Research Paper

S100A10 identified in a genome-wide gene × cannabis dependence interaction analysis of risky sexual behaviours

Renato Polimanti, PhD; Shashwath A. Meda, MSc; Godfrey D. Pearlson, MA, MBBS; Hongyu Zhao, PhD; Richard Sherva, PhD; Lindsay A. Farrer, PhD; Henry R. Kranzler, MD; Joel Gelernter, MD

Background: We conducted a genome-wide gene × environment interaction analysis to identify genetic variants that interact with cannabis dependence (CaD) in influencing risky sexual behaviours (RSB). **Methods:** Our sample included cannabis-exposed and sexually experienced African-American and European-American participants. A DSM-IV CaD diagnosis and RSB were evaluated using the Semi-Structured Assessment for Drug Dependence and Alcoholism. We analyzed RSBs as a score that takes into account experiences of unprotected sex and multiple sexual partners. **Results:** A total of 3350 people participated in our study; 43% had a CaD diagnosis, 56% were African-American and 33% were women. We identified a genome-wide significant locus in African-American participants (*S100A10* rs72993629, $p = 2.73 \times 10^{-8}$) and a potential transpopulation signal in women (*CLTC* rs12944716, $p = 5.27 \times 10^{-8}$). A resting-state fMRI follow-up analysis of *S100A10* rs72993629 conducted in an independent cohort showed 2 significant associations: reduced power of the left paracentral lobule in amplitude of low frequency fluctuations (ALFF) analysis ($p = 7.8 \times 10^{-3}$) and reduced power of the right pallidum in fractional ALFF analysis ($p = 4.6 \times 10^{-3}$). The activity of these brain regions is known to be involved in sexual functions and behaviours. The *S100A10* result functionally recapitulated our *S100B* finding observed in our previous genome-wide association study of CaD. The probability of identifying 2 *S100* genes in 2 independent genome-wide investigations by chance is approximately 1 in 1.1 million. **Limitations:** We were not able to identify any African-American cohort with appropriate sample size, and phenotypic assessment is available to replicate our findings. **Conclusion:** The *S100A10* and *S100B* genes, which are located on different chromosomes, encode specialized calcium-binding proteins. These data support a role for calcium homeostasis in individuals with CaD and its induced behaviours.

Introduction

Cannabis is the most widely used illicit drug worldwide, and in developed countries the number of cannabis users is rapidly increasing as a consequence of decriminalization/legalization and decreased risk perception.[1] Approximately 10% of occasional cannabis users develop craving, physiologic dependence and other drug-seeking behaviours, which often have physical, psychological, social and occupational consequences.[2] Owing to the growing number of occasional users, it can be predicted that the number of individuals with cannabis use disorders will also increase rapidly in the coming years. Among the adverse events associated with cannabis abuse, risky sexual behaviours (RSBs) are a serious public health concern, because their major downstream consequences include sexually transmitted diseases (STDs) and unwanted pregnancies. Cannabis users are more likely to report sexual intercourse without the use of birth control, casual sex and multiple sexual partners than nonusers.[3–8] As consequences of these behaviours, cannabis users have a greater risk than nonusers of STDs and unintended pregnancies.[9]

Many studies have shown that cannabis abusers tend to exhibit neurocognitive deficits in mechanisms associated with impulsivity, suggesting that cannabis abuse affects brain structures involved in inhibitory control[10] — effects that may persist after many days of abstinence.[11] Furthermore, individuals with cannabis dependence (CaD) have shown neurocognitive deficits before use, and these pre-existing deficits confer liability for cannabis use disorder.[12] Al-

Correspondence to: J. Gelernter, Yale University School of Medicine, Department of Psychiatry, 950 Campbell Ave., West Haven, CT 06516; joel.gelernter@yale.edu

Submitted Sept. 22, 2016; Revised Dec. 21, 2016; Accepted Feb. 5, 2017; Early-released Apr. 18, 2017

DOI: 10.1503/jpn.160189

though these neurocognitive data support the hypothesis that CaD affects inhibitory control, which could be a mechanism leading to RSB, the molecular basis of the association between CaD and RSB is poorly understood. Genome-wide association studies (GWAS) are powerful tools used to identify molecular pathways involved in human behaviours. Twin studies have estimated that the heritability of lifetime cannabis use is about 40%.[13] A GWAS from our group identified the first significant risk alleles for CaD; these implicated genes involved in neuronal calcium homeostasis and central nervous system development.[14] As GWAS are free of specific physiologic hypotheses, they are uninfluenced by previous biases. In the case of CaD and RSB, we believe that gene × environment interaction analysis is an appropriate approach. Indeed, in gene × environment analysis, the environmental risk factor can be an exposure, either physical, chemical, or biological; a behaviour pattern; or a life event.[15] In particular, genome-wide gene × environment analysis can be used to investigate the phenotypic variation associated with interactive processes, such as that between genetic variation and CaD on RSB. Recently, we conducted genome-wide investigations of RSB, identifying molecular mechanisms supporting the role of substance dependencies and other psychiatric disorders in moderating the genetic predisposition to RSB and its consequences.[16,17]

In the present study, we conducted a genome-wide gene × cannabis dependence analysis of RSB in a cohort of cannabis-exposed and sexually experienced African-American and European-American participants. Information regarding CaD and RSB was derived using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA).[18,19] We defined CaD in accordance with the diagnostic criteria of the DSM-IV, and we defined RSB as a score based on lifetime experiences of unprotected sex and multiple sexual partners. As also noted by the International Cannabis Consortium,[20] cannabis use and dependence are not commonly assessed in large-scale genetic studies, although, as demonstrated by our CaD GWAS,[14] they are more informative traits than cannabis exposure, which is largely environmentally determined. Accordingly, the findings of the present study are based on a kind of deep phenotypic assessment that is not usually conducted in the large cohorts needed for complex trait genetic investigations. For this reason, to our knowledge, no cohort of appropriate sample size is available to replicate our results, especially when these are identified in ancestry groups, such as African-Americans, that are not often investigated in genetic studies.

Methods

Participants and diagnostic procedures

The participants included in the present study were recruited at 5 sites in the eastern United States.[21] The institutional review board at each participating site approved the study, and we obtained written informed consent from each participant. Participants were protected by certificates of confidentiality issued by the National Institute on Drug Abuse (NIDA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA). We evaluated RSBs using questions from the SSADDA section on antisocial personality: I35B ("Have you ever had sex with 10 different people within a single year?") and I37 ("Have you more than once had unprotected sex [without a condom] with someone you believed could give you a disease, or when you had a disease that could be spread that way?"). Based on these 2 questions, we calculated an RSB score that ranged from 0 to 2 based on the number of affirmative responses. We included only those individuals who reported having ever used cannabis more than 10 times (i.e., cannabis exposed) and reported sexual intercourse with at least 10 sexual partners (i.e., sufficiently sexually experienced for RSB criteria to be relevant). These inclusion criteria were used to define controls who were exposed to cannabis but did not develop dependence and who engaged in more than minimal sexual behaviour without developing RSB. To determine the STD and HIV status of the participants, we used the response to the SSADDA items "Has a doctor ever told you that you have (had): a sexually transmitted disease?" and "Has a doctor ever told you that you have (had): HIV/AIDS?". Data from SSADDA interviews were also used to derive DSM-IV diagnoses of lifetime CaD and other major psychiatric traits. Further details are available in our published GWAS of CaD in African-Americans and European-Americans.[14] We used self-reported history of STD and HIV to evaluate the interactive effects of the loci identified with CaD on STD/HIV status.

Genotyping and imputation

We extracted DNA from immortalized cell lines, blood, or saliva. We genotyped the samples using 2 different arrays. We used the Illumina HumanOmni1-Quad v1.0 microarray containing 988 306 autosomal single-nucleotide polymorphisms (SNPs) to genotype 2263 participants at the Center for Inherited Disease Research (CIDR) or the Yale Center for Genome Analysis and the Illumina HumanCoreExome array to genotype 1087 additional participants in the Gelernter laboratory (West Haven, Conn.). Principal component analysis (PCA) was conducted based on each genotyping array and for each ancestry group (African-American and European-American) using Eigensoft and SNPs that were common to the GWAS data sets and HapMap panel (after pruning the genome-wide SNPs for linkage disequilibrium [LD], r^2 > 80%). Detailed information about the preimputation quality control pipeline is available in our published CaD GWAS.[14] Imputation was performed using Impute2 software and the 1000 Genomes phase 1 reference panel. After imputation, we included SNPs with minor allele frequency > 5% and high imputation quality (certainty > 0.9, info > 0.8), yielding ~5.4 million variants in African-Americans and ~4.3 million variants in European-Americans.

Statistical analysis

We performed a genome-wide gene × CaD interaction analysis using the R package GWAF to fit a generalized

estimating equations model to adjust for the relatedness among individuals included in the same families.[22] We used the geepack.quant.int.batch.imputed function (i.e., regression model including a main effect [SNP] and the effect of the interaction term [the product of allelic dosage and a covariate for interaction]) from the GWAF package to test the interaction between the imputed allele dosage and DSM-IV CaD diagnosis for the RSB score, after adjusting for DSM-IV alcohol, cocaine, opioid and nicotine dependence diagnoses; age; and the first 3 ancestry PCAs (to correct for within–ancestry group population stratification). The analysis was performed stratifying the samples by genotyping array, ancestry and sex, and the results were combined by meta-analysis using METAL.[23] Then we performed transpopulation, ancestry-specific and sex-specific meta-analyses. In all meta-analyses, we applied a genomic control correction to all input files. We included different stratification strategies; to ascertain whether we thereby increased the risk of false-positive results, we performed a permutation analysis. Specifically, we performed the same genome-wide investigation considering 10 phenotypes generated by random permutation of individual RSB scores. In this simulation, no genome-wide significant (GWS) signal ($p < 5 \times 10^{-8}$) was observed and our genome-wide hit in African-Americans was significantly different from the result distributions of the phenotypes generated by random permutations. Then considering the results of the gene × CaD in African-Americans and European-Americans, we performed gene-based analysis in each ancestry group using VEGAS2 software.[24] Reference panels of the 1000 Genomes Project phase 3 European and African samples were used to correct for LD patterns in European-Americans and African-Americans, respectively.

Neuroimaging analysis of S100A10 *rs72993629*

Twenty-four unrelated and alcohol-exposed African-American participants were selected from the Brain and Alcohol Research in College Students (BARCS) study.[25] All of them underwent a single 5-minute run of resting-state fMRI on a 3 T scanner, as described previously.[26] Following preprocessing, amplitude of low frequency fluctuations (ALFF) images were computed by extracting power spectra via a fast Fourier transform (FFT) and computing the sum of amplitudes in the 0.01–0.08 Hz frequency band. The ALFF measure at each voxel represents the averaged square root of the power in the above frequency windows normalized by the mean within-brain ALFF value for that individual. For fractional ALFF (fALFF), the measure was scaled by total power across all available frequencies. Next, we used the REX toolbox to extract the mean ALFF/fALFF signal from all anatomic atlas labelling regions of interest (ROIs). We assessed main effects of these conditions and then used selected (left and right) ROIs to extract primary eigenvariate values. The participants were genotyped at Yale using 1 of 2 arrays, the Illumina HumanOmni1-Quad or the Illumina HumanOmni2.5. We conducted PCA to confirm the African ancestry of the sample and to evaluate any cryptic relatedness. The best-guess genotypes of *S100A10* rs72993629 were estimated considering a genotype probability threshold above 0.8. Linear regression analysis was performed considering ALFF and fALFF task traits as phenotypes, and age, sex, and the first 3 ancestry PCAs as covariates. We conducted 10 000 random permutations of the most promising phenotypes to empirically assess the significance of their association with *S100A10* rs72993629.

Results

Participants

Our final sample included 3350 participants: 1906 African-Americans and 1444 European-Americans. Of these, 43% had a CaD diagnosis, and 33% were women. Table 1 shows the characteristics of the sample.

Cannabis dependence and its association with RSB

In our cohort of 3350 participants with high comorbidity for substance use disorders, we observed that CaD had a significant effect on behaviours related to unprotected sex and multiple sexual partners (Fig. 1). Participants with CaD had a 44% greater risk of having more than 10 sexual partners in a single year (odds ratio [OR] 1.44, 95% confidence interval [CI] 1.06–1.95, $p = 0.019$). They also had a 53% greater risk of

Table 1: Characteristics of the study population stratified by ancestry and sex

	Group; mean ± SD or no. (%)			
Characteristic	AA women (*n* = 616)	AA men (*n* = 1290)	EA women (*n* = 503)	EA men (*n* = 941)
Age, yr	40 ± 8.7	43 ± 9.1	38 ± 11	39 ± 11
DSM-IV CaD (lifetime)	190 (31)	566 (44)	188 (37)	497 (52)
Multiple sexual partners	200 (32)	607 (47)	205 (41)	391 (42)
Unprotected sex	173 (28)	346 (27)	134 (27)	232 (25)
RSB score	0.61 ± 0.75	0.74 ± 0.72	0.67 ± 0.73	0.66 ± 0.7
STD status (positive)	299 (49)	491 (38)	163 (32)	166 (18)
HIV status (positive)	48 (8)	100 (8)	19 (4)	38 (4)

AA = African-American; CaD = cannabis dependence; EA = European-American; HIV = human immunodeficiency virus; RSB = risky sexual behaviours; SD = standard deviation; STD = sexually transmitted disease.

having unprotected sex with a risky partner (OR 1.53, 95% CI 1.26–1.86, $p = 1.38 \times 10^{-5}$).

Genome-wide gene × CaD interaction analysis

We conducted a genome-wide gene × CaD ancestry- and sex-stratified analysis. We observed a GWS result for *S100A10* rs72993629 in the African-American sample (z = 5.558, $p = 2.73 \times 10^{-8}$; Fig. 2A, and Appendix 1, Table S1, available at jpn.ca) and a female-specific signal for *CLTC* rs12944716 (z = –5.442, $p = 5.27 \times 10^{-8}$; Fig. 2B, and Appendix 1, Table S2). In both analyses, negligible inflation of meta-analyzed *p* values was observed (Fig. 3). In African-Americans, the *S100A10* rs72993629 imputed dosage of the G allele was associated with more RSB in those with CaD (z = 4.631, $p = 3.64 \times 10^{-6}$) and reduced RSB in in those without CaD (z = –3.723, $p = 1.97 \times 10^{-4}$; Fig. 4A). This finding specific to African-Americans is notable because our previous CaD GWAS identified *S100B* (one of the other members of the *S100* gene family) in African-American participants.[14] The probability of identifying 2 *S100* genes (of the 19 in the gene family) in 2 independent genome-wide analyses by chance is 1 in 1.1 million, assuming no prior evidence and without correction for the number of "gene families" in the genome. Our female-specific analysis identified a strong interaction between *CLTC* rs12944716 and CaD in relation to RSB (Fig. 4B): the imputed dosage of the A allele was associated with fewer RSBs in those with CaD (z = –4.67, $p = 3.02 \times 10^{-6}$) and more RSBs in those without CaD (z = 3.145, $p = 1.66 \times 10^{-3}$). According to ENCODE data available at www.encodeproject.org,[27] both of these variants are located in experimentally validated elements involved in gene regulation. Rs72993629 is located in the *S100A10* upstream region and is involved in proximal transcriptional regulation of the *S100A10* gene and distal transcriptional regulation of the *S100A11* gene. *S100A10* proximal transcriptional regulation was observed with respect to histone-marked regions in multiple cell lines (Appendix 1, Table S2). *S100A11* distal transcriptional regulation was observed in MCF-7 cell lines (Appendix 1, Table S3). Rs12944716 is located in the *CLTC* upstream region. This polymorphism is in high LD ($r^2 > 0.8$) with 70 regulatory variants, and it is directly involved in both proximal and distal transcriptional regulation. Rs12944716 proximal transcriptional regulation was observed with respect to *CLTC* through multiple mechanisms, including open chromatin, transcription factor binding sites and histone-marked regions (Appendix 1, Table S4). Rs12944716 distal transcriptional regulation affects multiple genes located in cis- and trans-regulatory regions, and it was observed in several cell lines (Appendix 1, Table S5).

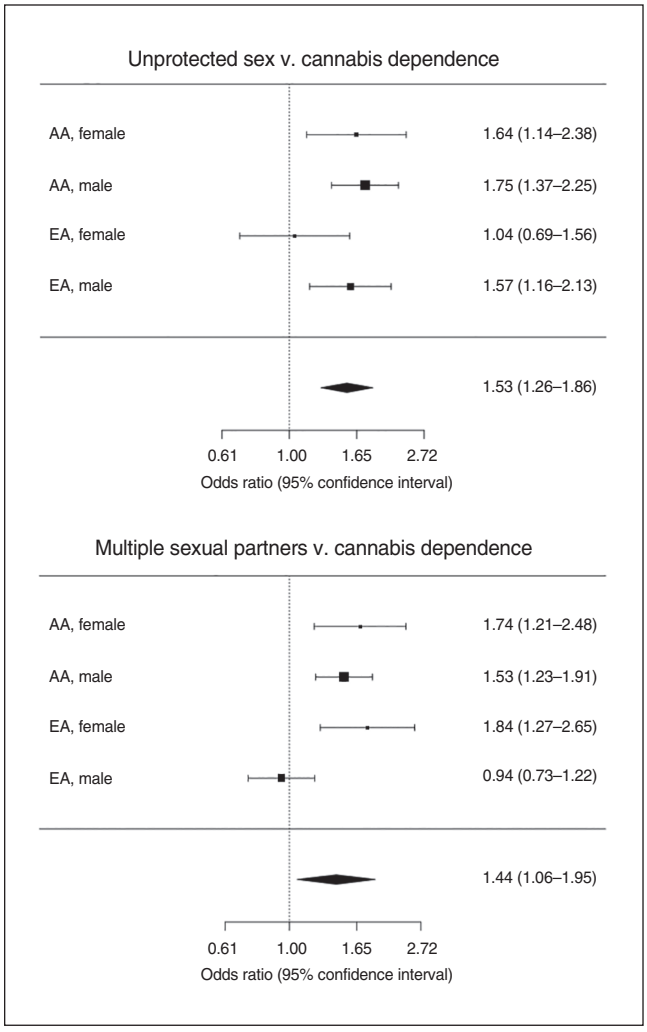

Fig. 1: Forest plots of associations between cannabis dependence (CaD) and positive reply to risky sexual behaviour (RSB) questions in ancestry × sex groups. AA = African-American; EA = European-American.

Neuroimaging follow-up analysis of LHPP *rs34997829*

Next, we conducted a follow-up fMRI analysis of *S100A10* rs72993629, the only GWS locus identified in our study, in an independent African-American sample. We investigated the resting ALFF and fALFF signals in all ROIs prescribed using the automatic anatomic labelling atlas. We observed that the G allele of *S100A10* rs72993629 (i.e., the imputed risk allele in our genome-wide investigation) was associated with reduced power of the left paracentral lobule ($t = -3.01$, $p = 7.8 \times 10^{-3}$) in the ALFF analysis and reduced fALFF signals of right pallidum ($t = -3.252$, $p = 4.6 \times 10^{-3}$). The subsequent permutation test ($n = 10\,000$) empirically confirmed these associations.

S100A10 *rs72993629*, CLTC *rs12944716 and the risk of STD and HIV*

To gain insight into the possible clinical significance of our main findings, we analyzed self-reported STD and HIV status in our cohort (in which we expect many false-negatives, but few false-positives). Because there were relatively few participants who reported these traits in our cohort, these results should be considered exploratory. In African-Americans, *S100A10* rs72993629 showed a trend interaction with CaD in association with both STD and HIV self-report status (z = 1.928, $p = 0.054$; and z = 1.802, $p = 0.071$, respectively) in a direction concordant with that observed for RSB.

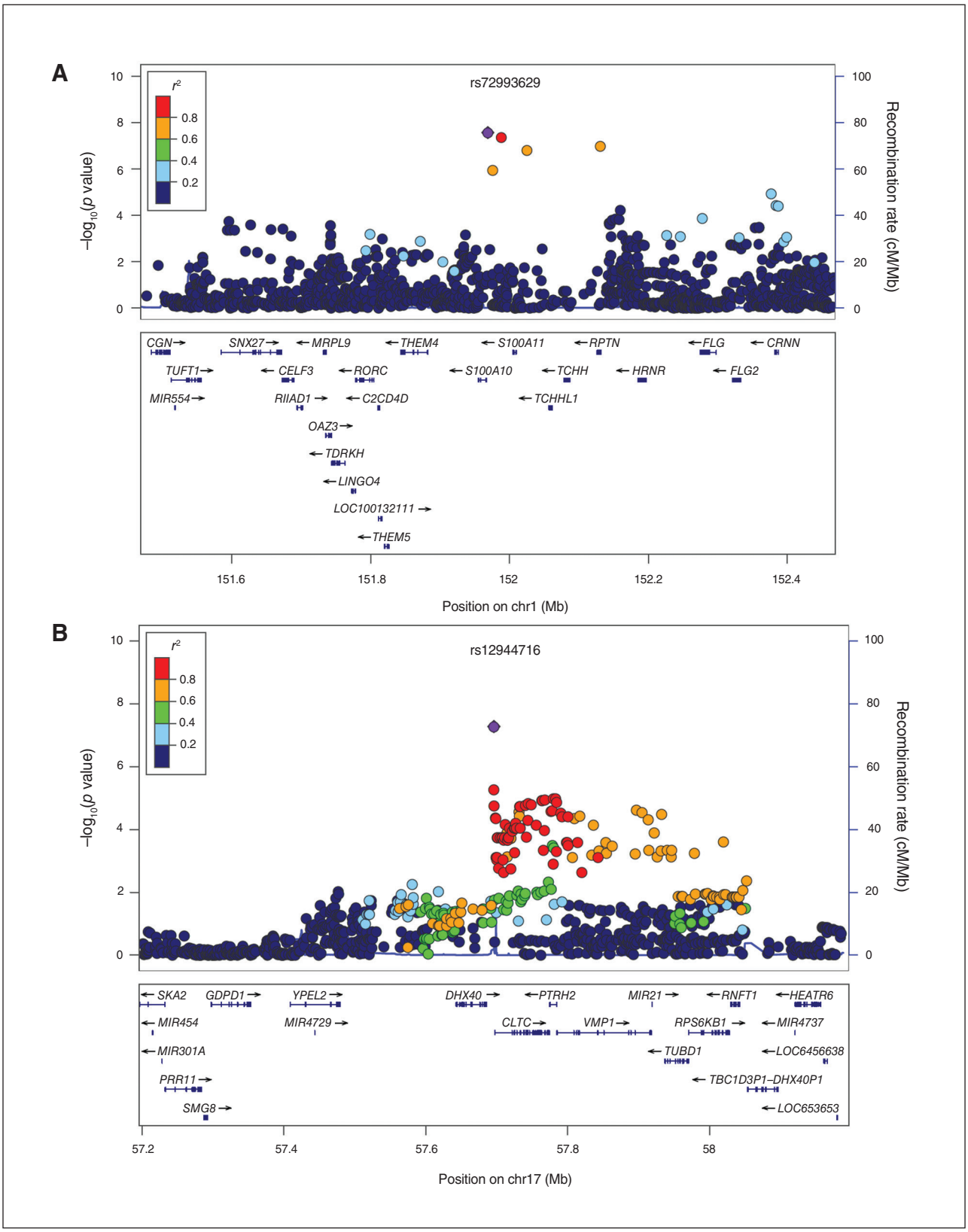

Fig. 2: Regional Manhattan plots of (**A**) *S100A10* rs72993629 and (**B**) *CLTC* rs12944716 in African-American–specific and female-specific meta-analyses, respectively.

In a female-specific analysis, we observed that *CLTC* rs12944716 interacted significantly with CaD in determining STD status (z = –2.366, $p = 0.018$).

Gene-based analysis

Finally, we conducted a gene-based analysis using the summary statistics of the ancestry-stratified analysis (because ancestry-specific LD patterns are used to identify the number of independent signals in each gene). No gene survived the gene-based GWS threshold. Considering the type I error rate at 1%, we observed that *TMOD2* is a potential candidate in both ancestry groups (African-Americans: gene-based $p = 9.49 \times 10^{-3}$, top-SNP [rs11630579] $p = 3.69 \times 10^{-4}$; European-Americans: gene-based $p = 6.10 \times 10^{-3}$, top-SNP [rs17525891] $p = 6.76 \times 10^{-4}$). An analysis of gene expression pattern based on Genotype-Tissue Expression (GTEx) data (available at www.gtexportal.org/home) indicated that *TMOD2* is a gene whose expression is brain-specific (Appendix 1, Fig. S1).

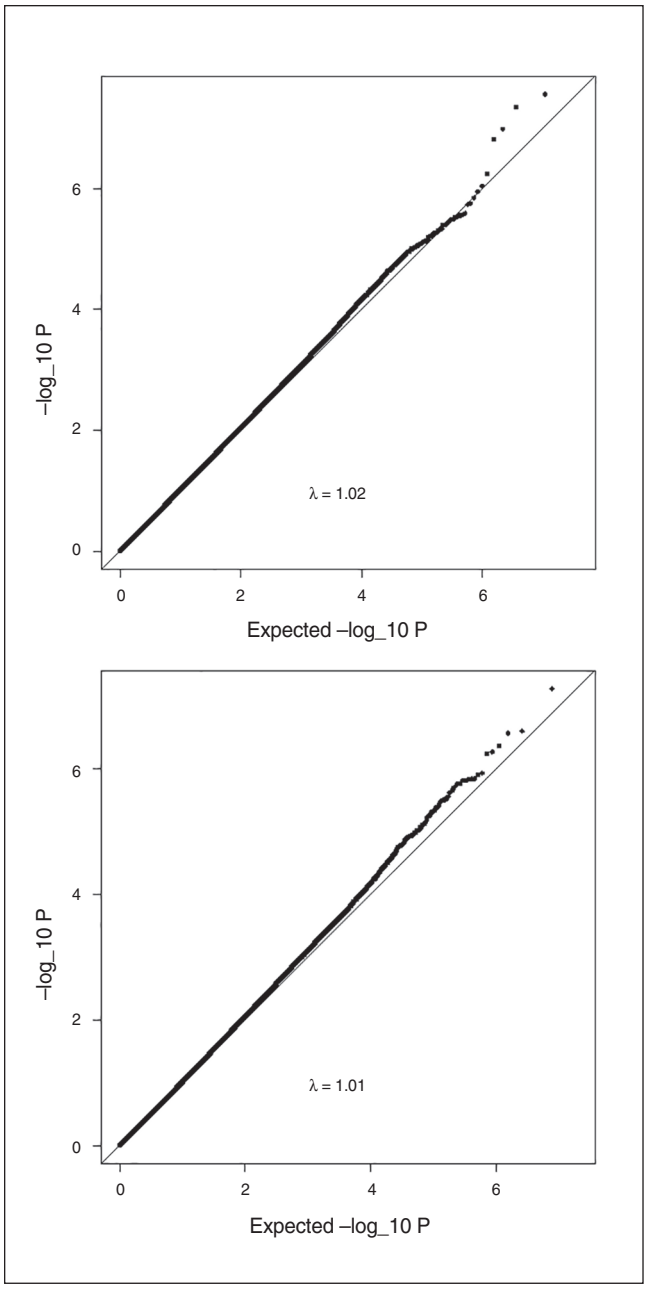

Fig. 3: Quantile–quantile plots of the genome-wide gene × cannabis dependence (**top**) African-American–specific and (**bottom**) female-specific meta-analyses.

Discussion

We conducted, to our knowledge, the first genome-wide investigation to understand the interplay of RSB and CaD and to identify specific genetic factors underlying that interaction. As observed in previous studies, CaD is associated with increased RSB in our cohort, which includes participants with high comorbidity for substance use disorders. This can be partially explained by the epidemiological correlation of these traits with a propensity for risk-taking behaviour.[28] From a genetic perspective, we would expect that a complex molecular network would also link these behaviours. Indeed, a recent GWAS of age at first sexual intercourse indicated that the onset of sexual activity is genetically correlated to risk-taking propensity.[29] A gene × CaD analysis can be a powerful approach to uncover the interactive molecular processes contributing to RSB. On this basis, our results represent an important first step toward understanding the biology of the interplay of RSB and CaD.

Our genome-wide gene × CaD analysis identified 2 loci, 1 that meets standard GWS and 1 that approaches GWS. These 2 findings are from sex- and ancestry-stratified analyses (females and African-Americans). Risky sexual behaviour differs greatly by sex, and it would be expected that different mechanisms and different risk loci could be involved in men and women.[30] Different population groups have different risk loci and alleles for many genetically complex traits,[31] so it is reasonable to have the same expectation for RSB.

In African-American participants, *S100A10* rs72993629 showed a GWS crossing interaction with CaD in association with RSB (i.e., the genetic association is in opposite direction with respect to DSM-IV CaD diagnosis). As mentioned previously, this variant is located in an experimentally validated regulatory element that affects transcriptional regulation of *S100A10* and *S100A11*. These genes are included in the *S100A* gene cluster on chromosome 1q21. *S100* genes encode a highly specialized family of calcium-binding proteins that function both intra- and intercellularly.[32] The S100A10 protein product (also known as P11) is downregulated in human and rodent depressive-like states[33] and may be involved in the mechanism of cocaine reward.[34] Regarding *S100A11*, a recent transcriptomic analysis of brains from individuals with psychiatric disorders indicated that this gene shows one of the most significant differences in coexpression between schizophrenia and bipolar disorders.[35] However, the most relevant published data come from our recent CaD GWAS.[14] In African-Americans, we observed a GWS association of a polymorphism (rs186825689; discovery cohort $p =$

1.86×10^{-8}) located in the upstream region of the gene encoding S100 calcium binding protein (*S100B*) with CaD symptom count. This result was also supported by a recent study that observed an increased *S100B* blood levels in participants with cannabis use disorder,[36] a result using completely different methodology that provides external validation. The *S100B* gene is located at 21q22.3, so our present finding regarding *S100A10* rs72993629 (on chromosome 1q21) shows a functional, rather than a positional, association. Multiple studies have demonstrated that the *S100B* protein is involved in several nervous system processes, including neurite extension, stimulation of Ca2+ fluxes, inhibition of protein kinase C (PKC)–mediated phosphorylation, astrocytosis and axonal proliferation, and inhibition of microtubule assembly, and it may also be involved in neurologic diseases, such Alzheimer disease, Down syndrome, epilepsy and amyotrophic lateral sclerosis.[37] Considering GTEx data,[38] we observed a different gene expression pattern between *S100B* and *S100A* genes (Appendix 1, Fig. S1). The *S100B* gene is predominantly expressed in brain tissues; *S100A10* and *S100A11* are less expressed in brain tissues than *S100B*, but they showed increased gene expression in other tissues, including those related to the female and male reproductive systems (i.e., vagina, fallopian tubes, cervix, uterus, prostate and testis). These observations support an interesting hypothesis. If the *S100* genes are relevant to the biology of CaD, as supported by our previous CaD GWAS and by the present results (as mentioned previously, the probability of a chance finding implicating 2 genes in this system in association with cannabis is extremely low), their gene expression distribution could reflect differences in their specific roles. *S100B* is mostly expressed in the central nervous system, and its effect on cannabis use may be more related to craving, dependence and other drug-seeking behaviours. Conversely, the wider tissue distribution of *S100A10* and *S100A11* expression may result in an involvement of these gene expression in downstream CaD-induced consequences. In particular, *S100A10* and *S100A11* may differentially moderate feedback mechanisms between genitalia and certain brain regions in individuals with and without CaD. Supporting evidence regarding this hypothesis is provided by our follow-up imaging analysis, where rs72993629 was associated with activities in brain regions involved in sexual behaviours through genitalia response. Specifically, we observed that rs72993629 was associated with reduced power at the left paracentral lobule in ALFF analysis and reduced power at the right pallidum in the fALFF analysis. Resting ALFF/fALFF analysis provides relevant data regarding spontaneous brain activity and signals originating in grey matter.[26] A previous independent fALFF analysis of sexual orientation showed increased activity of the right pallidum in homosexual men compared with heterosexual men,[39] and another study showed increased activation of the right pallidum during induced sexual arousal.[40] Regarding the paracentral lobule, numerous studies confirmed that this region is an important brain area for sexual function and behaviours.[41] Indeed, the genital sensory cortex, identified in the classical Penfield homunculus, is located in the paracentral lobule.[42] These previous findings confirm that the activity of brain regions associated with rs72993629 plays a key role in sexual behaviours. However, further brain imaging studies with larger samples and CaD information are needed to confirm and deepen our understanding of the role of the variant identified in the CaD–RSB interplay.

Our genome-wide investigation also identified a possible female-specific result for rs12944716, located in the *CLTC* up-

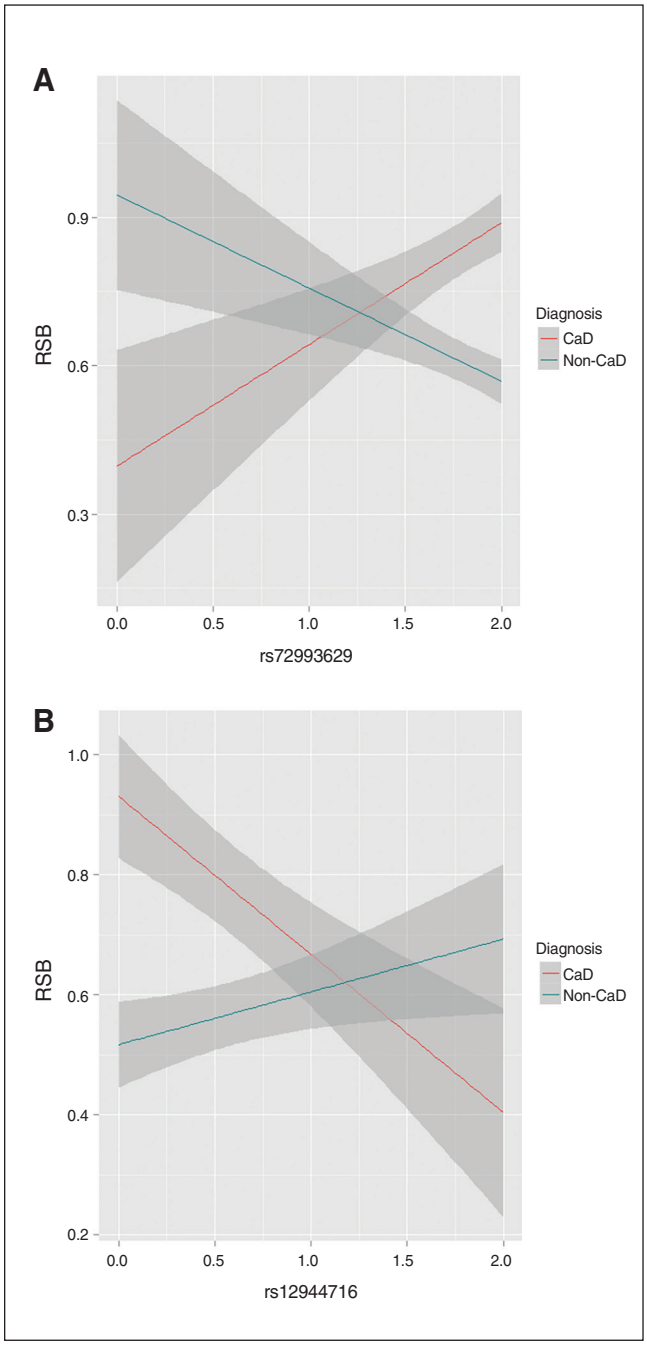

Fig. 4: Association between imputed allele dosage of (**A**) *S100A10* rs72993629 and (**B**) *CLTC* rs12944716 with risky sexual behaviour (RSB) in participants with and without cannabis dependence (CaD).

stream region, which nearly reached GWS. The SNP was involved in transcriptional regulation of the gene. *CLTC* encodes clathrin, a major protein component of the cytoplasmic face of intracellular organelles, which plays a key role in intracellular trafficking. In a previous study, morphine was found to regulate the expression of cytoskeleton genes, including *Cltc*, in rat striatum, causing a structural brain modification in chronic substance administration.[43] However, this result may be associated with other genes; ENCODE data indicate that rs12944716 is located in a complex regulatory element that affects the transcription of several genes located in cis- and transregulatory regions. Thus, further analysis is needed to confirm and follow up this sex-specific result.

Cannabis use has been proposed as a treatment for wasting syndrome in patients with HIV/AIDS,[44] and cannabis users with HIV/AIDS have been reported to have a high probability of RSB.[45–47] Thus, we investigated whether the variants identified may interact with CaD in association with STD and HIV status. We observed that both rs72993629 and rs12944716 may interact with CaD to link RSB to STD and HIV status. However, these results should be considered exploratory because of the low power available in this study given the low prevalence of STD and HIV in our sample and the lack of objective verification. The associations among CaD, RSB and STD/HIV risk are mediated by both environmental and biological factors. Although numerous social and behavioural factors have been identified to explain these associations, very few molecular mechanisms have been identified to help explain how the use of cannabis influences RSB consequences. To our knowledge, our data represent the first relevant genome-wide results in this area.

Gene-based analysis identified *TMOD2* as a candidate risk locus in both African-Americans and European-Americans. This gene encodes a neuronal-specific member of the tropomodulin family of actin-regulatory proteins. Knockout mice showed hyperactivity, reduced sensorimotor gating, impaired learning and memory, and enhanced long-term potentiation.[48] A recent study demonstrated that *TMOD2* is a positive regulator of dendritic complexity and spine morphology.[49] Cannabis use is well known to have a significant impact on brain structures that are enriched with cannabinoid receptors.[50] Although no other studies have verified this hypothesis yet (to date we can identify only 13 articles regarding *TMOD2* function), we speculate that *TMOD2* could be involved in the remodelling processes induced by cannabis use in brain areas relevant to sexual function and behaviours.

Limitations

The main limitation of our study is the lack of an African-American replication cohort for our key finding on the role of *S100* genes in individuals with CaD. This is emblematic of a major lack of African-American samples restricting the scope of many genetic investigations, particularly for psychiatric traits. Nevertheless, the identification of 2 members of the small *S100* gene family (19 genes) in 2 independent cannabis-related genome-wide investigations had very low a priori probability. We propose that this is unlikely to be due to chance, less so when considered in the context of the highly supportive imaging data. The absence of an adequate replication cohort for our study is also attributable to the fact that cannabis use and dependence are not commonly assessed in large-scale genetic studies that are designed to address other medical issues (notably, this is not the case for alcohol and nicotine use, which are widely recognized as important for many medical traits),[20] and most studies of cannabis use disorders are conducted in individuals with European ancestry. The generally reduced population diversity in genetic research is well-known.[51] Thus, our results are particularly relevant because they not only provide new insights into the biology of CaD and RSB, but they also contribute to our understanding of the genetics of psychiatric traits in African-Americans. Additional studies should investigate the temporal sequence of RSB–CaD interaction and how this interaction is moderated by genetic factors.

Conclusion

The key loci identified in the present study offer molecular insight into the interplay between CaD and RSB, highlighting mechanisms related to calcium homeostasis, brain activity and brain structure. Furthermore, the risk alleles identified may play a role in the risk of STD and HIV transmission through their effect on the RSB–CaD interplay. Although the shared genetic liability to addictive behaviours is surely correlated to other behaviours, such as impulsivity and sensation-seeking, our current findings appear to be specific to CaD. Indeed, there is no overlapping between the current findings and the results of our previous genome-wide gene × alcohol dependence analysis of RSB,[16] and the present *S100A10* result appears to be in line with our previous GWAS of CaD.[14] However, this hypothesis can be tested thoroughly only when much larger sample sizes can be attained.

Acknowledgements: This study was supported by National Institutes of Health grants RC2 DA028909, R01 DA12690, R01 DA12849, R01 DA18432, R01 AA11330, R01 AA017535, P50 AA012870, R01 AA016599, RC1 AA019036, the Connecticut and Crescenz Philadelphia VA MIRECCs, and a NARSAD Young Investigator Award (to R. Polimanti) from the Brain & Behavior Research Foundation. The authors appreciate the work in recruitment and assessment provided at Yale University School of Medicine and the APT Foundation, McLean Hospital, the Medical University of South Carolina and the University of Pennsylvania. Genotyping services for a part of our GWAS were provided by the Center for Inherited Disease Research (CIDR) and Yale University Center for Genome Analysis. CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University (contract number N01-HG-65403). The authors are grateful to Ann Marie Lacobelle and Christa Robinson for their excellent technical assistance, and to the SSADDA interviewers, led by Yari Nunez, who devoted substantial time and effort to phenotype the study sample.

Affiliations: From the Department of Psychiatry, Yale University School of Medicine and VA CT Healthcare Center, West Haven, Conn., USA (Polimanti, Pearlson, Gelernter); Olin Research Center, Institute of Living/Hartford Hospital, Hartford, Conn., USA (Meda, Pearlson); the Department of Neuroscience, Yale University School of Medicine, New Haven, Conn., USA (Pearlson, Gelernter); the Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, Mass., USA (Sherva, Farrer); the Department of

Biostatistics, Yale University School of Public Health, New Haven, Conn., USA (Zhao); the Department of Genetics, Yale University School of Medicine, New Haven, Conn., USA (Zhao, Gelernter); the Departments of Neurology, Ophthalmology, Biostatistics, and Epidemiology, Boston University Schools of Medicine and Public Health, Boston, Mass., USA (Farrer); and the Department of Psychiatry, University of Pennsylvania School of Medicine and VISN 4 MIRECC, Crescenz VAMC, Philadelphia, Pa., USA (Kranzler).

Competing interests: H. Kranzler has been an advisory board member, consultant, or CME speaker for Indivior, Lundbeck, and Otsuka. He is also a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which is supported by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Pfizer, and XenoPort. None declared by any other authors.

Contributors: R. Polimanti and J. Gelernter designed the study. S. Meda, G. Pearlson, L. Farrer, H. Kranzler and J. Gelernter acquired the data, which all authors analyzed. R. Polimanti wrote the article, which all authors reviewed and approved for publication.

References

1. Kalant H. Cannabis control policy: no rational basis yet for legalization. *Clin Pharmacol Ther* 2015;97:538-40.
2. Budney AJ, Roffman R, Stephens RS, et al. Marijuana dependence and its treatment. *Addict Sci Clin Pract* 2007;4:4-16.
3. Ross JM, Coxe S, Schuster RM, et al. The moderating effects of cannabis use and decision making on the relationship between conduct disorder and risky sexual behavior. *J Clin Exp Neuropsychol* 2015; 37:303-15.
4. Shorey RC, Fite PJ, Choi H, et al. Dating violence and substance use as longitudinal predictors of adolescents' risky sexual behavior. *Prev Sci* 2015;16:853-61.
5. Brodbeck J, Matter M, Moggi F. Association between cannabis use and sexual risk behavior among young heterosexual adults. *AIDS Behav* 2006;10:599-605.
6. Jackson JM, Seth P, DiClemente RJ, et al. Association of depressive symptoms and substance use with risky sexual behavior and sexually transmitted infections among African American female adolescents seeking sexual health care. *Am J Public Health* 2015;105: 2137-42.
7. Bryan AD, Schmiege SJ, Magnan RE. Marijuana use and risky sexual behavior among high-risk adolescents: trajectories, risk factors, and event-level relationships. *Dev Psychol* 2012;48:1429-42.
8. Auslander WF, Thompson RG Jr, Gerke DR. The moderating effect of marijuana use on the relationship between delinquent behavior and HIV risk among adolescents in foster care. *J HIV AIDS Soc Serv* 2014;13:179-97.
9. Schuster RM, Crane NA, Mermelstein R, et al. The influence of inhibitory control and episodic memory on the risky sexual behavior of young adult cannabis users. *J Int Neuropsychol Soc* 2012;18:827-33.
10. Crean RD, Crane NA, Mason BJ. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J Addict Med* 2011;5:1-8.
11. Bolla KI, Brown K, Eldreth D, et al. Dose-related neurocognitive effects of marijuana use. *Neurology* 2002;59:1337-43.
12. Panlilio LV, Goldberg SR, Justinova Z. Cannabinoid abuse and addiction: clinical and preclinical findings. *Clin Pharmacol Ther* 2015; 97:616-27.
13. Minica CC, Dolan CV, Hottenga JJ, et al. Heritability, SNP- and gene-based analyses of cannabis use initiation and age at onset. *Behav Genet* 2015;45:503-13.
14. Sherva R, Wang Q, Kranzler H, et al. Genome-wide association study of cannabis dependence severity, novel risk variants, and shared genetic risks. *JAMA Psychiatry* 2016;73:472-80.
15. Ottman R. Gene-environment interaction: definitions and study designs. *Prev Med* 1996;25:764-70.
16. Polimanti R, Wang Q, Meda SA, et al. The interplay between risky sexual behaviors and alcohol dependence: genome-wide association and neuroimaging support for LHPP as a risk gene. *Neuropsychopharmacology* 2017;42:598-605.
17. Wang Q, Polimanti R, Kranzler HR, et al. Genetic factor common to schizophrenia and HIV infection is associated with risky sexual behavior: antagonistic vs. synergistic pleiotropic SNPs enriched for distinctly different biological functions. *Hum Genet* 2016; 136:75-83.
18. Pierucci-Lagha A, Gelernter J, Feinn R, et al. Diagnostic reliability of the Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA). *Drug Alcohol Depend* 2005;80:303-12.
19. Pierucci-Lagha A, Gelernter J, Chan G, et al. Reliability of DSM-IV diagnostic criteria using the semi-structured assessment for drug dependence and alcoholism (SSADDA). *Drug Alcohol Depend* 2007;91:85-90.
20. Stringer S, Minica CC, Verweij KJ, et al. Genome-wide association study of lifetime cannabis use based on a large meta-analytic sample of 32 330 subjects from the International Cannabis Consortium. *Transl Psychiatry* 2016;6:e769.
21. Gelernter J, Sherva R, Koesterer R, et al. Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. *Mol Psychiatry* 2014;19:717-23.
22. Chen MH, Yang Q. GWAF: an R package for genome-wide association analyses with family data. *Bioinformatics* 2010;26:580-1.
23. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26:2190-1.
24. Mishra A, Macgregor S. VEGAS2: software for more flexible gene-based testing. *Twin Res Hum Genet* 2015;18:86-91.
25. Dager AD, Anderson BM, Stevens MC, et al. Influence of alcohol use and family history of alcoholism on neural response to alcohol cues in college drinkers. *Alcohol Clin Exp Res* 2013;37(Suppl 1): E161-71.
26. Meda SA, Wang Z, Ivleva EI, et al. Frequency-specific neural signatures of spontaneous low-frequency resting state fluctuations in psychosis: evidence from Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP) Consortium. *Schizophr Bull* 2015; 41:1336-48.
27. Kellis M, Wold B, Snyder MP, et al. Defining functional DNA elements in the human genome. *Proc Natl Acad Sci U S A* 2014;111: 6131-8.
28. Copeland J, Swift W. Cannabis use disorder: epidemiology and management. *Int Rev Psychiatry* 2009;21:96-103.
29. Day FR, Helgason H, Chasman DI, et al. Physical and neurobehavioral determinants of reproductive onset and success. *Nat Genet* 2016;48:617-23.
30. Yang BZ, Han S, Kranzler HR, et al. Sex-specific linkage scans in opioid dependence. *Am J Med Genet B Neuropsychiatr Genet* 2017; 174:261-68.
31. Polimanti R, Yang C, Zhao H, et al. Dissecting ancestry genomic background in substance dependence genome-wide association studies. *Pharmacogenomics* 2015;16:1487-98.
32. Zimmer DB, Chaplin J, Baldwin A, et al. S100-mediated signal transduction in the nervous system and neurological diseases. *Cell Mol Biol (Noisy-le-grand)* 2005;51:201-14.
33. Warner-Schmidt JL, Chen EY, Zhang X, et al. A role for p11 in the antidepressant action of brain-derived neurotrophic factor. *Biol Psychiatry* 2010;68:528-35.
34. Arango-Lievano M, Schwarz JT, Vernov M, et al. Cell-type specific expression of p11 controls cocaine reward. *Biol Psychiatry* 2014; 76:794-801.
35. de Baumont A, Maschietto M, Lima L, et al. Innate immune response is differentially dysregulated between bipolar disease and schizophrenia. *Schizophr Res* 2015;161:215-21.
36. Bayazit H, Cicek E, Selek S, et al. Increased S100B levels in cannabis use disorder. *Eur Addict Res* 2016;22:177-80.

37. Michetti F, Corvino V, Geloso MC, et al. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. *J Neurochem* 2012;120:644-59.

38. GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015;348:648-60.

39. Hu S, Xu D, Peterson BS, et al. Differing default mode network activities in men with homosexual or heterosexual preferences. *J Sex Med* 2014;11:2474-84.

40. Hu SH, Wei N, Wang QD, et al. Patterns of brain activation during visually evoked sexual arousal differ between homosexual and heterosexual men. *AJNR Am J Neuroradiol* 2008;29:1890-6.

41. Cazala F, Vienney N, Stoleru S. The cortical sensory representation of genitalia in women and men: a systematic review. *Socioaffect Neurosci Psychol* 2015;5:26428.

42. Komisaruk BR, Wise N, Frangos E, et al. Women's clitoris, vagina, and cervix mapped on the sensory cortex: fMRI evidence. *J Sex Med* 2011;8:2822-30.

43. Marie-Claire C, Courtin C, Roques BP, et al. Cytoskeletal genes regulation by chronic morphine treatment in rat striatum. *Neuropsychopharmacology* 2004;29:2208-15.

44. Aggarwal SK, Carter GT, Sullivan MD, et al. Medicinal use of cannabis in the United States: historical perspectives, current trends, and future directions. *J Opioid Manag* 2009;5:153-68.

45. Tyurina A, Krupitsky E, Cheng DM, et al. Is cannabis use associated with HIV drug and sex risk behaviors among Russian HIV-infected risky drinkers? *Drug Alcohol Depend* 2013;132:74-80.

46. Elkington KS, Bauermeister JA, Santamaria EK, et al. Substance use and the development of sexual risk behaviors in youth perinatally exposed to HIV. *J Pediatr Psychol* 2015;40:442-54.

47. Mimiaga MJ, Reisner SL, Grasso C, et al. Substance use among HIV-infected patients engaged in primary care in the United States: findings from the Centers for AIDS Research Network of Integrated Clinical Systems cohort. *Am J Public Health* 2013;103:1457-67.

48. Cox PR, Fowler V, Xu B, et al. Mice lacking Tropomodulin-2 show enhanced long-term potentiation, hyperactivity, and deficits in learning and memory. *Mol Cell Neurosci* 2003;23:1-12.

49. Gray KT, Suchowerska AK, Bland T, et al. Tropomodulin isoforms utilize specific binding functions to modulate dendrite development. *Cytoskeleton (Hoboken)* 2016;73:316-28.

50. Rocchetti M, Crescini A, Borgwardt S, et al. Is cannabis neurotoxic for the healthy brain? A meta-analytical review of structural brain alterations in non-psychotic users. *Psychiatry Clin Neurosci* 2013;67:483-92.

51. Haga SB. Impact of limited population diversity of genome-wide association studies. *Genet Med* 2010;12:81-4.

Service Information

Subscriptions and sales

Annual subscriptions are available at the following rates in 2017 (Canadian customers pay in Canadian funds and add applicable taxes; US and rest of world pay in US funds): Can$235 or US$270 individuals, Can$360 or US$420 institutions, Can$45 for Canadian students or residents, and Can$35 or US$45 for single or back issues. For more information or to subscribe, please contact the Joule Inc. Subscription Office, PO box 830350, Birmingham AL 35283-0350; phone 800 633-4931 (Canada and Continental US only) or 205 995-1567; fax 205 995-1588; cma@subscriptionoffice.com.

Change of address

Subscribers should send the new address and the effective date of change to the CMA Member Service Centre at cmamsc@cma.ca. Allow at least 8 weeks' notice to ensure uninterrupted service.

Reprints

Commercial and author reprints can be purchased through Sheridan Press. To purchase commercial article reprints and ePrints, or to request a quote, please contact Hope Robinson, Sheridan Content Sales Specialist; hope.robinson@sheridan.com. Authors can order reprints by submitting an author reprint order form available at the Sheridan Press Electronic Order Centre at www.sheridan.com/cma/eoc or by contacting Lori Laughman, Customer Service Representative; lori.laughman@sheridan.com.

Microform, abstracting and indexing

The journal is abstracted or indexed in: MEDLINE/Index Medicus, Current Contents, Bioresearch Index, Social Sciences Citation Index, Biological Abstracts, EMBASE/Excerpta Medica, e-psyche, Mental Health Abstracts, ISI Web of Science, Child Development Abstracts and Bibliography, Standard Periodical Directory, Arts and Humanities Citation Index, Research Alert (formerly Automated Subject Citation Alert), Criminal Justice Abstracts, Neuroscience Citation Index, Psychological Abstracts, Sociological Abstracts. *JPN* is available on microform from ProQuest Information and Learning, PO Box 1346, Ann Arbor MI 48106-1346; il.proquest.com.