

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

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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); *Am J Med Genet Part B* 141B:354–360; LOD = 2.7; Chr. 6 at 159 cM]. As part of an international consortium, genome-wide scans (using over 360 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 >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 ($P = 0.013$ with 1,000 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 NW. © 2009 Wiley-Liss, Inc.

Key words: genetics; linkage; nicotine; withdrawal

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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 (NW) [Cummings et al., 1985; Piasecki et al., 1998, 2003; Hughes, 2006]. The degree of NW-related dysphoria experienced during nicotine abstinence appears similar to the levels reported by psychiatric outpatients [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 NW after they quit or cut-down [Breslau et al., 1992; Pergadia et al., 2006a]. Uncovering both the environmental and genetic factors associated with NW could broaden our etiologic models of nicotine dependence (ND), and ultimately inform smoking cessation treatment.

Among smokers, ~45% of the variance in NW 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 NW [up to 23% of total variance; Pergadia et al., 2006a]. These findings suggest that there may be genetic variance in NW 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 NW 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; Vink et al., 2004; Ehlers and Wilhelmsen, 2006; Morley et al., 2006], quantity smoked [Bergen et al., 1999; Duggirala et al., 1999; Goode et al., 2003; Li et al., 2003, 2006; Saccone et al., 2003; Wang et al., 2005; Morley et al., 2006; Swan et al., 2006], habitual smoking [Bierut et al., 2004; Gelernter et al., 2004; Ehlers and Wilhelmsen, 2006], ND [FTND: Straub et al., 1999; Li et al., 2006; Swan et al., 2006; Gelernter et al., 2007; DSM-IV: Swan et al., 2006; Gelernter et al., 2007], short-term cessation [Swan et al., 2006], and maximum cigarettes smoked in a 24-hr 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-hr: 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 >3.0. Of note, only one linkage study to date has reported findings on NW, 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 ND, 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 article is to present our findings for DSM-IV NW, which prior research suggests is an important component of ND.

MATERIALS AND METHODS

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 ~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 sib-pair (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 sib-pair 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-hr 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 ND. 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 NW. 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 1,309 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 (3,425 individuals, 2,539 offspring) and 757 Finnish families (2,265 individuals, 2,090 offspring). Please see Saccone et al. [2007a] and Loukola et al. [2008] for further details on these samples.

Genotyping. The genome screen for the Australian NAG sample was comprised of 381 autosomal microsatellite markers spaced at

~10 cM across the genome. In the Finnish NAG sample, 362 of these were typed for the genome screen (19 additional markers were also typed for fine mapping only). 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, Foster City, CA) genotyping platform, and the FGC used both ABI and MegaBACE (Amersham Biosciences, Piscataway, NJ) 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 sib-pairs 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 sib-pairs with too low IBS-sharing were excluded. Please see Saccone et al. [2007a] and Loukola et al. [2008] for additional details on the final marker sets and 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 hr after they stopped or cut down on cigarettes. They were queried about each DSM-IV NW 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 hr of stopping or cutting down on cigarettes for the initially chosen episode were asked: "Has there been a time when you had four or more of the [NW] problems occur during the first 24 hr after you cut down or stopped smoking cigarettes?" if "yes," the subject was cycled through the questions on NW symptoms again. Individuals were classified according to whether they met criteria for DSM-IV NW using the substance dependence definition, that is, endorsement of four or more symptoms within 24 hr 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 NW 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 NW 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 NW 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 2 cM 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 NW. For LOD scores >3.0, we obtained genome-wide adjusted P-values using MERLIN both to generate 1,000 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 I depicts, the Australian and Finnish samples differed significantly across all NW-related measures, in addition to average number of cigarette smoked during heaviest period of smoking ($P < 0.01$), with the Australian sample generally exhibiting more severe levels of NW 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 NW 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 NW 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

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 ^a (N = 2,403)		Finnish ^a (N = 1,849)	
	Women (N = 1,204)	Men (N = 1,199)	Women (N = 749)	Men (N = 1,100)
DSM-IV nicotine withdrawal (NW)	54.6% ^b	49.4% ^b	42.7%	40.2%
NW-related impairment	25.6%	23.8%	13.6% ^c	9.3% ^c
NW symptoms				
Irritability	55.0% ^b	50.1% ^b	34.3%	34.6%
Restlessness	65.2%	63.6%	47.9%	52.1%
Concentration problems	38.6% ^b	34.0% ^b	30.8%	31.5%
Depressed mood	31.1% ^b	20.6% ^b	14.1% ^c	10.1% ^c
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] ^b	2.9 [2.7–3.0] ^b	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] ^b	26.9 [26.2–27.7] ^b	16.0 [15.3–16.8] ^c	21.4 [20.7–22.0] ^c

^aCross-country analyses: Australian and Finnish samples differ on all measures at $P < 0.01$.
^bIndicates sex differences within the Australian sample at $P < 0.05$.
^cIndicates sex differences within the Finnish sample at $P < 0.01$.

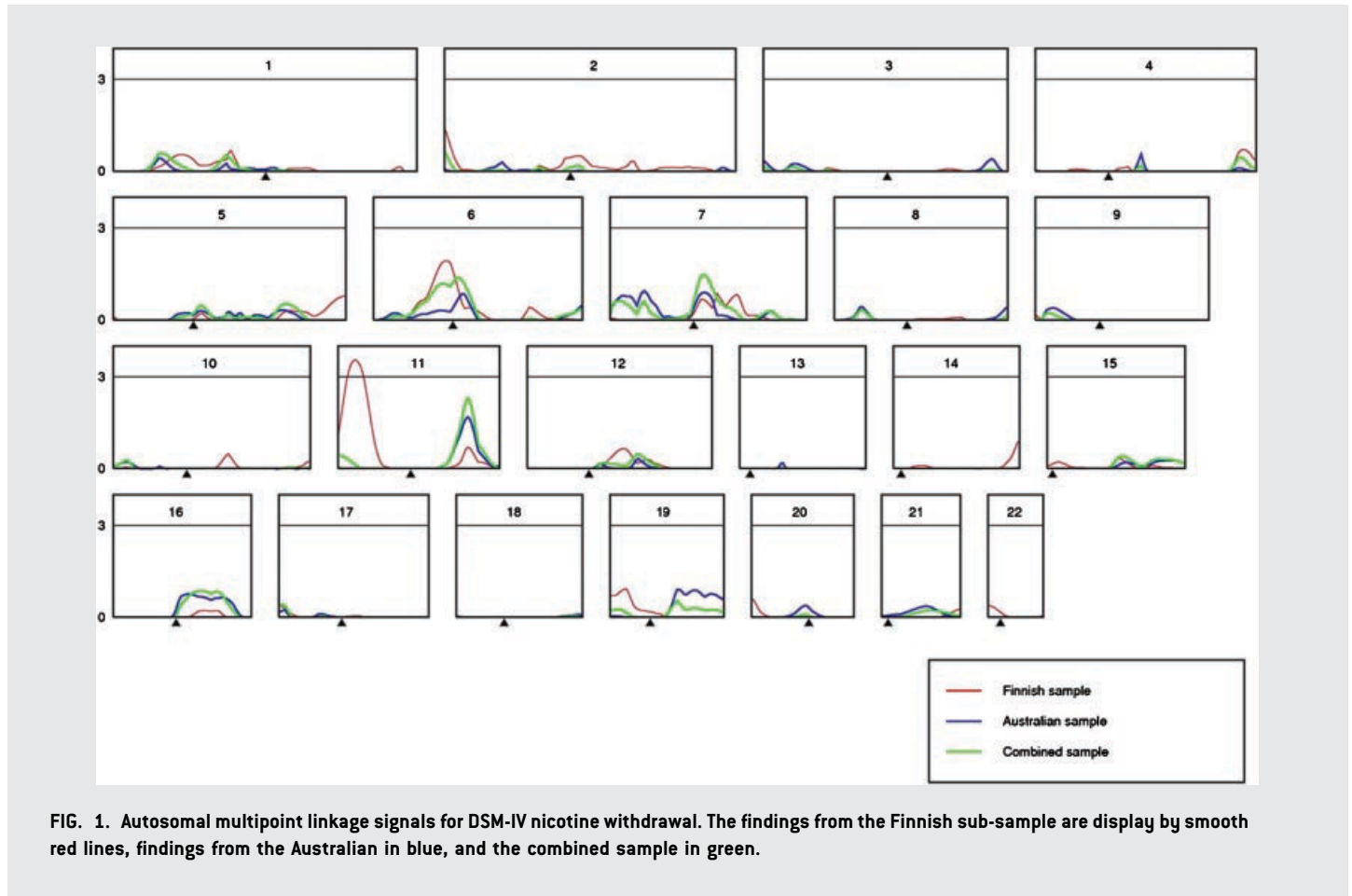


FIG. 1. Autosomal multipoint linkage signals for DSM-IV nicotine withdrawal. The findings from the Finnish sub-sample are display by smooth red lines, findings from the Australian in blue, and the combined sample in green.

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 ND (which includes NW as a symptom) were $r = 0.73$ and $r = 0.72$, but correlations between DSM-IV NW and a definition of ND using a cut-off of four or more on the Fagerström Test of ND [FTND; Heatherton et al., 1989, 1991; Breslau and Johnson, 2000; Bierut et al., 2007; Saccone et al., 2007b] 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 >1.5 for DSM-IV NW in sample specific or combined sample analyses are listed in Table II. 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 75 cM 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-

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 ^a	0.13
	123.3	1.68	0.70	2.30

^aMeets genome-wide significance at $P = 0.013$ with 1,000 simulations in MERLIN.

sample, was observed on chromosome 11 at 123 cM (see Figs. 1 and 2). The multipoint LOD of 3.55 at 11p15 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 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 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-hr [Saccone et al., 2007a] and other quantitative measures of ND did not show linkage on chromosome 11p15 in these samples. This pattern of findings suggest that there may be genetic variation influencing NW that is not easily detected using other measures of ND (including DSM-IV-based ND), 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 NW. Among sibling pairs concordant for DSM-IV NW, the range of number of cigarettes smoked per day (cpd) during peak lifetime use varies across sub-groups 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 NW 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 NW 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

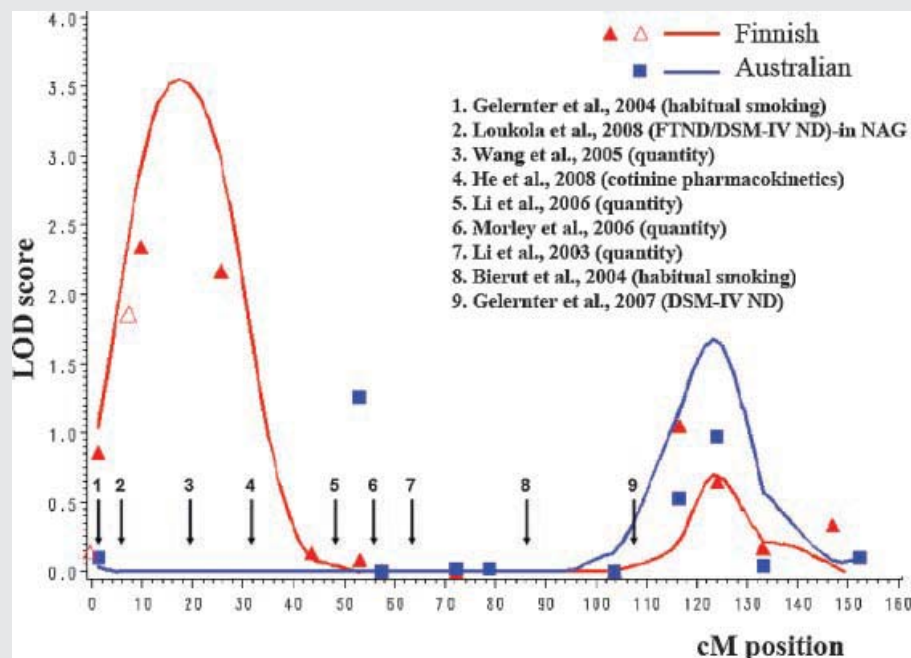


FIG. 2. The multipoint findings on chromosome 11 are represented by red and blue lines, and the single-point findings are represented by red triangles and blue squares, for the Finnish and Australian sub-samples, respectively. Two single-point markers, shown as translucent red triangles in Figure 2, 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.

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 ND 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 and 74 cM, He et al. [2008] recently reported suggestive linkage peaks using measures of cotinine pharmacokinetics. Between our linkage peaks from 40 to 100 cM on chromosome 11, at least four studies have reported linkage signals using smoking-related phenotypes [Li et al., 2003, 2006; Bierut et al., 2004; Morley et al., 2006]: 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 ND 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- α 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 [cf.; 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,b; 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; Swan et al., 2007; Ton et al., 2007]. Following-up on a modest linkage peak for ND on chromosome 11q23; Gelernter et al. [2006] found

significant associations between SNPs in *ANKK1* and ND 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 ND [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], ND [Greenbaum et al., 2006; Saccone et al., 2007b], 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 sub-sample 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 treat 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. [2006] linkage peak for NW 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 NW, 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 >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. One thousand simulations found that a LOD score of 3.1 was the threshold necessary for genomewide significance (e.g., genomewide adjusted P -value <0.05). 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 NW as one important aspect of smoking behavior that is partly under genetic influence.

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