Published in final edited form as:

Addiction. 2009 April ; 104(4): 518–532. doi:10.1111/j.1360-0443.2009.02504.x.

Candidate Genes for Cannabis Use Disorders: Findings, Challenges and Directions

Arpana Agrawal and Michael T. Lynskey

Washington University School of Medicine, Dept. of Psychiatry, St. Louis MO 63110

Abstract

Aim—Twin studies have shown that cannabis use disorders (abuse/dependence) are highly heritable. This review aims to: (i) review existing linkage studies of cannabis use disorders and (ii) review gene association studies, to identify potential candidate genes, including those that have been tested for composite substance use disorders, and (iii) to highlight challenges in the genomic study of cannabis use disorders.

Methods—Peer-reviewed linkage and candidate gene association studies are reviewed.

Results—Four linkage studies are reviewed: results from these have homed in on regions on chromosomes 1, 3, 4, 9, 14, 17 and 18, which harbor candidates of predicted biological relevance, such as monoglyceride lipase (*MGL*) on chromosome 3, but also novel genes, including *ELTD1* (EGF, latrophilin and seven transmembrane domain containing 1) on chromosome 1. Gene association studies are presented for (a) genes posited to have specific influences on cannabis use disorders: *CNR1*, *CB2*, *FAAH*, *MGL*, *TRPV1* and *GPR55* and (b) genes from various neurotransmitter systems that are likely to exert a non-specific influence on risk of cannabis use disorders e.g. *GABRA2*, *DRD2* and *OPRM1*.

Conclusions—There are challenges associated with (i) understanding biological complexity underlying cannabis use disorders (including the need to study gene-gene and gene-environment interactions), (ii) using diagnostic versus quantitative phenotypes, (iii) delineating which stage of cannabis involvement (e.g. use vs. misuse) genes influence and (iv) problems of sample ascertainment.

Keywords

Cannabis; genes; association

Glossary of genetic terms

1p36 or 6q14–15

Convention for denoting the location of a gene along a chromosome – the first digit (1 or 6) is the chromosome, the second letter (p or q) refers to the short (p-petit) arm or the long (q) arm and the remaining numbers refer to the chromosomal band (e.g. 1p36 refers to band 36 on the short arm of chromosome 1).

3' end/5' end of gene

The ends of a strand of DNA with conventional directionality going from the 5' end (upstream) to the 3' end (downstream) of a gene.

Basepairs

Adenine (A), Guanine (G), Thymine (T) and Cytosine (C) – the building blocks of the genome.

centiMorgan (cM)

a measure of genetic distance.

Conserved sequences/residues

Segments of the genome that have reduced variability across individuals and even across species, suggesting evolutionary importance.

Exons

Regions within a gene that code for proteins.

Family-based method

A genetic study that requires genetic and phenotypic data on related individuals.

Fine-mapping

Adding additional markers to a region identified by a linkage study and re-doing the analyses to further narrow the region down.

Gene-environment interaction

The expression of genetic susceptibility to cannabis use disorders in certain environmental contexts.

Genetic variant

A change in the genome, such as a change in basepairs (e.g. from A to C), which may induce changes in the amino acid the sequence codes for it, or may have no known function.

Heritability/heritable factors

The extent to which individual differences in cannabis use disorders are explained by unmeasured genetic influences;

Insertion-deletion

Also called indels, this refers to a mutation in which one or more basepairs are added or deleted.

Kilobases (kb)

1,000 basepairs – a measure of physical distance (e.g. the size of a gene).

Knock-out model

An animal model where an important region of a gene or the entire gene is deleted or inactivated (knocked-out).

Linkage analysis

Examines whether certain segments of the genome are shared more often (are identical-by-descent) by relative pairs who are affected than expected by chance alone.

Linkage peak

Visualization of evidence for linkage as LOD scores, plotting genetic distance by LOD score, a linkage peak occurs when LOD scores are increased, resulting in a peak in the plot.

Linkage region

A region of the genome, identified by genetic distance (cM) that is shared by affected relatives.

LOD score

Likelihood of Odds in favor of linkage – a LOD score of 3.0 suggests that it is a 1000 times ($\log_{10}(1000)=3$) more likely that the genomic region is shared by affected relatives than expected by chance alone.

Marker

A region of the genome comprised of one or more base pairs that naturally varies in frequency amongst members of a population, and may or may not have any functional significance.

Missense (or non-synonymous) mutation

A change in a basepair that causes the amino acid being coded by the 3 basepair sequence to change – these mutations have functional impact as they can change the protein that the gene codes for.

Multifactorial

The vulnerability to cannabis use disorders vary as a function of genes and environment (non-Mendelian characteristic).

Polygenic

Cannabis use disorders are influenced by multiple genes of modest additive influence (non-Mendelian inheritance).

Single Nucleotide Polymorphism (SNP)

A SNP is a change in a single base pair in a specific position along the genome that may or may not be functionally relevant.

Synonymous (or silent) mutation

A change in a basepair that does not cause the amino acid to change. This occurs due to redundancy in genetic code where multiple sets of 3 basepairs code for the same amino acid.

Trinucleotide repeats

a sequence of 3 basepairs (e.g. AAT) that is sequentially repeated a variable number of times.

Cannabis use disorders

Worldwide, the cumulative incidence of lifetime cannabis use ranges from 0.3% in China to 42% in the United States [1]. Of those who use cannabis in the United States, approximately 40% and 7% develop DSM-IV cannabis abuse and dependence respectively [2], both of which

are representative of cannabis use disorders. Cannabis abuse is defined by the endorsement of one of four DSM-IV criteria (recurrent legal problems, hazardous use, use despite social and interpersonal problems and failure to fulfill major role obligations) [3]. A diagnosis of cannabis dependence requires the experience of 3 or more of 6 DSM-IV criteria (tolerance, use in larger quantities or for longer than intended, repeated unsuccessful attempts to quit or cut back, giving up important activities, spending excessive time acquiring or using cannabis and recurrent use despite physical and/or emotional problems; DSM-IV currently does not recognize withdrawal as a dependence criterion for cannabis). Cannabis use disorders commonly represent the experience of these problems or abuse/dependence diagnoses. In 2006, cannabis use disorders contributed to 16% of the admissions to U.S. substance abuse treatment centers – second only to alcohol-related admissions [4].

Heritability of cannabis use disorders

Over the last decade or so, twin studies have firmly established that additive genetic influences contribute to the etiology of cannabis use disorders [5]. These heritable factors (h^2) explain in the region of between 30–80% of the total variance in risk of cannabis use disorders. These estimates have also been largely consistent across samples of adult Australians ($h^2 = 45\%$) [6], U.S. Virginia ($h^2 = 58\text{--}76\%$) [7,8], US Vietnam Era Veteran men ($h^2 = 33\%$) [9] and adult Norwegian twins ($h^2 = 77\%$) [10].

The next step: Genomic strategies

These twin studies have provided an important first step towards our comprehension of the etiology of cannabis use disorders by demonstrating the existence of heritable influences on these outcomes. However, the genetic influences estimated in these models refer to latent or unmeasured genetic factors that presumably represent the composite variance explained by hundreds of individual genes, without identifying any specific genes. There are several current efforts underway that have begun to scan the genome for regions, genes and variants in genes that influence the liability to develop cannabis use disorders. Several genomic strategies exist, the more common ones being genomewide linkage analysis, genomewide association analysis and candidate gene association analysis.

A review of gene identification strategies and initial findings in this area is accordingly timely. In this review we:

- i. Discuss findings from family-based linkage studies that have homed in on genomic regions that may harbor susceptibility genes for cannabis use disorders;
- ii. Discuss studies of candidate genes that are specifically related to cannabis use disorders;
- iii. Discuss studies of candidate genes that likely influence a number of substance use disorders, including cannabis use disorders;
- iv. Present challenges and directions for future genetically informative research into cannabis use disorders;

For (ii) and (iii), in addition to describing genomic methodologies and summarizing findings from the small number of studies that apply these methods to the study of cannabis use disorders, we summarize evidence in favor of candidate genes that are of theoretical interest, but have not yet been examined in human association studies.

Genomewide linkage analysis

In this family-based method, studies assess the likelihood that a genetic marker that is close to the causative genetic variant of functional importance is shared more often than by chance alone by sets of affected relatives [11-17]. The more genetic variants of common ancestral origin (i.e. the same allele inherited by each offspring from the same parent) that affected relatives share, the more evidence in support of linkage. Usually the causative genetic variant is unknown and evenly spaced markers are typed along the length of the genome (including the autosomes and sex chromosomes) and a test of linkage is carried out. The resulting likelihood of odds (LOD) score reflects evidence in favor of linkage, on a logarithmic scale. These LOD scores (particularly when they exceed 3.0[18]) imply that a segment of the genome is shared frequently by affected sets of relatives.

Linkage analysis rarely isolates a single gene. Rather, it narrows the investigator's search to a smaller region of the genome [19]. The search to identify specific genes (often more than one) within a linkage region (sometimes referred to as a 'LOD-support' region) requires information on the functional significance of genes in this chromosomal region and in some instances, fine-mapping of the region. There have been three published linkage studies of cannabis use disorders [20-22], and one of earlier stages of cannabis use (including frequency of use) [23] the key findings of which are presented in Table 1. The first of these, by Hopfer and colleagues [20], identified linkage regions for cannabis dependence symptoms in adolescents on chromosome 3 and 9. Under their linkage peak on chromosome 3 is the monoglyceride lipase gene (*MGLL*) which encodes an enzyme that has been found to hydrolyze the endocannabinoids 2-arachidonoyl ethanolamide and 2-arachidonoylglycerol (two naturally occurring substances that act on cannabinoid receptors on the brain). In other studies, genes were identified on chromosome 1 [22] and 14 [21]. The genes near these linkage peaks, however, have no known effects on cannabis use disorders but deserve further investigation. They include: *GPR68* (G-protein coupled receptor 68) which is involved in cAMP regulation and *ELTD1* (EGF, latrophilin and seven transmembrane domain containing 1) which encodes a G-protein coupled receptor that is part of the neuropeptide signaling pathway.

Thus, linkage studies have identified some novel candidate genes for future investigation. These include genes, such as the cannabinoid receptor 1 (*CNRI*) gene, which are known for their biological relevance in cannabis use disorders that are discussed in detail below. However, after identifying genes under a linkage peak investigators need to determine how far from the linkage peak they wish to look. They need to keep in mind that each centiMorgan (the units in which genetic distance in linkage analysis is measured) roughly corresponds to a million base pairs in humans. This means that a linkage region of 20–30 cM may harbor over 300 genes of medium size (100kb or so) and the selection of one gene as a biologically plausible candidate over another can be difficult because of our limited knowledge of cannabis use disorders. For these reasons, and others (including statistical power, the complexities of conducting family-based studies) many investigators prefer to do gene association studies.

Genomewide association studies (GWAS)

This is an exploratory method of study in which the genome is saturated with single nucleotide polymorphisms (SNPs). Researchers study association between these variants and the trait of interest (such as, cannabis use disorders). 'GWAS' are most commonly conducted by comparing cases (affected individuals) and controls (unaffected individuals) who are unrelated to each other. Family-based approaches can also be used. Rapid advances in technology have produced high density SNP chips (e.g. with one-million SNPs) that provide comprehensive coverage along the whole genome but coverage of specific genes may be less adequate. We are not aware of any GWAS of cannabis use disorders, although this design presents an avenue for progress.

Candidate gene association analysis

This is a more targeted way to examine the extent to which SNPs in a gene that has been selected for its biological relevance are associated with the trait of interest. Data may be either family-based or case-control [24]. For cannabis use disorders, we focus on:

- a. Genes that are specific to the endogenous cannabinoid system and hence likely to be specific to the etiology of cannabis use disorders;
- b. Biologically relevant genes that are largely non-specific and have been shortlisted for their association with other forms of substance abuse;

Specific genes of biological relevance

The identification of genes that specifically relate to the action and metabolism of exogenous cannabinoids is limited by the paucity of research in this area – most of the research draws heavily on parallels between the activity of endogenous cannabinoids and the presumed action of THC and other exogenous cannabinoids. The identification of cannabinoid receptors is an ongoing research effort, with the possibility of there being unknown receptors. We focus on 6 genes, two of which have been actively studied and the remainder that are leading contenders to explain individual differences in liability to cannabis use disorders. The majority of association studies conducted with these genes has been exploratory – hence, here we review all human association studies that have investigated the effects of these genes on any substance-related measure.

Cannabinoid receptors (*CNR1* and *CNR2*)

Arguably the most likely candidate genes for cannabis use disorders are those encoding the cannabinoid receptors. These receptors form the binding sites for endogenous cannabinoids, such as anandamide (N-arachidonyl-ethanolamine), 2-AG (2-arachidonyl-glycerol) [25-27] and the more recently identified noladin (2-arachidonyl-glycerol ether), virhodamine (O-arachidonyl-ethanolamine) and NADA (N-arachidonyl dopamine) [28-30].

So far two cannabinoid receptors have been identified in humans: CB1, which is highly expressed in the brain; and CB2, which is more prominently expressed in leukocytes and has largely been implicated in immune response. Both CB1 and CB2 are G-protein coupled receptors. Stimulation of these receptors by either endogenous or exogenous cannabinoids (e.g. THC) inhibits adenylate cyclase, activates mitogen-activated protein kinases and the inhibits and activates voltage-gated calcium and potassium channels respectively [31-35].

The gene encoding CB1 (*CNR1*) is on human chromosome 6q14–15. It is a 25 kb gene with 4 exons, of which the fourth is the largest and most commonly expressed in brain tissue [36]. Tests of association between substance dependence and polymorphisms in *CNR1*, including SNPs, but also a trinucleotide repeat (AAT)_n and an insertion-deletion (–3180T), and have shown conflicting results. As seen in Table 2, several of these studies that used a general measure of substance dependence reported associations with SNPs in *CNR1*, particularly rs2023239 and rs806368. Another SNP, rs806380 has been implicated in studies of cannabis dependence by Hopfer et al [37] and Agrawal [38] but Herman and colleagues [39] do not report association between polymorphisms in *CNR1* and cannabis dependence. In contrast, a majority of the studies of the (AAT)_n repeat have yielded negative results [40-42].

Given the modest number of studies in Table 2 of cannabis dependence, there is only limited evidence for the role of *CNR1* in cannabis dependence [43-45]. Moreover, the extent to which *CNR1* mediates reward related to earlier stages of cannabis involvement, such as initiation and regular use is largely unknown. When Chen and colleagues [46] examined the influence of *CNR1* on cigarette smoking initiation and dependence, they found stronger associations with

dependence. The hypothesis that the contribution of *CNR1* to cannabis-related behaviors is specific is complicated by positive associations with general substance dependence [47], nicotine dependence [46], the rewarding effects of alcohol [48] and for alcohol dependence in the absence of comorbid drug dependence [39]. None of these studies attempted to exclude cannabis dependence from their definition of 'substance dependence' (or nicotine and alcohol dependence) to examine whether the association signal was attributable to comorbid cannabis use problems. Therefore, while we can hypothesize that *CNR1* is related to a general vulnerability to substance dependence and not just to cannabis dependence, we cannot rule out the hypothesis that comorbid cannabis dependence is responsible for these associations.

The gene encoding CB2 (*CNR2*) lies on chromosome 1p36.11. The 39.4kb gene has 2 exons and has been actively investigated for its role in osteoporosis, inflammatory responses, leukemia and certain forms of cancer. There is now mounting evidence that CB2 receptors occur in the central nervous system and that the gene is expressed in the brain [49,50]. To date, only one study has examined an association between *CNR2* and substance-related behaviors. Ishiguro and colleagues [51] found an association between Q63R (glutamine to arginine), a two base pair substitution (rs2501432) and alcoholism (and also depression, see [52,53]) in a Japanese population.

Fatty acid amide hydrolase (*FAAH*)

A candidate with considerable promise for association studies of cannabis use disorders is the gene that encodes the fatty acid amide hydrolase enzyme (*FAAH*) [54-57]. *FAAH* catalyzes the conversion of both AEA (anandamide) and 2-AG to arachidonic acid and ethanolamine (AEA) or glycerol (2-AG) [58,59]. In animal studies, inhibiting *FAAH* activity (and thereby reducing breakdown of endogenous cannabinoids) increases non-opioid induced analgesia [60]. In murine knock-out models, *FAAH* $-/-$ mice are reported to be more responsive to exogenous cannabinoids and to have lower pain sensitivity [61].

Residing on human chromosome 1p35-34, the *FAAH* gene is 19.5kb in length and includes 15 exons. The gene is widely expressed in the central nervous system. There is evidence that while progesterone and leptin upregulate [62] the 674 bp *FAAH* promoter, estrogen and glucocorticoids [63] downregulate its activity. To date, there have been 4 association studies of *FAAH* and substance use disorders. A majority of these studies tested the effects of a missense mutation (C385A) that converts a conserved proline residue to threonine in exon 3 (rs324420, previously rs57947754). There is considerable evidence that this missense mutation is associated with risk for substance dependence (Table 3) in Caucasian and African-American, but not in Japanese or other Asian populations [64-66]. Sipe and colleagues [67,66] characterized this polymorphism and found that individuals homozygous for the minor A allele, were nearly 5 times more likely to be street drug users or to report problems with alcohol or drugs. The effects were strongest in those with comorbid drug and alcohol problems but there were no statistically significant effects in individuals with alcohol or nicotine dependence in the absence of illicit drug use. In contrast, when examining cannabis dependence alone (and not general substance dependence) Tyndale et al [68] found that A/A individuals were 0.25 times less likely to be cannabis dependent (but, potentially more likely to try cannabis). The authors argue that the reduced risk for cannabis dependence may stem from diminished *FAAH* activity leading to increased levels of endogenous cannabinoids which may ameliorate craving and withdrawal.

Monoglyceride lipase (*MGLL*)

In addition to *FAAH*, monoglyceride lipase (*MGL*) is responsible for the hydrolysis of 2-AG and other lipids [69-71]. The gene encoding this enzyme, *MGLL*, is 131 kb long, has 8 exons, lies on 3q21.3 and has been implicated in at least two independent studies of cannabis use

disorders [20,22]. In one association study, polymorphisms in *MGLL* were not found to be associated with alcoholism in Japanese adults [65].

The Transient Receptor Potential Vanilloid 1 (*TRPV1*)

AEA (anandamide) also activates the vanilloid receptor 1, a transient receptor potential cation channel. *TRPV1* is classically activated by hot chilli peppers (capsaicin) [72] and heat at noxious levels as well as by AEA whose affinity for it can be increased 10-fold during states of pathological stress [73-75]. The gene encoding the vanilloid receptor 1 (*TRPV1*) occurs on human chromosome 17p13.3, is 44kb long and has 17 exons. Research [76,77] suggests that *TRPV1* may serve as a unique receptor site for endogenous, and possibly exogenous, cannabinoids. We are not aware of any human association studies of *TRPV1* and substance dependence, although several functional SNPs have been characterized in this gene.

Orphan cannabinoid receptor (*GPR55*)

Several animal models (CB1 $-/-$ knockout) suggest that additional cannabinoid receptors may exist that mediate the effects of the 5 known endocannabinoids and other putative (e.g. palmitoylethanolamide) lipids. A recent study identified a G-protein coupled receptor, *GPR55*, which is expressed in some human brain regions and binds exogenous and endogenous cannabinoids [78-84]. The 17 kb gene encoding *GPR55* resides on 2q37 and has 3 exons.

Non-specific genes of biological relevance

Genes encoding the major neurotransmitter systems could potentially influence the biological basis of cannabis use disorders. Increased production of these neurotransmitters is potentially mediated by CB1 (cannabinoid receptor 1) activity. These systems include the glutamatergic, GABAergic, serotonergic, dopaminergic, opioidergic and acetylcholinergic systems. While it is likely that genes in these receptor families contribute in some way to the genetic underpinnings of cannabis-related behaviors, even abuse and dependence, their effects on cannabis use disorders are likely to be non-specific and similar to their effects on other psychoactive substance use behaviors. We briefly highlight three of these neurotransmitter systems, their general effects on substance-related behaviors and discuss their particular relationship with cannabis. We then select the most commonly investigated gene in each family and present evidence for association between this gene and cannabis use disorders.

The GABAergic System – *GABRA2*

Gamma-amino butyric acid is one of the major inhibitory neurotransmitters in humans. Genes encoding the GABA(A) receptor subunits, a family of ligand-gated ionotropic receptors, reside in clusters across several chromosomes, including chromosome 4, 5, 15 and X. GABA(C) is another family of ionotropic receptors but their impact on addictions in humans is largely unstudied [85-87]. Genes encoding subunits of the metabotropic GABA(B) receptors are being actively investigated in human studies of smoking cessation. Baclofen [88], a GABAB agonist, has also been found to have therapeutic potential in alcohol and cocaine dependence [89-91].

Within the substance use literature, the most widely studied GABAergic gene is *GABRA2* on chromosome arm 4p12 [85]. The most promising results for this gene have been with alcohol dependence although subsequent studies have also shown it influences liability to other substance use disorders. We are aware of two human association studies that specifically examined the association between SNPs in *GABRA2* and cannabis-related behaviors. In the first study, [92] conducted in the Collaborative Study of the Genetics of Alcoholism (COGA), a synonymous (a mutation that does not change the amino acid it codes for) polymorphism (rs279858) was associated with DSM-IV cannabis dependence. Recently, Lind et al. [93]

reported no association between rs279858 and a frequency of cannabis use across the lifetime. A third study has also identified polymorphisms in *GABRA2* for their influence on a composite measure of antisocial drug dependence [94].

The Dopaminergic System – *DRD2*

Studies suggest that THC increases dopamine firing release in several parts of the brain. Van der Stelt and Di Marzo [95] discuss several mechanisms that may inter-link the endocannabinoid and dopaminergic pathways. The possibility exists that cannabinoids indirectly activate mesolimbic dopaminergic pathways [96-100]. This makes a variety of dopaminergic receptor genes, such as *DRD2*, (which have been shown to be associated with alcoholism and drug addiction [101,102]) important candidate genes for earlier stages of cannabis involvement. A more intriguing, and controversial, hypothesis that links dopaminergic genes and cannabis posits that cannabinoids may increase dopamine system hyperactivity and thereby contribute to the link between cannabis use and psychotic symptoms [103-112]. Some forms of psychosis are attributed, in part, to excessive dopamine production in the nucleus accumbens, making the gene encoding the dopamine transporter (*DAT1*, *SLC6A3*), which is responsible for dopamine re-uptake, a good candidate. Genes that encode enzymes assisting in the production and metabolism of dopamine, such as *TH* (tyrosine hydroxylase, converting tyrosine to dopamine) and *DBH* (dopamine beta-hydroxylase, converting dopamine to norepinephrine) may also contribute to the etiology of cannabis-mediated reward.

Human association studies have found associations between *DRD2* and cannabis use. Conner et al [113] found that boys carrying one copy of the TaqA1 allele experienced their first cannabis-related 'high' at an earlier age than those who did not have this allele. Sakai et al [114] have also shown the TaqA1 allele to be associated with adolescent alcoholism. Since 93% of their alcohol dependent adolescents also reported comorbid cannabis abuse/dependence this sample may have identified an influence of Taq1A on comorbid alcohol and cannabis dependence. It is noteworthy that the TaqA1 polymorphism has recently been mapped to exon 8 of a neighboring gene *ANKK1* (ankyrin repeat and kinas domain containing 1) [115]. Therefore, it remains to be seen whether prior associations with *DRD2* are attributable to *ANKK1*.

The Opioidergic System – *OPRM1*

The endogenous opioid system consists of mu, delta and kappa receptors. The opioidergic system may play a role in negative affect regulation via substance use [116] and there is considerable evidence that the cannabinoid and opioid systems may interact [117,118]. The mu-receptors commonly co-localize with CB1 receptors in the central nervous system [119, 99,120]. Both opioids and cannabinoids produce antinociception and analgesia and animal studies have revealed considerable cross-tolerance and precipitation of cross-drug withdrawal between these two endogenous systems [121-123]. Furthermore, both cannabinoids and opioids target glutamatergic and GABAergic systems – for instance, Caille & Parsons [124] showed that a cannabinoid agonist-induced reduction in GABA could be reversed by naloxone, a competitive mu-opioid receptor antagonist. There is also mounting evidence that the motivational, cognitive and emotional responses associated with cannabinoids are cross-modulated (i.e. changes in activity of the receptor, such as CB1 when another substance, such as an opioid, binds to it) by opioids [125-128,117].

Given the primary importance of the mu-opioid receptors in cannabinoid-opioid interplay, the gene encoding the mu-opioid receptor 1 (*OPRM1*) on chromosome 6q24-q25, is an excellent candidate gene for cannabis use, abuse and dependence. Intriguingly, the gene lies 64.7 Mb (Megabases) downstream from the 3' end of the *CNR1* gene. *OPRM1* is a fairly large gene

(124kb), with 4 exons. It has been widely studied for its role in alcohol, nicotine and substance dependence [129-136]. The most actively investigated polymorphism in this gene is A118G (rs1799971) which leads to a change in the function of exon 1. Studies have failed to consistently find an association between A118G and liability to drug dependence. We are not aware of any published association studies of cannabis dependence and polymorphisms in *OPRM1*.

Challenges of genomic studies of cannabis use disorders

Despite the higher prevalence of cannabis use and cannabis use disorders than other illicit drug disorders in the general population [137] and the consistent evidence from twin and family studies of a heritable component to cannabis use and abuse/dependence, there has been less genomic research on cannabis use disorders than on cocaine and heroin disorders. There are several challenges facing genetic research into the etiology and course of cannabis (and other drug) use disorders. These include issues relating to research design, such as sample ascertainment, phenotype definition and statistical power (particularly an issue in the context of GWAS) as well as more fundamental issues relating to our understanding of risk for and development of cannabis (and other substance) use disorders

Biological mechanisms underlying genetic risk

Currently, our choice of candidate genes is driven by accumulating evidence from preclinical research on endogenous cannabinoids and clinical research into cannabis-related behaviors. Research into the complex neurochemistry and biochemistry of exogenous cannabinoids is still growing. The identification of novel genes associated with cannabis use disorders will undoubtedly improve our understanding of the biological processes underlying cannabis involvement. With increasing numbers of genetically informative samples and data repositories, such as dbGAP (<http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gap>), progress in the arena of cannabis-related genomic research is imminent. The challenge will be to integrate the existing and those emerging findings with our current knowledge of the biological systems influencing cannabis involvement. Two key aspects will require attention:

Gene-gene interactions

Cannabis and other substance use disorders are likely to be polygenic (i.e. to involve several genes of modest effect). It is unlikely that human association studies will uncover a single gene that explains more than a modest proportion of genetic variance [138,15]. Furthermore, as demonstrated by the recent discovery of *TRPV1* and *GPR55*, novel receptor sites for endo- and exogenous cannabinoids are continuously being identified. Hence, it is likely that networks of genes, both specific to cannabis involvement and non-specific candidates for drug abuse, will interactively predict vulnerability to cannabis use disorders. Recently, Haughey and colleagues demonstrated evidence for an interaction between the rs324420 and rs2023239 (in *CNR1*). In 18–25 year old daily cannabis users, individuals with C/A – C/T for *FAAH* – *CNR1* showed higher negative affect after cannabis withdrawal (both post-abstinence and post-cue exposure). Therefore, in addition to single gene identification, the analysis of families of genes and the interactions between their functional variants is necessary.

Gene-environment interactions

Cannabis use disorders are multifactorial and these factors (genes and environment) interact to shape its development [139]. Therefore, careful attention to the mechanisms by which changes in the environment modify genetic risk is required. To date, all gene-environment interaction studies of cannabis have treated cannabis use as the environmental exposure. For example, Caspi et al found that early exposure to cannabis use modified the genetic effects of COMT (catecholamine-o-methyl transferase) on psychosis [140]. There has been a recent

failure to replicate this result in a large UK case-control study by Zammit and others [141]. The genetic influences on cannabis use disorders itself may also be modified by environmental stimuli, such as early childhood adversity or stressful life events.

Definition of the Phenotype

Efforts are under way to improve existing DSM-IV criteria for cannabis use disorders, e.g. by the addition of withdrawal as a dependence criterion [142]. Quantitative assessments of liability to cannabis use disorders (e.g. cannabis abuse/dependence symptom counts) may also enhance statistical power for gene association studies. For example, there is overwhelming support for a unidimensional factor underlying DSM-IV cannabis abuse and dependence criteria [143-146]. Utilization of a factor score generated using the abuse and dependence symptoms would allow investigators to assess the role of genotype across a range of liability. Such quantitative measures, particularly those using factor analytic approaches, can incorporate additional measures of cannabis use (e.g. frequency or duration use, withdrawal) and also be used to identify cases and controls based on high or low scores on this liability spectrum.

Stages of cannabis involvement

When in the development of cannabis use disorders do genetic influences unfold? Twin studies suggest that a high proportion of the genetic influences on cannabis use disorders overlap with genetic influences on earlier stages of experimentation and regular cannabis use [147-150]. Twin analyses of the extent to which genetic influences on the liability to experiment with cannabis (lifetime use) are also responsible for genetic variation in the liability to cannabis use disorders, indicate that nearly 80–90% of these genetic factors were common to earlier and later stages of cannabis involvement [151]. Presumably, some of the genes described here specifically influence early stages of involvement (e.g. *DRD2* and early initiation of cannabis use) while others may contribute across several stages, including abuse/dependence (e.g. *CNR1*). To this end, genomic studies may need to include a range of controls, for instance, those who have never used cannabis (unexposed) as well as those who have used cannabis a few times but never developed problems (exposed controls). Such a stratified approach to cannabis involvement is vital to clarifying the genetic etiology of cannabis use disorders.

Sample ascertainment

One factor impeding genetic studies of cannabis use disorders may be problems in identifying and ascertaining cases. First, treatment seeking specifically for cannabis use disorders remains relatively rare [152,153]. Secondly, among those seeking treatment for cannabis-related problems a relatively large number of younger cases may be coerced into treatment either through contact with the justice system or as a result of workforce detection. This may result in a situation in some of those seeking treatment for “cannabis problems” report limited cannabis use and so may not be ideal cases for genetic studies [154]. Additionally, other substance use disorders and psychiatric disorders are highly prevalent in individuals seeking treatment for a cannabis use disorder. This suggests that treatment samples may not provide the optimal sampling frame for studying cannabis use disorders.

Implications

It is now time for research into the biological basis of cannabis use disorders, through the identification of genetic variants, their interplay with environmental change, and the integration of this knowledge. The possibility that our knowledge of cannabis use disorders will dramatically improve is perhaps the strongest scientific rationale for pursuing this line of genomic enquiry. However, an additional outcome of this research is the likelihood of enhancing strategies for treatment of cannabis use disorders, and herein lies the tremendous potential for public health. Given the identification of more endogenous cannabinoids, the

greatest outcomes of research into genetic vulnerabilities for cannabis use disorders may be a greater understanding of the actions of the endocannabinoids that can be exploited in the treatment of a range of substance related disorders [155,156].

Acknowledgements

DA023668 (AA), DA18267 and DA18660 (MTL). We thank Ms. Emily Balk for assistance with literature survey.

Reference List

1. Degenhardt L, Chiu WT, Sampson N, Kessler RC, Anthony JC, Angermeyer M, et al. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Med* 2008;5:e141. [PubMed: 18597549]
2. Agrawal A, Lynskey MT. Does gender contribute to heterogeneity in criteria for cannabis abuse and dependence? Results from the National Epidemiological Survey on Alcohol and Related Conditions. *Drug Alcohol Depend* 2007;88:300–307. [PubMed: 17084563]
3. American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association; Washington, DC: 1994.
4. Office of Applied Studies S. A. a. M. H. S. A. Treatment Episode Data Set (TEDS). 2008. [Http://Wwwdasis.Samhsa.Gov/Webt/Quicklink/US06.Htm](http://Wwwdasis.Samhsa.Gov/Webt/Quicklink/US06.Htm)
5. Agrawal A, Lynskey MT, Bucholz KK, Martin NG, Madden PA, Heath AC. Contrasting models of genetic co-morbidity for cannabis and other illicit drugs in adult Australian twins. *Psychol Med* 2007;37:49–60. [PubMed: 17076916]
6. Lynskey MT, Heath AC, Nelson EC, Bucholz KK, Madden PA, Slutske WS, et al. Genetic and environmental contributions to cannabis dependence in a national young adult twin sample. *Psychol Med* 2002;32:195–207. [PubMed: 11871371]
7. Kendler KS, Prescott CA. Cannabis use, abuse, and dependence in a population-based sample of female twins. *Am J Psychiatry* 1998;155:1016–1022. [PubMed: 9699687]
8. Kendler KS, Karkowski LM, Neale MC, Prescott CA. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch Gen Psychiatry* 2000;57:261–269. [PubMed: 10711912]
9. Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, et al. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* 1998;55:967–972. [PubMed: 9819064]
10. Kendler KS, Aggen SH, Tambs K, Reichborn-Kjennerud T. Illicit psychoactive substance use, abuse and dependence in a population-based sample of Norwegian twins. *Psychol Med* 2006;36:955–962. [PubMed: 16650346]
11. Sham, P. John Wiley and Sons; London, UK: 1998. *Statistics in Human Genetics* ..
12. Terwilliger, JD.; Ott, J. Johns Hopkins University Press; Baltimore, MD: 1994. *Handbook of Human Linkage Analysis* ..
13. Risch N. Linkage strategies for genetically complex traits. III. The effect of marker polymorphism on analysis of affected relative pairs. *Am J Hum Genet* 1990;46:242–253. [PubMed: 2301394]
14. Risch N. Linkage strategies for genetically complex traits. II. The power of affected relative pairs. *Am J Hum Genet* 1990;46:229–241. [PubMed: 2301393]
15. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. *Am J Hum Genet* 1990;46:222–228. [PubMed: 2301392]
16. Rice JP, Saccone NL, Corbett J. Model-based methods for linkage analysis. *Adv Genet* 2008;60:155–73. 155–173. [PubMed: 18358320]
17. Almasy L, Blangero J. Contemporary model-free methods for linkage analysis. *Adv Genet* 2008;60:175–93. 175–193. [PubMed: 18358321]
18. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995;11. [PubMed: 7647778]
19. Thomson G, Barcellos LF, Valdes AM. Searching for additional disease loci in a genomic region. *Adv Genet* 2008;60:253–92. 253–292. [PubMed: 18358324]

20. Hopfer CJ, Lessem JM, Hartman CA, Stallings MC, Cherny SS, Corley RP, et al. A genome-wide scan for loci influencing adolescent cannabis dependence symptoms: Evidence for linkage on chromosomes 3 and 9. *Drug Alcohol Depend*. 2006
21. Agrawal A, Hinrichs A, Dunn G, Bertelsen S, Dick D, Saccone SF, et al. Linkage scan for quantitative traits identifies new regions of interest for substance dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) Sample. *Drug Alcohol Depend* 2008;93:12–20. [PubMed: 17942244]
22. Agrawal A, Pergadia ML, Saccone SF, Lynskey MT, Wang JC, Martin NG, et al. An Autosomal Linkage Scan for Cannabis Use Disorders in the Nicotine Addiction Genetics Project. *Arch Gen Psychiatry* 2008;65
23. Agrawal A, Morley KI, Hansell NK, Pergadia ML, Montgomery GW, Statham DB, et al. Autosomal linkage analysis of cannabis use behaviors in Australian Adults. In Review. 2007
24. Daly AK, Day CP. Candidate gene case-control association studies: advantages and potential pitfalls. *Br J Clin Pharmacol* 2001;52:489–499. [PubMed: 11736857]
25. Mechoulam R, Hanus L, Martin BR. Search for endogenous ligands of the cannabinoid receptor. *Biochem Pharmacol* 1994;48:1537–1544. [PubMed: 7980618]
26. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258:1946–1949. [PubMed: 1470919]
27. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 1995;215:89–97. [PubMed: 7575630]
28. Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J, et al. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* 2002;301:1020–1024. [PubMed: 12023533]
29. Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE, et al. Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. *J Biol Chem* 2001;276:42639–42644. [PubMed: 11518719]
30. Bisogno T, Melck D, Bobrov MY, Gretskaya NM, Bezuglov VV, De PL, et al. N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. *Biochem J* 2000;(351)(Pt 3):817–24. 817–824. [PubMed: 11042139]
31. Bisogno T, Ligresti A, Di Marzo V. The endocannabinoid signalling system: biochemical aspects. *Pharmacol Biochem Behav* 2005;81:224–238. [PubMed: 15935454]
32. Di Marzo V, De PL, Fezza F, Ligresti A, Bisogno T. Anandamide receptors. *Prostaglandins Leukot Essent Fatty Acids* 2002;66:377–391. [PubMed: 12052051]
33. De PL, Cascio MG, Di Marzo V. The endocannabinoid system: a general view and latest additions. *Br J Pharmacol* 2004;141:765–774. [PubMed: 14744801]
34. Di Marzo V. A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists. *Trends Pharmacol Sci* 2006;27:134–140. [PubMed: 16476494]
35. Di Marzo V, Deutsch DG. Biochemistry of the endogenous ligands of cannabinoid receptors. *Neurobiol Dis* 1998;5:386–404. [PubMed: 9974173]
36. Zhang PW, Ishiguro H, Ohtsuki T, Hess J, Carillo F, Walther D, et al. Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. *Mol Psychiatry* 2004;9:916–931. [PubMed: 15289816]
37. Hopfer CJ, Young SE, Purcell S, Crowley TJ, Stallings MC, Corley RP, et al. Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. *Am J Med Genet B Neuropsychiatr Genet* 2006;141:895–901. [PubMed: 16917946]
38. Agrawal A, Wetherill L, Dick DM, Xuei X, Hinrichs A, Hesselbrock V, et al. Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. *Am J Med Genet B Neuropsychiatr Genet*. 2008
39. Herman AI, Kranzler HR, Cubells JF, Gelernter J, Covault J. Association study of the CNR1 gene exon 3 alternative promoter region polymorphisms and substance dependence. *Am J Med Genet B Neuropsychiatr Genet* 2006;141:499–503. [PubMed: 16741937]

40. Covault J, Gelernter J, Kranzler H. Association study of cannabinoid receptor gene (CNR1) alleles and drug dependence. *Mol Psychiatry* 2001;6:501–502. [PubMed: 11526463]
41. Li T, Liu X, Zhu ZH, Zhao J, Hu X, Ball DM, et al. No association between (AAT)_n repeats in the cannabinoid receptor gene (CNR1) and heroin abuse in a Chinese population. *Mol Psychiatry* 2000;5:128–130. [PubMed: 10822338]
42. Heller D, Schneider U, Seifert J, Cimander KF, Stuhmann M. The cannabinoid receptor gene (CNR1) is not affected in German i.v. drug users. *Addict Biol* 2001;6:183–187. [PubMed: 11341859]
43. Comings DE, Muhleman D, Gade R, Johnson P, Verde R, Saucier G, et al. Cannabinoid receptor gene (CNR1): association with i.v. drug use. *Mol Psychiatry* 1997;2:161–168. [PubMed: 9106242]
44. Ballon N, Leroy S, Roy C, Bourdel MC, Charles-Nicolas A, Krebs MO, et al. (AAT)_n repeat in the cannabinoid receptor gene (CNR1): association with cocaine addiction in an African-Caribbean population. *Pharmacogenomics J* 2006;6:126–130. [PubMed: 16314880]
45. Haughey HM, Marshall E, Schacht JP, Louis A, Hutchison KE. Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. *Addiction* 2008;103:1678–1686. [PubMed: 18705688]
46. Chen X, Williamson VS, An SS, Hettema JM, Aggen SH, Neale MC, et al. Cannabinoid receptor 1 gene association with nicotine dependence. *Arch Gen Psychiatry* 2008;65:816–824. [PubMed: 18606954]
47. Zuo L, Kranzler HR, Luo X, Covault J, Gelernter J. CNR1 Variation Modulates Risk for Drug and Alcohol Dependence. *Biol Psychiatry*. 2007
48. Hutchison KE, Haughey H, Niculescu M, Schacht J, Kaiser A, Stitzel J, et al. The incentive salience of alcohol: translating the effects of genetic variant in CNR1. *Arch Gen Psychiatry* 2008;65:841–850. [PubMed: 18606956]
49. Onaivi ES, Leonard CM, Ishiguro H, Zhang PW, Lin Z, Akinshola BE, et al. Endocannabinoids and cannabinoid receptor genetics. *Prog Neurobiol* 2002;66:307–344. [PubMed: 12015198]
50. Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, et al. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann N Y Acad Sci* 2006;1074:514–536. [PubMed: 17105950]
51. Ishiguro H, Iwasaki S, Teasenfitz L, Higuchi S, Horiuchi Y, Saito T, et al. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. *Pharmacogenomics J* 2007;7:380–385. [PubMed: 17189959]
52. Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, et al. Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS ONE* 2008;3:e1640. [PubMed: 18286196]
53. Onaivi ES, Ishiguro H, Sejal P, Meozzi PA, Myers L, Tagliaferro P, et al. Methods to study the behavioral effects and expression of CB2 cannabinoid receptor and its gene transcripts in the chronic mild stress model of depression. *Methods Mol Med* 2006;123:291–298. [PubMed: 16506415]
54. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 1996;384:83–87. [PubMed: 8900284]
55. Di Marzo V, De PL, Bisogno T, Melck D. Metabolism of anandamide and 2-arachidonoylglycerol: an historical overview and some recent developments. *Lipids* 1999;34(Suppl):S319–25. S319–S325. [PubMed: 10419192]
56. Bisogno T, De PL, Di Marzo V. Fatty acid amide hydrolase, an enzyme with many bioactive substrates. Possible therapeutic implications. *Curr Pharm Des* 2002;8:533–547. [PubMed: 11945157]
57. Ligresti A, Cascio MG, Di Marzo V. Endocannabinoid metabolic pathways and enzymes. *Curr Drug Targets CNS Neurol Disord* 2005;4:615–623. [PubMed: 16375679]
58. Ben-Shabat S, Frider E, Sheskin T, Tamiri T, Rhee MH, Vogel Z, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* 1998;353:23–31. [PubMed: 9721036]
59. Di Marzo V, Bisogno T, Sugiura T, Melck D, De PL. The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. *Biochem J* 1998;331:15–19. [PubMed: 9512456]

60. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A* 2001;98:9371–9376. [PubMed: 11470906]
61. Lichtman AH, Hawkins EG, Griffin G, Cravatt BF. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. *J Pharmacol Exp Ther* 2002;302:73–79. [PubMed: 12065702]
62. Maccarrone M, Bari M, Di RM, Finazzi-Agro A, Rossi A. Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin. *J Biol Chem* 2003;278:32726–32732. [PubMed: 12799380]
63. Waleh NS, Cravatt BF, pte-Deshpande A, Terao A, Kilduff TS. Transcriptional regulation of the mouse fatty acid amide hydrolase gene. *Gene* 2002;291:203–210. [PubMed: 12095693]
64. Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, et al. A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. *Neurosci Lett* 2005;376:182–187. [PubMed: 15721218]
65. Iwasaki S, Ishiguro H, Higuchi S, Onaivi ES, Arinami T. Association study between alcoholism and endocannabinoid metabolic enzyme genes encoding fatty acid amide hydrolase and monoglyceride lipase in a Japanese population. *Psychiatr Genet* 2007;17:215–220. [PubMed: 17621164]
66. Flanagan JM, Gerber AL, Cadet JL, Beutler E, Sipe JC. The fatty acid amide hydrolase 385 A/A (P129T) variant: haplotype analysis of an ancient missense mutation and validation of risk for drug addiction. *Hum Genet* 2006;120:581–588. [PubMed: 16972078]
67. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci U S A* 2002;99:8394–8399. [PubMed: 12060782]
68. Tyndale RF, Payne JI, Gerber AL, Sipe JC. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. *Am J Med Genet B Neuropsychiatr Genet* 2007;144:660–666. [PubMed: 17290447]
69. Dinh TP, Freund TF, Piomelli D. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* 2002;121:149–158. [PubMed: 12505697]
70. Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem* 1997;272:27218–27223. [PubMed: 9341166]
71. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* 2002;99:10819–10824. [PubMed: 12136125]
72. Szallasi A, Blumberg PM. Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol Rev* 1999;51:159–212. [PubMed: 10353985]
73. van der SM, Di Marzo V. Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. *Eur J Biochem* 2004;271:1827–1834. [PubMed: 15128293]
74. Di Marzo V, Blumberg PM, Szallasi A. Endovanilloid signaling in pain. *Curr Opin Neurobiol* 2002;12:372–379. [PubMed: 12139983]
75. Di Marzo V, Bisogno T, De PL. Anandamide: some like it hot. *Trends Pharmacol Sci* 2001;22:346–349. [PubMed: 11431028]
76. Starowicz K, Nigam S, Di Marzo V. Biochemistry and pharmacology of endovanilloids. *Pharmacol Ther* 2007;114:13–33. [PubMed: 17349697]
77. Smart D, Jerman JC. Anandamide: an endogenous activator of the vanilloid receptor. *Trends Pharmacol Sci* 2000;21:134. [PubMed: 10740287]
78. Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–1101. [PubMed: 17876302]
79. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem Biophys Res Commun* 2007;362:928–934. [PubMed: 17765871]
80. Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HH, et al. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is

- extensively expressed in human brain. *Brain Res Mol Brain Res* 1999;64:193–198. [PubMed: 9931487]
81. Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci U S A* 2008;105:2699–2704. [PubMed: 18263732]
 82. Petitet F, Donlan M, Michel A. GPR55 as a new cannabinoid receptor: still a long way to prove it. *Chem Biol Drug Des* 2006;67:252–253. [PubMed: 16611220]
 83. Hiley CR, Kaup SS. GPR55 and the vascular receptors for cannabinoids. *Br J Pharmacol* 2007;152:559–561. [PubMed: 17704825]
 84. Pertwee RG. GPR55: a new member of the cannabinoid receptor clan? *Br J Pharmacol* 2007;152:984–986. [PubMed: 17876300]
 85. McLean PJ, Farb DH, Russek SJ. Mapping of the alpha 4 subunit gene (GABRA4) to human chromosome 4 defines an alpha 2-alpha 4-beta 1-gamma 1 gene cluster: further evidence that modern GABAA receptor gene clusters are derived from an ancestral cluster. *Genomics* 1995;26:580–586. [PubMed: 7607683]
 86. Bailey ME, Matthews DA, Riley BP, Albrecht BE, Kostrzewa M, Hicks AA, et al. Genomic mapping and evolution of human GABA(A) receptor subunit gene clusters. *Mamm Genome* 1999;10:839–843. [PubMed: 10430673]
 87. Russek SJ. Evolution of GABA(A) receptor diversity in the human genome. *Gene* 1999;227:213–222. [PubMed: 10023064]
 88. Paterson NE, Froestl W, Markou A. The GABAB receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat. *Psychopharmacology (Berl)* 2004;172:179–186. [PubMed: 14610636]
 89. Haney M, Hart CL, Foltin RW. Effects of baclofen on cocaine self-administration: opioid- and nonopioid-dependent volunteers. *Neuropsychopharmacology* 2006;31:1814–1821. [PubMed: 16407903]
 90. Roberts DC. Preclinical evidence for GABAB agonists as a pharmacotherapy for cocaine addiction. *Physiol Behav* 2005;86:18–20. [PubMed: 16061264]
 91. Slattery DA, Markou A, Froestl W, Cryan JF. The GABAB receptor-positive modulator GS39783 and the GABAB receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* 2005;30:2065–2072. [PubMed: 15841108]
 92. Agrawal A, Edenberg HJ, Foroud T, Bierut LJ, Dunne G, Hinrichs AL, et al. Association of GABRA2 with Drug Dependence in the Collaborative Study of the Genetics of Alcoholism Sample. *Behav Genet* 2006;36:640–650. [PubMed: 16622805]
 93. Lind P, MacGregor S, Agrawal A, Heath AC, Martin NG, Whitfield JB. The Role of GABRA2 in Alcohol Dependence, Smoking and Illicit Drug Use in an Australian Population Sample. *Alcohol Clin Exp Res*. 2008in press
 94. Corley RP, Zeiger JS, Crowley T, Ehringer MA, Hewitt JK, Hopfer CJ, et al. Association of candidate genes with antisocial drug dependence in adolescents. *Drug Alcohol Depend* 2008;96:90–98. [PubMed: 18384978]
 95. van der SM, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 2003;480:133–150. [PubMed: 14623357]
 96. French ED. delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci Lett* 1997;226:159–162. [PubMed: 9175591]
 97. French ED, Dillon K, Wu X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport* 1997;8:649–652. [PubMed: 9106740]
 98. Gardner EL, Vorel SR. Cannabinoid transmission and reward-related events. *Neurobiol Dis* 1998;5:502–533. [PubMed: 9974181]
 99. Maldonado R, Rodriguez de FF. Cannabinoid addiction: behavioral models and neural correlates. *J Neurosci* 2002;22:3326–3331. [PubMed: 11978807]
 100. Tanda G, Loddo P, Di CG. Dependence of mesolimbic dopamine transmission on delta9-tetrahydrocannabinol. *Eur J Pharmacol* 1999;376:23–26. [PubMed: 10440085]

101. Noble EP, Zhang X, Ritchie TL, Sparkes RS. Haplotypes at the DRD2 locus and severe alcoholism. *Am J Med Genet* 2000;96:622–631. [PubMed: 11054769]
102. Berman SM, Ozkaragoz T, Noble EP, Antolin T, Sheen C, Siddarth P, et al. Differential associations of sex and D2 dopamine receptor (DRD2) genotype with negative affect and other substance abuse risk markers in children of alcoholics. *Alcohol* 2003;30:201–210. [PubMed: 13679114]
103. Hall W, Degenhardt L, Teesson M. Cannabis use and psychotic disorders: an update. *Drug Alcohol Rev* 2004;23:433–443. [PubMed: 15763748]
104. Degenhardt L, Hall W. Is cannabis use a contributory cause of psychosis? *Can J Psychiatry* 2006;51:556–565. [PubMed: 17007222]
105. Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. *Pharmacol Biochem Behav* 1997;56:803–807. [PubMed: 9130308]
106. Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrani B, Tast D, et al. The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA* 1991;266:1793–1800. [PubMed: 1832466]
107. Di FM, Morrison PD, Butt A, Murray RM. Cannabis use and psychiatric and cognitive disorders: the chicken or the egg? *Curr Opin Psychiatry* 2007;20:228–234. [PubMed: 17415074]
108. Cohen M, Solowij N, Carr V. Cannabis, cannabinoids and schizophrenia: integration of the evidence. *Aust N Z J Psychiatry* 2008;42:357–368. [PubMed: 18473254]
109. Semple DM, McIntosh AM, Lawrie SM. Cannabis as a risk factor for psychosis: systematic review. *J Psychopharmacol* 2005;19:187–194. [PubMed: 15871146]
110. Muller-Vahl KR, Emrich HM. Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev Neurother* 2008;8:1037–1048. [PubMed: 18590475]
111. Ben AM, Potvin S. Cannabis and psychosis: what is the link? *J Psychoactive Drugs* 2007;39:131–142. [PubMed: 17703707]
112. Linszen D, van AT. Cannabis and psychosis: an update on course and biological plausible mechanisms. *Curr Opin Psychiatry* 2007;20:116–120. [PubMed: 17278907]
113. Conner BT, Noble EP, Berman SM, Ozkaragoz T, Ritchie T, Antolin T, et al. DRD2 genotypes and substance use in adolescent children of alcoholics. *Drug Alcohol Depend* 2005;79:379–387. [PubMed: 16102380]
114. Sakai JT, Hopfer CJ, Hartman C, Haberstick BC, Smolen A, Corley RP, et al. Test of association between TaqIA A1 allele and alcohol use disorder phenotypes in a sample of adolescent patients with serious substance and behavioral problems. *Drug Alcohol Depend* 2007;88:130–137. [PubMed: 17069991]
115. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 2004;23:540–545. [PubMed: 15146457]
116. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry* 2007;164:1149–1159. [PubMed: 17671276]
117. Robledo P, Berrendero F, Ozaita A, Maldonado R. Advances in the field of cannabinoid–opioid cross-talk. *Addict Biol* 2008;13:213–224. [PubMed: 18482431]
118. Lopez-Moreno JA, Gonzalez-Cuevas G, Moreno G, Navarro M. The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict Biol* 2008;13:160–187. [PubMed: 18422831]
119. Rodriguez JJ, Mackie K, Pickel VM. Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. *J Neurosci* 2001;21:823–833. [PubMed: 11157068]
120. Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, Conrath M. CB1-cannabinoid and mu-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *Neuroreport* 2001;12:3689–3692. [PubMed: 11726775]
121. Lichtman AH, Sheikh SM, Loh HH, Martin BR. Opioid and cannabinoid modulation of precipitated withdrawal in delta(9)-tetrahydrocannabinol and morphine-dependent mice. *J Pharmacol Exp Ther* 2001;298:1007–1014. [PubMed: 11504797]

122. Cichewicz DL. Synergistic interactions between cannabinoid and opioid analgesics. *Life Sci* 2004;74:1317–1324. [PubMed: 14706563]
123. Castane A, Robledo P, Matifas A, Kieffer BL, Maldonado R. Cannabinoid withdrawal syndrome is reduced in double mu and delta opioid receptor knockout mice. *Eur J Neurosci* 2003;17:155–159. [PubMed: 12534979]
124. Caille S, Parsons LH. Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. *Neuropsychopharmacology* 2006;31:804–813. [PubMed: 16123766]
125. Paldyova E, Bereczki E, Santha M, Wenger T, Borsodi A, Benyhe S. Naladin ether, a putative endocannabinoid, inhibits mu-opioid receptor activation via CB2 cannabinoid receptors. *Neurochem Int* 2008;52:321–328. [PubMed: 17698254]
126. Paldyova E, Bereczki E, Santha M, Wenger T, Borsodi A, Benyhe S. Altered gene expression and functional activity of opioid receptors in the cerebellum of CB1 cannabinoid receptor knockout mice after acute treatments with cannabinoids. *Acta Biol Hung* 2007;58(Suppl):113–29. 113–129. [PubMed: 18297798]
127. Kathmann M, Flau K, Redmer A, Trankle C, Schlicker E. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* 2006;372:354–361. [PubMed: 16489449]
128. Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, Maldonado R. Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. *J Neurosci* 2002;22:1146–1154. [PubMed: 11826143]
129. Ray LA, Hutchison KE. A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res* 2004;28:1789–1795. [PubMed: 15608594]
130. Kranzler HR, Gelernter J, O'Malley S, Hernandez-Avila CA, Kaufman D. Association of alcohol or other drug dependence with alleles of the mu opioid receptor gene (OPRM1). *Alcohol Clin Exp Res* 1998;22:1359–1362. [PubMed: 9756053]
131. Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, et al. Association between two {micro}-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet* 2006;15:807–819. [PubMed: 16476706]
132. Nishizawa D, Han W, Hasegawa J, Ishida T, Numata Y, Sato T, et al. Association of micro-opioid receptor gene polymorphism A118G with alcohol dependence in a Japanese population. *Neuropsychobiology* 2006;53:137–141. [PubMed: 16679777]
133. Arias A, Feinn R, Kranzler HR. Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. *Drug Alcohol Depend* 2006;83:262–268. [PubMed: 16387451]
134. Luo X, Zuo L, Kranzler H, Zhang H, Wang S, Gelernter J. Multiple OPR genes influence personality traits in substance dependent and healthy subjects in two American populations. *Am J Med Genet B Neuropsychiatr Genet*. 2008
135. Luo X, Kranzler HR, Zhao H, Gelernter J. Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. *Am J Med Genet B Neuropsychiatr Genet* 2003;120B:97–108. [PubMed: 12815747]
136. Gelernter J, Kranzler H, Cubells J. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* 1999;4:476–483. [PubMed: 10523821]
137. Stinson FS, Grant BF, Dawson DA, Ruan WJ, Huang B, Saha T. Comorbidity between DSM-IV alcohol and specific drug use disorders in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Drug Alcohol Depend* 2005;80:105–116. [PubMed: 16157233]
138. Risch NJ. Searching for genetic determinants in the new millennium. *Nature* 2000;405:847–856. [PubMed: 10866211]
139. Moffitt TE, Caspi A, Rutter M. Strategy for Investigating Interactions Between Measured Genes and Measured Environments. *Arch Gen Psychiatry* 2005;62:473–481. [PubMed: 15867100]
140. Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, et al. Moderation of the Effect of Adolescent-Onset Cannabis Use on Adult Psychosis by a Functional Polymorphism in the

- Catechol-O-Methyltransferase Gene: Longitudinal Evidence of a Gene X Environment Interaction. *Biol Psychiatry* 2005;57:1117–1127. [PubMed: 15866551]
141. Zammit S, Spurlock G, Williams H, Norton N, Williams N, O'Donovan MC, et al. Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *Br J Psychiatry* 2007;191:402–407. [PubMed: 17978319]
 142. Budney AJ. Are specific dependence criteria necessary for different substances: how can research on cannabis inform this issue? *Addiction* 2006;101(Suppl 1):125–33. 125–133. [PubMed: 16930169]
 143. Lynskey MT, Agrawal A. Psychometric properties of DSM assessments of illicit drug abuse and dependence: Results from the National Epidemiological Survey on Alcohol and Related Conditions. *Psychological Medicine*. 2007In Press
 144. Teesson M, Lynskey M, Manor B, Baillie A. The structure of cannabis dependence in the community. *Drug Alcohol Depend* 2002;68:255–262. [PubMed: 12393220]
 145. Langenbucher J, Labouvie EW, Martin C, Sanjuan PM, Bavly L, Kirisci L, et al. An Application of Item Response Theory Analysis to Alcohol, Cannabis and Cocaine Criteria in DSM-IV. *J Abnorm Psychol* 2004;113:72–80. [PubMed: 14992659]
 146. Gillespie NA, Neale MC, Prescott CA, Aggen SH, Kendler KS. Factor and Item-Response Analysis of DSM-IV Criteria for Abuse and Dependence on Cannabis, Cocaine, Hallucinogens, Sedatives, Stimulants and Opiates. *Addiction* 2007;102:920–930. [PubMed: 17523987]
 147. Karkowski LM, Prescott CA, Kendler KS. Multivariate assessment of factors influencing illicit substance use in twins from female-female pairs. *Am J Med Genet* 2000;96:665–670. [PubMed: 11054775]
 148. Agrawal A, Neale M, Jacobson K, Prescott CA, Kendler KS. Illicit drug use and abuse/dependence: modeling of two-stage variables using the CCC approach. *Addict Behav* 2005;30:1043–1048. [PubMed: 15893102]
 149. Lynskey MT, Grant JD, Nelson EC, Bucholz KK, Madden PA, Statham DJ, et al. Duration of cannabis use--a novel phenotype? *Addict Behav* 2006;31:984–994. [PubMed: 16713122]
 150. Fowler T, Lifford K, Shelton K, Rice F, Thapar A, Neale MC, et al. Exploring the relationship between genetic and environmental influences on initiation and progression of substance use. *Addiction* 2007;102:413–422. [PubMed: 17298649]
 151. Agrawal A, Lynskey M. The Genetic Epidemiology of Cannabis Use, Abuse and Dependence: A Review. *Addiction* 2006;101:801–812. [PubMed: 16696624]
 152. McRae AL, Budney AJ, Brady KT. Treatment of marijuana dependence: a review of the literature. *J Subst Abuse Treat* 2003;24:369–376. [PubMed: 12867212]
 153. Nordstrom BR, Levin FR. Treatment of cannabis use disorders: a review of the literature. *Am J Addict* 2007;16:331–342. [PubMed: 17882603]
 154. Copeland J, Maxwell JC. Cannabis treatment outcomes among legally coerced and non-coerced adults. *BMC Public Health* 2007;7:111. [PubMed: 17567917]
 155. Parolaro D, Vigano D, Rubino T. Endocannabinoids and drug dependence. *Curr Drug Targets CNS Neurol Disord* 2005;4:643–655. [PubMed: 16375682]
 156. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006;58:389–462. [PubMed: 16968947]
 157. Schmidt LG, Samochowiec J, Finckh U, Fiszer-Piosik E, Horodnicki J, Wendel B, et al. Association of a CB1 cannabinoid receptor gene (CNR1) polymorphism with severe alcohol dependence. *Drug Alcohol Depend* 2002;65:221–224. [PubMed: 11841893]

Table 1

Linkage studies of cannabis use disorders

Author (Date)	Phenotype	Sample	Highest LOD (chromosome, cM, LOD)	Closest marker(s)	Proposed gene(s)
Hopfer [20]	DSM-IV cannabis dependence symptom count	Adolescents from treatment centers and general population	3, 137 cM, 2.61 9, 156 cM, 2.57	D3S1267 D9S1826	Chr 3: <i>MGL</i>
Agrawal [21]	DSM-IV cannabis dependence	U.S. adults from dense multiplex alcoholic families	14, 95 cM, 1.90	rs759364-rs872945	Chr 14: <i>GPR68</i> , <i>CKB</i> , <i>SERPINA1</i> , <i>SERPINA2</i>
Agrawal [22]	(i)DSM-IV cannabis dependence (ii)Cannabis abuse/dependence factor score	Australian adults from families ascertained for heavy cigarette smoking	1, 102 cM, 3.36 4, 38 cM, 2.22	D1S2841 D4S419	Chr 1: <i>ELTD1</i> Chr 4: <i>GABRA2</i>
Agrawal [23]	Lifetime frequency of cannabis use	Australian adults from families from multiple sources	18, 96.8 cM, 2.10	GATA129F05_M	Chr 18: Family of <i>SERPINB</i> genes, <i>DOK6</i>

Table 2

Human association studies of the Cannabinoid receptor 1 (CNR1) gene

Authors (Date)	Phenotype	Significant Polymorphism	Summary
*Comings [43]	Alcohol, cocaine, cannabis, amphetamine, intravenous drug abuse	AAT repeat	5+ repeats associated with drug dependence
Li [41]	Heroin abuse	AAT repeat	No association in this Chinese population
Heller [42]	Intravenous drug addiction	AAT repeat	No association
		1359G/A	
Covault [40]	Drug dependence	AAT repeat	No association
Schmidt [157]	Severe alcohol dependence	1359G/A	A/A genotype associated with a history of alcohol delirium
Zhang [36]	Polysubstance abuse	rs2023239	TAG haplotype strongly associated with polysubstance abuse; TAG is associated with reduced mRNA expression in cerebral cortex and midbrain
		rs806379	
		rs1535255	
		-3180T indel	
Ballon [44]	Cocaine dependence with and without schizophrenia	AAT repeat	AAT (12) repeat was higher in cocaine dependent cases, particularly those with schizophrenia, compared to controls in this African-Caribbean sample
*Herman [39]	Cannabis, cocaine, opioid, alcohol and polysubstance dependence	rs1535255	Modest association with alcohol dependence (no comorbid drug dependence)
*Hopfer [37]	Cannabis dependence symptoms	rs806380	Rs806380 & haplotypes of rs6454674-rs806380-rs806377-rs1049353 (GGCC, TACC, GACC) associated with cannabis problems
Zuo [47]	Alcohol dependence, drug dependence, comorbid	rs6454674	Interaction between rs6454674 (G/G or G/T) and rs806368 (T/T) influence alcohol and drug dependence
		rs806368	
Chen [46]	Smoking initiation, Nicotine	rs2023239	Single SNP and haplotype effects on nicotine dependence in women
	Dependence	rs12720071	
		rs806368	
Hutchison [48]	CB1 receptor density in postmortem brain, Imaging outcomes; Rewarding effects of alcohol	rs2023239	Individuals with the CT genotype report greater CB1 binding, greater activation of mesocorticolimbic activation and greater reward and positive affect after drinking
*Haughey [45]	Marijuana Dependency Checklist	rs2023239	Individuals with CT genotype report higher dependency scores, smoke greater quantities and more often and also report heavier alcohol use.
	Marijuana Withdrawal Checklist		
	Profile of Mood States (POMS)		CT Individuals also indicate greater craving at abstinence, post-abstinence and post cue exposure.
	Craving and Mood Questionnaire		
	Alcohol consumption		
*Agrawal [38]	Cannabis dependence	rs806380,	Associated with DSM-III-R cannabis dependence in alcoholic families
		rs806368,	
		rs806379	

* Studied cannabis dependence specifically.

Table 3

Human association studies of the C385A (P129T, rs324420) promoter missense mutation in FAAH and substance-related phenotypes

Authors (Date)	Phenotype	Summary
Sipe [67]	Problem drug or alcohol use Street drug use Street drug + problem drug/alcohol use	A/A genotype associated with problem alcohol, drug and street drug use with odds-ratios ranging from 2.15 – 4.54
Morita [64]	Methamphetamine dependence Multi-substance abuse	No association in this Japanese population
Flanagan [66]	Drug addiction (alcohol or illicit)	A/A genotype associated with drug addiction in Caucasians (combined with Sipe et al gave odds-ratio of 3.2), in African Americans; underpowered in Asians;
*Tyndale [68]	In all and those who tried cannabis: Cannabis dependence Regular alcohol, hallucinogen, nicotine, opiate, sedative, stimulant use	A/A genotype is associated with a 0.25 decrease in odds of cannabis dependence in those who try cannabis; A/A genotype associated with increased odds of regular sedative use
Iwasaki [65]	DSM-III-R alcohol dependence	No association in this Japanese population
*Haughey [45]	Marijuana Dependency Checklist Marijuana Withdrawal Checklist Profile of Mood States (POMS) Craving and Mood Questionnaire Alcohol consumption	No association in 18–25 year old marijuana smokers for dependency, withdrawal, alcohol. C/C group have increased craving post-abstinence.

* Studied cannabis dependence specifically.