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Interleukin-23 receptor polymorphisms are associated with Alzheimer's disease in Han Chinese

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ABSTRACT

Inhibition of interleukin-23 (IL-23) signaling was reported to reduce AD pathology, and IL-23 receptor gene (IL23R) which encodes IL-23 receptor may represent a candidate susceptibility gene for AD. Here, we conducted a case–control association study to assess the effect of IL23R genetic polymorphisms on the risk of AD in a Northern Han Chinese population. Two tag functional single polymorphisms (SNPs), rs10889677 and rs1884444 were selected, and their associations with AD risk factors were assessed in 1133 AD patients and 1156 matched controls. Our association analysis showed that C allele of rs10889677 was significantly associated with decreased AD risk even after adjusting for age, gender, and apolipoprotein E gene (APOE) ε4 status. The G allele of rs1884444 polymorphism is significantly associated with a higher risk of AD in APOE ε4 carriers. Our results demonstrate that IL23R genetic polymorphisms are associated with AD in a Northern Han Chinese population.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, typically presenting with a progressive loss of cognitive function and memory. It is neuropathologically characterized by the formation of extracellular senile plaques containing the amyloid-β (Aβ) within the parenchyma of the brain (Wilson et al., 2002). Considerable evidence identifies that the innate immune response and neuroinflammation may play critical roles in the pathological process of AD (Eikelenboom et al., 2006; Yu et al., 2011; Tan et al., 2013). Besides, some epidemiological evidence of a potential protective effect of nonsteroidal anti-inflammatory drugs (NSAID) for AD further indicates the harmful effect of the inflammatory response in AD (Townsend and Pratico, 2005).

Interleukin-23 (IL-23) is a member of the IL-12 family; it is a heterodimeric proinflammatory cytokine composed of a p19 subunit and a p40 subunit that is shared with IL-12. Vom Berg and colleagues identified that the IL-23a mRNA was up-regulated in microglia of Alzheimer's disease-prone mice (Vom Berg et al., 2012), suggesting a possible involvement of these mediators in the pathology of AD. They found that the genetic ablation of the IL-12/IL-23 signaling molecules p40, p35 or p19 resulted in the decreased cerebral amyloid burden in Alzheimer's disease-prone mice which lack IL-12/IL-23 subunits. Besides, our group also identified decreased cerebral amyloid-beta levels in p40-deficient mice (Tan et al., 2014b). All these clues may indicate the potential role of IL-23 cytokines in Alzheimer's disease. Normally, IL-23 typically participates in cell-mediated immunity by binding to the IL-23 receptor (IL23R) complex and mediates its effects by signaling through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) and NF-κB pathways (Parham et al., 2002; Cho et al., 2006). *IL23R* gene encodes a subunit of the receptor for IL23a/IL23, which is composed of interleukin-12 receptor β1 (IL-12Rβ1) shared with IL-12 receptor (IL-12R) (Parham et al., 2002). Meanwhile, a robust reduction in amyloid burden was reported to be associated with a decrease in the expression of IL12Rβ1 receptor by T cell after vaccination with Aβ 1–42, as part of a reduction in Th1 immunity after Aβ-peptide vaccination (Town et al., 2002). Thus, the IL-23 receptor has the potential to influence the amyloid burden and the pathology of AD.

Based on the above data, and in view of the important role of immunology in the pathogenesis of AD, we hypothesized that interleukin-23 receptor gene (*IL23R*) which encodes IL-23 receptor may represent a candidate susceptibility gene for AD. Here, we conducted a case–control association study to assess the effect of *IL23R* genetic polymorphisms on the risk of AD in a large Northern Han Chinese population.

Abbreviations: AD, Alzheimer's disease; IL, Interleukin; SNP, single-nucleotide polymorphism; ApoE, apolipoprotein E; LOAD, late-onset Alzheimer's disease; Aβ, amyloid-β; MMSE, Mini Mental State Examination; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

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2. Materials and methods

2.1. Subjects

We investigated 2289 subjects comprising 1133 sporadic AD patients (women 669, mean age onset: 75.01 ± 7.99, range 65–108) and 1156 healthy control subjects (women 641, mean age at examination: 74.47 ± 6.29, range 65–98) matched for gender and age. All the subjects were Northern Han Chinese in origin. Our case group was enrolled from the Department of Neurology at Qingdao Municipal Hospital, the Affiliated Hospital of the Medical College of Qingdao University, Qingdao Hiser Hospital, Qingdao Central Hospital, and Qingdao Mental Health Center. All patients in case group were clinically diagnosed as "probable AD" according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) (McKhann et al., 1984). No AD patients were reported with a family history of neurodegenerative disorders or other dementias. The age of onset was estimated from the time of the first symptoms of AD and determined from caregivers. All individuals of control group were collected from the Health Examination Center of each collaborating hospitals and they were all confirmed healthy and neurologically normal after neurological and medical examinations. No participants were reported with tumor or autoimmune diseases. Demographic details of the sample set are shown in Table 1. Informed consent to participate in this study was obtained from each subject or from a guardian and this study protocol was approved by the Ethical Committee of Qingdao Municipal Hospital (2009-05-06-003).

2.2. SNP selecting and genotyping

Initially, we consulted the Han Chinese from Beijing (CHB) genotype data in HapMap database (release#27, February 2009, http://www.hapmap.org/) covering the *IL23R* gene and selected SNPs within IL23R with minor allele frequencies (MAF) >0.1. After evaluating by Haploview 4.2, we found these SNPs in *IL23R* were located in 2 haplotype blocks. We further selected one functional SNP [rs10889677, located at the 3′-untranslated region (3′-UTR); rs1884444, located at codon 3 of exon2] which conform to the criteria that minor allele frequencies (MAF > 0.1) and pairwise tagging ($r^2 > 0.80$) from each block (Fig. 1). Our selection is in accord with another study which was performed under the same criteria in CHB (Wang et al., 2012). Thus, we chose rs10889677 and rs1884444 for the subsequent genotyping in the full cohort. Genomic DNA was extracted from venous blood of patients and healthy individuals using the Wizard genomic DNA purification kit (Catalog no. A1125, Promega, USA). Genotyping of *IL23R* (rs10889677, rs1884444) polymorphisms was determined by polymerase chain reaction–ligase detection reaction (PCR–LDR) (TaqMan Assay) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA) (Favis et al., 2000; Xiao et al., 2006), with technical support from the Shanghai Genesky Biotechnology Company. PCR and extension primers (rs10889677, F: TCTGTGCTCCTACCATCACCATGT, R: CATGTTCCACCTTC GGGACCTT; rs1884444, F: TCCCTAATCAAAGGTTCCCATCAA, R: CCTCCA TGACACCAGCYGAAGA) were designed using the Sequenom Mass ARRAY assay-design software. Randomly selected DNA samples from each genotype were analyzed in duplicate using the ligation detection reaction method. Two allele specific probes and one fluorescently labeled probe were designed for the LDR reaction (Table 2). Results of the ligation detection reaction method corresponded with the results of sequencing.

2.3. Statistical analysis

Hardy–Weinberg equilibrium (HWE) version 1.20 (Columbia University, New York, NY) was used to exclude deviations from HWE in both patients and control subjects. Differences in allele and genotype frequencies between patients with AD and control subjects were assessed using the Pearson χ^2 test or Fisher's exact test. The relative risk associated with rare alleles, genotypes and haplotypes was estimated as an odds ratio (OR) with 95% confidence intervals (CI), calculated by logistic regression after adjustment for age of onset (age at examination for control subjects), gender, and *APOE* ε4 status. The two SNPs (minor allele homozygote counts of no less than 14) were tested as dominant, additive, and recessive coding in separate logistic regression models; the optimal genetic model for the two SNPs were selected using the Schwarz–Bayesian information criterion with the respective minimum Wald test *P* value (Lettre et al., 2007). All the statistical analysis was implemented using SPSS 13.0 software. A Bonferroni-corrected *P* value of 0.05 (based on the number of SNPs analyzed) was considered significant. The population allowed the study to achieve more than 90% power to detect a common variant with modest risk (Genetic Power Calculator, King's College London, De Crespigny Park, UK) (Purcell et al., 2003). Power calculations at significance level of 0.05 were also assessed with STPLAN 4.3 software based on the allele frequency observed in the current study and a predicted OR (OR = 1.5) for both SNP (power > 0.90 separately). We used Haploview 4.2 to calculate the linkage disequilibrium (LD). To obtain a measure of significance that was corrected for multiple testing bias, we ran 10,000 permutations to compute *P* values using Haploview 4.2 program. All statistical tests were two-sided, and the statistical significance was defined as $P < 0.05$.

3. Results

3.1. Characteristics of the study groups

We studied 2289 ethnic northern Han Chinese subjects including a total of 1133 subjects (49.5%) with probable AD. No statistically

Table 1
Characteristics of the study groups.

Characteristic	Patients with AD (n = 1133)	Control subjects (n = 1156)	*P* value
Age[a], year; mean ± SD (age range)	75.01 ± 7.99 (65–108)	74.47 ± 6.29 (65–98)	0.073
Sex, n (%)			
Male	464 (41.0)	515 (44.6)	0.082
Female	669 (59.0)	641 (55.4)	
MMSE score, mean ± SD	10.06 ± 3.82	28.26 ± 1.08	<0.001
APOE genotypes, n (%)			
4 carrier	315 (27.8)	158 (13.7)	<0.001
4 non-carrier	818 (72.2)	998 (86.3)	

Abbreviations: AD, Alzheimer's disease; OR, odd ratio; CI, confidence interval; SD, standard deviation; MMSE, Mini-Mental State Examination.

[a] Age of the patients with AD was the age of onset; age of control group was the age at examination. Differences in the characteristics of age and MMSE score between the two groups were examined using the Student's *t* test. Differences in sex and genotype frequencies of apolipoprotein E (*APOE*) between AD cases and control subjects were assessed using the Pearson Chi-square test. Distribution of the *APOE* polymorphisms was in Hardy–Weinberg equilibrium in both cases and control subjects.