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Association of interleukin-4 genetic polymorphisms with sporadic Alzheimer's disease in Chinese Han population

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HIGHLIGHTS

- A cohort of controls and patients with Alzheimer's disease was genotyped for IL-4 −590C > T, −33C > T and −1098T > G SNPs.
- The −590C and −1098G alleles of IL-4 are associated with the increased risk for Alzheimer's disease.
- The CCG haplotype occurred at significantly higher frequencies in patients with Alzheimer's disease.
- The −590C > T and −1098T > G SNPs have a linkage disequilibrium with multiple potentially functional SNPs inside *IL-4* gene.
- Genetic variation in IL-4 plays a role in the susceptibility of Alzheimer's disease.

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ABSTRACT

Cytokine interleukin-4 (IL-4) is thought to play a role in the pathogenesis of Alzheimer's disease (AD). This study aimed to evaluate the potential association between single nucleotide polymorphisms (SNP) of *IL-4* gene and AD susceptibility. This case-control study was conducted in Chinese Han populations consisting of 203 AD patients and 205 controls. Three common SNPs of *IL-4* gene, including −590C > T (rs2243250), −33C > T (rs2070874), and −1098T > G (rs2243248), were determined by the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and verified using DNA sequencing methods. Our data show that −590C and −1098G alleles of *IL-4* were more common in AD patients (30.5% vs 22.2% $p = 0.007$; 14.3% vs 3.4% $p < 0.0001$) and significantly associated with elevated risk for AD (OR = 1.51 95% CI 1.05–2.23; OR = 4.78 95% CI 2.37–7.67). Haplotype analysis revealed five common haplotypes CCG (OR = 4.41), CCT (OR = 1.22), TTT (OR = 1.02), CTT (OR = 0.7), and TCT (OR = 0.14), from highest to lowest risk for AD. None of the associations appeared to be modified by APOE ε4 genetic variant. Bioinformatic analysis shows that −590C > T and −1098T > G have a linkage disequilibrium (LD) with multiple potentially functional SNPs inside *IL-4* gene. Our findings indicate that the −590C and −1098G alleles located in the promoter of *IL-4* may increase the susceptibility to AD among the Han Chinese and might be used as molecular markers for AD risk evaluation.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly characterized by neuronal loss, atrophy, gliosis, and clinically by progressive cognitive impairment, however, its precise etiology still remains enigmatic. Accumulating evidence indicates that inflammation plays a pivotal role in the pathogenesis of AD [1]. Inflammatory manifestations, such as microglial activation, reactive astrocytosis, over-expression of inflammatory mediators, are prominent pathological hallmark of AD in brain [3]. In this regard, cytokines seem critical in the pathobiology of AD as perturbations of their levels in brain, blood and cerebrospinal fluid were iteratively reported and cytokine modulating therapies can improve AD pathology in experimental models [30].

Interleukin (IL)-4 is one of the anti-inflammatory cytokines produced by mature T type 2 helper cells (Th2), mast cells or basophils, which is able to drive Th2 responses, mediates the recruitment

Abbreviations: IL, Interleukin; SNP, single nucleotide polymorphisms; AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association; LD, linkage disequilibrium.

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and activation of mast cells, and stimulates the production of IgE antibodies [16]. Previous studies have indicated an involvement of IL-4 in the pathogenesis of AD [5]. IL-4 and its receptors have been found to be present in the human brain [17,32] and IL-4 production were reduced in basal and mitogen-stimulated PBMC from AD patients compared with that from control subjects [21]. IL-4 has also been reported to decrease amyloid-β (Aβ) induced production of the inflammatory cytokines, IL-1 and IL-6 [32] and inhibit the expression of mouse formyl peptide receptor 2, a receptor for Aβ, in TNF-α-activated microglia [9]. In addition, Nakayama et al. demonstrated that IL-4 induced the clearance of oligomeric Aβ by primary rat microglial cells via increased expression of scavenger receptor CD36 and the Aβ-degrading enzymes neprilysin (NEP) [29], and they further showed that *in vivo* injection of IL-4/IL-13 mixture reduced the Aβ levels in the brain and improved the cognitive capacity in amyloid precursor protein (APP23) transgenic mice [12]. In fact, IL-4-based treatment and immunization have been reported to reverse certain aspects of AD pathology and elicit a neuroprotective effect in animal models [4,12,13,36]. Several studies found that acetylcholinesterase inhibitor (AChEI) treatment ameliorates clinical symptoms of patients with AD via increased IL-4 expression [6,15,22,23], supporting a pivotal immunomodulatory effect of IL-4 in AD.

The gene for *IL-4* has been mapped to the q arm (q23-31) of chromosome 5 [5]. Multiple single nucleotide polymorphisms (SNPs) within the *IL-4* gene have been reported, including −590C > T (rs2243250), −33C > T (rs2070874), −1098T > G (rs2243248G > T), +2979T > G (rs2227284), +3437C > G (rs2227282) and the variable number of tandem repeat (VNTR) region in intron 3 [31,33,37]. SNPs in the *IL-4* gene may alter its expression and its downstream signaling, which may contribute to an individual's susceptibility to inflammation-related diseases [5]. Therefore, it was hypothesized that SNPs in the *IL-4* gene could be functional and be associated with AD development. In fact, although Shibata et al. found no association between −33C > T variant of *IL-4* and risk for AD in Japanese population [28], the study from Ribizzi et al. showed that −590C > T and −1098T > G polymorphisms of *IL-4* were significantly correlated with AD susceptibility in a small cohort of Caucasian with a significantly higher percentage of C allele and T allele for these two SNPs appeared in AD patients (19 cases) as compared with controls (20 cases) [25].

In view of the potential discrepancy of SNPs in different ethnic groups, the purpose of this study was to confirm whether particular alleles, genotypes of the *IL-4* gene would modify the occurrence of AD in Chinese Han population. In this study, we examined a group of 203 AD patients and compared their IL-4 polymorphisms with a healthy control population sample (205 cases).

2. Material and methods

2.1. Study subjects

We studied a total sample of 408 subjects in a Chinese Han population from Jiangsu Province, China. The AD group consisted of 203 patients (96 females and 107 males; mean age 79.8 ± 5.6 years; mean age at onset 70.3 ± 7.6 years), consecutively examined in Nanjing Medical University Affiliated to Nanjing Brain Hospital, China between January 2011 and January 2013. Clinical diagnosis of probable AD required that the patient has performed magnetic resonance imaging (MRI) or a computed tomographic (CT) scan consistent with a primary diagnosis of AD. All participants met the NINCDS-ADRDA criteria for probable AD; in addition, all met the diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV) criteria for AD. The mini-mental state examination (MMSE) was performed in all participants as a global bedside measure of their cognitive function. None of these patients reported a family history of AD. Patients were excluded if they had any of the following: active infection, multiple sclerosis (or any other demyelinating disorder), vascular dementia, clinically significant neurological disease other than AD or a score greater than four on the modified Hachinski Ischemic Rating Scale, uncontrolled diabetes mellitus, tuberculosis, history of lymphoma, or congestive heart failure.

A total of 205 subjects were included as controls (97 females and 108 males). The control subjects were not related to each other or to the patients with AD; in particular, we selected only those subjects who were the same age as AD patients (mean age 80.2 ± 6.8 years), with no known diagnosis of dementia or other chronic neurological diseases or psychiatric syndromes with cognitive impairment, cerebrovascular disease, nephropathy or end-stage renal disease, and no severe functional limitations.

Written informed consent was obtained from all study participants according to the Declaration of Helsinki, and the study protocol was approved by the Research Ethics Committees of the Nanjing Medical University Affiliated to Nanjing Brain Hospital.

2.2. Genotype determination

Genomic DNA was extracted from peripheral blood leukocytes in 10 ml of peripheral blood using a commercially available QIAamp DNA Mini Kit (QIAGEN, Beijing, China). Three SNPs of the *IL-4* gene (−590C > T, −33C > T and −1098T > G) were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Supplementary Table 1). APOE genotypes were determined using the restriction enzyme digestion approach as described previously [27].

See Supp Table 1 as supplementary file. Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2014.01.019.

2.3. Bioinformatic analysis of associated SNPs

We used HaploReg (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) to investigate the bioinformatics on associated SNPs [35]. The tool provides information on SNPs that are in LD with the queried SNP (r2 was set to 0.8 for these analyses and the population chosen for analyses was of Asian descent) and also gives information on which of these SNPs lie in promoter histone marks, enhancer histone marks, DNase sites and protein binding regions. It can also inform on the conservation of the relevant sequence and any changes to regulatory motifs based on the SNP allele changes. Each of the three genotyped SNPs were entered into HaploReg and analysis was completed with r2 = 0.8.

2.4. Statistical analyses

We performed power calculations for the genetic association analyses using online genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html) [20]. Simulation analysis yielded more than 80% power to achieve ~1.3–1.5 risk effect at $p = 0.05$, indicating that our case-control sample size was sufficient to find moderate genetic association with AD.

The frequencies of allele and genotype in AD patients and controls were calculated by gene-counting method. Differences in allelic and genotypic distributions between AD patients and controls were assessed by chi-square ($\chi 2$) test. Genotype and allele frequencies for each SNP were also stratified by the presence of the APOE ε4 allele. The results were adjusted for age, gender and APOE ε4 status using multiple logistic regression models. The Hardy–Weinberg equilibrium (HWE) was checked for AD patients and controls with the $\chi 2$ test. The above statistical analyses were