Original Investigation

Early Cannabis Use, Polygenic Risk Score for Schizophrenia, and Brain Maturation in Adolescence

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IMPORTANCE Cannabis use during adolescence is known to increase the risk for schizophrenia in men. Sex differences in the dynamics of brain maturation during adolescence may be of particular importance with regard to vulnerability of the male brain to cannabis exposure.

OBJECTIVE To evaluate whether the association between cannabis use and cortical maturation in adolescents is moderated by a polygenic risk score for schizophrenia.

DESIGN, SETTING, AND PARTICIPANTS Observation of 3 population-based samples included initial analysis in 1024 adolescents of both sexes from the Canadian Saguenay Youth Study (SYS) and follow-up in 426 adolescents of both sexes from the IMAGEN Study from 8 European cities and 504 male youth from the Avon Longitudinal Study of Parents and Children (ALSPAC) based in England. A total of 1577 participants (aged 12-21 years; 899 [57.0%] male) had (1) information about cannabis use; (2) imaging studies of the brain; and (3) a polygenic risk score for schizophrenia across 108 genetic loci identified by the Psychiatric Genomics Consortium. Data analysis was performed from March 1 through December 31, 2014.

MAIN OUTCOMES AND MEASURES Cortical thickness derived from T1-weighted magnetic resonance images. Linear regression tests were used to assess the relationships between cannabis use, cortical thickness, and risk score.

RESULTS Across the 3 samples of 1574 participants, a negative association was observed between cannabis use in early adolescence and cortical thickness in male participants with a high polygenic risk score. This observation was not the case for low-risk male participants or for the low- or high-risk female participants. Thus, in SYS male participants, cannabis use interacted with risk score vis-à-vis cortical thickness (P = .009); higher scores were associated with lower thickness only in males who used cannabis. Similarly, in the IMAGEN male participants, cannabis use interacted with increased risk score vis-à-vis a change in decreasing cortical thickness from 14.5 to 18.5 years of age (t_{137} = −2.36; P = .02). Finally, in the ALSPAC high-risk group of male participants, those who used cannabis most frequently (≥61 occasions) had lower cortical thickness than those who never used cannabis (difference in cortical thickness, 0.07 [95% CI, 0.01-0.12]; P = .02) and those with light use (<5 occasions) (difference in cortical thickness, 0.11 [95% CI, 0.03-0.18]; P = .004).

CONCLUSIONS AND RELEVANCE Cannabis use in early adolescence moderates the association between the genetic risk for schizophrenia and cortical maturation among male individuals. This finding implicates processes underlying cortical maturation in mediating the link between cannabis use and liability to schizophrenia.

JAMA Psychiatry. 2015;72(10):1002-1011. doi:10.1001/jamapsychiatry.2015.1131
Published online August 26, 2015.

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Cannabis is the most common illicit substance used across the world, with the 2012 annual prevalence of cannabis use reaching 3.8% (177.63 million users) among people aged 15 to 64 years.[1] Globally, more than 13 million people were dependent on cannabis in 2010; annual prevalence of cannabis dependence appears to peak between 20 and 24 years of age and is higher in males than females and in high-income countries.[2] As with any other illicit substance, cannabis use emerges during adolescence. Based on the 2011 European School Survey Project on Alcohol and Other Drugs,[3] a mean lifetime prevalence of cannabis use among high school students aged 15 to 16 years was 17%, with large variations across the 36 participating countries (eg, 19% in Germany, 25% in the United Kingdom, and 39% in France). The 2014 Monitoring the Future survey[4] has reported a lifetime prevalence of cannabis use of 35.8% among youth aged 15 to 16 years living in the United States in 2013. Thus, a large proportion of individuals are exposed to cannabis during early to middle adolescence, a developmental period characterized by the continuing maturation of neural circuits.

Adolescence is a period of transition that involves a large number of age-related changes in physiological processes (eg, sex hormones) and social environment (eg, peer-peer interactions).[5,6] Such influences—often in interaction with genetic variations—shape the neurobiological features that underly maturation of the adolescent brain, as quantified in vivo with magnetic resonance imaging (MRI). A number of large-scale MRI studies of typically developing adolescents[7-12] have identified age-related changes in gray and white matter volumes, cortical thickness, white-matter microstructure, and brain response to various stimuli and cognitive processes. Many of these brain metrics show sex differences in their trajectories, such as steeper slopes of age-related increases in white matter and decreases in (cortical) gray matter in male compared with female adolescents.[13,14] These sex differences in the dynamics of brain maturation during adolescence may be of particular importance with regard to vulnerability of the male brain to external factors, such as cannabis exposure, during this period of development. In this context, we note previous observations of an earlier onset of schizophrenia in men compared with women; the first signs of schizophrenia, the first positive symptoms, and the first admissions occur 3 to 5 years earlier in men, and the age range of the first sign of mental disorder is from 15 to 24 years for men (compared with 20-29 years for women).[15] Given the solid epidemiologic evidence supporting a link between cannabis exposure during adolescence and schizophrenia,[16] we investigate whether the use of cannabis during early adolescence (by 16 years of age) is associated with variations in brain maturation as a function of genetic risk for schizophrenia, as assessed with the recently developed polygenic risk score.[17] We address this question in 3 samples of typically developing youth for whom we have obtained (1) information about their cannabis use during adolescence; (2) structural T1-weighted MRI of the brain; and (3) their polygenic risk score for schizophrenia.[17]

Methods

Samples and Overall Strategy

The initial analysis was performed in a sample of 1024 adolescents recruited in the context of the Saguenay Youth Study (SYS).[18] This sample comes from the Saguenay Lac-Saint-Jean region of Quebec, Canada.[19] Magnetic resonance imaging of the brain and information about cannabis use were collected at 1 point in a cross-sectional manner from participants aged 12 to 18 years.

Follow-up analyses were performed in 2 other population-based samples. The first replication sample consisted of 504 male youth recruited from the Avon Longitudinal Study of Parents and Children (ALSPAC)[20] based in England. The use of cannabis was assessed repeatedly throughout adolescence, and MRIs of the brain were collected at 1 point when the participants reached 18 to 21 years of age. The second replication sample consisted of 426 adolescents recruited in 8 European cities in the context of the IMAGEN Study.[21] Magnetic resonance images of the brain and information about cannabis use were collected when the participants entered the study (time 1; approximately 14.5 years of age) and 4 years later (time 2; approximately 18.5 years of age). In addition, cannabis use was assessed in the same participants between the 2 MRI sessions (at approximately 16 years of age). Characteristics of the study participants for all 3 samples are summarized in eTable 1 in the Supplement. Given the known sex differences in brain maturation during adolescence, we performed all analyses (SYS and IMAGEN samples) for male and female adolescents separately; in the ALSPAC sample, MRIs were available in male participants only. The institutional review boards of all participating institutions approved all studies reported herein. The parents and adolescents provided written informed consent and assent, respectively. All data were deidentified.

In all samples, we used exposure to cannabis by 16 years of age as the main independent variable; this choice is consistent with the epidemiologic findings on cannabis use during adolescence, with the high dynamics of brain development in early to middle adolescence, and with the previous work on the association between cannabis use by 16 years of age and structural properties of the adolescent[22] and adult[23,24] brains. In the SYS sample, we classified adolescents as having ever or never used cannabis based on their answer to a question about lifetime cannabis use; information about the number of occasions of cannabis use in their lives was not available. In the ALSPAC and IMAGEN samples, we were able to address the latter question using (ordinal) data on the number of occasions of cannabis use by 16 years of age.

We used the mean cortical thickness (across the entire cortical mantle) as the main dependent variable. We believe that cortical thickness is a useful metric for capturing the cumulative effects of various experiential factors on cortical neurobiological features, especially neuropil (ie, dendrites, glial cells) and capillary densities.[25] In addition to the mean thickness, we have related regional variations in the group differences (users vs nonusers) in thickness across 34 cortical regions to those in the expression of the cannabinoid receptor 1

gene (*CNR1* [NCBI Entrez Gene 1268]) derived from the Allen Brain Atlas in the same regions.[26] This atlas provides postmortem measurements of gene expression obtained in 6 adult brains (1269 cortical samples were used to calculate an average for each of the 34 regions). We used *CNR1* expression as a proxy of the cannabinoid type 1 receptor density to evaluate whether the extent of the relationship between cannabis use and cortical thickness varies as a function of this receptor's density in the cerebral cortex, thus testing for the level of specificity in this relationship.

Finally, we asked whether the genetic risk for schizophrenia moderates the relationship between cannabis use and cortical thickness. To answer this question, we used imputations from genome-wide single-nucleotide polymorphisms (SNPs) obtained in each of the 3 samples to calculate a polygenic risk score/profile from 108 loci identified by the Psychiatric Genomics Consortium in a genome-wide comparison of 36 989 patients with schizophrenia and 113 075 controls.[17] Risk scores ranged from −2.45 to 2.06 across the 3 samples, with greater scores indicating higher genetic risk for schizophrenia. Additional details of the study methods are provided in the eMethods in the Supplement.

Statistical Analysis

All statistical analyses were performed with JMP (version 17 10.0; SAS Institute Inc). Effect sizes were calculated using R software (version 3.1.2).[27] Linear regression was the primary statistical test used. The Cohen *d* statistic, Pearson correlation, Spearman correlation, and *t* tests were also used as specified in the Results section below and in the eResults in the Supplement.

Results

SYS Sample

In male adolescents (459 with available data) in the SYS sample, we observed an interaction between cannabis use (never/ever) and the risk score on age-adjusted cortical thickness (t_{455} = −2.60; P = .009); as shown in **Figure 1**A, age-adjusted cortical thickness decreases with the increasing risk score in cannabis users (R^2 = 0.06; P = .002) but not in nonusers ($R^2 = 8.4 \times 10^{-5}$, P = .87). We observed main effects of cannabis use (t_{455} = −2.69; P = .008) but not the risk score (t_{455} = 0.16; P = .87). As expected, those who ever used cannabis were older than those who never used cannabis, but this relationship does not vary between participants with low and high polygenic scores (P = .59, logistic regression). Figure 1B shows the differences in thickness across risk score deciles and cannabis use in male adolescents; Figure 1C shows the interaction between cannabis use and risk score on age-adjusted cortical thickness in female adolescents. Results of a vertex-based analysis of the interaction between Schizophrenia Risk Score and cannabis groups (ever vs never) vis-à-vis cortical thickness are shown in eTable 2 in the Supplement.

The above results are comparable to those obtained when using sex-specific median values of the risk score to classify adolescents in the high (ie, above the median) and low (below the median) risk groups. We use this strategy in the ALSPAC and IMAGEN samples to evaluate the possible effects of cumulative cannabis frequency, given the small number of individuals in the different cannabis frequency cells. To allow a comparison of the 3 samples using this approach, we have reanalyzed the SYS data using the median-based risk groups (**Figure 2**A and eFigure and eResults in the Supplement).

IMAGEN Sample

In the IMAGEN sample of adolescents (145 male and 188 female participants with available data), we were able to evaluate a relationship between frequency of cannabis use (by 16 years of age) and change in cortical thickness during adolescence (from time 1 [approximately 14.5 years] to time 2 [approximately 18.5 years] adjusted for scanner manufacturer). We observed an interaction between cannabis use (never/ever) and the risk score on the adjusted change in cortical thickness (t_{137} = −2.36; P = .02). In this model, we also observed main effects of cannabis use (t_{137} = −2.29; P = .02) and risk score (t_{137} = 2.76; P = .007). In female participants, we observed a main effect of risk score (t_{181} = −2.75; P = .007) but not of cannabis use (t_{181} = 0.90; P = .37) or the interaction between them (t_{181} = 1.36; P = .18). We were able to evaluate a relationship between frequency of cannabis use (by 16 years of age) and change in cortical thickness using the median-based groups (Figure 2C and eFigure and eResults in the Supplement).

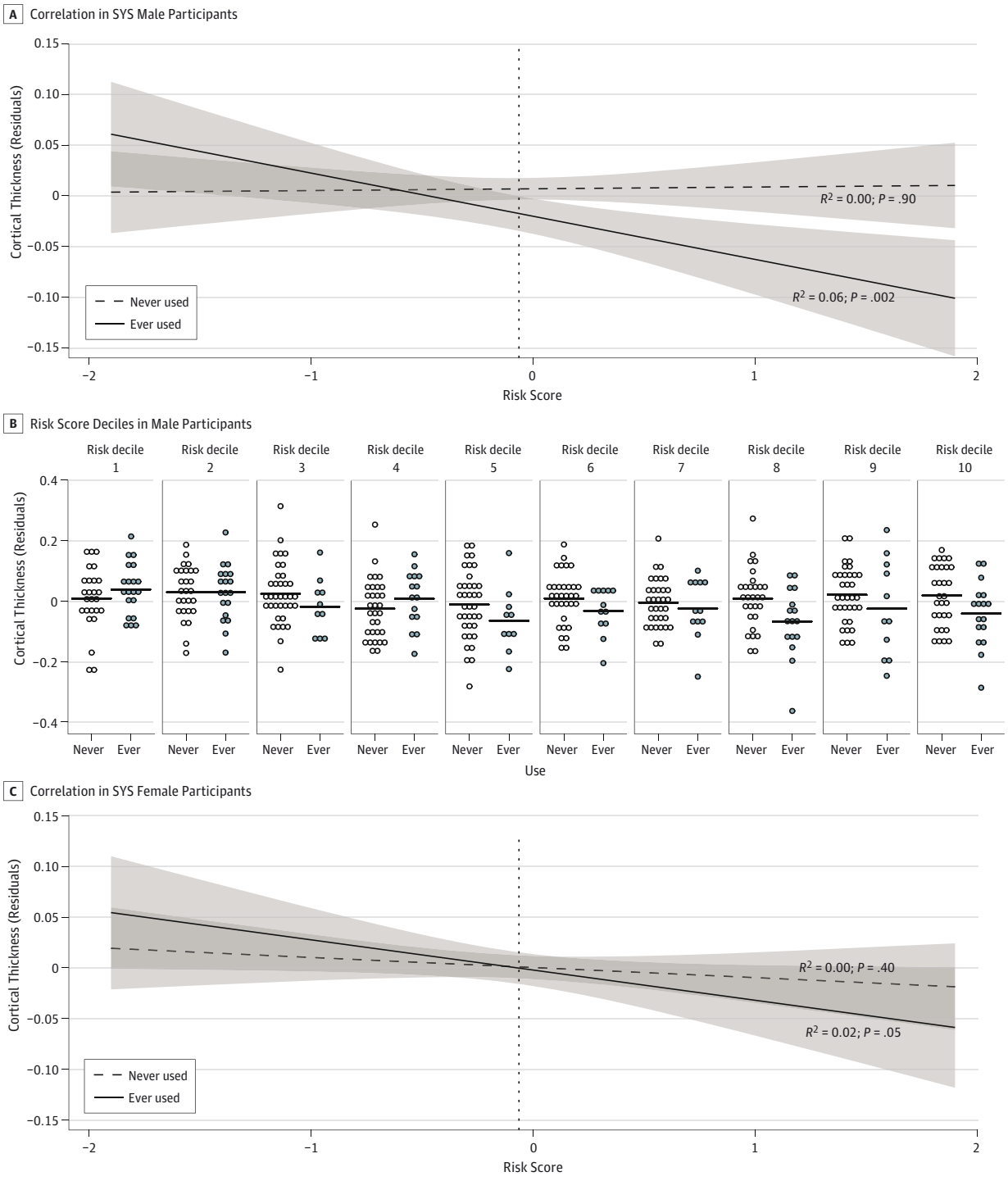
ALSPAC Sample

In this sample of male youth (295 with available data), we were able to evaluate again a relationship between the frequency of cannabis use (by 16 years of age) and age-adjusted cortical thickness measured from 18 to 21 years of age. First, we found no difference in cortical thickness between those who never and those who ever used cannabis, with the latter consisting of those who reported cannabis use with any frequency, in the high-risk (P = .78) and in the low-risk (P = .61) groups. Second, using the median-split approach (Figure 2C), we observed a difference in the high-risk group in age-adjusted cortical thickness (in arbitrary units) between those who never used cannabis and the most frequent users (ie, ≥61 occasions), with a difference of 0.07 (95% CI, 0.01-0.12; P = .02; Cohen d = 0.8). We also observed a similar difference between light users (<5 occasions) and the most frequent users (difference, 0.11 [95% CI, 0.03-0.18]; P = .004; d = 1.9). No such differences were observed in the low-risk group.

Relationship Between Cannabis-Related Differences in Thickness and *CNR1* Expression

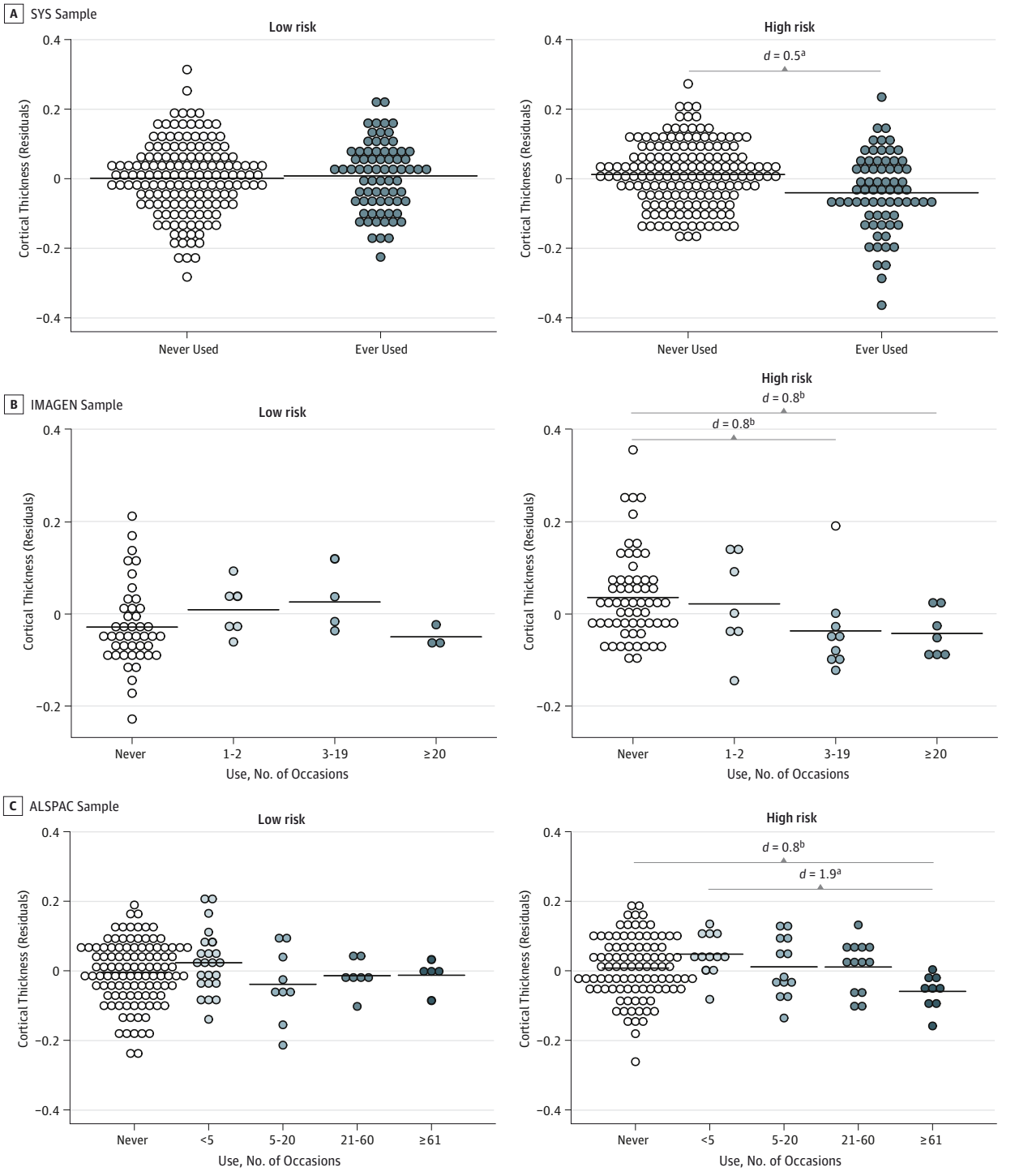
Expression of *CNR1* varies across the 34 cortical regions segmented by FreeSurfer[28]; as shown in **Figure 3**A, these regional variations are consistent across the 6 donors for whom expression data were available (left hemisphere). Figure 3B depicts group differences between those who never and ever used cannabis (SYS male participants) as a function of *CNR1* expression (eTable 3 in the Supplement). We observed high rank-order correlations between the group difference in cortical

Figure 1. Age-Adjusted Cortical Thickness and Polygenic Risk Score for Schizophrenia in the Saguenay Youth Study (SYS) Participants



The SYS participants are stratified by cannabis use as never and ever having used. A, Among SYS male participants, 317 had never and 142 had ever used cannabis. Regression lines for those who never and ever used are plotted with shaded 95% CIs. Median risk score is marked with the dotted vertical line. Risk scores range from −1.86 to 1.53, with greater scores indicating higher risk. B, Dot plots show age-adjusted cortical thickness across risk score deciles of male adolescents who never and ever used cannabis. Mean thickness values are marked with solid bars. The Schizophrenia Working Group of the Psychiatric Genomics Consortium[17] found that the top decile (based on the top 108 loci) contained about 3 times more cases of schizophrenia than the bottom decile (mean odds ratio across 39 samples, 3.21). C, Among SYS female participants, 319 had never and 171 had ever used cannabis. A weak albeit significant relationship between cortical thickness and risk score is seen with cannabis exposure. Lines and risk scores are described in part A. Cortical thickness is presented in arbitrary units (residuals).

Figure 2. Dot Plots of Mean Cortical Thickness for Different Groups of Male Cannabis Users at High and Low Risk

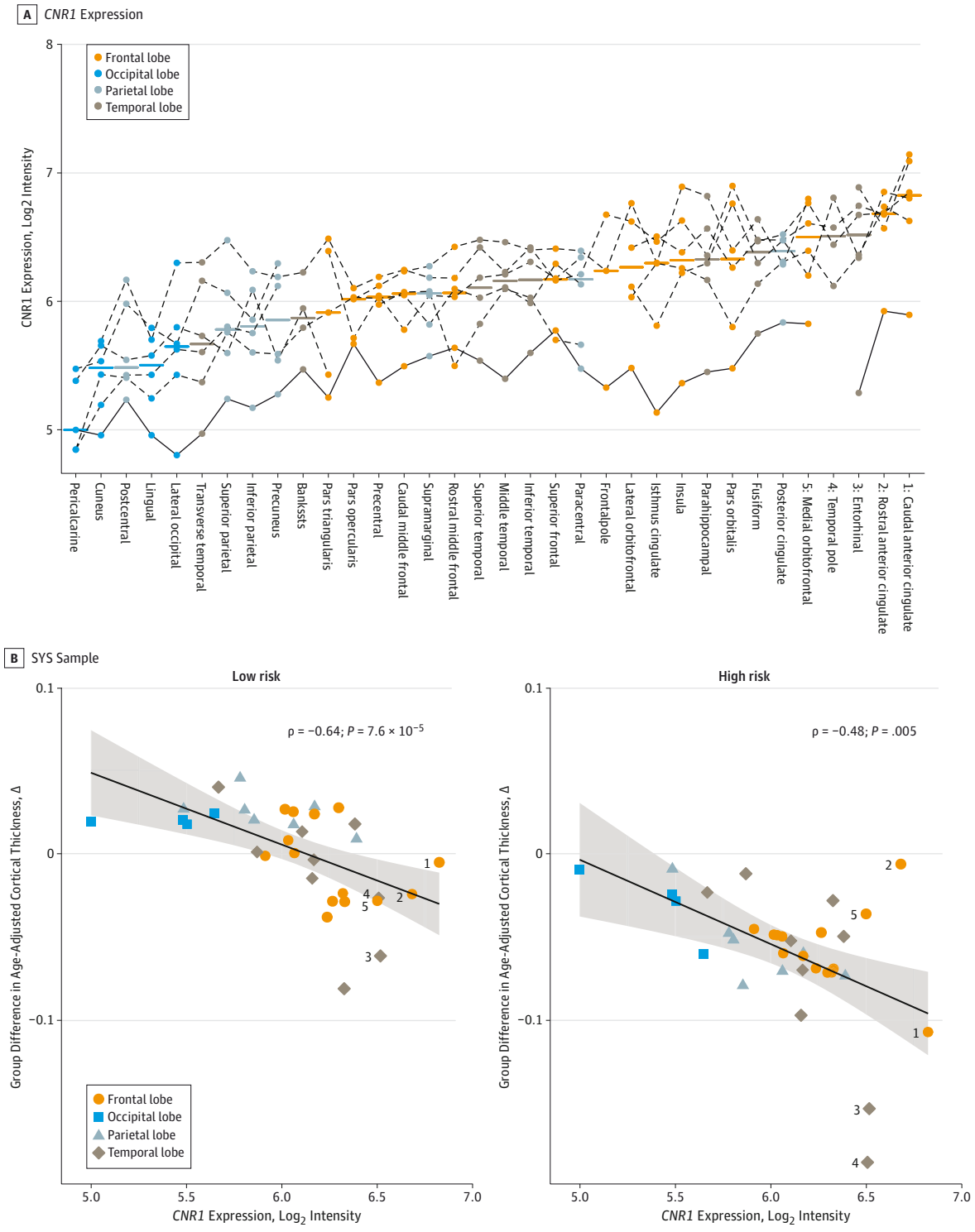


Thickness values are binned and stacked horizontally within each grouping. Mean thickness values are marked with thick black lines. Significant group differences are marked with lines and Cohen *d* statistics. A, Age-adjusted cortical thickness is presented in male participants who ever and never used cannabis. B, Change in cortical thickness (time 2 − time 1) by number of occasions of use. C, Age-adjusted cortical thickness is presented by number of occasions of use. ALSPAC indicates Avon Longitudinal Study of Parents and Children; SYS, Saguenay Youth Study. Cortical thickness is presented in arbitrary units (residuals).

[a] $P < .005$, *t* test.

[b] $P < .05$, *t* test.

Figure 3. Regional Variations in Group Differences in Cortical Thickness and *CNR1* Expression



A. Median values of *CNR1* expression (across 6 donors) are plotted as bars for the 34 cortical regions (left hemisphere); regions are ordered according to the expression values (lowest [left] to highest [right]). Median values obtained in each donor (median of all samples available for a given cortical region) are indicated by individual points. Lines connect expression values belonging to the same donor; solid line connects values contributed by a donor with relatively low (flat) expression values. (donor ID:H0351.2002; 39-year old male).
B. Group differences in age-adjusted cortical thickness between male adolescent participants who never and ever used cannabis as a function of *CNR1* expression in groups at low (left) and high (right) risk from the Saguenay Youth Study (SYS). Regression lines are plotted with shaded 95% CIs; correlation statistics are provided. All corresponding (mean) values are provided in eTable 3 in the Supplement. The 5 regions with highest *CNR1* expression are identified by their rank; corresponding names are provided in the x-axis of part A. Bankssts indicates banks of superior temporal sulcus.

thickness and *CNR1* expression across the 34 regions in the low-risk (Figure 3B, left; $\rho = -0.64$; $P = 7.6 \times 10^{-5}$) and high-risk (Figure 3B, right; $\rho = -0.48$; $P = .005$) male SYS participants. Thus, the largest group differences between those who never and ever used cannabis were found in regions that showed high *CNR1* expression (eg, entorhinal and anterior cingulate cortex).

Discussion

Across 3 population-based samples of typically developing youth, we observed a negative association between cannabis use in early adolescence and cortical thickness in male adolescents with a high genetic risk for schizophrenia, as indicated by their risk profiles across 108 genetic loci identified by the Psychiatric Genomics Consortium in a large genome-wide comparison of patients with schizophrenia and control individuals.[17] This association appears to vary with the cumulative frequency of cannabis use before 16 years of age, as evaluated in two of the samples. The association may emerge during adolescence, as evidenced by the longitudinal MRI data obtained in one of the samples. Male participants with low polygenic risk scores and all female participants did not present similar associations in our data sets.

Observational studies such as ours cannot attribute causality to the observed relationships. Even the longitudinal design does not rule out the possibility that individuals with a particular developmental trajectory may be more likely to experiment with cannabis rather than the cannabis exposure affecting the trajectory. Although genetic approaches, such as mendelian randomization,[29] may address this issue to some extent, only studies in model systems allow one to assess the true consequences of cannabis exposure in organisms randomized experimentally into different treatments.

Unlike the SYS and IMAGEN samples, the high-risk male participants in the ALSPAC sample do not show a difference in cortical thickness between those who never and ever used cannabis; only the high-frequency users do. We can only speculate that, with a given sample size, the association between less-frequent cannabis use and cortical thickness is less robust and, therefore, sensitive to other (confounding) effects that may accumulate with age; the ALSPAC sample is almost 5 years older than the SYS sample.

Adolescence is a period of vulnerability with regard to the emergence of psychotic disorders,[30] perhaps especially in boys.[15] Cannabis use during adolescence may be a contributing factor; high odds ratios were found for schizophrenia in a 35-year prospective study of men[16] when the investigators compared frequent cannabis users (>50 occasions by those aged 18-19 years) with nonusers. Our findings suggest that cannabis use might interfere with the maturation of the cerebral cortex in male adolescents at high risk for schizophrenia by virtue of their polygenic risk score. The overall volume of cortical gray matter and cortical thickness decrease with age in typically developing male adolescents.[13,14] Our longitudinal findings suggest that cannabis exposure might accelerate such processes, including cortical thinning, in male adolescents with a high polygenic risk score. A profound thinning of cortical gray matter was observed during adolescence in patients with childhood-onset schizophrenia (onset of symptoms by 12 years of age)[31,32] and, to a much lesser extent, in their nonpsychotic siblings.[33] Patients with childhood-onset schizophrenia have higher polygenic risk scores for schizophrenia than their siblings.[34] Several studies suggest that associations between cannabis use and various outcomes may be particularly pronounced during early (<16 years) adolescence.[35-38] Follow-up observations of the adolescents in the SYS and IMAGEN samples will allow us to evaluate whether this association applies for those who initiate the use of cannabis during late adolescence.

What might underlie cannabis-related thinning of cerebral cortex in male adolescents? In general, the following 2 processes may play a key role in shaping cortical thickness during male adolescence: (1) experience-driven plasticity and related growth of neuropil, which increases cortical thickness over time; and (2) testosterone-induced restructuring of neuropil, which decreases cortical thickness over time.

The first process, namely, experience-related plasticity, has been shown to drive changes in brain structure, as measured with MRI.[39,40] Cannabis may interfere with this process at pharmacologic and psychosocial levels. The former possibility is supported by the role of cannabinoid type 1 receptors in long-term potentiation[41-43] and in various neurotrophic events.[44] Chronic exposure to cannabis is associated with lower plasma levels of neurotrophins, such as brain-derived neurotrophic factor[45] and nerve growth factor.[46] The latter possibility is supported by studies suggesting that cannabis use during adolescence is associated with a number of psychosocial phenomena that may limit the richness of educational (eg, dropping out of high school[47-49]) and extracurricular (eg, lower engagement in sports[50]) experiences during this period of development. These pharmacologic and psychosocial pathways together may attenuate over time experience-related increases in cortical thickness during adolescence.

The second process, namely, testosterone-driven variations in cortical gray matter, has been demonstrated in a number of MRI studies of typically developing male adolescents.[13,51,52] Using a functional polymorphism in the androgen-receptor gene, we showed that testosterone-related decreases in cortical gray matter during male adolescence are, at least in part, mediated by the androgen receptor.[13] Which cellular compartments contribute to this phenomenon remains unclear; for example, testosterone may influence spine density[53] or the diameter of intracortical axons.[54,55] Interindividual variations in plasma levels of testosterone during early adolescence predict cannabis use in late adolescence and cannabis dependence in young adulthood.[56] Rising levels of testosterone during male adolescence and the associated high dynamics in the neurobiological features underlying cortical maturation may represent a risk factor with regard to other external (eg, cannabis) and/or internal (eg, genetic risk) perturbations. Furthermore, limited evidence supports the possible effects of testosterone on potentiating the action of cannabinoid type 1 receptor agonists on presynaptic inhibi-

tion of excitatory inputs in vitro[57] and on transcriptional upregulation of the *CNR1* gene.[58,59]

In this report, the polygenic risk score for schizophrenia calculated with the genome-wide significant SNPs ($P < 5 \times 10^{-8}$) showed an association with cortical thickness. This association was not in evidence when we calculated the score with the 24 727 nominally significant SNPs ($P < .05$) (eTable 4 in the Supplement). Nevertheless, the latter score is superior to the former in predicting liability to schizophrenia.[17] This discrepancy may be owing to the fact that our study examines the relationship of the polygenic risk score with a brain phenotype (cortical thickness) rather than a liability to schizophrenia. This phenotype may represent a vulnerability trait that is not specific to a particular psychiatric disorder. Similarly, genes have pleiotropic effects on psychopathologic features.[60] Herein we show that cortical thickness (in male cannabis users) is related only to a risk score based on genetic variations most strongly associated with schizophrenia, possibly by virtue of their involvement in relevant biological pathways (see below). We speculate that the top SNPs relate to brain vulnerability (a first "hit"[61]), whereas the nominal SNPs contribute to a broad array of factors underlying heritability of specific clinical manifestations (disorders), such as schizophrenia.

With this evidence, we speculate that the moderating influence of cannabis use on the association between the genetic risk for schizophrenia and cortical thickness may represent a combination of reduced experience-related brain plasticity taking place on the background of testosterone-associated decreases in cortical gray matter. The absence of the latter in female adolescents may represent a brain reserve that protects them to a certain extent (Figure 1C) from the cannabis-related perturbation of the brain-plasticity pathway. Genetic variations in the approximately 20 genes captured by the genetic risk score for schizophrenia (±5000 base pairs at each of the 114 SNPs) may increase vulnerability of their bearers by reducing the efficiency of neurotransmission (*CLCN3* [NCBI Entrez Gene 1182], *CHRNA3* [NCBI Entrez Gene 1136], *HCN1* [NCBI Entrez Gene 348980], *CACNB2* [NCBI Entrez Gene 783], and *GPM6A* [NCBI Entrez Gene 2823]), by making the brain more sensitive to immunity-related stressors (genes in the major histocompatibility complex), or by their involvement in early brain development (*CNTN4* [NCBI Entrez Gene 152330], *FES* [NCBI Entrez Gene 2242], *BCL11B* [NCBI Entrez Gene 64919] and *CACNB2* [NCBI Entrez Gene 783]). The fact that the group differences in regional cortical thickness between those who never and ever used cannabis show a gradient as a function of the regional differences in *CNR1* expression in the same set of cortical regions suggests that the above influences indeed interact with the cannabinoid system. Nonetheless, only experimental studies can confirm the causal role of the above molecular pathways in mediating the observed statistical relationships.

Conclusions

Cannabis use in early adolescence moderates the association between the genetic risk for schizophrenia and cortical maturation among male individuals. This finding implicates processes underlying cortical maturation in mediating the link between cannabis use and liability to schizophrenia.

ARTICLE INFORMATION

Submitted for Publication: February 11, 2015; final revision received May 21, 2015; accepted May 22, 2015.

Published Online: August 26, 2015. doi:10.1001/jamapsychiatry.2015.1131.

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Acquisition, analysis, or interpretation of data: French, Gray, Leonard, Pike, Richer, Séguin, Veillette, Evans, Artiges, Banaschewski, Bokde, Bruehl, Buchel, Cattrell, Conrod, Flor, Frouin, Gallinat, Garavan, Gowland, Heinz, Lemaitre, Martinot, Nees, Orfanos, Pangelinan, Poustka, Rietschel, Smolka, Walter, Whelan, Schumann, Smith, Pausova, Paus.
Drafting of the manuscript: French, Bromberg, Frouin, Smith, Paus.
Critical revision of the manuscript for important intellectual content: French, Gray, Leonard, Perron, Pike, Richer, Séguin, Veillette, Evans, Artiges, Banaschewski, Bokde, Bromberg, Bruehl, Buchel, Cattrell, Conrod, Flor, Gallinat, Garavan, Gowland, Heinz, Lemaitre, Martinot, Nees, Orfanos, Pangelinan, Poustka, Rietschel, Smolka, Walter, Whelan, Timpson, Schumann, Smith, Pausova, Paus.
Statistical analysis: French, Gray, Orfanos, Pangelinan.
Obtained funding: Richer, Séguin, Buchel, Conrod, Flor, Gallinat, Garavan, Rietschel, Smolka, Schumann, Paus.
Administrative, technical, or material support: French, Leonard, Perron, Pike, Richer, Veillette, Evans, Bokde, Buchel, Cattrell, Conrod, Garavan, Gowland, Lemaitre, Martinot, Nees, Orfanos, Pangelinan, Rietschel, Smolka, Walter, Whelan, Schumann, Smith.
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Conflict of Interest Disclosures: Dr Banaschewski served in an advisory or consultancy role for Hexal Pharma, Lilly, Medice, Novartis, Otsuka, Oxford outcomes, PCM scientific, Shire, and Viforpharma; has received conference attendance support and conference support or speaker's fees from Lilly, Medice, Novartis, and Shire; and has been involved in clinical trials conducted by Lilly, Shire, and Viforpharma. The present work is unrelated to these grants and relationships. Dr Paus is the Tanenbaum Chair in Population Neuroscience at the Rotman Research Institute. No other disclosures were reported.

Funding/Support: The Saguenay Youth Study (SYS) was supported by the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada (Drs Pausova and Paus). A National Sciences and Engineering Research Council of Canada Postdoctoral fellowship award supports Dr French.The IMAGEN Study was supported by contract LSHM-CT- 2007-037286 for the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-Related Behaviour in Normal Brain Function and Psychopathology); by contract 602450 for the FP7 projects IMAGEMEND (Imaging Genetics for Mental Disorders); by contract 603016 for Multidisciplinary Approaches to Translational Research In Conduct Syndromes; by contract 115300-2 for the Innovative Medicine Initiative Project EU-AIMS; by Medical Research Council (MRC) Programme grant 93558 (Developmental Pathways into Adolescent Substance Abuse); by the Swedish funding agency FORMAS; by the MRC and the Wellcome Trust (Behavioural and Clinical Neuroscience Institute, University of Cambridge); by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London, Maudsley National Health Service Foundation Trust, and King's College London; by Bundesministeriumfur Bildung und Forschung grants 01GS08152, 01EV0711, eMED SysAlc01ZX1311A, and Forschungsnetz AERIAL; by grant RO1 MH085772-01A1 from the National Institutes of Health (Axon, Testosterone and Mental Health during Adolescence) (Dr Paus). The MRC Integrative Epidemiology Unit at the University of Bristol is supported by the MRC and the University of Bristol (Drs Smith and Timpson). The Avon Longitudinal Study of Parents and Children (ALSPAC) was supported by grant 092731 from the MRC and the Wellcome Trust and by the University of Bristol.

Role of the Funder/Sponsor: The funding sources had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: Nick Qiu, MSc, Philips Healthcare, Toronto, Canada, Anja Klasnja, BSc, Rotman Research Institute, Klara Mareckova, PhD, Central European Institute of Technology, Brno, Czech Republic, and Deborah Schwartz, PhD, Canadian Institute for Health Information, Toronto, Ontario, Canada, assisted with processing and quality control of the magnetic resonance images. The following individuals contributed to data acquisition in the SYS: Manon Bernard, BSc, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada (database architect) and Helene Simard, MA, and her team of research assistants (Cégep de Jonquière). The entire ALSPAC team, including interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses, made this study possible. Larissa Duffy, BSc, University College London, Emma Evans, BSc, Sarah Thompson, Janine Fletcher, BSc, Elizabeth Jane Sanders, MA, Jonathan Oslow, and Amanda Hill, MA, ALSPAC, University of Bristol, Brisol, England, Spiro Stathakis, MSc, School of Psychology, Cardiff University, Cardiff, Wales, and Ding Qianqian, MSc, and Rosanne Aleong, PhD, Rotman Research Institute, assisted with recruitment and testing of the participants, technical assistance, and coordination of the imaging project in the ALSPAC. Dave Evans, PhD, University of Queensland Diamantina Institute, Translational Research Institute, Brisbane Australia, prepared the ALSPAC genome-wide association study dataset. None of these contributors received any compensation for their role.

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