Association of the Choline Acetyltransferase Gene with Responsiveness to Acetylcholinesterase Inhibitors in Alzheimer's Disease

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Key words
- Alzheimer's disease
- acetylcholinesterase inhibitors
- drug response
- *CHAT*

Abstract

Introduction: The response to acetylcholinesterase inhibitors (AChEIs) of Alzheimer's disease (AD) patients varies depending on the genetic characteristics of the patient. We have examined the association of response to AChEIs and genetic polymorphisms in AD patients.

Methods: 158 patients with AD underwent treatment with AChEIs, and the therapeutic effect was assessed with the Korean version of the Mini Mental State Examination (K-MMSE). The association of 25 SNPs located in 3 genes (*CHAT, CHT* and *ACHE*) with changes in the K-MMSE score was analyzed.

Results: The response to AChEIs in AD patients was significantly associated with 2 SNPs on the intronic region of *CHAT* rs2177370 (uncorrected P=0.0025, FDR controlled P=0.026) and rs3793790 (uncorrected P=0.0024, FDR controlled P=0.026).

Conclusion: The results of our study confirmed again that genetic polymorphism of *CHAT* has an influence on drug response in AD.

Introduction

Dementia is a progressive neurodegenerative disease with a rising prevalence and societal burden [1,2]. Alzheimer's disease (AD) is the most common form in dementia and it accounts for 60–70% of all cases [3].

AD is associated with widespread degeneration of cholinergic neurons, and acetylcholinesterase inhibitor (AChEI) drugs are approved for symptomatic treatment, with the aim of restoring the cholinergic deficit [4]. However, therapeutic response rates vary from 40–70% [5]. If the response to the drug initially selected is insufficient, a change of drugs can be considered. However, the recognition of non-response requires prolonged observation. Thus, an ability to predict response early in the course of AD is an important therapeutic objective.

One promising approach is pharmacogenomics [6]. Several preliminary pharmacogenomic studies [7,8] have reported that the clinical response to donepezil is highest in carriers of the APOE epsilon4 allele, although a recent large study obtained a negative result [9].

The pathology of the brain cholinergic system is prominent in AD and AChEI drugs are widely used. Thus, the cholinergic system is a logical target for pharmacogenomic studies. There have been several studies on possible associations between genetic polymorphisms of cholinergic-related genes and the therapeutic effect of AChEIs [10,11]. However, there are limitations as follows in those previous studies. First, the results are inconsistent. Second, the selection of SNPs was limited, so the entire candidate gene region was not covered. Moreover, ethnic heterogeneity was not explored in these studies. In the present study we have examined the polymorphic variations of the genes encoding 3 enzymes involved in the synthesis, transport, and metabolism of acetylcholine in the cholinergic system (**Fig. 1**) [11,12]. Choline acetyltransferase (ChAT) encoded by the gene *CHAT* synthesizes acetylcholine, using choline and acetyl-CoA as substrates [11]. The choline transporter (ChT) catalyzes the uptake of choline from the extracellular space to the neuronal cytoplasm, and is encoded by *SLC5A7* [12]. Acetylcholine esterase (AChE) is encoded by *ACHE* and acts to hydrolyze acetylcholine, thereby inactivating the neurotransmitter [11]. This study extends previous reports by the simultaneous coverage of 3 genes important for function of the brain cholinergic system. We

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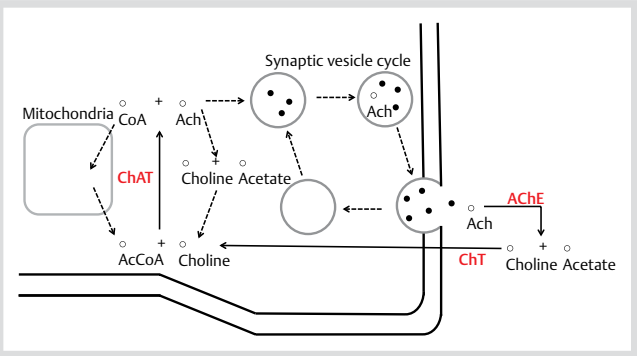

Fig. 1 The function of ChAT, AChE and ChT involved in synthesis and movement of acetylcholine in the cholinergic system. (from the KEGG database, http://www.genome.jp/kegg/). (Color figure available online only).

also aimed to assess in our Asian (Korean) population the replicability of previous reports in Caucasians [10,11]. The hypothesis of this exploratory study is that SNPs of genes involved in the synthesis and movement of acetylcholine may affect the response of AChEIs in AD.

Patients and Methods

Subjects

Subjects were 158 patients diagnosed with AD from the Clinical Trial Program in the Geropsychiatry Clinic at the Samsung Medical Center. All were of unrelated Korean ancestry. Patients were registered between November 2001 and January 2012. Subjects were eligible for this clinical trial only if they satisfied all following criteria: All patients were diagnosed as AD or probable AD according to the standards of the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association) [13]; They had a score of 26 or less in the Korean version of the Mini-Mental State Examination (K-MMSE) [14]; They had a history of cognitive decline which was gradual in onset and progressive for more than 6 months; they had a reliable caregiver who helped them to take their medication, participate in the assessment, and provide ongoing information about them [13]. Patients were excluded if any of the following conditions was present: other neurodegenerative diseases except AD (i.e., Parkinson's disease or Huntington's disease), psychiatric disorder or severe behavioral disturbances requiring psychotropic medications, cerebral injuries induced by trauma, hypoxia, and/or ischemia, clinically active cerebrovascular disease, medical history of seizure disorder, and other physical conditions requiring acute treatments.

All subjects underwent brain magnetic resonance imaging (MRI), neurological evaluation, and routine laboratory tests prior to this clinical trial in order to screen for other possible causes of dementia. The Institutional Review Board (IRB) at Samsung Medical Center approved the protocol. Written informed consent was obtained from both caregiver and patient. The study is registered (NCT01198093) in ClinicalTrials.gov.

Procedures

Subjects were assigned to receive monotherapy for 26 weeks with an acetylcholinesterase inhibitor (donepezil, galantamine or rivastigmine) as determined by a clinician. In this semi-naturalistic clinical trial, the choice of drug was based on the anticipated side effects in at-risk individuals and on current clinical practice guidelines. Donepezil was administered to 84 patients, galantamine to 52 patients and rivastigmine to 22 patients.

Doses were titrated into the usual range based on tolerability and side effects. All subjects were assessed in clinic visits after 1 week and 4 weeks on drug to adjust the dosage and evaluate adverse events. Psychotropic medications except acetylcholinesterase inhibitors were not allowed with one exception. Benzodiazepines could be used only as a short-term adjunctive for insomnia. If the subjects did not show any significant changes or serious adverse events, the interval for clinic visits was increased to 13 weeks. Experienced geriatric psychiatrists performed the assessment at each visit for clinical review of cognitive status, to examine physical and neurological status, and to review adverse events. Vital sign checks, physical examinations, laboratory tests including complete blood counts, blood chemistry profiles, vitamin B12/folate levels, syphilis serology, thyroid function tests, and ECG at baseline were carried out in all subjects.

Selection of SNP markers and genotyping

These SNPs were genotyped using the MassARRAY system (Sequenom, Inc., San Diego, Calif). 25 SNPs were discovered and selected as candidate genes with the computer program Tagger [15] with criteria of $r^2 > 0.65$ and minor allele frequency > 0.05 in combined Asian population (JPT/HCB). 21 for *CHAT*, 3 for *SLC5A7* and one for *ACHE* were genotyped. The total missing genotype counts were 50 (total call rate: 98.7%), these genotyping data were not included in the SNP association analyses. All investigators and raters were blinded to the results of genotyping throughout the study. The laboratory worker who performed the genotyping was blind to clinical data of the subjects. The organization and selected SNP locations of *CHAT* gene are shown in **Fig. 2**. There were no significant differences in genotype distribution of the 25 SNPs according to drug choice.

Measures

The response rate was assessed and compared at 26 weeks of treatment. Response was defined as no change (i.e., no deterioration) or improvement on the score of the Korean version of the Mini-Mental State Examination (K-MMSE) [16,17]. Global severity of disease was assessed according to the Clinical Dementia Rating (CDR) [18]. These research assessments of cognitive outcome were performed by a single, trained rater.

Data analysis

Continuous variables were presented as mean ± standard deviation (SD) or as median and interquartile range. Categorical variables were summarized as frequencies and proportions. Wilcoxon rank-sum test or Student's t test was performed to compare continuous variables between 2 groups according to the normality of the distribution. The association of categorical variables was determined based on the chi-squared test in all subjects.

We assessed the associations between each SNP and responsiveness by using the exact Cochran-Armitage test for trend (a genotypic trend model) [19]. Chi-squared testing was used to examine deviation from Hardy-Weinberg equilibrium [20]. The four-gamete rule by Haploview was used to check linkage disequilibrium (LD) structure [21]. Phasing haplotypes were conducted using PHASE 2.1.1 for each of the haplotype blocks individually [22]. The exact Cochran-Armitage test for a trend was used to examine the associations between a haplotype

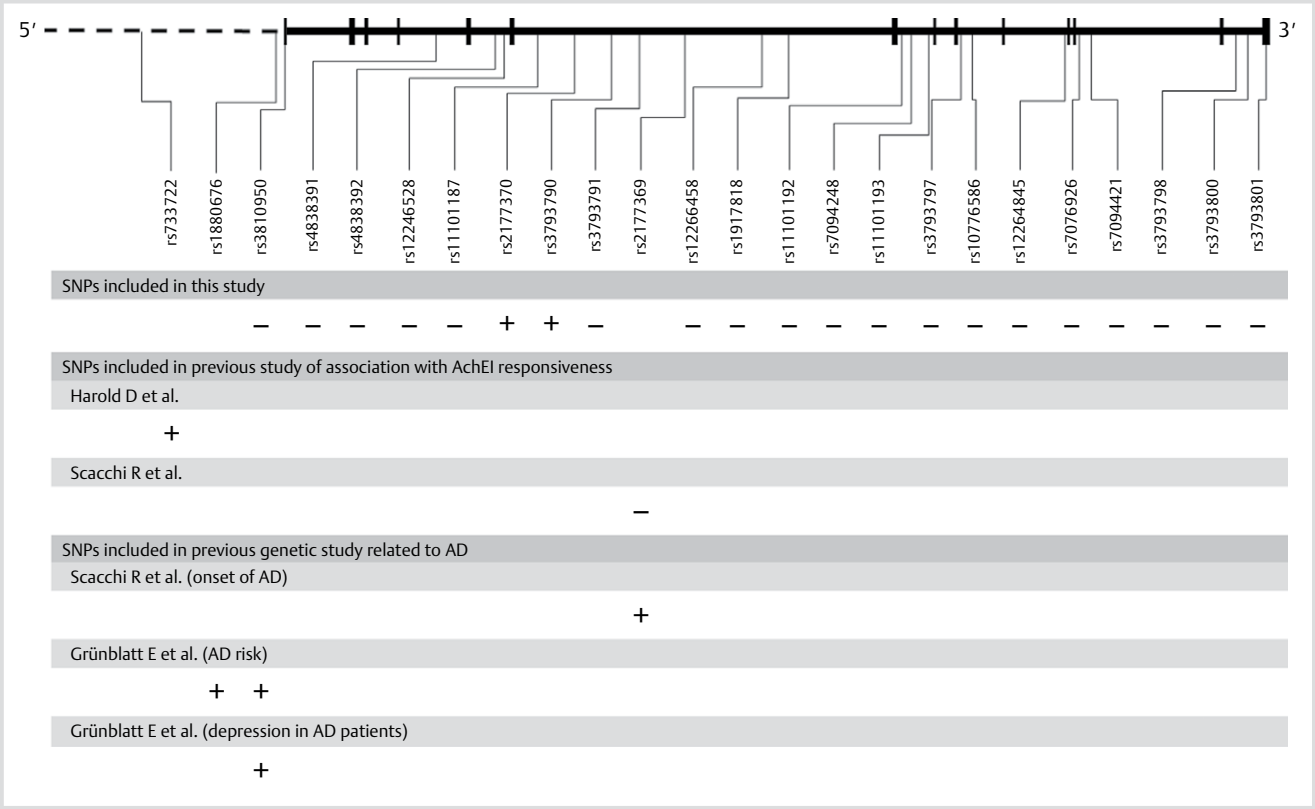

Fig. 2 *CHAT* organization and single-nucleotide polymorphism (SNP) locations (from National Center for Biotechnology Information Gene Database, http://www.ncbi.nlm.nih.gov/gene/). The horizontal line represents the genomic sequence and vertical bars represent exons. Plus signs and minus signs denote SNPs with significant association and SNPs with negative results, respectively.

Table 1 Clinical and demographic characteristics (n = 158).

	Total	Responder (n = 102)	Non-Responder (n = 56)	Statistics	P
Gender, male (%)	64 (40.5%)	39 (38.2%)	25 (44.6%)	$X^2_1 = 0.62$	0.43[a.]
Age (year, mean ± SD)	72.66 ± 8.31	73.47 ± 8.18	71.18 ± 8.41	$t_{156} = -1.67$	0.10[b.]
Education (year, median and interquartile)	8 (6, 12)	6 (6, 12)	9 (6, 12)	$Z = 1.19$	0.23[c.]
Drug (%)					
Donepezil	84 (53.2%)	57 (55.9%)	27 (48.2%)		
Galantamine	52 (32.9%)	33 (32.4%)	19 (33.9%)	$X^2_2 = 1.39$	0.50[a.]
Rivastigmine	22 (13.9%)	12 (11.8%)	10 (17.9%)		
Baseline Dementia Severity					
K-MMSE score (mean ± SD)	19.11 ± 4.73	18.55 ± 4.70	20.13 ± 4.64	$t_{156} = 2.02$	**0.04**[b.]
CDR (%)					
0.5	54 (34.2%)	32 (31.4%)	22 (39.3%)		
1	74 (46.8%)	51 (50.0%)	23 (41.1%)	$X^2_2 = 1.30$	0.52[a.]
2	30 (19.0%)	19 (18.6%)	11 (19.6%)		

SD, standard deviation; K-MMSE score, Korean Mini Mental State Examination score; CDR, Clinical Dementia Rating

[a.] Chi-squared test was used; [b.] Student's t test was used; [c.] Wilcoxon rank-sum test was used

allele and response. For the significance of association of a SNP or haplotype allele, the false discovery rate (FDR) control was used to correct each P-value [23].

The associated SNPs and haplotype alleles were entered into a multiple logistic regression model to evaluate the impact of each genetic variable on response, adjusting for other variables. In this model, the genetic variable represented the minor allele count for a subject (0, 1 or 2) and the dependent variable represented the treatment outcome (1 = response and 0 = non-response). Results were considered as significant with a threshold of $P < 0.05$. All statistical tests were performed using SAS 9.1 (SAS Institute, Inc., Cary, North Carolina).

Results

Subject characteristics

Clinical and demographic characteristics are shown in **Table 1**. Mean age of the subjects was 72.66 (SD = 8.31) years and most were in the early stage of Alzheimer's disease. The rate of response to acetylcholinesterase inhibitors was 102 of 158 (64.6%). There was no significant difference between responders and non-responders with respect to gender, age, education level and baseline global severity (CDR). The rate of response was not affected by choice of drug (donepezil, galantamine and rivastigmine). However, there was a marginally significant difference

Table 2 SNP association analysis with responsiveness.

SNP by Group	Genotype Count			Location [a.]	Statistics for HWE [b.]	P [c.]	FDR Corrected P
CHAT (chromosome 10)							
rs3810950	GG	GA	AA				
Responder	75	24	1	50824619	$X^2_1 = 0.34$	0.73	1
Non-Responder	40	14	1		$P = 0.56$		
rs4838391	CC	TC	TT				
Responder	58	39	4	50832109	$X^2_1 = 0.09$	0.14	1
Non-Responder	27	23	6		$P = 0.77$		
rs4838392	AA	GA	GG				
Responder	34	49	15	50834978	$X^2_1 = 0.46$	0.61	1
Non-Responder	19	26	6		$P = 0.50$		
rs12246528	GA	GG	AA				
Responder	12	89	0	50835264	$X^2_1 = 103.35$	0.42	1
Non-Responder	4	51	0		$P = 2.80 \times 10^{-24}$		
rs11101187	CC	CT	TT				
Responder	93	9	0	50837034	$X^2_1 = 2.18$	0.79	1
Non-Responder	53	2	1		$P = 0.14$		
rs2177370	CC	TC	TT				
Responder	48	47	6	50838874	$X^2_1 = 0.29$	**0.003**	**0.03**
Non-Responder	44	7	4		$P = 0.59$		
rs3793790	AA	GA	GG				
Responder	46	52	4	50840736	$X^2_1 = 0.91$	**0.002**	**0.03**
Non-Responder	42	10	3		$P = 0.34$		
rs3793791	CC	TC	CC				
Responder	49	45	8	50841704	$X^2_1 = 0.25$	0.90	1
Non-Responder	31	18	7		$P = 0.61$		
rs12266458	CC	TC	TT				
Responder	36	47	19	50847997	$X^2_1 = 0.81$	0.21	1
Non-Responder	15	25	15		$P = 0.37$		
rs1917818	AA	CA	CC				
Responder	62	30	10	50849342	$X^2_1 = 6.36$	0.20	1
Non-Responder	39	13	3		$P = 0.01$		
rs11101192	GG	GA	AA				
Responder	60	32	8	50854767	$X^2_1 = 4.66$	1	1
Non-Responder	34	15	6		$P = 0.03$		
rs7094248	CC	GC	GG				
Responder	52	36	12	50855368	$X^2_1 = 4.34$	0.55	1
Non-Responder	33	17	6		$P = 0.04$		
rs11101193	GG	GT	TT				
Responder	71	24	7	50856138	$X^2_1 = 7.56$	0.68	1
Non-Responder	41	12	3		$P = 0.01$		
rs3793797	TT	CT	CC				
Responder	64	27	11	50857849	$X^2_1 = 3.15$	1	1
Non-Responder	31	22	2		$P = 0.08$		
rs10776586	TT	TC	CC				
Responder	53	36	8	50858346	$X^2_1 = 0.08$	0.89	1
Non-Responder	27	20	3		$P = 0.78$		
rs12264845	CC	CA	AA				
Responder	37	54	11	50863083	$X^2_1 = 0.69$	0.90	1
Non-Responder	23	24	8		$P = 0.41$		
rs7076926	TT	CT	CC				
Responder	55	43	4	50863565	$X^2_1 = 1.42$	0.49	1
Non-Responder	28	24	4		$P = 0.23$		
rs7094421	AA	GA	GG				
Responder	76	23	1	50863623	$X^2_1 = 0.49$	0.25	1
Non-Responder	47	9	0		$P = 0.48$		
rs3793798	TT	AT	AA				
Responder	51	39	10	50871466	$X^2_1 = 0.21$	0.53	1
Non-Responder	25	25	6		$P = 0.64$		
rs3793800	AA	AG	GG				
Responder	80	21	1	50871716	$X^2_1 = 0.29$	0.43	1
Non-Responder	47	9	0		$P = 0.59$		
rs3793801	CC	TC	TT				
Responder	44	47	10	50872912	$X^2_1 = 0.67$	0.61	1
Non-Responder	26	26	4		$P = 0.41$		

Table 2 Continued.

SNP by Group	Genotype Count			Location [a.]	Statistics for HWE [b.]	P [c.]	FDR Corrected P
***SLC5A7* (chromosome 2)**							
rs6542746	CC	TC	TT				
Responder	50	41	10	13279665	$X^2_1 = 0.02$	0.37	1
Non-Responder	31	22	3		$P = 0.88$		
rs6720783	GG	GT	TT				
Responder	48	46	6	13297151	$X^2_1 = 2.64$	1	1
Non-Responder	25	26	3		$P = 0.11$		
rs11685873	GG	AG	AA				
Responder	73	27	1	13285348	$X^2_1 = 0.86$	0.75	1
Non-Responder	42	10	4		$P = 0.35$		
***ACHE* (chromosome 7)**							
rs6942609	GG	AG	AA				
Responder	40	50	11	38928323	$X^2_1 = 1.06$	0.70	1
Non-Responder	24	27	5		$P = 0.30$		

SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; FDR, false discovery rate

[a.] Genomic position (NCBI Build 37)

[b.] Chi-squared test was used

[c.] Exact Cochran-Armitage test for trend was used

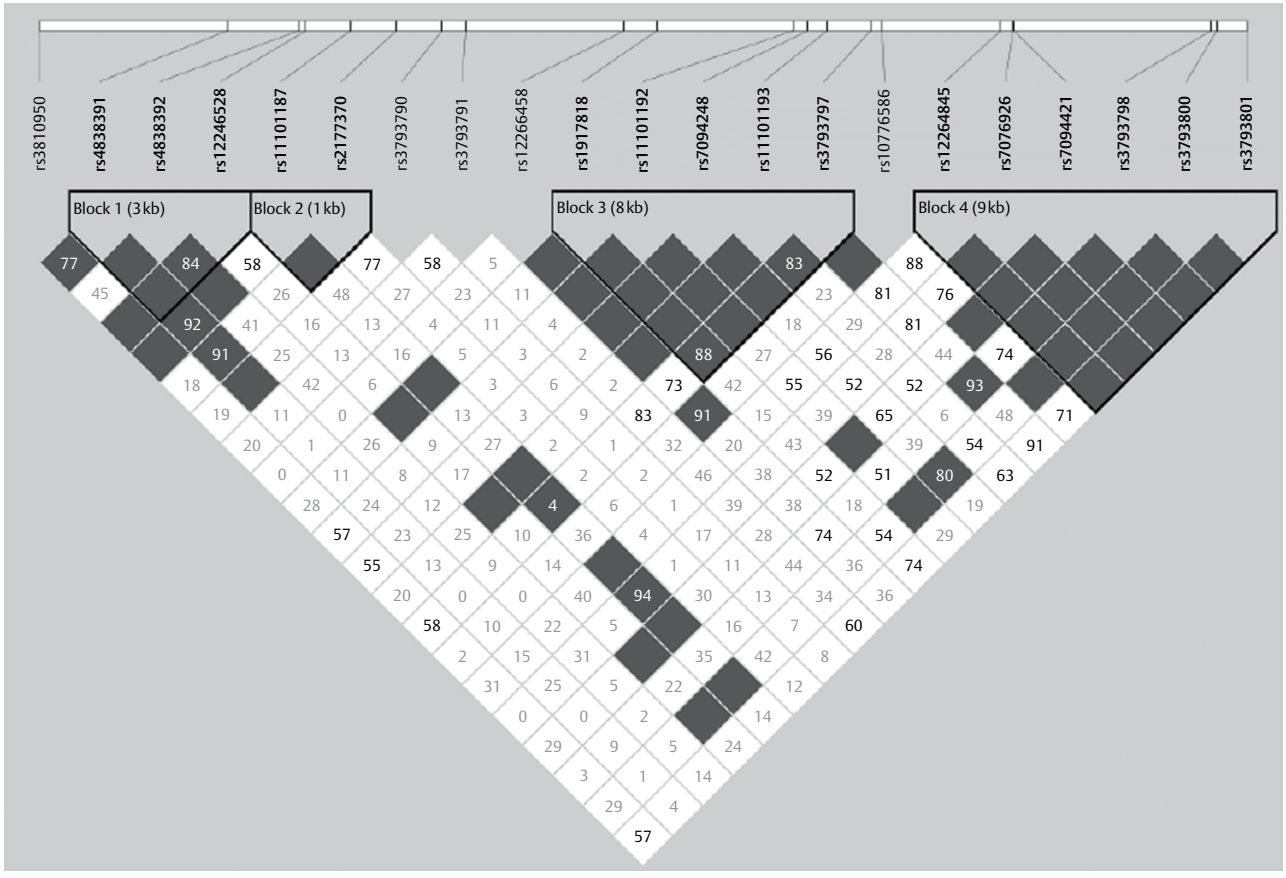

Fig. 3 Linkage disequilibrium (LD) and haplotype structure of *CHAT*. Pairwise SNP |D′| values (×100) of linkage (|D′| = 1 not shown) are shown together with haplotype blocks. Black squares represent less than 4 distinct 2-marker haplotypes and white squares represent 4 distinct 2-marker haplotypes by the 4 gamete rule. Triangles surrounding the markers represent haplotype blocks identified using the default 4-gamete rule algorithm of Haploview 4.2.

between responders and non-responders in baseline K-MMSE score (P = 0.04).

SNP association analysis with responder of acetylcholinesterase inhibitors

The results of SNP association analysis are shown in **Table 2**. The observed genotype frequencies in each case fitted the ones expected according to the Hardy-Weinberg equilibrium, except one SNP, rs12246528 ($P = 2.80 \times 10^{-24}$). However, we did not

exclude this SNP, because this pharmacogenetic study was conducted in AD patients and did not have a normal control group [19,24]. Moreover, 2 adjacent SNPs (rs2177370 and rs3793790) were significantly associated with response.

The rs2177370 in the intronic region of *CHAT* gene was significantly associated with response (uncorrected P=0.0025, FDR controlled P=0.026). The rs3793790 located in the same intron of the rs2177370 showed a significant association with responsiveness (uncorrected P=0.0024, FDR controlled P=0.026). These associations were preserved after controlling for gender, age, education year, drug and baseline K-MMSE score (for rs2177370, P=0.0065, odds ratio=2.45, 95% confidence interval=1.28–4.68; for rs3793790, P=0.0039, odds ratio=2.73, 95% confidence interval=1.38–5.38).

Haplotype association analysis with responder of acetylcholinesterase inhibitors

We discovered 4 haplotype blocks in the *CHAT* gene (**Fig. 3**). Among the 13 haplotype allele, 2 alleles in block 2 that included rs2177370 had significant associations with response (for haplotype CC, uncorrected P=0.004, FDR controlled P=0.023; for haplotype CT, uncorrected P=0.003, FDR controlled P=0.023). These haplotypes were also associated with response after controlling for gender, age, education year, drug and baseline K-MMSE score (for haplotype CC, P=0.006, odds ratio=0.44, 95% confidence interval=0.24–0.79; for haplotype CT, P=0.006, odds ratio=2.47, 95% confidence interval=1.29–4.72). However, no haplotype blocks were found to be significantly associated with response in the *SLC5A7* gene or the *ACHE* gene (**Table 3**).

Table 3 Haplotype association analysis with responsiveness in *CHAT* gene.

Haplotype by Group	Allele count			P [a.]	FDR Corrected P
	0	1	2		
Block 1 (rs4838391-rs4838392-rs12246528)					
CGG					
Responder	44	46	12	0.70	0.83
Non-Responder	23	30	3		
CAG					
Responder	41	48	13	0.61	0.83
Non-Responder	23	29	4		
TAG					
Responder	58	40	4	0.14	0.45
Non-Responder	27	23	6		
Block 2 (rs11101187-rs2177370)					
CC					
Responder	10	48	44	**0.004**	**0.023**
Non-Responder	5	9	42		
CT					
Responder	49	47	6	**0.003**	**0.023**
Non-Responder	45	7	4		
Block 3 (rs1917818-rs11101192-rs7094248-rs11101193-rs3793797)					
CGCTT					
Responder	72	23	7	0.78	0.85
Non-Responder	41	12	3		
AGCGC					
Responder	64	28	10	1	1
Non-Responder	31	23	2		
AAGGT					
Responder	61	32	9	0.62	0.83
Non-Responder	34	16	6		
AGCGT					
Responder	60	38	4	0.07	0.32
Non-Responder	25	26	5		
Block 4 (rs12264845-rs7076926-rs7094421-rs3793798-rs3793800-rs3793801)					
ATGTGC					
Responder	80	21	1	0.43	0.83
Non-Responder	47	9	0		
ACATAC					
Responder	55	43	4	0.49	0.83
Non-Responder	28	24	4		
CTAAAC					
Responder	51	41	10	0.62	0.83
Non-Responder	25	25	6		
CTATAT					
Responder	45	47	10	0.70	0.83
Non-Responder	26	26	4		

FDR, False discovery rate

[a.] Exact Cochran-Armitage test for trend was used

Discussion

In this study we assessed 25 SNPs of 3 cholinergic system genes (*CHAT, SLC5A7 and ACHE*) for association with response to AChEI drugs in AD. We found that 2 SNPs in the intronic region of *CHAT*, rs2177370 and rs3793790 had a significant association with drug response. Haplotype association analysis which was additionally performed showed that block 2 including rs2177370 among 4 haplotype blocks of the *CHAT* gene had a significant association with drug response. However, the *ACHE* and *SLC5A7* genes did not contain SNPs or haplotypes that were significantly associated with response. From this we conclude that the brain's ability to synthesize ACh in AD is a critical factor for response to AChEIs, whereas transport and inactivation of the transmitter are less important factors.

The association of *CHAT* gene polymorphisms with response is consistent with a previous study [10], but the association with *CHAT* rs2177370 has not been previously described. In one previous study *CHAT* rs733722 had a significant association with AChEI drug response in AD patients [10]. In a second study, no association of *CHAT* rs2177369 with response was reported [11]. The *CHAT* gene has also been studied for association with AD onset [11], AD risk factor [25], and depression in AD [25] (**Fig. 2**). Although the significantly associated SNPs in our study differ from those in previous studies, these other results suggest convergent evidence for the importance of the *CHAT* gene as a significant gene marker that affects the response of AChEIs. Our results call attention to the role of ChAT in the synthesis of acetylcholine and to the mechanism of action of AChEIs in patients with AD.

Acetylcholinesterase inhibitors are drugs that inhibit the acetylcholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine [26]. However, AChEI drugs depend for their efficacy on an adequate synthesis of ACh. When ACh synthesis already is impaired by degeneration of cholinergic neurons in AD, then a genetically determined relatively high synthesis capacity in the remaining neurons would be expected to favor response to AChEI drugs, and vice versa. We might infer that haplotype CC, with an odds ratio for response of 0.44, is associated with a relatively reduced rate of ACh synthesis, whereas haplotype CT, with an OR of 2.47, is associated with a relatively high rate of ACh synthesis. Additional studies are needed to establish the functional direction of influence that rs2177370 and rs3793790 exert on the activity of *CHAT*.

We did not confirm the report of Scacchi et al. that the *ACHE* rs2571598 had a significant association with drug response in AD patients treated with rivastigmine [11]. We found no association between the *ACHE* gene and response to AChEI drugs. The discrepancies between our study and Scacchi's may due to differences of SNP selection, ethnicity and the genetic models adopted. In a previous study examining the *CHAT* gene, rs3810950 had a significant association with both depression [25] and disease progression [27] in AD. However, there was no association with drug response in this study. Because both comorbid depression and disease stage can influence cognitive function in AD, these factors will need to be considered in future pharmacogenetic studies.

We conducted this study in Korean patients. In our previous pharmacogenetic study of the serotonin transporter in patients with depression, conflicting results were reported according to ethnicity (Caucasian, Asian) [28]. Most of the existing genetic studies for drug response in AD patients have been limited to Caucasian populations. Thus, replication studies in different ethnic populations will be required. Our study is the first haplotype association study of response of AChEIs and found that haplotype blocks located on *CHAT* may affect response in both favorable and unfavorable directions. A possible limitation or a potential advantage in this study was the use of 3 members of the AChEI drug class. On the one hand we lack statistical power to examine SNP associations with response to individual drugs. On the other hand, by using all the common members of the AChEI class our results may be generalizable to the clinical setting.

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Author Contributions

The individual authors contributed as follows: Doh Kwan Kim, Woojae Myung, Shin-Won Lim and Hyeyeon Yoon were involved in study planning and the writing of the manuscript; Doh Kwan Kim conducted the clinical parts of the study. Hyo Shin Kang was involved in data acquisition, Seonwoo Kim, Woojae Myung, Hong-Hee Won and Hyeyeon Yoon performed the statistical analyses; Bernard J. Carroll edited the manuscript and assisted with interpretation of the data.

Conflict of Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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