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Genetic Linkage Findings for DSM-IV Nicotine Withdrawal in Two Populations

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Abstract

Nicotine withdrawal (NW) is both an important contributor to difficulty quitting cigarettes and because of mood-related withdrawal symptoms a problem of particular relevance to psychiatry. Twin-studies suggest that genetic factors influence NW (heritability= 45%). Only one previous linkage study has published findings on NW (Swan et al., 2006; LOD=2.7; Chr. 6 at 159 cM). As part of an international consortium, genome-wide scans (using 381 autosomal microsatellite markers) and telephone diagnostic interviews were conducted on 289 Australian (AUS) and 161 Finnish (FIN, combined (COMB) N=450 families) families ascertained from twin registries through index-cases with a lifetime history of cigarette smoking. The statistical approach used an affected-sib pair design (at least two adult full siblings reported a history of DSM-IV NW) and conducted the linkage analyses using MERLIN. Linkage signals with LOD scores greater than 1.5 were found on two chromosomes: 6 (FIN: LOD= 1.93 at 75 cM) and 11 at two different locations (FIN: LOD= 3.55 at 17 cM, and AUS: LOD= 1.68 with a COMB: LOD= 2.30 at 123 cM). The multipoint LOD score of 3.55 on chromosome 11p15 in FIN met genomewide significance ($p = .013$ with 1000 simulations). At least four strong candidate genes lie within or near this peak on chromosome 11: *DRD4*, *TPH*, *TH*, and *CHRNA10*. Other studies have reported that chromosome 11 may harbor genes associated with various aspects of smoking behavior. This study adds to that literature by highlighting evidence for nicotine withdrawal.

Keywords

genetics; linkage; nicotine; withdrawal

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#This paper is a tribute to the memory of Dr. Richard Todd, a senior investigator on this project, who died on August 22, 2008.

Introduction

Approximately 20% of the US (CDC, 2006), Australian (PHAA, 2004), and Finnish (Helakorpi et al., 2007) adult population continues to smoke, and estimates suggest that over 50% of continuing smokers will die of a smoking-related illness (Peto et al., 1992). A shift in thinking about the problem of smoking cessation, from one of “will-power” to a potentially treatable disorder may be dated to around 1988, when the U.S. Surgeon General released the report which concluded that nicotine “is a powerfully addicting drug... We must recognize both the potential for behavioral and pharmacological treatment of the addicted tobacco user and the problems of withdrawal” (USDHHS, 1988). Curbing tobacco use will reduce population rates of morbidity and mortality, yet, at least for some, a limiting step to quitting cigarettes are symptoms of nicotine withdrawal (Cummings et al., 1985; Hughes, 2006; Piasecki et al., 1998; Piasecki et al., 2003). The degree of nicotine withdrawal-related dysphoria experienced during nicotine abstinence appears similar to the levels reported by psychiatric out-patients (Hughes, 2006). While a third of U.S. smokers try to quit each year, only 3-5% stay quit over a year (CDC, 2002), which may partially be accounted for by over 50 % of smokers experiencing symptoms of nicotine withdrawal after they quit or cut-down (Breslau et al., 1992; Pergadia et al., 2006a). Uncovering both the environmental and genetic factors associated with nicotine withdrawal could broaden our etiologic models of nicotine dependence, and ultimately inform smoking cessation treatment.

Among smokers, approximately 45% of the variance in nicotine withdrawal can be accounted for by genetic factors (Pergadia et al., 2006a). Controlling for both cigarette experimentation and for quantity smoked during heaviest period of use, there are residual genetic influences on nicotine withdrawal (up to 23% of total variance; Pergadia et al., 2006a). These findings suggest that there may be genetic variance in nicotine withdrawal that is independent of genetic influences contributing to the development of tolerance to nicotine (Saccone et al., 2007a) and to experimentation with cigarettes. Even after controlling for early social factors, such as cigarette experimentation with a co-twin, previous research suggest that substantial genetic influences on nicotine withdrawal remain that cannot be attributable to twin social environmental influences (Pergadia et al., 2006b).

While twin-studies can help to elucidate the extent to which an observed behavior is heritable, genomic research approaches can begin to identify chromosomal regions or specific genes associated with the behavioral phenotype (e.g., nicotine withdrawal). To date, linkage studies using smoking-related phenotypes have included behaviors such as smoking initiation (Bergen et al., 2003; Ehlers and Wilhelmsen, 2006; Morley et al., 2006; Vink et al., 2004), quantity smoked (Swan et al., 2006; Morley et al., 2006; Bergen et al., 1999; Duggirala et al., 1999; Goode et al., 2003; Li et al., 2003; Li et al., 2006; Saccone et al., 2003; Wang et al., 2005), habitual smoking (Ehlers and Wilhelmsen, 2006; Bierut et al., 2004; Gelernter et al., 2004), nicotine dependence (FTND: Swan et al., 2006; Li et al., 2006; Gelernter et al., 2007; Straub et al., 1999; DSM-IV: Swan et al., 2006; Gelernter et al., 2007), short-term cessation (Swan et al., 2006), and maximum cigarettes smoked in a 24-hour period (Saccone et al., 2007a). Suggestive linkage has been reported at multiple locations across the genome, but with most LOD scores not exceeding 3.0 and indeed most samples used were not selected for informativeness for cigarette smoking, with a few notable exceptions: 1) quantity smoked: LOD = 4.17 on chromosome 10 (Li et al., 2006), 2) short-term cessation: LOD = 4.0 on chromosome 16 (Swan et al., 2006), and 3) maximum cigarettes consumed in 24-hours: LOD = 5.98 on chromosome 22 (Saccone et al., 2007a). Each of these latter three studies included samples that were ascertained for smoking-behavior and reported LOD scores for cigarette smoking phenotypes greater than 3.0. Of note, only one linkage study to date has reported findings on nicotine withdrawal, and found

the highest peak for withdrawal symptoms on chromosome 6 (LOD = 2.7; Swan et al., 2006).

As part of an international consortium known as “The Nicotine Addiction Genetics (NAG) Project”, investigators from Australia, Finland and the United States are using population-based samples selected to be informative for smoking outcomes to identify genes harboring risk loci for heavy cigarette smoking and nicotine dependence, and contributing to risk of becoming a long-term nicotine dependent cigarette smoker (Saccone et al., 2007a). Towards this goal, we conducted genome scans using data from 450 Australian and Finnish families. Our aim in this paper is to present our findings for DSM-IV nicotine withdrawal, which prior research suggests is an important component of nicotine dependence.

Methods and Materials

Participants

Family Ascertainment—The study participants for the NAG project (Saccone et al., 2007a; Loukola et al., 2008) were enrolled at two different sites: the Queensland Institute of Medical Research in Australia, and the University of Helsinki in Finland. Families were identified through smoking index cases selected using previously administered interview and questionnaire surveys of the community-based Australian and population-based Finnish cohorts of twins. The Finnish arm of the NAG project recruited families of Finnish ancestry through twin pairs from the Finnish Twin Cohort, born between 1938 and 1957. Families chosen for the Australian arm of the NAG study were identified from two cohorts of the Australian Twin Panel, which included opposite-sexed twins and spouses of the older of these two cohorts, for a total of approximately 12,500 families with information about smoking. The ancestry of the Australian samples is predominantly Anglo-Celtic and Northern European (> 90%), while the Finnish sample is all of Finnish ancestry. Further details about these samples are given elsewhere (Saccone et al., 2007a). All data collection procedures were approved by institutional review boards at the lead institution, Washington University, the Queensland Institute of Medical Research, and at the University of Helsinki, including the use of appropriate and approved informed consent procedures. Written informed consent was provided by all participants. If the subject was an index case, permission was obtained from them to contact other family members.

Clinical Assessment—The data collection protocol included two phases, a telephone screening and a diagnostic interview phase, after which a sample of blood for DNA extraction was obtained (via phlebotomy lab or public health clinic selected by the respondent). Eligible families across both sites were required to have at least one adult sibpair (including no more than one monozygotic twin per pair) concordant for a history of cigarette smoking, based on earlier questionnaire or interview surveys. At the Australian site, families were targeted for screening using the criteria that they have at least one sibpair possibly concordant for a lifetime history of heavy smoking (defined as either a history of smoking 20 cigarettes per day during the period of heaviest smoking, or smoking at least 40 cigarettes in any 24-hour period). Priority was given to families with available biological parents. On the Finnish site, due to the absence of information on cigarette smoking history in non-twin siblings (from previous surveys), families with dizygotic co-twins concordant for a lifetime history of regular smoking were prioritized. Some families with monozygotic co-twins concordant for lifetime smoking were also enrolled in the study, but only one monozygotic twin per pair was included for linkage analyses. Priority was given to families of dizygotic twins with histories of heavier cigarette consumption, particularly where the female twin was a regular smoker (in a cohort where smoking by women was rare). On both sites, during the screening interview, sufficient information on lifetime history of cigarette

smoking was obtained from the index case to confirm family eligibility, and permission was requested to contact available parents and full biological siblings for participation in the study.

Clinical data were collected using a computer-assisted telephone diagnostic interview (CATI) based on an adaptation of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994; Hesselbrock et al., 1999) and the Composite International Diagnostic Interview (CIDI; Cottler et al., 1991) for telephone administration, and also a mailed self-report questionnaire. The tobacco section for the CATI was derived from the CIDI (Cottler et al., 1991), and incorporated FTND (Heatherton et al., 1991), DSM-III-R (APA, 1987) and DSM-IV (APA, 1994) assessments of nicotine dependence. It also included a detailed history of the first use of cigarettes and other tobacco products, the quantity and frequency of use for current or most recent (if ex-smoker) and heaviest period of use, supplemental items concerning attempts at cessation, and DSM-IV symptoms of nicotine withdrawal. Information on other comorbid psychiatric disorders such as major depression and anxiety disorders, and conduct and anti-social personality disorder were also elicited by the CATI. Interviewers were mostly selected from highly trained panels of interviewers at Queensland Institute of Medical Research and the University of Helsinki that included nurses or graduates in psychology or a related field.

The average sibling age was 42.6 years and 54.6 years for the Australian and Finnish samples, respectively. From Australia, 289 families were selected for genome scan, which included 1309 individuals (of which 917 were offspring) who provided both genotypic and phenotypic data. From the Finnish site, 161 families were selected for genome scan, which included 541 individuals (of which 522 were offspring) with both genotypic and phenotypic data. For phenotypic analyses, we used all available data, which included 946 Australian families (3425 individuals, 2539 offspring) and 757 Finnish families (2265 individuals, 2090 offspring). Please see Saccone et al. (2007a) and Loukola et al. (2008) for further details on these samples.

Genotyping—The genome screen for both the Australian and Finnish NAG samples was comprised of 381 autosomal microsatellite markers spaced at approximately 10 cM across the genome. Our analyses are based on the deCODE genetic map (Kong et al., 2002). The few markers included that were not a part of the deCODE set were assigned genetic map positions based on interpolation of the physical map obtained from build 36.2 of the human reference genome [National Center for Biotechnology Information (NCBI)]. The Australian NAG sample was genotyped at the Australian Genome Research Facility (AGRF) and the Finnish NAG sample was genotyped at the Finnish Genome Center (FGC). The AGRF used an ABI (Applied Biosystems) genotyping platform, and the FGC used both ABI and MegaBACE (Amersham Biosciences) platforms (Saccone et al., 2007a; Loukola et al., 2008). PedCheck (version 1.1; O'Connell and Weeks, 1998), RelCheck (version 0.67; Boehnke and Cox, 1997; Broman and Weber, 1998) and PREST (version 3.0; McPeck and Sun, 2000) were used to screen for Mendelian errors and familial misspecifications, such as cases of non-paternity, and genotypes producing Mendelian errors were deleted. In the Finnish sample, three markers and six individuals with success rates below 70% were excluded. Two nuclear families were excluded due to an excess of Mendelian errors. Two MZ pairs were detected and one individual from each pair deleted. Two sibpairs with too low IBS-sharing were excluded. In the Australian sample, one marker with an error rate of 38% was excluded. Four MZ pairs were detected and one individual from each pair deleted, and five sibpairs with too low IBS-sharing were excluded. Please see Saccone et al. (2007a) and Loukola et al. (2008) for additional details on genotyping procedures.

Phenotype: DSM-IV Nicotine Withdrawal

Smokers (individuals who smoked 100 or more cigarettes lifetime, or 26 or more cigarettes lifetime and weekly for two consecutive months) were asked about problems they might have had in the first 24 hours after they stopped or cut down on cigarettes. They were queried about each DSM-IV nicotine withdrawal symptom (see Table 1): irritability, restlessness, concentration problems, depressed mood, increased appetite, sleep problems, nervousness and decreased heart rate, within the context of a smoking cessation (or reduction) attempt that they remember most clearly (if more than one attempt). The interview was designed to help the respondent to focus on one particular episode of nicotine abstinence/reduction and asked "At that particular time, did you cut down, or did you completely stop smoking cigarettes? Please tell me more about that time. What do you remember most clearly about that time?" Subjects not endorsing four or more withdrawal symptoms within 24 hours of stopping or cutting down on cigarettes for the initially chosen episode were asked: "Has there been a time when you had 4 or more of the [nicotine withdrawal] problems occur during the first 24 hours after you cut down or stopped smoking cigarettes?", if 'yes', the subject was cycled through the questions on nicotine withdrawal symptoms again. Individuals were classified according to whether they met criteria for DSM-IV nicotine withdrawal (NW) using the substance dependence definition, i.e. endorsement of four or more symptoms within 24 hours of quitting/reducing use of cigarettes, or endorsement of smoking cigarettes, or any other tobacco product, nicotine substitute or medication to relieve or avoid withdrawal symptoms (APA, 1994). For the affected sib-pair linkage analyses, all NW affected sib-pairs reported smoking 100 or more cigarettes lifetime. Thus for all phenotypic analyses reported, individuals who reported smoking fewer than 100 times lifetime were set to missing (0.01% of smokers). The use of 100 or more cigarettes lifetime is widely used to define a lifetime history of adult cigarette smoking (CDC, 2006). In the broader Australian sample, 51.9% of siblings with a history of smoking met criteria for DSM-IV NW (79.8% of these cases reported four or more symptoms of nicotine withdrawal and the remaining 20.2% reported using nicotine to avoid withdrawal). There were 232 affected sib-pairs (n-1 formula) from 154 families in the Australian linkage sample. In the broader Finnish sample, 41.2% of siblings with history of smoking met criteria for DSM-IV NW (78.4% of these cases reported four or more symptoms of nicotine withdrawal and the remaining 21.6% reported using nicotine to avoid withdrawal). Sixty-eight affected sib-pairs (n-1) were identified from 45 families in the Finnish linkage sample.

Statistical Approach

Chi-square tests, ANOVA, and tetrachoric correlations were conducted to examine variation in nicotine withdrawal symptoms and related-smoking characteristics across the Australian and Finnish samples using Stata (2003), which adjusts standard errors for the non-independence of measures within family members. Multipoint and single-point non-parametric linkage was conducted in MERLIN (Abecasis et al., 2002), first separately in each sample and then after combining both samples. In the combined analyses, allele assignments and frequencies were calculated separately for the Australian and Finnish samples using a slight modification to the program's source code (G. Abecasis, personal communication; see Saccone et al., 2007a). For the multi-point linkage analyses, the 2cM grid option was used. Evidence for linkage was supported in a particular genomic region, if the number of alleles shared identical by descent was greater than would be expected by chance in sib-pairs concordant for DSM-IV nicotine withdrawal. For LOD scores greater than 3.0, we obtained genome-wide adjusted p-values using MERLIN both to generate 1000 simulated datasets and to test that data for linkage. This provided a threshold for determining the degree to which we would obtain such a LOD score due to just chance alone.

Results

As table 1 depicts, the Australian and Finnish samples differed significantly across all nicotine withdrawal (NW)-related measures, in addition to average number of cigarette smoked during heaviest period of smoking ($p < .01$), with the Australian sample generally exhibiting more severe levels of nicotine withdrawal and heavier smoking than the Finnish sample. The Australian sample was significantly more likely to report almost all individual symptoms of DSM-IV NW, a greater number of NW symptoms, and more cigarettes per day at the time of peak lifetime cigarette use; the Australian smokers were also more likely to meet criteria for DSM-IV NW. Only nervousness during nicotine abstinence was reported at significantly lower levels in the Australian sample (26%) than in the Finnish sample (39%). In terms of sex differences, across both the Australian (AUS) and Finnish (FIN) sample, women (AUS: 31%, FIN: 14%) were significantly more likely to report depressed mood as a symptom of nicotine withdrawal than men (AUS: 21%, FIN: 10%) and fewer cigarettes per day at peak lifetime use. In the Australian sample, women were also more likely than men to report irritability (AUS Women: 55%, AUS Men: 50%) and problems with concentration (AUS Women: 39%, AUS Men: 34%), while in the Finnish sample women were more likely to report impairment related to nicotine withdrawal compared to men (FIN Women: 14%, FIN Men: 9%). No other important sex differences were found. For the NW affected sib-pairs that were included in the non-parametric linkage analyses, rates of restlessness, concentration problems, decreased heart rate and increased appetite no longer differed significantly across the samples, and the number of NW symptoms were more comparable (mean = 4.4 FIN, 4.7 AUS). Tetrachoric correlations between DSM-IV NW and DSM-IV nicotine dependence (which includes NW as a symptom) were $r = 0.73$ and $r = 0.72$, but correlations between DSM-IV NW and a definition of nicotine dependence using a cut-off of 4 or more on the Fagerström Test of Nicotine Dependence (FTND; Heatherton et al., 1991; Heatherton et al., 1989; Breslau and Johnson, 2000; Saccone et al., 2007b; Bierut et al., 2007) were a more modest $r = 0.35$ and $r = 0.40$ in the Australian and Finnish samples, respectively.

Linkage analyses revealed several loci with suggestive or significant linkage for DSM-IV NW. The genome-wide autosomal linkage results for DSM-IV NW in the Finnish sample, Australian sample, and the combined sample are depicted in Figure 1.

Chromosomal regions with multipoint LODs greater than 1.5 for DSM-IV NW in sample specific or combined sample analyses are listed in Table 2. For the Australian sample a multipoint LOD of 1.68 was found on chromosome 11 at 123 cM. For the Finnish sample, a multipoint LOD of 1.93 was found on chromosome 6 at 75cM and a LOD of 3.55 on chromosome 11 at 17 cM. Combining the samples a LOD of 2.30, that was larger than LOD scores found in either sub-sample, was observed on chromosome 11 at 123 cM (see Figure 1 and Figure 2). The multipoint LOD of 3.55 at 11p15.5 in the Finnish sample met genomewide significance ($p = 0.013$). The highest single point marker in this region yielded a LOD = 2.34 (at D11S1338). The chromosome 11 findings are plotted in Figure 2.

Discussion

The most notable finding from this report on DSM-IV Nicotine Withdrawal (NW) is a linkage signal (LOD = 3.6) that meets genomewide significance on chromosome 11p15 in the Finnish families. Smaller signals in the same area on 11p15 were detected in the same Finnish sample using binary phenotypes for DSM-IV nicotine dependence (ND) and FTND using two-point non-parametric linkage (LOD = 1.8; Loukola et al., 2008), which is consistent with the modest to high correlations we found between DSM-IV NW and DSM-IV ND and DSM-IV NW and the FTND in this same Finnish sample. Maximum cigarettes

in 24-hours (Saccone et al., 2007a) and other quantitative measures of nicotine dependence did not show linkage on chromosome 11p15 in these samples. This pattern of findings suggest that there may be genetic variation influencing nicotine withdrawal that is not easily detected using other measures of nicotine dependence (including DSM-IV-based nicotine dependence), especially in the Finnish sample. While bivariate linkage analysis using both FTND and DSM-IV NW might illuminate common versus specific genetic effects, the small number of NW-affected Finnish sib-pairs available precludes this approach.

Inclusion of samples with lower and higher levels of tobacco use may have increased our ability to detect an empirically significant linkage peak associated with nicotine withdrawal. Among sibling pairs concordant for DSM-IV NW, the range of number of cigarettes smoked per day (cpd) during peak lifetime use varies across subgroups stratified by sex and society, with the Finnish women of pairs concordant for NW at the lower end of the distribution (Finnish women: mean cpd = 18.6) and Australian men of concordant sib-pairs on the upper end of the severity continuum (Australian men: mean cpd = 29.2; Finnish men: mean cpd = 24.3; Australian women: mean cpd = 25.6); suggesting that Finnish women, who are smoking at a lower level, but still part of a sib-pair concordant for NW, might be particularly informative in identifying genetic loci that may contain genes contributing independently to NW vulnerability. Across societies, women were significantly more likely to report depressed mood as a symptom of nicotine withdrawal than men, which appears to be a common phenomenon (Pergadia et al., 2006a). One other notable difference in the Finnish sample was that women were also more likely to report impairment related to nicotine withdrawal relative to men. Previous analyses of the individual symptoms of NW found: that most items were familial (even after controlling for regular smoking), variation within the items could be accounted for by a single factor, and that additive genetic variance significantly influenced categorically defined DSM-IV NW (accounting for over 45% of the variance; Pergadia et al., 2006a). Thus, our approach in this study was to conduct genetic linkage using only the categorical measure of DSM-IV NW. This approach also circumvented multiple testing.

There is evidence from other studies that chromosome 11 may harbor genes associated with smoking behavior (Li, 2008). Figure 2 displays approximate areas of overlap (using the deCODE genetic map) across studies reporting linkage findings with smoking-related phenotypes. The first three findings as we move along chromosome 11 in Figure 2 are most consistent with the highest peak we found for DSM-IV NW on 11p. The first finding was reported by Gelernter et al. (2004) for habitual smoking in a small sample ascertained for panic disorder, where the highest peak for habitual smoking was located at 11p15 at D11S4046 ($Z_{lr} = 3.4$). As mentioned above, using a binary measure of FTND or DSM-IV nicotine dependence in the same NAG Finnish sample reported on here for DSM-IV NW, a signal was detected in the same area (two-point LOD = 1.8; Loukola et al., 2008). Interestingly, at the height of our Finnish multipoint peak, around 20 cM, Wang et al. (2005), reported a genomewide significant linkage peak, using a quantity smoked phenotype measured in the Framingham Heart Study. At both 31 cM and 74 cM He et al. (2008), recently reported suggestive linkage peaks using measures of cotinine pharmacokinetics. Between our linkage peaks from 40 cM to 100 cM on chromosome 11, at least four studies have reported linkage signals using smoking-related phenotypes (Morley et al., 2006; Li et al., 2003; Li et al., 2006; Bierut et al., 2004): with the highest linkage signals detected in this region using quantity smoked measures in African American, Australian and the US-based Framingham Heart Study families (the later two being predominantly Caucasian ancestry), respectively. Bierut et al. (2004) found a LOD = 1.6 at 87 cM using a habitual smoking phenotype in families targeted for alcohol dependence. Finally, near our peak for the Australian sample on 11q, Gelernter et al. (2007) reported a two-point LOD = 1.97 at 109

cM for DSM-IV nicotine dependence in a U.S. Caucasian sample ascertained for cocaine or heroin dependence.

The positions for at least nine possible candidate genes lie within or near the two regions identified by linkage on chromosome 11. These include for 11p: *DRD4* (dopamine receptor 4), *TPH* (tryptophan hydroxylase 1), *TH* (tyrosine hydroxylase), and *CHRNA10* (nicotinic cholinergic receptor-alpha 10), and for 11q23: *HTR3A* (hydroxytryptamine receptor 3A), *HTR3B* (hydroxytryptamine receptor 3B), *DRD2* (dopamine D2 receptor gene), *ANKK1* (ankyrin repeat and kinase domain containing 1), and *GRIK4* (ionotropic kainate glutamate receptor 4 gene). The suggestive linkage peak at 123 cM in the Australian (and combined samples) occurs close to *DRD2*, which has long been considered as a candidate gene in addiction research (c.f.; Blum et al., 1991, and to *ANKK1*; Neville et al., 2004), with the Taq 1A RFLP polymorphism formally assumed to lie within the *DRD2* gene now known to occur within an exon of the protein kinase gene *ANKK1*. Positive reports of association with various aspects of smoking behavior—including withdrawal symptoms (Robinson et al., 2007), persistent smoking (Morton et al., 2006), and pharmacotherapy response (David et al., 2007a; David et al., 2007b; Han et al., 2008)—have been reported for polymorphic markers within the *DRD2*, sometimes in interaction with other loci (e.g., for pharmacotherapy response: Morton et al., 2006; Ton et al., 2007; Swan et al., 2007). Following-up on a modest linkage peak for nicotine dependence on chromosome 11q23; Gelernter et al. (2006) found significant associations between SNPs in *ANKK1* and nicotine dependence using family-based association, but not for SNPs within *DRD2*.

Reports suggest that variations in *TPH* are associated with impulse control problems (New et al., 1998), suicidal behavior (Nielsen et al., 1998), and earlier stages of smoking, such as smoking initiation (Lerman et al., 2001; Sullivan et al., 2001), but not nicotine dependence (Lerman et al., 2001; Sullivan et al., 2001). Three studies have examined polymorphisms within *CHRNA10* and smoking related behavior, and found no associations with smoking initiation (Greenbaum et al., 2006), nicotine dependence (Saccone et al., 2007b; Greenbaum et al., 2006), or smoking cessation (Heitjan et al., 2007). The ionotropic kainate glutamate receptor 4 gene (*GRIK4*), which is located directly under the highest position of the multipoint peak associated with DSM-IV NW in our Australian subsample and in our combined analyses at 123 cM on chromosome 11, was recently found to be associated with response to a selective serotonin reuptake inhibitor (citalopram) used to depressive symptoms (Paddock et al., 2007).

In this study, we also found modest multipoint elevations for DSM-IV NW on chromosomes 6 (for the Finnish sample) and 7 (after combining the samples). While Swan et al.'s (2006) linkage peak for nicotine withdrawal was also located on chromosome 6 at 159 cM (near the *OPRM1*) this was far from our location at 75 cM. The two studies used rather different phenotypic definitions. Perhaps most notably, in this study we used a diagnostic definition of nicotine withdrawal, while Swan et al. (2006) used a quantitative measure of withdrawal severity.

There are a number of limitations to our findings that temper our conclusions. Given that we used non-parametric linkage, which requires affected sib-pairs, from the 161 Finnish families for which we had both genotypic and phenotypic data, only a smaller sub-set of families (N= 45 families) had an affected sib-pair contributing to the significant linkage peak. While the two microsatellite marker flanking this linkage peak on 11p were both greater than 2.0, it is possible that this LOD score may be inflated due to interpolation. On the other hand, given that linkage rests on the assumption of genetic effect sizes that are at least modest (Risch, 2000), suggests that our genomewide significant linkage finding is less likely to be a false positive. 1000 simulations found that a LOD score of 3.1 was the

threshold necessary for genomewide significance (e.g. genomewide adjusted p value $< .05$). The lack of consistency between Australian and Finnish samples may also be due to the greater genetic homogeneity of a Finnish sample relative to other Caucasian samples (Peltonen et al., 2000). On 11q, the combined linkage peak of LOD = 2.30 resulted from 199 families with 300 sib-pairs, and while the peak is smaller in magnitude, it may eventually prove to be a true signal. Thus, these findings will need to be extended and replicated using other samples. The examination of possible sex differences associated with these effects should be pursued in future work, with larger sample sizes, using SNPs within specific genes of interest. This linkage panel set includes markers at approximately every 10 cM. Thus, fine-mapping of this region will be necessary to narrow this linkage region. Despite limitations, these findings contribute to the growing evidence that chromosome 11 may harbor genes associated with smoking behavior (Li, 2008), and highlights the potential value of focusing specifically on nicotine withdrawal as one important aspect of smoking behavior that is partly under genetic influence.

Acknowledgments

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Figure 1. Autosomal multipoint linkage signals for DSM-IV nicotine withdrawal. The findings from the Finnish sub-sample are display by smooth red lines, findings from the Australian in blue, and the combined sample in green.

Figure 2. the multipoint findings on chromosome 11 are represented by red and blue lines, and the single-point findings are represented by red triangles and blue squares, for the Finnish and Australian sub-samples, respectively. Two single-point markers, shown as translucent red triangles in Figure 2, were typed in the Finnish sample only. Single point findings are not plotted for LOD scores less than zero. Other studies reporting linkage findings on chromosome 11 using smoking related phenotypes are depicted by centimorgan (cM) position and numerated 1-9.

Table I

Lifetime Prevalence Rates and Mean Number of Symptoms: DSM-IV Nicotine Withdrawal Measures and Related Phenotypes in siblings with lifetime cigarette smoking

	Australian * (N= 2403)		Finnish* (N= 1849)	
	N= 1204 Women	N= 1199 Men	N= 749 Women	N= 1100 Men
DSM-IV Nicotine Withdrawal (NW)	54.6% ^a	49.4% ^a	42.7%	40.2%
NW-related impairment	25.6%	23.8%	13.6% ^b	9.3% ^b
NW symptoms-				
Irritability	55.0% ^a	50.1% ^a	34.3%	34.6%
Restlessness	65.2%	63.6%	47.9%	52.1%
Concentration Problems	38.6% ^a	34.0% ^a	30.8%	31.5%
Depressed Mood	31.1% ^a	20.6% ^a	14.1% ^b	10.1% ^b
Increased Appetite	56.9%	55.6%	43.7%	41.5%
Sleep Problems	27.1%	28.5%	16.0%	16.0%
Nervousness	27.9%	24.5%	38.0%	40.1%
Decreased Heart Rate	11.1%	10.0%	8.1%	7.1%
NW symptom count (and 95% CI)	3.1 (3.0-3.2) ^a	2.9 (2.7-3.0) ^a	2.3 (2.1-2.5)	2.3 (2.2-2.5)
Cigs/day (at peak use, and 95% CI)	23.0 (22.2-23.7) ^a	26.9 (26.2-27.7) ^a	16.0 (15.3-16.8) ^b	21.4 (20.7-22.0) ^b

* Cross-country analyses: Australian and Finnish samples differ on all measures at $p < 0.01$;

^a indicates sex differences within the Australian sample at $p < 0.05$;

^b indicates sex differences within the Finnish sample at $p < 0.01$;

Table II

Multipoint linkage findings for DSM-IV Nicotine Withdrawal in NAG project: LOD scores greater than 1.5 from affected sib-pair analyses using non-parametric linkage

Chromosome	Position (cM)	LOD Score		
		Australian	Finnish	Combined
6	75.2	0.29	1.93	1.17
11	17.3	-0.19	3.55*	0.13
	123.3	1.68	0.70	2.30

* Meets genome-wide significance at $p = 0.013$ with 1000 simulations in MERLIN;