No Genetic Association between Polymorphisms of Heme Oxygenase 1 and 2 and Alzheimer's Disease in a Japanese Population

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Key Words

Alzheimer's disease · Apolipoprotein E · Heme oxygenases · Polymorphisms

Abstract

Background/Aims: Recently, it has been shown that the heme oxygenases HMOX1 and HMOX2 might play a role in the pathogenesis of Alzheimer's disease (AD). **Methods:** To investigate whether there is any association between the HMOX1 and HMOX2 genes and AD, five single nucleotide polymorphisms (SNPs) in each gene were genotyped in 180 AD patients and 132 age-matched controls using TaqMan technology. **Results:** Our study failed to detect any association between the SNPs of the HMOX1 and HMOX2 genes and AD. In addition, we did not observe any synergetic association between the SNPs studied and apolipoprotein E in our AD patients. **Conclusion:** Further genetic studies are needed to clarify the relationship between the two genes and AD.

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease causing dementia. The main pathological feature of AD is the formation of senile plaques

containing aggregated amyloid β peptide. Three genes have been identified as causative genes in familial AD, the amyloid precursor protein (APP), presenilin-1 and presenilin-2. In addition, the apolipoprotein E (ApoE) ϵ 4 allele is the only recognized genetic risk factor for familial as well as sporadic AD. Although many studies have reported associations between genes involved in cholesterol metabolism and AD, the functional connection between the cholesterol pathway and AD pathology has not been clearly resolved.

Several studies have reported that changes in heme metabolism and oxidative stress may be involved in the pathogenesis of AD [1, 2]. Previously, it has also been shown that the accumulation of iron in neurons is associated with neuritic plaques in AD. Furthermore, iron might affect plaque formation through its effect on APP processing by modifying the function of α -secretase [3–6]. Polymorphisms in several iron-related genes have been reported to increase the risk of sporadic AD [7]. Mutations in the PLA2G6 gene, which encodes phospholipase A2, were found in AD patients with high levels of brain iron [8]. Two polymorphisms, C282Y and H63D, of the hemochromatosis (HFE) gene, as well as an increase in the C2 variant of the transferrin gene have also been implicated in AD development [9–15].

The heme oxygenases HMOX1 and HMOX2 play important roles as antioxidant enzymes and are involved in heme catabolism [16]. Moreover, several studies have

Table 1. Distribution of HMOX1 polymorphisms in AD and control groups

All cases	HMOX1		AD	Con- trols	χ^2	p value
rs2071747	exon 1 (H/D)	C/C	1	2	1.37	0.50
		C/G	32	19		
		G/G	145	111		
rs2071748	intron 1	A/A	37	34	1.60	0.45
		A/G	102	66		
		G/G	41	32		
rs2071749	intron 3	A/A	12	17	3.49	0.17
		A/G	91	62		
		G/G	77	53		
rs5755720	intron 4	A/A	47	39	6.63	0.08
		A/G	98	62		
		G/G	30	31		
rs5995098	intron 4	C/C	48	42	3.69	0.16
		C/G	100	59		
		G/G	32	31		

shown a synergetic association between HMOX1 and APP [17–19], while HMOX2 has been reported to play an active role in neuroprotection [20].

Although one study of a microsatellite marker within the HMOX1 gene promoter region failed to find any association with AD [21], polymorphisms in the HMOX1 and HMOX2 genes have not been fully studied in AD patients. We therefore investigated the possible relationship between polymorphisms of the HMOX1 and HMOX2 genes and sporadic AD.

Materials and Methods

DNA was extracted from white blood cells using a standard method. Sporadic Japanese AD cases ($n = 180$, male:female ratio = 79:101) were recruited from the in-/outpatients of our hospital. The mean age ($\pm$ SD) at the time of examination of the AD group (67.4 ± 6.2 years) was not significantly different from that of the control group (64.4 ± 6.7 years). All the AD cases were diagnosed according to the NINCDS-ADRDA criteria and none had a familial history of AD. The control cases ($n = 132$, male:female ratio = 63:69) were obtained from healthy volunteers with no history of dementia or any other neuropsychiatric disease. We could not confirm whether these individuals have any family history of dementia. The purpose and significance of the study were explained in detail to each patient and his/her family, and all subjects provided their informed consent. The study protocol was

Table 2. Distribution of HMOX2 polymorphisms in AD and control groups

All cases	HMOX2		AD	Con- trols	χ^2	p value
rs11639998	intron 1	C/C	86	67	0.39	0.94
		C/G	74	50		
		G/G	19	14		
rs11643057	intron 1	C/C	21	14	2.04	0.56
		C/T	68	48		
		T/T	86	69		
rs1362626	intron 1	C/C	19	12	0.93	0.82
		C/G	75	50		
		G/G	83	67		
rs11076834	intron 1	C/C	87	69	1.09	0.78
		C/G	65	47		
		G/G	23	14		
rs1051308	3' UTR	A/A	91	71	3.40	0.33
		A/G	66	45		
		G/G	19	16		

approved by the Ethics Committee of the Juntendo University School of Medicine.

Information on the single nucleotide polymorphisms (SNPs) was obtained from the SNP database established by the National Center for Biotechnology Information. Five SNPs of the HMOX1 gene and five SNPs of the HMOX2 gene were genotyped using TaqMan technology on an ABI7500 system (Applied Biosystems, Foster City, Calif., USA). All probes and primers were designed by the Assay-by-Design™ service of Applied Biosystems. The standard polymerase chain reaction was carried out using the TaqMan Universal PCR Master Mix reagent kit in a 10- μ l volume. For all the samples, ApoE genotypes were determined as described previously [22].

Hardy-Weinberg equilibrium tests were carried out for all SNPs for both cases and controls. Statistical analyses of the AD cases divided into the subgroups, ApoE4 positive and negative, were also carried out. The genotypic distribution of the AD groups and their respective controls were compared using Fisher's exact probability test ($p < 0.05$ was considered statistically significant). Linkage disequilibrium between the SNPs was analyzed using the estimated haplotype program.

Results

Our sample set has the power to detect an odds ratio of at least 1.90, assuming a significance level of 0.05, power of 0.80 and an exposure frequency of 0.30 in controls. Five SNPs of the HMOX1 gene and five SNPs of the HMOX2

Table 3. Frequencies of the polymorphisms of the HMOX1 and the HMOX2 genes stratified according to ApoE status

			ApoE4 positive				ApoE4 negative			
			AD	controls	χ^2	p value	AD	controls	χ^2	p value
HMOX1										
rs2071747	exon 1 (H/D)	C/C	0	0	0.19	0.66	1	2	2.55	0.28
		C/G	11	6			21	12		
		G/G	63	27			82	83		
rs2071748	intron 1	A/A	16	11	2.97	0.23	21	22	0.26	0.88
		A/G	45	14			57	52		
		G/G	14	8			27	23		
rs2071749	intron 3	A/A	4	6	4.79	0.09	8	11	0.82	0.66
		A/G	36	12			55	49		
		G/G	35	15			42	37		
rs5755720	intron 4	A/A	18	6	1.16	0.56	29	32	7.31	0.06
		A/G	41	17			57	45		
		G/G	16	10			14	20		
rs5995098	intron 4	C/C	17	8	0.66	0.72	31	33	3.27	0.19
		C/G	40	15			60	44		
		G/G	18	10			14	20		
HMOX2										
rs11639998	intron 1	C/C	36	14	0.89	0.83	50	53	2.32	0.51
		C/G	33	17			41	31		
		G/G	5	2			14	12		
rs11643057	intron 1	C/C	6	1	2.14	0.54	15	13	3.19	0.36
		C/T	30	17			38	29		
		T/T	38	15			48	54		
rs1362626	intron 1	C/C	5	1	1.32	0.72	14	11	1.83	0.61
		C/G	35	18			40	30		
		G/G	34	14			49	53		
rs11076834	intron 1	C/C	39	15	1.96	0.58	48	54	3.13	0.37
		C/G	29	16			36	29		
		G/G	6	1			17	13		
rs1051308	3' UTR	A/A	40	15	1.71	0.63	51	56	3.52	0.32
		A/G	29	16			37	27		
		G/G	4	2			15	14		

gene were genotyped in our Japanese samples. The genotypes of all the SNPs were in Hardy-Weinberg equilibrium in our Japanese cases. The genotypic frequencies of each SNP for our cases and controls are shown in tables 1 and 2. There was no significant difference in the genotypic distribution for any polymorphism of the HMOX1 and HMOX2 genes. In addition, both the ApoE4-positive and -negative subgroups failed to show any genetic association between the SNPs and disease (table 3).

Discussion

Our case-control dataset has the statistical power to detect risk factors, such as ApoE4. For the HMOX1 gene, our results indicate that none of the polymorphisms, including one non-synonymous SNP, altered the risk for AD in our Japanese cohort. Our study also failed to detect any positive genetic association between HMOX1, ApoE and the disease. This is in agreement with a recent study,

which showed that a (GT)_n repeat microsatellite polymorphism of the HMOX1 gene did not affect the AD risk in a Japanese population [21]. However, a positive synergistic association was recently reported between an SNP in the promoter of the HMOX1 gene (rs2071746) together with SNPs of the liver X receptor- β (LXR- β) gene and AD [23]. We speculate that several factors may account for the different findings, such as differences in ethnic origin and differences in the location of the chosen SNPs. Although the datasets used in the microsatellite study and our study are from the same ethnic group, the pathological consequences of SNPs might differ from that of a microsatellite polymorphism. Although our study showed negative results, the non-synonymous (H>D) SNP in exon 1 (rs2071747) has been validated, is functionally important and is located close to the SNP rs2071746 mentioned above. Furthermore, a very recent study reported a positive synergistic association between rs2071747 with tau polymorphisms and AD [24]. Therefore, combined with the fact that AD is a genetically complex disease, further genetic studies of rs2071747 and the surrounding region would be important.

To the best of our knowledge, this is the first study evaluating the association between SNPs of the HMOX2 gene and AD. The five intronic SNPs chosen cover almost the entire region of the gene. We confirmed the tight linkage disequilibrium among the five SNPs in our Japanese cohort (data not shown). Recent papers have suggested that decreased HMOX2 activity might be associated with chronic neurodegenerative disorders [25, 26]. Although we did not find any positive results for the HMOX2 gene and AD, replication studies with other ethnic groups are needed to clarify the relationship between the HMOX2 gene and AD.

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