.008*		
	PH KW	p=0.129	p=0.017*	p=0.086
<b>p-tau<sub>231</sub></b>	KW	H test=10.203, df=2, p=0.006*		
	PH KW	p=0.604	p=0.065	p=0.015*
<b>VILIP-1</b>	KW	H test=17.298, df=2, p<0.001*		
	PH KW	p=0.106	p=0.003*	p=0.002*

A $\beta$ <sub>1-42</sub>, amyloid  $\beta$ 1-42 protein; AD, Alzheimer's disease; AD + VaD, mixed dementia; CBS, corticobasal syndrome; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; HC, healthy control; KW, Kruskal-Wallis test; MCI, mild cognitive impairment; PH KW, Kruskal-Wallis post hoc; p-tau<sub>181</sub>, tau protein phosphorylated at threonine 181; p-tau<sub>231</sub>, tau protein phosphorylated at threonine 231; p-tau<sub>199</sub>, tau protein phosphorylated at serine 199; PD, Parkinson's disease; TNF $\alpha$ , tumor necrosis factor alpha; VaD, vascular dementia; VILIP-1, visinin-like protein 1. \*p<0.05.

## Figures

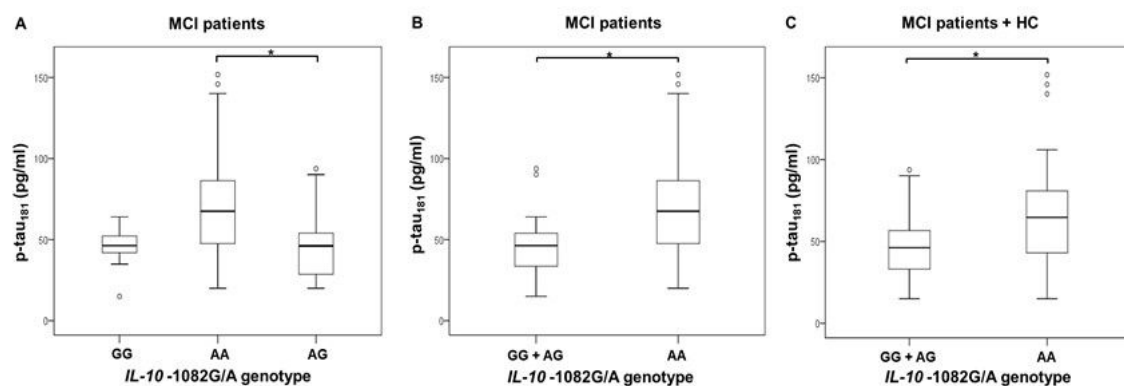


Figure 1

Levels of p-tau<sub>181</sub> in (A-B) MCI patients and (C) MCI patients and HC with different IL-10 -1082G/A (rs1800896) genotypes. \*p<0.05.

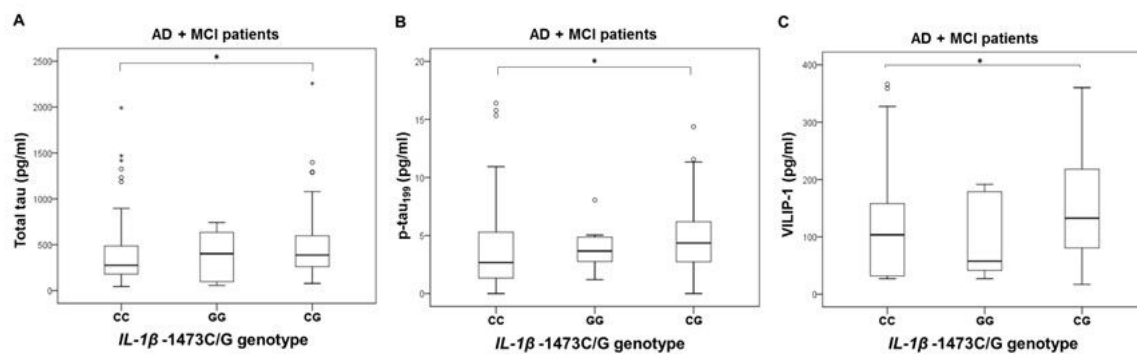


Figure 2

(A) T-tau, (B) p-tau<sub>199</sub> and (C) VILIP-1 levels in AD and MCI patients with different IL-1β -1473C/G (rs1143623) genotypes. \*p<0.05.



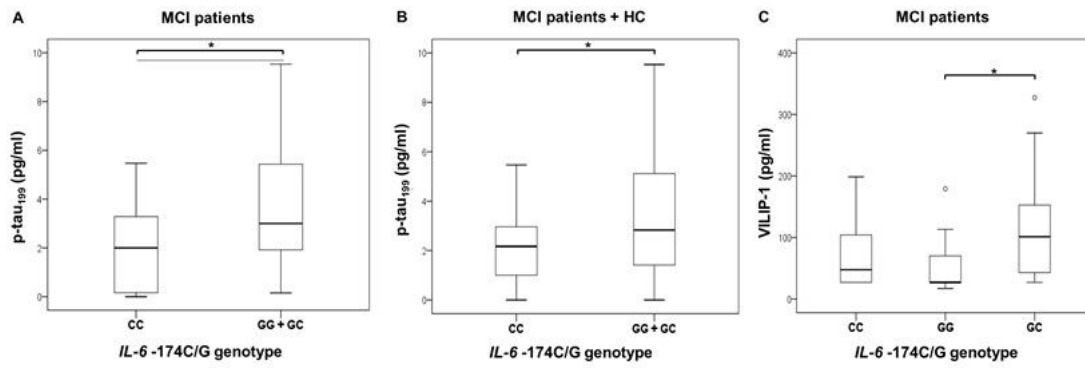


Figure 5

(A-B) P-tau<sub>199</sub> and (C) VILIP-1 levels in subjects with different IL-6 -174C/G (rs1800795) genotypes. \*p<0.05.

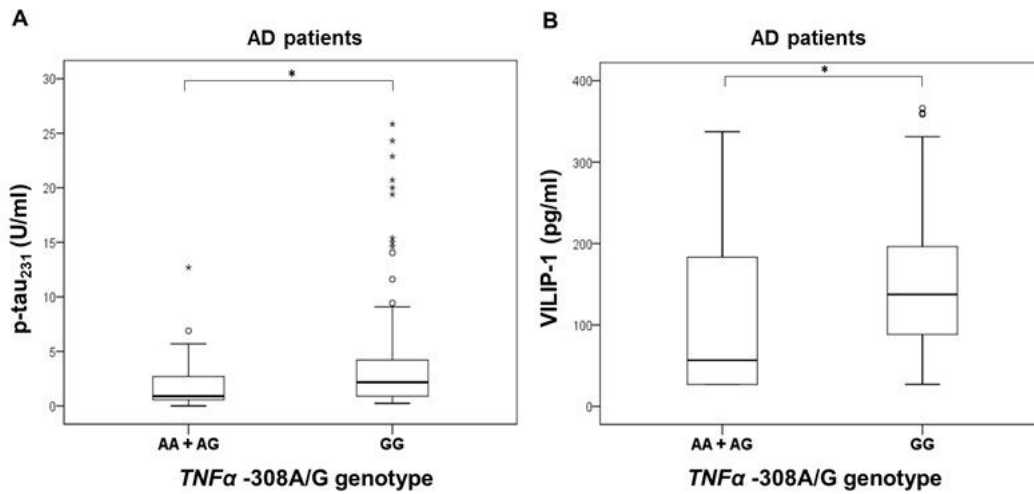
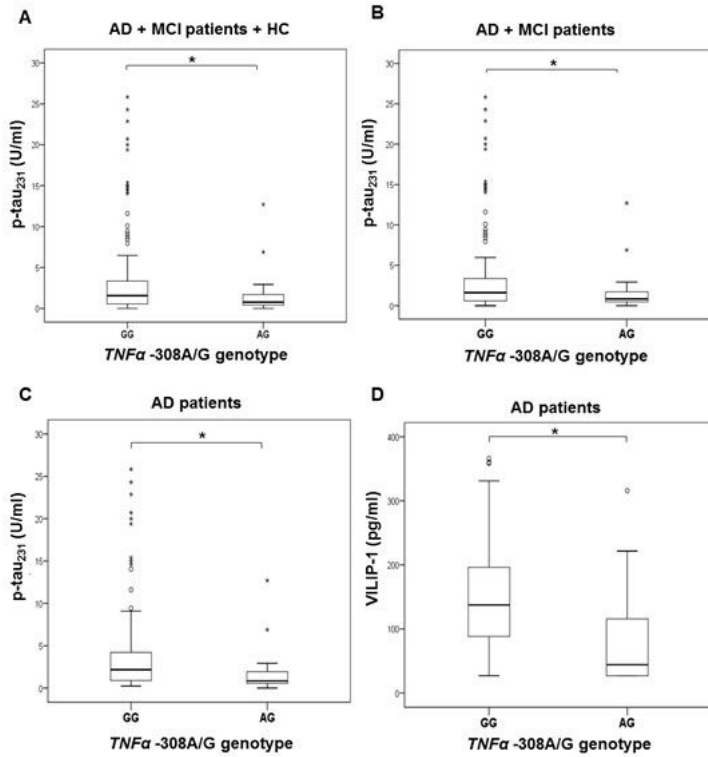


Figure 6

Levels of (A) p-tau<sub>231</sub> and (B) VILIP-1 in AD patients with different TNFα -308A/G (rs1800629) genotypes. \*p<0.05.

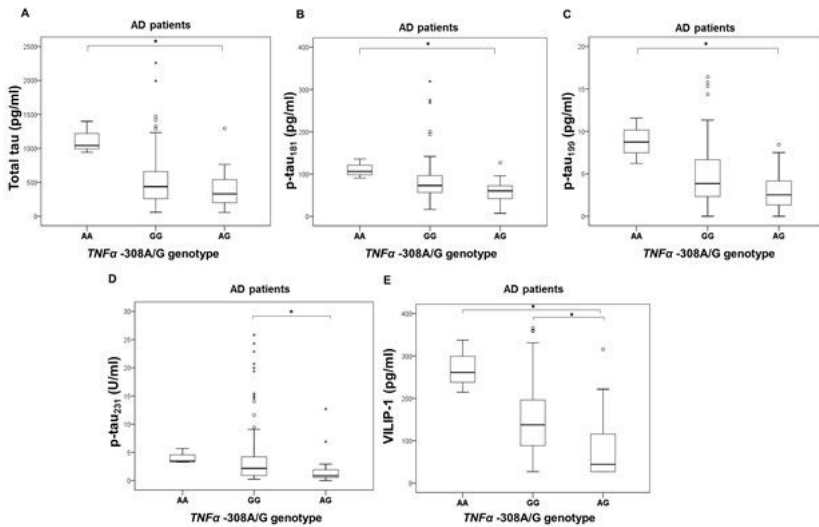




**Figure 7.** Levels of (A-C) p-tau<sub>231</sub> and (D) VILIP-1 in subjects with different *TNFα* -308A/G (rs1800629) genotypes. \*p<0.05.

**Figure 7**

. Levels of (A-C) p-tau<sub>231</sub> and (D) VILIP-1 in subjects with different *TNFα* -308A/G (rs1800629) genotypes. \*p<0.05.



**Figure 8.** Levels of (A) Aβ<sub>1-42</sub>, (B) t-tau, (C) p-tau<sub>181</sub>, (D) p-tau<sub>199</sub>, (E) p-tau<sub>231</sub> and (F) VILIP-1 in AD patients with different *TNFα* -308A/G (rs1800629) genotypes. \*p<0.05.

**Figure 8**

Levels of (A) Aβ1-42, (B) t-tau, (C) p-tau181, (D) p-tau199, (E) p-tau231 and (F) VILIP-1 in AD patients with different TNFα -308A/G (rs1800629) genotypes.  
\*p<0.05.