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# Monoacylglycerol lipase (MGLL) polymorphism rs604300 interacts with childhood adversity to predict cannabis dependence symptoms and amygdala habituation: Evidence from an endocannabinoid system-level analysis

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## Abstract

Despite evidence for heritable variation in cannabis involvement and the discovery of cannabinoid receptors and their endogenous ligands, no consistent patterns have emerged from candidate endocannabinoid (eCB) genetic association studies of cannabis involvement. Given interactions between eCB and stress systems and associations between childhood stress and cannabis involvement, it may be important to consider childhood adversity in the context of eCB-related genetic variation. We employed a system-level gene-based analysis of data from the Comorbidity and Trauma Study ($N$ = 1,558) to examine whether genetic variation in 6 eCB genes (anabolism: *DAGLA*, *DAGLB*, *NAPEPLD*, catabolism: *MGLL*, *FAAH*, binding: *CNR1*; SNPs $N$ = 65) and childhood sexual abuse (CSA) predicts cannabis dependence symptoms. Significant interactions with CSA emerged for *MGLL* at the gene-level ($p$ = .009), and for rs604300 within *MGLL* ($\Delta R^2$ = .007, $p$ < .001), the latter of which survived SNP-level Bonferroni correction and was significant in an additional sample with similar directional effects ($N$ = 859; $\Delta R^2$ = .005, $p$ = .026). Furthermore, in a third sample ($N$ = 312), there was evidence that rs604300 genotype interacts with early life adversity to predict threat-related basolateral amygdala habituation, a neural phenotype linked to the eCB system and addiction ($\Delta R^2$ = .013, $p$ = .047). Rs604300 may be related to epigenetic modulation of *MGLL* expression. These results are consistent with rodent models implicating 2-arachidonoylglycerol (2-AG), an endogenous cannabinoid metabolized by the enzyme encoded by *MGLL*, in the etiology of stress adaptation related to cannabis dependence, but require further replication.

### Keywords

cannabis; endocannabinoid; childhood abuse; *MGLL*; amygdala

## Endocannabinoid Variants and Childhood Adversity Predict Cannabis Dependence Symptoms and Amygdala Habituation

After alcohol, cannabis is the second most widely used recreational drug in developed nations (Degenhardt & Hall, 2012). In the United States, 45% of adults report using cannabis at some point in their lives, with 12% using in the past 12 months (Substance Abuse and Mental Health Services Administration, 2012). Cannabis dependence was formerly determined in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR; 4th ed., text rev.; American Psychiatric Association, 2000)[1] by the endorsement of

[1]The DSM-5 no longer diagnoses cannabis dependence. Instead, the 6 DSM-IV dependence criteria were combined with 3 abuse criteria (use in hazardous situations, failure to fulfil major role obligations and social/interpersonal problems due to recurrent use), as well as withdrawal and craving to define cannabis use disorders in individuals experiencing 2 or more of these problems in a 12 month period. However, as the data used in this study precede DSM-5 by several years, we utilize the DSM-IV definitions.

at least 3 of the following 6 criteria within a 12-month period: 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. Estimates suggest that 1.3% of the U.S. population meets the criteria for DSM-IV-TR cannabis dependence at some point during their lives (Stinson, Ruan, Pickering, & Grant, 2006). Among users, 9% qualify for dependence; of daily users, that number rises to roughly 25-50% (Hall & Degenhardt, 2009; Lopez-Quintero et al., 2011).

## Genetic Basis for Cannabis Dependence

Even though twin studies suggest thatapproximately 50-60% of variance in cannabis use disorders (abuse and dependence) is attributable to additive genetic influences (Verweij et al., 2010), molecular genetic studies have had limited success identifying variants implicated in the emergence of problematic cannabis involvement. For instance, a genome-wide association study (GWAS) of cannabis dependence, performed in the replication sample used in the current study, failed to find any genome-wide significant (i.e., $p < 5E\text{-}8$) associations (Agrawal et al., 2011). While GWAS is a powerful tool for SNP discovery, it is fairly agnostic with regard to the putative mechanisms underlying the behavior under study, equally weighting, arguably unduly, variants in all gene systems. Those interested in hypothesis-driven data analyses have frequently reverted to candidate gene approaches, which, with rare exception, have yielded null or nonreplicable results (Agrawal & Lynskey, 2009; Flint & Munafò, 2013).

## Challenges with A Priori Selection of Candidate Variants

Traditional candidate gene analyses, historically conducted in modestly sized samples of less than 500 participants, relied on a handful of genotypes selected for their purported functional effects on behavior. Reliance on such restricted sets of variants was primarily due to an oversimplification of genotype-phenotype relationships. For instance, if positive reinforcement is a core feature of substance use and is related to the release of dopamine, then variants associated with differential dopamine function (e.g., rs1800497 in *DRD2/ANKK1*) must be of importance to cannabis dependence (Maldonado & Rodriquez de Forseca, 2002). While the hypothesis that dopaminergic mechanisms, even specifically DRD2 receptors, are at play is fairly reasonable, a recent meta-analysis suggests no association between this polymorphism and cannabis dependence (Deng et al., 2015). Prior limitations with annotation of the human genome, compounded by the prohibitive costs associated with large-scale genotyping, likely relegated the implementation of early genetic association studies to a limited number of handpicked variants across genes and/or gene-tagging single nucleotide polymorphisms (SNPs). However, recent improvements in feasibility, customizability, and affordability of genome-wide arrays, the practicality of imputing, and the efficiency of data analysis (particularly with software packages such as PLINK; Purcell et al., 2007; Chang et al., 2015) have rendered some custom candidate gene panels obsolete. The availability of high density arrays with superior gene coverage allow for the study of candidate gene systems, an approach more amenable to hypothesis-testing.

## Modern Methods for Gene-Based Analyses

With the increasing accessibility of genome-wide data, there has been a corresponding surge of novel methods that leverage its high dimensionality. Typically, because approximately 1 million SNPs are examined individually, GWAS incur a high cost for multiple comparisons ($p < 5E-8$). In response, and consistent with the polygenic basis underlying behavioral traits (Plomin, Haworth, & Davis, 2009), recent research has begun to examine aggregate genetic influence and effects across genes and biological systems (Holmans, 2010; Neale & Sham, 2004; Purcell et al., 2009; Ramanan, Shen, Moore, & Saykin, 2012; Wang, Li, & Bucan, 2007; Wang, Li, & Hakonarson, 2010). Such gene- and system-level association analyses not only reduce the likelihood of false negatives by lowering the threshold for significance, but are also more compatible with the resolution (i.e., downstream neural, behavioral, and self-report measures) at which clinical, behavioral, and neural genetics research is conducted (Dudbridge, Gusnanto, & Koeleman, 2006; Nikolova, Ferrell, Manuck, & Hariri, 2011; Plomin et al., 2009).

When there is ample evidence for the involvement of a given gene or system but little prior knowledge regarding specific polymorphisms within it, methods have been introduced that average across associations for all variants within the gene/system, typically regardless of function, to create a summary statistic (Holmans, 2010; Li, Gui, Kwan, & Sham, 2011; Liu et al., 2010; Neale & Sham, 2004; Ramanan et al., 2012; Wang et al., 2007, 2010). Numerous approaches have been proposed to mine GWAS data in this manner. For instance, the Versatile Gene-Based Association Analysis (VEGAS; Liu et al., 2010) software package utilizes *p*-values for each SNP from a typical GWAS, assigns them to one of 17,787 autosomal genes, and creates a sum statistic representing gene-based association whose empirical *p*-value is calculated via simulations. This approach has successfully identified genes associated with cannabis dependence when single-SNP GWAS have failed (Agrawal et al., 2014).

While the above represents a data-driven approach for assignment of SNPs to genes/systems, other methods allow investigators to ascribe SNPs to a gene set and conduct set-based association tests. Using such an approach, one study linked variants in the norepinephrine, glutamatergic, GABAergic, and corticotropin-releasing hormone systems to alcohol dependence (Reimers, Riley, Kalsi, Kertes, & Kendler, 2012). The present study uses a similar approach to examine the relationship between all SNPs in genes within the endocannabinoid system and cannabis dependence. Due to the relatively recent characterization of cannabinoids' actions in the brain and the limited exploration of individual SNPs in this system, prior robust association findings are limited, making system-level and gene-based analyses an appealing alternative to traditional single-SNP or additive multilocus candidate gene approaches (e.g., Nikolova, Ferrell, Manuck, & Hariri, 2011).

## The Endocannabinoid System and Cannabis Involvement

In this study, we propose that genes comprising the endocannabinoid system may play a particularly important role in the etiology of cannabis dependence, especially in the context of childhood stress. In the past 25 years, receptors for exogenous cannabinoids such as Δ9-tetrahydrocannabinol (THC) in *Cannabis*, along with endogenous ligands, have been

identified (for a review, see De Petrocellis & Di Marzo, 2009). The endogenous cannabinoid, or "endocannabinoid" (eCB), system plays a critical role in reward, anxiety, and stress responsiveness (Hill, McLaughlin, et al., 2010; Solinas, Goldberg, & Piomelli, 2008). The molecular aspects of the eCB system have been extensively reviewed elsewhere (see Bisogno, 2008; Bisogno, Ligresti, & Di Marzo, 2005; Di Marzo, 2011; Di Marzo, 2009; Piomelli, 2003; Wilson & Nicoll, 2002) and are briefly described below (see also Table 1).

Unlike most transmitter systems, endocannabinoid receptors have two major ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), as well as a host of other putative ligands (e.g., noladin, virodhamine). AEA and 2-AG are synthesized postsynaptically and released "on-demand," presumably by an unknown endocannabinoid membrane transporter. Anandamide is synthesized directly from the phospholipid precursor N-arachidonoyl phosphatidylethanolamine (NAPE) by N-acyl-phosphatidylethanolamine-selective phosphodiesterase (NAPE-PLD); alternative indirect synthesis routes exist but have not yet been fully characterized. The other eCB ligand, 2-AG, is formed by the hydrolysis of diacylglycerols (DAGs) by sn-1-selective diacylglycerol lipases (DAGLs). Once released into the synapse, eCB ligands bind to cannabinoid type 1 (CB1) and type 2 (CB2) receptors. As with eCB ligands, other putative receptors exist (e.g., GPR55, TRPV1) but have yet to be well characterized. AEA is broken down primarily by fatty acid amide hydrolase-1 (FAAH), while 2-AG is mostly catabolized by monoacylglycerol lipase (MAGL).

Given that eCB receptors bind the psychoactive component, THC, in cannabis as well as endogenous ligands similar in structure to THC, it is a promising candidate system for probing genetic vulnerability to cannabis dependence. Chronic cannabis users, for example, show global decreases in CB1 receptor availability (Ceccarini et al., 2013). Correspondingly, animal models have persuasively documented that the psychoactive effects of cannabis are exerted via their interface with these eCB receptors in the central nervous system, particularly CB1 (Lichtman & Martin, 1997; Wiley et al., 2014). For instance, impairment of short term spatial memory is a frequently observed outcome of acute THC administration (Wise et al., 2012), and CB1 receptor antagonism in the hippocampus attenuates this memory disruption, while elevation of AEA and 2-AG levels via simultaneous FAAH and MAGL blockade recapitulates this phenotype (Wise, Thorpe, & Lichtman, 2009). Despite this strong preclinical evidence, much of the extant literature on specific genes and polymorphisms within the eCB system influencing cannabis phenotypes (e.g., Filbey, Schacht, Myers, Chavez, & Hutchison, 2010; Haughey, Marshall, Schacht, Louis, & Hutchison, 2008; Schacht, Selling, & Hutchison, 2009) remains unreplicated or has yielded null results (Agrawal & Lynskey, 2009). Furthermore, variation in eCB genes along the anabolic and catabolic pathways has been largely neglected.

## The Role of Environment

Independent lines of research linking the eCB system to stress responsiveness (Gunduz-Cinar, Hill, McEwen, & Holmes, 2013), and early life adversity to cannabis dependence risk (Duncan et al., 2008), suggest that it is important to consider the environment with regard to eCB-related genetic risk for cannabis dependence. The eCB system is modulated by stress and has reciprocal interactions with the hypothalamic-pituitary-adrenal (HPA) axis, a central

regulator of stress responsiveness. Non-human animal models have demonstrated that stress exposure increases the eCB ligand 2-AG while decreasing AEA levels, with pharmacologic evidence that these changes mediate effects of stress on HPA axis activation (Hill et al., 2009; Patel, Kingsley, Mackie, Marnett, & Winder, 2009; Rademacher et al., 2008). Moreover, there is accumulating preclinical evidence that, unlike adult-onset stressors, chronic stress exposure during early life and adolescence results in sustained stress-related effects on eCB signaling and gene expression that do not recover following enrichment during adulthood (Buwembo, Long, & Walker, 2013; El Rawas et al., 2011; Lee & Hill, 2013; Malone, Kearn, Chongue, Mackie, & Taylor, 2008; Reich, Mihalik, Iskander, Seckler, & Weiss, 2013; Sciolino et al., 2010). These sustained differences in the eCB system may leave individuals vulnerable to later psychopathology, including cannabis dependence.

Independent of genetic considerations, environmental factors are thought to contribute to 40-50% of variation in problematic cannabis use (Verweij et al., 2010). Abuse, particularly during childhood, has been reliably associated with substance use problems in both adolescence and adulthood (see Simpson & Miller, 2002, for review). Childhood sexual abuse (CSA), in particular, is associated with increased adolescent and adult substance use and misuse, with one study suggesting a 2-fold increased hazard of cannabis use disorders in individuals exposed to CSA (Duncan et al., 2008). Similarly, a longitudinal study found that, even after controlling for covariates (including socioeconomic status, other childhood adversity, parental separation, and parental history of offending), exposure to CSA was significantly predictive of alcohol and other substance use disorders by age 16 to 18 years (Fergusson, Horwood, & Lynskey, 1996). Furthermore, risk for substance use and disorders is elevated in twins exposed to CSA relative to their genetically related unexposed co-twin, underscoring the importance of environmental experience (Myers & Prescott, 2000; Nelson et al., 2006).

Given interactions between the eCB and stress regulatory systems, as well as documented main effects of early life adversity on cannabis use disorders and HPA-axis and eCB-related function, it is possible that eCB-related genetic variation only confers risk for cannabis involvement in the context of stress exposure. Such Gene × Environment interactions (GxE) can emerge in a variety of forms. For instance, genetic effects might be potentiated or exacerbated in the context of environmental exposure. As an example, Meyers and colleagues found that the G allele of *ADH1B* rs1229984, which is associated with decreased conversion of alcohol to acetaldehyde and increased problematic alcohol use, has a stronger influence on heavy drinking and alcohol dependence in individuals with a prior history of CSA (Meyers et al., 2013). In contrast, certain genotypes might suppress risk or protection afforded by the environment. For instance, a synonymous polymorphism within *CNR1* (rs1049353) has been found to attenuate the pathogenic effects of childhood physical abuse on later vulnerability to anhedonia (Agrawal, Nelson, et al., 2012; but see also Pearson et al., 2013). In that study, carriers of the minor allele were protected from the pathogenic effects of childhood abuse on anhedonia.

## Endocannabinoids and the Basolateral Amygdala

In addition to guiding GxE research, neuroscience can be used to disentangle the neural mechanisms through which genetic variation and the environment promote individual differences in behavior (Caspi & Moffitt, 2006). Cross-species research on the effects of stress on the eCB system as well as pharmacologic eCB manipulation suggest that individual differences in amygdala function, and in particular its diminished response to repeated threat-related stimuli (i.e., habituation), may play a key role in mediating associations between the eCB system, stress exposure, and behavior (Ramikie & Patel, 2012). First, stress-induced endocannabinoid changes (e.g., reduced AEA, increased 2-AG) within the basolateral amygdala (BLA) facilitate HPA axis activation and increase anxiety (Rademacher et al., 2008). Moreover, chronic early life stress results in sustained eCB differences within the BLA that may provide a lasting environmental signature conferring vulnerability to cannabis dependence symptoms (Lee & Hill, 2013; Sciolino et al., 2010). Second, THC and CB1 agonist administration reduce subjective anxiety, blunt threat-related amygdala function, and facilitate fear extinction (Gruber, Rogowska, & Yurgelun-Todd, 2009; Phan et al., 2008; Rabinak et al., 2013). Importantly, knockout and pharmacologic manipulation studies in rodents suggest that eCB signaling is critical for fear extinction, but not conditioning, which is consistent with recent genetic work in humans linking an eCB polymorphism (rs324420 in *FAAH*) to amygdala habituation as well as stress-related negative emotionality (Gunduz-Cinar, MacPherson, et al., 2013). Collectively, these independent lines of research suggest that the effects of eCB-related genetic variation and stress exposure may exert behavioral effects by influencing amygdala habituation to threat-related stimuli. Such differences may lead to cannabis dependence symptoms through self-medication of cannabinoid signaling, impulsivity, and/or the removal of negative affect, consistent with recent theories of the transition to dependence (Koob & Volkow, 2010).

## The Present Study

Based on prior evidence (a) supporting the role of the eCB system and related genes in cannabis dependence, (b) that childhood adversity confers risk for substance use disorders, and (c) of interactions between the eCB system and neural systems underlying threat and stress responsivity, we first examined whether CSA moderates associations between genetic variation across the eCB system and cannabis dependence symptoms (Sample 1 $N = 1,558$). Second, we attempted to replicate any interactions with sexual/physical abuse in an independent sample (Sample 2 $N = 859$). Third, in light of evidence that the eCB system plays a prominent role in fear extinction and amygdala-driven stress reactivity, we examined whether any variants associated with cannabis dependence symptoms in the context of CSA predicted early life stress (ELS)-related differences in amygdala habituation (Sample 3 $N = 312$). Independent and dependent variables used across studies are described in Table 2. In addition to traditional single-SNP analyses within the eCB system, we employed gene-based analyses in our discovery sample that aggregated effects across SNPs within a gene.

## Methods

### Comorbidity and Trauma Study (CATS; *N* = 1,558)

Australian adults of European ancestry who completed the Comorbidity and Trauma Study (CATS) and had data on childhood sexual abuse and cannabis use were considered for analyses (*N* = 1,621). Of these participants, 96.1% reported having ever used cannabis; as such, those who did not report using cannabis were excluded from subsequent analyses, leaving a final *N* of 1,558 (Table 3). CATS is a case-control study of opioid-dependent individuals (primarily heroin, *n* = 1,189), aged 18 or older, who were recruited from clinics in the greater Sydney region at which they received opioid substitution therapy (for additional details: Nelson et al., 2013, 2014; Shand, Degenhardt, Slade, & Nelson, 2011) Neighborhood controls (*n* = 369) who had little or no lifetime history of recreational opioid use were recruited from socially disadvantaged neighborhoods in geographic proximity to locations where cases had been recruited. Participants were excluded for recent suicidal intent and current psychosis. Institutional Review Board (IRB) approval was obtained from University of New South Wales, Washington University School of Medicine, QIMR Berghofer Medical Research Institute, and Sydney area health service ethics committees.

**Measures—**A modified version of the Semi-Structured Assessment for the Genetics of Alcoholism was used to assess DSM-IV psychiatric diagnosis (SSAGA-OZ; Bucholz et al., 1994; Heath et al., 2011). CSA was defined using 6 items that assessed unwanted exposure to another person's genitals, touching of breasts and/or genitalia, threats regarding sexual activity, and attempting to or having vaginal, oral, or anal sex, all prior to age 18. Items were averaged and then log-transformed to create a continuous CSA score (log-transformed: *M* = 0.09, *SD* = 0.11; untransformed: *M* = 0.26, *SD* = 0.34; raw: *M* = 1.57, *SD* = 2.04; distribution: 0 *n* = 806, 1 *n* = 184, 2 *n* = 114, 3*n* = 111, 4 *n* = 114, 5 *n* = 127, 6 *n* = 102). Our outcome variable of interest, cannabis dependence symptoms, was defined as the number of lifetime DSM-IV dependence symptoms reported[2] (Table 3; *M* = 2.95, *SD* = 2.15). The distributions of dependence symptoms across genotype and CSA groups are given in the Supplement (Table S1).

**Gene and SNP selection and gene-level testing—**Details regarding genotyping using a GWAS array are available in supplemental text. SNPs (MAF > .05; call rate > 95%) within six endocannabinoid-related genes (*CNR1*, *FAAH*, *MGLL*, *DAGLA*, *DAGLB*, and *NAPEPLD*), ±10kbps to include both promoter and flanker regions, were extracted into a gene set using PLINK (v1.07; http://pngu.mgh.harvard.edu/purcell/plink/; Purcell et al., 2007). *CNR2* was not included in our analysis due to lack of coverage on the array. The resulting 79 SNPs were then pruned for independence (50-SNP window shifted 5 SNPs at each step, linkage disequilibrium $r^2$ threshold = .80), which resulted in a pruned set of 65 independent SNPs (excluding *CNR2*), ranging from 3 (*NAPEPLD*) to 24 (*MGLL*) within each gene (Table S2).

[2]DSM-IV cannabis dependence symptoms: 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.

The procedure for set-based testing in PLINK was modified and adapted for these analyses[3] (Perlis et al., 2008; Purcell et al., 2007). In the modified set-based approach used here, a null distribution was formed via 100,000 label-swapping permutations, in which the outcome phenotype (dependence symptoms), CSA score, and covariates of no interest were permuted together. A participant's full genome was left intact to preserve the linkage structure across individual SNPs during permutations.

Once the relevant data were permuted, statistical tests were performed for each permuted dataset. Ordinary least squares regression was used to test whether the interaction between CSA and each SNP within each gene was associated with cannabis dependence symptoms. Analyses of main effects were conducted in the same manner and were controlled for in interaction testing. Case status (opioid dependent vs. non-dependent), sex, age quintile, and three ancestrally informative principal components were entered as covariates for all analyses. In accordance with recommendations for GxE analyses, additional covariates were entered representing the interactions between the SNP and each covariate of no interest as well as CSA score and each covariate of no interest (Keller, 2014). In order to restrict the gene-level test statistic to only potentially informative SNPs and thus minimize the influence of gene size and within-gene linkage disequilibrium patterns on significance, the maximum number of SNPs within each gene passing the nominal uncorrected significance threshold of $p < .05$ in a single permutation was used as the number of SNPs ($N_{maxSNPs}$) across which the SNP-level $\Delta R^2$ values were averaged to form gene-level statistics (Gene-Stats). For example, the gene *MGLL* contained 24 SNPs, but the maximum number of nominally significant SNPs obtained in any one of the 100,000 permutations was 14, so only the 14 highest $\Delta R^2$ values in the original analysis and each permutation were averaged to obtain the gene-level -statistic. In PLINK, in contrast, an arbitrary SNP cutoff across all genes would have been pre-specified.

The gene-level empirical *p*-value was defined as the proportion of times the original Gene-Stat exceeded a permuted Gene-Stat. Bonferroni correction was then used to adjust for the 6 genes under study, with a final *p*-threshold $p < .0083$ (0.05/6). As a corollary to these gene-based analyses, individual SNPs were also tested for significance using a significance threshold of $p < .0008$ ($\alpha$=.05/65 SNPs). All statistical analyses were coded in Python (v. 2.7.6) using the Numerical Python (“NumPy”, v.1.7.1), StatsModels (v.0.5.0), and Python Data Analysis (“pandas”, v.0.12.0) libraries. Follow-up analyses of individual SNPs were conducted using the PROCESS (Hayes, 2013) and MODPROBE (Hayes & Matthes, 2009) macros in SPSS (v.22).

### Study of Addiction: Genetics and Environment (SAGE; *N* = 859)

European-American[4] participants with genotypic and interview data were obtained from the Study of Addiction: Genetics and Environment (SAGE; Bierut et al., 2010). SAGE was funded as part of the Gene Environment Association Studies (GENEVA) initiative

[3]The set-based testing procedure in PLINK v 1.07 does not allow for tests of GxE interactions and uses an averaging procedure that is sensitive to differences in gene size, which was undesirable for the eCB gene set, in which the number of tagged SNPs within each gene, post-pruning for quality assurance and linkage disequilibrium, ranged from 3 to 24.

[4]Only participants of European ancestry were selected in our replication and follow-up samples in order to be consistent with the ancestral composition of CATS.

supported by the National Human Genome Research Institute (dbGaP study accession phs000092.v1.p1). For this study, alcohol dependent and control participants were recruited from three large, complementary datasets ascertained for alcohol (Collaborative Study of the Genetics of Alcoholism; COGA; Foroud et al., 2000; T. Reich et al., 1998), nicotine (Collaborative Study of the Genetics of Nicotine Dependence; COGEND; Bierut et al., 2007) and cocaine (Family Study of Cocaine Dependence; FSCD; Bierut, Strickland, Thompson, Afful, & Cottler, 2008) dependence. Childhood abuse data were only collected in FSCD and from a subset of COGA participants, constraining our analyses to 859 participants (Table 3). Genotyping details are available in the Supplement. The top SNP from CATS, rs604300 in *MGLL* (call rate = 100%, HWE $p = 0.60$, MAF = 0.11), was the only variant examined for the purposes of replication within SAGE and was coded as major allele homozygotes (GG) and A allele carriers (AA/AG) due to the rareness of A allele homozygosity ($n = 8$). The Institutional Review Board at each contributing institution reviewed and approved the protocols for genetic studies under which all participants were recruited.

**Measures—**All three participating studies (COGA, COGEND, and FSCD) used the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994). Childhood abuse (CA) was dichotomously coded into participants reporting no physical or sexual abuse ($n = 549$) or at least one instance of physical or sexual abuse, defined as physical injury by or forced sexual contact with a relative or someone other than a family member before the age of 16 ($n = 310$). Physical abuse was included due to the smaller sample and limited sexual abuse of the sample. Of our final participants, 65.7% reported having ever used cannabis. As in CATS, dependence symptoms were defined as the number of lifetime DSM-IV dependence symptoms reported (Table 3; $M = 1.22$, $SD = 2.00$); due to the relatively small sample size, individuals who had never used cannabis were included in these analyses and coded as “0” dependence symptoms. Distributions of cannabis dependence symptoms across genotype and CA groups are provided in the Supplement (Table S3).

**Statistical analyses—**Ordinary least squares regression was used to test the association of cannabis dependence symptoms with the interaction between rs604300 genotype and CA. Sex, age quartiles, one ancestrally informative principal component, and study of origin (FSCD or COGA) were included as covariates. As in CATS, additional covariates were entered representing the interactions between rs604300 and each covariate of no interest as well as CA and each covariate of no interest (Keller, 2014).

### Duke Neurogenetics Study (DNS; *N* = 312)

The Duke Neurogenetics Study (DNS) assesses a wide range of behavioral, experiential, and biological phenotypes among young-adult (aged 18-22) college students (Gorka, Knodt, & Hariri, 2014; Corral-Frías et al., 2015). European-American participants who completed the ongoing DNS for whom overlapping fMRI threat-related amygdala reactivity and genetic data were available as of January 6, 2014, were included in analyses ($N = 325$). Participants provided informed written consent prior to participation and were in good general health and free of DNS exclusion criteria: (1) medical diagnosis of cancer, stroke, diabetes requiring

insulin treatment, chronic kidney or liver disease or lifetime psychotic symptoms; (2) use of psychotropic, glucocorticoid or hypolipidemic medication, and (3) conditions affecting cerebral blood flow and metabolism (e.g., hypertension). Current DSM-IV Axis I and select Axis II disorders (i.e., Antisocial Personality Disorder and Borderline Personality Disorder) were assessed with the electronic Mini International Neuropsychiatric Interview (Sheehan, 1998) and Structured Clinical Interview for the DSM-IV Axis II (SCID-II; First, Gibbon, Spitzer, Williams, & Benjamin, 1997). These disorders are not exclusionary, as the DNS seeks to establish broad variability in multiple behavioral phenotypes related to psychopathology. Participants were excluded ($n$ = 13) for quality issues in data collection: a large number of movement outliers in fMRI data ($n$ = 3), inadequate signal in our regions of interest ($n$ = 6), and poor behavioral performance ($n$ = 4). This resulted in a final sample for analysis of 312 (Table 3). Details regarding genotyping are available in the Supplement. The top SNP from CATS, rs604300 in *MGLL* (call rate = 100%, HWE $p$ = 0.75, MAF = 0.10), was the only variant examined for the purposes of these analyses; due to its low minor allele frequency and the resulting small number of minor homozygotes in DNS ($n$ = 2), AA/AG participants were combined to form a minor allele carrier group ($n$ = 61).

**Self-report measures—**Participants completed a battery of self-report questionnaires to assess past and current experiences and behavior. For the present analyses, the Childhood Trauma Questionnaire: Short Form (CTQ-SF; Bernstein et al., 2003) was used to measure ELS. The CTQ-SF is a 28-item, retrospective screening tool used to detect the occurrence and frequency of emotional, physical, and sexual abuse, as well as emotional and physical neglect before the age of 17. The CTQ has high test-retest reliability (i.e., coefficients ranging from .79 to .86; Bernstein & Fink, 1998) and internal consistency (i.e., coefficients ranging from .66 to .92; Bernstein & Fink, 1998; Scher, Stein, Mccrearyp, & Forde, 2001), and it correlates with both a clinician-rated interview of childhood abuse and independent therapists' ratings of abuse (Bernstein & Fink, 1998; Bernstein et al., 2003). Though a sexual abuse subscore was available, only 3.8% ($n$ = 12) of the final sample reported any form of sexual abuse. As such, an aggregate score of all CTQ-SF questions was used as an index of ELS. As in CATS, this measure was log-transformed to reduce skew (log-transformed: $M$ = 1.49, $SD$ = 0.08; untransformed: $M$ = 31.18, $SD$ = 6.68, where a score of 25 indicates no endorsement of any stressor). These scores are comparable to those obtained in other community (e.g., metropolitan Memphis, Tennessee area, $N$ = 1,007, $M$ = 31.7; Scher et al., 2001) and college samples (e.g., UCSD; $N$ = 949, $M$ = 35.2; Wright et al., 2001). DSM-IV diagnoses of cannabis dependence were available for participants; however, only 1.6% of the sample met diagnostic criteria, so an analysis of cannabis dependence symptomatology was not feasible (Table 3).

**BOLD fMRI corticolimbic reactivity paradigm—**A widely used and reliable challenge paradigm was employed to elicit amygdala reactivity. The paradigm consists of 4 task blocks requiring face-matching interleaved with 5 control blocks requiring shape-matching. In each face-matching trial within a block, participants view a trio of faces derived from a standard set of facial affect pictures (Ekman & Friesen, 1975; expressing angry, fearful, surprised, or neutral emotions) and select which of 2 faces presented on the bottom row of the display matches the target stimulus presented on the top row. Each emotion-specific

block (e.g., fearful facial expressions only) consists of 6 individual trials, balanced for gender of the face. Block order is pseudo-randomized across participants. Each of the 6 face trios is presented for 4 seconds with a variable inter-stimulus interval of 2-6 seconds; total block length is 48 seconds. In the shape-matching control blocks, participants view a trio of geometric shapes (i.e., circles, horizontal and vertical ellipses) and select which of 2 shapes displayed on the bottom matches the target shape presented on top. Each control block consists of 6 different shape trios presented for 4 seconds with a fixed inter-stimulus interval of 2 seconds, comprising a total block length of 36 seconds. The total paradigm is 390 seconds in duration. Reaction times and accuracy are recorded through an MR-compatible button box.

Details regarding BOLD fMRI acquisition and analysis may be found in the Supplement. Briefly, amygdala habituation to threat-related stimuli, our outcome variable of interest in this analysis, was calculated as the linear decrease in reactivity over successive face-matching blocks (i.e., block 1 > block 2 > block 3 > block 4). BOLD paramete estimates from clusters within the left and right basolateral amygdala ROIs exhibiting a main effect for the habituation contrast were extracted using the VOI tool in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and exported for regression analyses. Extracting parameter estimates from clusters activated by our fMRI paradigm, rather than those specifically correlated with our independent variables of interest, precludes the possibility of any correlation coefficient inflation that may result when an explanatory covariate is used to select a region of interest. We have successfully used this strategy in prior studies (Bogdan, Williamson, & Hariri, 2012). The distribution of this outcome measure across genotype and ELS groups is reported in the Supplement (Table S4).

**Statistical analyses—**Ordinary least squares regression was used to test the association of rs604300, ELS, and threat-related amygdala habituation. To maintain variability but constrain the influence of extreme outliers, prior to analyses all variables were Winsorized (i.e., outliers more than ± 3 standard deviations from the mean were set at ± 3 standard deviations from the mean; habituation $n = 1$; ELS $n = 2$). Sex and two ancestrally informative principal components were entered as covariates for all analyses. As in CATS and SAGE, additional covariates were entered representing the interactions between rs604300 and each covariate of no interest as well as ELS and each covariate of no interest (Keller, 2014).

## Results

### CATS

No nominally significant gene-level or SNP-level main effects on cannabis dependence emerged. Of the 6 eCB genes tested, only *MGLL* demonstrated a significant interaction with CSA predicting symptoms of cannabis dependence (Table 4; Figure S1; Gene-Stat = 0.002 using top 14 SNPs; 6 of 24 SNPs nominally significant; $p = .0085$. Inspection of individual SNPs across the entire eCB gene set revealed one SNP, rs604300 within *MGLL*, that survived SNP-level Bonferroni correction (Table 5; Table S5; Figure 1; $b_{GxE} = -4.37$, 95% CI [-6.69, -2.05], $\Delta R^2 = .007$, $\Delta F(1,1527) = 13.69$, $p = .0002$). Within the full rs604300

model, and consistent with prior literature (Duncan et al., 2008), there was an overall main effect of CSA on cannabis dependence symptoms, such that increasing exposure to CSA was associated with a greater number of dependence symptoms ($b_E$ = 2.73, 95% CI [1.72, 3.73], $p$ < .001). However, this effect was only present in G allele homozygotes ($b_E$ = 3.68, 95% CI [2.56, 4.81], $p$ < .001); there was no association between CSA and cannabis dependence symptoms in heterozygotes ($b_E$ = -0.69, 95% CI [-2.75, 1.38], $p$ = .515), and a negative relationship between CSA and cannabis dependence symptoms in A allele homozygotes ($b_E$ = -5.06, 95% CI [-9.30, -0.81], $p$ = .020). Post-hoc Johnson-Neyman tests revealed that the association between genotype and cannabis dependence symptoms was significant at mean-centered log-transformed CSA values of 0.11 and above, corresponding to the endorsement of 1.69 or more forms of childhood sexual abuse. There was no main effect of rs604300 genotype on cannabis dependence symptoms ($b_G$ = -0.14, 95% CI [-0.37, 0.08], $p$ = .218), nor was there evidence that genotype was associated with CSA ($b_G$ = 0.002, 95% CI [-0.01, 0.01], $p$ = .745).

## SAGE

The interaction between rs604300 genotype and CA was significantly associated with cannabis dependence symptoms in SAGE (Table S6; Figure 2; $b_{GxE}$ = -0.75, 95% CI [-1.42, -0.09], $\Delta R^2$ = .005, $\Delta F(1,837)$ = 4.95, $p$ = 0.026). As in CATS, CA was associated with greater endorsement of DSM-IV cannabis dependence criteria ($b_E$ = 0.98, 95% CI [0.72, 1.24], $p$ < .001), but this effect was observed only in G homozygotes ($b_E$ = 1.13, 95% CI [0.84, 1.42], $p$ < .001), with no association between CA and cannabis dependence symptoms among carriers of the minor A allele ($b_E$ = 0.38, 95% CI [-0.22, 0.98], $p$ = .215). Genotype did not predict CA ($b_G$ = -0.02, 95% CI [-0.10, 0.61], $p$ = .640), but, unlike in CATS, there was a significant main effect of genotype on cannabis dependence symptoms, such that A allele carriers had relatively greater symptoms ($b_G$ = 0.32, 95% CI [0.02, 0.63], $p$ = .039), though post-hoc tests indicated this effect was significant only among participants reporting no CA ($b_G$ = 0.59, 95% CI [0.20, 0.98], $p$ = .003)[5].

## DNS

Consistent with prior work, we found evidence of threat-related amygdala habituation, whereby amygdala activation decreased across blocks (Figure 3a). A Genotype × ELS interaction predicted threat-related habituation in the right, but not left, basolateral amygdala (Right: Table S7; Figure 3b; $b_{GxE}$ = 0.77, 95% CI [0.01, 1.53], $\Delta R^2$ = .013, $\Delta F(1,299)$ = 3.97, $p$ = .047; Left: $b_{GxE}$ = -0.05, 95% CI [-0.70, 1.70], $\Delta R^2$ < .001, $\Delta F(1, 299)$ = 0.01, $p$ = 0.915). A allele carriers, who were protected against the effects of CA on cannabis dependence symptoms within CATS and SAGE, displayed heightened right basolateral amygdala habituation in the context of increased ELS ($b_E$ = 0.82, 95% CI [0.14, 1.51], $p$ = .018); G homozygotes, who were vulnerable to cannabis dependence in the context of childhood abuse in the prior two samples, did not exhibit a relationship between ELS and amygdala habituation ($b_E$ = -0.20, 95% CI [-0.28, 0.40], $p$ = .744). Post-hoc Johnson-Neyman tests revealed that the association between genotype and amygdala habituation was

[5]A similar pattern was observed in CATS, wherein the A allele was associated with greater cannabis dependence symptoms in participants reporting no CSA, but this effect was not significant ($b_G$ = 0.24, 95% CI [-0.06, 0.54], $p$ = .120).

significant at mean-centered log-transformed CTQ values of -0.06 (i.e., raw score of 26.68, where the minimum possible score is 25) and below. This pattern is consistent with SAGE, in which, among individuals reporting no CA, A allele carriers had relatively higher cannabis dependence symptoms. The main effects of ELS and genotype on habituation were not significant, and, as in both CATS and SAGE, genotype was not associated with ELS (all *p*s > 0.10).

## Post-hoc Structural Equation Modelling

Given the replicated associations between rs603600 and childhood adversity predicting cannabis dependence symptoms and the biological extension of that interaction to predict right basolateral amygdala habituation, a post-hoc structural equation model (SEM) was tested in an opioid-dependent subset of CATS (*n* = 1,182) that integrated a dichotomous measure of using cannabis to control mood[6] (see Supplement for additional methodological details; Figure 4). The rs604300 × CSA interaction significantly predicted using cannabis to control mood ($b_{GxE}$ = -2.04, 95% CI [-3.78, -0.20], *p* = .025), which, in turn, significantly predicted cannabis dependence symptoms ($b_M$ = 0.84, 95% CI [0.71, 0.96], *p* < .001). The rs604300 × CSA interaction was specific to cannabis: it did not predict the use of other types of substances, or of substances in general, to control mood (all *ps*> 0.20). The SEM overall demonstrated good fit (relative $\chi^2$< 0.001, RMSEA < 0.001, WRMR = 0.40, CFI = 1.00). The indirect pathway from the GxE interaction to cannabis dependence symptoms through using cannabis to control mood was also significant ($b_{IND}$ = -1.71, 95% CI [-3.26, -0.21], *p* = .029). Analyses of indirect effects within genotype groups revealed that the indirect effect of CSA on cannabis dependence symptoms through using cannabis to control mood was significant only among G allele homozygotes (GG: $b_{IND}$ = 1.85, 95% CI [1.17, 2.47], *p* < .001; AA/AG: $b_{IND}$ = 0.13, 95% CI [-1.24, 1.27], *p* = .851). These results are consistent with the proposed hypothesis of differential sensitization to stress across rs604300 genotypes leading to stress-related coping with cannabis and thus susceptibility to dependence.

## Epigenetic Annotation

The WashU EpiGenome Browser (http://epigenomegateway.wustl.edu/browser/; Zhou et al., 2011, 2013, in press) was used to annotate the epigenetic landscape surrounding rs604300. As described in detail in the Supplement, we found evidence that this SNP resides just downstream of epigenetic histone modifications (i.e., H3K4me1, H3K27ac) within a functional enhancer that likely upregulates gene expression. There was evidence of tissue specificity wherein enrichment is highest in neural tissue. Moreover, there was evidence that methylation and histone modification in this epigenetically-regulated enhancer region of *MGLL* may play role in *MGLL* transcription (Figure 5 Figure S8). As such, it is possible that environmentally-mediated individual differences in methylation and histone modification-related enhancement of this region may produce differential *MGLL* expression in the brain. Rs604300 is located near this epigenetically-regulated enhancer; while we are unable to test for genotype-dependent differences in methylation and histone modification with the data available to us, this annotation suggests that rs604300 genotype itself or a SNP in LD with it

[6]The question of interest ("Have you frequently used [cannabis] to control your mood or to chase another drug?") was asked in a section of the interview only completed by participants meeting criteria for opioid dependence. See Supplement for full context.

may impact *MGLL* expression by affecting epigenetic modification within this *MGLL* enhancer.

## Discussion

Despite the fundamental link between cannabis and the eCB system, human genetics studies have not reliably linked eCB genetic polymorphisms to cannabis-related outcomes (Agrawal & Lynskey, 2009; Verweij et al., 2012). Here, we found evidence that rs604300 genotype in *MGLL* moderates the relationship between CSA and cannabis dependence symptoms. Those who carried two copies of the major G allele were at increased risk for cannabis dependence as a function of increasing exposure to CSA events. This finding was not surprising, as CSA has been widely documented as a potent contributor to the etiology of a host of addictive behaviors, including cannabis dependence (Duncan et al., 2008; Fergusson et al., 1996; Myers & Prescott, 2000; Nelson et al., 2006; Simpson & Miller, 2002). What was particularly intriguing was that rs604300 minor A allele carriers did not demonstrate increased vulnerability to cannabis dependence symptoms in the presence of CSA exposure. This alludes to the potential buffering effects of the A allele in rs604300 on the pathogenic effects of CSA. This is highly congruent not only with one prior study of eCB genotypes and their interplay with childhood trauma to predict anhedonia (Agrawal, Nelson, et al., 2012), but also complies with observations in rodent paradigms documenting the role of eCBs in stress adaptation (e.g., Gunduz-Cinar, MacPherson, et al., 2013; Hill & Gorzalka, 2006; Hill & McEwen, 2010; Hill, Patel, et al., 2010; Patel, Roelke, Rademacher, Cullinan, & Hillard, 2004; Viveros, Marco, & File, 2005). Importantly, our finding of increased amygdala habituation as a function of ELS in minor A allele carriers, but not in GG individuals, reinforces the possibility that *MGLL* rs604300 genotype may play a key role in decoupling the neurobiological link between ELS and mental health outcomes in later life. Such increased amygdala habituation to threatening stimuli may be reflective of an adaptive response that could potentially promote fear extinction when a threat is no longer imminent and, speculatively, a decreased reliance on substances for affect regulation. In support of this hypothesis, a post-hoc SEM demonstrated that the pathway from CSA to cannabis dependence symptoms was partially mediated by using cannabis to control one's mood in GG individuals only. Notably, however, this interpretation remains speculative and must be tempered by our inability to temporally disentangle using marijuana to change one's mood from cannabis dependence symptoms.

In interpreting these results, it is important to note that, consistent with prior behavioral, psychiatric, and neuro-genetics studies (Duncan & Keller, 2011; Flint & Munafo, 2013; Hibar et al., 2015), the effects reported in this study are small, explaining only 0.5-1.3% of variation in our outcome phenotypes. As such, these findings alone will not be practically informative at an individual level. Additionally, evidence suggests that many published GxE results may represent type-I errors, leading to low replication rates (Duncan & Keller, 2011). Interactions between genes and environment, relative to main effects of genotype, are particularly susceptible to increased rates of false positives due to low prior probabilities of interaction, reduced variance of the interaction term, and the potential for unmodeled nonlinearity (see Dick et al., 2015, for review). However, within the current study, the convergence of evidence across three samples implicating rs604300 in individual

differences in both cannabis dependence symptoms and a psychiatrically relevant neural phenotype in the context of childhood adversity pinpoints a target within the endocannabinoid system worthy of future replication and extension.

## Sensitivity to Stress and Vulnerability to Problematic Cannabis Use: The Importance of 2-AG and MAGL

A majority of research evaluating the role of eCB signaling in stress responsiveness has focused on CB1-AEA activity (Gunduz-Cinar, Hill, et al., 2013; Gunduz-Cinar, MacPherson, et al., 2013). However, emerging rodent research also suggests that 2-AG, which is increased following stress exposure, may be particularly important for recovery from stress's anxiogenic effects (Patel et al., 2009). Consistent with this idea, selective MAGL inhibitors acutely reduce anxiety (Busquets-Garcia et al., 2011; Kinsey, O'Neal, Long, Cravatt, & Lichtman, 2011), with evidence that chronic MAGL inhibition prevents the development of stress-related anxiety, potentially by blocking stress-induced long-term depression of inhibitory signaling in the BLA (Sumislawski, Ramikie, & Patel, 2011).

Interestingly, MAGL inhibition also mimics THC, particularly its anxiolytic and antidepressant-like properties (Wiley et al., 2014), and attenuates cannabis withdrawal in rodents (Schlosburg et al., 2009). Moreover, much like stress, chronic exposure to THC in rodents is associated with desensitization of CB1 receptors and reduction in AEA and 2-AG. Consistent with the potential importance of MAGL in problematic cannabis use, two independent prior cannabis dependence linkage studies have identified the chromosomal region on 3p where *MGLL* resides (Agrawal et al., 2008; Hopfer et al., 2007). Thus, there is considerable evidence that MAGL, in concert with FAAH and CB1, plays a critical role in the behavioral experiences associated with THC, particularly its effects on mood.

Childhood stress, particularly sexual abuse, is amongst the most prominent risk factors for problems related to mood and anxiety as well as cannabis dependence (Duncan et al., 2008; Lindert et al., 2014). The persistent use and misuse of drugs in individuals exposed to CSA, as well as to childhood physical abuse, may reflect coping behavior (Bujarski et al., 2012; Potter, Vujanovic, Marshall-Berenz, Bernstein, & Bonn-Miller, 2011; Vilhena-Churchill & Goldstein, 2014; Walsh et al., 2014), and this relationship may be susceptible to eCB-regulated modulation in the BLA. Speculatively, individuals with increased MAGL function, corresponding to reduced availability of 2-AG, may be more prone to poorer stress recovery, which could lead to stress-induced elevations in anxiety and resulting emotion regulation with cannabis. Conversely, if the minor A allele of rs604300 is associated with MAGL reductions, then this stress-adaptive state, as reflected by enhanced amygdala habituation in the context of prior stress exposure, might result in stress adaptation, and downstream, with less problematic cannabis use. This also fits well with our pathway analysis, as well as with rodent studies showing the involvement of the eCB in extinction of aversive memories (de Bitencourt, Pamplona, & Takahashi, 2013).

A challenge associated with this interpretation is that the functional consequences of rs604300 are not understood. Our examination of gene expression data (publically available at: http://www.braineac.org/) suggest that rs604300 genotype is not associated with global differences in *MGLL* expression (Hardy, Trabzuni, & Ryten, 2009; Ryten, Trabzuni, &

Hardy, 2009). However, if rs604300-related reductions in MGLL expression only occur in the context of childhood adversity, then its main effects may not be observable in curated expression datasets from the general population. Given the exquisite plasticity of eCB signaling in the context of stress, we speculate that the action of rs604300 on cannabis dependence may be epigenetic in nature. While intronic, rs604300 is located just downstream of an enhancer site that is heavily influenced by epigenetic modification (see Supplement). As such, it is possible that rs604300 may result in epigenetically-dependent differences in expression. Future epigenetic research in the context of early life adversity with this locus may be promising.

## Limitations

While the interaction between rs604300 genotype and CA reached significance in SAGE, there are several caveats to interpreting this replication. First, due to the low prevalence of CSA (20.0%), childhood abuse was defined in SAGE as exposure to sexual or physical abuse. This is in contrast to CATS, where individuals reported experiencing 1.57 CSA events on average. These substantial differences in childhood abuse experiences across samples could have resulted in the regions of significance differing across studies (i.e., genotype effects only at higher levels of CSA in CATS and only among the no-CA group in SAGE and low ELS group in DNS). Furthermore, due to heterogeneous measurement of CA across the studies contributing to the SAGE sample, CA was dichotomized (present versus absent), thus resulting in a loss of power when compared to the continuous measure used in CATS. Relatedly, as the minor allele is rare, AA and AG individuals were combined in SAGE. As shown in Figures S5 and S6, restricting the sample to CSA alone or examining AA and AG individuals separately did not alter the nature of the interaction; however, we did not consider these findings to be reliable due to the modest sample size (Table S3). Conversely, assuming a dominant model in CATS (i.e., examining AA/AG individuals together) or using a combined measure of childhood physical and sexual abuse resulted in little change to the nature or significance of the interaction (dominant model: $b_{GxE}$ = -4.62, 95% CI [-7.01, -2.22], $\Delta R^2$ = .008, $\Delta F(1,1527) = 14.29$, $p$ = .0002; combined CSA and CPA: $b_{GxE}$ = -3.18, 95% CI [-6.23, -0.13], $\Delta R^2$ = .002, $\Delta F(1,1527) = 4.18$, $p$ = .041).

A second important distinction between CATS and SAGE relates to the distribution of cannabis dependence symptoms. Due to study design (i.e. opioid dependent cases and controls from high risk neighborhoods), the rate of lifetime cannabis use in CATS was 96.1%, and a majority of the participants reported experiencing cannabis dependence symptoms. Despite the oversampling for alcohol dependence in SAGE, rates of cannabis use and endorsement of dependence symptoms were lower, presumably due to the control group, which was not environmentally matched to the alcoholic cases. This necessitated the inclusion of never-users of cannabis in the replication analyses; however, restricting analyses to ever users produced a similar, albeit non-significant, pattern of results (Figure S7). The distinction between the phenotypes used in CATS and SAGE is nontrivial. By including never-users in SAGE, the effects of genotype and environment and their interplay on later, more problematic stages of cannabis involvement (i.e. dependence symptoms) cannot be disentangled from their effects on cannabis use. As a corollary, the high rate of cannabis use in CATS precluded any study of contributors to its variance. Therefore, we can

only conclude that the interaction between rs604300 and childhood abuse may be related to both onset of cannabis use as well as misuse. This is consistent with twin epidemiological studies that document a high degree of genetic overlap between these stages of cannabis involvement (Agrawal et al., 2012).

Third, we were unable to link individual differences in amygdala habituation as a result of rs604300 and childhood trauma interplay to cannabis dependence. The extent of problematic cannabis use is limited in this college-attending sample – only 48.4% of the EA participants report using cannabis even once in their lifetime, and, of those, only 23.7% report using it >10 times, with 4 individuals meeting criteria for cannabis dependence. Because THC administration reduces anxiety in rodents and amygdala reactivity to threatening stimuli, it is possible that individuals who show prolonged amygdala response (i.e., decreased amygdala habituation), may be more sensitive to the coping-related effects of cannabis and thus more likely to develop problematic cannabis usage (Phan et al., 2009; Koob & Volkow, 2010). Evidence that fear extinction and drug-seeking extinction rely on similar neural pathways also suggests that individuals who can more readily adapt to threatening stimuli may also be more able to extinguish substance use (Peters, Kalivas, & Quirk, 2009). On the other hand, it is also entirely possible that increased amygdala habituation might lead to more exploratory behavior and exposure to substances, but not necessarily increased problematic use (Lissek et al., 2005).

Fourth, while we conducted a system-level analysis, our gene-based testing did not reach statistical significance after Bonferroni correction. The single SNP that did survive correction, rs604300, is unlikely to be the only important variant within the eCB system. The balance between AEA (metabolized by FAAH) and 2-AG may be paramount to understanding eCB system contributions to psychiatric disorders and related brain function. A particularly fruitful future approach may be to examine evidence for epistasis using system-level analytic methods that do not rely on SNP-level priors or on the additive nature of the current approach (e.g., random forest regression trees; for review, see Upstill-Goddard, Eccles, Fliege, & Collins, 2013).

## Summary

Limitations notwithstanding, this study finds evidence across two samples that the minor A allele of rs604300 within *MGLL* exerts protective effects against childhood abuse-related increases in cannabis dependence. We further extend these findings to show that rs604300 genotype interacts with early life stress to predict amygdala habituation, providing a neural mechanism for future study in the context of cannabis dependence symptoms. Consistent with the proposed hypothesis that cannabis dependence symptoms may arise from using cannabis to cope with stress, a post-hoc structural equation model showed that reporting that one uses cannabis to alter one's mood indirectly linked the interaction between rs604300 and childhood sexual abuse to cannabis dependence symptoms only among G allele homozygotes. These findings await further replication and validation from independent studies.

This study highlights the importance of considering the role of genotype in stress adaptation or resilience. In addition to its clearly demonstrated impact on energy regulation/obesity and

pain modulation (André & Gonthier, 2010; Iversen & Chapman, 2002; Li, Jones, & Persaud, 2011; Lynch & Ware, 2015), there is considerable research underway exploring the therapeutic role of eCB signaling in treatment of mental health problems (Hill & Gorzalka, 2009), including addiction (e.g., Pava & Woodward, 2012). For example, MAGL inhibition attenuates the effects of withdrawal in opioid-, nicotine-, and THC-dependent rodents (Muldoon et al., 2015; Ramesh et al., 2011, 2013; Schlosburg et al., 2009). If eCB signaling is associated with individual differences in stress adaptation, then medications directed at enhancing these effects may be a valuable resource in the treatment arsenal. However, manipulation of the eCB system is challenging and prior failed clinical trials documenting serious adverse psychiatric events, including suicide, in cardiovascular risk patients treated with CB1 antagonist, rimonabant, serves as a cautionary tale (Boekholdt & Peters, 2010; Topol et al., 2010).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## General Scientific Summary

This study suggests that genetic variation within the endocannabinoid system confers differential susceptibility to cannabis dependence symptoms in the context of early life adversity. These effects may arise through associations with threat-related brain function and substance-related coping.

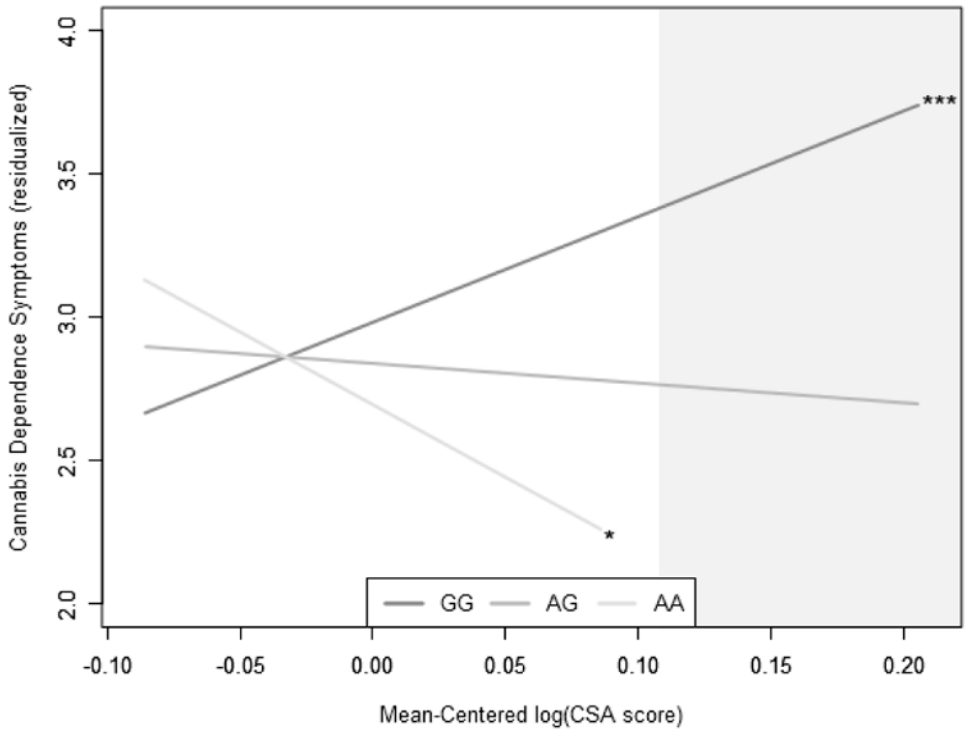


**Figure 1.**

Interaction of rs604300 with childhood sexual abuse to predict cannabis dependence symptoms in CATS. X-axis denotes the log of a participant's CSA score, while y-axis indicates the residualized number of DSM-IV cannabis dependence symptoms endorsed by a participant. Shaded area indicates Johnson-Neyman region of significance. For raw data, see Figure S2. CSA = childhood sexual abuse.

* $p < .05$. ** $p < .01$. *** $p < .001$.

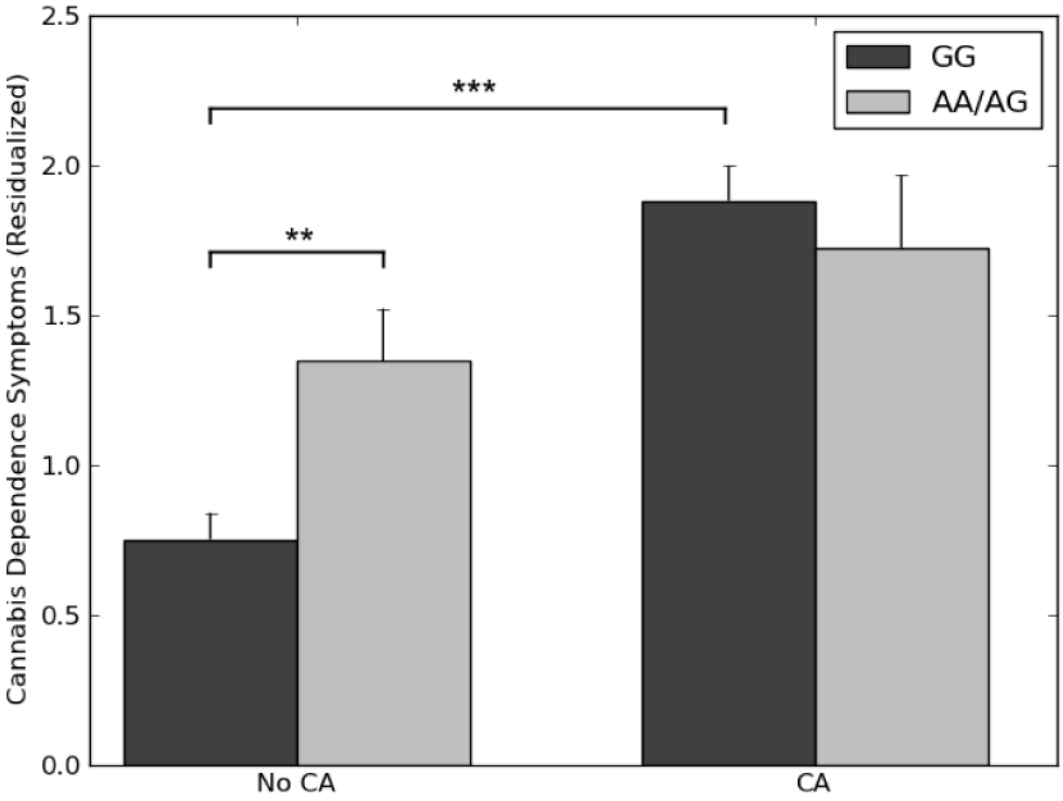


**Figure 2.**

Interaction of rs604300 with childhood abuse to predict cannabis dependence symptoms in SAGE. X-axis denotes whether or not a participant experienced abuse before the age of 16, while y-axis indicates residualized number of DSM-IV cannabis dependence symptoms endorsed by that participant. Error bars represent the SEM. For raw data, see Figure S3. CA = childhood abuse.

* $p < .05$. ** $p < .01$. *** $p < .001$.

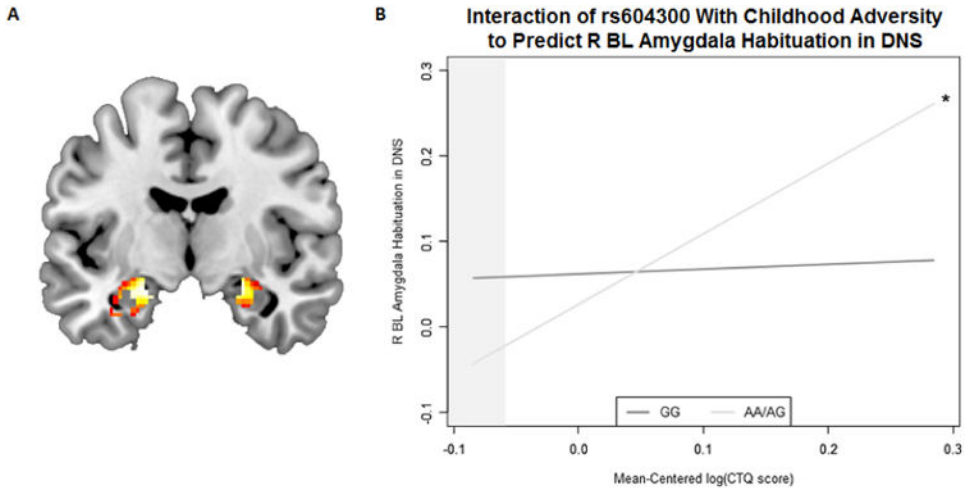


**Figure 3.**

Main effects of fMRI task and interactions with rs604300 to predict threat-related amygdala habituation. A) Bilateral threat-related basolateral amygdala habituation (block 1 > block 2 > block 3 > block 4) across all participants. Right hemisphere: Montreal Neurological Institute (MNI) coordinates = 24, -8, and -16 ($t$ = 9.25, P < .05 FWE), cluster size = 103 voxels. Left hemisphere: MNI coordinates = -22, -8, and -16 ($t$ = 9.00, P < .05 FWE), cluster size = 161 voxels. B) Graph of the interaction between the most significant SNP from the Comorbidity and Trauma Study (CATS), rs604300 in *MGLL*, with childhood adversity to predict habituation to threat-related stimuli in the right basolateral amygdala in the Duke Neurogenetics Study (DNS). X-axis denotes the log of a participant's score on the Childhood Trauma Questionnaire—Short Form (CTQ), while y-axis indicates amount of neural reactivity to socially-relevant stimuli early in the course of the task relative to later in the task. Shaded area indicates Johnson-Neyman region of significance. For raw data, see Figure S4. R BL = right basolateral.

* $p$ < .05. ** $p$ < .01. *** $p$ < .001.

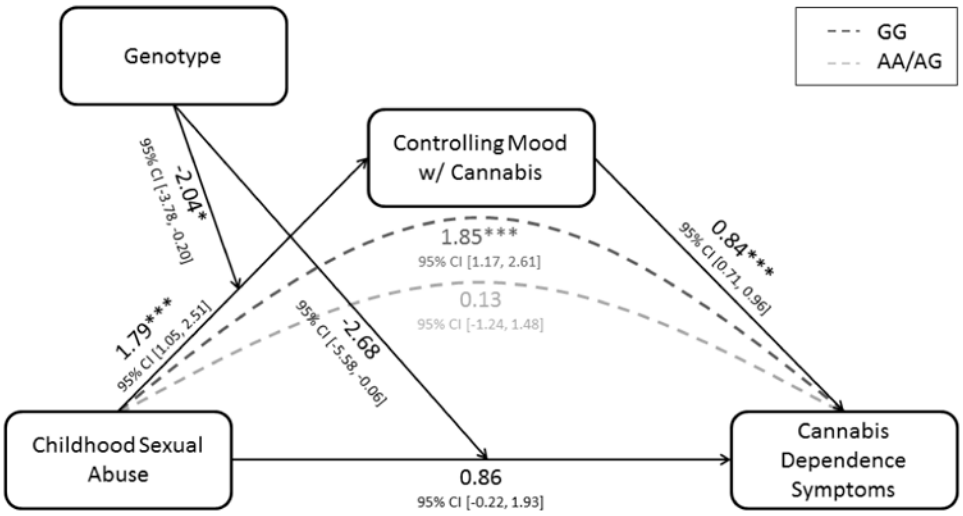


**Figure 4.**

Full structural equation model predicting the effect of childhood sexual abuse (CSA) on cannabis dependence symptoms through using cannabis to control mood, as moderated by rs604300 genotype. The dashed lines represent the indirect effects of CSA on dependence symptoms through the mediator for each genotype group.

* $p < .05$. ** $p < .01$. *** $p < .001$.

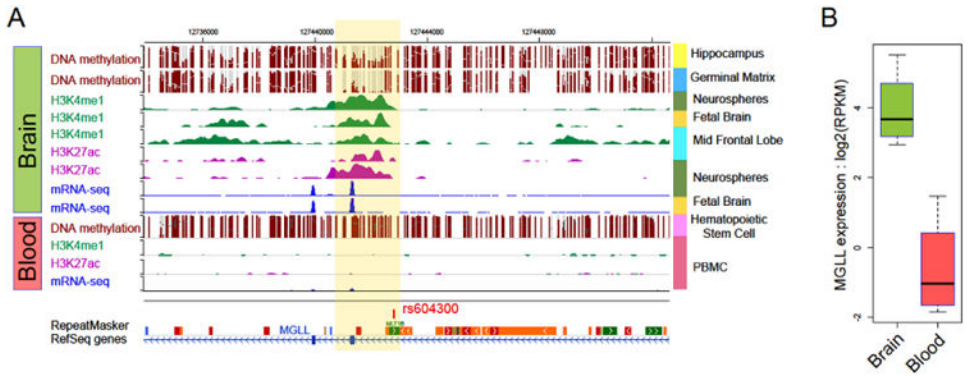


**Figure 5.**

Methylation and histone-mediated epigenetic signatures and *MGLL* mRNA expression in brain and blood samples. A) There is little methylation in neurospheres in this region accompanied by H3k4me1 and H3K27ac enrichment. Such enrichment, would serve to enhance *MGLL* gene expression. In contrast, in blood cells, this region is heavily methylated with no H3k4me1 and H3K27ac enrichment. B) These epigenetic markers of methylation and histone-modification-related enhancement may serve to drive *MGLL* expression in the brain while suppressing it in blood. Additional details may be found in the Supplement.

## Table 1

### Endocannabinoid System Genes

| Gene | Protein | Role | Rationale |
|---|---|---|---|
| *CNR1* | CB1 | Receptor | primary endocannabinoid receptor |
| *CNR2* | CB2 | Receptor | secondary endocannabinoid receptor |
| *FAAH* | FAAH | Catabolism | responsible for the breakdown of AEA and a small percentage of the breakdown of 2-AG |
| *MGLL* | MAGL | Catabolism | responsible for ~85% of the breakdown of 2-AG |
| *DAGLA* | DAGL-α | Anabolism | with DAGL-β, responsible for the hydrolosis of DAGs to 2-AG |
| *DAGLB* | DAGL-β | Anabolism | with DAGL-α, responsible for the hydrolosis of DAGs to 2-AG |
| *NAPEPLD* | NAPE-PLD | Anabolism | enzyme in the most direct synthesis pathway of AEA |

*Note*. CB1 = cannabinoid type 1 receptor; CB2 = cannabinoid type 2 receptor; AEA = anandamide; FAAH = fatty acid amide hydrolase-1; 2-AG = 2-arachidonoylglycerol; MAGL = monoacylglycerol lipase; DAGL-α = sn-1-selective diacylglycerol lipase- α; DAGs = diacylglycerols; DAGL-β = sn-1-selective diacylglycerol lipase- β; NAPE-PLD = N-acyl-phosphatidylethanolamine-selective phosphodiesterase.

## Table 2
## Participant Characteristics and Variables Across Studies

| Study | Subjects | Genotype Groups | Childhood Adversity Measure | Outcome |
|---|---|---|---|---|
| CATS | Opioid-dependent cases and neighborhood controls who ever used cannabis (*N* = 1558) | GG, AG, AA[a] | Number of types of childhood sexual abuse (i.e., unwanted exposure, touching, threats, vaginal sex, oral sex, anal sex) endorsed | DSM-IV cannabis dependence symptoms among cannabis users |
| SAGE | Alcohol-dependent cases and non-dependent controls (*N* = 859) | GG, AX[b] | Whether ever experienced childhood physical or sexual abuse (yes = 1, no = 0) | DSM-IV cannabis dependence symptoms (nonusers coded as "0") |
| DNS | Young adult college students (*N* = 312) | GG, AX[b] | Occurrence and frequency of emotional, physical, and sexual abuse, as wellas emotional and physical neglect | Right and left amygdala habituation to emotional faces |

*Note*. CATS = Comorbidity and Trauma Study; SAGE = Study of Addiction: Genetics and Environment; DNS = Duke Neurogenetics Study

[a] Analyses using carrier coding, as in the other studies, yielded consistent results.

[b] Heterozygotes and A allele homozygotes treated as one group.

## Table 3

### Sample Demographics

| Variable | CATS | SAGE | DNS |
|---|---|---|---|
| *N* | 1558 | 859 | 312 |
| Age | 36.11 (8.92) | 37.39 (10.90) | 19.71 (1.23) |
| Female (%) | 42.2% | 52.3% | 51.6% |
| Can. Dep. Sx | 2.95 (2.15) | 1.23 (2.00) | N/A |
| Cannabis Dependent (%) | 55.5% | 23.6% | 1.6% |
| CSA/CA/CTQ | 1.57 (2.04)[a] | 0.21 (N/A)[b] | 31.18 (6.68)[c] |

*Note*. CATS = Comorbidity and Trauma Study; SAGE = Study of Addiction: Genetics and Environment; DNS = Duke Neurogenetics Study; CSA = childhood sexual abuse; CA = childhood abuse (physical or sexual); CTQ = Childhood Trauma Questionnaire.

[a] Average number of types of CSA reported, out of 6.

[b] Proportion reporting any CA.

[c] Minimum score for CTQ is 25, indicating no childhood trauma.

## Table 4

### Gene-Level Analysis Results in CATS

| Gene | $N_{maxSNPS}$ | Gene-Stat | Within-Gene *P* |
|---|---|---|---|
| *CNR1* | 11 | .0006 | .605 |
| *DAGLA* | 9 | .0002 | .926 |
| *DAGLB* | 4 | .0006 | .348 |
| *FAAH* | 5 | .0010 | .167 |
| *MGLL* | 14 | .0024 | .009 |
| *NAPEPLD* | 3 | .0009 | .178 |

*Note*. $N_{maxSNPS}$ = maximum number of nominally significant ($p < 0.05$) SNPs obtained in permutations; Gene-Stat = average of the $N_{maxSNPS}$most significant SNP $\Delta R^2$ values; Within-Gene *P* = empirical *p*-value obtained for each gene.

**Table 5**

**SNP-Level Analysis Significant Results in CATS**

| Gene | SNP | A1 | A2 | MAF | HWE *P* | *T* | $\Delta R^2$ | *P* (uncorr.) |
|---|---|---|---|---|---|---|---|---|
| *FAAH* | rs4660928 | A | C | .28 | .41 | -1.97 | .002 | .049 |
| *MGLL* | rs604300 | A | G | .11 | .79 | -3.70 | .007 | < .001 |
| | rs507961 | A | G | .21 | .94 | -3.23 | .006 | .001 |
| | rs497897 | A | G | .10 | .38 | -3.01 | .005 | .003 |
| | rs13066225 | A | G | .18 | .50 | 2.54 | .004 | .011 |
| | rs664910 | G | A | .31 | .72 | -2.37 | .003 | .018 |
| | rs782446 | C | A | .22 | 1.00 | -2.11 | .002 | .035 |

*Note*. A1 = sample minor allele; A2 = sample major allele; MAF = minor allele frequencies; HWE *P* = Hardy-Weinberg test statistics.