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Association analysis of NAD(P)H:quinone oxidoreductase gene 609 C/T polymorphism with Alzheimer's disease☆

Binbin Wang[a,f], Feng Jin[a,b], Yanchen Xie[c], Ying Tang[a,f], Rui Kan[a,f], Chenguang Zheng[e], Ze Yang[d], Li Wang[a,*]

[a] *Center for Human and Animal Genetics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*
[b] *Research Laboratory for Behavior Biology, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China*
[c] *Department of Neurology, Beijing Friendship Hospital, Affiliate of Capital University of Medical Sciences, Beijing 100050, China*
[d] *Laboratory for Medical Genetics, Institute of Geriatrics, Beijing Hospital, Ministry of Health, Beijing 100730, China*
[e] *Jiangbin Hospital, Nanning, Guangxi 530021, China*
[f] *Graduate School of the Chinese Academy of Sciences, Beijing 100101, China*

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Abstract

Alterations of the NAD(P)H:quinone oxidoreductase (NQO1) activity are associated with Alzheimer's disease (AD). A polymorphism consisting of a single nucleotide (C → T) change at position 609 of *NQO1* influences the NQO1 activity. Therefore the *NQO1* C609T polymorphism may confer susceptibility for AD developing. To test the hypothesis, we have performed an association study between the *NQO1* gene polymorphism C609T and late-onset Alzheimer's disease (LOAD) in Chinese population. Totally 104 LOAD patients and 128 controls were enrolled in our data set. All subjects were genotyped for *NQO1* and Apolipoprotein E (*APOE*). There were no significant differences in *NQO1* genotype or allele frequencies between cases and controls. Likewise, with the stratification of *APOE* ε4 status, no statistical difference was observed between cases and controls. Our findings suggested that this polymorphism might not represent additional genetic risk factor for LOAD. However, the present study cannot exclude *NQO1* as a possible candidate for LOAD. Further study in a larger population and biological functional analysis of *NQO1* gene is required to verify the role of *NQO1* in LOAD.

Keywords: Late-onset Alzheimer's disease; *APOE* ε4; NQO1; Case-control study; Chinese

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to dementia. AD is characterized by accumulation of neurofibrillary tangles and amyloid deposition resulting in the formation of senile plaques in the brain [9,21]. While the early-onset, autosomal dominant AD is relatively well characterized, our understanding of the more common late-onset forms of the disorder (LOAD) remains much less complete [13]. The only confirmed genetic risk factor for LOAD is apolipoprotein E allele 4 (*APOE* ε4), but it is neither necessary nor sufficient for LOAD [2,18]. This has prompted a search for other genetic factors that may confer susceptibility to LOAD.

Several studies have suggested a role of oxidative stress in the toxicity induced by A-beta in AD [12]. The A-beta toxicity might be due to the formation of reactive oxygen species (ROS), which can cause lipid and protein oxidation in brain [4]. As a result, the antioxidant enzymes that can prevent the formation of ROS may be involved in the progression of AD. NAD(P)H:quinone oxidoreductase (NQO1), as DT-diaphorose, is such an enzyme. NQO1 catalyzes the reduction of two-electron of quinones, preventing their participation in redox cycle and subsequent generation of ROS [3,5]. More directly, NQO1 activity has been found increased in hippocampal pyramidal neurons of AD patients [22]. Therefore the polymorphism of *NQO1* gene, which can influence the NQO1 activity, may confer susceptibility for AD developing. There is such a polymorphism consisting of a single nucleotide (C → T) change at position 609 of *NQO1* cDNA. The transition leads to the substitution of serine for proline in the mature protein [20]. NQO1 activity is not detected in cells due to a lack of protein in the TT genotype [19]. As a result,

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* Corresponding author. Tel.: +86 10 6486 5569; fax: +86 10 8272 7652.
E-mail address: lwang@genetics.ac.cn (L. Wang).

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Table 1
Genotype and allele frequencies of *NQO1* gene polymorphism C609T in LOAD patients and controls

Group	No.	Genotype frequency (%)			Allele frequency (%)	
		C/C	C/T	T/T	C	T
LOAD	104	27 (26.0)	53 (51.0)	24 (23.1)	107 (51.4)	101 (48.6)
Controls	128	31 (24.2)	70 (54.7)	27 (21.1)	132 (51.6)	124 (48.4)
		$\chi^2 = 0.32$, d.f. = 2, $P = 0.85$			$\chi^2 = 0.0007$, d.f. = 1, $P = 0.979$	

NQO1 T cannot detoxify the A-beta. To investigate whether the *NQO1* polymorphism was associated with LOAD and their possible synergic effect with the *APOE* ε4 on the risk of LOAD, in the present study, we examined the *NQO1* and *APOE* genotypes in a Chinese sample.

Totally 104 sporadic LOAD patients (mean age 79.2 ± 6.3; range 64–97 years; 46% female) and 128 unrelated healthy controls (mean age 68.1 ± 2.8; range 65–77 years; 30% female) were recruited from Chinese population in Guangxi, China. Individuals affected with LOAD were diagnosed following the DSM-III-R criteria [1] and clinically examined based on the NINCDS-ADRDA criteria to exclude vascular dementia [11]. All LOAD patients were measured through MRI [23]. Healthy controls were selected by the assessment of a full medical history and a physical examination. Cognitive function was assessed using the Mini Mental State Examination (score ≥ 27).

Genomic DNA was extracted from peripheral blood leukocytes using standard method. The genotypes for *APOE* were determined as previously described [6]. The sequences of primers were 5′-AACAACTGACCCCGGTGGCG-3′ as the upstream primer and 5′-ATGGCGCTGAGGCCGCGCTC-3′ as downstream one, respectively. The PCR product was digested with *Hha*I. The *APOE* ε2 allele and ε4 allele corresponded to 83 bp fragment and 72 bp fragment, respectively, while the ε3 allele was characterized by two fragments of 91 bp and 48 bp.

The *NQO1* gene C609T polymorphism was genotyped using the method as reported elsewhere with minor modification [15]. The region containing the C609T polymorphism was amplified by polymerase chain reaction (PCR) using the following primer set: 5′-CCTCTCTGTGCTTTCTGTATCCT-3′ and 5′-GGTGTCTCATCCCAAATATTCTC-3′. The PCR reaction generated a fragment of 166 bp, which contains the nucleotide 609. The C609T substitution creates a *Hinf*I recognition sequence with resulting 103 bp and 63 bp fragments.

Allele frequencies and genotype distribution of LOAD patients and controls were determined by allele-counting method. The association of *NQO1* polymorphism C609T with AD was investigated by Pearson chi-square test with odds ratios (OR). Logistic regression analysis was performed to examine the possible interaction between *NQO1* and *APOE* polymorphisms on the risk for AD using Statistic Package for the Social Science (SPSS). The statistical significance was set at $P < 0.05$.

As expected, the distribution of *APOE* genotypes was significantly different between LOAD subjects and controls ($\chi^2 = 12.99$, d.f. = 5, $P = 0.023$). The *APOE* ε4 allele frequency in patients with LOAD was significantly higher than that in controls ($\chi^2 = 9.80$, d.f. = 1, $P = 0.0017$).

The allele and genotype frequencies of the *NQO1* polymorphism C609T in LOAD subjects and controls were shown in Table 1. The genotype distributions were in Hardy–Weinberg equilibrium in both AD patients and controls (data not shown). There was no statistical difference in *NQO1* genotype and allele frequencies between AD cases and controls ($\chi^2 = 0.32$, d.f. = 2, $P = 0.85$ by genotype; $\chi^2 = 0.0007$, d.f. = 1, $P = 0.979$ by allele). Compared with controls, there was a higher T/T genotype frequency in the LOAD cases (23.1% versus 21.1%), but the association to LOAD did not reach significance ($\chi^2 = 0.13$, d.f. = 1, $P = 0.72$). Taking into consideration the studies in other populations, the prevalence of TT genotype in Hmong population (34%) and other Asian populations (Chinese, 21.1%; Japanese, 17.0%; Korean, 18.8%) is obviously higher than that in African-American (5.2%) and Caucasians (3.5%). This suggested an ethnic difference [7,8,14].

Logistic regression analysis revealed no effect of the interaction between *NQO1* TT genotype and *APOE* ε4 on the risk for LOAD ($\chi^2 = 2.13$, d.f. = 1, $P = 0.14$, OR = 0.43, 95% CI 0.14–1.34). When stratifying all samples by the *APOE* ε4 status, genotypic and allelic distributions of *NQO1* gene in subgroups

Table 2
Genotype and allele distributions of *NQO1* gene polymorphism C609T in *APOE* ε4 carriers and non-carriers

Group	No.	Genotype frequency (%)			Allele frequency (%)	
		C/C	C/T	T/T	C	T
APOE ε4 carriers						
LOAD	28	6 (21.4)	14 (50.0)	8 (28.6)	26 (46.4)	30 (53.6)
Controls	14	3 (21.4)	7 (50.0)	4 (28.6)	13 (46.4)	15 (53.6)
		$\chi^2 = 0.00$, d.f. = 2, $P = 1.00$			$\chi^2 = 0.00$, d.f. = 2, $P = 1.00$	
APOE ε4 non-carriers						
LOAD	76	21 (27.6)	39 (51.3)	16 (21.1)	81 (53.3)	71 (46.7)
Controls	114	28 (24.6)	63 (55.3)	23 (20.2)	119 (52.2)	109 (47.8)
		$\chi^2 = 0.32$, d.f. = 2, $P = 0.85$			$\chi^2 = 0.044$, d.f. = 1, $P = 0.834$	

were shown in Table 2. No obvious differences of genotype or allele frequencies between patients and controls were observed in either group of *APOE* ε4 carriers or non-*APOE* ε4 carriers (shown in Table 2).

Increasing evidence suggested that the oxidative stress played a role for AD developing [10]. As an antioxidant enzyme, NQO1 activity was found increased in astrocytes and neurons of the hippocampus of AD patients [16,22]. Furthermore, the NQO1 activity co-localizes closely with AD pathology [17]. These evidences showed that the NQO1 activity has a potential neuroprotective role in AD.

Although *NQO1* may represent a good candidate, our study failed to establish a statistically significant association between the previously described functional C/T polymorphism in the *NQO1* gene and AD developing, nor did we detect any association after stratification of the patient sample according to *APOE* genotype.

However, *NQO1* cannot be excluded as a possible candidate for AD too. There may be several reasons for that. Firstly, there may be other genetic variations which are not in linkage disequilibrium with C609T polymorphism and still can be associated with the disease. Secondly, the investigated sample of our study is small and not completely matched for sex and age, which may lead false negative results. Finally, it is worth noting that more than one study have confirmed the association between NQO1 activity and AD [16,17,22].

In summary, our results suggest that the C609T polymorphism may not play a major role in AD pathogenesis in Chinese population. To confirm our findings, this analysis should be repeated in a larger and matched sample, especially in other ethnic populations. And more studies on the relationship between other polymorphisms/mutations in the gene and AD are required to understand the substantial role of *NQO1* gene in the etiology of AD.

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