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Association study of 45 candidate genes in nicotine dependence in Han Chinese

Jinxue Wei [a,b,1], Chengjing Chu [a,c,1], Yingcheng Wang [a,b], Yanchun Yang [a], Qiang Wang [a,b], Tao Li [a,b], Lan Zhang [a,*], Xiaohong Ma [a,b,**]

[a] Psychiatric Laboratory and Department of Psychiatry, West China Hospital, Sichuan University, Chengdu 610041, PR China
[b] National Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, PR China
[c] Department of Psychology, Guangdong Medical College, Dongguan 523808, PR China

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ABSTRACT

Numerous genetic linkages, association studies have been performed in different ethnic groups and revealed many susceptibility loci and genes for nicotine dependence. However, limited similar researches were performed in Han Chinese. This study was designed to investigate the association of candidate genes with nicotine dependence in Han Chinese. We genotyped 384 SNPs within 45 candidate genes with nicotine dependence in a Han Chinese population consisting 223 high nicotine dependent subjects and 257 low nicotine dependent subjects by employing GoldenGate genotyping assay (Illumina). Following association analysis was performed using PLINK software. Individual SNP-based association analysis revealed that nine SNPs located in *DRD3* (rs2630351), *DRD5* (rs1967550), *MAP3K4* (rs2314378), *DDC* (rs11575461), *CHRNB3* (rs4954), *GABBR2* (rs2779562), *DRD2* (rs11214613 and rs6589377) and *CHRNA4* (rs2236196) were significantly associated with FTND after correction for multiple testing with the *p* values from 2.59×10^{-7} to 9.99×10^{-5}. Haplotype-based association analysis revealed haplotype G-A-A formed by rs2630351, rs167771 and rs324032 and haplotype G-G-G-A formed by rs3773678, rs2630349, rs2630351 and rs167771 in *DRD3*; haplotype of G-A formed by rs2779562 and rs2808566 in *GABBR2* and haplotype of T-T-A-G-A formed by rs6832644, rs4057797, rs9764, rs4552421 and rs10033119 in *NPY1R* are associated with FTND ($p=3.61\times10^{-7}$–8.78×10^{-6}). Our results provided confirmation of the previous findings that DRD2, *DRD3*, *DDC*, *CHRNB3*, *GABBR2* and *CHRNA4* are associated with nicotine dependence. Furthermore, we for the first time report a significant association between nicotine dependence and *DRD5*, *MAP3K4* and *NPY1R*. These findings need independent replication in the future studies.

1. Introduction

Tobacco use causes severe health problems worldwide. According to the World Health Organization, more than 1.2 billion people smoke in the world (WHO, 2008). Smoking-related diseases, e.g. cancer, cardiovascular and respiratory diseases and susceptibility to infectious diseases, are responsible each year for 5.4 million deaths worldwide (Benowitz, 2008; CDC, 2005; Friedman, Pross, & Klein, 2006); this number is predicted to increase to 8.3 million at 2030 (WHO, 2008). The western pacific region of the WHO which covers East Asia has the highest smoking rate. The smoking rates in Chinese aged 15 or older are 66.0% in males and 3.1% in females (MOHC, 2006). Accordingly, more than 350 million Chinese people use tobacco, almost one third of the total smokers in the world (CCDC, 2009).

Substantial evidence indicates that nicotine is the main psychoactive substance in the smoke of tobacco (Benowitz, 2008; Benowitz et al., 1999). Data from a meta-analysis of 17 twin studies have shown that the weighted mean heritability for nicotine dependence is 0.56 for adult smokers (Li, Cheng, Ma, & Swan, 2003). These results indicate that nicotine dependence is affected by both environmental and genetic factors.

Considerable efforts have been made to explore the genetics factors that affect nicotine dependence. Genome-wide linkage studies have identified many genomic regions located on chromosomes 3–7, 9–11, 17, 20, and 22 that are associated with nicotine dependence (Li, 2008). Candidate gene and genome-wide SNP-based association studies have identified numerous susceptibility genes for nicotine dependence in the past few years (Sullivan et al., 2004). High-throughput expression analysis also identified many genes related to nicotine administration in cell or animal models (Hwang & Li, 2006; Konneker et al., 2008; Li, Konu, Kane, & Becker, 2002; Wang et al., 2007). These genes are involved in multiple biological pathways and functions, e.g. neurotransmitter systems, metabolic enzymes and growth factors. The susceptibility loci and candidate genes for nicotine dependence that identified in

* Correspondence to: L. Zhang, No. 1 Keyuan Si Road, High Tech Parkm, Chengdu 610041, PR China. Tel./fax: +86 28 85164019.
** Correspondence to: X. Ma, Psychiatric Laboratory and Department of Psychiatry, West China Hospital, Sichuan University, Chengdu 610041, PR China. Tel./fax: +86 28 85164019.
E-mail addresses: lanzhang3@gmail.com (L. Zhang), maxiaohong2002@yahoo.com.cn (X. Ma).
[1] These authors contributed equally to this work.

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previous genetic, bioinformatics and other biological analysis have been very well summarized (Li, 2008; Sullivan et al., 2004). Although these findings greatly improved our understanding of the etiology of nicotine dependence, much is still unknown. More genetic studies of nicotine dependence are greatly needed, especially in different ethnic populations.

The large number of smokers and limited genetic studies of nicotine dependence in China prompted us to perform a systematic association analysis of genetic variation with nicotine dependence in this population. In the current study, we examined the association of 384 SNPs located in 45 candidate genes with nicotine dependence in a case/control-based sample consisting 480 Han Chinese participants.

2. Methods

2.1. Subject

We recruited Han Chinese participants aged 25 years or older in the south-west of China from the local communities in Mianyang, Sichuan, PR China in 2007. Individuals with other psychiatric diagnosis, such as psychosis were excluded. Due to the very low smoking rates in female Chinese, only males were recruited. Data collected from each participant included demographic information (e.g., age, weight, height, years of education, and marital status), drug or substance use history and nicotine dependence measures. The participants who had a smoking history of at least 5 years with a cigarette consumption rate minimum of 5 cigarettes per week were used for this study. All the participants signed an informed consent form prior to the participation in the study. This consent form had been approved by the Ethics Committee of the West China Hospital of Sichuan University.

2.2. Assessment of nicotine dependence

The Fagerström Test for Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) was used for the measure of nicotine dependence. The scores of FTND >=6 was determined as high dependence and the scores <=3 of FTND were determined as low dependence (Rios-Bedoya, Snedecor, Pomerleau, & Pomerleau, 2008).

2.3. DNA preparation, SNP selection and genotyping

Genomic DNA was extracted from peripheral blood cells from each participant using the phenol-chloroform method. In brief, 10 ml lysis solution (1 M Tris·Cl (pH 7.0), 1 M $MgCl_2$, 10% sucrose and 1% triton X-100) was added to 4 ml blood and centrifuged at 3000 rpm for 20 min. The supernatant was discarded and 2.5 ml STE buffer containing 250 μl 10% SDS and 25 μl proteinase K was added. The mixture was incubated at 37 °C for 4 h and then was extracted by phenol, phenol/chloroform (1:1) and chloroform/isoamyl alcohol (24:1) separately. The supernatant was transferred to a fresh tube and DNA was precipitated by adding 2 volumes of cold ethanol and 1/10 volume sodium acetate (3 M). The precipitated DNA was washed by 70% cold ethanol and dissolved in TE buffer and stocked in −20 °C for future use. Concentration of each DNA sample was determined by the optical density (OD) at 260 nm and the purification was evaluated by OD 260/280 ratio.

The candidate genes in this study meet at least one of the following criteria: 1, the genes previously were reported to be associated with nicotine dependence; 2, the genes were located in the susceptibility loci for nicotine dependence that were identified in linkage studies; 3, the genes regulated by nicotine as indicated by some high-throughput expression analyses, e.g. microarray; 4, the genes located adjacent to the susceptibility loci for nicotine dependence. Totally, 45 genes were selected for association analysis (Table S1).

When selecting SNPs within the candidate genes, we first picked out tag SNPs in the HapMap database by using the tagger program (with minor allele frequency >5%, r2 = 0.8). Then, the SNPs located in the exons were included. Last, to make the selected SNPs distributed evenly in a candidate gene, some SNPs located in the introns and the flanking areas of the genes were added. The total number of selected SNPs was 384 (Table S2).

A total of 250 ng DNA was applied to SNP typing using the GoldenGate genotyping assay (Illumina, San Diego, USA) under the manufacturer's instructions.

2.4. Statistical and association analysis

The Haploview program (Barrett, Fry, Maller, & Daly, 2005) was used to analyze pair-wise linkage disequilibrium (LD) between all SNP markers in each chromosome. Individual SNP and haplotype-based case/control association tests were carried out using PLINK v1.07. Samples with genotyping rates <90% and SNPs with minor allele frequency <=0.05 and Hardy–Weinberg equilibrium (HWE) <=0.001 were excluded from the individual SNP-based association tests. In haplotype-based association test, we employed a sliding window approach by examining all possible haplotypes consisting of 2–6 consecutive SNPs. The haplotypes with frequency <=0.05 were excluded from the analysis. All the associations were designated as significant when corrected for the multiple testing numbers of SNPs analyzed in individual SNP-based association test and the number of sliding windows in haplotype-based SNP association test.

3. Results

3.1. The clinical characteristic of participants

We recruited a total of 680 Han Chinese participants in southwest China who have a smoking history of at least 5 years with a smoking consumption of 5 or more cigarettes per week. Of these, 257 participants are low nicotine dependence (LND, FTND<=3) and 223 participants are high nicotine dependence (HND, FTND>=6). 200 participants with FTND = 4 or 5 are excluded from current analyses. The clinical characteristics of the samples are presented in Table 1. There were no significant difference of age, education years and years for smoking between LND and HND samples. Age of smoking onset was higher in LND than in HND ($p<0.01$).

3.2. Association analysis of individual SNPs

24 SNPs and 20 samples were excluded from our analysis using the exclusion criteria described in Methods. In the individual SNP-based association test, 9 SNPs within 8 genes were found to be significantly associated with FTND after correction for multiple testing by SNPs numbers, i.e. rs2630351 in *DRD3*, rs1967550 in *DRD5*, rs2314378 in *MAP3K4*, rs11575461 in *DDC*, rs4954 in *CHRNB3*, rs2779562 in *GABBR2*, rs11214613 and rs6589377 in *DRD2* and rs2236196 in *CHRNA4* (Table 2).

Table 1
Clinical characteristics (mean ± SD) of the samples.

Characteristics	LND	HND	*P* value
Number of subjects	257	223	–
Mean age	49.6 ± 11.0	49.5 ± 11.7	0.93
Education years	10.4 ± 3.1	10.2 ± 3.1	0.52
FTND score	1.4 ± 1.0	7.6 ± 1.0	–
Age of smoking onset	25.0 ± 7.7	20.8 ± 6.3	<0.01
Years for smoking	24.4 ± 11.5	24.7 ± 12.3	0.77