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Significant association of *CHRNB3* variants with nicotine dependence in multiple ethnic populations

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Smoking dependence is a complex trait that is significantly influenced by genetics, with an estimated heritability of 0.56. Previous genome-wide association meta-analysis revealed that rs6474412, located about 2.1 kb from the 5′ end of *CHRNB3*, is significantly associated with the number of cigarettes smoked per day.[1] In addition, nominal associations of several variants in or near the *CHRNB3* gene with smoking behavior have been reported.[2–5] However, almost all subjects used in these studies were of European ancestry. Given the demonstrated differences across ethnicities with respect to genetics, physiological processes and behavior underlying nicotine dependence (ND),[6–11] it is of great interest to examine whether this gene also is associated with ND in smokers of other ancestries. To attack this issue, we performed a meta-analysis of variants in *CHRNB3* in relation to ND by combining data from the studies of subjects of different ethnicities.

Techniques for ascertainment, diagnostic assessments, genotyping, quality control and analysis are detailed elsewhere.[12–14] The four samples included in this study can be described briefly as follows. (1) Mid-South Tobacco Case Control Study (MSTCC): This population consists of 4548 smokers and non-smokers aged 18 years or older of either African American (AA) ($N = 3161$) or European American (EA) ($N = 1387$) origin, who were recruited primarily from the city of Jackson, Mississippi during 2005–2011. Although questionnaires assessing various smoking-related behaviors were administered to each participant, only the Fagerström Test for Nicotine Dependence (FTND) data were analyzed in this study. (2) Study of Addiction: Genetics and Environment (SAGE):[12] This study involves three samples: the Collaborative Study on the Genetics of Alcoholism, the Family Study of Cocaine Dependence (FSCD) and the Collaborative Genetic Study of Nicotine Dependence. The FTND score was available for all case subjects; some of them were addicted to cocaine, alcohol, marijuana, opiate and/or other substances as well as tobacco. To minimize the effects of other addictive phenotypes on the association results of *CHRNB3* variants with ND, all the phenotypes were included as covariates in our association analysis. For the FSCD sample, only one subject from each family was used. Together, a total of 2428 subjects with EA ancestry and 1136 with AA ancestry were included. (3) A Genome Wide Scan of Lung Cancer and Smoking (CGEMS):[13] This study involves two samples. The first contains about 2000 patients with newly diagnosed lung cancer and around 2000 age-, sex- and region-matched controls, all recruited from the Lombardy region of Italy.[15] The second sample contains about 850 lung cancer patients and about 850 age- and sex-matched controls.[16] Almost all subjects of this study were assessed by a variety of smoking measures, including FTND score, and were of European ancestry. (4) Korea Association Resource (KARE) study:[14] All subjects in this study were recruited from two areas, Ansung and Ansan, in South Korea. After appropriate quality control and filtering, 8842 individual samples (4183 men and 4659 women) were eligible. Because no FTND score was available for these smokers, the ND phenotype was analyzed as an ordinal trait with five categories (1–5), as we did previously on this sample[17,18] according to the number of cigarettes smoked per day: non-smoking, <10 cigarettes/day, 11–20 cigarettes/day, 21–30 cigarettes/day, and >31 cigarettes/day, respectively. All studies were conducted under the appropriate ethical approvals, and all subjects provided written informed consent.

Except for the MSTCC DNA samples, which were genotyped with *Taq*Man assay in a 384-well microplate format, genotyping results for the SAGE and CGEMS samples came from the National Institutes of Health database of Genotypes and Phenotypes. Except for Single Nucleotide Polymorphism (SNP) rs6474412 selected from the reported study,[1] other six SNPs were selected according to our preliminary association analysis results of SAGE data. Quality control was performed in each sample separately with the goal of removing those individuals with sex anomalies, low call rate or first- or second-degree relatedness. For those non-genotyped SNPs in the original SAGE, CGEMS and KARE data sets, we conducted imputation using MaCH[19] and IMPUTE (v2).[20] To make the SNPs consistent across different samples, we imputed rs6474412 for SAGE, 6 SNPs except rs10958725 for CGEMS and all 7 SNPs for KARE using the 1000 Genome EUR v2 (2010–11 release) for samples of European origin, the 1000 Genome AFR v2 (2010–11 release) for samples of African origin, and the HapMap Phase II CHB + JPT for samples of Asian origin as reference panels.

Following appropriate quality control and imputation, we conducted a meta-analysis of six samples of African, European or Asian ancestry. For each sample, association analysis was performed on smokers only using a linear regression model by regressing FTND scores for the MSTCC, SAGE and CGEMS samples and indexed CPD for the KARE sample on age, sex, SNP allele dosage and other cohort-specific covariates in PLINK,[21] and all non-smokers were excluded from the regression analysis. Supplementary Table 1 shows detailed information on each sample, including descriptions, the covariates included in the regression model, sample size of original study, and sample size of smokers used in the current analysis and their corresponding ND measure and related ND statistics. Supplementary Table 2 presents detailed association analysis results of each SNP among all samples, which include allele frequency for positive effect allele, beta value, and *P* value. Fixed-effects meta-analysis was conducted in METAL[22] using the inverse variance-weighted method, which has the advantage of calculating the effect size and corresponding standard error for each SNP of interest. Heterogeneity within each ethnic sample(s) or among different ethnic samples was assessed with I^2 in METAL, which measures the degree of inconsistency among the results from different samples.[23]

As shown in Table 1, our meta-analysis of the association of *CHRNB3* variants with ND in the three ethnic populations revealed the following main findings. (A) All alleles with positive effects for the seven SNPs in *CHRNB3* are the same in the three populations,

Table 1. Meta-analysis results for association of seven SNPs in *CHRNB3* gene with ND based on inverse variance

dbSNP ID	*Allele (+/−)*	*African-American samples*				*European-American samples*				*Asian Sample*				*All samples*		
		Freq (s.e.) (+)	*Beta (s.e.)*	P-*value*	I^2	*Freq (s.e.) (+)*	*Beta (s.e.)*	P-*value*	I^2	*Freq (+)*	*Beta (s.e.)*	P-*value*	I^2	*Beta (s.e.)*	P-*value*	I^2
rs10958725	G/T	0.307 (0.0006)	0.1196 (0.0423)	0.0047	0	0.777 (0.0146)	0.2242 (0.0542)	3.55E-05	0	0.837	0.1784 (0.0724)	0.0137	0	0.1626 (0.0303)	8.09E-08	0
rs10958726	T/G	0.398 (0.0003)	0.1018 (0.0398)	0.0105	0	0.776 (0.0136)	0.2354 (0.0542)	1.39E-05	0	0.849	0.1847 (0.0714)	0.0097	0	0.1546 (0.0292)	1.24E-07	22.8
rs4736835	C/T	0.348 (0.001)	0.1105 (0.0402)	0.0060	0	0.775 (0.0142)	0.2378 (0.0541)	1.10E-05	0	0.849	0.1813 (0.0713)	0.0110	0	0.1602 (0.0294)	5.07E-08	8.5
rs6474412	T/C	0.344 (0.0104)	0.1068 (0.0407)	0.0087	0	0.775 (0.015)	0.2306 (0.0541)	2.05E-05	8	0.914	0.1844 (0.0979)	0.0597	0	0.1548 (0.0309)	5.34E-07	23.5
rs4950	T/C	0.272 (0.0009)	0.05 (0.0433)	0.2485	0	0.772 (0.0172)	0.2374 (0.0539)	1.07E-05	0	0.849	0.1825 (0.0713)	0.0104	0	0.1343 (0.0305)	1.08E-05	40.4
rs13280604	A/G	0.275 (0.0015)	0.0567 (0.0432)	0.1892	0	0.773 (0.0175)	0.2337 (0.0538)	1.41E-05	0	0.848	0.1811 (0.0711)	0.0109	0	0.1362 (0.0305)	7.77E-06	38.2
rs6474415	A/G	0.232 (0.0039)	0.0606 (0.0452)	0.1803	0	0.772 (0.0185)	0.2294 (0.0538)	1.97E-05	0	0.848	0.18 (0.0711)	0.0113	0	0.14 (0.0311)	6.81E-06	39.9

except that allele frequencies in the samples of African ancestry are different from those of European and Asian ancestry, where the last two ethnic samples appear to be similar. (B) There exists no heterogeneity among samples within each ethnic population for all seven SNPs, and low-to-moderate heterogeneity among the three populations for a few SNPs in the *CHRNB3* gene. (C) All seven SNPs in or near the *CHRNB3* gene showed significant association with ND, with *P*-values ranging from 5.1×10^{-8} for rs4736835 to 1.1×10^{-5} for rs4950. Of the SNPs, rs10958725 and rs4736835 reached the genome-significance level. [24] Supplementary Figure 1 shows forest plots for the seven SNPs among these samples. For each ethnic sample, significant association with ND was detected for all seven SNPs in the samples of European and Asian ancestry and for four SNPs in the samples of African origin. (D) As shown in Supplementary Figure 2, these seven SNPs show a high linkage disequilibrium (LD) in each ethnic sample. Thus, it remains to be determined, which SNP(s) contributes to the significant association of *CHRNB3* variants with ND.

In conclusion, this study provides convincing evidence for a role of *CHRNB3* in ND. Most importantly, we derived this conclusion by analyzing samples of three representative ancestries from throughout the world, which represents a significant extension of earlier reports where only subjects of European origin were analyzed. Further, our results indicate this region is rather homogeneous across the three ethnic populations, implying any causative variants identified in this gene could be important for almost all smokers, regardless of ancestry. Unfortunately, because of the high LD of the genomic region where these causative variants are located, what they are remains to be further examined.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Age-dependent effect of the *MAOA* gene on childhood physical aggression

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Quantitative genetic studies suggest that genetic contributions account for a substantive part of a range of disruptive behaviors.[1] At a molecular level, Caspi *et al.*[2] reported no main effect of the monoamine oxidase A (*MAOA*) gene but an interactive effect with early maltreatment in the prediction of aggressive behavior. Later studies using different research designs as well as various measures of disruptive behavior and early adversity have yielded mixed results. These studies mainly relied on one or two time point assessments in adolescence or adulthood; few studies focused on childhood.[3,4] As such, no study has yet modeled explicitly an age-dependent contribution of MAOA. Suggestions have been made as to why the effect of genotype may decrease or increase with age.[5] Accordingly, we examined the age-dependent contribution of MAOA alone or in interaction with family socioeconomic adversity to the frequency of physical aggression during the elementary school years.

We selected 436 boys from a longitudinal study of kindergarten children in Quebec (Canada). The study sample has been described elsewhere.[6] The boys' frequency of physical aggression was rated annually from age 6 to 12 years by teachers with the Social Behavior Questionnaire[7] (each item rated on a three-point scale (0–2, from 'never applies' to 'frequently applies'). Three items were used: (1) fights, (2) bullies and (3) kicks, bites, or hits (alphas ranging from 0.79 to 0.86). We used an index of family socioeconomic adversity based on information collected at the start of the study[6] about: (1) family structure, (2) parents' levels of education, (3) parents' occupational status and 4) parents' age at the birth of the first child. We imputed the values for 20 participants from the constituent variables of the index and from behavioral characteristics of the boys at 6 years. We assessed common tag single-nucleotide polymorphisms (SNPs; minor allele frequency $>5\%$) and SNPs located up to 5-kbp upstream of the transcription site. Tag SNPs were obtained using HapMap and Tagger's multimarker-tagging procedure ($r^2 > 0.8$).[6] To reduce the number of statistical comparisons, only the most informative SNPs were selected from our genetic database using an algorithm based on r^2 linkage disequilibrium.[8] Table 1 presents the selected SNPs and their frequencies. Details on genetic assessments for this sample are provided elsewhere.[6]

We utilized a Latent Growth Modeling framework to model age-dependent effects (Full Information Maximum Likelihood and Maximum Likelihood Robust estimator). The baseline model without predictors indicated a linear decline of physical aggression ($P<0.001$), with a slope estimated to -0.06 each year, corresponding to a decline from a mean score of 1.04 at 6 years to 0.68 at 12 years (coherent with the well-documented decline of physical aggression from 3 to 4 years onwards).[9] Then, we estimated one model for each SNP by entering a given SNP and the adversity index as predictors. Adversity made a significant positive contribution to the initial level (but not the slope) of physical aggression in every model (β between 0.15 and 0.16; all $P<0.01$). The SNP rs5906957 had a significant main effect on the slope of physical aggression, meaning that levels of aggression for T carriers decreased less (linear decline of -0.01 each year) than for C carriers (-0.08 each year). T carriers also had a trend toward lower initial levels of physical aggression than C carriers ($P=0.05$). Therefore, T carriers tended to have lower initial level of physical aggression but this initial level remained relatively stable, whereas it decreased for C carriers. Table 1 shows similar results for rs5953385 and rs2283725. All models fitted well: standardized root mean square residual <0.10; root mean square error of approximation <0.05; comparative fit index >0.95. No interaction was detected between adversity and any SNP for initial level or slope.

The results suggest that the *MAOA* gene may have a role in the development of physical aggression prior to adolescence. For example, the stable physical aggression levels of T carriers for rs5906957, compared with the declining levels of C carriers, suggest that T carriers do not take advantage of the socialization forces exerted on physical aggression during the elementary school years. A previous study[4] assessed children at one point (7 years) and reported a significant genetic main effect (but no significant interaction) for antisocial behavior. Unexpectedly, children with the *high* activity MAOA allele had higher levels of antisocial behavior. Our study shows that a developmental approach may shed light on these findings: an initial genetic trend in one direction may be progressively overridden by a developmental genetic effect (as exemplified by C carriers above). We did not detect any significant interaction for either the initial level or the slope of physical aggression. Previous investigations conducted in adolescence and adulthood suggest that interactions may emerge during adolescence.[3]

Our measure of family socioeconomic adversity did not include maltreatment: the present study should not be directly compared with findings using maltreatment, and was not designed to replicate earlier findings.[2] Although not all SNPs reached the significance threshold, all effects were in the same direction. However, caution is required until the present findings are replicated. Overall, these results call for a more systematic