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A Genome-Wide Scan for Loci Influencing Adolescent Cannabis Dependence Symptoms: Evidence for Linkage on Chromosomes 3 and 9

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Abstract

Objective: Cannabis is the most frequently abused illicit substance among adolescents and young adults. Genetic risk factors account for part of the variation in the development of Cannabis Dependence symptoms; however, no linkage studies have been performed for Cannabis Dependence symptoms. This study aimed to identify such loci.

Method: 324 sibling pairs from 192 families were assessed for Cannabis Dependence symptoms. Proband (13–19 years of age) were recruited from consecutive admissions to substance abuse treatment facilities. The siblings of the probands ranged in age from 12–25 years. A community-based sample of 4843 adolescents and young adults was utilized to define an age- and sex-corrected index of Cannabis Dependence vulnerability. DSM-IV Cannabis Dependence symptoms were assessed in youth and their family members with the Composite International Diagnostic Instrument -Substance Abuse Module. Siblings and parents were genotyped for 374 microsatellite markers distributed across the 22 autosomes (average inter-marker distance = 9.2 cM). Cannabis Dependence symptoms were analyzed using Merlin-regress, a regression-based method that is robust to sample selection.

Results: Evidence for suggestive linkage was found on chromosome 3q21 near marker D3S1267 (LOD = 2.61), and on chromosome 9q34 near marker D9S1826 (LOD = 2.57).

Conclusions: This is the first reported linkage study of cannabis dependence symptoms. Other reports of linkage regions for illicit substance dependence have been reported near 3q21, suggesting that this region may contain a quantitative trait loci influencing cannabis dependence and other substance use disorders.

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Keywords

genetics; Cannabis; antisocial behavior; adolescence; linkage study

1. Introduction

Cannabis is the commonly used illicit substance in developed countries and its use is increasing in developing countries (WHO, 1997). Of users who experiment with cannabis only a portion develop Cannabis Dependence, which is defined in the Diagnostic and Statistical Manual of Mental Disorder, 4th edition, text revision (DSM –IVTR) as having at least three out of seven symptoms (American Psychiatric Association, 2000). These include physiological symptoms, such as tolerance or withdrawal, cognitive symptoms such as being preoccupied with obtaining or using cannabis, and symptoms of psychosocial impairment such as continuing to use despite impairment in school or relationship functioning. Studies from multiple countries demonstrate that Cannabis Dependence or Cannabis Use Disorders are highly prevalent. In the US, approximately 4% of population develops Cannabis Dependence in their lifetime (Compton et al. 2004) and 8.5% a Cannabis Use Disorder (Stinson et al. 2006). Twelve percent of a New Zealand cohort had developed Cannabis Dependence by age 25 (Boden et al., 2006). Thirty percent of a German young adult sample were cannabis users and 35% of those reported at least one dependence symptom within a four-year period (Nocon et al. 2005). The 12-month prevalence of Cannabis Use Disorders in Australia is 2.2% (Swift et al., 2001). Thus, Cannabis Dependence and Cannabis Use Disorders are highly prevalent in the developed world.

Cannabis Use Disorders are associated with substantial morbidity and mortality and are frequently associated with other substance use disorders. Cannabis use is associated with lower educational attainment (Lynskey & Hall, 2000), the development of other illicit drug use (Lessem et al., 2006; Lynskey et al., 2006; Young et al., 2002; Crowley et al. 1998), the development of serious psychiatric conditions (Lynskey et al., 2004), motor vehicle accidents (NHSTA, 2001), and medical complications (Hashibe et al., 2005; Tashkin, 2005). Cannabis is the most common reason for admissions for adolescents to publicly funded substance abuse treatment facilities in the United States and accounts for two-thirds of such admissions (SAMHSA, 2002). The World Health Organization has noted that Cannabis Use Disorders have a major, worldwide, public health impact (WHO, 1997).

Experimentation with cannabis typically begins in adolescence or young adulthood and the peak risk period for developing Cannabis Use Disorders is during late adolescence. In the United States, in 2003, 46% of 12th graders reported having tried cannabis at some point in their lifetime, 35% used within the past month, and 6% smoked cannabis daily (Johnston et al., 2004). Using survival-analysis techniques on data from the National Comorbidity Survey, Wagner and Anthony (2002) demonstrated that the peak period for developing Cannabis Dependence symptoms was 17-18. Similarly, Stinson et al (2006), using data from the National Epidemiologic Survey on Alcohol and Related Conditions found that the peak age for the onset of cannabis abuse and dependence symptoms was around 18. There is also some evidence to suggest that adolescent-onset users are more likely to develop cannabis dependence than adult-onset users (Chen et al., 1997; Chen et al. 2005). Thus, adolescence and young adulthood are periods of initiation into cannabis use and the symptoms of Cannabis Use Disorders usually occur by age 25.

Twin studies have demonstrated that genetic factors influence the liability to develop Cannabis Dependence in both adults and adolescents (Kendler and Prescott, 1998; Tsuang et al., 1998; Maes et al., 1999; Kendler et al., 2000; Miles et al., 2001; Lynskey et al., 2002; Rhee et al., 2003). As reviewed by Agrawal and Lynskey (2006), heritability estimates range between .45

to .78 for cannabis abuse or dependence. Twin studies of the comorbidity between dependence on multiple substances of abuse have suggested both an underlying genetic factor that influences liability to drug dependence across multiple substances and substance-specific genetic risks (Tsuang et al., 1998; Kendler et al., 2003; Agrawal and Lynskey 2006). Additionally, substance use disorders and antisocial behavior are influenced by common familial influences (Young et al., 2000; Hicks et al., 2004), including possibly common genetic loci (Stallings et al., 2005). Thus, twin studies suggest that there are both cannabis-specific risk genes and those that confer vulnerability to a broader range of substance use disorders and co-occurring antisocial behaviors.

Although no published linkage studies have examined Cannabis Dependence symptoms, several studies have reported results of genome scans that sought to identify genetic loci for risk for dependence on other substances, including nicotine (Straub et al., 1999; Bierut et al., 2003; Li et al., 2003), alcohol (Long et al., 1998; Reich et al., 1998; Foroud et al., 2000; Saccone et al., 2000; Ehlers and Wilhelmsen, 2005), cocaine (Gelernter et al., 2005), and polysubstance abuse (Uhl et al., 2001; Stallings et al., 2003; Stallings et al., 2005). These studies detected signals for a variety of loci across the genome. A recent review by Uhl (2004) identified 16 regions of interest that contribute to substance abuse vulnerability.

Adolescence and young adulthood are critical periods for the development of symptoms of Cannabis Dependence, and represent the primary period of risk when this disorder develops (Stinson, et al., 2006; Wagner and Anthony, 2002). To our knowledge, no studies have examined quantitative trait loci (QTL) that increase risk for Cannabis Dependence in samples of either adolescents or young adults, and, thus, we performed a genome scan to search for loci contributing to Cannabis Dependence in adolescents recruited from a substance abuse treatment facility.

2. Method

2.1 Sample

Subjects for this study were participants in a linked set of family, twin, and adoption studies that are part of the Colorado Center on Antisocial Drug Dependence (CADD; PI: Thomas J. Crowley), funded by the National Institute on Drug Abuse. Details of the studies involved in this Center are described in Stallings et al. (2003). Youth in treatment for substance use disorders and their siblings were recruited as subjects for this study. A comparison general population sample from ongoing twin, community control, and adoption studies, was used to define an age and sex-corrected comparison group to account for marked age trends in the initiation and development of Cannabis Dependence symptoms.

2.1.1 Treatment Sample—Adolescent probands were recruited from an adolescent substance abuse treatment program in the Denver metropolitan area, associated with the University of Colorado at Denver and Health Sciences Center. Youth in treatment were 13 to 19 years of age ($M = 15.9$, $SD = 1.3$) at the time of assessment and were recruited from consecutive admissions between February 1993 and June 2001 to the program. Approximately 12.5% of consecutive admissions refused participation in the study. An additional 4.5% were interviewed but the data excluded because their IQ scores were less than 80. In addition, only youth with a full sibling between the ages of 12 and 25 were studied. A total of 324 sibling pairs from 192 families were assessed and included in the analysis (after omission of 28 individuals where paternity and/or full-sibling status was questioned based on genetic analysis). The ethnicity distribution of the 192 families was 7.8% African-American, 36.5% Hispanic, 52.1% Caucasian, and 3.6% other. Previous reports on other issues in this sample have been reported by Stallings et al. (2003).

2.1.2 Comparison Population Sample—Because cannabis use, abuse, and dependence show marked age trends (Young et al., 2002), we used data from community adolescent and young adult participants in the CADD family, twin, and adoption samples to determine age and sex-corrected norms for cannabis use and dependence symptoms. Adolescent twin pairs and their non-twin siblings (age 12-18) were drawn from the Colorado Twin Registry, a community-based sample of twin families residing in Colorado. Adolescents and young adults (age 12-25) were also drawn from two other Colorado community-based family samples: the Colorado Adoption Project (CAP) and the Colorado Adolescent Substance Abuse (ASA) family study. The CAP is an ongoing, longitudinal adoptive family study of genetic and environmental influences on behavioral, cognitive, and emotional development (DeFries et al., 1994). The ASA family study is a study of the familial transmission of substance abuse risk and associated psychopathology (Miles et al., 1998). The design of the ASA family study includes families of adolescents in treatment for substance abuse and delinquency (clinical sample described above) and the families of community adolescents who were age- and ethnically-matched to the treatment probands. The community controls of the ASA were included in the pool of community controls which was used to define age and sex corrected norms for the development of cannabis dependence symptoms.

The total community-based sample included 4843 individuals between 12 and 25 years of age ($M=15.9$; $SD=2.1$); 54% were female, 46% were male. The ethnicity distribution of the sample was 81.6% Caucasian, 12.1% Hispanic, 2.3% African-American, and 4% of mixed or unknown ethnicity. A recent report on this sample by Young et al. (2002) demonstrated that its substance use patterns and prevalence rates are comparable to those reported in national studies such as the Monitoring the Future Study (Johnston et al., 2000; Johnston et al., 2001) and the National Household Survey of Drug Abuse (SAMHSA, 2004).

2.2 Assessment

2.2.1 Assessment procedures—Participants were assessed with a range of cognitive, psychiatric, and socio-demographic instruments that included structured diagnostic interviews as well as self-report questionnaires. Youth recruited from the treatment program were assessed in the treatment facilities within two weeks of admission. Siblings of the treatment probands and all community adolescents were assessed privately in their homes by trained lay interviewers. Siblings of probands were assessed within 6 months of proband admission, and individual members of sibling pairs were assessed by different interviewers to minimize correlated errors of measurement. All participants who were at least 18 years of age gave informed written consent to participate. A parent or legal guardian gave written consent for juvenile participation and assent was obtained from all juveniles. All research protocols and consent forms were approved by institutional review boards of the University of Colorado.

2.2.2 Substance use—Cannabis use and dependence data were obtained using the Composite International Diagnostic Interview-Substance Abuse Module (CIDI-SAM; Cottler and Keating, 1990), a structured diagnostic interview designed to be administered by lay interviewers. The CIDI-SAM has been shown to be valid and reliable in both clinical and epidemiological samples (Cottler and Keating, 1990; Horton et al., 2000), and in adolescent populations (Crowley et al., 2001). The structured interview assesses DSM-IV (American Psychiatric Association, 1994) symptoms and diagnoses of substance abuse and dependence for Cannabis as well as other substances. In order to be asked follow-up questions regarding abuse and dependence symptoms, subjects must report “repeated use,” defined by the CIDI-SAM as using Cannabis at least six times during their lifetime.

2.3 Definition of Cannabis Dependence Vulnerability

We used counts of lifetime DSM-IV cannabis dependence symptoms to create a quantitative phenotype for cannabis dependence vulnerability. The community sample was used to age- and sex-correct the scores using standard regression procedures (i.e., residual scores were obtained); the correction weights were then applied to the selected sample. The scores were standardized within sex groups in order to be expressed in relation to the population means for adolescent males and females. Each youth received a score equal to his or her deviation in standard deviation units from the mean of youths of the same age and gender in the community sample. Thus, for example, a 14-year-old who reported three symptoms of Cannabis Dependence would have a higher score than an 18-year-old who meets three Cannabis Dependence symptoms, since the threshold of three symptoms is met less commonly by younger adolescents.

2.4 DNA Collection and Genotyping

Genomic DNA was isolated from buccal cells using a modification of published procedures (Lench et al., 1988; Meulenbelt et al., 1995; Krauter et al., 2001). Briefly, our method involved collecting buccal cells by rubbing the cheeks with cotton swabs followed by a rinse with Scope® mouthwash. DNA was isolated by solvent extraction, quantified with PicoGreen® (Molecular Probes, Eugene, OR), and a working stock of 20 ng/μL was prepared in TE buffer. The average yield of DNA was 20 μg. Primer Extension Preamplification (PEP) was performed on 1 μL aliquots of the genomic DNA using a modification (Krauter et al., 2001) of the method of Zhang et al. (1992), which resulted in an approximately 100-fold amplification of the DNA. The PEP procedure was originally to be used only on those samples that had poor yields. However, after confirming that allele calls for all markers were identical when comparing PEP DNA with DNA purified from cell lines in two CEPH individuals, PEP was routinely used for all of the DNA samples.

Parents and all sibling pairs from our selected families (i.e. families of clinical probands) were genotyped for 374 microsatellite (STR) markers (ABI PRISM LMS2-MD10 panels, PE-Biosystems, Foster City, CA) spanning all 22 autosomes. Genotypes were available from both parents for 47% of the families, 49% had genotypes from one parent, and 4% had no parental genotypes available. Sibships included 147 pairs, 35 trios, 7 sibships of four, and 3 sibships of five. Genotypes were determined by PCR amplification of polymorphic markers using primers labeled with fluorescent probes. DNA fragments were analyzed using an ABI 377 DNA sequencing instrument and GeneScan and Genotyper software. Allele calling was performed by technicians blind to subject identity and phenotype. Sex-averaged marker maps were obtained from the Marshfield Center for Medical Genetics database (<http://research.marshfieldclinic.org/genetics>), and allele frequencies were estimated from the full sample. The average inter-marker distance was approximately 9.24 cM, with an average marker heterozygosity of 78.3% and average multipoint polymorphism information content (PIC) of 0.55.

2.5 Genotype and Relationship Validation

The Discovery Manager® (Genomica; Boulder, CO) database system was used to store genetic and phenotypic data and to perform initial error checking. Additional genotype and relationship validation was performed using the Graphical Relationship Representation (GRR) program (Abecasis et al., 2001), PEDSTATS (Abecasis et al., 2000), Merlin (Abecasis et al., 2002), and GENEHUNTER version 2.0 (Kruglyak et al., 1996). Merlin was utilized to estimate identity-by-descent (IBD) sharing for all sib pairs at 1 cM intervals across the 22 autosomes, as well as at each marker for single-point analysis. GRR plots the average allele sharing by its standard deviation for all available markers for all pairs of individuals, and was used to confirm the family pedigree relationships. This analysis identified 24 individuals with questionable

paternity and/or sibling status as well as 2 pairs of monozygotic (MZ) twins. These individuals were excluded from linkage analysis, reducing the final sample size to 324 pairs of siblings. Comparison of the allele calls in the two pairs of MZ twins indicated that our undetectable genotyping error rate was less than 1%.

2.6 Data Analysis

The linkage analysis was performed using the Sham et al. (2002) regression method as implemented in the Merlin and Merlin-regress software packages (Abecasis et al., 2002). The Sham et al. (2002) method regresses the IBD of a sibling pair on the squared sums and differences of the pair's trait being analyzed. This method has the power of variance component methods, but is robust to selected samples (Johnston et al., 2000). Although the selected probands from the CADD study were not directly selected on Cannabis Dependence, their levels of Cannabis Dependence were higher than that of the comparison population sample. Merlin-regress (Abecasis et al., 2002) and the Sham et al. (2002) method require the input of the population trait distribution parameters. The comparison population sample was used to estimate the heritability of Cannabis Dependence at 0.40 and shared environmental variance at .2, implying a sib correlation of .4. Merlin-regress requires estimates of sib correlations which were derived from our comparison population. It also models non-independence among sibships in the case of multiple sibships. Multipoint linkage analysis was performed at 1-cM intervals across the genome.

Sensitivity analyses were conducted at all locations where we obtained suggestive evidence for linkage (LOD scores >2.0) to insure that the peak LOD scores were not due to the effect of a single family by iteratively dropping each family (with replacement) from the analyses at the two locations (3q21.1 and 9q34).

Additionally, simulated data sets were constructed using Merlin to determine empirical p-values for the observed LOD scores. We also computed IBDs independently for each ethnic group, and then combined the results for doing linkage analyses.

3. Results

3.1 Descriptive data

Cannabis use (six or more times) was reported by 99% of the youth in treatment, compared to only 55% of their siblings. The prevalence of lifetime DSM-IV Cannabis Dependence was 59.9% for the probands and 14.4% for their siblings. Lifetime Cannabis Dependence symptoms were distributed as follows in the probands: 9.38% had none, 15.63% one, 14.58% two, 11.46% three, 18.23% four, 14.58% five, 10.42% six, and 5.73% seven. In the siblings the distribution was: 64.80% had none, 13.60% one, 7.20% two, 6.40% three, 4.40% four, 1.60% five, and 2.00% six.

In the community sample, the prevalence of Cannabis Dependence was 5.1% for control probands (matched to the clinical probands by age, sex, ethnicity, and zip code) and 6.7% for their siblings (Young et al., 2002). Thus, the siblings of the treatment probands reported higher prevalence rates for Cannabis Dependence than observed in the community sample, as expected. Once the phenotypic scores for Cannabis Dependence were age/sex-corrected and standardized, the proband mean (z-score) was 2.84 and the mean for their siblings was 0.48, compared to a mean of zero for the community sample. That is, among adolescent patient probands, the mean number of reported cannabis dependence symptoms was 2.84 standard deviations above the mean of the community sample.

3.2 Linkage results

Figure 1 displays the resulting LOD scores for Cannabis Dependence across all 22 autosomes. Multipoint linkage mapping indicated two quantitative trait loci (QTL) with LOD scores meeting criteria for “suggestive” linkage (Lander and Kruglyak, 1995). The first was on chromosome 3q21.1, with a maximum LOD of 2.61 ($p = .0003$) near marker D3S1267 (Table 1). The second peak was located on chromosome 9q34, near the telomere, with the maximum LOD near marker D9S1826 (LOD = 2.57, $p = .0003$; Table 2). The LOD peaks for these two QTL are shown in graphical detail in Figures 2 and 3.

Sensitivity analyses for these two QTSs revealed that one family had a particularly large influence at the 3q21.1 peak, accounting for about twice the LOD score change as the next most influential family. Dropping that family from the analysis increased the LOD score from 2.6 to 3.55 at 3q21.1, but only had a small effect at 9q34, decreasing the LOD score from 2.57 to 2.36. No other family showed such a large influence, either positive or negative, on the LOD score at either peak location. Dropping of any other single family could not reduce the LOD score at 3q21.1 lower than 2.12 or raise it higher than 2.99. At 9q34 dropping of any single family did not reduce the LOD score lower than 1.99 or raise it higher than 3.02. In all cases the empirical p -value derived from the simulations was close to the calculated p -value (shown in Tables 1 and 2). Furthermore, after putting families into a “best-guess” ethnic group, and computing IBDs independently for each group, mean IBD at any given locus barely changed, and the interesting LOD scores only changed at the second or third decimal place.

4. Discussion

This study detected two quantitative trait loci (QTL), one on chromosome 3q21 (LOD = 2.61) and another on chromosome 9q34 (LOD = 2.57) for Cannabis Dependence vulnerability in adolescents. These results meet the criteria for “suggestive” linkage (Lander and Kruglyak, 1995). The peak for chromosome 3q, mapped to the 137 centiMorgan position, is located near loci detected by Uhl et al. (2001) for polysubstance abuse, at positions 130 and 140. It also fell within the confidence interval of a peak detected by Stallings et al. (2005) at position 173, for a measure of polysubstance dependence vulnerability in the same sample used in this study. Other studies found evidence for linkage in this area as well, including Long et al. (1998) at positions 165, 174, and 181 for alcohol dependence, Bergen et al. (1999) at position 171 for smoking, and Uhl et al. (2001) at position 177 for polysubstance abuse. Taken together, these results suggest that one or more QTL on chromosome 3q increases the risk for dependence on a variety of substances, including cannabis.

The peak we detected near the telomere of chromosome 9q is located near the same marker (D9S1826, ~ position 160) as the peak previously found in this sample for both polysubstance dependence (LOD = 1.92) as well as the comorbidity between substance dependence and conduct disorder (LOD = 2.65). Bergen et al. (1999) also reported a peak for smoking very nearby at position 161. Overall, these results converge with other linkage studies examining other substance-related phenotypes, including alcohol, smoking, and polysubstance abuse. This analysis, focused on cannabis dependence, was motivated by a realization that no prior studies had examined cannabis dependence as a phenotype, and as Agrawal and Lynskey (2006) noted in their review of the genetic epidemiology of cannabis dependence, that despite ample evidence that cannabis dependence is heritable, “the scientific community awaits the elucidation of genomic regions and even candidate genes that may be involved in the genetic etiology of cannabis involvement.”

The adolescents and young adults examined in this study were ascertained for general substance and conduct problems, not specifically for cannabis use. However, recent epidemiological data from the U.S. (Wagner and Anthony, 2002; Stinson et al., 2006) suggests that in general-

population samples, the mean age of the emergence of cannabis dependence is around 17-19, with the majority of the age of risk for the development of cannabis dependence being passed by 25. Furthermore, comorbidity is the rule rather than the exception, with 72.1% of lifetime cannabis dependent individuals reporting lifetime alcohol dependence and 69.6% reporting lifetime nicotine dependence (Stinson et al, 2006). Thus, this “clinical” sample of adolescents with cannabis use disorders demonstrates patterns of comorbidity remarkably similar to cannabis dependent samples drawn from general population surveys. In this sample, 59.9% of probands met criteria for a diagnosis of Cannabis Dependence, 56.3% were dependent on tobacco, 36% on alcohol, and 26.8% on other illicit drugs; 56.7% met criteria for two or more dependence diagnoses, and 34.8% met criteria for three or more. Thus, patterns of comorbidity are similar to reported comorbidity patterns in cannabis dependent individuals assessed through national epidemiological surveys (Stinson et al., 2006) and the loci detected are consistent with the view that many of the genetic influences on substance use problems are common to multiple substances (Tsuang et al., 1998;Uhl, 2004).

One potential candidate gene that may be associated with cannabis dependence is monoglyceride lipase. This enzyme, as indicated in figure 2, is located at position 3q21.3, under the linkage peak on chromosome three. This enzyme is part of the endocannabinoid pathway and is involved in the inactivation of endogenous cannabinoids (Dinh et al. 2002;Saario et al, 2004). Substantial evidence now implicates the endogenous cannabinoid system in the reinforcing effects of substances of abuse (see review by Gardner, 2002). Thus, in conjunction with other evidence that variation in genes associated with the endogenous cannabinoid system may be associated with cannabis dependence (Hopfer et al., in Press), monoglyceride lipase could be tested in future studies for association with Cannabis Dependence or other substance use disorders.

One strength of this study may have been its use of a continuous measure of cannabis dependence severity. Another strength of this study was the use of a selected sample for the linkage analyses, which provided considerably more power than using a population sample. However, the power to detect the small effect sizes commonly found with psychiatric disorders is still limited in linkage analysis. In addition, although the adolescent probands selected from a substance abuse treatment facility yielded high levels of Cannabis Dependence, there are likely some subjects who have not yet passed through the period of risk, and may become dependent on cannabis in the future.

In conclusion, the first genome scan to examine Cannabis Dependence vulnerability resulted in suggestive linkage on chromosomes 3q and 9q. Future studies that further examine linkage in these specific regions, as well as studies that examine candidate genes in other areas of the genome, would increase understanding of the etiology of Cannabis Dependence.

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Figure 1.
Multipoint linkage results for entire genome for Cannabis Dependence Symptoms

Figure 2. Multipoint linkage results for chromosome 3 for Cannabis Dependence Symptoms. MGL indicates the position of the enzyme Monglyceride Lipase

Figure 3.
Multipoint linkage results for chromosome 9 for Cannabis Dependence Symptoms

Table 1

LOD scores for peak on chromosome 3q

Position ^a	Info ^b	LOD	p-value	Empirical-p ^c
131	58.9%	2.119	.0009	.0010
132	57.1%	2.260	.0006	.0008
133	56.0%	2.384	.0005	.0006
134	55.7%	2.483	.0004	.0005
135	56.2%	2.555	.0003	.0004
136	57.5%	2.598	.0003	.0004
137	59.5%	2.610	.0003	.0004
138	62.4%	2.596	.0003	.0004
139	66.1%	2.557	.0003	.0004
140	63.0%	2.439	.0004	.0005
141	60.3%	2.272	.0006	.0008
142	58.9%	2.067	.0010	.0011

^aPosition in centiMorgans.^bInfo = proportion of linkage information extracted at this location.^cEmpirical-p was determined by simulation in Merlin

Table 2

LOD scores for peak on chromosome 9q

Position ^a	Info ^b	LOD	p-value	Empirical-p
150	64.6%	2.078	.0010	.0011
151	61.5%	2.213	.0007	.0008
152	59.2%	2.334	.0005	.0007
153	57.7%	2.435	.0004	.0005
154	57.1%	2.510	.0003	.0004
155	57.2%	2.556	.0003	.0004
156	58.1%	2.571	.0003	.0004
157	59.8%	2.557	.0003	.0004
158	62.4%	2.515	.0003	.0004
159	65.7%	2.451	.0004	.0005
160	68.0%	2.389	.0005	.0006
161	68.5%	2.328	.0005	.0007
162	70.3%	2.233	.0007	.0008
163	73.3%	2.109	.0009	.0010

^aPosition in centiMorgans.^bInfo = proportion of linkage information extracted at this location.