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CANNABIS (F FILBEY, SECTION EDITOR)

Genetic and Environmental Factors Associated with Cannabis Involvement

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Abstract Approximately 50–70 % of the variation in cannabis use and use disorders can be attributed to heritable factors. For cannabis use, the remaining variance can be parsed in to familial and person-specific environmental factors while for use disorders, only the latter contribute. While numerous candidate gene studies have identified the role of common variation influencing liability to cannabis involvement, replication has been elusive. To date, no genome-wide association study has been sufficiently powered to identify significant loci. Despite this, studies adopting polygenic techniques and integrating genetic variation with neural phenotypes and measures of environmental risk, such as childhood adversity, are providing promising new leads. It is likely that the small effect sizes associated with variants related to cannabis involvement will only be robustly identified in substantially larger samples. Results of such large-scale efforts will provide valuable single variant targets for translational research in neurogenetic, pharmacogenetic, and non-human animal models as well as polygenic risk indices that can be used to explore a host of other genetic hypotheses related to cannabis use and misuse.

Keywords Cannabis · Endocannabinoid · Gene · Adversity · Neurogenetics · Imaging genetics

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Introduction

According to the Monitoring the Future survey, 44 % of US 12th graders report a lifetime history of cannabis use [1•]. A recent iteration of the National Epidemiological Survey of Alcohol and Related Conditions indicates that the past year incidence of cannabis use has more than doubled in the last decade [2•]. This survey also found that nearly 36 % of cannabis users subsequently developed cannabis use disorder (CUD), a serious psychiatric diagnosis associated with emergency department visits particularly with comorbid mental health problems [3]. As a climate of progressive efforts towards legalization sweep across the USA, research is turning to aggressively accelerating the pace of the identification of risk and protective influences on cannabis involvement. Here, we review classical observations regarding the role of genetic and environmental influences on cannabis use and CUD and discuss recent studies that have advanced our understanding of the genetic underpinnings of cannabis involvement

Evidence for Genetic and Environmental Influences

Twin studies indicate that 50–70 % of individual differences in cannabis use and misuse are attributable to the additive effects of genetic variation [4•]. Environmental factors, broadly categorized as person-specific and those shared by members of twin pairs, account for the remainder of the variance.

Shared environmental factors (≈20 %), which are an alternate source of familial resemblance and can range in context from in utero exposures to parenting practices and sharing the same classroom, are more likely to impact onset of and experimentation with cannabis. For instance, Kendler and colleagues [5•] found that familial resemblance for cannabis consumption can be almost entirely attributed to these shared environmental sources during adolescence and early adulthood, with genes becoming increasingly relevant during the late 20s. This developmental attenuation of shared environmental factors from adolescence to adulthood has also been observed for self-reported availability of cannabis [5•]. Additionally, while most aspects of early cannabis involvement are heritable in nature, a recent study reported that the speed of transition from first use to trying cannabis a second time, a potential indicator of addiction vulnerability, was largely influenced by shared (25 %) and person-specific (75 %) environmental factors [6]. For cannabis use disorder (CUD), however, genetic and person-specific environmental factors appear to be important with smaller effects of shared environment [4•].

Cannabis involvement is a multi-stage process. For genetic studies, this creates an interesting problem. When examining CUD, or other later stages of cannabis involvement, those who did not experiment with cannabis are often treated in the same manner as those who do not meet criteria for CUD (e.g., never user = 0; use without problems = 0; problems but no diagnosis = 0; diagnosis = 1). However, when using this approach, the resulting estimate of heritability confounds estimates for CUD with estimates for early stages (i.e., use). Twin methodology can disentangle the sources of variance in CUD from those on earlier stages by utilizing models that account for this contingent, multi-stage nature of addiction and by treating the liability to addiction in a lifetime never user as unknown (i.e., structurally missing data point). Using such approaches, several studies report a generous overlap in genetic influences on cannabis use and CUD. Most recently, Gillespie and colleagues [7•] reported that 67 % of the genetic variance in CUD is attributable to genetic factors that also influence cannabis use. Further, these investigators found that an overwhelming majority of the familial influences on cannabis use were related to those factors that shape availability to cannabis, even before use was initiated.

Given the notable familial co-aggregation of cannabis involvement with other forms of substance use and misuse [8, 9], twin studies have investigated the extent to which genetic influences on cannabis involvement index a general versus specific liability. Numerous studies point to the strong commonality across genetic factors influencing multiple psychoactive substances with limited evidence for drug-specific genetic influences on cannabis involvement (e.g., [10–12]). Two recent studies have further elucidated the nature of this overlap. First, Vrieze and colleagues [13] found that as individuals enter adulthood, not only does the correlated nature of their substance involvement, including cannabis dependence symptoms, decline but this decline is primarily due to an attenuation of genetic influences on this general liability to all forms of substance involvement. Thus, in older individuals, specific genetic (and environmental) factors govern variation in cannabis involvement. Second, this general liability to substance involvement also encompasses vulnerability to other disinhibited behaviors, such as conduct disorder. Our recent research [14] documents the extent to which conduct disorder explains the genetic comorbidity across alcohol, nicotine, and cannabis use disorders. Two results were noteworthy—first, consistent with the findings of Vrieze and colleagues, substance-specific genetic influences were responsible for 40–73 % of the variance in each substance dependence phenotype in this adult sample. Second, all the genetic overlap across the three substances was attributable to genes that were also shared with liability to conduct disorder, suggesting that the bulk of the well-documented genetic overlap across substances is due to co-occurring problem behaviors.

Identification of Genetic Influences

The substantial heritability of cannabis involvement phenotypes has spurred efforts to identify the molecular genetic differences conferring risk. The vast majority of this research has been conducted within a candidate gene framework and has focused on: (1) the endocannabinoid system, where cannabis achieves its effects and (2) polymorphisms associated with other substance use disorders given strong evidence for a heritable generalized liability to substance involvement. More recently, a handful of genome-wide association studies (GWAS) have examined whether commonly occurring genetic variation across the genome is associated with various measures of cannabis involvement.

Endocannabinoid Candidate Gene Studies The psychoactive component of cannabis, tetrahydrocannabinol (THC), was described in 1964 but it was not until the 1980s and 1990s that its binding sites were identified and characterized [15–17]. These discoveries and the ongoing characterization of the endocannabinoid system has provided the blueprint for modern investigation of the mechanisms through which cannabis influences the body as well as the diverse roles of the endocannabinoid system in a host of behavioral and neural phenotypes such as anxiety, obesity, inflammation, stress recovery, and brain maturation [18]. Further, this research ushered in multiple candidate gene studies investigating whether genetic variation within the endocannabinoid system confers susceptibility to cannabis initiation, use, and CUD.

Candidate gene association studies of cannabis involvement have primarily focused on variation within the cannabinoid type 1 (CB1) receptor gene (*CNR1*) in light of evidence

suggesting that the psychoactive effects of cannabis are primarily achieved through THC-CB1 binding [19–21] and linkage analysis associating the genomic region in which *CNR1* resides to cannabis use disorder [22]. However, despite these promising leads, research on *CNR1* polymorphisms has largely produced inconsistent associations with cannabis involvement phenotypes (Table 1). For instance, nominally significant associations have been reported for the *CNR1* single nucleotide polymorphism (SNP) rs806380 (such that the A allele has been associated with elevated cannabis dependence [23, 30]); however, these are contrasted with null association reports [27]. Indeed, a meta-analysis of three *CNR1* polymorphisms in existing data concluded that only the AAT repeat polymorphisms in *CNR1* was putatively associated with general illicit drug dependence [34]. Whether these equivocal results are the result of methodological differences (e.g., the control population being cannabis exposed, e.g., [27] or a mix of individuals who never initiated cannabis use and those who have but have not developed cannabis dependence symptoms, e.g., [23]), small studies providing imprecise estimates for small effects, or are merely false positives remains an open question.

A somewhat overlapping line of research has probed whether genetic variation within fatty acid amide hydrolase (*FAAH*), and in particular, the common functional SNP, rs324420, is associated with marijuana-related phenotypes (Table 1). FAAH is a membrane enzyme that degrades endocannabinoids (particularly anandamide). The A allele at rs324420 is associated with reduced FAAH expression and activity presumably resulting in heightened functional anandamide (AEA) and AEA-CB1 binding [35, 36]. While the candidate gene literature on *FAAH* and marijuana-related phenotypes is much smaller than that on *CNR1*, it has, arguably, produced more consistent results. Indeed, evidence suggests that the C allele at rs324420 may confer vulnerability to cannabis dependence [33], potentially through several mechanisms such as increased withdrawal symptoms, greater reward-related brain activation to marijuana cues [37•], and increased positive responsiveness (i.e., increased happiness and reduced heart rate) following acute marijuana use [28, 32]. However, it must be cautioned that there are a limited number of genetic studies of *FAAH* and cannabis involvement and those that are available have been primarily conducted within small samples (e.g., [38]).

Other Variants Associated with Cannabis Involvement Numerous variants in other genes, particularly in dopamine and opioid pathways, have been implicated in the etiology of cannabis involvement; however, results have been fairly inconsistent [39, 40]. The principal concern with the selection of these variants is the weak a priori support for their role in the etiology of cannabis involvement and the relatively modest sample sizes in which they are being tested, the combination of which results in an inflated false discovery rate [41]. The most encouraging findings have resulted from analyses of genetic variants associated with tobacco smoking for which genome-wide significant loci exist. In particular, two recent studies have found that SNPs associated with tobacco smoking also play a role in cannabis involvement. Both studies utilized results from one of the largest genome-wide association studies of smoking initiation and cigarettes per day (Tobacco and Genetics Consortium (TAG) [42]; $n = 143,023$). In the first study, investigators created polygenic scores, representing the additive and cumulative effects of multiple loci associated with tobacco smoking, based on the results from TAG to predict cannabis use in an independent cohort of Dutch adolescents and adults [43]. Polygenic risk scores (PRS) for cigarettes smoked per day significantly predicted cannabis initiation in the independent sample suggesting the role of common genetic underpinnings to both these substance use behaviors, as has been suggested by twin studies [12]. Using a different dataset, another study found that rs6265 in the brain-derived neurotrophic factor gene (*BDNF*) previously associated with smoking initiation was also related to cannabis use while a variant (rs1451240) related to cigarette smoking at genome-wide significant levels in another large genome-wide meta-analysis [44] was associated with liability to cannabis dependence [45]. However, these studies were unable to pinpoint the extent to which these associations of tobacco-related SNPs with cannabis involvement were specific to cannabis or related to its severe comorbidity with tobacco smoking.

Genome-Wide Association Studies Four published GWAS of cannabis involvement have failed to identify any single variant reaching genome-wide significance for DSM-IV cannabis dependence [46], DSM-5 cannabis use disorder [47] use initiation [48•, 49], and age of onset for cannabis use [49]. Notably, while these studies have failed to identify single variants, they have still yielded important clues for the genetic basis of cannabis involvement. For instance, using gene-based association testing, Agrawal and colleagues [47] linked variation across *C17orf58*, *BPTF*, and *PPM1D* to cannabis use disorder at a gene-wide association level of significance. Moreover, these studies estimate that, in aggregate, common genetic variation may account for 6–25 % of the variance in cannabis use-related phenotypes [47, 48•, 49, 50], suggesting that larger samples may eventually identify genome significant loci and that polygenic approaches may provide predictive utility. Indeed, recent evidence suggests that increased polygenic risk for schizophrenia is associated with cannabis use initiation and the quantity of use [51•].

Probing Neural Mechanisms Neuroscience may be used alongside traditional psychiatric molecular genetic association research to uncover neural mechanisms through which genetic variation and the environment promote individual differences

Table 1 Summary of endocannabinoid and cannabis involvement genetic research

Study reference	Sample	Polymorphisms	Phenotypes	Result summary
Agrawal et al., 2009 [23]	1923 individuals from 219 families with alcohol-dependent probands	*CNR1*: rs806368, rs12720071, rs4707436, rs1049353, rs2023239, rs1535255, rs806379, rs806380, rs754387	Cannabis dependence	rs806380: A allele more common among individuals with cannabis dependence relative to the G allele. rs806368: T allele more common among individuals with cannabis dependence relative to the C allele. No significant effects of any other SNP (trend level of association for rs806379).
Bidwell et al., 2013 [24]	151 regular marijuana users	*CNR1*: *rs2023239*, *rs1049353*, *rs806368* *FAAH*: *rs4141964*, *rs324420*, *rs11576941*, *rs6703669*, *rs6429600*	Trait and behavioral impulsivity, marijuana-related problems	*CNR1*: *No main effects for any genotype*. rs2023239 × trait impulsivity interaction: TT genotype and higher impulsivity associated with elevated marijuana use problems; qualified by interaction with haplotype. No additional interaction effects. *FAAH*: No individual SNP associations. rs4141964, rs324420, and rs1 1576941 haplotype TAG was associated with elevated marijuana use problems.
Carey et al., 2015 [25•]	Discovery sample: 1558 marijuana exposed individuals Replication sample: 859 Neurogenetic extension sample: 312	Tagging SNPs across *CNR1*, *FAAH*, *MGLL*, *DAGLA*, *DAGLB*, *NAPEPLD*	Cannabis dependence symptoms, basolateral amygdala habituation, childhood sexual abuse, childhood abuse, childhood adversity	*MGLL*: Identification and replication of an interaction between *MGLL* rs604300 and childhood adversity. The minor A allele at rs604300 conferred protection against early life stress-related elevations in cannabis dependence symptoms in two samples. Further the A allele was associated with increased amygdala habituation among those exposed to early life stress. No other associations remained significant after accounting for multiple testing.
Comings et al., 1997 [26]	92 patients being treated for addiction; 114 controls	*CNR1* AAT repeat	Cannabis dependence	AAT ≥5 repeats associated with greater cannabis dependence (not significant when correcting across substances tested for dependence).
Hartman et al., 2009 [27]	224 individuals endorsing at least one cannabis dependence symptoms; 108 cannabis-exposed controls. Additional family study of 219	*CNR1*: rs6454674, rs806380, rs806379, rs1535255, rs2023239, rs806377, rs1049353	Presence of at least one cannabis dependence symptom	rs1049353: C allele associated with the presence of at least one cannabis dependence symptom. But not significant when data are meta-analyzed with an additional sample (Hopfer et al., 2006). Trend level replication in family study. No significant effects of any other SNP observed.

Table 1 (continued)

Study reference	Sample	Polymorphisms	Phenotypes	Result summary
Haughey et al., 2008 [28]	105 student daily users	*CNR1* rs2023239 *FAAH* rs324420	Self-reported withdrawal and craving pre and post 5 days of abstinence	*CNR1* rs2023239: C/T genotype associated with elevated craving as well as abstinence-related withdrawal and depression relative to T/T genotype. *FAAH* rs324420: C homozygotes experienced higher withdrawal and craving symptoms. A allele associated with trend level increases in negative affect following abstinence and cue exposure.
Herman et al., 2006 [29]	895 individuals with drug dependence; 472 controls	*CNR1*: rs6928499, rs806379, rs1535255, rs2023239	Cannabis dependence	No associations with cannabis dependence.
Hopfer et al., 2006 [30]	327 individuals who used marijuana and developed at least one dependence symptom; 214 controls who tried marijuana but did not develop any dependence symptoms	*CNR1*: rs2273512, rs6454674, rs806380, rs806377, rs104935	Exposure to cannabis and developing at least one cannabis symptom	rs806380: A allele associated with the development of at least one dependence symptom among individuals exposed to cannabis. However, when meta-analyzed with a replication sample, this result was no longer significant as reported in Hartman et al., 2009. rs6454674, rs806380, rs806377, rs104935 haplotypes: GGCC associated with reduced likelihood of developing at least one cannabis dependence symptom. TACC and GACC were associated with increased risk of cannabis dependence symptoms.
Krebs et al., 2014 [31]	3807 students	*CNR1*: rs806379, rs1535255, rs2023239, rs1049353, rs12720071, AAT repeat	Psychotomimetic effects when first using cannabis	rs202329, rs1535255, rs806379 haplotype: AAA haplotype associated with reduced psychotomimetic effects upon first using cannabis.
Schacht et al., 2009 [32]	40 abstaining (24 h) daily marijuana users	*FAAH* rs324420	Craving following cue exposure, acute effects of MJ administration, withdrawal	C allele associated with elevated withdrawal, increased happiness and reduced heart rate following acute administration.
Tyndale et al., 2007 [33]	749 adults	*FAAH* rs324420	Cannabis initiation, regular use, or dependence	C allele carrier more likely to be dependent.

Studies are alphabetically by the last name of the first author. Unless, otherwise noted, results reflect nominally significant associations that were not corrected for multiple comparisons

in behavior [52, 53]. This approach, known as neurogenetics, has recently been implemented in the context of candidate studies of the endocannabinoid system and cannabis involvement and has identified several polymorphisms within *CNR1* and *FAAH* that are associated with individual differences in threat-, reward-, and working memory-related brain function as well as resting state connectivity, and brain structure that may be relevant to cannabis phenotypes (Table 2). Notably, the vast majority of neurogenetics research has used different tasks and has studied different polymorphisms in typically small samples; as a result, the stability and strength of these associations are presently unclear.

The most consistently investigated polymorphism, rs324420 within *FAAH*, has been associated with differential threat and reward-related brain function as well as functional and structural connectivity [37•, 58, 59•, 60]. A wealth of rodent research (for review, see [66]), suggests that endocannabinoid signaling, and in particular anandamide, within the basolateral amygdala, protects against the development of anxiety-related behavior. Consistent with these observations, in humans, the A allele at rs324420, which has been associated with protection from cannabis use disorder [33], as well as reduced FAAH expression and presumably increased anandamide signaling, has been linked to reduced threat-related amygdala reactivity and greater amygdala habituation [59•, 60]; but see also evidence of enhanced startle among A allele carriers [54]. Similar effects have been observed within a rodent knock-in model of this polymorphism [58]. While the direct application of this research in healthy controls to cannabis use has not thoroughly been investigated, we have observed that other genetic polymorphisms conferring reduced amygdala habituation predict cannabis dependence symptoms, as well as using cannabis to regulate one's mood [25•]. Collectively, these results raise the possibility that rs324420 C homozygotes may potentially use cannabis to regulate emotion, which is consistent with evidence that these individuals have a more positive response to acute marijuana use as well as more intensive withdrawal symptoms following abstinence and cue exposure in the context of abstinence [28, 32]. An additional, though not mutually exclusive, pathway that this polymorphism may affect cannabis dependence is reward-related activation to cannabis cues. Indeed, while C homozygosity has been associated with relative reduced activation in the ventral striatum to monetary-related positive feedback [60], it has been associated with elevated ventral striatum response to marijuana cues following abstinence, a pattern of activation seen across substances of abuse that may potentiate the development of dependence [67].

Interplay Between Genetic and Environmental Factors

Twin studies disarticulate environmental sources of variance into those that make members of a twin pair similar (i.e., shared environment) and those that are person-specific. The identification of individual risk and protective environmental influences for cannabis use and misuse, however, have primarily centered on those factors that relate to social control (e.g., availability, peer deviance, parental monitoring) [68•] and onset (and, potentially, also to CUD, e.g., [69]) and those that relate to childhood adversity (e.g., abuse, family history) [70] and impact both onset of use and persistence to dependence. Within the latent genetic paradigm, most studies have combined cannabis with alcohol and tobacco when examining gene-environment interplay. However, specifically examining cannabis use in the context of peer deviance, Gillespie and colleagues [71] concluded that the genes influencing CU also influenced liability to affiliate with deviant drug-using peers and further, that cannabis use was associated with selecting into such substance-using peer groups. Importantly, this and other studies highlight the heritable nature of respondent reports of peer substance use [72], ($h^2 = 30–50$ %) which is among the most robust risk factors associated with onset of cannabis use. Parental monitoring has also been linked to likelihood of cannabis initiation [73]. While studies of cannabis specifically are absent, most of the substance use literature suggests that heritable influences on early stages of substance use are dampened in the presence of increased parental monitoring (e.g., [74]). Broadly, these factors relate to environmental permissivity—environments conducive to substance use are likely to enhance the role of heritable variation [75]. Despite this, studies of cannabis use in the Netherlands, which adopts a more legislatively tolerant view towards cannabis than the USA, have reported similar estimates of the heritability of cannabis use [76, 77•]. More recently, US trends show a shift towards more social acceptance of recreational cannabis use, particularly in youth [1•]—future twin studies will be highly informative in accounting for the effects of this cultural shift in heritability estimation.

Childhood adversity is another major contributor to cannabis involvement with at least one study noting its relevance for both onset and maintenance of use, particularly in European-American women [70]. Given its strong main effects, it is hypothesized that in the presence of childhood adversity, the role of genetic variance may be less consequential [78]. Alternatively, exposure to childhood adversity may potentiate or exacerbate the role of certain genetic polymorphisms (e.g., [79]).

The role of childhood adversity may be a particularly important consideration in the context of genetic variation within the endocannabinoid system. Indeed, evidence suggests that the endocannabinoid system regulates stress responsiveness

Table 2 Imaging genetics studies of endocannabinoid genetic variation

Study reference	Sample	Polymorphisms	Phenotypes	Results summary
Carey et al., 2015 [54]	312 students	*MGLL* rs604300	BOLD fMRI basolateral amygdala habituation to emotional faces	rs604300 genotype interacted with childhood adversity to predict amygdala habituation. A allele carriers had a positive association between childhood adversity and amygdala habituation that was absent among G homozygotes.
Chakrabarti et al., 2006 [55]	19 student participants	*CNR1* rs1049353, rs806377, rs806380, rs6454674	BOLD fMRI response to happy faces (happy > neutral)	rs1049353 T allele, rs806377 C allele, rs806380 G allele, rs6454674 G allele associated with elevated striatum response to happy faces.
Colizzi et al., 2015 [56]	208 healthy individuals	*CNR1* rs1406977	BOLD fMRI during 2-back working memory task	rs1406977 G allele associated with reduced prefrontal mRNA. G allele carriers who were also cannabis users had greater functional connectivity in the left centrolateral PFC reduced working memory accuracy during 2-back relative to other groups.
Demers et al., in press [57]	661 students	*FAAH* rs324420 *CRHR1* rs110402	BOLD fMRI basolateral amygdala habituation to emotional faces	*FAAH* rs324420 × *CRHR1* rs110402 interactions: FAAH A allele carriers who were also CRHR1 A allele homozygotes showed the least amount of amygdala habituation, which was associated with increased anxiety disorder risk.
Dincheva et al., 2015 [58]	35 healthy participants	*FAAH* rs324420	Resting state functional connectivity	rs324420 A allele relatively increased resting state functional correlation between bilateral amygdala and ventromedial PFC.
Filbey et al., 2010 [37•]	37 3-day-abstinent regular marijuana users (4 times or greater/week for the last 6 months)	*CNR1* rs2023239 *FAAH* rs324420	fMRI BOLD response to marijuana cues (i.e., pictures of a marijuana pipe > pencil)	*CNR1* rs2023239: Individuals with C/T genotype had greater response to marijuana cues in the orbitofrontal cortex, anterior cingulate cortex, inferior frontal gyrus. *FAAH* rs324420: C homozygotes had greater response to marijuana cues in the ventromedial PFC, orbitofrontal cortex, thalamus, anterior cingulate cortex, ventral striatum. Additive genetic profile combining the number of *FAAH* rs324420 C alleles and *CNR1* rs2023239 C alleles was associated with greater response to marijuana cues in ventral striatum, thalamus, anterior cingulate cortex, and inferior frontal gyrus.
Gunduz-Cinar et al., 2013 [59•]	103 participants (same sample as used in Hariri et al., 2009)	*FAAH* rs324420	BOLD fMRI habituation to emotional faces	rs324420 A allele carriers had greater amygdala habituation to emotional faces.
Hariri et al., 2009 [60]	109 participants	*FAAH* rs324420	BOLD fMRI response to monetary wins and emotional faces	rs324420 A allele carriers had blunted amygdala response to emotional faces and heightened ventral striatum response to monetary gains relative to losses.
Ho et al., 2011 [61]	235 patients with schizophrenia spectrum disorders (52 with	12 *CNR1* tagging SNPs: rs806365, rs7766029, rs806366, rs806368,	White matter volume (structural MRI) and cognitive function	rs7766029 C homozygotes had smaller temporal and parietal white matter volumes.

Table 2 (continued)

Study reference	Sample	Polymorphisms	Phenotypes	Results summary
	marijuana abuse or dependence)	rs12720071, rs1049353, rs806374, rs806375, rs806376, rs6454672, rs9450898, rs806380		rs12720071 G allele carriers had smaller frontal and temporal white matter volume and worse speed/attention and problem solving abilities. rs12720071 × marijuana use interaction on white matter volume and cognitive function. G allele carriers with marijuana abuse/dependence had smallest parietal white matter volume and worst problem solving performance relative to G allele carriers without marijuana abuse or dependence and A homozygotes regardless of marijuana abuse/dependence. rs9450898 C allele homozygotes had smaller frontal and parietal white matter volume. No nominally significance associations with marijuana abuse/dependence.
Onwuameze et al., 2013 [62]	235 patients with schizophrenia spectrum disorders (same sample as Ho et al., 2011)	*CNR1* rs12720071 *MAPK14* tagging SNPs: rs3804454, rs2237094, rs12199654, rs851007, rs851006, rs3804452, rs8510, rs7757672, rs916346	White matter volume (structural MRI) and cognitive function	Only effects involving *CNR1* are summarized here; refer to the paper for *MAPK14* effects. Effects reported in Ho et al., 2011 remain after controlling for genetic variation in *MAPK14*. Diplotype grouping: number of *CNR1* rs12720071 G alleles and *MAPK14* rs12199654 A alleles was associated with reduced white matter brain volume. Further, this interacted with marijuana abuse/dependence whereby reduced white matter volume among larger genetic profiles was only significant among those with marijuana abuse and dependence.
Schacht et al., 2012 [63]	94 heavy cannabis users and 37 controls	*CNR1* rs2023239	Structural MRI: Hippocampus and amygdala volume	rs2023239 × group interaction: G allele with cannabis use associated with reduced bilateral hippocampus volume relative to control participants regardless of genotype.
Shollenbarger et al., 2015 [64]	33 cannabis users (>50 lifetime joints or >25 in past year) and 34 non-regular users (<5 joints in last year or 10 joints lifetime)	*FAAH* rs324420	DTI Fractional anisotropy	rs324420 genotype × cannabis group interaction: rs324420 C homozygote cannabis users and A allele carrier controls had reduced FA in forceps minor. C homozygote cannabis users had reduced bilateral anterior thalamic radiation FA.
Stadelman et al., 2011 [65]	20 Healthy volunteers administered placebo, cannabis extract, 9-THC (within subject design)	*CNR1* AAT repeat	P300 amplitude and latency during auditory choice reaction task	AAT 10 repeats and greater: reduced P300 amplitude and prolonged latency under all experimental manipulations. Effects largest in the THC only condition.

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and recovery by modulating hypothalamic-pituitary-adrenal axis activity as well as amygdala responsiveness to threat [57, 66, 80•]. Moreover, emerging evidence from non-human animal models suggests that stress exposure during early life and adolescence, but not adulthood, results in sustained effects on endocannabinoid (eCB) signaling that do not recover following enrichment and may promote cannabis dependence [25•, 81–83]. Alongside evidence that childhood adversity is associated with cannabis dependence, these data suggest that genetic variation within the endocannabinoid system may moderate cannabis-related phenotypes. We recently tested this hypothesis by conducting an endocannabinoid system-level analysis examining whether genetic variation across the endocannabinoid system moderates the effects of childhood adversity on cannabis dependence symptoms [25•]. A SNP within the monoacylglycerol lipase gene (*MGLL*), rs600304, moderated the association between childhood adversity and cannabis dependence, after conservative correction for multiple testing, and was further replicated in an independent sample, and shown to moderate associations between childhood adversity and amygdala habituation within a third sample, providing a putative neural mechanism through which these effects may at least partially arise. While these preliminary findings provide promising leads to gene-environment interplay and neural mechanisms that may contribute to cannabis-related phenotypes, they require further replication.

Cannabis use as an Environmental Influence

Adolescent exposure to cannabis has been posited as a "risk factor" for a variety of outcomes, most notably use of hard drugs, psychosis, educational achievement, and, more broadly, brain maturation. Excellent reviews on the relationship between cannabis use and neurodevelopment exist (e.g., [84–86]). There are also several in-depth overviews of the nature of the relationship between early cannabis use and educational outcomes and of the putative gateway effects of early onset cannabis use [87]. Thus, we focus here on a genetic perspective to the contentious relationship between cannabis exposure and psychotic illnesses.

Individuals with schizophrenia and other psychotic illnesses are highly likely to report a history of adolescent-onset cannabis use [88]. This early onset use typically escalates to CUD representing a fairly well established comorbidity. The hypothesis that adolescent-onset cannabis use causes psychotic illness is not well supported by the extant epidemiological literature as escalation in cannabis use has not corresponded with increases in the incidence of psychotic illness [88, 89]. The predominant alternative hypothesis has been that early cannabis use acts as a provocateur of genetic vulnerability to psychosis. An early study by Caspi and colleagues [90] found that individuals who carried one or more copies of the Valine (Val) allele for rs4680 in catechol-o-methyltransferase (*COMT*) and reported using cannabis prior to age 17 were more likely to exhibit a variety of psychosis-related outcomes later in life. COMT regulates the degradation of endogenous amines, such as dopamine; thus, any deficiency in COMT activity is related to excess dopamine in the synaptic cleft. The Val (relative to Met or methionine) allele in rs4680 has been associated with enhanced enzymatic efficiency and consequently, lower dopamine levels. While the *COMT* × cannabis analysis has not been systematically replicated, a recent neuroimaging study [91] found that a polygenic risk score for loci associated with schizophrenia from the largest meta-analysis of genome-wide association studies [92] (*n* cases = 36,989; *n* controls = 113,075) was associated with cortical thickness in cannabis users. Boys, but not girls, who had used cannabis showed relatively thinner cortex as a function of increasing polygenic liability to schizophrenia, indicating that the relationship between early exposure to cannabis and later schizophrenia may be via the activation of a polygenic vulnerability that impacts brain maturation.

Evidence for a third alternative has begun to accumulate—that cannabis use and psychosis share common genetic underpinnings. Until recently, the primary challenge with testing this hypothesis was that the low rate of psychosis in general population twin studies and the absence of data on cannabis involvement in the older family studies of schizophrenia precluded estimation of their genetic correlation. However, with the availability of GWAS data, one study [51•] found that polygenic risk associated with schizophrenia is also related to cannabis use. Furthermore, polygenic risk was greatest in twins who were concordant for cannabis use indicating genetic commonality.

Finally, a recent co-relative control study [93] that leverages the vast and rich data available from the Swedish national registries reported that cannabis abuse and schizophrenia may be related via causal pathways. In the Swedish population, cannabis abuse was associated with a 10 times increased odds of schizophrenia with the magnitude of the association diminishing as time elapsed between diagnoses, but remaining significant. As confounding due to genetic factors was accounted for (i.e., comparing unrelated subjects who share no genetic background versus cousins or siblings who share some of their genetic background identical-by-descent), the likelihood of schizophrenia remained elevated in cannabis abusers. Consistent with Power and colleagues, [51•], this study also concluded that shared genetic liability was an important contributor—the association between unrelated (odds ratio of 10) was substantially greater than the association in sibling pairs (odds ratio of 1.98) indicating that shared genes explained much of this relationship. However, based on the significance of the association in full siblings (who share 50 % of their genes) and extrapolating to identical twin pairs (who

share 100 % of their genes), these investigators concluded that even after accounting for shared genetic variance, individuals with a history of cannabis abuse were at increased risk for schizophrenia, relative to their genetically identical non-abuser co-twins, thus suggesting a causal role of cannabis abuse on schizophrenia.

Future Directions

1. Improve gene identification. Despite the robust heritability estimates for cannabis phenotypes, GWAS to date have not identified significant loci. This is most likely attributable to the rather modest sample size, specifically of cannabis-dependent cases, for existing GWAS. The extraordinary success that was experienced by genome-wide association studies of schizophrenia, which contained over 36,000 cases and 113,000 controls [92] suggests that increasing sample sizes of cannabis use disorder GWAS, which presently has not exceeded 4000 cannabis-exposed individuals, might yield replicable genome-wide significant signals for CUD. This is a priority for multiple substance use consortia. An additional factor that may improve gene identification is optimizing the phenotype under study. For example, by studying severe recurrent, and primarily melancholic, depression in China, the CONVERGE consortium [94] was able to identify genome-wide significant associations with major depressive disorder despite a lower sample size (5303 cases) than previous major depression GWAS (9000 cases) that failed to identify genome-wide significant loci [95]. As such, similar approaches focusing on severe cannabis dependence may prove fruitful. Notably, phenotypes such as cannabis initiation, which are more common and therefore, more readily available across large international population-based surveys (e.g., one prior meta-analysis had >10,000 subjects [4•]) may allow for larger samples to be accumulated. However, understanding of genetic contributions to this phenotype may be clouded by sociocultural trends which underpin worldwide rates of cannabis use creating substantial heterogeneity in the extent to which heritable factors are able to impact variance in cannabis use. For instance, heritability of tobacco smoking has been shown to be moderated by cohort effects [96], among other social factors, and cannabis may be subject to similar variability. If there are true genetic loci associated with cannabis use, one might argue that large samples would uncover these. However, the impact of covariates and confounders will likely play an important part in this discovery.

2. Understanding the polygenic nature of cannabis involvement and comorbidity. As sample sizes accumulate, novel genetic approaches, such as LD regression [97], could be readily implemented to estimate the genetic correlation between CUD and a variety of substance-related and comorbid mental health as well as neuroimaging phenotypes, using effect sizes generated by large-scale consortia such as the Psychiatric Genomics Consortium (PGC) and ENIGMA [92, 98]. In addition to discovery of novel variants and co-analysis with results from other large-scale consortia, cannabis-related polygenic risk scores (PRS) could be computed from genome-wide effect sizes to predict a variety of substance-related and comorbid mental health as well as neuroimaging phenotypes in independent samples of smaller size [51•]. Further, PRS can also be modeled in the context of environmental factors (polygenic G × E) [99]. However, how small these samples may be to be able to reliably detect polygenic associations with novel phenotypes will be dependent upon the strength of associations observed in the discovery GWAS studies as well as the penetrance of these genetic variants on related phenotypes under study (see the following item).

3. Identifying mechanisms of risk. The identification of genetic determinates of cannabis involvement provides a foundation for further molecular (e.g., interactions between the eCB and HPA axis [80•]), neural (e.g., neural response to marijuana cues [37•]), and behavioral (e.g., cue-induced craving of marijuana [28]) mechanistic investigation. Unlike initial expectations, emerging evidence suggests that some intermediate phenotype approaches (e.g., brain structure [100]) may be associated with effect sizes similar to those observed for traditional psychiatric diagnoses [98], but see also [101]. As a result, such intensive and expensive mechanistic studies may be best used to better understand biologically characterized polymorphisms (e.g., *FAAH* rs324420), psychiatrically associated risk markers, as well as polygenic profiles, as opposed to GWAS-based gene identification [102]. Notably, large consortia, such as ENIGMA, have begun to successfully identify novel genetic associations with brain volume [98]; however, acquiring large enough samples through collaborative efforts for more specific cannabis-related phenotypes (e.g., cue-related reactivity) is likely years or decades away. We can comfortably assert that genome-wide significant loci and resulting PRS will serve as targets for the next era of neurogenetics enquiry but the sample size needed to reliably detect these associations will depend on assumptions of expected effect size for neural outcomes. PRS may augment this power; for instance, schizophrenia PRS (for all SNPs associated at $p \leq 0.05$) were related to a Nagelkerke R^2 of 0.18 when predicting schizophrenia in independent smaller samples. One inherent limitation confronting PRS application to neural phenotypes is that because this approach aggregates information across the entire genome, its usefulness for understanding system-level mechanisms (e.g., the role of endocannabinoid signaling) alongside other neuroscience tools (e.g., pharmacologic challenge [103]) is absent. Several additional polygenic methods can be used to confront this limitation including conducting system level based investigation of PRS (while controlling for overall polygenic

risk), using biologically informed multilocus profiles, and enrichment analyses that have begun to produce promising results [53].

4. Incorporating the environment. The environment, and in particular adversity during childhood, are among the most potent predictors of cannabis involvement phenotypes [104]. Moreover, given that the endocannabinoid system is intricately involved in regulating stress response and recovery through its modulatory effect on amygdala and hypothalamic-pituitary-adrenal axis function [66], incorporating measures of the environment may advance genetic discovery. Further, it will be important to understand the molecular and neural mechanisms through which such effects emerge (e.g., epigenetic modification [105], stress-related gene expression [106]). Indeed, such mechanistic understanding will facilitate the development of treatments that may target links within the etiologic chain. Additionally, as we witness a wave of legalization of recreational cannabis use, the impact of social change on reshaping the sources of variance in cannabis use and misuse should not be underestimated. As discussed previously, studies of alcohol and tobacco document the sensitivity of genetic influences to changes in permissivity surrounding their use (e.g., [75]). For instance, if we postulate that genetic variants associated with trying cannabis are likely to relate to general disinhibitory circuitry (e.g., novelty-seeking), then it is possible that as experimentation with cannabis becomes more normative, the importance of such loci will be attenuated.

Conclusions

Twin and family studies clearly demonstrate that cannabis involvement has a substantive genetic and environmental basis. Candidate molecular genetic association research has generally produced mixed and unreliable results (Table 1). The most consistent effects appear to be from a small body of literature implicating a functional polymorphism within *FAAH* to cannabis dependence, as well as withdrawal symptoms, and responses to acute THC administration. This evidence is buttressed by a growing neurogenetic literature linking this polymorphism to psychiatrically relevant neuroimaging outcomes such as threat-related amygdala function and habituation, and ventral striatum response to reward, including marijuana cues (Table 2). Notably, however, all of these studies to date have been underpowered and await further clarification of association. GWAS studies have identified novel genes that may play a role in cannabis-related phenotypes [47], but these have yet to be replicated nor has any single polymorphism reached genome-wide significance. These findings are to be expected given accumulating evidence suggesting that common genetic variation will have at most small effects on psychiatrically relevant phenotypes [92, 98]. Adopting polygenic techniques has begun to provide replicable associations [51•] and new research suggests that it may be fruitful to consider G × E, particularly in investigations of the endocannabinoid system [25•]. Future large consortia projects may accumulate large enough samples to identify small of effects of common genetic variation on cannabis involvement. Such psychiatric genetic research, in concert with neurogenetic research, may be able to usefully inform our understanding of the neural mechanisms through which genetic variation influences susceptibility (e.g., [25•]).

Compliance with Ethical Standards

Conflict of Interest Ryan Bogdan received support from the Klingenstein Third Generation Foundation and the National Institute on Aging (NIA; AG045231). Arpana Agrawal received support from the National Institute on Drug Abuse (NIDA; DA23668, DA32573) and during the conduct of the study; grants and other from ABMRF/Foundation for Alcohol Research, outside the submitted work. Jonathan Winstone declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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