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Linkage Analyses of Stimulant Dependence, Craving and Heavy Use in American Indians

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Abstract

Amphetamine-type substances are the second most widely used illicit drugs in the United States. There is evidence to suggest that stimulant use (cocaine and methamphetamine) has a heritable component, yet the areas of the genome underlying these use disorders are yet to be identified. This study's aims were to map loci linked to stimulant dependence, heavy use, and craving in an American Indian community at high risk for substance dependence. DSM diagnosis of stimulant dependence, as well as indices of stimulant "craving" and "heavy use", were obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). Genotypes were determined for a panel of 791 micro-satellite polymorphisms in 381 members of multiplex families using SOLAR. Stimulant dependence, stimulant "craving" and "heavy stimulant use", were all found to be heritable. Analyses of multipoint variance component LOD scores, failed to yield evidence of linkage for stimulant dependence. For the stimulant "craving" phenotype, linkage analysis revealed a locus that had a LOD score of 3.02 on chromosome 15q25.3-26.1 near the nicotinic receptor gene cluster. A LOD score of 2.05 was found at this same site for "heavy stimulant use". Additional loci with LOD scores above 2.00 were found for stimulant "craving" on chromosomes 12p13.33-13.32 and 18q22.3. These results corroborate the importance of "craving" as an important phenotype that is associated with regions on chromosome 12, 15 and 18, that have been highlighted in prior segregation studies in this and other populations for substance dependence-related phenotypes.

Keywords

Amphetamine dependence; Native American; heritability; genome scan; linkage analyses

Introduction

Stimulants (STIM methamphetamine (MA and cocaine COC are the most commonly used illicit drugs world-wide second to cannabis use Compton et al., 2004; Maxwell and Rutkowski, 2008. Recent surveys indicate that MA is the fastest-growing illicit drug of

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choice, particularly in the Western United States and Canada, leading some to describe the MA problem as an “epidemic” Barr et al., 2006; Tanne, 2006. MA has been demonstrated to produce psychomotor and cognitive impairments, as well as chronic health problems [Richards et al., 1999; Paulus et al., 2002; Gonzalez et al., 2004; Ersche and Sahakian, 2007; Darke et al., 2008; Ersche and Sahakian, 2007; Gonzalez et al., 2004; Paulus et al., 2002; Richards et al., 1999; Shetty et al., 2010). National surveys suggest that stimulant dependence also differs among ethnic groups with Native Americans having the highest rates among all groups evaluated (Iritani et al., 2007; SAMHSA, 2005a,b). Among Native Americans in drug treatment, the rate of primary amphetamine use has been shown to be higher than that for other illicit drugs (Evans et al., 2006). From 1997 to 2004, the number of Indian Health Service outpatient treatment visits attributed to stimulants increased by 30 times (Indian Health Service, 2005). Thus, focusing efforts on understanding the causes of drug dependence in this minority population is critically needed in order to address health disparities (Need and Goldstein, 2009).

Twin and family studies have consistently found that stimulant use and use disorders appear to in part have a genetic basis. Studies that have evaluated the role of genetic and environmental risk factors on stimulant abuse or stimulant dependence in twin samples have found heritability estimates that range from 0.39 to 0.79 (see Kendler and Prescott, 1998a,b; Kendler et al., 2003; Tsuang et al., 1996,1998). Despite these substantial heritability estimates, identifying genetic loci that confer risk for stimulant misuse disorders has been difficult given that the genetic architecture underlying these disorders and substance use disorders in general appears to be polygenic (Barr et al., 2006; Tyrfingsson et al., 2010; Uhl et al., 2009). Nonetheless, these studies suggest identifying genes that contribute to involvement with stimulants may be warranted.

Given that disorders of stimulant use likely represent genetically complex traits that are influenced by a number of genes each of small effect, the genes contributing to the development of these disorders might be detected if more narrowly defined phenotypes or subgroups of stimulant dependent individuals can be identified that show an oligogenic inheritance pattern (i.e., influenced by a small set of genes of moderate effect). For example, Kranzler et al. (2008) used data reduction methods and an empirical cluster-analytic approach to identify subgroups of individuals with cocaine dependence based on measures of cocaine use, cocaine-related effects and treatment history. In their population of small nuclear families they found a 6 cluster solution, and 4 of the 6 clusters were found to yield heritability estimates in excess of 0.3. A linkage analysis of the three clusters that contained >80% of the cocaine dependent subjects revealed a LOD score of 4.66 for membership in the “Heavy Use, Cocaine predominant” cluster on chromosome 12 and a LOD score of 3.35 for membership in the “Moderate Cocaine and Opioid Abuse” cluster on chromosome 18 (Gelernter et al., 2005). This could indicate that loci of moderate effect are contributing to the development of these cocaine dependence subtypes.

Of direct relevance to the present study, we have demonstrated that using “craving” or “strong desire to take a drug” as a phenotype in linkage analyses in populations with drug dependence can produce genomewide significant LOD scores (Ehlers and Wilhelmsen, 2005; Ehlers et al., 2010a). In one study of an American Indian group, analyses of multipoint variance component LOD scores for the dichotomous variable “strong desire for alcohol” revealed evidence for linkage on chromosome 3 with a maximal LOD score of 2.2 and on chromosome 5 with a maximal LOD score of 4.5 (Ehlers and Wilhelmsen, 2005). In another study of families (The San Francisco Family Study), linkage analyses were conducted for a phenotype indexing cannabis “craving” (Ehlers et al., 2010a). The symptom of cannabis “craving” yielded evidence for linkage on chromosome 7 (LOD = 5.7), on chromosome 3 (LOD=4.4), on chromosome 1 (LOD =3.6), and on chromosome 6 (LOD=

3.2). Yet no studies to date have conducted linkage analyses specifically on amphetamine dependence, heavy use, and/or craving phenotypes.

In addition to identifying refined phenotypes, the power of genetic studies of complex phenotypes, can also be increased when they are conducted in well-defined populations such as Native American tribes living on reservations (Lander and Schork, 1994). The present report is part of a larger study exploring risk factors for substance dependence among Native American Indians (see Ehlers et al., 2001a,b,c,d; 2004a, 2008c; Gilder et al., 2004, 2006, 2007, 2009). The lifetime prevalence of substance dependence in this Indian population is high and evidence for heritability and linkage to specific chromosome locations and associations with candidate genes have been demonstrated (see Ehlers and Wilhelmsen, 2005, 2007; Ehlers et al., 2004b, 2006b, 2007a,b,c, 2008a,b, 2009a,b, 2010a,b; Wall et al., 2003; Wilhelmsen and Ehlers, 2005). The current study's aims were to: (1) map loci linked to STIM phenotypes and (2) to determine if there was overlap of the loci identified for STIM phenotypes and loci previously mapped for alcohol and other substance dependence in this American Indian community.

Methods

Participants were recruited from eight geographically contiguous reservations, with a total population of about 3,000 individuals, using a combination of a venue-based method for sampling hard-to-reach populations (Kalton and Anderson, 1986; Muhib et al., 2001), as well as a respondent-driven procedure (Heckathorn, 1997) as previously described (Ehlers et al., 2004a; Gilder et al., 2004). The venues for recruitment included: tribal halls and culture centers, health clinics, tribal libraries, and stores on the reservations. A 10–25% rate of refusal was found depending on venue. Refusal rates were higher at tribal libraries and stores than health clinics and tribal halls/culture centers. Transportation from participants' homes to The Scripps Research Institute was provided by the study.

To be included in the study, participants had to be a Native American Indian indigenous to the catchment area, at least 1/16th Native American Heritage (NAH), between the age of 18 and 70 years, and be mobile enough to be transported from his or her home to The Scripps Research Institute (TSRI). The protocol for the study was approved by the Institutional Review Board (IRB) of TSRI, and the Indian Health Council, a tribal review group overseeing health issues for the reservations where recruitment was undertaken.

Potential participants first met individually with research staff to have the study explained and give written informed consent. During a screening period, participants had blood pressure and pulse taken, and completed a questionnaire that was used to gather information on demographics, personal medical history, ethnicity, and drinking history (Schuckit, 1985). Participants were asked to refrain from alcohol and drug usage for 24 hours prior to the testing. No individuals with detectable breath alcohol levels were included in the study dataset (n=3). During the screening period, the study coordinator also noted whether the participant was agitated, tremulous, or diaphoretic and their data were eliminated from subsequent analyses. Each participant also completed an interview with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) and the family history assessment module (FHAM) (Bucholz et al., 1994), which was used to make substance use disorder and psychiatric disorder diagnoses according to Diagnostic and Statistical Manual (DSM-III-R) criteria in the probands and their family members (American Psychiatric Association, 1987). The SSAGA is a semi-structured, poly-diagnostic psychiatric interview that has undergone both reliability and validity testing (Bucholz et al., 1994; Hesselbrock et al., 1999). It has been used in another Native American sample (Hesselbrock et al., 2000, 2003). Personnel from the Collaborative Study on the Genetics of Alcoholism (COGA) trained all

interviewers. The SSAGA interview includes retrospective lifetime assessments of alcohol use, abuse, and dependence. A research psychiatrist/addiction specialist made all best final diagnoses.

The phenotypes chosen for the present linkage analyses, based on having significant heritability were: (1) a DSM–III-R stimulant (amphetamine or cocaine) dependence diagnosis, (2) stimulant "craving" defined as endorsing: "In situations where you couldn't use stimulants, did you ever have such a strong desire for it that you couldn't think of anything else," and (3) A measure of a period of heavy use of stimulants defined as: "Was there ever a period of a month or more when a great deal of your time was spent using stimulants, getting stimulants, or getting over its effects."

One hundred and eighty-one pedigrees containing 1600 individuals were used in the genetic analyses. Sixty-six families have only a single individual with phenotype data. All these individuals were included within some analyses to the extent that they contribute information about trait means and variance and the impact of covariates. The family sizes for the remaining families ranged between 4 and 41 subjects (average 12.19 ± 8.19). Eighty-one families were genetically informative. The data includes 142 parent-child, 260 sibling, 53 half sibling, 11 grandparent-grandchild, 235 avuncular, and 240 cousin relative pairs. Only sibling, half-sibling, avuncular and cousin pairs were included as being potentially genetically informative. Several pedigrees contained large numbers of individuals and/or complex loops that could not be analyzed due to the high computational demands required. These pedigrees were thus broken using procedures originally described by Lange and Elston (1975), and treated as independent to allow for their inclusion in the linkage analysis.

DNA was isolated from whole blood using an automated DNA extraction procedure, genotyping was done as previously described (Wilhelmsen et al., 2003). Genotypes were determined for a panel of 791 autosomal microsatellite polymorphisms (Weber and May, 1989) using fluorescently labeled PCR primers under conditions recommended by the manufacturer (HD5 version 2.0; Applied Biosystems, Foster City, CA). The HD5 panel set has an average marker-to-marker distance of 4.6 cM, and an average heterozygosity of greater than 77% in a Caucasian population. Allele frequencies observed in the unrelated founders were used for linkage analysis.

Genotypes were determined for 381 subjects. The PREST software program, which assesses degree of allele sharing among relative-pairs, was used to identify potential errors in pedigree structure (McPeek and Sun, 2000). Six individuals were identified as problematic and removed from further analyses. Pedcheck was then used to detect non-Mendelian inheritance patterns (O'Connell and Weeks, 1998). When a Mendelian inconsistency was observed, genotypes for the nuclear family at that polymorphism were removed. This resulted in the removal of 772 genotypes (0.3%). To further reduce errors, the maximum-likelihood error-checking algorithm implemented in Merlin (Abecasis et al., 2002) was used to identify genotypes that had a probability of less than 0.025 of being correct. A total of 508 genotypes (0.2%) were removed in this step. Ultimately 273,598 genotypes (99.5%) were accepted.

Analyses were conducted to estimate the heritability of the three phenotypes of interest: DSM-III-R stimulant dependence, stimulant craving, and heavy use using SOLAR (Almasy and Blangero, 1998) as previously described (see Ehlers et al., 2009). Participant's age at the time of evaluation and sex were evaluated as potential covariates and retained if they accounted for at least 5% of the total variance. The total additive genetic heritability (h^2) and its standard error were estimated, and the probability that h^2 was greater than zero was determined using a Student's t-test for each scale. All three phenotypes were found to be

heritable and as such suitable for linkage analyses. There were 684 individuals with full phenotype data included in these analyses.

For linkage analysis, a variance components approach was used to calculate multipoint LOD scores at 1 cM intervals across the genome for the three stimulant phenotypes using SOLAR v4.2.0 (Almasy and Blangero, 1998; S.F.B.R, 2011). Because the Native American Mission Indian sample contains large extended pedigrees, a variance components approach to linkage analysis allowing for multiple pedigree types was preferred over sibling pair approaches (i.e., Kong and Cox statistic (1997)) due to the greater statistical power afforded by the former (Amos et al., 1997; Duggirala et al., 1997). All traits were analyzed using a latent threshold model in which a normally distributed trait is assumed along with a threshold in the distribution above which an individual is designated affected.

Variance components linkage analysis assumes that phenotypes are normally distributed, and violations of this assumption can result in inflated LOD scores. To protect against this possibility, simulations were conducted in which a single genetic locus was simulated under the null hypothesis of no linkage across 100,000 trials to derive pointwise empirical p-values. These p-values were used to determine the significance of the reported LOD scores (Blangero et al., 2000) with a $p<2.2\times10^{-5}$ used to identify genome-wide significance as suggested by Lander and Kruglyak (1995), and $p<0.001$ to identify suggestive evidence for linkage. These simulations suggested some negative bias in LOD scores for the stimulant dependence diagnosis though little bias for the remaining phenotypes as 17, 105, and 72 simulations out of 100,000 for the stimulant dependence, “craving,” and “heavy use” phenotypes, respectively, yielded LOD scores greater than 2.00 compared to an expected 100 simulations for each phenotype and 0, 4, and 3 out of 100,000 simulations for the stimulant dependence, “craving,” and “heavy use” phenotypes, respectively, yielded LOD scores greater than 3.00 compared to an expected 10 simulations for each phenotype.

To better characterize the evidence for linkage across families at the reported peaks, heterogeneity tests of the family-specific LOD scores were performed using the SOLAR HLOD (Goring, 2002) test. This test contrasts a null model in which families belong to a single distribution exhibiting genetic linkage to the tested locus against an alternative model in which families belong to one of two distributions only one of which shows evidence of genetic linkage to the tested locus.

Results

Three hundred eighty-one participants out of a larger population of 720 had completed a SSAGA and had genotyping data that were available for these analyses. Two hundred and twelve participants met criteria for amphetamine dependence, 17 met criteria for cocaine dependence and 51 met criteria for both cocaine and amphetamine dependence, for a total number of participants with either diagnosis (STIM DEP) of 280 which was 40 percent of the sample. Demographics of this sample are presented in Table 1. There were no significant differences in the demographics between the participants with phenotyping data and genotyping available (e.g. the linkage sample, n=381) and the entire sample of participants in the study with valid SSAGA data (n=720) but no genotyping, at the $p<0.01$ level.

The phenotype of DSM-III-R STIM DEP (e.g. amphetamine and/or cocaine) was found to be significantly heritable ($h^2 = 0.21 \pm 0.13, p<0.05$), as were the symptoms of STIM “craving” ($h^2 = 0.5 \pm 0.20, p<0.003$), and STIM heavy use ($h^2 = 0.36 \pm 0.36, p<0.006$). Analyses of multipoint variance component LOD scores did not reveal any significant loci for stimulant dependence. An inspection of the results for the stimulant dependence phenotype showed that 51.7% of loci yielded a LOD score ≤0, 48.0% of loci yielded a LOD

score between 0 and 1.00, and 0.3% of loci yielded a LOD >1.00. Analysis of the "craving" phenotype revealed one locus that had a LOD score greater than 3.0 on chromosome 15q25.3-26.1 at 83 cM (LOD= 3.02) (pointwise empirical p-value=0.00004) and two loci with LOD scores greater than 2 on chromosomes 12p13.33-13.32 at 5 cM (LOD= 2.11) (pointwise empirical p-value=0.0009) and 18q22.2 at 113 cM (LOD=2.55) (pointwise empirical p-value=0.00032). An inspection of the results for the "craving" phenotype showed that 49.6% of loci yielded a LOD score ≤0, 47.2% of loci yielded a LOD score between 0 and 1.00, 1.9% of loci yielded a LOD between 1.00 and 2.00, and 1.3% of loci yielded a LOD score >2.00. One locus was found with a LOD score over 2.0 for the "heavy use" phenotype on chromosome 1515q25.3-26.1 at 82 cM (LOD=2.04, pointwise empirical p-value=0.0007). An inspection of the results for the "heavy use" phenotype showed that 52.2% of loci yielded a LOD score ≤0, 46.6% of loci yielded a LOD score between 0 and 1.00, 1.2% of loci yielded a LOD between 1.00 and 2.00, and <0.1% of loci yielded a LOD score >2.00.

Figure 1 presents the linkage peaks generated by these analyses across the genome. Figure 2 presents data for the three phenotypes for chromosome 15. Table 2 presents the peak LOD scores, the closest marker location for the loci identified, pointwise empirical p values, and additionally gives information of other findings in the literature for substance-related phenotypes observed at or near those locations. Notably, none of the reported peaks exhibited heterogeneity in LOD scores across pedigrees. The estimated alpha scores, which can be interpreted as the probability of a given family belonging to a single population yielding evidence for linkage, were >0.97 for all families at each peak.

Discussion

It has been suggested that the effort to identify genetic factors and the mechanisms whereby they influence addiction may be aided by the use of phenotypes that may be more closely related to the biological processes underlying risk for use disorders (Gottesman and Gould, 2003). One phenotype that most substance dependence syndromes have in common is craving. A general theory of addiction posits that the neurobiological mechanisms underlying the homeostatic regulation of appetitive drives and instincts becomes dysregulated during the process of drug exposure (Koob, 2000). Some measures of the strength of this process include an increase or strong desire to take the drug often called 'drug craving' (see Anton, 1999). Human and animal studies have demonstrated that craving is an important element in the addictive process and that control of craving may improve efforts at abstinence (see Anton, 1999; Field et al., 2004; Haughey et al., 2008; Heishman and Singleton, 2006; Robinson and Berridge, 1993; Sinha and O'Malley, 1999; Wise, 1988). Evaluation of the heritability of stimulant craving (h^2 =0.5) and heavy use (h^2 =0.36) demonstrated that these two phenotypes were more heritable than the DSM diagnosis of stimulant dependence (h^2 =0.20). Thus it is notable that three sites in the genome, chromosomes 12p13.33-13.32, 15q25.3-26.1 and 18q22.3, suggested evidence for linkage to these latter phenotypes, whereas there was no suggestive evidence for linkage observed for the stimulant dependence diagnosis.

One location that provided suggestive evidence of linkage was on chromosome 12p13.33-13.32 at 5 cM that had a LOD score of 2.11. A number of previous studies in this Indian population have found evidence or suggested evidence for linkage for a number of phenotypes associated with substance dependence including alcohol dependence phenotypes (Ehlers et al., 2004b), alcohol craving (Ehlers and Wilhelmsen, 2005), tobacco usage (Ehlers and Wilhelmsen, 2006), cannabis dependence phenotypes (Ehlers et al., 2009), externalizing diagnoses (Ehlers et al., 2008a), Body Mass Index (Ehlers and Wilhelmsen, 2007), EEG phenotypes (Ehlers et al., 2010c), and level of response to alcohol (Ehlers et al., 2010d).

None of these studies found evidence or suggestive evidence for linkage at the site on chromosome 12 identified for the STIM craving phenotype in the present study suggesting that it may be unique to this phenotype in this population. However, this site was identified by Li and colleagues (2008) for a phenotype indexing the number of cigarettes smoked per day. In that study, a LOD score of 2.49 was found using the variance component method at 6 cM in a EuroAmerican sample, and a LOD score of 4.4 was found at that same site in a combined sample of EuroAmericans and African Americans.

A second area of the genome that was identified in the present study for the STIM heavy use and STIM craving phenotypes was on chromosome 15q25.3-26.1 at80 cM (LOD score: 2.05 and 3.02, respectively). This site is near a location that was reported previously as linked to alcohol withdrawal in this Indian population (Ehlers et al., 2004b), alcohol dependence with late onset and harm avoidance personality features in the COGA study (Dick et al., 2002), and a cannabis craving phenotype in the San Francisco Family Alcoholism study (Ehlers et al., 2009). It contains some promising candidate genes such as *NTRK3*, which belongs to a family of genes that encode for neurotrophic tyrosine kinase receptors and is involved in striatal neuronal development. Notably, *NTRK3* expression is increased following cocaine administration in rats (Freeman et al., 2003; Jung et al., 1996) and is also altered following prenatal ethanol administration to rat pups (Light et al. 2002; Moore et al., 2004).

The site we identified on human chromosome 15q25.3-26.1 for the STIM phenotypes in the present study is also approximately 10 Mb telomeric of the human alpha 3 (*CHRNA3*), alpha 5 (*CHRNA5*), and beta 4 (*CHRNB4*) neuronal nicotinic receptor subunit genes on the long arm of chromosome 15 (15q24) (Raimondi et al., 1992). These receptor genes have been found to be associated with numerous substance dependence phenotypes including: heavy smoking and nicotine dependence (Berrettini et al., 2008; Bierut, 2010, a review; Li et al., 2010; Saccone et al., 2009), opioid dependence severity (Erlich et al., 2010), multiple dependence phenotypes (Sherva et al., 2010), level of response to alcohol (Joslyn et al., 2008), and tobacco related cancers (Lips et al., 2010; Truong et al., 2010). Using syntenic mapping (Ehlers et al., 2010e), this site on human chromosome 15 was found to map to a region on mouse chromosome 9 where the *CHRNA3*, *CHRNA5*, *CHRNB4* are located (Bessis et al., 1990; Eng et al., 1991), as well as genes encoding for cytochrome P45, subfamily I (CYPlal), mannose phosphate isomerase (MP-1) and the muscle form of pyruvate kinase (Pk-3) (Cox and Donlon, 1989). Multiple studies performed with mice have found quantitative trait loci for alcohol preference within this region (Phillips et al., 1994, 1998; Tarantino et al., 1998) as well as associations with nicotine intake (Fowler et al., 2011). These findings suggest that a large group of homologous sequences may eventually be found on human chromosome 15 and mouse chromosome 9 that may be important for substance dependence and that the search for additional candidate genes within this location may be productive in identifying general mechanisms underlying addiction-related phenotypes.

One additional site provided suggested evidence for linkage to STIM craving on chromosome 18q22.3 at 113 cM with a LOD score of 2.55. A few other studies have identified linkage peaks in this general location on chromosome 18. For instance, Agrawal et al., (2008) have reported a site on chromosome 18 at 97 cM (LOD=2.14) that was linked to the frequency of use of cannabis. Additionally, Li et al., (2008) found a broad peak in this region of chromosome 18 for tobacco use phenotypes in both a EuroAmerican and African American sample. This area of the genome has not been previously found to be linked to other substance use phenotypes, including cannabis and tobacco, in this American Indian population.

In conclusion, these data represent the first linkage analysis of amphetamine-related phenotypes in any population. The results suggest that several areas of the genome may harbor genes that modulate level of addiction to stimulants. Loci highlighted in prior studies in this population as well as other populations for substance dependence phenotypes were identified including a site on chromosome 15q25.3-26.1. The results of this study should, however, be interpreted in the context of several limitations. First, stimulant dependence was defined by DSM-III-R, and thus, the use of DSM-IV criteria might have produced different results. For example, the DSM-III-R criteria considered failures to fulfill role obligations and the use of the substance under hazardous conditions as symptoms of dependence, whereas the DSM-IV considers these as symptoms of abuse. Such differences in diagnostic criteria could have influenced the results. Second, the heritability estimate for stimulant dependence in the present study was lower than has been previously reported, and this may have contributed to the lack of linkage findings for this phenotype. Third, although dense coverage was achieved across the genome using microsatellites (average marker-to-marker distance of 4.6 cM), high throughput genotyping methods can now be used to generate high-density SNP data for linkage analysis, which might have improved our ability to detect risk loci. Fourth, the findings of this study may not generalize to other Native Americans or represent all Native American Indians of the tribes studied, and comparisons of linkage findings to non-Indian populations may be limited by differences in a host of potential genetic and environmental variables. Despite these limitations, this report represents an important step in an ongoing investigation to understand the genetic determinants associated with the development of substance use disorders in this high risk and understudied ethnic group.

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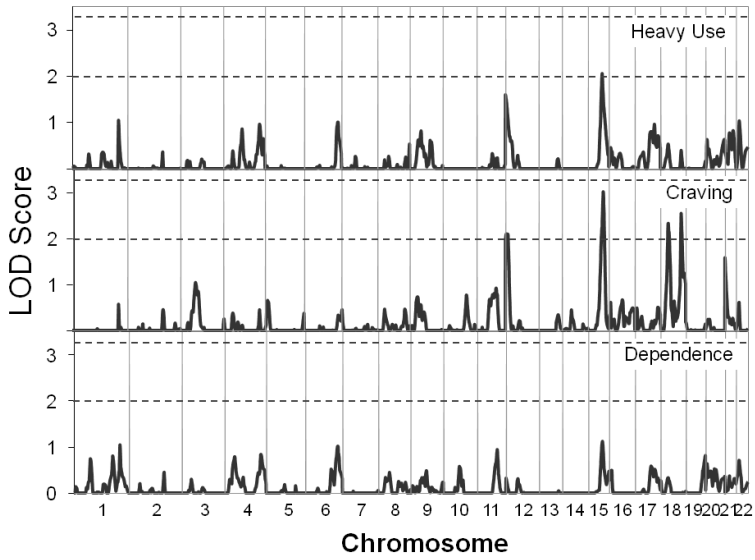

Figure 1.

Multipoint Linkage Analysis for the heavy stimulant usage (HEAVY USE) stimulant craving (CRAVING) and stimulant dependence (DEPENDENCE) phenotypes for the entire genome. Results for each chromosome are aligned end to end with the p terminus on the left. Log of the Odds (LOD) score is plotted on the Y-axis. Horizontal dashed lines indicated the cutoffs for suggestive evidence of linkage (LOD > 2.00) and the empirically determined threshold for genomewide significant evidence of linkage (LOD > 3.33). The numbers above on the X-axis indicate the chromosome number. Vertical lines indicate the boundaries between the chromosomes.

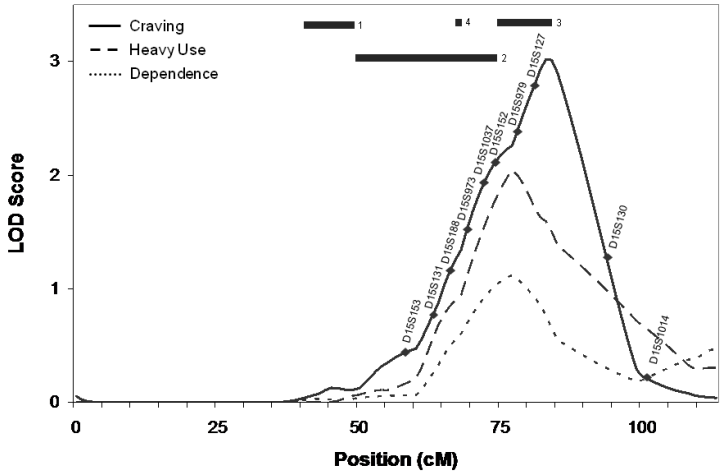

Figure 2.

Multipoint Linkage Analysis for the heavy stimulant usage (HEAVY USE) stimulant craving (CRAVING) and stimulant dependence (DEPENDENCE) phenotypes for chromosome 15. Log of the Odds (LOD) score (Y-axis) is plotted for the chromosome location map (in centimorgans (cM), X-axis). Locations of the markers across the peak are presented. The following numbers indicate the location of previous linkage and association findings: 1 - Alcohol Withdrawal (Ehlers et al., 2004b), 2 - Anxious Drinking (Dick et al., 2002), 3 - Cannabis Craving (Ehlers et al., 2010a), 4 - CHRN gene cluster: Lung Cancer (Truong et al., 2010), Cigarette Smoking (Bierut, 2010; Saccone et al., 2009).

Table 1

Demographics

	Linkage Sample (n = 381)	Entire Sample (n = 720)
Gender	Male = 149 Female = 232	Male = 299 Female = 421
Married (n)	81	126
Employed (n)	177	286
Income ≥ $20,000 yr. (n)	182	366
Native American Heritage, n ≥ 50%	157	323
Age (yrs)	30.1 ± 0.6	31.1 ± 0.5
Education (yrs)	11.6 ± 0.1	11.6 ± 0.1
Stimulant dependence	157	282
Stimulant craving	98	192
Heavy stimulant use	122	229

Table 2

Genetic loci for methamphetamine use traits in an American Indian community

CHR	Trait	LOC (cM)	LOD	Nearest Marker	Pointwise Empirical p-value	Supporting References (phenotype)
12	STIM Craving	5	2.11	D12S352/ D12S1725	0.00099	Li et al., (2008) (nicotine dep)
15	STIM Heavy Use	77	2.05	D15S979	0.00078	Ehlers et al. 2004b, (alc withdrawal), Dick et al.,(2002) (alc dep subtype) Ehlers et al., 2010a (cannabis craving), Joslyn et al., (2008) (level of response to alcohol)
15	STIM Craving	83	3.02	D15S127	0.00004	Truong et al., (2010) (lung cancer, pooled analysis), Bierut (2010) (nicotine dep, a review),
18	STIM Craving	113	2.55	D18S469	0.00032	Li et al., (2008) (nicotine dep), Agrawal et al., (2008) (cannabis use behaviors)