Published in final edited form as:

Hum Genet. 2010 June ; 127(6): 691–698. doi:10.1007/s00439-010-0818-3.

Replication and extension of association of choline acetyltransferase with nicotine dependence in European and African American smokers

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Abstract

Choline acetyltransferase is critical in the synthesis of acetylcholine and regulation of cholinergic neuron functions. We recently reported association of the encoding gene *ChAT* with both smoking cessation and nicotine dependence (ND) in two independent European American (EA) samples; however, in the replication sample, only limited SNPs partially covering the gene were examined. In this study, we examined the association of 14 SNPs, which cover the entire gene, with ND, assessed by smoking quantity (SQ), heaviness of smoking index (HSI), and Fagerström Test for ND (FTND), in 2,037 subjects from 602 families of African American (AA) or EA origin. Individual SNP-based association analysis revealed that five SNPs showed nominal association

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with at least one ND measure in one of the samples (P = 0.022–0.042); none remained significant after correction for multiple testing. Haplotype-based association analysis revealed that haplotypes G–G–A–C, formed by rs1880676–rs3810950–rs10082479–rs8178990 (P = 0.005–0.0178), and G–G–T–C–G–C, formed by rs1880676–rs3810950–rs10082479–rs8178990–rs3793790–rs12266458 (P = 0.00247–0.00468), displayed significant association with all three ND measures in the AA sample, as did haplotype T–C–G–A–T, formed by rs12266458–rs11101191–rs8178991–rs4838544–rs4838547 (P = 0.00741–0.0103), in the EA sample. All these detected haplotype-based associations remained significant after correction for all major haplotypes for a given SNP combination. Together, our findings, in conjunction with the previous report of the association, warrant further investigation of *ChAT* in ND.

Introduction

Tobacco smoking causes significant problems, including cardiovascular and respiratory diseases, cancer, and susceptibility to infectious diseases (Benowitz 2008; CDC 2005; Friedman et al. 2006). Tobacco-attributable deaths are projected to rise from 5.4 million in 2004 to 8.3 million in 2030: approximately 10% of all deaths worldwide (WHO 2008). In the USA, 20.8% of adults use tobacco products, and approximately 438,000 deaths are caused by tobacco-induced diseases with more than $157.7 billion in costs each year (CDC 2007).

Nicotine is the main psychoactive component of tobacco that results in addiction (Benowitz 2008; Benowitz et al. 1999). Numerous studies have demonstrated that nicotine dependence (ND) is influenced by both genetic and environmental factors, with a mean heritability of 0.5 in all smokers, suggesting that ND is a complex trait that involves multiple genes and environmental risk factors (Li et al. 2003).

The cholinergic system is one of the neurotransmitter systems involved in nicotine's rewarding effects (Maskos 2008; Ray et al. 2009). Many conventional and high-throughput expression studies have revealed that nicotine modulates the expression of multiple genes and exerts its effects on the neuronal system by binding to nicotinic acetylcholine receptors (Dunckley and Lukas 2003; Gutala et al. 2006; Hwang and Li 2006; Konu et al. 2001; Li and Kane 2003; Serres and Carney 2006). Nicotine administration also causes and sensitizes release of acetylcholine in the brain (Hernandez and Terry 2005; Reid et al. 1999). Modulation of the cholinergic system by nicotine improves learning and memory, and is thought to contribute to the regulation of central reward mechanisms (Maskos 2008; Uzum et al. 2004). Thus, investigation of the associations of variants in genes within the cholinergic system may provide important insights into the etiology of ND.

The human choline acetyltransferase gene (*ChAT*) is located on chromosome 10q11.23. Six transcript variants have been identified in humans, which result from differential utilization and alternative splicing of five noncoding exons at the 5′ end of mRNA (Dobransky and Rylett 2003). The longest transcript has 15 exons and spans about 51 kb. These transcripts encode 69, 74, and 82-kDa isoforms of the ChAT protein (Dobransky and Rylett 2003, 2005), which is the key enzyme that catalyzes and regulates the synthesis of acetylcholine (Dobransky and Rylett 2005). In brain, ChAT is selectively expressed and traditionally used

as a phenotypic marker for cholinergic neurons (Dobransky and Rylett 2003). Consistent with its regulatory function in these neurons, *ChAT* has been implicated in cholinergic-related neurological disorders, including Alzheimer's disease (Kim et al. 2004; Mubumbila et al. 2002).

In a recent study, we reported that *ChAT* was associated with smoking cessation in a discovery sample of treatment-seeking smokers and a replication sample of community-based smokers of all European origin (Ray et al. 2010). However, the seven SNPs that were genotyped in the replication sample for association with ND are located in only two regions, between exons 1 and 2 and exons 11 and 15, and no SNPs between exons 3 and 11, which spans about 40 kb, were examined. Moreover, association studies of *ChAT* with ND in independent samples, especially in other ethnic groups, are needed to better understand the role of *ChAT* in smoking. Thus, in the present study, we performed association analysis of 14 SNPs that cover the entire *ChAT* gene and found significant association between *ChAT* and ND in a family-based sample consisting of 2,037 participants of either African American (AA) or European American (EA) origin.

Methods

Subjects

All participants involved in this study were recruited during 1999–2004, primarily from the mid-south states of Tennessee, Mississippi, and Arkansas. Proband smokers were required to be at least 21 years old, to have smoked for at least 5 years, and to have smoked at least 20 cigarettes per day for the last 12 months. Following recruitment of a qualified proband smoker, all his or her siblings and biological parents were recruited whenever possible, regardless of their smoking status.

The study included 2,037 participants in 602 nuclear families, with 671 subjects in 200 EA families and 1,366 subjects in 402 AA families. Data collected from each participant included demographic information, medical history, current smoking behavior, ND measures, and personality traits (Beuten et al. 2005; Beuten et al. 2006; Li et al. 2008; Li et al. 2006). All participants provided informed consent. The study protocol, forms, and procedures were approved by all participating institutional review boards.

The degree of ND of each smoker was ascertained by the three most commonly used measures: smoking quantity (SQ; defined as the number of cigarettes smoked per day), the heaviness of smoking index (HSI; 0–6 scale), and the Fagerström Test for ND score (FTND; 0–10 scale). All three measures have been used consistently in our previous studies on ND for these samples (Beuten et al. 2005; Huang et al. 2009; Li et al. 2005; Li et al. 2007). Given the overlap in the content of the three ND measures, fairly robust correlations among them (r = 0.88–0.94) exist in both the AA and EA samples (Li et al. 2008; Li et al. 2006). The primary reasons for employing all three measures were that there was a lack of consensus regarding the best approach to assess ND as a phenotype and to permit maximum cross-referencing with previous studies of ND.

DNA extraction, SNP selection, and genotyping

Genomic DNA was extracted from peripheral blood cells of each participant using a Maxi blood DNA extraction kit from Qiagen (Valencia, CA, USA). Fourteen SNPs were selected to cover the whole *ChAT* gene. We tried to include as many missense SNPs as possible, because they are more likely to affect the function of a gene of interest. Other factors, such as minor allele frequency (MAF) and number of nucleotides between each adjacent SNP pair, were also considered when we selected the SNPs for genotyping. Information on the 14 chosen SNPs is shown in Table 1.

All SNPs were genotyped using the *Taq*Man assay. Primers/probe sets for each SNP were purchased from Applied Biosystems (Foster City, CA, USA). The PCRs for genotyping were performed in 384-well plates using a standard protocol, as described in our previous reports (Beuten et al. 2007; Beuten et al. 2006). To ensure the quality of genotyping, we included four non-template controls and eight positive controls in each 384-well plate. Allelic discrimination analysis was performed on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems).

Statistical and association analysis

The Haploview program (Barrett et al. 2005) was used to identify any inconsistent Mendelian inheritance, nonpaternity, or other errors and to analyze pairwise linkage disequilibrium (LD) between all SNP markers. Association of individual SNPs with each ND measure was determined by the Pedigree-Based Association Test (PBAT, v.3.6) (Lange et al. 2004). Haplotype association was determined by the Family-Based Association Test (FBAT, v2.0.3) with the option of computing *P* values of the *Z* statistic based on Monte Carlo sampling (Horvath et al. 2004). In the consideration of low LD among these SNPs, especially in the AA sample, we employed a sliding window approach by examining all possible haplotypes consisting of three to six consecutive SNPs (Lin et al. 2004). Both additive and dominant models were tested, with sex and age as covariates, for the AA or EA sample. All associations designated significant were corrected for multiple testing of the number of SNPs analyzed using the SNP spectral decomposition (SNPSpD) approach (Nyholt 2004) for individual SNP analysis and Bonferroni correction by dividing the number of major haplotypes (>5% frequency) for a given SNP combination.

Results

Association analysis of individual SNPs

In our previous study, we reported association of *ChAT* with smoking cessation and ND in two samples of European origin (Ray et al. 2010). In consideration of the presence of genetic differences for nicotine metabolism and other smoking behaviors in different ethnic populations (Benowitz et al. 1999), it is important to determine whether *ChAT* is associated with ND in other ethnic groups, such as AAs.

Individual SNP-based association analysis revealed nominal association of three SNPs with at least one ND measure in the EA or the AA sample (Table 2). SNP rs3793790 was nominally associated with SQ ($P = 0.033$), HSI ($P = 0.042$), and FTND ($P = 0.040$) in the

AA sample, whereas SNP rs11101191 was associated with SQ ($P = 0.022$) and FTND ($P = 0.039$) in the EA sample. SNPs rs4838547 ($P = 0.042$), rs11101202 ($P = 0.040$), and SNP rs867687 ($P = 0.039$) were associated with HSI in the EA sample. However, none of these associations remained significant after correction for multiple testing.

The pairwise D values for the 14 SNPs within *ChAT* were calculated using Haploview (Barrett et al. 2005). We observed two LD blocks in the AA sample and three LD blocks in the EA sample. As shown in Fig. 1, the block containing rs12266458 and rs11101191 was found in both the AA and EA samples. Another block in the AA sample containing rs6537546 and rs1917810 differed from the one in the EA sample, which contained rs4838547, rs6537546, rs1917810, rs11101202, and rs867687. The block containing rs1880676 and rs3810950 was found only in the EA sample.

Haplotype analysis

The FBAT program was employed to analyze the association between haplotypes and ND measures. To identify statistically significant haplotypes associated with different ND measures and considering weak LD among most SNPs examined in this study on our samples, we adopted a window-sliding approach by examining all possible haplotypes consisting of three to six consecutive SNPs in the EA and AA samples separately. This approach revealed multiple major haplotypes (defined as >5.0%) to be significantly associated with ND measures. In the AA sample, haplotype G–G–A–C, formed by rs1880676–rs3810950–rs10082479–rs8178990, showed a significant association with all three ND measures in the AA sample (frequency 53.2%, $Z = 2.369$, and $P = 0.0178$ with SQ; $Z = 2.589$ and $P = 0.00962$ with HSI; $Z = 2.810$ and $P = 0.00496$ with FTND; Table 3). We also found that another haplotype, G–G–T–C–G–C, with a frequency of 11.6%, formed by rs1880676–rs3810950–rs10082479–rs8178990–rs3793790–rs12266458, showed a significant inverse association with all three ND measures ($Z = -2.828$ and $P = 0.00468$ with SQ; $Z = -2.945$ and $P = 0.00323$ with HSI; $Z = -3.027$ and $P = 0.00247$ with FTND) (Table 4). In the EA sample, haplotype T–C–G–A–T with a frequency of 6.4%, formed by rs12266458–rs11101191–rs8178991–rs4838544–rs4838547, showed a significant association with all three ND measures ($Z = 2.564$ and $P = 0.0103$ with SQ; $Z = 2.627$ and $P = 0.00861$ with HSI; $Z = 2.678$ and $P = 0.00741$ with FTND) (Table 5). All these associations remained significant after Bonferroni correction for multiple testing of major haplotypes for each given SNP combination.

Discussion

In this study, we first confirmed the association of *ChAT* with ND with the EA sample used for the replication sample in our previous study (Ray et al. 2010) by genotyping additional SNPs to provide a better coverage of the entire gene. Further, we replicated significant associations of the *ChAT* gene with ND in an independent AA sample. Three haplotypes were found to be significantly associated with all the three ND measures in these two ethnic groups. The first haplotype, G–G–A–C, formed by rs1880676–rs3810950–rs10082479–rs8178990, was significantly associated with ND in the AA sample. For this sample, we also found another haplotype, G–G–T–C–G–C, formed by the four SNPs contained in the first

haplotype plus two additional SNPs, rs3793790–rs12266458, showed significant association with the three ND measures. The third haplotype, T–C–G–A–T, formed by rs12266458–rs11101191–rs8178991–rs4838544–rs4838547, showed significant association with the three ND measures in the EA sample.

As a key enzyme for acetylcholine synthesis, ChAT is widely used as a phenotypic marker for cholinergic neurons (Dobransky and Rylett 2005). The cholinergic system has been reported to modulate the mesolimbic and mesocortical dopamine systems, which are key elements in the central reward circuitry (Ikemoto 2007). Cholinergic projection from the mesopontine tegmentum (MPT) modulates dopamine neuron activity in the ventral tegmental area (VTA) (Maskos 2008). Nicotine acts as a cholinergic agonist to modulate cholinergic projection from MPT to VTA by both direct regulation of MPT neurons and stimulation of nicotinic receptors located on VTA neurons; the latter has been considered the primary mechanism underlying the rewarding effect of nicotine (Maskos 2008; Ray et al. 2009). In the developing brain, nicotine reduces expression of *ChAT*, indicating a direct effect of the drug on the central cholinergic system (Abreu-Villaca et al. 2003). Endogenous cholinergic signaling also is involved in modulation of nicotine-induced plasticity during development (Heath and Picciotto 2009). Alteration of the cholinergic system may be related to vulnerability to drug addiction in offspring whose mothers smoke during pregnancy (Abreu-Villaca et al. 2003; Franke et al. 2008; Heath and Picciotto 2009). In adults, an increase in cholinergic activity in response to nicotine is involved in improvement of learning and memory (Hernandez and Terry 2005; Uzum et al. 2004). Thus, effects on the cholinergic system caused by nicotine support the plausibility of association of *ChAT* with ND.

Effects of nicotine on *ChAT* expression in other brain regions have been reported as well. Chronic nicotine administration alters *ChAT* expression in both adult (Hernandez and Terry 2005) and adolescent (Abreu-Villaca et al. 2003) animals. Moreover, recent studies indicate that phosphorylation of the ChAT protein regulates its function by modulating its catalytic activity and subcellular location in cholinergic neurons (Dobransky and Rylett 2005), suggesting that ChAT acts as an important regulator of the synthesis of endogenous acetylcholine and functions of cholinergic neurons. Protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII), both involved in calcium signaling, are the two best-documented kinases that phosphorylate ChAT (Dobransky and Rylett 2005). Because calcium signaling plays an important role in mediating the effects of nicotine, it is reasonable to assume that nicotine regulates ChAT activity through phosphorylation. Thus, further research on the effects of nicotine in regulating ChAT activity and expression will be helpful to understand the association between *ChAT* and ND.

In summary, our individual SNP- and haplotype-based association analyses revealed that *ChAT* is associated with ND in both the AA and EA samples, which represents not only a replication of previous association reports in EA populations, but also an extension to an independent ethnic population. These genetic associations, along with the possible biological role of *ChAT* in ND, make *ChAT* an appropriate target for further research into the mechanisms underlying association with ND.

Acknowledgments

This study was supported by the National Institutes of Health grants DA-12844 and DA-13783 (to MDL). We are grateful for the invaluable contributions of clinical information and blood by all participants in the genetic study, as well as the dedicated work of many research staff at different clinical sites.

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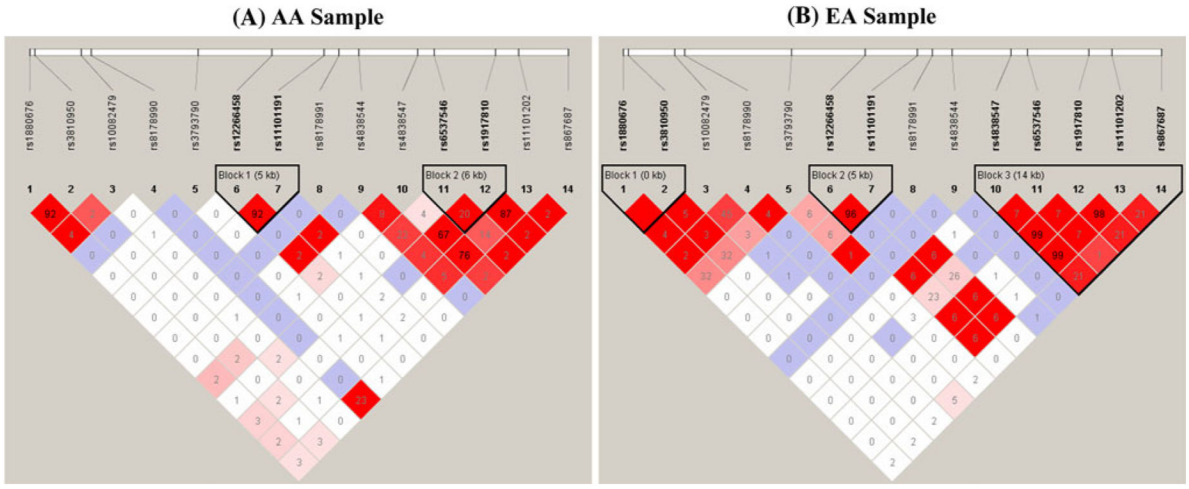

Fig. 1.

Haploview-generated LD patterns for 14 SNPs within the *ChAT* in the AA (**a**) and EA (**b**) samples. Pairwise LD between all SNPs was evaluated using the Haploview program (Barrett et al. 2005) with the option of determining haplotype blocks according to the criteria defined by Gabriel et al. (2002). The *number* in each *box* represents the R^2 value for each SNP pair

Table 1

Information of SNPs and Hardy–Weinberg equilibrium (HWE) in the AA and EA samples and a comparison of their minor allele frequencies in the NCBI dbSNP database (build 126) and in this study

dbSNP ID	Alleles	Chromosome position	SNP location	MAF (NCBI dbSNP)		MAF (this study)		HWE	
				YRI	CEU	AA	EA	AA	EA
rs1880676	A/G	50494123	Intron	0.004 (A)	0.230 (A)	0.052 (A)	0.281 (A)	0.858	0.821
rs3810950	A/G	50494625	Exon	0.000 (A)	0.230 (A)	0.057 (A)	0.275 (A)	0.934	0.935
rs10082479	A/T	50499225	Intron	0.417 (T)	0.170 (T)	0.422 (T)	0.129 (T)	0.831	0.465
rs8178990	C/T	50500177	Exon	0.000 (T)	0.097 (T)	0.011 (T)	0.072 (T)	0.153	1.000
rs3793790	A/G	50510742	Intron	0.403 (G)	0.327 (G)	0.383 (G)	0.355 (G)	0.793	0.752
rs12266458	C/T	50518003	Intron	0.093 (T)	0.108 (T)	0.127 (T)	0.076 (T)	0.278	0.160
rs11101191	C/T	50523198	Intron	0.142 (C)	0.093 (C)	0.135 (C)	0.077 (C)	0.004	0.165
rs8178991	A/G	50524643	Exon	N/A	N/A	0.010 (A)	0.028 (A)	0.004	0.415
rs4838544	A/G	50526658	Exon	0.204 (G)	0.000 (G)	0.114 (G)	0.002 (G)	0.522	1.000
rs4838547	C/T	50532410	Intron	0.304 (C)	0.415 (C)	0.410 (C)	0.445 (C)	0.591	0.997
rs6537546	A/T	50534049	Intron	0.375 (T)	0.136 (T)	0.300 (T)	0.078 (T)	0.846	0.998
rs1917810	A/G	50540077	Intron	0.190 (G)	0.407 (G)	0.311 (G)	0.448 (G)	0.538	1.000
rs11101202	C/G	50542408	Intron	0.241 (G)	0.407 (G)	0.344 (G)	0.448 (G)	0.857	0.996
rs867687	C/T	50547271	3′ Flanking	0.004 (C)	0.243 (C)	0.052 (C)	0.215 (C)	0.856	0.409

Nucleotide given in each parenthesis represents the minor allele of the studied sample

N/A not available, *YRI* sub-Saharan African sample from NCBI dbSNP database, *CEU* European sample from NCBI dbSNP database

Table 2

P value of minor alleles for association of individual SNPs with three ND measures in the EA and AA samples under the dominant model

SNP ID	Minor allele	EA sample				AA sample			
		MAF	SQ	HSI	FTND	MAF	SQ	HSI	FTND
rs1880676	A	0.281	−0.779	−0.694	−0.775	0.052	−0.475	−0.449	−0.652
rs3810950	A	0.275	−0.956	−0.808	−0.867	0.057	−0.563	−0.700	0.821
rs10082479	T	0.129	0.749	0.379	0.313	0.422	−0.702	−0.510	−0.422
rs8178990	T	0.072	−0.743	0.939	−0.931	0.011	0.856	−0.998	0.897
rs3793790	G	0.355	0.409	0.624	0.574	0.383	**−0.033**	**−0.042**	**−0.040**
rs12266458	T	0.076	0.721	0.384	0.569	0.127	−0.518	−0.462	−0.689
rs11101191	C	0.077	**0.022**	0.056	**0.039**	0.135	−0.407	−0.426	−0.592
rs8178991	A	0.028	−0.517	−0.672	−0.914	0.010	0.893	0.703	0.662
rs4838544	G	0.002	−0.920	0.635	0.785	0.114	0.773	0.659	0.768
rs4838547	C	0.445	−0.167	**−0.042**	**−0.050**	0.410	−0.655	−0.863	0.802
rs6537546	T	0.078	−0.830	−0.881	−0.834	0.300	−0.204	−0.247	−0.259
rs1917810	G	0.448	−0.188	−0.072	−0.092	0.311	0.424	0.402	0.197
rs11101202	G	0.448	−0.125	**−0.040**	−0.051	0.344	−0.642	−0.709	0.522
rs867687*	C	0.215	−0.459	**0.039** (**T***)	−0.912	0.052	0.373	0.705	−0.584

Significant association at a 0.05 significance level is given in bold

A *P* value with a negative sign indicates an inverse association of the allele with the ND measure

*This *P* value is for the major allele ‘T’ of SNP rs867687

Table 3

Association of major haplotypes formed by rs1880676, rs3810950, rs10082479, and rs8178990 in *ChAT*

Haplotype	EA				AA			
	Frequency (%)	SQ	HSI	FTND	Frequency (%)	SQ	HSI	FTND
G–G–A–C	59.5	2.106 (0.0352, 41)	2.158 (0.0309, 41)	2.061 (0.0393, 41)	53.2	**2.369 (0.0178, 112)**	**2.589 (0.00962, 109)**	**2.810 (0.00496, 113)**
G–G–T–C	6.5	0.595 (0.552, 27)	0.724 (0.469, 27)	1.055 (0.292, 27)	40.8	−0.819 (0.413, 159)	−1.152 (0.249, 158)	−1.316 (0.188, 163)
A–A–A–C	26.3	−0.205 (0.838, 57)	−0.256 (0.798, 57)	−0.184 (0.854, 60)	4.1	−0.452 (0.651, 42)	−0.445 (0.656, 44)	−0.104 (0.917, 45)
G–G–T–T	6.2	−0.211 (0.833, 21)	0.182 (0.856, 20)	0.022 (0.982, 22)	0.8	0.034 (0.973, 10)	Not detected	0.388 (0.698, 11)
Global *P* value		6.276 (0.179)	7.623 (0.106)	8.202 (0.0845)		5.717 (0.221)	6.989 (0.072)	8.479 (0.076)

Three numbers within each cell are *Z* score, *P* value, and informative family size (last two are in parentheses), respectively

Cells in bold were significant after correction for multiple testing at the 0.05 level

For the SNP combination, the adjusted *P* value at 0.05 level are 0.0125 (i.e., 0.05/4 major haplotypes) and 0.0250 (i.e., 0.05/2 major haplotypes) for the AA and EA samples, respectively. Only the results of the dominant model are shown

Table 4

Association of major haplotypes formed by rs1880676, rs3810950, rs10082479, rs8178990, rs3793790, and rs12266458 in *CHAT*

Haplotype	EA				AA			
	Frequency (%)	SQ	HSI	FTND	Frequency (%)	SQ	HSI	FTND
G–G–A–C–A–C	46.5	0.566 (0.571, 54)	0.596 (0.551, 53)	0.472 (0.637, 55)	27.3	0.533 (0.594, 147)	0.778 (0.437, 150)	1.069 (0.285,154)
G–G–T–C–A–C	4.5	0.898 (0.369, 20)	1.479 (0.139, 20)	1.462 (0.144, 20)	24.7	0.622 (0.534, 140)	0.520 (0.603, 140)	0.061 (0.951,143)
G–G–A–C–G–C	8.6	2.297 (0.0216, 33)	2.246 (0.0247, 34)	2.083 (0.0372, 34)	19.1	0.234 (0.815, 140)	0.311 (0.756, 138)	0.440 (0.660,147)
A-A-A-C-G-T	17.8	−1.287 (0.198, 55)	−1.037 (0.300, 55)	−0.950 (0.342, 57)	2.6	−0.212 (0.832, 23)	−0.471 (0.638, 24)	−0.043 (0.966,24)
G-G-T-C-G-C	1.5	Not shown	Not shown	Not shown	11.6	**−2.828 (0.00468, 96)**	**−2.945 (0.00323, 97)**	**−3.027 (0.00247, 100)**
A–A–A–C–A–C	6.5	0.835 (0.404, 32)	0.881 (0.378, 32)	0.535 (0.593, 33)	1.4	−0.684 (0.494, 17)	−0.472 (0.637, 17)	−0.424 (0.672, 18)
G–G–T–T–A–C	5.9	−1.038 (0.299, 19)	−0.462 (0.644, 18)	−0.552 (0.581, 20)	0.4	Not shown	Not shown	Not shown
Global *P* value		13.583 (0.059)	16.536 (0.021)	12.443 (0.087)		13.210 (0.212)	13.769 (0.184)	14.195 (0.164)

Three numbers within each cell are *Z* score, *P* value, and informative family size (last two are in parentheses), respectively

Cells in bold were significant after correction for multiple testing at 0.05 level

For the SNP combination, the adjusted *P* value at the 0.05 level are 0.0100 (i.e., 0.05/5 major haplotypes) and 0.0125 (i.e., 0.05/4 major haplotpes) for the AA and EA samples, respectively. Only the results of the dominant model are shown

Table 5

Association of major haplotypes formed by rs12266458, rs11101191, rs8178991, rs4838544, and rs4838547 in *ChAT*

Haplotype	EA				AA			
	Frequency (%)	SQ	HSI	FTND	Frequency (%)	SQ	HSI	FTND
C–T–G–A–T	47	0.017 (0.986, 49)	0.126 (0.900, 50)	0.001 (0.999, 50)	38.7	−0.615 (0.538,138)	−0.211 (0.832,141)	−0.287 (0.774,146)
C–T–G–A–C	43.3	−1.105 (0.269, 59)	−1.701 (0.0889, 58)	−1.653 (0.0984, 59)	36.1	−0.299 (0.765,144)	−0.041 (0.968,144)	0.279 (0.780,150)
T–C–G–A–T	6.4	**2.564 (0.0103, 25)**	**2.627 (0.00861, 24)**	**2.678 (0.00741, 25)**	9.2	−0.212 (0.832,68)	−0.039 (0.969,67)	−0.013 (0.990,70)
C–T–G–G–T	0.3	Not shown	Not shown	Not shown	10.6	0.298 (0.766,84)	0.238 (0.812,85)	−0.055 (0.956,91)
Global *P* value		6.975 (0.073)	8.157 (0.043)	8.157 (0.043)		1.303 (0.935)	0.620 (0.987)	0.383 (0.996)

Three numbers within each cell are *Z* score, *P* value, and informative family size (last two are in parentheses), respectively

Cells in bold were significant after correction for multiple testing at the 0.05 level

For the SNP combination, the adjusted *P* value at the 0.05 level are 0.0167 (i.e., 0.05/3 major haplotypes) and 0.0125 (i.e., 0.05/4 major haplotypes) for the AA and EA samples, respectively. Only the results of the dominant model are shown