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Addict Biol. Author manuscript; available in PMC 2010 April 6.

Published in final edited form as:
Addict Biol. 2009 July ; 14(3): 338–348. doi:10.1111/j.1369-1600.2009.00160.x.

Heritability and a genome-wide linkage analysis of a Type II/B Cluster Construct for cannabis dependence in an American Indian community

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Abstract

Subtyping of substance dependence disorders holds promise for a number of important research areas including phenotyping for genetic studies, characterizing clinical course, and matching treatment and prevention strategies. This study sought to investigate whether a dichotomous construct similar to Babor's Types A/B and Cloninger's Types I/II for alcohol dependence can be identified for cannabis dependence in a Native American sample. In addition, heritability of this construct and its behavior in a genetic linkage analyses were evaluated. Information on cannabis use and dependence symptoms and other psychiatric disorders was obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) from a community sample of 606 American Indians. Hierarchical average linkage and K means cluster analysis was used, and a 3-cluster solution was found to generate the best separation of variables. Ninety-one percent of cannabis dependent participants fell into one of the two subtypes: Type A/I cluster (N=114, 56%) and Type B/II cluster (N=70, 35%). Heritability (estimated using SOLAR) was only significant for the Type B/II cluster (h^2 =0.44, S.E.=0.18, p<0.01). Evidence for linkage was found for the Type B/II cluster (vs. no diagnosis) on chromosome 16 (@139 cM, LOD score= 4.4), and on chromosome 19 (@74 cM, LOD score= 6.4). Regions of interest for this phenotype (LOD > 1.5) were also located on chromosomes 14,21,22. These findings suggest that a Type B/II cannabis dependence phenotype can be identified in this population and that it is in part heritable and linked to areas of the genome identified previously for drug dependence phenotypes in this population as well as in other studies.

Keywords

American Indians; cannabis dependence; heritability

INTRODUCTION

Cannabis use and use disorders are prevalent in the industrialized world, including in Europe, the United States, Canada, and Australia (Anthony *et al.* 1994; Swift *et al.* 2001; Vega *et al.* 2002; SAMHSA 2003). In the U.S., cannabis use disorders (abuse and dependence) rank third, behind only tobacco and alcohol, in frequency of substance use disorders (Anthony *et al.* 1994; SAMHSA 2003). The 2003 National Survey on Drug Use and Health (SAMHSA

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2003) reported that 40.6% of the U.S. population had used cannabis or hashish during their lifetime and that 4.2 million individuals age 12 years and older met criteria for past year cannabis abuse or dependence using DSM-IV (American Psychiatric Association 1994) criteria. The 2001–2002 National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) found that 8.45% of the population had a lifetime DSM-IV diagnosis of either cannabis dependence (1.30%) or abuse (7.16%) (Conway *et al.* 2006). Using DSM-III-R criteria, the National Co morbidity Survey (NCS) found that 46.3% of the population had a lifetime history of extra medical use of cannabis and 4.2 % of the population aged 18–54 years had a lifetime DSM-III-R (American Psychiatric Association 1987) history of cannabis dependence (Anthony *et al.* 1994).

Persistent cannabis use is associated with significant morbidity. Persistent use poses health problems similar to those of tobacco (Taylor *et al.* 2000; Mittleman *et al.* 2001; Fisher *et al.* 2005; Hashibe *et al.* 2005; Tashkin 2005), is implicated in syndrome characterized by apathy, loss of goal-directed behavior, and cognitive impairment termed the “amotivational syndrome” (Sharma 1975; Pope *et al.* 2001; Solowij *et al.* 2002; Schuckit 2006), and is associated with impaired educational and work performance (Kandel & Chen 2000; Lynskey & Hall 2000; Swift *et al.* 2001; Schuckit 2006). Cannabis use, particularly by adolescents and young adults, may also facilitate progression to other illicit drug use (the “gateway” drug hypothesis) (Fergusson & Horwood 2000; Lynskey *et al.* 2003). In the general U.S. population, cannabis dependence is significantly co-morbid, not only with alcohol and other drug dependence, but also with anxiety, depression, and personality disorders (Regier *et al.* 1990; Troisi *et al.* 1998; Agosti *et al.* 2002; Conway *et al.* 2006; Stinson *et al.* 2006) suggesting that cannabis dependence shares etiological relationships with other substance use and psychiatric disorders.

Work to date suggests that there are significant genetic as well as environmental risk factors for substance dependence disorders (Tsuang *et al.* 1996, 1998; Kendler & Prescott 1998; Maes *et al.* 1999; Kendler *et al.* 2000, [2003], [2007]; Miles *et al.* 2001; Lynskey *et al.* 2002; Rhee *et al.* 2003; Wilhelmsen & Ehlers 2005; Ehlers *et al.* 2007; Hopfer *et al.* 2007; Agrawal *et al.* 2008; Kranzler *et al.* 2008). Multiple genetic and environmental risk factors for substance dependence disorders raise the possibility that a substance dependence diagnosis is a phenotypically similar but etiologically heterogeneous diagnosis. Etiologic heterogeneity also raises the possibility that subtyping of dependence disorders with more homogeneous environmental and genetic determinants might lead to more successful characterization of etiological risk and protective factors.

Most of the work on subtyping substance dependence disorders has focused on alcohol dependence. These efforts have produced several sub typing schemes (Hesselbrock & Hesselbrock 2006). The best known of these are Cloninger’s Types I and II (Cloninger *et al.* 1981) and Babor’s Types A and B (Babor *et al.* 1992; Brown *et al.* 1994). Cloninger’s Type II and Babor’s Type B are similar in having earlier onset of drinking problems, more antisocial and other psychiatric co-morbidity, a more severe course as opposed to Type I and Type A. A Type A/B dichotomy has been found for alcohol dependence in several ethnicities (Hesselbrock & Hesselbrock 2006). Evidence suggests that type II/B might be more heritable than type I/A (Cloninger *et al.* 1981; Foroud *et al.* 1998), raising the possibility that Type II/B is more genetically based and Type I/A more environmentally based (Hesselbrock & Hesselbrock 2006).

Recently, efforts have been made to subtype drug use disorders using cluster analysis. Ball and colleagues (1995) adduced evidence for a Type A/B in cocaine abusers. Feingold and colleagues (1996) adduced evidence for a Type A/B dichotomy from a group of alcohol, cocaine, marijuana, and opiate abusers approximately 60% of whom were recruited from

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treatment programs. Kranzler and colleagues (2008), in a study of cocaine dependence in 1393 subjects from 660 small nuclear families, identified 6 clusters, four of which showed rates of cocaine dependence of 78–100% with heritability ranging from 0.32 to 0.50. In those 4 clusters, rates of ASPD ranged from 9.3 to 19.9% and major depression episode from 6.7 to 20.1%. Gelernter and colleagues (2006) used cluster analytic methods to identify three clusters of moderate to heavy opioid users with heritability ranging from 0.40 to 0.65. These studies are producing specific genome wide scan information (Gelernter *et al.* 2005, 2006).

Efforts to subtype substance dependence disorders in select ethnic minority populations are important for several reasons. First, ethnic minority populations may be more homogeneous in terms of both genetic and environmental risk factors and so they may offer advantages for characterizing these risk and protective factors for substance use disorders over more heterogeneous samples taken from the general population. Second, some ethnic minority populations bear a higher burden from some substance use disorders than the general population. Characterizing the specific risk and protective factors in these groups likely to lead to more effective treatment and prevention strategies is an important public health objective.

Some Native American tribes have been reported to have high rates of substance use disorders, including cannabis dependence (Mitchell *et al.* 2003; Gilder *et al.* 2006, 2007). In previous work with a Native American community, Ehlers and colleagues have found high rates of lifetime DSM-III-R cannabis dependence (43% for men, 24% for women) (Gilder *et al.* 2006) and have characterized some of the risk factors associated with cannabis dependence. Early cannabis use was found to be significantly associated with cannabis dependence (Ehlers *et al.* 2007). Unlike the general population, independent anxiety and affective disorders were not comorbid with cannabis dependence (Gilder *et al.* 2006). The aims of the present study were to: 1) further characterize cannabis dependence in this sample by subtyping the participants with cannabis dependence using cluster analytic techniques; 2) determine if those subtypes have differential heritability; and 3) conduct a linkage analysis of heritable subtypes of cannabis dependence.

METHODS

Participants

Participants, known collectively as Mission Indians, were recruited from eight geographically contiguous reservations with a total population of about 3,000 individuals. Participants were recruited using a combination of a venue-based method for sampling hard-to-reach populations (Kalton & Anderson 1986; Muhib *et al.* 2001) as well as a respondent-driven procedure (Heckathorn 1997). The venues included: tribal halls, health clinics, tribal libraries, and stores on the reservations. Fliers advertising the study were placed in each venue with the telephone number of the tribal recruitment coordinator. The venues were also regularly visited by the tribal recruitment coordinator who approached potential participants to offer information about and enrollment in the study. Approximately half of the participants were recruited using each method. A 10–25% rate of refusal using the venue method occurred depending on venue. The refusal rate in the respondent-driven procedure is not known. Potential participants contacted the tribal recruitment coordinator or the study coordinator at the research facility. At that time, interested participants were given a brief description of the study, told transportation would be provided, and informed as to the amount they would be paid for participation. Individuals who elected to participate by the venue method were encouraged to inform other eligible participants about the study (respondent-driven procedure). Transportation from home to The Scripps Research Institute was provided by the study.

To be included in the study, participants had to be Mission Indian, at least 1/16th Native American Heritage (NAH), between the age of 18 and 70 years, and be mobile enough to be

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transported from his or her home to the General Clinical Research Center (GCRC) of The Scripps Research Institute (TSRI). The protocol for the study was approved by the Institutional Review Board (IRB) of TSRI, the Scientific Advisory Committee of the GCRC, 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, took an alcohol breathalyzer test to assess blood alcohol concentration, and completed a questionnaire that was used to gather information on demographics, personal medical history, ethnicity, and substance use history (Schuckit 1985). No individuals with detectable blood alcohol levels were included in the study dataset. During the screening period, the study coordinator noted whether the participant was agitated, tremulous, or diaphoretic.

Psychiatric diagnoses and symptom history

Each participant also completed an interview with the SSAGA (Bucholz *et al.* 1994), which was used to collect demographic information, make lifetime substance dependence and psychiatric disorder diagnoses according to DSM-III-R criteria (American Psychiatric Association, 1987), and collect other information on cannabis use and use related symptomatology. The SSAGA is a fully 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; Hesselbrock *et al.* 2003). Interviewers were trained by personnel from COGA. All best final diagnoses were made by a research psychiatrist/addiction specialist (DAG).

Diagnostic criteria and lifetime rates of cannabis dependence, four anxiety disorders (panic disorder with or without agoraphobia, agoraphobia without panic, social phobia, and obsessive-compulsive disorder), three affective disorders (major depressive disorder, bipolar I disorder, and dysthymic disorder), childhood conduct disorder (onset of three or more conduct disorder symptoms before age 15), and adult antisocial personality disorder (ASPD) were evaluated. In the present study, diagnoses of anxiety and affective disorders were considered only if they were independent of alcohol and drug use. Criteria for diagnosing anxiety and affective disorders independent of, as opposed to induced by, cannabis, alcohol, or other substances followed those developed by Schuckit and colleagues (1997a, 1997b), Hesselbrock and colleagues (2000), and DSM-IV-TR (American Psychiatric Association 2000). These criteria have been described previously (Gilder *et al.* 2004, 2006). Severity of the most severe lifetime depression episode was calculated as the number of DSM-III-R symptoms in addition to depressed mood during that episode, whether or not that episode was deemed independent of alcohol or drug use and whether or not the depression episode met criteria for major depression episode. In addition, the number and kind of cannabis withdrawal symptoms, the severity of cannabis use and age of onset of cannabis use were determined.

Clinical Data Analysis

SPSS (version 11 for Mac, SPSS, Inc., Chicago, IL) was used to generate hierarchical average linkage and K means cluster analysis from clinical variables selected a priori as likely to contribute to the heritability of cannabis dependence. These variables were: age of first cannabis use, the number of childhood (<15 years) and adult (>=15 years) antisocial symptoms, the number of co-morbid lifetime anxiety and affective disorders, the number of DSM-III-R cannabis dependence criteria, the number of cannabis withdrawal symptoms, and severity of cannabis use. The number of cluster solutions was also specified a priori as two, three, and four. The number of cluster solutions was kept low in order to generate cluster solutions likely to be clinically meaningful.

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Subsequently, comparisons of each clinical variable in each pair of clusters in each cluster solution were undertaken. For example, age of onset of use was compared in each pair of clusters in the two-cluster solution (1 vs. 2), the three-cluster solution (1 vs. 2, 1 vs. 3, and 2 vs. 3), and the four-cluster solution (1 vs. 2, 1 vs. 3, 1vs. 4, 2 vs. 3, 2 vs. 4, 3 vs. 4). The three-cluster solution generated the best separation of the data (see *Results*, below). Demographic variables, which had not been used to generate the clusters, were then compared in each pair of clusters in the three-cluster solution. Continuous variables, including all clinical variables, age, and years of education, were compared using analysis of variance (ANOVA). Dichotomous variables, including gender, current employment (yes vs. no), annual household income (<$20K vs. >=$20K), Native American Heritage (<50% vs. >=50%), and whether currently married (yes vs. no), were compared using chi-square and Fisher exact test. Significance was set at $p<0.05$.

Genetic data analyses

One hundred and seventy-two pedigrees containing 1548 individuals were used in the genetic analyses. Of these, 584 individuals were from 106 extended families where multiple family members were directly interviewed, data from these individuals were used to calculate heritability of the phenotypes. Sixty-six additional families have only a single individual with direct interview data. The individuals in these 66 families were not included in the linkage analyses but were included in the co-morbidity analyses and in the calculation of trait means and variance in order to determine the impact of covariates. Four hundred and sixteen individuals from 81 extended pedigree families have both genotype and phenotype data and were used in the linkage analyses. The family sizes for the 81 families ranged between 4 and 41 members (average 13.9±8.9) with between 2 and 15 individuals having both genotype and phenotype data (average 5.0±3.3). The 416 individuals within the 81 families that were genetically informative include: 142 parent-child, 260 sibling, 53 half-sibling, 11 grandparent-grandchild, 235 avuncular, and 240 cousin relative pairs. Only sib, half-sib, avuncular and cousin pairs were included as being potentially genetically informative. Family structure and genotype consistency were verified and described previously (Ehlers *et al.* 2004; Ehlers & Wilhelmsen 2005, 2006, 2007).

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 & 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 were estimated from the entire Mission Indian population with genotype data. Gender and age accounted for greater than 5% of the phenotypic variance for each of the phenotypes. Therefore, age and gender were included as covariates in the analyses.

Genotypes were ultimately determined for 416 participants. The total additive genetic variance (heritability, h^2) and its standard error were estimated for the CD, ASPD, ASPD/CD phenotypes using SOLAR (http://solar.sfbrgenetics.org/). Two approaches to estimating heritability were used for the three traits. In the first approach the trait was modeled as a latent normally distributed variable with a threshold above which an individual is considered "affected". Using a second approach, the same trait was modeled as if it was a normally distributed variable. In this case heritability is higher for the trait modeled as if it was a normally distributed variable. These methods can occasionally give very dissimilar results presumably because of factors related to convergence. In this analysis it was required that both methodologies provide support for heritability prior to linkage analyses and the lower estimate

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is presented. Variance component estimate methods were used to calculate LOD scores using SOLAR v2.0.4 (Almasy & Blangero, 1998). Simulation analysis was used to estimate empirical LOD scores and make appropriate genome wide adjustments for non-normality (Blangero *et al.* 2000).

RESULTS

Demographics

Six hundred and six individuals completed an interview with the SSAGA. Overall, the demographic characteristics of the sample, including mean age (30.4 ± 11.85 yrs), years of education (11.6 ± 1.6 yrs), economic status (47% of the sample had an annual household income < $20K), employment (44% of the sample had any current employment and 32% of the sample had current fulltime employment), and current marital status (single= 65%, married =19%, divorced=8%, separated=7%, widowed=2%), are similar to available information for the tribe from U.S. census data (United States Bureau of the Census 1990). Forty-four percent of men and 26% of women had lifetime DSM-III-R cannabis dependence. Demographic characteristics of participants with and without DSM-III-R cannabis dependence were compared. There were no significant differences between the groups for age, economic status, employment, marital status, and Native American Heritage. Compared to participants without cannabis dependence, participants with cannabis dependence were more likely to be male (p<0.001), younger (p<0.05), and have fewer years of education (p<0.01).

Cluster Analysis

Two hundred and two individuals with lifetime DSM-III-R cannabis dependence provided the information on variables associated with cannabis use and cannabis dependence used to generate clusters. Two, 3, and 4 cluster solutions were considered. The three-cluster solution showed the best separation of the data as evidenced by significant separation of a larger number of variables than was seen in the two and four cluster solutions. Therefore the three cluster solution was used in subsequent analyses including estimates of heritability and linkage.

In the three cluster solution, the most common cluster was a Type A/I cluster (N=114, 56%), and the second most common was a Type B/II (N=70, 35%). The Type B/II cluster, as compared to the Type A/I cluster, was characterized by a earlier age of onset of use (mean 11.5 compared to 13.9 years), more conduct and adult antisocial behaviors, and more cannabis dependence and withdrawal symptoms. The third cluster, called "Cluster 3" in this analysis, was the least common (N=18, 9%); it was not different from the Type A/I cluster on any variable except age of onset of use (mean 19.9 compared to 13.9 years). The Type B/II cluster, as compared to Cluster 3, was characterized by earlier age of onset of use (mean 11.5 compared to 19.4 years), more conduct and adult antisocial behaviors, and more cannabis dependence symptoms, but not more withdrawal symptoms. Numbers of independent anxiety and affective disorders did not differ in any pairwise cluster comparison.

Demographic characteristics of the three clusters and post hoc comparisons of pairs of clusters for each demographic variable are shown in Table 2. Pairs of clusters did not differ in any demographic variable except age and gender. Cluster 3 participants (mean age 38.3 years) were significantly older than participants in both the Type II/B (mean age 28.2 years, p<0.001) and Type I/A (mean age 26.8 years, p<0.001) clusters. The Type II/B and Type I/A clusters did not significantly differ on age. Both the Type I/A cluster (p<0.03) and the Type II/B (p<0.002)) differed in the proportion of women in the cluster based on the total number of women/men ascertained.

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Heritability and Linkage

Heritability of each of the three clusters was tested and found to be significant only for the Type B/II cluster (h^2 =.44, S.E. = 0.18, p<0.01). In the Type B/II cluster, age at interview and gender were significant covariates. Gender (p=0.000006) and age (p= 0.02) together accounted for 5% of the variance respectively. As seen in Fig. 1 and Fig. 2, evidence for linkage was found for the Type B/II cluster vs. no diagnosis, on chromosome 16 (@139 cM, LOD score= 4.4), and on chromosome 19 (@74 cM, LOD score= 6.4). Regions of interest for this phenotype (LOD > 1.5) were also located on chromosome 14, at 122 cM, on chromosome, 21 at 7 cM and on chromosome 22 at 17 cM as seen in Fig. 3 and listed in Table 3.

DISCUSSION

The results of this study suggest that, in general, a dichotomous typology similar to Babor's Types A/B (Babor *et al.* 1992) and Cloninger's Types I/II (Cloninger *et al.* 1981) for alcohol dependence characterizes cannabis dependence in this Indian community with the exception of an infrequent late onset cluster that may represent a temporal cohort effect. In this sample, the Type B/II cluster (in comparison to the Type A/I cluster) for cannabis dependence had an earlier age of onset of use, more childhood conduct and adult antisocial behaviors, and more cannabis dependence and withdrawal symptoms. Numbers of independent anxiety and affective disorders did not differ between the clusters. The Type B/II cluster was found to be heritable (h^2 = 0.44, S.E. = 0.18, p<0.01), while the Type A/I and Cluster 3 were not found to be heritable.

These results are similar to the B/II and A/I typology established in previous studies of alcoholics (Cloninger *et al.* 1981; Babor *et al.* 1992; Schuckit *et al.* 1995; Feingold *et al.* 1996; Babor & Caetano 2006; Hesselbrock & Hesselbrock 2006) in a variety of studies using inpatient, outpatient, and community samples. Using cluster analytic techniques, a similar Type B/II vs. a Type A/I typology has been identified in substance use disorders, including cocaine, opiates, and cannabis (Ball *et al.* 1995; Feingold *et al.* 1996; Kranzler *et al.* 2008). To our knowledge, Feingold and colleagues (1996) have made the only other attempt to examine a sample of cannabis dependent participants for the Type B/II vs. A/I subtypes. Their sample consisted of predominantly treatment seeking substance users and psychiatric patients. Mean age in that sample was 35.7 years (S.D. = 11.5) and ethnicity reported as 32% African American, 6% Hispanic, and 63% White. Variables which were highly correlated with cluster membership in their study were withdrawal avoidance, dependence severity, polydrug use, higher scores on the Drug Abuse Screening Test and Michigan Alcoholism Screening Test, and an anxiety index from the Addiction Severity Index.

Feingold and colleagues (1996) also compared their Type B and Type A cannabis use disorder clusters using variables not used to establish the clusters. They found an approximately 40% – 60% ratio between Type B and Type A clusters in all four substance classes, a ratio similar to that found in this study. Feingold and colleagues (1996) also found no significant gender differences between Types B and A in any of their substance use disorder clusters. In the present study while the type I/A cluster had an almost equal distribution of men and women the type II/B cluster contained approximately 40% women and 60% men.

In addition to the Type II/B and Type I/A clusters, the cluster analysis used in the present study also generated a third cannabis dependence cluster. This third cluster was infrequent (n=18, 8.9% of the total sample) and differed from the Type I/A cluster only in having an age of onset of first cannabis intoxication of 19.4 years (as opposed to 13.9 years, p < 0.001) and a mean age at interview of 38.3 years (as opposed to 26.8 years, p < 0.001) (Table 2). We believe that the most parsimonious explanation for the third cluster is that it represents a temporal cohort effect, possibly marking a period of increased cannabis availability in the 1980s. Alternatively,

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or in addition, this small third cluster may represent individuals who had onset of cannabis use later in life due to other environmental variables.

This cluster analytic approach may also be useful in identifying etiological relationships between disorders. As an example in this study, the numbers of DSM-III-R anxiety and affective disorders did not differ between heritable and non-heritable subtypes of cannabis dependence. This finding is consistent with a previous finding in this sample that cannabis dependence was not co-morbid with anxiety and affective disorders (Gilder *et al.* 2006). Taken together, these findings suggest that anxiety and affective disorders may not be as etiologically related to cannabis dependence in this population as they may be in other populations where co-morbidity is more pronounced (Regier *et al.* 1990; Grant 1995; Grant & Pickering 1998; Agosti *et al.* 2002).

Several studies suggest that there is a moderate genetic influence on cannabis dependence and recently there have been several reports that have identified regions in the genome that may be linked to cannabis dependence (Kendler & Prescott 1998; Tsuang *et al.* 1998; Maes *et al.* 1999; Miles *et al.* 2001; Lynskey *et al.* 2002; Kendler *et al.* 2003; Rhee *et al.* 2003; Wilhelmsen & Ehlers 2005; Agrawal & Lynskey 2006; Hopfer *et al.* 2007; Agrawal *et al.* 2008). Hopfer and colleagues (2007) conducted a genome wide scan for loci influencing adolescent cannabis dependence in 324 sibling pairs from 192 families. In that study, probands (52.1% of whom were EuroAmerican, 36.5% of whom were Hispanic, and 7.8% of whom were African-American) were identified from consecutive admissions to substance abuse treatment facilities. The authors found evidence for suggestive linkage on chromosomes 3q21 (LOD = 2.61) and 9q34 (LOD = 2.57). These areas of the genome were not identified in the current linkage scan for Cannabis dependence II/B, however, a region on chromosome 3 was previously identified in a genome-wide linkage analysis for loci associated with the syndromic diagnoses of childhood conduct disorder (CD), ASPD, and the combined phenotype ASPD/CD in this Native American community. Agrawal and colleagues (2008) conducted a linkage scan in a sample from the COGA study for six DSM-IV cannabis dependence criteria considered as a continuous variable (0–6) and found a maximum LOD score of 1.9 at 95 cM on chromosome 14. A “region of interest” on chromosome 14 at 122 cM was also found for the type B/II cannabis subtype in the present study. This region on chromosome 14 was also identified previously in a genome-wide linkage analysis for ASPD/CD in this Native American community (Ehlers et al., 2008).

Two areas of the genome were identified in the present study with LOD scores that provide evidence for linkage on chromosome 16 (@139 cM, LOD score= 4.4), and on chromosome 19 (@74 cM, LOD score= 6.4). There are no published studies that have identified a region on chromosome 16 for cannabis dependence. However, there have been several reports of linkage findings within a 35 cM region on chromosome 16 for other drug dependence phenotypes. In this American Indian sample a region of interest was identified on chromosome 16 at 95 cM for a phenotype of “Any drug dependence /regular tobacco usage”. In this general area of chromosome 16, three other studies have also found regions of interest for alcohol dependence (Sheffield *et al.* 1999; Hill *et al.* 2004), as well as for the number of grams of alcohol consumed a day (Ma *et al.* 2003). There have been some findings in genome scans for cannabis dependence and other drug use related phenotypes on chromosome 19. A region has been identified on chromosome 19 at 17 cM for early-onset cannabis use in a study of 2314 Australian families (Agrawal et al. 2008). Closer to the region identified in the present study were the findings from the COGA study for a genome-wide screen for genes influencing conduct disorder where a region was identified on chromosome 19 at 35 cM.

Taken together the results of our study suggest that a Type B/II cannabis dependence phenotype can be identified in this Indian population that it is in part heritable. Additionally, a genome-

wide linkage analysis has identified regions of the genome that are linked to this cannabis dependence phenotype in this population. Some of the locations identified for the TypeB/II cannabis dependence subtype on chromosomes 14, 16 and 22 were previously found to be associated with drug/tobacco dependence and/or ASPD/CD, but not alcohol dependence, phenotypes in this Indian population. Additionally, all of the sites identified have some supportive data for substance dependence related phenotypes in published genome scans in other population samples. The results of this study should, however, be assessed in the light of several limitations. Our venue-based and respondent driven ascertainment strategies may not have yielded a sample representative of the Indian population assessed. Given the diversity of Native American populations, our findings may not generalize to other Native Americans. Our study consisted of a cross-sectional sample, which contained individuals who may not have yet passed through the age of risk for developing cannabis dependence and the clinical symptoms used in the cluster analysis. A prospective study would address these limitations. It is important to point out that heritability of a cannabis dependence cluster does not necessarily arise from genetic variation since shared family environments may have contributed to its heritability. Comparisons of linkage findings to non-Indian populations may be limited by differences in a host of potential genetic and environmental variables. The underlying assumption that these phenotypes are normally distributed, an assumption of variance component analyses, may not be warranted. Finally, because this population has significant admixture, estimates of allele frequencies may produce biased LOD scores.

Despite these limitations, we believe that this study illustrates a potentially useful approach to characterizing heritable vs. non-heritable subtypes of cannabis dependence whose differentiation is useful for genotyping for genetic studies and may also be important for characterizing clinical course, and matching treatment and prevention strategies.

Acknowledgments

Supported in part by the National Institute on Drug Abuse Grant: DA019333, National Institute on Alcohol Abuse and Alcoholism Grants: AA00269, AA010201, the National Center on Minority Health and Health Disparities (NCMHD), and the Stein Endowment Fund. The authors thank Linda Corey, Michelle Dixon, Abigail Gross, Lilach Harris, Philip Lau, Susan Lopez, Evelyn Phillips, Shirley Sanchez, Gina Stouffer, Derek Wills, and Vincent Wong for assistance in data collection and analysis.

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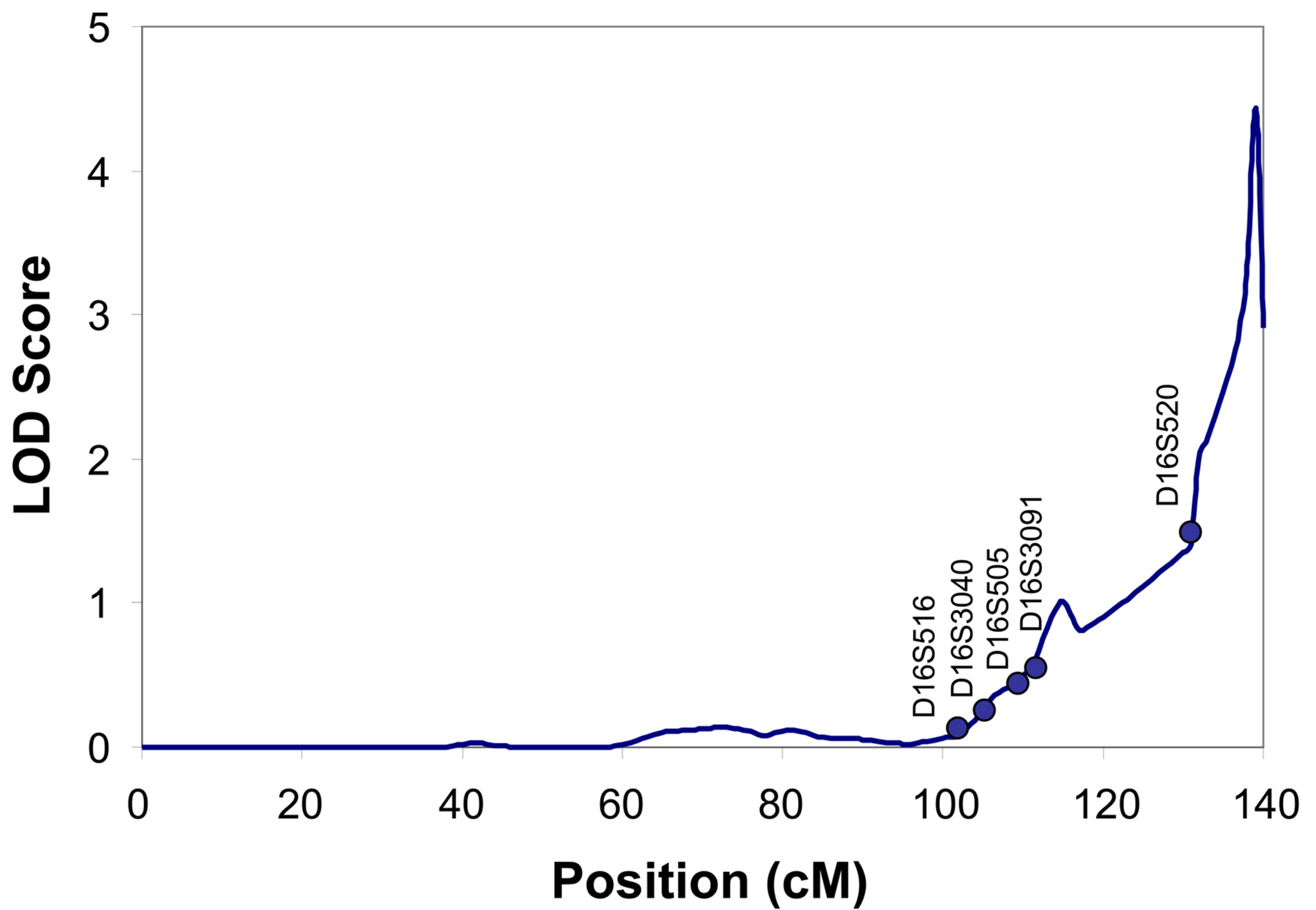

Figure 1.
Multipoint Linkage Analysis for Type II/B Cannabis cluster phenotype for chromosome 16. The analysis assumes a latent normally distributed variable with a threshold above which an individual is affected. Log of the Odds (LOD) score (Y-axis) is plotted for the chromosome location map (in centimorgans (cM), X-axis).

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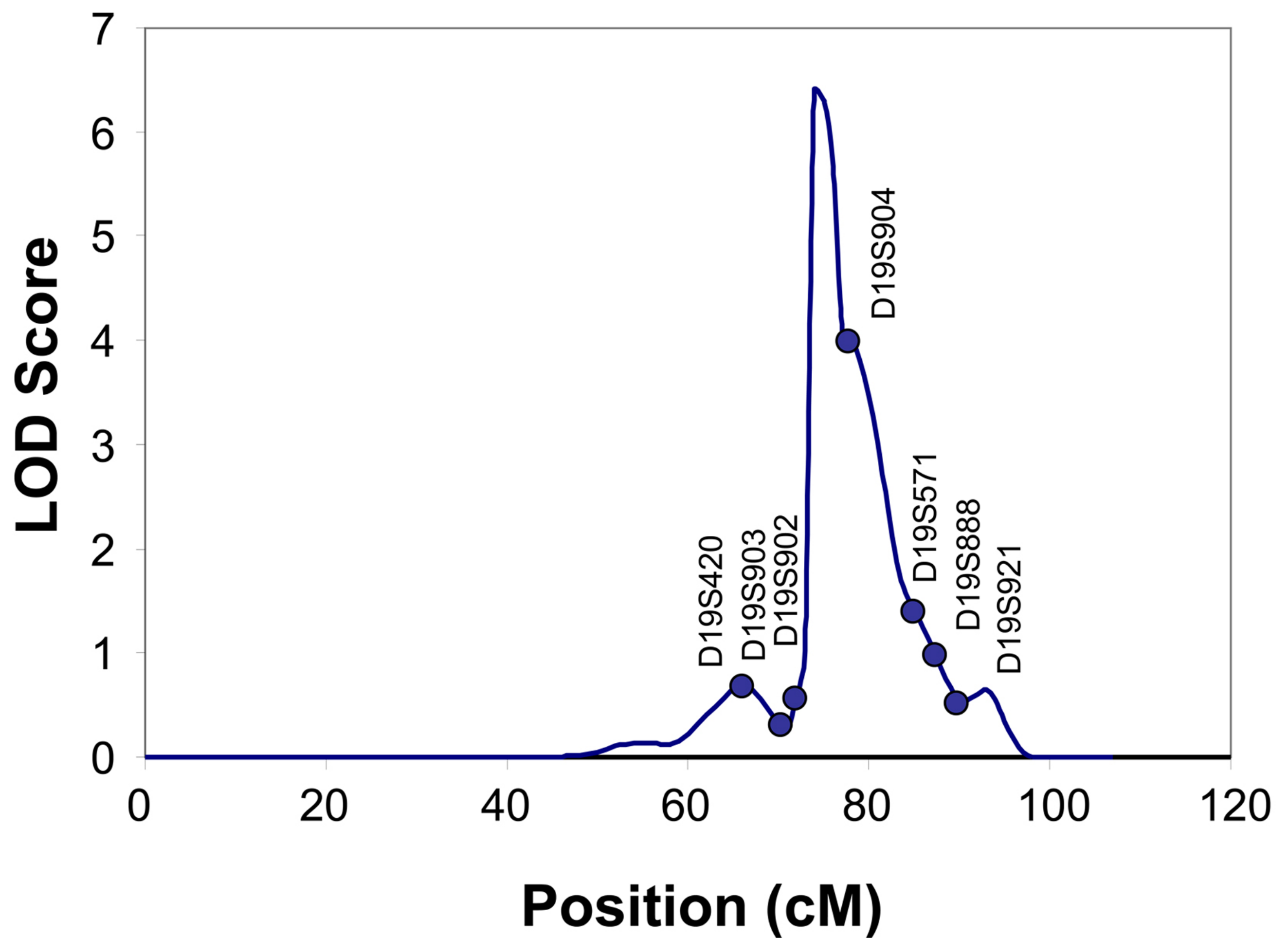

Figure 2.
Multipoint Linkage Analysis for the Type II/B Cannabis cluster phenotype for chromosome 19. The analysis assumes a latent normally distributed variable with a threshold above which an individual is affected. Log of the Odds (LOD) score (Y-axis) is plotted for the chromosome location map (in centimorgans (cM), X-axis).

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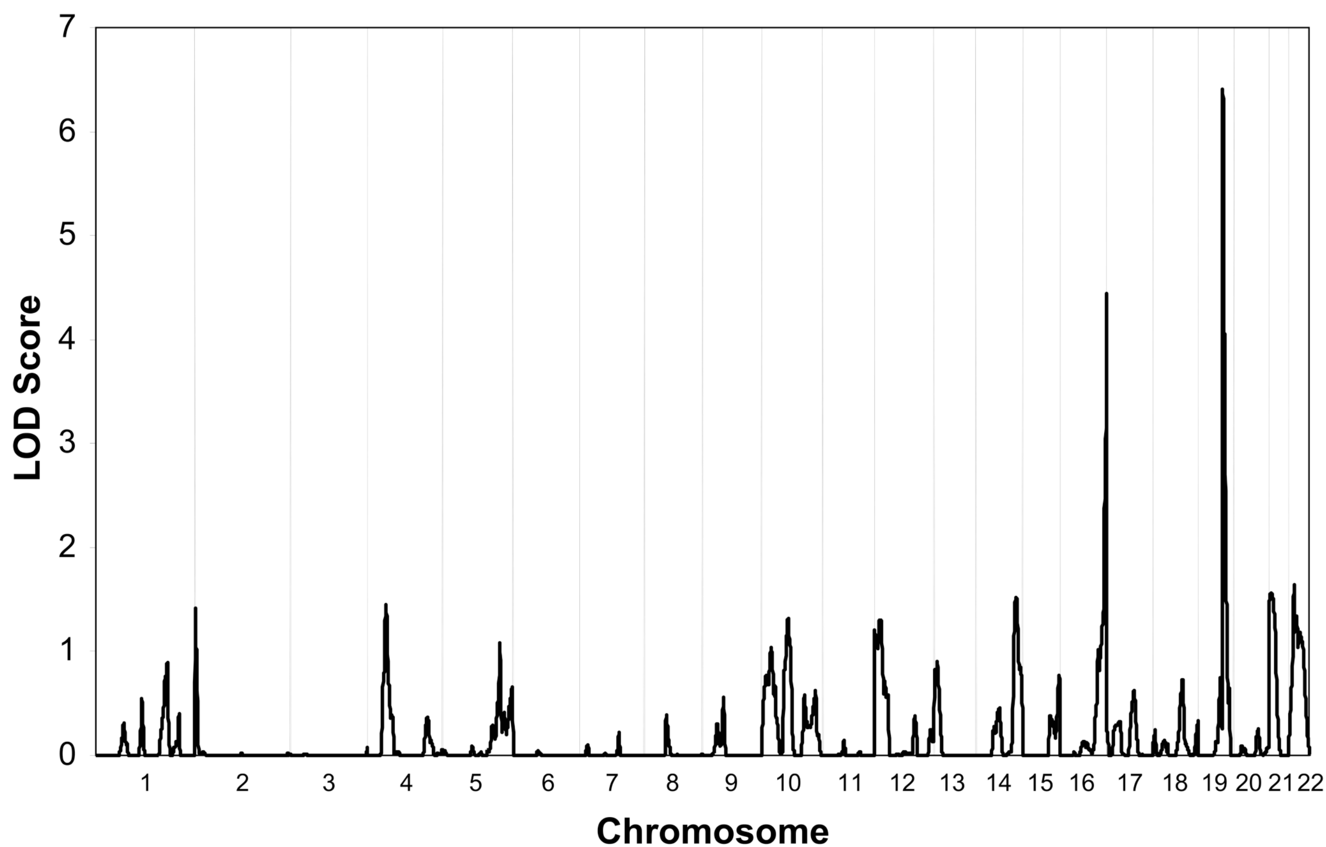

Figure 3.

Multipoint Linkage Analysis for Type II/B Cannabis cluster phenotype for the entire genome. Results for each chromosome are aligned end to end with the p terminus on the left. Vertical lines indicate the boundaries between the chromosomes. The numbers above on the X-axis indicate the chromosome locations.

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Table 1

Average count of cluster variables and age onset of use in each cluster and comparison of variables in each pair.

Variable	Cluster 1	Cluster 2	Cluster 3	1 vs 2 p-value	1 vs 3 p-value	2 vs 3 p-value
Adult antisocial symptoms	2.84	5.03	3.06	**0.001**	0.89	**0.001**
CD symptoms	1.29	3.84	0.83	**0.001**	0.45	**0.001**
Psychiatric Disorders	0.39	0.53	0.28	0.49	0.86	0.48
Cannabis use severity count	2.61	2.73	2.89	0.45	0.23	0.64
Withdrawal symptoms	1.10	1.80	1.61	**0.01**	0.37	0.88
Dependence symptoms	4.77	6.21	4.83	**0.001**	0.99	**0.01**
Age onset use (yrs)	13.94	11.46	19.44	**0.001**	**0.001**	**0.001**

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Table 2

Means and counts of demographic variables in each cluster and comparison of variables in each pair.

Variable	Cluster 1	Cluster 2	Cluster 3	1 vs 2 p-value	1 vs 3 p-value	2 vs 3 p-value
	Mean; se	**Mean; se**	**Mean; se**			
Age	26.8; 0.83	28.2; 1.16	38.3; 1.65	0.60	**0.001**	**0.001**
Number of years of education	11.5; 0.13	11.0; 0.18	11.3; 0.42	0.10	0.84	0.80
	N, % of total population	**N, % of total population**	**N, % of total population**			
Male	59, 23	42, 16	10, 04	0.03	0.002	0.229
Female	55, 16	28, 08	8, 02			
	N, %	**N, %**	**N, %**			
Un-Employed	62, 56	41, 63	8, 44	0.428	0.447	0.182
Employed	49, 44	24, 37	10, 56			
Income				0.641	0.804	1.000
< 20 K/year	53, 48	29, 44	8, 44			
≥ 20 K/year	57, 52	37, 56	10, 56			
Native American Ancestry				0.446	0.316	0.792
< 50	66, 58	36, 51	8, 44			
≥ 50	48, 42	34, 49	10, 56			
Not married	98, 86	60, 87	12, 67	1.000	0.080	0.074
Married	16, 14	9, 13	6, 33			

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Table 3

Chromosome locations for a type II/B cannabis cluster

CHR	LOC (cm)	LOD	Nearest marker	Supporting references (phenotype)
14	122	1.5	D14S985	Agrawal et al., 2008 (cannabis dep) Ehlers et al., 2008 (ASPD/CD)
16	139	4.4	D16S520	Ehlers et al., 2007 (Any drug dep) Hill et al., 2004 (Alc dep) Ma et al., 2003 (Drinking) Sheffield et al., 1999 (Alc Dep)
19	74	6.4	D19S902	Dick et al., 2004 (Conduct symptoms)
21	7	1.6	D21S1899/ D2151904	Ehlers et al., 2007 (Any drug dep) Uhl et al., 2001 (Drug dep)
22	17	1.6	D22S315	Ehlers & Wilhelmsen, 2006 (Reg. tobacco use) Saccone et al., 2000 (Max drinks)

Abbreviations: CHR (chromosome number), LOC (location), dep (dependence), ASPD/CD (antisocial personality disorder/conduct disorder), Alc dep (Alcohol dependence), Max drinks (maximum number of drinks in a 24 hour period). Supporting references are best estimates of proximity within 35 cM considering the use of different maps and populations.