Association of Specific Haplotypes of Neurotrophic Tyrosine Kinase Receptor 2 Gene (*NTRK2*) with Vulnerability to Nicotine Dependence in African-Americans and European-Americans

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Background: *The gene encoding neurotrophic tyrosine kinase receptor 2 (NTRK2) has been localized to a region on chromosome 9q22-q23 that showed a "suggestive" linkage to nicotine dependence (ND) in our previous linkage analyses. However, no association of NTRK2 with ND has been identified.*

Methods: *Family-based association analyses of 2037 participants (1366 African Americans [AA], 671 European Americans [EA]) representing 602 nuclear families were performed to evaluate association of nine single nucleotide polymorphisms (SNPs) within NTRK2 with ND.*

Results: *Individual SNP-based association analysis indicated that in the EA sample, SNPs rs1659400 and rs1187272 were significantly associated with at least one adjusted ND measure. Haplotype analysis revealed that even after Bonferroni correction, the haplotype T-T-A of rs1659400-rs1187272-rs1122530 had a highly significant positive association, with adjusted ND measures in the EA sample (max Z = 3.78; p = .0001, frequency 59.9%). We further identified a major haplotype, T-G-C-A-A (26%), formed by rs993315-rs736744-rs920776-rs4075274-rs729560, which showed a significant positive association (max Z = 2.97, p = .003) with adjusted ND measures in the AA sample.*

Conclusions: *These results strongly suggest that NTRK2 is a susceptibility gene for ND. These findings imply that NTRK2 plays a role in the etiology of ND and represents an important biological candidate for further investigation.*

Key Words: Haplotype, SNP, *TrkB*, association analysis, nicotine dependence, tobacco smoking

Nicotine dependence (ND) is a complex disorder determined by both genetic and environmental factors (Hughes 1986; Li et al 2003a; Sullivan and Kendler 1999). Nearly one third of the global adult population smokes tobacco-related products, making tobacco use a major worldwide health problem (Ezzati and Lopez 2003). On the basis of 17 reported twin studies, we estimated a weighted mean polygenic heritability of .59 in male smokers and .46 in female smokers, with an average of .56 for the whole population (Li et al 2003a). These findings indicate that genetic factors play an important role in determining this complex behavior. To identify susceptibility genes for ND, many association studies have been reported (for reviews, see Tyndale 2003). However, only limited associations of various polymorphisms with ND have been replicated in multiple independent studies (for reviews, see Li et al 2004; Munafo et al 2004).

Previously, we reported that several chromosomal regions are likely to harbor susceptibility loci for ND (Li et al 2003b). A genomic region showing "suggestive" linkage with smoking quantity is located within a 13-cM segment on chromosome 9q22-q33 (Figure 1A; Li et al 2003b). Furthermore, three independent studies reported linkage, at a nominally significant level, of smoking to a region on chromosome 9 overlapping our linked region (Bergen et al 1999; Bierut et al 2004; Gelernter et al 2004). The gene encoding the neurotrophic tyrosine kinase receptor 2 (*NTRK2*; also known as the tyrosine kinase receptor gene; *TrkB*, Online Mendelian Inheritance in Man [OMIM] 191315; National Center for Biotechnology Information [NCBI] Locus ID4915) has been localized to the linked region for ND on chromosome 9q (Nakagawara et al 1995; Valent et al 1997) (Figure 1B). Molecular studies indicate *NTRK2* spans approximately 350 kilobase (kb), contains 19 exons, and encodes a transcript of about 4.0 kb (Nakagawara et al 1995). The *NTRK* genes encode tyrosine kinase transmembrane receptors that are stimulated by neurotrophins and are responsible for the transduction of signals controlling neuropoiesis and neuron survival in the central and peripheral nervous systems (Valent et al 1997). Three forms of *Trk* receptors (*TrkA*, *TrkB*, and *TrkC*) have been identified, each of which binds with high affinity to its specific growth factor ligand: *TrkA* binds to nerve growth factor, *TrkB* to brain-derived neurotrophic factor (*BDNF*) and neurotrophins (*NT*)-4/5, and *TrkC* to neurotrophin-3 (Kerschensteiner et al 2003). The binding of *NTRK2* to *BDNF* regulates short-term synaptic functions and long-term potentiation of brain synapses (Soppet et al 1991). Furthermore, *NTRK2* is essential for the development of gamma-aminobutyric acid (GABA)ergic neurons and regulates synapse formation in addition to its role in the development of axon terminals (Rico et al 2002).

Although, so far, there is no immediate evidence for a role of *NTRK2* in substance dependence, studies in both animals and humans have shown that the gene might be involved in the etiology of various brain disorders. For example, mouse mutants that express decreased amounts of the *BDNF* receptor *TrkB* show hyperphagia and maturity-onset obesity (Xu et al 2003). Furthermore, *TrkB* knockout mice, in which both *BDNF* and *NT-4/5* function are abolished, die within 48 hours of birth from a variety

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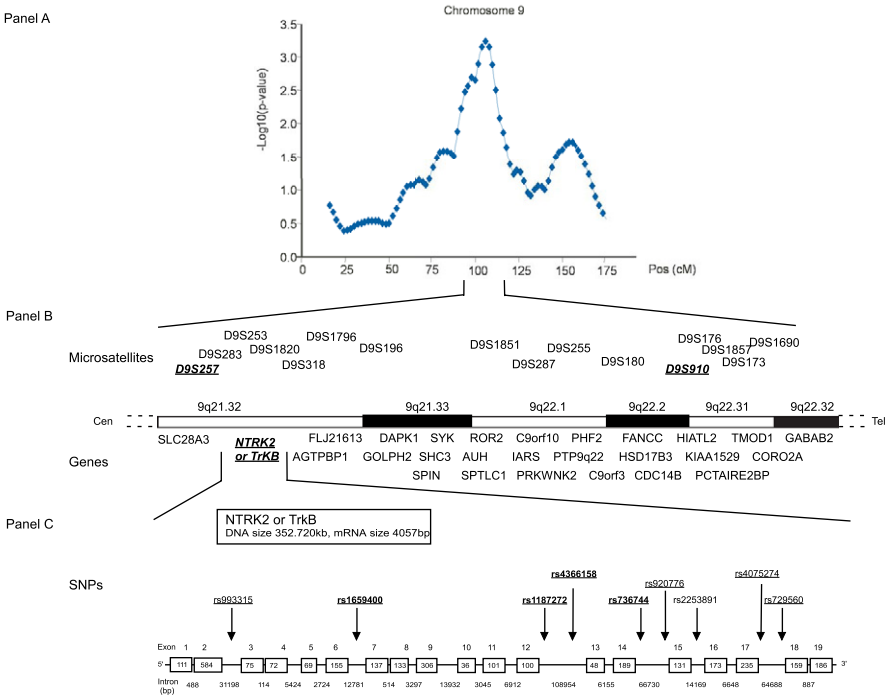

Figure 1. Schematic view of the chromosome 9 linkage region showing position and structure of *NTRK2* and the SNPs selected for association analysis. Panel A shows linkage analysis results for three types of transformed SQ in the Framingham Heart Study population (Li et al 2004; Li et al 2003b). Both EM and traditional Haseman-Elston methods implemented in GeneHunter were used. Panel B shows the relative position of a number of microsatellite markers and genes, including *NTRK2*, within the 9q linkage region. The microsatellite markers used in the linkage analysis shown in Panel A (i.e., D9S257 and D9S910) are marked in bold and underlined. Panel C shows *NTRK2* structure and the positions of the SNPs (presented from 5′ to 3′). Exons are represented as open boxes, with the size of the exon shown in the box, and introns are depicted by a horizontal line between the exons. Single nucleotide polymorphisms showing significant association with ND in the single-SNP analysis are marked in bold, and the SNPs used for haplotype analysis are underlined. *NTRK2*, neurotrophic tyrosine kinase receptor 2; SNP, single nucleotide polymorphism; SQ, smoking quantity; EM, expectation maximization; ND, nicotine dependence.

of neuronal deficits (Kernie et al 2000). Other mouse models in which the *TrkB* receptor has been disrupted demonstrate hyperlocomotion, stereotyped behaviors, and cognitive impairments, similar to the behavioral symptoms postulated for mouse models of attention-deficit disorder (Zorner et al 2003). Kageyama et al (1996) found a missense mutation in the *TrkB* gene in substrains of stroke-prone spontaneously hypertensive rats. Immunohistochemical studies in humans indicate differences in *BDNF* and *TrkB* in the brains of schizophrenic patients and healthy individuals, and these differences may be one of the factors in the pathogenesis of schizophrenia (Iritani et al 2003).

It is well documented that the mesolimbic dopaminergic system of the brain plays an important role in the reinforcing effects of nicotine and other substances (Uhl et al 1998). Brain-derived neurotrophic factor is involved in the neurodevelopment of dopaminergic-related systems and interacts with the mesolimbic dopaminergic systems involved in the therapeutic response to antipsychotic drugs and substance abuse (Krebs et al 2000). It further has been suggested that *BDNF* stimulates the release of dopamine by activation of the *TrkB* receptor (Blochl and Sirrenberg 1996). All these lines of evidence, together with the fact that we have reported an association between alleles of multiple markers in *BDNF* and ND (Beuten et al 2005), motivated us to investigate whether the *NTRK2* gene, encoding *TrkB*, is associated with ND. In this study, we performed an association analysis of single and multiple single nucleotide polymorphisms (SNPs) within *NTRK2* in a large sample set of smokers and their family members and found a significant association between ND and *NTRK2* variants.

Methods and Materials

Study Population

The subjects used in this study were of either African American (AA) or European American (EA) origin and were recruited primarily from the mid South states in the United States during 1999 to 2004. Extensive clinical data were collected on each participant, including demographics (e.g., sex, age, race, biological relationships, weight, height, years of education, and marital status), medical history, smoking history and current smoking behavior, ND, and personality traits, assessed by various questionnaires, which are available at National Institute of Drug Abuse (NIDA) Genetics Consortium website (http://zork.wustl.edu/nida). Individuals were excluded on the basis of current psychiatric diagnosis, including other substance abuse. All participants provided informed consent. The study protocol and forms/procedures were approved by the participating Institutional Review Boards.

In the present study, the ND of each smoker was ascertained by the three measures most commonly used in the literature: smoking quantity (SQ; defined as the number of cigarettes smoked per day, which was also used in our previous linkage study for ND in the Framingham Heart Study [FHS] samples) (Li et al 2003b); the Heaviness of Smoking Index (HSI; 0–6 scale), which includes SQ and smoking urgency (i.e., how soon after waking up does the subject smoke the first cigarette); and the Fagerstrõm Test for ND score (FTND) on a 0 to 10 scale (Heatherton et al 1991). Given the presence of overlap in the contents of the three ND measures, there exists a fairly robust correlation among them (r = .88−.94). Of the 2037 participants in this study, the average age was 39.4 ± 14.4 (SD) years for the AA group and 40.5 ± 15.5 years for the EA group. The average nuclear family size was 3.14 ± .75 for AAs and 3.17 ± .69 for EAs. The average FTND score for smokers was 6.26 ± 2.15 for AAs and 6.33 ± 2.22 for EAs. The average number of cigarettes smoked per day was 19.4 ± 13.3 for AA smokers and 19.5 ± 13.4 for EA smokers. A detailed description of the demographic and clinical characteristics is presented in Table 1.

DNA Extraction, SNP Selection, and Genotyping

Deoxyribonucleic acid was extracted from peripheral blood samples of each participant using a kit from Qiagen Inc. (Valencia, California). Selection of the SNPs for association analysis was based on 1) high heterozygosity (minor allele frequency ≥ .15 as