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The genetic basis of the comorbidity between cannabis use and major depression

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Abstract

Background and aims—While the prevalence of major depression is elevated amongst cannabis users, the role of genetics in this pattern of comorbidity is not clear. This study aimed to estimate the heritability of cannabis use and major depression, quantify the genetic overlap between these two traits, and localize regions of the genome that segregate in families with cannabis use and major depression.

Design—Family-based univariate and bivariate genetic analysis.

Setting—San Antonio, Texas, USA

Participants—Genetics of Brain Structure and Function study (GOBS) participants: 1,284 Mexican-Americans from 75 large multi-generation families and an additional 57 genetically unrelated spouses.

Measurements—Phenotypes of lifetime history of cannabis use and major depression, measured using the semi-structured MINI-Plus interview. Genotypes measured using ~1M single nucleotide polymorphisms (SNPs) on Illumina BeadChips. A sub-selection of these SNPs were used to build multipoint identity-by-descent matrices for linkage analysis.

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DISCLOSURES

The authors declare no conflict of interest.

Findings—Both cannabis use (h^2=0.614, p=1.00×10^{-6}, SE=0.151) and major depression (h^2=0.349, p=1.06×10^{-5}, SE=0.100) are heritable traits, and there is significant genetic correlation between the two (ρ_g=0.424, p=0.0364, SE=0.195). Genome-wide linkage scans identify a significant univariate linkage peak for major depression on chromosome 22 (LOD=3.144 at 2cM), with a suggestive peak for cannabis use on chromosome 21 (LOD=2.123 at 37cM). A significant pleiotropic linkage peak influencing both cannabis use and major depression was identified on chromosome 11, using a bivariate model (LOD=3.229 at 112cM). Follow-up of this pleiotropic signal identified a SNP 20kb upstream of *NCAM1* (rs7932341) that shows significant bivariate association (p=3.10×10^{-5}). However this SNP is rare (7 minor allele carriers) and does not drive the linkage signal observed.

Conclusions—There appears to be significant genetic overlap between cannabis use and major depression among Mexican-Americans, a pleiotropy that appears to be localized to a region on chromosome 11q23 that has been previously linked to these phenotypes.

Introduction

Cannabis is one of the most commonly used psychoactive substances globally, approximately 181.8 million people used the drug in 2013 [1]. In the same year, it was estimated 43.7% of the US population had tried cannabis at some point and 4.2 million Amercians reached criteria for cannabis abuse or dependence [2]. There is substantial evidence from a number of different populations indicating that cannabis use is associated with a modest but significant increase in risk of major depression (MDD); the degree of risk is also known to increase as the level of cannabis use increases [3–5]. Furthermore, for patients with MDD, comorbid drug use and abuse is associated with a poorer prognosis [6].

There is debate over the mechanism that underlies the comorbidity between cannabis use and MDD [7]. The self-medication hypothesis proposes that depression leads to cannabis use as a method to manage depressive symptoms [8,9], but there is longitudinal evidence suggesting that cannabis use younger in life increases the likelihood of later depression [3,10]. A third hypothesis is that there are common genetic etiological factors that increase the risk for both traits. If genes that exert pleiotropic effects on both cannabis use and MDD can be identified, this will provide an insight into the neurobiological basis of the connection between these two disorders.

Both MDD and cannabis use are well-established as heritable traits; for MDD 31-42% of liability to the disorder is due to additive genetic factors [11]. The heritability for initiation of cannabis use has been estimated at 40-48%, whilst cannabis abuse/dependence show a higher heritability of 51-59% [12]. Considering the relationship between these two heritable traits, reports from family studies show a significant role for genetic factors in the comorbidity of MDD and cannabis dependence [13–15], and a recent GWAS (genome-wide association study) reported SNP-based evidence of pleiotropy in European-American, although not African-American samples [16].

However, identifying the specific genes involved in MDD and cannabis has proved complex. The largest GWAS in MDD published to date found no significant associations [17]. This may be due to lack of statistical power, but heterogeneity could play an important role. If so,

increasing sample size through the combination of a large number of different cohorts may increase heterogeneity and compound the problem further. A recent study using low coverage genome-sequencing focused specifically on Han Chinese females with recurrent MDD, and identified two replicated loci on chromosome 10 [18]. Alternatively, family-based analyses can also reduce heterogeneity, as the analysis is constrained for genetic background and environmental exposures [19]. Indeed using linkage methods a genome-wide linkage signal on 3p has been identified in two independent cohorts [20,21], although the specific variants underlying this signal remain elusive.

Cannabis use has been less extensively studied from a genetic perspective; but there has been recent success in identifying significant associations in a GWAS of cannabis dependence symptoms (on chromosomes 3, 8 and 10, [16]). Further, gene-based tests showed significant associations with cannabis use and four genes (*NCAM1*, *CADM2*, *SCOC* and *KCNT2*) in a meta-analysis [22]. This meta-analysis also observed SNP-based heritability estimates (between 13-20%) lower than twin-based estimations [22], possibly indicating rare variants are also important in this phenotype. Nevertheless, linkage studies (which are ostensibly able to capture both common and rarer variation due to the family-based design) are yet to identify replicated linkage signals for cannabis use [23–25].

Despite the reports of shared genetic influences outlined above, to our knowledge there has been no previous research investigating the chromosomal loci underpinning the comorbidity between cannabis use and MDD on a genome-wide scale. To do this, here we use an extended pedigree sample. We apply bivariate linkage scans that not only allow the identification of pleiotropic loci influencing both cannabis use and MDD, but have also been shown to give increased power and localization for the mapping of correlated traits [26]. The identification of pleiotropic influences also allows us to focus on the neurobiological pathways shared between MDD and cannabis use, thus tackling the issue of phenotypic heterogeneity. These family-based methods also allow us to both decrease the genetic heterogeneity of the sample and capture rarer genetic variation. By using a randomly ascertained sample, we avoid the potential bias of clinically ascertained samples, where greater symptom severity and higher rates of comorbidity are likely to occur [27,28].

The individuals in our study are Mexican-American, representing a relatively understudied population. In the USA, Mexican-Americans make up approximately 64% of the Hispanic and Latino population, and approximately 11% of the total population [29]. In genetic terms, the sample shows admixture from European and Native American populations (with small proportions of West African ancestry) [30].

Aims

The aims of this study are 1) estimate the heritability of MDD and cannabis use within this sample, 2) quantify the genetic correlation between these two traits, 3) localize regions of the genome that segregate in families with MDD and cannabis use using univariate and bivariate linkage models to identify both specific and pleiotropic risk loci, 4) follow up any identified genome-wide significant linkage regions using available SNP data to try to localize the genetic signal further.

Methods

PARTICIPANTS

The Genetics of Brain Structure and Function study (GOBS) consists of 1,284 Mexican-Americans from 75 large multi-generation families (containing between 2-132 subjects, mean pedigree size=16.36, SD=19.41) and an additional 57 genetically unrelated spouses. The mean age of the sample was 46.08 years (SD=14.89, age range=18-97 years), and 62.7% of the sample was female. Familial relationships are shown in **Table 1**. Stated pedigree relationships were confirmed using PREST and available autosomal markers [31].

SAMPLE

GOBS is a subset of the San Antonio Family Study cohort of individuals who were pseudo-randomly ascertained with the constraints that participants must live within the San Antonio region, be Mexican-American in ancestry, and part of a large family (at least 6 1st degree relatives). Full recruitment details are available elsewhere [32,33]. All subjects provided informed written consent (as approved by Institutional Review Boards at the University of Texas Health Science Center San Antonio and Yale University) and the cohort have been actively participating in research for over 18 years.

MEASURES

PHENOTYPIC MEASURES—The semi-structured Mini International Psychiatric Interview Plus (MINI-Plus [34]) was administered to all participants. Further details on the MINI-Plus are in the **Supplementary Materials**. To assess cannabis use, participants were asked to whether they had taken cannabis more than once, in order to get high, to feel better or to change their mood. Measures refer to lifetime history of MDD and cannabis use.

GENOTYPIC MEASURES—Genotyping was performed following the Illumina Infinitum protocol, using Illumina BeadChips covering ~1 million SNPs, capturing approximately 90% of the common variants in humans. This was either achieved using the 1M-Duo BeadChip (which covers ~1 million SNPs, n=714) or the HumanMap550 BeadChip (which covers ~550,000 SNPs) in tandem with a supplementary Human 450S BeadChip (n=570) to give matching content. Full quality control details given in the **Supplementary Materials**.

For linkage analysis, a subset of 28,387 SNPs were selected using genotypes from 345 founders. These SNPs were selected in order to maximize the information content across the genome but avoid potential bias from linkage disequilibrium (LD) patterns [35]. Therefore SNPs were selected with a minimum spacing of 1KB and a MAF>5% and a LD limit of pairwise r^2<0.0225 within a 100KB sliding window was used. Build NCBI36/hg18 was used for all SNP locations. The selected subset of SNPs gave an average of 7-8 SNPs per centimorgan (cM). Using these 28,387 SNPs, multipoint identity-by-descent matrices were constructed at each cM location, using a stochastic Markov Chain Monte Carlo procedure within LOKI [36].

STATISTICAL ANALYSIS—Within SOLAR [37], maximum likelihood decomposition methods were used to model the patterns of trait covariance between family members as a

function of genetic relationship, allowing the estimation of the genetic and environmental contributions to phenotypes within a family structure. As binary traits are used, the standard threshold model for dichotomous phenotypes was employed [38]. In the simplest case of univariate variance decomposition, the additive genetic contribution to a trait is signified by the heritability (h^2). This was calculated for both cannabis use and MDD. To decompose the phenotypic covariation between the two traits, bivariate analyses were [39]; genetic correlations determine if there is evidence of shared genetic influences (pleiotropy) [26]. The covariates of age, age^2, sex and the interactions between these were included in all analyses.

Genome-wide multipoint linkage analysis was then undertaken, to identify genetic loci involved in each phenotype. Using a model which incorporates the specific marker density and pedigree complexity of the sample [40]; it has previously been established in this sample that a LOD score >2.9 indicates a genome-wide significant linkage peak, whilst a LOD score over 1.67 is indicative of a suggestive peak (likely to occur by chance less than once per genome-wide scan [41]). For further details on the selection of these genome-wide thresholds, see **Supplementary Materials**. We conducted univariate linkage of cannabis use and MDD followed by a bivariate linkage scan to identify chromosomal locations influencing both traits. In additional to LOD scores, empirical p-values were also estimated via simulations for each model (see **Supplementary Materials**). To test whether any linkage peaks identified within this scan were truly bivariate in nature, nested models were compared to test whether the model could be explained in the absence of any shared genetic effect; that is where the co-occurrence of linkage peaks in the two traits occurred by chance [42]. This test is referred to as the “pleiotropy test”.

Finally, to follow-up any significant linkage signals, we performed measured genotype association (mga) analyses beneath significant loci, which in this case were defined as the interval of maximum LOD score–1 surrounding the linkage peak. SNPs within these loci were selected from the full set of ~1M SNPs available from the Illumina BeadChips, and tested for association using the mga test within SOLAR, where models include the fixed effect of the SNP as well as random effects of local and polygenic heritability. An additive genetic model was assumed, and the first four principal components of the GWAS data were included as covariates to account for population stratification. To test for significance, a likelihood ratio test was employed, comparing models where the genotype parameter is allowed to vary freely or is fixed to zero. Given patterns of LD, the pairwise correlations between SNPs were used to calculate the effective number of independent tests within each region (effSNPS, [43]) for an appropriate multiple-testing correction.

Results

DESCRIPTIVE STATISTICS AND TRAIT HERITABILITY

Details of cannabis use and MDD in the sample are shown in **Table 2**. Considering the relationship between the two phenotypes, 35.9% of cannabis users had comorbid MDD. This represents a modest but significant increase in the likelihood of MDD amongst cannabis users (OR=1.530, 95%CI=1.056-2.209, p=0.024). The two traits were both heritable and

showed significant genetic correlation with no evidence of environmental correlation (**Table 3 and 4**).

UNIVARIATE GENOME-WIDE LINKAGE

Genome-wide multipoint linkage scans were conducted for both cannabis use and MDD. A suggestive linkage peak was identified for cannabis use (21q22, LOD=2.123, p=0.0004), whilst a significant linkage peak was identified for MDD (22q11, LOD=3.144, p<0.0001). Further details are within **Table 3 and Figures 1&2**.

To follow up the significant linkage signal for MDD, association analyses were performed for all SNPs underneath this 2.3MB peak. This region included 676 SNPs (effSNPs=423.27, adjusted p-threshold=1.21×10^{-4}), but none showed significant association with MDD (minimum p-value observed=2.24×10^{-3}).

BIVARIATE LINKAGE BETWEEN MDD AND CANNABIS USE

Genome-wide bivariate linkage models of cannabis use and MDD were then analyzed. One region of genome-wide significance was identified on 11q23 (LOD=3.229, p<0.0001). Within the 3.3MB region surrounding the peak, 1,166 SNPs were tested for bivariate association with cannabis use and MDD (effSNPs=613.75, adjusted p-threshold=8.36×10^{-5}). One SNP exceeded this threshold; rs7932341 (A/C, n=7). This SNP is intergenic; it lies 20kb upstream from *NCAM1* (neural cell adhesion molecule 1). When looking at the univariate associations for this SNP, suggestive association was seen for both traits when considered separately. See **Table 4**, **Figure 3** & S4 for further details.

To test whether the identified SNP can account the linkage signal on chromosome 11, we repeated our linkage analysis, covarying for rs7932341. However, the linkage signal at 112cM on chromosome 11 increased in significance (LOD=3.572). This indicates that rs7932341 is not driving our linkage signal; there may be additional variants within this region that are associated with cannabis use and MDD but are not captured on the SNP BeadChips.

Discussion

SUMMARY OF FINDINGS

We observe a significant relationship between cannabis use and MDD (OR=1.530 for MDD amongst cannabis users) and demonstrate significant genetic correlation between the two heritable traits. Localizing the chromosomal regions involved, a genome-wide significant linkage peak was observed for MDD at 22q11.1-q11.21, with a suggestive signal for cannabis use noted at 21q22.11-q22.12. Using a bivariate linkage model, a pleiotropic genome-wide significant trait locus was identified on 11q23.1-q23.2, which spans the *NCAM1-TTC12-ANKK1-DRD2* gene cluster.

REPLICATION OF PREVIOUS FINDINGS

The heritability estimates [11,12], elevated incidence of MDD amongst cannabis users [3–5] and the shared genetic etiology [13,14,16] of these traits within our sample are consistent

with previous studies. The linkage results also show convergence with previous findings. First, the signal for MDD on chromosome 22 is within the location of 22q11.2 deletion syndrome, where hemizygous deletions of up to 3MB are associated with a range of symptoms including heart abnormalities, immune system dysfunction, developmental delays, and various psychiatric disorders especially schizophrenia [44]. Many of the genes located at this site of micro-deletion are brain-expressed genes [45] and a recent survey of 1400 individuals with this deletion showed increased prevalence of psychiatric disorders, particularly schizophrenia, autism, anxiety and MDD [46]. However, using SNPs from the available genotype data, we are unable to localize this univariate linkage signal of MDD more precisely within our sample.

Second, the bivariate cannabis-MDD peak at 11q23 spans the *NCAM1-TTC12-ANKK1-DRD2* gene cluster. *DRD2* encodes the D2 dopamine receptor and dopamine has previously been implicated in both MDD and addition phenotypes, via its role in motivation and reward [47] and the effects of abused substances (including cannabis) on dopamine levels in the nucleus accumbens [48,49]. *NCAM1* encodes a brain-expressed cell adhesion protein [50], known to be involved in the development of the nervous system and neuroplasticity (important in learning and memory, as reviewed by [51]). This is pertinent for addiction, as it is posited to involve the subversion of reward-related learning and there is also evidence that addiction phenotypes alter expression levels of polysialylated NCAM in the brain [52–54]. Similarly, depressive episodes have been linked to neuronal atrophy, whilst efficacious antidepressant treatment increases neurogenesis and enhances neural plasticity [55,56] and there is evidence that NCAM levels may be associated with MDD (and related behaviors) [57–59].

In terms of previous genetic evidence within this genomic location, *DRD2* and in particular the *DRD2* Taq1A polymorphism (rs1800497, located in the coding region of *ANKK1* [60]) have been frequently interrogated in relation to substance use. However, candidate gene studies are known to be prone to type I errors. When systematic meta-analysis of the literature is conducted, evidence of association with substance use/abuse is equivocal; meta-analyses looking at rs1800497 in relation to smoking [61,62] and alcohol [63,64] have been mixed, with effects possibly being population, gender or substance-specific.

Nevertheless, Gelernter and colleagues propose that there are multiple variants across the *NCAM1-TTC12-ANKK1-DRD2* gene cluster that impact on addiction-related phenotypes (such as alcohol, nicotine and comorbid alcohol and drug dependence) in both African-American and European-American samples [65–67]. In line with this, a meta-analysis by the International Cannabis Consortium [22] found that using a gene-based approach *NCAM1* (along with three other genes) showed significant association with lifetime cannabis use, although no individual SNP reached genome-wide significance thresholds. This is consistent with a number of variants within the gene influencing the complex trait of cannabis use.

Using association analysis to follow up the significant linkage signal, no evidence of association was found for the *DRD2* Taq1A polymorphism (using univariate or bivariate models, $p>0.25$ in all cases), but we note a peak-wide bivariate association between a SNP in the region (rs7932341) and cannabis-MDD. The identified intergenic SNP is 20KB

upstream of *NCAM1* and shows interesting population-specific variation. In this sample it is a rare SNP (n=7, estimated MAF=0.0016); using the 1000 Genomes Phase 3 data [68] we note that the minor allele is found within American admixture (MAF=0.01) and African (MAF=0.09) populations, but is not observed in Asian or European samples. These differences between populations highlight the importance of studying populations from a wide range of different ancestral backgrounds. Further analysis in our sample indicates this rare variant is not driving the linkage signal observed in this region, consistent with the presence of additional variants. As rs7932341 is both rare in this sample and does not drive the linkage signal, the observed association with this SNP should be considered tentative; replication attempts, particularly in populations where the variant is more common, would be of interest. Therefore, the bivariate linkage signal occurs in a previously highlighted region of interest for both MDD and cannabis use on chromosome 11. Nevertheless, additional work is needed to identify the relevant causal genetic variants involved.

SPECIFIC AND PLEIOTROPIC GENETIC INFLUENCES

Whilst we observe evidence of pleiotropic influences on cannabis use and MDD on chromosome 11, the univariate signals on chromosome 21 (cannabis use) and 22 (MDD) identified here appear to be phenotype-specific; LOD scores are very low (LOD<0.5) for the alternative phenotype. We cannot rule out the presence of pleiotropic effects that are too small to be detected in this sample, but the observed pattern suggests that the genetic architecture of cannabis use and MDD consists of a combination of phenotype-specific and shared genetic influences, where there are genetic loci that influence only MDD with no effects on cannabis use, and vice versa, as well as loci exerting pleiotropic effects on both phenotypes. This model would be in agreement with the pattern of genetic overlap between cannabis use and MDD, accounting for some but not all of the heritability of each trait.

Evidence of genetic correlation between cannabis use and MDD is not sufficient to determine the biological pathways involved in their comorbidity. Shared genetic etiology could reflect genes exerting effects on both cannabis use and MDD, or alternatively genetic influences could increase liability to one trait, which then in turn increases risk of the second trait. Identification of the specific genes involved offers a starting point to untangle these factors. Through our linkage and association analysis we have highlighted 11q23 as a region of interest. Further replication is needed, but once validated genes connected to both cannabis use and MDD are established, researchers will have a tool with which to investigate the underlying neurobiology and begin to determine the directionality of the relationship between these two phenotypes. We suggest that the picture is likely to be complex with multiple interacting pathways and a high degree of inter-individual heterogeneity.

STUDY LIMITATIONS

Whilst a number of our findings align well with previous literature, we do not replicate previous findings in MDD on chromosome 3p [20,21] or chromosome 10 [18]. Similarly, the highest linkage peak that we observe for cannabis use on chromosome 21 is not a region identified in prior genomic studies of cannabis-related phenotypes [16,23,25,69]. This may

be due sample differences in ancestry, phenotype definitions or limitations in statistical power. See **Supplementary Materials** for power calculations.

Nonetheless, there is evidence that rare variants play an important role in cannabis-related phenotypes [70] and so linkage studies are valuable in the search for the genetics underpinning cannabis use, as they are able to capture effects from both common and rare variants (in contrast to GWAS which focus on common SNPs). There is evidence that the heritability of cannabis phenotypes increases with the severity of use [12], so focusing on a more severe phenotype of cannabis dependence could be beneficial in identifying linkage regions. Nevertheless, the merits of a more narrowly defined phenotype must balanced with the associated decrease in available cases: in the case of the GOBS sample only 4.0% report cannabis dependence, whilst 13.2% report cannabis use.

Given the family-based design of the sample, when estimating the degree of genetic influence for traits, we are not able to disentangle environmental influences that make family members similar to each other (“shared environmental effects”) from genetic effects, as twin studies can. However, these shared environmental effects are less likely to inflate heritability estimates within a multigenerational extended pedigree sample such as GOBS [71] and this family study design enables the localization of trait loci.

Finally, we note that whilst the lifetime prevalence of cannabis use in this sample (13.2%) matches closely with that given for the USA in a recent United Nations report (13.7% [72]), the sample prevalence for MDD (33.8%) is greater than the ~17% estimated in US-based epidemiological studies [73,74]. Whilst US-based epidemiology surveys note that Hispanics generally have lower rates of psychiatric illness than the USA as a whole, as previously mentioned the term “Hispanic” encompasses a wide heterogeneity in bio-geographic ancestry. Factors such acculturation, relative social status and level of perceived discrimination are known to play an important role in variability in vulnerability to psychiatric illness within Hispanic minority subgroups [75–78]. Research focusing on understudied populations such as these are important in helping to build a understanding of vulnerability to psychiatric illness and how this may vary across the globe.

CONCLUSIONS

Genetic correlations between cannabis use and MDD indicate that the pattern of comorbidity between the two phenotypes is (in part) due to a shared genetic etiology. We identify a linkage signal for MDD on 22q11 as well as a pleiotropic locus on 11q23, which spans the *NCAM1-TTC12-ANKK1-DRDR2* gene cluster. The genes within this region have previously been linked to both addiction and MDD phenotypes. Further work is need to confirm the causal variants within this locus, but we have identified genomic regions of interest that may act as a starting point for understanding the relationship between cannabis use and MDD, and the neurobiology underpinning their shared etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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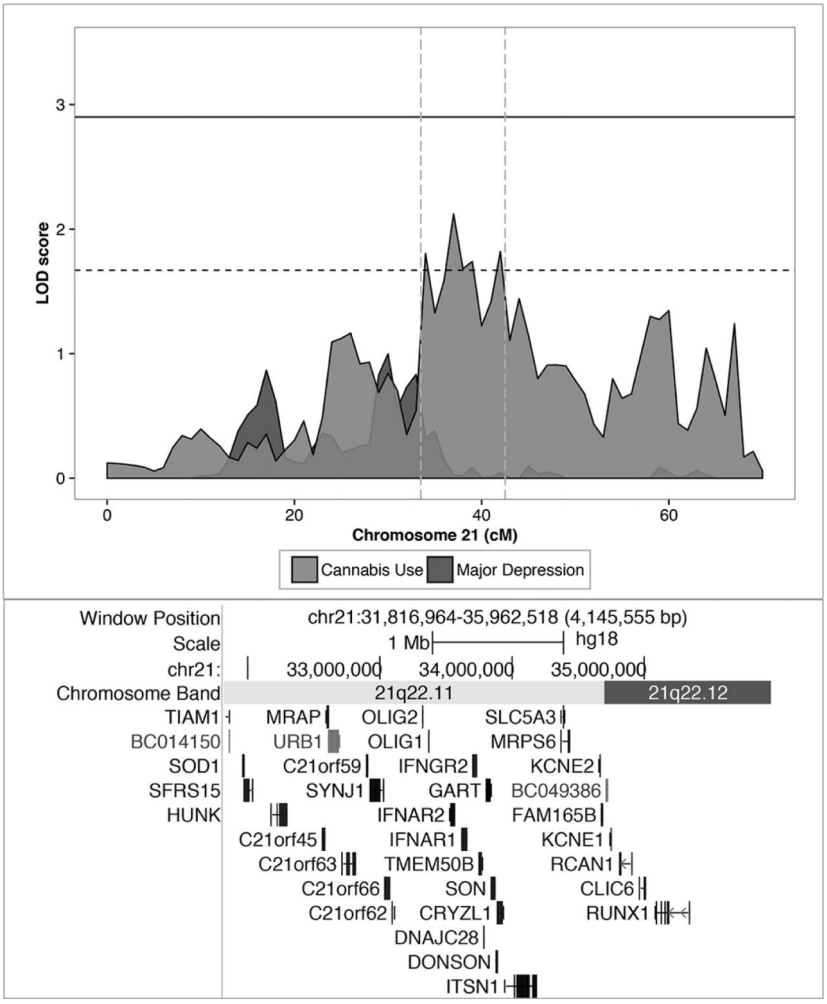

Figure 1.

Upper panel: suggestive univariate linkage peak for cannabis use at 21q22.11- q22.12, logarithm of the odds (LOD) = 2.123. Horizontal black solid and dashed lines indicate significant and suggestive thresholds for LOD scores. Vertical dashed grey lines define the linkage region, using (maximum LOD-1) confidence interval. Lower panel: Genes within the 21q22.11-q22.12 suggestive linkage peak for cannabis use. Drawn using the UCSC Genome Browser

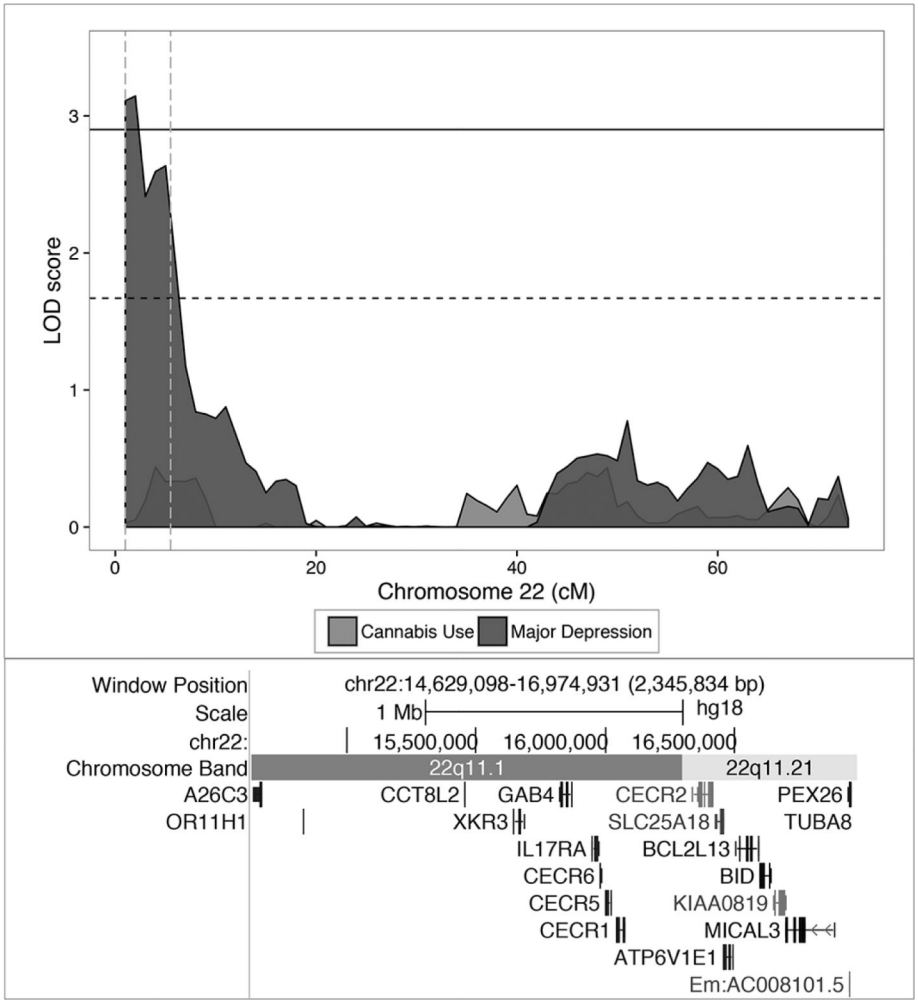

Figure 2.

Upper panel: significant univariate linkage peak for major depression at 22q11.1- q11.21, limit of detection (LOD) = 3.144. Horizontal black solid and dashed lines indicate significant and suggestive thresholds for LOD scores. Vertical dashed grey lines define the linkage region, using (maximum LOD-1) confidence interval. Lower panel: genes within the 22q11.1-q11.21 significant linkage peak for major depression. Drawn using the UCSC Genome Browser

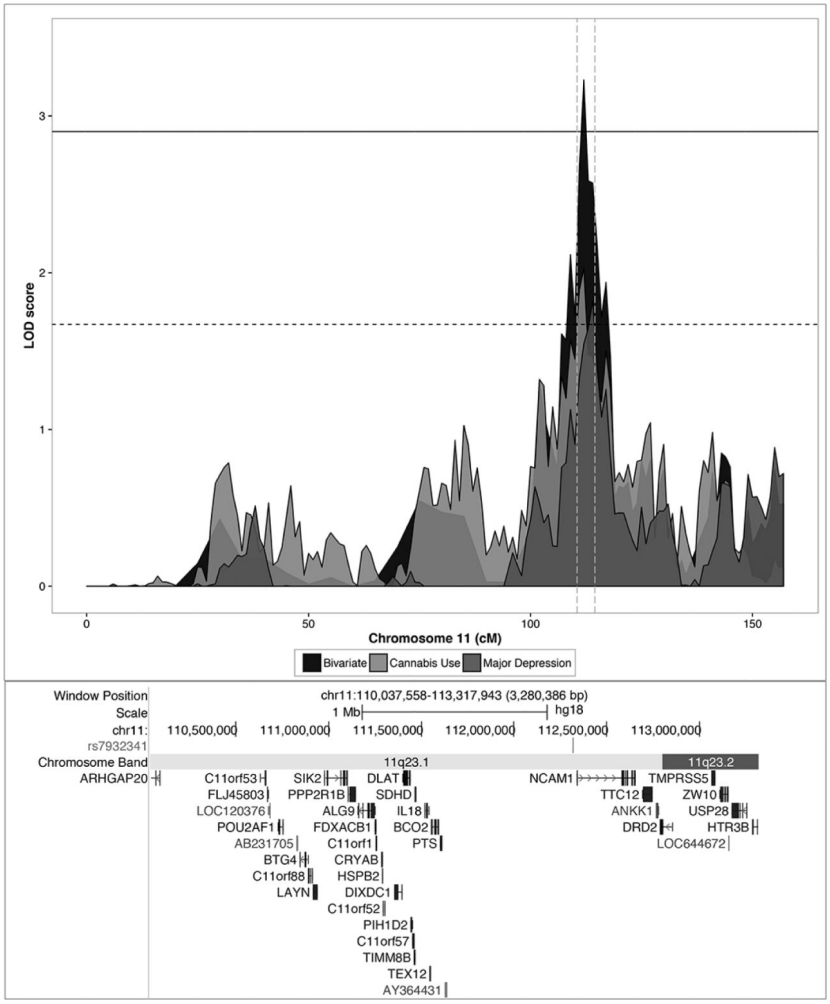

Figure 3.

Upper panel: significant bivariate linkage peak for cannabis use-major depression at 11q23.1-q23.2, limit of detection (LOD) = 3.229, pleiotropy test $B∓V (J2 (1) = 9.77, P = 8.86 $B!_(J 10-4). Horizontal black solid and dashed lines indicate significant and suggestive thresholds for LOD scores. Vertical dashed grey lines define the linkage region, using (maximum LOD-1) confidence interval. Lower panel: genes within the 11q23.1-q23.2 significant bivariate linkage peak for cannabis use-major depression. The position of single nucleotide polymorphism (SNP) s7932341 is also shown. Drawn using the UCSC Genome Browser

TABLE 1

RELATIONSHIPS WITHIN PEDIGREES

No. of pairs	Relationship
1284	Self
1	Identical sib-pair
841	Parent-offspring
917	Siblings
185	Grandparent-grandchild
1590	Avuncular
176	Half siblings
6	Double 1st cousins
2764	3rd degree
3103	4th degree
2180	5th degree
1062	6th degree
563	7th degree
130	8th degree
9980	Unrelated

TABLE 2

SAMPLE DEMOGRAPHICS

	Prevalence			
	N	**% of sample**	**Mean age in years (SD)**	**% female**
Cannabis Use	170	13.2%	36.32 (11.56)	40%
MDD	434	33.8%	46.85 (13.15)	73.5%

TABLE 3

RESULTS FROM UNIVARIATE ANALYSES

	Heritability			Linkage	
	H^2	P value	SE	Location	LOD
Cannabis Use	**0.614**	**1.00×10^{-6}**	**0.151**	Chr 21, 37cM	2.123
MDD	**0.349**	**1.06×10^{-5}**	**0.100**	**Chr 22, 2cM**	**3.144**

Significant heritability and linkage results highlighted in bold

TABLE 4

RESULTS FROM BIVARIATE ANALYSES

Analysis		Bivariate model	Univariate models	
		Cannabis use-MDD	**Cannabis use**	**MDD**
Genetic correlation		ρ_g=0.424, p=0.036, SE=0.195		
Environmental correlation		ρ_E=−0.086, p=0.655, SE=0.195		
Linkage at Chr 11, 112cM	**LOD**	**3.229**	2.028	1.545
Association at rs7932341	**Pval**	**3.10×10^{-5}**	5.75×10^{-4}	1.86×10^{-3}
	Beta	Cannabis; −2.638 MDD; −4.89	−2.554	−5.275

(Univariate findings are also shown at the location of interest)