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Serotonin Transporter and Receptor Genes Significantly Impact Nicotine Dependence through Genetic Interactions in both European American and African American Smokers*

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Abstract

Background—Pharmacologic studies implicate a significant role of genes encoding the serotonin transporter (*SLC6A4*) and the 5-HT_{3AB} subunits *HTR3A* and *HTR3B* in nicotine dependence (ND). However, whether they are involved in ND remains largely unknown.

Methods—Here, we examined the impact of variations in the three genes on ND in 1366 individuals from 402 African American (AA) and 671 individuals from 200 European American (EA) families. The ND of each smoker was assessed with smoking quantity (SQ), Heaviness of Smoking Index (HSI), and Fagerström Test for Nicotine Dependence (FTND).

Results—Association analysis revealed marginal association of rs10160548 in *HTR3A* with SQ and HSI in AA, 5-HTTLPR in *SLC6A4* with FTND in EA, and rs11606194 in *HTR3B* with SQ and FTND in the pooled sample. Haplotype-based association analysis revealed a few major haplotypes in *HTR3A* that were significantly associated with ND in the AA, EA, and pooled samples. However, none of these associations remained significant after correcting for multiple testing except for a haplotype GC-C-T-A-T formed by SNPs rs1150226, rs1062613, rs33940208, rs1985242, rs2276302, and rs10160548 in *HTR3A* for the AA sample. Considering biological functions of the three genes, we examined interactive effects of variants in the three genes, which revealed significant interactions among rs1062613 and rs10160548 in *HTR3A*, rs1176744 in *HTR3B*, and 5-HTTLPR and rs1042173 in *SLC6A4* in affecting ND in the three samples.

Conclusions—We conclude that *SLC6A4*, *HTR3A* and *HTR3B* play a significant role in ND through genetic interactions.

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Keywords

Serotonin transporter; serotonin receptor 3_{AB}; nicotine dependence; African and European American; gene-by-gene interactions; epistatic

1. INTRODUCTION

Tobacco use continues to be a foremost worldwide health concern. In the United States, 46.0 million adults were cigarette smokers in 2008, and the number of deaths annually from smoking-related illnesses was estimated to be around 440,000 in 2008 (CDC, 2009). The annual economic burden of smoking is also substantial with a staggering \$193 billion in medical costs and productivity losses (CDC, 2008). It has been estimated by the World Health Organization that tobacco-related deaths would rise from approximately 5.0 million in 2004 to 8.0 million by 2030 (WHO, 2008).

Cigarette smoking is a complex behavior, with both genetic and environmental components (Al Koudsi and Tyndale, 2005; Sullivan and Kendler, 1999). Many family, adoption, and twin studies of smoking addiction have indicated a heritability of 11% - 78%, with an average heritability of 57% for both male and female smokers (Kendler et al., 1999; Li et al., 2003; Maes et al., 2004; Vink et al., 2005).

Nicotine, the primary psychoactive ingredient in cigarette smoke, exerts its effects by readily crossing the blood-brain barrier and binding with nicotinic acetylcholine receptors (nAChRs) located in various brain structures (Wonnacott, 1997). Activation of nAChRs on dopaminergic terminals induces dopamine release in the mesolimbic brain reward system (Kleijn et al., 2011; Wonnacott et al., 2000). In addition to dopamine, nicotine can induce the release of a variety of other neurotransmitters that regulate the complex brain circuitry modulating addictive behaviors (Mihailescu et al., 1998; Ribeiro et al., 1993). Of these neurotransmitter systems, the serotonin (5-HT) system regulates self-administration of nicotine (Levin et al., 2008), nicotine-induced cognitive enhancement (Levin and Rezvani, 2007), memory (Levin et al., 2005), and smoking progression and cessation (Wang and Li, 2010).

The serotonergic system consists of seven major receptor classes; among these, all but the 5-HT₃ receptors are G-protein coupled (Barnes et al., 2009; Boess and Martin, 1994; Connolly and Wafford, 2004). The 5-HT₃ receptors belong to the Cys-loop super-family of pentameric neurotransmitter-gated ion channels that also includes nicotinic acetylcholine (nACh), GABA_A, and glycine receptors (Enoch et al., 2011). Within this super-family, the closest resemblance to 5-HT₃ receptors is shown by nAChRs, which share about 30% structural homology (Maricq et al., 1991). There are several other significant similarities between 5-HT₃ and nACh receptors. For example, activation of both pre-synaptically located nACh and 5-HT₃ receptors results in Ca²⁺-dependent neurotransmitter release (Yamauchi et al., 2011) and mediates common physiological functions such as blood pressure and pain (Drisdell et al., 2008). Moreover, they are co-localized on nerve terminals in several brain pathways of reward processing, including dopaminergic terminals in the striatum (Nayak et al., 2000). However, the co-localized endogenous 5-HT₃ and nAChR receptors do not appear to interact physically; rather, they cross-regulate each other at a down-stream molecular level, possibly through regulation of intracellular Ca²⁺ concentrations (Dougherty and Nichols, 2009; Nayak et al., 2000). Together, these findings highlight the important role of the serotonergic pathway in the development of ND.

The 5-HT_{3A} subunit exists as a homomeric structure in the central nervous system. When the 5-HT_{3A} subunit combines with the 5-HT_{3B} subunit, they form pharmacologically more potent 5-HT_{3AB} heteropentameric receptor complexes, which are distributed throughout the limbic structures implicated in addiction (Davies et al., 1999; Dubin et al., 1999; Enoch et al., 2011). On the other hand, serotonin transporter (5-HTT) is the only molecule known to regulate synaptic serotonin concentrations through re-uptake into presynaptic nerve terminals. Thus, it self-modulates the availability of serotonin molecules for binding with the 5-HT_{3AB} receptors. For these reasons, in addition to perform association analysis at both the individual SNP and haplotype levels, we also examined gene-gene interactive effects on ND in this study, with the goal of determining whether variants in the genes encoding the serotonin transporter (*SLC6A4*) and receptor subunits 3A and 3B (*HTR3A* and *HTR3B*) play any role in ND.

2. MATERIALS AND METHODS

2.1 Subjects and ND Measures

Subjects of both African American (AA) and European American (EA) origin were recruited primarily from the US mid-South states of Tennessee, Mississippi, and Arkansas during 1999 to 2004. Proband smokers were required to be at least 21 years old, to have smoked for at least the last 5 years, and to have smoked at least 20 cigarettes per day during the last 12 months. Once proband smokers were identified, their biological parents and siblings were invited to participate whenever possible. Together, a total of 1366 individuals from 402 AA families and 671 individuals from 200 EA families were included. Table 1 provides the detailed characteristics of the two ethnic samples. All participants provided informed written consent, and the study was approved by the Institutional Review Boards of the University of Virginia and the University of Mississippi Medical Center.

The ND of each smoker was assessed with the three commonly used measures of Smoking Quantity (SQ; the number of cigarettes smoked per day), the Heaviness of Smoking Index (HSI; 0-6 scale), and the Fagerström Test for ND (FTND; 0-10 scale; Fagerstrom, 1978). Because of the overlap of the contents of the three measures, a fairly robust correlation exists among them in both populations ($r = 0.88-0.94$).

2.2 DNA Sample Processing, and Selection and Genotyping of Polymorphic Variants

Genomic DNA was extracted from the peripheral venous blood of each participant using a Maxi kit (Qiagen Inc, Valencia, CA) according to the manufacturer's protocol. Eight SNPs in *HTR3A* and seven SNPs in *HTR3B* were selected based on the following characteristics: minor allele frequency ≥ 0.05 as provided on NCBI website (<http://www.ncbi.nlm.nih.gov/snp/>) and known or putative functional importance of the variants. Detailed information on the 15 SNPs is provided in Supplementary Table 1¹. All 15 SNPs in *HTR3A* and *HTR3B* were genotyped using *TaqMan* assays in the 384-well microplate format (Applied Biosystems Inc., Foster City, CA) as reported previously (Beuten et al., 2005; Li et al., 2005; Sun et al., 2008). Briefly, 15 ng of DNA was amplified in a total volume of 7 μ l containing an MGB probe and 2.5 μ l of *TaqMan* universal PCR master mix. Allelic discrimination analyses were performed on the ABI Prism 7900HT Sequence Detection System. To ensure the quality of genotyping, four no-template negative controls and four positive controls were added to each 384-well plate.

We also selected two known functional polymorphisms in *SLC6A4* to examine the association of variations of serotonin transporters with ND. They are rs1042173, located in

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the 3'-UTR region, and the 5'-serotonin transporter-linked polymorphic region (5'-HTTLPR), located in the promoter region of *SLC6A4* (Lesch et al., 1999; Seneviratne et al., 2009). The 5'-HTTLPR long (L) and short (S) alleles were genotyped by PCR amplification of genomic DNA (15 ng) in a final volume of 20 μ l containing 5 $\times$ My *Taq* Reaction Buffer, *Taq* DNA polymerase, and 20 μ M each of the forward (5'-TCCTCCGCTTTGGCGCCTCTTCC-3') and reverse (5'-TGGGGGTTGCAGGGGAGATCCTG-3') primers. The PCR cycling conditions were: initial denaturation at 95°C for 1 min, followed by 30 cycles at 95°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 10 seconds. The PCR products were electrophoresed on a 3.5% agarose gel, and the amplicons were identified using ethidium bromide staining. The fragments at the 484- and 528-bp levels were identified as "S" and "L" alleles, respectively. The T/G alleles of SNP rs1042173 were genotyped using a *Taq*Man SNP assay with the same procedure used for SNPs in *HTR3A* and *HTR3B* as described above.

2.3 Statistical Analysis

To test for genotyping quality, we assessed Mendelian inconsistencies and departure from Hardy-Weinberg Equilibrium (HWE) using Haploview (v. 4.0) software (Barrett et al., 2005). Subjects with any inconsistent SNP data for a given genetic variant were excluded from further analysis.

2.3.1. Individual and haplotype-based association analysis—Associations between the 17 individual polymorphisms in *HTR3A*, *HTR3B*, and *SLC6A4* and the three ND measures were determined using the Pedigree-Based Association Test (PBAT; v. 3.5) based on the generalized estimating equation approach (Lange et al., 2003). Pair-wise linkage disequilibrium (LD) and haplotype blocks for the 15 SNPs in *HTR3A* and *HTR3B* were assessed by Haploview (v. 4.0) software (Barrett et al., 2005; Gabriel et al., 2002). Association analysis for haplotypes located within these LD blocks with the three ND measures was performed using the Family-Based Association Test (FBAT; v.1.7.3; Horvath et al., 2004). Three genetic models (additive, dominant, and recessive) were tested for all PBAT and FBAT association analyses, with sex and age included as covariates in the AA and EA samples and sex, age and ethnicity in the pooled sample. Statistically significant results ($p < 0.05$) for individual SNPs and major haplotypes (frequency $\geq 5\%$) were corrected for multiple testing using Bonferroni correction.

2.3.2. Gene-by-gene interaction analysis of HTR3A, HTR3B, and SLC6A4 variants—Gene-by-gene interactions among polymorphisms in *HTR3A*, *HTR3B*, and *SLC6A4* were analyzed using Pedigree-Based Generalized Multifactor Dimensionality Reduction (PGMDR) program (Lou et al., 2008). As with the association analysis described above, the three ND measures were covariant-adjusted for age and sex in the models for the AA and EA samples and for age, sex, and ethnicity in the models for the pooled sample. We performed an exhaustive search for all two- to five-locus polymorphic combinations within and among the three genes with interactive effects on ND measures. The best statistical gene-by-gene interaction model for a given order of interaction was determined by three factors: (1) its empirical P value for prediction accuracy (PA) evaluated by a permutation test based on 10^6 shuffles of the adjusted phenotypic values; (2) the P -value and cross-validation consistency obtained by the sign-test for PA implemented in MDR software (Hahn et al., 2003); and (3) association of the polymorphic combination with all three ND measures examined.

3. RESULTS

3.1 Quality control and pair-wise LD tests

Prior to performing association and genetic interaction analysis, we conducted Hardy-Weinberg Equilibrium tests on all 17 polymorphisms in *HTR3A*, *HTR3B*, and *SLC6A4*, which showed significant deviations ($P < 0.001$) for SNPs rs33940208 in the EA and rs2276305 in the AA samples. Thus, rs33940208 and rs2276305 were excluded from the association and interaction analyses within EA and AA samples, respectively. For the remaining polymorphisms, the HWE P values ranged from 0.015 to 1.0 in the AA and 0.293 to 1.0 in the EA sample.

3.2 Individual SNP-Based Association Analysis

Results from the individual SNP-based association analyses in the two ethnic samples and the pooled sample are shown in Table 2. Of the *HTR3A* polymorphisms, SNP rs10160548 showed marginal associations with SQ and HSI ($P = 0.030$ and 0.042 , respectively) in the AAs under an additive model. Of the *HTR3B* polymorphisms, the only variant significantly associated with SQ and FTND in the pooled samples was rs11606194, with a P value of 0.039 and 0.028 , under the additive model. Further, the polymorphism 5-HTTLPR in *SLC6A4* showed a marginal association with FTND ($P = 0.030$) in the EAs under the additive model. However, none of these associations remained significant after Bonferroni correction for multiple testing for 17 variants in the three genes (the corrected P value at 0.05 is 0.003).

3.3 Haplotype-Based Association Analysis

According to the haplotype block definition by Gabriel et al. (2002), we found four blocks within *HTR3A* and *HTR3B* in the AA, EA, and pooled samples (Figure 1) and no LD block between the two polymorphisms in *SLC6A4*.

We employed the FBAT program to perform haplotype-based association analysis for all different major haplotypes in each abovementioned LD block with the three ND measures in the AA (Table 3), EA (Table 4), and pooled (Table 5) samples. In AAs, there were two major haplotypes located in the 5' region of *HTR3A* that were significantly associated with the three ND measures: (1) G-C-C-T-A-T, formed by SNPs rs1150226, rs1062613, rs33940208, rs1985242, rs2276302, and rs10160548 (LD block 3; Figure 1), with a frequency of 19.5%, was associated significantly with SQ ($Z = 2.596$; $P = 0.009$), HSI ($Z = 3.027$; $P = 0.002$), and FTND ($Z = 2.824$; $P = 0.004$) in a dominant model; and (2) G-A, formed by SNPs rs1150220 and rs1176713 (LD block 4; Figure 1), with a frequency of 66.6%, was significantly associated with SQ ($Z = 3.041$; $P = 0.002$), HSI ($Z = 3.011$; $P = 0.003$), and FTND ($Z = 2.863$; $P = 0.004$). All abovementioned haplotype-based associations remained significant after Bonferroni correction for multiple testing for each LD block. We also detected a nominally significant association of haplotype A-T-C-A-G-G in the LD block 3 of *HTR3A* with a frequency of 24.1% with SQ under the dominant model ($Z = 1.996$; $P = 0.046$).

For the EA sample, we found no haplotypes in *HTR3A* or *HTR3B* showing significant association with ND (Table 4). In the pooled sample (Table 5), we found one haplotype, G-G-G formed by SNPs rs10160548, rs1150220, and rs1176713 of *HTR3A*, with a frequency of 13.5%, significantly associated with SQ ($Z = -2.377$; $P = 0.017$), HSI ($Z = -2.310$; $P = 0.021$) and FTND ($Z = -2.190$; $P = 0.029$). However, none of them remained significant after Bonferroni correction.

3.4 Gene-by-Gene Interaction Analysis of *HTR3A*, *HTR3B*, and *SLC6A4*

Considering the biological and pharmacological functions of the three genes in regulating serotonin signaling, we performed an exhaustive search of all possible two- to five-locus interaction models among the 17 polymorphisms in *HTR3A*, *HTR3B*, and *SLC6A4* for their epistatic effect on the three ND measures in the AA, EA, and pooled samples. As shown in Table 6, the detected best interaction model for each sample shows a significant genetic interaction effect on all three ND measures, with an empirical $P < 0.01$, cross-validation consistency (CVC) of at least 7 of 10, and test accuracies (TA) $> 50\%$ based on 10^6 permutation tests except for the model on FTND in the AA sample, where the empirical P value is 0.057. Of the three samples, the epistatic effect of the detected best interaction model on the three ND measures in the pooled sample appeared to be the strongest, with an empirical P value of 0.00025 to 0.00085.

4. DISCUSSION

By employing a series of association analyses at both the individual polymorphic and haplotype levels and genetic interaction analyses among 17 variants in *HTR3A*, *HTR3B*, and *SLC6A4*, we revealed significant interactions among the variants of these genes in affecting ND in the AA, EA, and pooled samples. This finding is highly significant and novel, as it highlights the significance of variations in genes encoding the serotonin transporter and receptors involved in governing trans-synaptic serotonergic signaling underlying the pathophysiology of ND.

In the pooled sample, an interaction model consisting of five loci in *HTR3A*, *HTR3B*, and *SLC6A4* showed significant epistatic effects on all the three ND measures examined. These five loci were rs1062613 and rs10160548 in *HTR3A*, rs1176744 in *HTR3B*, and 5-HTTLPR and rs1042173 in *SLC6A4*. Interestingly, the minor allele frequencies of these five polymorphisms are considerably high, with the lowest frequency being 0.211 for rs1042173 in *SLC6A4* in the AA sample and 0.244 for rs1062613 in *HTR3A* in the EA sample. Of them, three polymorphisms have been demonstrated to alter the expression of the RNA and/or protein encoded by the respective genes (Niesler et al., 2001). For example, the rs1062613 is a translation regulatory variant located in an open reading frame upstream of the translation initiation site of *HTR3A* mRNA (Niesler et al., 2001). The two polymorphisms in *SLC6A4* were shown to alter 5-HTT expression through transcription regulation for 5-HTTLPR and degradation of mRNA transcripts for rs1042173 (Heils et al., 1997, 1996; Seneviratne et al., 2009; Vallender et al., 2008). Of the remaining SNPs, rs10160548 is located in intron 6 near an intron-exon boundary. It is thus reasonable to speculate that it alters the expression of functional *HTR3A* transcripts through alternative splicing. The rs1176744 in *HTR3B* does not alter expression, but it was shown to substantially change serotonergic signaling through altered gating kinetics of the 5-HT_{3AB} receptor complex (Krzywkowski et al., 2008).

By analyzing the AA and EA samples independently, we revealed slightly different interaction models for each ethnic sample. In the AA sample, there was a significant interactive effect of SNPs rs10160548 in *HTR3A* and 5-HTTLPR and rs1042173 in *SLC6A4* on all three ND measures. Although the two *SLC6A4* polymorphisms were also included in the best interaction model detected in the EA sample, the model contained three additional loci: rs1062613 and rs1150220 in *HTR3A* and rs1176744 in *HTR3B*. In previous studies by other research groups, SNP rs1062613 in *HTR3A* has been associated with several psychiatric disorders in individuals of European descent (Gatt et al., 2010; Walstab et al., 2010). Yet, whether rs1062613 has ethnicity-specific *cis*-acting effects on the differential translation levels of *HTR3A* in AAs and EAs remains to be characterized. However, inclusion of rs1062613 in the best interaction model in the pooled samples, with

even stronger interaction effects than were seen in EAs only, argues against this possibility. The other *HTR3A* SNP, detected only in the EA sample, was rs1150220, which is moderately correlated with rs10160548 in both EAs and AAs ($r^2 = 0.42$ in AAs and $r^2 = 0.51$ in EAs) in an LD block located at the 3'-end of the *HTR3A* gene. The second main difference between AA and EA samples was the absence of *HTR3B* rs1176744 in the best model for AAs. Although SNPs rs1176744 and 5-HTTLPR in *SLC6A4* have been found to be significantly associated with alcohol dependence in AAs (Enoch et al., 2011), we found no significant association of these two polymorphisms with ND in our AA, EA, or pooled samples, except for 5-HTTLPR, which showed a marginal association with FTND in the EA sample. However, our genetic interaction analysis demonstrated the two polymorphisms in *SLC6A4* play an important role in ND through interactions with other SNPs in *HTR3A* and *HTR3B* in the AA, EA, and pooled samples.

Another noteworthy consideration of our results is that none of the polymorphisms included in the above-mentioned epistatic models was significant at the individual locus level. Significant epistatic effects of variants without major genetic effect have become an increasingly identified phenomenon in studies of complex disorders (Li et al., 2008; Steen, 2012; Zuk et al., 2012). For example, several other studies and the present study have shown marginal or no association of the polymorphism 5-HTTLPR in *SLC6A4* with ND (Gerra et al., 2005; Trummer et al., 2006). Yet, as clearly demonstrated by our gene-by-gene interaction analysis, the effect of 5-HTTLPR on ND is highly significant when its epistatic effect is taken into consideration. Another unique strength of our findings is that the interaction models detected in AAs, EAs, and the pooled samples were highly significant across multiple ND measures, providing further support for their role in smoking-related behaviors.

The biological basis for the genetic interaction effect we detected can be explained by the effects of nicotine on serotonergic signaling. Nicotine competes with its natural ligand serotonin for 5-HT₃ receptors (Breitinger et al., 2001). Depending on whether the 5-HT₃ receptors are located pre- or post-synaptically, nicotine binding either can result in the release of various neurotransmitters or affect the propagation of fast-acting serotonergic signal along the post-synaptic neuron. The availability of synaptic serotonin for binding to the 5-HT₃ receptors is modulated by the presynaptic 5-HTTs. Prior studies have reported mixed effects of chronic nicotine exposure on the density of 5-HTTs, thus regulating the amount of synaptic serotonin available for 5-HT₃ receptors. For example, Semba and Wakuta (2008) reported a reduction in the density of 5-HTTs in the rat brain whilst two other studies reported an elevation in 5-HTTs (Awtry and Werling, 2003; Slotkin and Seidler, 2010). On the other hand, Staley et al. (2001) reported an elevation 5-HTTs in the human brain, and in human platelets they were reported to be reduced (Patkar et al., 2003). Serotonin plays a crucial role mediating cognitive behavioral functions, stress response, mood, appetite, and motor functions (Jasinska et al., 2012). Thus, the interactions among the three genes may represent the interacting biological effects of nicotine on fast-acting serotonergic signaling in ND.

In summary, by examining association of variants in *HTR3A*, *HTR3B*, and *SLC6A4* with three ND measures at both individual SNP and haplotype levels, we only found marginal association of rs10160548 in *HTR3A*, rs11606194 in *HTR3B*, and 5-HTTLPR in *SLC6A4* with one or two ND measures in one of the three samples. However, when we examined these variants interactively through gene-gene interaction approach, we identified a combination of functional polymorphisms in the three genes with significant genetic interaction effects on ND. This indicates these genetic variants play a significant role in ND through a genetic epistatic effect. This suggests it is important to investigate genetic epistatic

effect when one searches susceptibility loci for a complex trait such as ND investigated in this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. LD structures for *HTR3B* and *HTR3A* SNPs in the AA, EA, and Pooled samples. Haploview (v. 3.2; Barrett et al., 2005) was used to calculate all D' values, and haplotype blocks were defined according to Gabriel et al. (2002). The number in each box represents the D' value for each SNP pair surrounding that box.

Table 1

Clinical Characteristics of AA, EA, and Pooled Samples

Characteristic	African-American	European-American	Pooled
No. of nuclear families	402	200	602
Avg. members/family	3.14±0.75	3.17±0.69	3.15±0.73
No. of subjects	1,366	671	2,037
Female (%)	66.1	69.5	67.2
Age (years)	39.4±14.4	40.5±15.5	39.7±14.8
No. of smokers	1,053	515	1,568
Age of smoking onset	17.3±4.7	15.5±4.4	16.7±4.7
Years smoked	20.4±12.5	23.2±13.5	21.3±12.9
Smoking quantity/day	19.4±13.3	19.5±13.4	19.5±13.3
HSI	3.7±1.4	3.9±1.4	3.8±1.4
FTND score	6.26±2.15	6.33±2.22	6.29±2.17

Notes: HSI = Heaviness of Smoking Index; FTND = Fagerstrom Test for ND.

Table 2

Minor Allele Frequencies and P values for Associations of Individual SNPs/Polymorphism with Three ND measures in the AA, EA and Pooled Samples

GENE	SNP ID/Polymorphism	African-American sample				European-American sample				Pooled sample			
		Minor Allele (MAF)	SQ	HSI	FTND	Minor Allele (MAF)	SQ	HSI	FTND	Minor Allele (MAF)	SQ	HSI	FTND
HTR3B	rs3758987	T(0.371)	0.170 ^d	0.214 ^d	0.305 ^d	T(0.302)	0.691 ^a	-0.936 ^a	0.989 ^a	T(0.350)	0.191 ^d	0.321 ^d	0.365 ^d
	rs11606194	T(0.016)	-0.149 ^f	-0.151 ^f	-0.153 ^f	T(0.074)	-0.088 ^a	-0.156 ^a	-0.163 ^a	T(0.034)	-0.039^a	-0.070 ^a	-0.028^a
	rs4938056	T(0.371)	-0.293 ^d	-0.573 ^d	-0.860 ^d	T(0.470)	-0.239 ^d	-0.183 ^d	-0.308 ^d	T(0.420)	0.292 ^f	0.266 ^f	0.431 ^f
	rs1176744	C(0.476)	-0.582 ^f	-0.479 ^f	-0.518 ^f	C(0.326)	0.496 ^f	0.721 ^f	0.787 ^f	C(0.430)	-0.895 ^a	-0.579 ^a	-0.664 ^a
	rs2276305	Excluded from analysis as HWE p<0.001				G(0.006)	-0.988 ^a	-0.868 ^a	-0.789 ^a	G(0.035)	0.388 ^a	0.127 ^a	0.347 ^a
	rs1672717	G(0.083)	-0.220 ^f	-0.181 ^f	-0.141 ^f	G(0.350)	0.892 ^a	0.336 ^d	0.588 ^a	G(0.165)	-0.545 ^a	-0.493 ^f	-0.640 ^f
HTR3A	rs17614942	C(0.104)	-0.336 ^f	-0.469 ^f	-0.831 ^f	C(0.074)	-0.736 ^a	-0.252 ^a	-0.260 ^a	C(0.095)	-0.338 ^f	-0.396 ^a	-0.492 ^a
	rs1150226	G(0.314)	-0.081 ^f	-0.086 ^f	-0.106 ^f	G(0.089)	-0.550 ^d	-0.235 ^d	-0.221 ^d	G(0.244)	-0.133 ^f	-0.097 ^f	-0.129 ^f
	rs1062613	T(0.463)	-0.060 ^a	-0.066 ^a	-0.116 ^a	T(0.244)	0.681 ^d	-0.967 ^d	-0.798 ^d	T(0.395)	-0.147 ^a	-0.098 ^a	-0.151 ^a
	rs33940208	T(0.146)	0.685 ^d	-0.717 ^f	-0.732 ^f	Excluded from analysis as HWE p<0.001				T(0.113)	-0.356 ^f	-0.308 ^f	-0.385 ^f
	rs1985242	T(0.333)	0.109 ^a	0.161 ^a	0.283 ^a	T(0.322)	-0.503 ^d	-0.349 ^d	-0.216 ^d	T(0.441)	0.148 ^a	0.133 ^a	0.183 ^a
	rs2276302	G(0.496)	0.135 ^a	0.175 ^a	0.366 ^a	G(0.338)	0.595 ^f	0.690 ^f	0.735 ^f	G(0.452)	-0.259 ^a	-0.234 ^a	-0.292 ^d
SLC6A4	rs10160548	T(0.262)	0.030^d	0.042^a	0.090 ^a	T(0.309)	0.724 ^d	0.595 ^d	0.521 ^d	T(0.396)	0.063 ^d	0.092 ^d	0.174 ^d
	rs1150220	G(0.132)	-0.390 ^a	-0.678 ^a	-0.840 ^a	G(0.202)	-0.491 ^f	-0.340 ^f	-0.193 ^f	G(0.154)	-0.377 ^f	-0.359 ^f	-0.295 ^f
	rs1176713	G(0.321)	-0.421 ^f	-0.417 ^f	-0.449 ^f	G(0.216)	-0.231 ^f	-0.105 ^f	-0.062 ^f	G(0.288)	-0.201 ^f	-0.161 ^f	-0.154 ^f
	5-HTTLPR	S(0.227)	-0.528 ^d	-0.615 ^a	-0.597 ^a	S(0.433)	-0.058 ^a	-0.095 ^a	-0.030^a	S(0.290)	-0.132 ^a	-0.190 ^a	-0.105 ^a
	rs1042173	T(0.211)	-0.080 ^d	-0.154 ^d	-0.130 ^d	T(0.441)	-0.368 ^a	-0.304 ^a	-0.259 ^a	T(0.281)	-0.065 ^d	-0.118 ^d	-0.087 ^d

Notes: 1) MAF: minor allele frequency. 2) Significant associations at the 0.05 level before correction for multiple testing are given in bold. 3) Superscripts indicate genetic model used for analysis: 4) For each ethnic-specific sample, age and sex were used as covariates; for the combined sample, age, sex, and ethnicity were used as covariates. Negative signs indicate protective effect with the model specified in superscripted letters.

^a additive

d_{dominant}
 $r_{\text{recessive}}$

Table 3
Haplotypes within HTR3B and HTR3A Associated with Three ND Measures in AA Sample

rs3758987-rs11606194 (HTR3B)										SQ				HSI				FTND				
1	2	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
T	T	0.617	197	1.164	0.244 ^a		210	1.283	0.200 ^a		226	1.309	0.190 ^a		226	1.309	0.190 ^a		226	1.309	0.190 ^a	
C	T	0.366	79	-0.665	0.506 ^r	0.072 ^a	212	-0.652	0.514 ^a	0.050 ^d	171	-0.577	0.564 ^d	0.013 ^a								

rs1176744-rs1672717 (HTR3B)										SQ				HSI				FTND				
4	6	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
C	A	0.471	104	-0.605	0.545 ^r		111	-0.734	0.463 ^r		117	-0.841	0.400 ^r		117	-0.841	0.400 ^r		117	-0.841	0.400 ^r	
A	A	0.448	191	1.765	0.078 ^a		205	1.814	0.070 ^a		214	1.825	0.068 ^a		214	1.825	0.068 ^a		214	1.825	0.068 ^a	
A	G	0.067	74	-1.637	0.102 ^a	0.092 ^a	79	-1.485	0.138 ^a	0.112 ^a	82	-1.638	0.101 ^a	0.115 ^a								

rs1150226-.....-rs10160548 (HTR3A)										SQ				HSI				FTND				
8	9	10	11	12	13	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
A	T	C	A	G	G	0.241	102	1.996	0.046 ^d		112	1.460	0.144 ^d		119	1.110	0.267 ^d		119	1.110	0.267 ^d	
G	C	C	T	A	T	0.195	107	1.964	0.050 ^a		117	2.542	0.011 ^a		124	2.290	0.022 ^a		124	2.290	0.022 ^a	
G	T	C	A	G	G	0.145	103	2.596	0.009 ^d	0.006 ^d	114	3.027	0.002 ^d		120	2.824	0.004 ^d	0.015 ^d	120	2.824	0.004 ^d	
G	C	T	A	A	G	0.105	75	-0.696	0.486 ^d		14	-0.812	0.417 ^r		114	0.871	0.384 ^d		114	0.871	0.384 ^d	
G	C	C	T	A	G	0.079	72	-0.749	0.454 ^d		80	-0.972	0.331 ^a		85	-1.250	0.211 ^d		85	-1.250	0.211 ^d	
											79	-0.835	0.404 ^d		83	-0.726	0.468 ^d		83	-0.726	0.468 ^d	

rs1150220-rs1176713 (HTR3A)										SQ				HSI				FTND				
14	15	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
G	A	0.666	187	2.560	0.010 ^a		202	2.663	0.008 ^a		209	2.677	0.007 ^a									
G	G	0.202	110	3.041	0.002 ^d	0.037 ^a	119	3.011	0.003 ^d	0.035 ^a	123	2.863	0.004 ^d	0.033 ^a								
G	G	0.202	25	-1.971	0.049 ^r	0.017 ^d	26	-2.025	0.043 ^r	0.024 ^d	26	-1.934	0.053 ^r	0.037 ^d								

rs1150220-rs1176713 (<i>HTR3A</i>)															
				SQ				HSI				FTND			
14	15	G	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
A		G	0.123	112	-1.956	0.050^a		121	-1.695	0.090 ^a		123	-1.486	0.137 ^a	

Notes: 1) Only major haplotypes (>5%) are shown; the one with a P value<0.05 is given in bold. 2) Statistically significant P values after Bonferroni correction for individual haplotypes within each haplotype block (given in separate panels) are shown bold italics. 3) Superscripts indicate genetic model used in the analysis; 4) ND measures were corrected for age and sex.

^a additive

^d dominant

^r recessive.

Table 4

Haplotypes within *HTR3A* Associated with Three ND Measures in EA Sample

rs10160548-...-rs1176713				SQ			HSI			FTND		
13	14	15	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z Score	P haplotype	P global	P haplotype
T	G	A	0.680	22	1.494	0.135 ^d		25	1.626	0.104 ^d		0.141 ^d
G	A	G	0.190	56	0.612	0.541 ^a	0.035^a	60	0.560	0.576 ^a	0.036^a	0.792 ^a
G	G	A	0.111	38	-0.924	0.355 ^a		37	-0.966	0.334 ^d		0.495 ^d

Notes: 1) Only major haplotypes with a frequency of >5% are shown; those with a P value <0.05 are given in bold. 2) Statistically significant P values after Bonferroni correction for individual haplotypes within each haplotype block (given in separate panels) are shown bold italics. 3) Superscripts indicate genetic model used in the analysis: r = recessive. 4) ND measures were corrected for age and sex.

^a additive
^d dominant

Table 5
Haplotypes within *HTR3A* Associated with Three ND Measures in Pooled Sample

rs1150226-....-rs2276302						SQ			HSI			FTND					
8	9	10	11	12	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
G	C	C	T	A	0.408	182	1.823	0.068 ^d		200	1.853	0.064 ^d		202	2.209	0.027 ^d	
A	T	C	A	G	0.187	41	-1.153	0.249 ^r		42	-1.008	0.314 ^r		43	-0.907	0.365 ^r	
G	T	C	A	G	0.142	20	-1.420	0.156 ^r	0.080 ^d	22	-1.372	0.170 ^r	0.113 ^a	180	1.631	0.103 ^d	0.049 ^d
G	C	C	A	A	0.075	87	-0.817	0.414 ^a		93	-1.164	0.244 ^a		99	-1.262	0.207 ^a	
G	C	C	A	G	0.060	73	0.572	0.568 ^a		85	0.675	0.500 ^a		98	0.650	0.516 ^a	

rs10160548--rs1176713						SQ			HSI			FTND			
13	14	15	Freq	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global	No. of families	Z score	P haplotype	P global
T	G	A	0.390	231	1.464	0.143 ^a		199	1.386	0.166 ^d		208	1.235	0.217 ^d	
G	G	A	0.318	219	1.411	0.158 ^a		190	2.240	0.025 ^a		201	2.244	0.025 ^a	
G	A	G	0.141	154	-0.637	0.524 ^a	0.011 ^a	106	-1.313	0.189 ^a	0.028 ^a	109	-1.108	0.268 ^a	0.023 ^a
G	G	G	0.135	22	-2.377	0.017 ^r		23	-2.310	0.021 ^r		167	-2.190	0.029 ^a	

Notes: 1) Only major haplotypes with a frequency of >5% are shown; those with a P value <0.05 are given in bold. 2) Superscripts indicate genetic model used in the analysis: 3) ND measures were corrected for age, sex, and ethnicity.

^a additive

^d dominant

^r recessive.

Table 6

Detected Best SNP Combination of SLC6A4, HTR3A, and HTR3B Associated with ND Measures in EA, AA, and Pooled Sample on Basis of Test Accuracy and Empirical P value from 10^6 Permutations

Sample	SNP Combination	ND Measure	Test Accuracy	Cross-Validation Consistency (CVC)	Permutated P value
EA	<i>HTR3A</i> : rs1062613, rs1150220;	SQ	0.5678	7	0.003
	<i>HTR3B</i> : rs1176744;	HSI	0.5699	9	0.002
	<i>SLC6A4</i> : 5-HTTLPR, rs1042173	FTND	0.5703	10	0.002
AA	<i>HTR3A</i> : rs10160548;	SQ	0.5500	10	0.005
	<i>SLC6A4</i> : 5-HTTLPR, rs1042173	HSI	0.5458	10	0.009
		FTND	0.5317	8	0.057
Pooled	<i>HTR3A</i> : rs1062613, rs10160548;	SQ	0.5516	8	0.00051
	<i>HTR3B</i> : rs1176744;	HSI	0.5547	8	0.00025
	<i>SLC6A4</i> : 5-HTTLPR, rs1042173	FTND	0.5479	10	0.00085