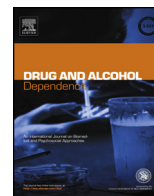




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DSM-5 cannabis use disorder: A phenotypic and genomic perspective[☆]

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ABSTRACT

Background: We explore the factor structure of DSM-5 cannabis use disorders, examine its prevalence across European- and African-American respondents as well as its genetic underpinnings, utilizing data from a genome-wide study of single nucleotide polymorphisms (SNPs). We also estimate the heritability of DSM-5 cannabis use disorders explained by these common SNPs.

Methods: Data on 3053 subjects reporting a lifetime history of cannabis use were utilized. Exploratory and confirmatory factor analyses were conducted to create a factor score, which was used in a genome-wide association analysis. *p*-values from the single SNP analysis were examined for evidence of gene-based association. The aggregate effect of all SNPs was also estimated using Genome-Wide Complex Traits Analysis.

Results: The unidimensionality of DSM-5 cannabis use disorder criteria was demonstrated. Comparing DSM-IV to DSM-5, a decrease in prevalence of cannabis use disorders was only noted in European-American respondents and was exceedingly modest. For the DSM-5 cannabis use disorders factor score, no SNP surpassed the genome-wide significance testing threshold. However, in the European-American subsample, gene-based association testing resulted in significant associations in 3 genes (*C17orf58*, *BPTF* and *PPM1D*) on chromosome 17q24. In aggregate, 21% of the variance in DSM-5 cannabis use disorders was explained by the genome-wide SNPs; however, this estimate was not statistically significant.

Conclusions: DSM-5 cannabis use disorder represents a unidimensional construct, the prevalence of which is only modestly elevated above the DSM-IV version. Considerably larger sample sizes will be required to identify individual SNPs associated with cannabis use disorders and unequivocally establish its polygenic underpinnings.

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1. Introduction

Cannabis is the most commonly used illicit psychoactive substance in developed nations (Degenhardt and Hall, 2012). While a majority of cannabis users do not report problems, 10–30% of those who ever use cannabis meet criteria for a lifetime history of cannabis abuse or dependence as defined by the fourth edition of the Diagnostic and Statistical Manual (DSM-IV; American Psychiatric Association, 1994). Recently, changes to the

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diagnostic criteria for substance use disorder have been made in DSM-5 (American Psychiatric Association, 2013), including several for the diagnosis of cannabis use disorders (Hasin et al., 2013). Across the broad range of substance use disorders, (i) the distinction between abuse and dependence has been replaced by a unidimensional symptom count, with endorsement of 2 or more symptoms resulting in a DSM-5 diagnosis of substance use disorder (endorsement of specific numbers of symptoms define a mild, moderate or severe diagnosis); (ii) the DSM-IV criterion of legal problems has been eliminated from the diagnostic repertoire; and (iii) a new criterion for the DSM-5, craving (a long held substance dependence criterion in the International Classification of Disease, ICD) has been added. More specifically for cannabis, withdrawal is now a criterion. A wealth of psychometric evaluations in epidemiological (Agrawal et al., 2008; Compton et al., 2009; Gillespie et al., 2005; Hartman et al., 2008; Hasin et al., 2012, 2008; Langenbucher et al., 2004; Lynskey and Agrawal, 2007; Martin et al., 2006; Piontek et al., 2011; Wu et al., 2009, 2012) and clinical samples (Budney, 2006; Budney and Hughes, 2006) support these recommendations; however, the impact of these revisions on the prevalence of cannabis use disorders under the new DSM-5 classification remains largely unexplored. A recent study of Australian adults found a modest reduction in the rate of cannabis use disorder with the transition from DSM-IV to DSM-5 (Mewton et al., 2013), while another study of individuals with substance use disorders noted a modest increase of 4% (Peer et al., 2013).

Twin studies indicate that 50–60% of the variation in cannabis use disorders (abuse/dependence, variously defined using DSM-III-R, DSM-IV and ICD) can be attributed to heritable influences (Verweij et al., 2010). Despite this robust heritability estimate, association studies for cannabis use disorders have largely failed to identify genetic variants of significant and replicable effect. A prior genome-wide association study (GWAS) of DSM-IV cannabis dependence, conducted in the sample used in this study, failed to identify genetic variants at a statistically significant level (Agrawal et al., 2011b). This has resulted in speculation regarding the biological underpinnings of cannabis use disorders; in particular, the question of whether common variation available in commercially available genome-wide arrays captures it (Sullivan et al., 2012).

Aggregating the effects of all single nucleotide polymorphisms (SNPs) on commercial arrays might quantify the overall role of common SNPs as well as causal variants in linkage disequilibrium (LD)

Table 1

Prevalence (%) of individual DSM-IV and proposed DSM-5 criteria for cannabis use disorder in 3053 lifetime cannabis users of European-American (EA) and African-American (AA) ancestry.

	Males		Females	
	EA	AA	EA	AA
Role obligations	26.0 ^a	25.8 ^a	10.0 ^b	13.2 ^b
Hazard	49.2	37.3	21.8	16.8
Legal	4.8 ^a	3.2 ^a	1.4 ^b	1.4 ^b
Social/Interpersonal	31.3 ^a	26.9 ^a	14.2 ^b	13.8 ^b
Tolerance	34.0 ^a	32.5 ^a	13.2	17.2
Withdrawal	21.2 ^a	24.8 ^a	9.6	14.6
Larger/Longer	26.5	35.8	13.7	19.4
Quit	29.1	35.1	13.9	23.8
Time spent	34.4 ^a	37.3 ^a	12.9	20.8
Give up	24.3 ^a	21.6 ^a	8.4 ^b	10.4 ^b
Problems	26.1 ^a	27.2 ^a	14.6 ^b	16.8 ^b
Craving	17.9 ^a	19.8 ^a	7.0	12.0
DSM-IV abuse/dependence	55.4 ^a	52.6 ^a	28.1 ^b	30.2 ^b
DSM-5 use disorder	51.1 ^a	52.6 ^a	25.1	31.8

^a Prevalence with the same superscripts could be statistically equated to each other at $p > 0.05$.

^b Prevalence with the same superscripts could be statistically equated to each other at $p > 0.05$.

with these SNPs on the trait of interest (Yang et al., 2010, 2011b). When significant, this would indicate that heritable variation in the trait is at least partially captured by these SNPs in a highly polygenic manner. Applying this methodology, investigators have successfully attributed 23–51% of the variation in current smoking, major depression, schizophrenia and human intelligence to genetic influences (Davies et al., 2011; Lee et al., 2012; Lubke et al., 2012).

The present study uses a multi-pronged phenotypic and genomic approach to evaluate, respectively, the architecture and genetic underpinnings of DSM-5 cannabis use disorders, defined as a quantitative phenotype. Instead of relying on a diagnostic measure, we first utilize item response models to construct a factor representing liability to DSM-5 cannabis use disorders, while accounting for sex and ethnic differences. Second, we use this psychometrically constructed factor score in a genome-wide association analysis. Finally, we evaluate whether genome-wide SNPs and putative causal variants in linkage disequilibrium with them explain a significant proportion of the heritable variation in DSM-5 cannabis use disorders.

2. Methods

2.1. Sample

The Study of Addictions: Genes and Environment (SAGE) includes 3988 individuals ascertained from 3 study sources: the Collaborative Study of the Genetics of Alcoholism ($N = 1410$; Begleiter et al., 1995; Reich et al., 1998), the Collaborative Study of the Genetics of Nicotine Dependence ($N = 1406$; Bierut et al., 2007) and the Family Study of Cocaine Dependence ($N = 1172$; Bierut et al., 2008). Further details regarding the study are available elsewhere (Bierut et al., 2010). The study includes substantial numbers of individuals who have used cannabis and experience problem use. For these analyses, data on 3053 (77% of the sample) individuals reporting a history of ever using cannabis were used.

2.2. Measures

2.2.1 DSM criteria. Twelve criteria from DSM-IV and DSM-5 (American Psychiatric Association, 1994) were utilized (Table 1). These included DSM-IV abuse criteria of (i) failure to fulfill major role obligations (role failure), (ii) recurrent use in hazardous situations (hazard), (iii) recurrent social/interpersonal problems because of use (social/interpersonal), and (iv) legal problems (legal), as well as the six DSM-IV dependence criteria of (v) tolerance, (vi) using in larger quantities or for longer than intended (larger/longer), (vii) persistent failed quit attempts (quit), (viii) spending a great deal of time using cannabis (time spent), (ix) giving up important activities to use cannabis (give up) and (x) experiencing physical or psychological problems because of cannabis use (problems). In addition, the two DSM-5 criteria of (xi) withdrawal and (xii) craving were also used.

2.2.2 DSM-5 factor score

Based on the factor analyses described below, a score representing liability to DSM-5 cannabis use disorders was used as a quantitative index.

2.3. Genotyping

The genotyping and quality control procedures applied to these data are explained in detail in earlier publications (Bierut et al., 2010; Laurie et al., 2010). In brief, DNA samples from 3988 individuals were genotyped on the Illumina Human 1 M beadchip by the Center for Inherited Diseases Research (CIDR) at Johns Hopkins University. As described earlier, 948,658 SNPs passed data cleaning protocols. No imputed data were used for these analyses. HapMap genotyping controls, duplicates, related subjects, and outliers were removed. For the current analyses, data on 3053 (77% of the sample) individuals reporting a lifetime history of cannabis use were used. Self-identified ethnicity (consistent with analysis of genetic data) was 2018 European Americans and 1035 African Americans.

2.4. Statistical analysis

2.4.1. Phenotypic factor analysis. We used MPlus (v5; Muthen and Muthen, 2007) to conduct exploratory and confirmatory factor analyses of the 12 DSM-IV/DSM-5 criteria in the same sample. Exploratory analyses were conducted in the full sample, while subsequent confirmatory factor analyses were conducted in African-American (AA) and European-Americans (EA), separately by sex, using a multi-group framework. Initially, factor loadings and thresholds were constrained across the ethnic groups and across sexes. Individual submodels were tested to determine whether allowing the factor loading and threshold for each criterion to vary across the groups resulted in a significant improvement in model fit. The model that accommodated

all statistically significant differences was used to generate factor scores that were subsequently used for genome-wide association analysis.

2.4.2. Genetic analyses. GWAS: A linear regression model, in the PLINK (Purcell et al., 2007) software package, was used. Genotype was coded log-additively (i.e. increasing copies of the minor allele, selected from the full sample with ethnicities combined). Analyses were conducted separately in the EA ($N = 2018$) and AA ($N = 1035$) subsamples, adjusting for further ethnic differences via the inclusion of 2 principal components (generated via EIGENSTRAT; Price et al., 2006). Other covariates included age at interview (dummy-coded to represent the lower three quartiles with the oldest age group used as a reference), sex and study source (whether the participants were drawn from COGA, COGEND or FSCD). The results from the EA and AA subsamples were meta-analyzed in METAL (Willer et al., 2010) using effect size weighting by the inverse of the standard errors.

2.4.3. Gene-based association analysis. We used the Versatile Gene-based Association Study (VEGAS) program to conduct gene-based tests of association (Liu et al., 2010). VEGAS assigns individual SNPs to each of the 17,787 autosomal genes (by physical position on the UCSC hg18 Genome Browser assembly). p -Values from the GWAS are converted to upper-tailed chi-square statistics and then used to examine whether the chi-square distribution for each gene deviates from the null distribution. Due to differing linkage disequilibrium patterns across ethnicities, gene-based association was conducted on results when EA (using CEU) and AA (using YRI) subjects were analyzed separately.

2.4.4. Estimation of total genomic variation (heritability). Genome-wide Complex Trait Analysis (GCTA; Yang et al., 2011a) was used to estimate the proportion of genomic variation explained by all SNPs available from the GWAS. Univariate analyses were conducted for the factor score, and covariates (sex, age, study site and ethnicity as indexed by principal components) were included in all computations. Analyses were restricted to those of self-reported EA ancestry. Analyses were not conducted in the AA subset because the modest sample size would likely have resulted in a large standard error.

3. Results

3.1. Sample characteristics

The sample used for analyses was restricted to those who reported at least one lifetime use of cannabis ($N = 3053$; 49% male; 32.5% from COGA, 38.5% from COGEND, 29% from FSCD; 66% self-reported EA; mean age of 38.1 [18–68 years]). These individuals are characterized with respect to the 12 individual DSM-IV/DSM-5 criteria in Table 1. Prevalence of each criterion was higher in males than females for both ethnic groups, and males, regardless of ethnicity, were more likely than females to meet criteria for DSM-IV and DSM-5 diagnoses. However, several intriguing ethnic differences emerged. For both sexes, hazardous use, use of larger amounts or for a longer period of time and desire to quit or multiple failed quit attempts were differentially endorsed by EA and AA. EA men and women were more likely to endorse hazardous use and less likely to endorse using larger amounts or for longer than intended and failed quit attempts than their AA counterparts. In addition, tolerance, time spent using cannabis and the DSM-5 criteria of withdrawal and craving were more commonly reported by AA women than their EA counterparts—similar differences were not noted for men. The prevalence of DSM-IV cannabis abuse/dependence was higher in men compared with women, but no within-sex ethnic differences were noted. For DSM-5, cannabis use disorder was again more common in men than women, and there were no ethnic differences in men. However, AA women were more likely to meet criteria compared with their EA counterparts (31.8% vs. 25.1%). Comparing the prevalence of DSM-IV vs. DSM-5 cannabis use disorders—within each group, very modest changes were observed. Decrease in overall prevalence was noted for EA, while AA women showed a slight increase and AA men remained unchanged. Examining the [95%] confidence limits for the point estimates, only the decrease in prevalence in the EA was statistically significant (for men: 55.4% [52.2–58.6] vs. 51.1% [47.9–54.1]; for women: 28.1 [25.4–30.9] vs. 25.1% [22.6–27.9]) while the estimates in AA subjects could be equated across diagnostic

classification scheme (for men: 52.6% [48.3–56.9] vs. 52.6 [47.2–55.8]; for women: 30.2% [26.2–34.4] vs. 31.8% [27.7–36.1]).

3.2. Factor analysis

An exploratory factor analysis of the full sample revealed that a single factor solution provided a reasonable fit to the data (comparative fit index (CFI): 0.996, root mean square error of approximation (RMSEA): 0.054). While a 2-factor exploratory solution modestly improved these fit indices (e.g., 2 factor solution: CFI: 0.999, RMSEA: 0.036), the inter-factor correlation was 0.90. Hence, we proceeded with the more parsimonious single factor confirmatory analysis, which readily approximates item response parameters. Confirmatory factor analysis of the 4 DSM-IV abuse, 6 DSM-IV dependence and the DSM-5 withdrawal and craving criteria revealed high factor loadings (0.75–0.90) for all criteria except legal problems (0.23), which was excluded (consistent with DSM-5) from further analyses comparing factor loadings and thresholds for each individual criterion across EA and AA males and females. The factor loadings and thresholds (all significant at $p < 0.0001$) from the model allowing for statistically significant differences across individual items are shown in Table 2. Factor loadings and thresholds could not be constrained across the groups for hazardous use, interpersonal problems, withdrawal, using more than intended (larger/longer), repeated/failed quit attempts, time spent and physical/psychological problems (please see supplemental eTable 1 for fit indices¹). Factor scores that accommodated these differing thresholds and factor loadings were created for each of the four subgroups and used for genomic analyses.

3.3. GWAS

Individual signals did not surpass the Bonferroni corrected genome-wide significance threshold of $p < 5 \times 10^{-8}$. The results for the top 20 SNPs are presented in Table 3 (the top 100 results for the EA and AA subsamples are available in eTables 2 and 3, respectively²). For the EA subsample, 11 SNPs on 17q23-24 appeared to be associated at nominal levels of significance although none surpassed the genome-wide threshold of 5×10^{-8} . The top SNP, rs6504555, was an intronic variant in the bromodomain PHD finger transcription factor (*BPTF*) gene—a regional association plot for this region of chromosome 17 is shown in Fig. 1, indicating a high degree of linkage disequilibrium across the associated SNPs. With the exception of rs11870068, the remaining chromosome 17 SNPs were in moderate to high linkage disequilibrium (r^2 ranging 0.66 to 1.0). In the AA subsample, results did not aggregate in any particular chromosomal region. The most significant SNP, rs4364205, on chromosome 3, was intergenic.

Meta-analysis of the results from the EA and AA subsamples did not yield a boost in statistical significance (Table 3). This was evident from a comparison of results in the EA and AA subsamples. Of all SNPs with p -values < 0.05 in EA subsample, only 5% had corresponding p -values < 0.05 in AA subsample. However, particularly for the SNPs for the EA subsample shown in Table 2, the direction of effect in the AA subsample predominantly (with the exception of 5 of 20 SNPs) concurred with the EA subsample.

3.4. Gene-based association results

Three genes surpassed the conservative gene-based Bonferroni threshold of 2.8×10^{-6} in the EA, but not the AA subsample

¹ Supplementary material can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:...

² Supplementary material can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:...

Table 2
Standardized factor loadings [95% confidence intervals] from one factor confirmatory factor analysis in 3053 lifetime cannabis users of European-American (EA) and African-American (AA) ancestry.

	Males		Females	
	EA	AA	EA	AA
Role obligations	0.90 [0.87–0.92]	0.90 [0.87–0.92]	0.90 [0.87–0.92]	0.90 [0.87–0.92]
Hazard	0.83 [0.78–0.88]	0.73 [0.64–0.82]	0.83 [0.78–0.88]	0.71 [0.60–0.82]
Social/Interpersonal	0.92 [0.88–0.95]	0.90 [0.85–0.95]	0.89 [0.85–0.94]	0.89 [0.82–0.96]
Tolerance	0.87 [0.85–0.90]	0.87 [0.85–0.90]	0.87 [0.85–0.90]	0.87 [0.85–0.90]
Withdrawal	0.88 [0.83–0.93]	0.85 [0.78–0.92]	0.92 [0.88–0.96]	0.93 [0.89–0.98]
Larger/Longer	0.84 [0.79–0.90]	0.89 [0.84–0.94]	0.91 [0.87–0.95]	0.91 [0.86–0.97]
Quit	0.75* [0.69–0.82]	0.73* [0.63–0.83]	0.85 [0.80–0.90]	0.79* [0.70–0.88]
Time Spent	0.87 [0.82–0.91]	0.90 [0.84–0.94]	0.92 [0.88–0.96]	0.94 [0.89–0.93]
Give up	0.92 [0.89–0.94]	0.92 [0.89–0.92]	0.92 [0.89–0.94]	0.92 [0.89–0.94]
Problems	0.91 [0.88–0.95]	0.90 [0.84–0.95]	0.92 [0.88–0.96]	0.84 [0.75–0.92]
Craving	0.90 [0.88–0.93]	0.90 [0.88–0.93]	0.90 [0.88–0.93]	0.90 [0.88–0.93]
Mean Factor Score	0.49 [0.31–0.67]	0.50 [0.30–0.69]	–0.23 [–0.42–0.05]	0.00 [Reference]

Note: If factor loadings could be constrained, then they are shown as such. Differing factor loadings may not statistically differ from each other [i.e. as indicated by overlapping confidence limits] but may have been unconstrained in models because of differing thresholds.
* Those factor loadings with an * could be equated to each other but not to other estimates.

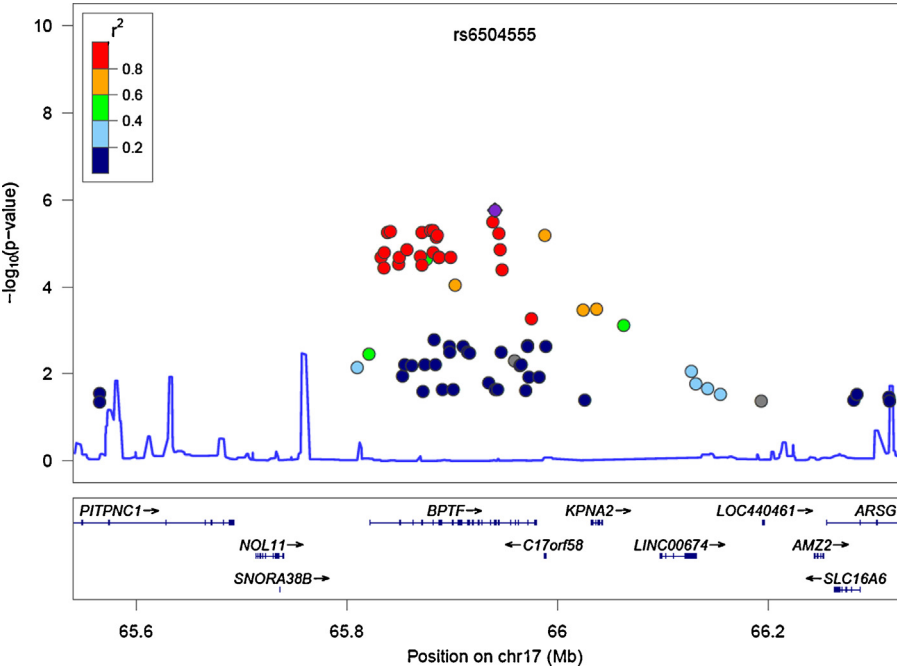


Fig. 1. Regional association plot of chromosome 17 results from the European-American subsample. SNP with lowest *p*-value (diamond-shape, in purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Table 4). In the EA subsample, association was noted on chromosome 17q23–24 for *C17orf158* (chromosome 17 open reading frame 58), and the adjacent genes *BPTF* and *PPM1D* (protein phosphatase Mg^{2+}/Mn^{2+} dependent, 1D). Multiple other neighboring genes also showed aggregation of association signals although none surpassed gene-based correction. As VEGAS allows for SNPs to be assigned to the 50 kb region flanking the gene (footprint), this clustering of

genes might be attributed to SNPs being assigned to the footprints of multiple neighboring genes. The association appeared to be specific to the EA subsample with corresponding *p*-values >0.05 in the AA subsample. For results from the AA subsample, the lowest *p*-value for the gene-based association test was 0.00013 for Patched domain containing 3 (*PTCHD3*), which had a corresponding *p*-value of 0.61 in the EA results.

Table 3

Top 20 association results from genome wide association study of the DSM-5 cannabis use disorders factor scores in 2018 European-American and 1035 African-American lifetime cannabis users. Also shown are *p*-values from a meta-analysis of the results from both ethnic groups.

Chromosome	SNP	Gene	Allele	Frequency	Beta	Lower 95%	Upper 95%	<i>p</i> -value	<i>p</i> -value other ^a	Dir.	Meta <i>p</i> -value
European-Americans											
17	rs6504555	<i>BPTF</i>	A	0.22	0.15	0.09	0.20	1.73E-06	0.35	++	2.00E-05
X	rs7884312	<i>DMD</i>	A	0.12	0.21	0.13	0.30	1.73E-06	0.87	+-	1.59E-03
X	rs7880016	<i>DMD</i>	A	0.11	0.22	0.13	0.31	2.65E-06	0.53	+-	6.43E-03
17	rs8071463	<i>BPTF</i>	G	0.22	0.14	0.08	0.20	3.25E-06	0.29	--	2.16E-05
2	rs3731808	<i>PDE11A</i>	T	0.01	-0.58	-0.83	-0.33	4.82E-06	0.47	+-	5.14E-04
12	rs10082916	<i>RARG</i>	T	0.04	-0.27	-0.39	-0.15	4.84E-06	0.60	--	4.93E-04
12	rs12307672	<i>RARG</i>	A	0.04	-0.27	-0.39	-0.15	4.84E-06	0.71	--	9.12E-04
17	rs9897982	<i>BPTF</i>	T	0.22	0.14	0.08	0.20	5.10E-06	0.60	++	1.29E-04
17	rs3935969	<i>BPTF</i>	C	0.22	0.14	0.08	0.20	5.13E-06	0.16	--	1.35E-05
17	rs2365468	<i>BPTF</i>	T	0.22	0.14	0.08	0.20	5.40E-06	0.55	++	1.12E-04
17	rs9890629	<i>BPTF</i>	A	0.22	0.14	0.08	0.20	5.52E-06	0.15	++	1.26E-05
17	rs8074078	<i>BPTF</i>	G	0.22	0.14	0.08	0.20	5.65E-06	0.15	--	1.19E-05
17	rs11870068	<i>BPTF</i>	C	0.22	0.14	0.08	0.20	5.80E-06	0.17	+-	1.84E-03
12	rs11065202		C	0.42	0.11	0.06	0.16	5.91E-06	0.95	+-	2.39E-04
17	rs9891146	<i>C17orf58</i>	T	0.28	0.13	0.07	0.18	6.42E-06	0.98	++	3.04E-04
17	rs6504548	<i>BPTF</i>	C	0.22	0.14	0.08	0.20	6.49E-06	0.59	--	1.41E-04
12	rs2066938	<i>MGC5139</i>	G	0.27	0.13	0.07	0.18	6.86E-06	0.09	--	3.16E-06
17	rs7208663	<i>BPTF</i>	C	0.22	0.14	0.08	0.20	7.13E-06	0.17	--	1.85E-05
8	rs12056774		T	0.23	0.13	0.07	0.19	7.71E-06	0.46	++	9.16E-05
22	rs165685	<i>PCQAP</i>	T	0.18	0.15	0.08	0.21	9.31E-06	0.65	++	2.16E-05
African-Americans											
3	rs4364205		T	0.41	-0.19	-0.26	-0.12	1.30E-07	0.06	+-	1.10E-01
1	rs16853258		G	0.07	-0.34	-0.47	-0.20	1.06E-06	0.16	+-	1.87E-06
11	rs1981990		A	0.30	0.19	0.11	0.26	2.62E-06	0.80	+-	8.70E-03
2	rs7601137	<i>AFF3</i>	C	0.14	0.25	0.14	0.35	3.39E-06	0.62	++	2.61E-03
8	rs2410545	<i>NAT1</i>	A	0.12	-0.27	-0.39	-0.16	4.08E-06	0.87	+-	7.50E-02
2	rs12479422		A	0.09	0.30	0.17	0.42	4.57E-06	0.29	--	2.18E-04
8	rs11203943	<i>NAT1</i>	A	0.05	-0.38	-0.54	-0.22	4.67E-06	0.32	++	2.85E-03
10	rs493965		A	0.48	-0.17	-0.24	-0.10	5.83E-06	0.52	+-	3.11E-02
8	rs6586712	<i>NAT1</i>	G	0.12	-0.26	-0.37	-0.15	8.95E-06	0.64	+-	1.56E-01
8	rs16871627		T	0.17	-0.21	-0.31	-0.12	9.41E-06	0.43	+-	1.40E-05
2	rs865108	<i>STEAP3</i>	G	0.47	0.17	0.09	0.24	9.56E-06	0.54	+-	4.74E-02
22	rs9627601	<i>TBC1D22A</i>	C	0.15	-0.23	-0.33	-0.13	1.01E-05	0.23	+-	2.47E-05
14	rs11850171		G	0.23	0.19	0.10	0.27	1.18E-05	0.92	++	1.31E-05
3	rs6774262		A	0.43	0.16	0.09	0.24	1.30E-05	0.19	--	2.13E-04
1	rs950601		A	0.16	-0.22	-0.32	-0.12	1.63E-05	0.20	++	1.35E-03
1	rs6677326		G	0.04	0.40	0.22	0.58	1.69E-05	0.87	+-	2.30E-05
10	rs2815527		G	0.42	0.16	0.09	0.24	2.00E-05	0.67	++	6.75E-03
22	rs12170279	<i>TBC1D22A</i>	G	0.20	-0.20	-0.29	-0.11	2.20E-05	0.30	+-	4.59E-05
7	rs12668723		A	0.21	0.19	0.10	0.27	2.33E-05	0.12	--	1.41E-04
10	rs2815523		G	0.42	0.16	0.09	0.24	2.54E-05	0.68	++	7.58E-03
2	rs7557254		C	0.37	-0.16	-0.24	-0.09	2.60E-05	0.14	--	2.50E-05

^a *p*-value other indicates the *p*-value for that SNP in the other ethnicity (i.e. AA *p*-value for analyses conducted in European-Americans and vice versa).

Table 4

Top 10 genes showing association via gene-based association analysis in European- and African-American subsamples analyzed separately.

Gene	Chromosome	Start basepair	End basepair	<i>p</i> -value (EA)	<i>p</i> -value (AA)
European-American subsample (<i>N</i> = 2018)					
<i>C17orf58</i>	17	63,417,678	63,420,227	<1E-6	0.422
<i>PPM1D</i>	17	56,032,335	56,096,818	<1E-6	0.149
<i>BPTF</i>	17	63,252,241	63,410,956	0.000001	0.351
<i>UNC119B</i>	12	11,963,221	11,964,582	0.000016	0.11
<i>LRRC37A3</i>	17	60,280,949	60,345,365	0.000026	0.618
<i>ACADS</i>	12	11,964,795	11,966,219	0.000027	0.119
<i>KIAA0152</i>	12	11,960,933	11,962,405	0.000033	0.136
<i>MYEF2</i>	15	46,218,920	46,257,850	0.000137	0.944
<i>CABP1</i>	12	11,956,280	11,958,951	0.000161	0.288
<i>ZNF681</i>	19	23,713,836	23,733,533	0.000164	0.24
African-American subsample (<i>N</i> = 1035)					
<i>PTCHD3</i>	10	27,727,122	27,743,303	0.000125	0.61
<i>AFF3</i>	2	99,530,147	10,012,546	0.000381	0.466
<i>FILIP1L</i>	3	10,103,467	10,131,603	0.000415	0.224
<i>C3orf26</i>	3	10,101,937	10,138,013	0.000527	0.215
<i>CNOT10</i>	3	32,701,701	32,790,358	0.000556	0.171
<i>FANCM</i>	14	44,674,885	44,739,843	0.000565	0.407
<i>IAPP</i>	12	21,417,084	21,423,683	0.000588	0.493
<i>NMUR2</i>	5	15,175,129	15,176,503	0.000603	0.674
<i>FKBP3</i>	14	44,654,858	44,674,272	0.000668	0.311
<i>CCDC91</i>	12	28,301,399	28,594,366	0.000686	0.257

3.5. Total genomic variation (heritability)

Genomic variation was responsible for 21% (SE = 17.7) of the phenotypic variance in the factor score. However, the estimate was not statistically significant ($p = 0.13$).

4. Discussion

We sought to examine the phenotypic and genomic architecture of a continuously distributed cannabis use disorders factor, psychometrically derived from DSM-5 criteria, in samples ascertained for alcohol, nicotine and cocaine dependence. Our analyses revealed a high degree of support for the unidimensionality of cannabis use disorders. Analysis of ethnic differences indicated a modest reduction in the prevalence of DSM-5 cannabis use disorders, relative to DSM-IV, in EA. Genomic analyses, using a genome-wide scan, failed to identify SNPs that satisfied statistical thresholds for significance; however, gene-based association implicated genes on the q-arm of chromosome 17. A genome wide variance calculation revealed that 21% of the phenotypic variance in cannabis use disorders was captured by the available common variation on the genome-wide array, but this estimate had a large standard error and was not significant.

We used the factor score as our phenotype for genomic analyses. Incorporating withdrawal and craving, excluding legal problems and combining across DSM-IV abuse and dependence criteria, this factor embodies the ‘spirit’ of the new DSM-5 diagnostic scheme while not being encumbered by concerns that the threshold of 2 or more criteria for diagnosis of disorder is too lax (Martin et al., 2011b). From a psychometric perspective, our results are consistent with the extant literature (see Hasin et al., 2013 for a comprehensive overview). For instance, despite our sample being ascertained for alcohol, nicotine and cocaine dependence, which inflated endorsement rates of individual criteria (i.e. due to the high comorbidity across substance use disorders), our high rates of hazardous use were comparable with those reported for lifetime cannabis users from the general population as reflected in data from the National Epidemiological Survey of Alcohol and Related Conditions (Agrawal and Lynskey, 2007; Compton et al., 2009). Likewise, broadly consistent with numerous other studies, the DSM-IV abuse criterion of legal problems was infrequently endorsed and had a weak factor loading, affirming its proposed exclusion from DSM-5. The overall prevalence of the remaining criteria, although much higher than in general population cohorts, supports the presence of a unidimensional construct across sexes and ethnicities. Craving and withdrawal, both of which have been added to DSM-5, performed well, with high factor loadings supporting their inclusion.

Overall, rates of diagnostic DSM-5 cannabis use disorders appear to be modestly lower than those for DSM-IV abuse/dependence, but only in EA, particularly men. This finding is highly comparable with epidemiological analyses of alcohol symptomatology in U.S. (Agrawal et al., 2010; Martin et al., 2011a; Verges et al., 2011) and with results from the 2007 Australian National Survey of Mental Health and Wellbeing, which reported a decrease in the lifetime rate of cannabis use disorder from 6.2% to 5.4% when transitioning from DSM-IV to DSM-5 (Mewton et al., 2013). In our sample, this decrease was uniformly attributable to individuals who endorsed hazardous use alone, which results in a DSM-IV diagnosis of cannabis abuse but not a DSM-5 diagnosis of cannabis use disorder, because it falls below the latter's minimum two-symptom threshold. No differences were noted in AA men (or women), and this is also not surprising. Individuals endorsing this criterion alone tend to be of higher socio-economic standing (Keyes and Hasin, 2008) and tend to, overwhelmingly, endorse this criterion due to a history of drinking and driving (Agrawal et al., 2011a). That

socio-economic status may correlate with ethnicity is expected—in our data, 45.9% of AA participants reported a gross annual income of less than \$20,000, vs. 15.4% of their EA counterparts.

Upon examining gender and ethnic differences within classification version (e.g., DSM-5 diagnoses across males and females), the only significant variation was noted for DSM-5 diagnoses in AA women who were more likely to receive a diagnosis of DSM-5, but not DSM-IV cannabis use disorder, relative to their EA female counterparts. Intriguingly, also relative to their EA counterparts, they were less likely to endorse hazardous use but more likely to endorse numerous other criteria, with the exception of giving up important activities and use despite physical/psychological problems. This finding may be attributable to the larger number of AA women that were ascertained from the cocaine dependence study (46% AA vs. 18.5% EA women are drawn from FSCD) vs. other studies. Although this observation holds true for the men as well, and the prevalence (or mean number of symptoms endorsed) did not vary across AA and EA women, it is possible that AA women (but not men or EA women) from the FSCD study represent a high-risk group. For instance, when compared to the alcohol and nicotine dependence studies, AA women from the cocaine (FSCD) study were more likely to report lower household income (59.6% vs. 34.4%) and a greater likelihood of less than a high school education (32.6% vs. 17.4%). Thus, this vulnerability might reflect environmental adversity rather than increased genetic susceptibility, and in any case, is accounted for in the genomic analyses by incorporating study sample and gender as covariates.

From a genetic perspective, the single SNP analyses did not reveal any genome wide significant signals. This is likely because our sample is underpowered, even with a quantitative trait, to detect single variants of modest effect size. Using GWAPower (Feng et al., 2011), we estimated power available in our dataset to identify SNPs of varying effect size. Power was 80% when an effect size of 0.01 (1%) was anticipated (with covariates explaining about 20% of the variance, and Type 1 error set at 5×10^{-8}). Increasing efforts to amass larger samples with comparable cannabis-related data would afford greater power to detect variants of more modest effect size via meta- and mega-analyses. However, few current studies have DSM-5 criteria data. In this regard, factor scores (or symptom counts) such as ours may prove to be useful phenotypes as they can accommodate DSM-IV and DSM-5 based assessments of vulnerability to cannabis use disorders.

In contrast, the gene-based analyses conducted with the EA subsample identified a cluster of genes, of varied function, on the q-arm of chromosome 17 that appeared to contain an aggregation of variants associated with DSM-5 cannabis use disorders. The genes that surpassed gene-based correction were *C17orf58*, *BPTF* and *PPM1D*. *PPM1D* is in a region of chromosome 17 that is well documented to be amplified in breast cancer (Bernards, 2004), and the gene itself belongs to a family of serine/threonine phosphatases that are involved in stress signaling (Lowe et al., 2012). On the other hand, *BPTF* was originally identified in brain homogenates from deceased Alzheimer's patients (Jordan-Sciutto et al., 2000). It is putatively involved in chromatin remodeling (Landry et al., 2008). We hesitate to speculate about the potential role of these genes in the etiology of cannabis use disorders.

Contradictory to the extant twin literature positing 50% heritable variation in cannabis use disorders, the aggregate effects of SNPs on the array captured 21% of genetic variation; however, this estimate was not statistically significant. The lack of significance is primarily due to our sample size. For instance, with cigarette smoking, a sample size of 4181 yielded a heritability estimate of 19% at $p = 0.024$ (Lubke et al., 2012). It is, however, worth noting that similar to other major psychiatric disorders (Lee et al., 2013), common variation on commercial arrays does not capture all the postulated heritability

in complex traits. This may be attributable to imperfect linkage disequilibrium between these SNPs and rarer causal variants or due to other factors, such as epistasis, gene-environment interplay and other variation (e.g., copy number variants).

Some other limitations of the present study are worth noting. First and foremost, the present sample was ascertained from three family studies of substance use disorders for the express purpose of identifying genetic variants for alcoholism, nicotine and cocaine dependence and related psychopathology. Hence, the psychometric analyses may not generalize to other cohorts with different ascertainment criteria. Second, while we were able to include a measure of cannabis withdrawal in the analysis, the symptoms and diagnostic scheme (i.e. 3 or more of 7 withdrawal symptoms) used to assess withdrawal do not conform to those in DSM-5. This was unavoidable since all studies predated the DSM-5 by a considerable number of years. However, analyses using the DSM-5 criteria in an independent twin sample do not indicate any evidence for genetic influences on DSM-5 withdrawal that do not overlap with DSM-IV cannabis abuse/dependence (Verweij et al., 2012).

From a clinical and public health standpoint, it is also reassuring to note that a transition from DSM-IV to DSM-5 will likely involve only a modest alteration in prevalence of diagnosed individuals. However, future studies, particularly those aggregating individual-level genotypic and phenotypic data across multiple samples should explore the extent to which individual DSM-IV, and in particularly, the new DSM-5 criteria contribute to specificity of genetic signals identified.

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Contributors

AA, MTL, KKB and LJB conceived of analyses. AA conducted all analyses with statistical support from LA, DMD, HJE, TF, AG, DBH and SH. Data were collected via funding to LA, DMD, HJE, TF, AG, EOJ, VH, JRK, SK, JIN, MS and LJB. Phenotypic expertise was provided by MTL, KKB, DMD, EOJ, VH, JRK, SK, MS. Support with genetic analyses was provided by LA, DMD, HJE, TF, DBH, SH, AG and JIN. All authors read and critically reviewed drafts of the manuscript.

Conflict of interest

Laura J. Bierut is listed as an inventor on Issued U.S. Patent 8080,371, "Markers for Addiction" covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.drugalcdep.2013.11.008>.

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