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Family-Based Analysis of Genetic Variation Underlying Psychosis-Inducing Effects of Cannabis

Sibling Analysis and Proband Follow-up

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Context: Individual differences exist in sensitivity to the psychotomimetic effect of cannabis; the molecular genetic basis underlying differential sensitivity remains elusive.

Objective: To investigate whether selected schizophrenia candidate single-nucleotide polymorphisms (SNPs) moderate effects of cannabis use.

Design: Interactions between recent cannabis use, determined by urinalysis results, and 152 SNPs in 42 candidate genes were examined in 740 unaffected siblings of 801 patients with psychosis to examine genetic moderation of the association between Structured Interview for Schizotypy–Revised positive schizotypy and recent cannabis use (at-risk paradigm). The SNPs showing Bonferroni-adjusted association in the at-risk paradigm were used in a case-only analysis in the 801 patients, as well as in a case-sibling and case-control analysis (using 419 controls) focusing on genetic moderation of developmental effects of cannabis on later psychotic disorder.

Setting: The Netherlands and Flanders, Belgium.

Participants: Eight hundred one patients with psychosis and their 740 unaffected siblings.

Main Outcome Measure: Significant interaction between any of the selected SNPs and cannabis in the at-risk paradigm, followed by selective case-only, case-sibling, and case-control analyses.

Results: In the unaffected siblings, 16 SNPs in 12 genes showed significant interaction at $P < .05$, 3 of which survived correction for multiple testing ($P < .0003$), situated in *AKT1* (rs2494732 and rs1130233) and *LRRTM1* (rs673871). Follow-up analysis supported *AKT1* rs2494732 × cannabis interaction in the case-only ($\beta = 0.20$; $P = .007$), case-sibling (interaction $P = .040$), and case-control (interaction $P = .057$) analyses, with individuals with C/C genotypes having an approximately 2-fold odds of being diagnosed with a psychotic disorder when having used cannabis. In the unaffected siblings, the *AKT1* × cannabis interaction explained 2.2% additional variance in schizotypy in the whole sample and 19.0% additional variance in the exposed siblings with recent cannabis use.

Conclusions: Genetic variation in *AKT1* may mediate both short-term as well as longer-term effects on psychosis expression associated with use of cannabis, possibly through a mechanism of cannabinoid-regulated AKT1/GSK-3 signaling downstream of the dopamine D_2 receptor.

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GENETIC RISK FOR PSYCHOTIC disorder may be expressed in part as sensitivity to the psychotomimetic effect of cannabis,[1] but which genes underlie differential sensitivity remains unknown. An earlier study by Caspi and coworkers[2] suggested that a functional Val/Met polymorphism in the gene encoding

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catechol-*O*-methyltransferase (*COMT*) may mediate differential sensitivity to cannabis, with some support from 2 (semi)experimental studies assessing acute psychotomimetic effects of tetrahydrocannabinol (THC).[3,4] However, a case-only study in 493 patients with a psychotic disorder did not support a *COMT* Val158Met × cannabis interaction.[5]

Given evidence that common polymorphisms of small effect likely confer a major part of the genetic vulnerability for schizophrenia,[6] many other common variants may play a role in differential sensitivity to cannabis underlying psychotic symptoms. Since the report by Caspi and colleagues, however, little progress has been made in identifying additional risk polymorphisms involved in differential cannabis sensitivity. One major reason is methodological problems associated with the nature of cannabis as an environmental exposure, given that cannabis may also be used to cope with psychotic symptoms (reverse causality).[7] Furthermore, the

retrospective assessment of cannabis use in patients with a diagnosed psychotic disorder may yield biased estimates. Even if measurement error induced by retrospective assessment could be overcome, the pathway from the hypothesized psychotomimetic effect of cannabis in vulnerable individuals to the disease end point (a diagnosis of a psychotic disorder) is long and tortuous and may involve many factors under varying genetic control, such as stress associated with emerging psychotic symptoms, changes in patterns of use, co-occurring use of other substances, and differences in help seeking.

Cannabis use is not only associated with psychotic disorder,[8,9] however, but also with enhanced risk of interview-based measures of positive schizotypy in first-degree relatives of patients.[1] In addition, there is evidence that differential sensitivity to the psychotomimetic response of cannabis in first-degree relatives is greatest for measures of recent cannabis use assessed by urinalysis, suggesting that the differences between controls and relatives of patients are caused predominantly by contrasts in (sub)acute response to cannabis, in the form of positive schizotypal experiences.[1]

Therefore, studying the effects of THC on positive schizotypy in unaffected siblings of patients with psychosis, who are at higher than average genetic risk for psychosis,[10] using an experimental design, may be a powerful and valid approach to examine which genes confer psychosis risk following cannabis use. However, experimental gene × environment (G × E) cannabis studies require impractically large numbers of participants and, for ethical reasons, are not possible in cannabis-naive individuals. An alternative is to examine not the experimental but the natural variation of cannabis use in unaffected siblings in relation to schizotypy. Studying the effects of recent cannabis use in this at-risk population, and genetic moderation thereof, may be used to study short-term gene × cannabis interactions in an ethically acceptable fashion, not confounded by antipsychotic treatment effects. An additional important advantage is that to the degree that the effect of any genetic factor involved in cannabis sensitivity may depend on the copresence of other genetic factors involved in the etiology of schizophrenia,[11] siblings of patients are more likely to carry such additional variants because they share 50% of their genes with their patient relative.

The at-risk G × E interaction paradigm as described earlier, using positive schizotypy as the outcome, was used in a sample of 740 unaffected siblings recruited as a part of the Genetic Risk and Outcome in Psychosis (GROUP) study, a longitudinal study focusing on G × E interactions relevant to psychotic disorders.[1] Genetic moderation of the effect of recent cannabis use, as established by urine toxicology results, was examined for a range of a priori candidate single-nucleotide polymorphisms (SNPs).

If molecular genetic variation can be shown to mediate the altered psychotomimetic response to recent cannabis use in siblings of patients, the natural subsequent hypothesis is that the same molecular genetic variation may also underlie the developmental effect of lifetime cannabis use on risk of psychotic disorder in the patients. Therefore, to provide for a within-study follow-up and show relevance of any identified interactions for the schizotypy psychosis phenotype at the level of psychotic disorder, significant (conservatively Bonferroni-adjusted) SNPs in the at-risk study were reexamined using different epidemiological models of G × E interaction in a sample consisting of patients who were siblings of the at-risk group.

METHODS

SAMPLE

In selected representative geographical areas in the Netherlands and Belgium, patients were identified through representative clinicians working in regional psychotic disorder services, whose case load was screened for inclusion criteria. Subsequently, a group of patients presenting consecutively at these services either as outpatients or inpatients were recruited for the study. Controls were selected through a system of random mailings to addresses in the catchment areas of the cases.

The full GROUP sample consisted of 1120 patients with nonaffective psychotic disorder, 1057 siblings of these 1120 patients, 919 parents of the patients and their siblings, and 590 unrelated controls. Inclusion criteria were (1) age range 16 to 50 years, (2) diagnosis of nonaffective psychotic disorder, and (3) good command of the Dutch language. Controls had no first-degree relative with a psychotic disorder as established by the Family Interview for Genetic Studies,[12] with the control as the informant. Diagnosis was based on *DSM-IV* criteria,[13] assessed with the Comprehensive Assessment of Symptoms and History interview[14] or Schedules for Clinical Assessment in Neuropsychiatry (version 2.1).[15] *DSM-IV* diagnoses of the patients were schizophrenia and related disorders (*DSM-IV* 295.x; n=945; 84%), other psychotic disorders (*DSM-IV* 297/298; n=149; 13%), and psychotic illness in the context of substance abuse or somatic illness (n=9; 1%).[1]

MEASURES

The Structured Interview for Schizotypy–Revised (SIS-R)[16,17] was administered to controls and siblings. The SIS-R is a semistructured interview containing 20 schizotypal symptoms and 11 schizotypal signs rated on a 4-point scale. Symptoms are defined as verbal responses to standardized questions concerning, for example, magical ideation, illusions, and referential thinking. Signs refer to behaviors that are rated by the interviewer, such as goal directedness of thinking and flatness of affect. Questions and rating procedures are standardized.

Cannabis measures were chosen a priori and consistently used in the current article as well as in a companion article.[1] These were recent cannabis use, as established by urinalysis results (the exposure variable in the at-risk paradigm), and Composite International Diagnostic Interview (CIDI) cannabis pattern of use during the lifetime period of heaviest use, restricted to those individuals where the age at most heavy use preceded onset of psychosis (hereafter, CIDI lifetime use; none, 0; less than weekly, 1; weekly, 2; and daily, 3). Onset of psychosis was defined as the first mental health contact for psychosis. Urinalysis was carried out as a screen for the presence of cannabis at the Jellinek Clinic laboratory. The method used was immunoassay with a cutoff of 50 ng/mL. In addition, as an integrity parameter, the creatinine level of every sample was measured. Cannabis urine screening has a detection window up to 30 days, but the detection time has been documented in the literature to be even longer (up to 3 months), depending on the level of

cannabis use.[18] Given the relatively high cutoff level of 50 ng/mL, a conservative detection window of 1 month can be inferred.

GENETIC VARIATION

Based on published findings up to April 2009,[19-24] the use of a hypothesis-based approach toward gene selection for G × E interaction was attempted. The selection of genes was based on a 2-stage review of the literature. First, at the level of the gene, genes were selected that (1) were previously suggested to be associated with schizophrenia (*RGS4*, *NRG1*, *DTNBP1*, *PIP5K2A*, *G72/DAOA*, *DISC1*, *HT2A*, *AKT1*, *LRRTM1*, *FGF2*, *FGFR1*, *GPM6A*, *PRODH*, *GRM3*, *GABRA6*, *GAD1*, *NOS1*, *RGS2*, *ROBO1*, *CHRM3*, and *TBX1*); (2) are important for dopaminergic neurotransmission given the hypothesis that cannabis may increase psychosis risk by impacting dopamine neurotransmission (*COMT*, *ANKK1*, *DRD1*, *DRD2*, *DRD3*, *SLC6A3*, *PPP1R1B*, and *SLC18A2*); (3) are directly related to cannabinoid signaling (*CNR1*); (4) have a role in regulating differential sensitivity to broadly defined environmental influences, particularly with regard to responsivity to environmental stress (*ADRA2C* and *FKBP5*) and adaptive neuronal survival (*BDNF*, *P2RX7*, *NPY*, *NQO1*, *GST-1*, and *GST-2*); and (5) may be involved in epigenetic regulation of environmental influences (*MTHFR*, *MTR*, *MTRR*, *DNMT3B*, *EHMT1*, *EHMT2*, and *PRDM2*). Subsequently, SNPs within these genes were identified that were previously associated with (1) schizophrenia or (2) possible functional impact.

Thus, a total of 179 SNPs in 46 genes were selected for the current study. These SNPs were selectively determined by Sequenom (Hamburg, Germany) using the Sequenom Mass ARRAY iPLEX platform at the facilities of the manufacturer; SNPs, therefore, were not selected from a larger set of genome-wide markers. According to quality control criteria of the GROUP study, SNPs with more than 10% genotyping errors are excluded, as are SNPs in severe Hardy-Weinberg disequilibrium ($P < .001$). Of the 179 SNPs originally included, 22 SNPs were excluded because they had more than 10% genotyping errors in the sibling sample, and an additional 2 SNPs were excluded because they were in severe Hardy-Weinberg disequilibrium in the siblings and no variation was found for 1 variant (eTable, http://www.archgenpsychiatry.com). A further 2 SNPs were excluded because they had more than 10% genotyping errors in the healthy controls (rs1360780 in *FKBP5*) or because of violation of Hardy-Weinberg equilibrium in controls (rs1047552 in *APH1B*), leaving a final set of 152 SNPs in 42 genes suitable for analysis.

STATISTICAL ANALYSIS

At-Risk Paradigm

The outcome of interest in the at-risk paradigm was positive schizotypy. The choice for positive schizotypy was based on a previous factor analysis of the SIS-R[25] and on evidence that recent cannabis use impacts positive psychotic experiences.[4] Genetic main effects (marginal effects) were investigated by regressing continuous SIS-R positive schizotypy on each SNP. Given that some families contributed more than 1 sibling, hierarchical clustering of data at the level of family was taken into account using the multilevel random regression xtreg command in Stata, version 11.[26]

To examine G × E interaction, continuous SIS-R positive schizotypy was regressed, using the xtreg command, on recent cannabis use, the SNP (genotypes coded as 0, 1, or 2 and modeled as a linear effect), and their interaction. Analyses additionally were adjusted for the following a priori confounders: age, sex, amphetamine use (by urinalysis results), and cocaine use (by urinalysis results).[1] Furthermore, since the effects of recent use may be influenced by the degree of previous exposure,[27] analyses were also controlled for CIDI lifetime use of cannabis. The mean of SIS-R positive schizotypy items (referential thinking, psychotic phenomena, derealization, magical ideation, illusions, and suspiciousness; range, 0-2.7) was used as the outcome measure.

Since positive schizotypy may be expected to display a nonnormal distribution with many individuals scoring zero, which may give rise to false-positive evidence for interaction, zero-inflated count models were used to investigate the robustness of interactions surviving Bonferroni correction. Zero-inflated models were not used as the primary analysis, however, since they did not display better model fit than traditional count models and because of the violation of underlying assumptions in the current data set. Specifically, the underlying assumption of zero-inflated models is that individuals with a zero score exist in 2 states: nonaffected individuals who are inherently not at risk of developing the outcome ("true zeros") and individuals at inherent risk but with an absence of expression of the outcome.[28,29] In a population specifically selected for being at higher than average genetic risk, such as the sample used herein, this assumption is problematic. An important further consideration is that treating schizotypy as a count variable may be problematic as well, because every point increase on a certain item is statistically treated as a new incident symptom of schizotypy, violating the proportional odds assumption.[30] Zero-inflated negative binomial regression displayed better model fit than zero-inflated Poisson regression and was thus used, with robust standard errors to account for familial clustering of observations.

To adjust for multiple testing, Bonferroni correction was applied. The Bonferroni procedure refers to all applied independent statistical tests, ie, the number of SNPs as well as the number of phenotypes applied. It does not take into account linkage disequilibrium between SNPs but assumes independence of the different hypotheses tested. Since linkage disequilibrium effectively reduces the number of independent hypotheses tested, the Bonferroni correction can be considered conservative. This approach was chosen because it allows for stringent control for multiple testing and a reduction of type II errors, associated with testing a large number of hypotheses with relatively low prior probability.[31,32] Since we tested 152 hypotheses of SNP × cannabis interaction, the Bonferroni-adjusted significance level was set at $P = .0003$.

Follow-up Analysis in Patient-Siblings of the At-Risk Group

In addition to stringent control for multiple testing, supportive evidence from different studies or designs is a valuable tool in distinguishing "true" from "false" interactions. Therefore, selected SNPs were followed up in the sample of patients who were relatives of the sibling at-risk group, using case-only, case-sibling, and case-control designs.

CASE-ONLY DESIGN

A case-only design determines presence of G × E interaction on the basis of an association between SNP and exposure, while assuming independence between SNP and exposure.[33] This assumption cannot hold when using a mass-marker approach[34] but is acceptable in the case of selective follow-up of previously established interactions with high prior probability. A case-only design provides greater statistical power than case-sibling or case-control designs,[35,36] while the nature

Table 1. Marginal Effects (at $P < .05$) on SIS-R Positive Schizotypy in 740 Unaffected Siblings

SNP	Gene	Risk Allele	HWE *P* Value	Effect Size, β	*P* Value
rs907094	*PPP1R1B*	C	.82	0.05	.037
rs1049353	*CNR1*	A	.27	0.05	.044
snp8nrg241930	*NRG1*	T	.76	0.07	.004
rs909706	*DTNBP1*	G	.78	0.05	.029
rs2619528	*DTNBP1*	A	.53	0.06	.019
rs3213207	*DTNBP1*	G	.12	0.08	.025
rs760761	*DTNBP1*	T	.33	0.06	.018
rs2619522	*DTNBP1*	G	.31	0.07	.010
rs308420	*FGF2*	G	.12	0.07	.033
rs2269726	*TBX1*	T	.74	0.04	.049

Abbreviations: HWE, Hardy-Weinberg equilibrium; SIS-R, Structured Interview for Schizotypy–Revised; SNP, single-nucleotide polymorphism.

of the cohort under study allows for direct examination of the assumption of independence between genes and exposure to cannabis in controls and unaffected siblings. Thus, SNPs surviving Bonferroni correction in the at-risk sample were examined in the patient sample for association with CIDI lifetime use to corroborate short-term genetic moderation of cannabis response in an at-risk population with long-term developmental effects on psychotic disorder. Both SNP (coded as 0, 1, or 2) and CIDI lifetime use (coded as 0, 1, 2, or 3) were modeled as linear effects, thus examining the hypothesis that increased risk allele loading was associated with increasing levels of lifetime use (linear trend). To obtain an estimation of effect size of the case-only analysis in patients, multinomial logistic regression was used, with the different levels of CIDI lifetime use as the dependent variable and SNP, recent cannabis use, and the confounders (described earlier) as independent variables.

CASE-SIBLING AND CASE-CONTROL DESIGN

Case-sibling and case-control designs were additionally used to investigate G × E interaction in the SNPs surviving Bonferroni correction in the at-risk paradigm. These designs do not rely on the gene-environment independence assumption, as the case-only design does, but have lower statistical power.[36] An advantage of the case-sibling design over the case-control design is that it may have greater power to detect G × E interaction while it is immune to bias related to population stratification.[36] Case-sibling and case-control analyses examine the odds of being a case as a function of genotype and exposure to the environmental factor. For this purpose, logistic regression with robust standard errors was used with genotype (coded as 0, 1, or 2 and modeled as a linear effect) and CIDI lifetime use (dichotomized to no use [0] vs any period of cannabis use preceding onset of psychosis [1] to preserve maximal statistical power) as independent variables and case-control status as the dependent variable.

Meaningful estimates of population impact for significant SNPs in both the at-risk and the follow-up paradigms were calculated. In the at-risk paradigm, a measure of impact was obtained by deducting explained variance of the model without the interaction term from the model with the interaction term to estimate the additional variance in schizotypy attributable to the interaction in the entire population of unaffected siblings. Furthermore, to estimate the explained variance attributable to the SNP in the exposed (ie, cannabis-using siblings), the variance explained by the model of confounders only in siblings with recent use was deducted from the variance explained by the model of SNP and confounders in siblings with recent use.

RESULTS

AT-RISK PARADIGM

Of the 1057 unaffected siblings of patients with a psychotic disorder included in the GROUP sample, genetic data were available for 813 (mean [SD] age, 27.4 [8.0] years; 46.3% male). Siblings who agreed to provide DNA displayed no large or significant differences in sex, CIDI lifetime use, or recent use of cannabis, cocaine, or amphetamines and were slightly younger than siblings who did not provide DNA (27.4 years vs 29.1 years; SE, 0.60; $P = .005$). Of the siblings for whom DNA was available, 749 also provided a urine sample, of whom 7.6% screened positive for recent cannabis use. Of the 749 unaffected siblings, SIS-R data were not available for 9, leaving a final sample of 740 individuals for analysis.

Recent cannabis use was significantly associated with positive schizotypy (β = 0.22; SE, 0.06; $P < .0001$). Marginal effects in models of SIS-R positive schizotypy at $P < .05$ were found for SNPs in *PPP1R1B*, *CNR1*, *NRG1*, *DTBNP1*, *FGF2*, and *TBX1* (**Table 1**). None of these SNPs was associated with recent use of cannabis at $P < .05$.

Sixteen SNPs in 12 different genes showed significant interaction at $P < .05$ with recent cannabis use. Implicated genes included *DRD2*, *GAD1*, *MTHFR*, *CNR1*, *DTNBP1*, *G72/DAOA*, *AKT1*, *LRRTM1*, *PRODH*, *TBX1*, *NPY*, and *RGS2* (**Table 2**). Three of these 16 SNPs showed significant interaction at the Bonferroni-corrected threshold of significance ($P = .0003$). Two SNPs were situated in *AKT1* and 1 SNP was situated in *LRRTM1* (Table 2). Zero-inflated negative binomial regression provided support for the robustness of these interactions (*AKT1* rs2494732 × cannabis interaction, $P = .0013$; *AKT1* rs1130233 × cannabis interaction, $P = .0146$; *LRRTM1* rs673871 × cannabis interaction, $P = .010$). None of these SNPs displayed a significant marginal effect or an association with recent cannabis use at $P < .05$.

CASE-ONLY FOLLOW-UP

Genetic data were available in 801 patients (76.8% male, mean [SD] age, 27.9 [8.2] years). No large or significant differences in age, sex, CIDI lifetime use, or recent use of cannabis, cocaine, or amphetamines were found for

Table 2. Significant SNP × Cannabis Interactions (at $P < .05$) in 740 Unaffected Siblings

SNP	Gene	Risk Variant	HWE *P* Value	Effect Size, β	*P* Value
rs1799732	*DRD2*	Deletion	.65	0.24	.0312
rs1800498	*DRD2*	C	.94	0.20	.0147
rs2058725	*GAD1*	G	.20	0.26	.0349
rs379850	*GAD1*	G	.83	0.22	.0113
rs1801133	*MTHFR*	C	.07	0.19	.0339
rs806379	*CNR1*	T	.04	0.16	.0428
rs806308	*CNR1*	T	.86	0.25	.0036
rs1018381	*DTNBP1*	T	.40	0.34	.0119
rs1421292	*G72/DAOA*	A	.30	0.21	.0112
rs1130233	*AKT1*	A	.47	0.37	.0003[a]
rs2494732	*AKT1*	C	.43	0.42	.0001[a]
rs673871	*LRRTM1*	T	.74	1.17	.0001[a]
rs372055	*PRODH*	A	.20	0.24	.0246
rs5746832	*TBX1*	G	.40	0.21	.0343
rs3037354	*NPY*	Deletion	.41	0.19	.0368
rs4606	*RGS2*	G	.23	0.25	.0340

Abbreviations: HWE, Hardy-Weinberg equilibrium; SNP, single-nucleotide polymorphism.
[a] The SNPs showing interaction at the Bonferroni-adjusted threshold ($P = .0003$).

Table 3. Case-Only Follow-up of Significant SNPs in the At-Risk Paradigm

		At-Risk Paradigm (n=740)					Case-Only Paradigm (n=689)[a]				
SNP	Gene	Distribution, %	Effect Size, β[b]	SE	*P* Value	Risk Variant	Distribution, %	Effect Size, β[b]	SE	*P* Value	Risk Variant
rs1130233	*AKT1*	G/G: 54.9 A/G: 39.0 A/A: 6.1	0.37	0.17	.0003	A	G/G: 54.9 A/G: 39.0 A/A: 6.1	0.14	0.09	.097	
rs2494732[c]	*AKT1*	T/T: 32.5 C/T: 50.2 C/C: 17.3	0.42	0.22	.0001	C	T/T: 34.4 C/T: 46.6 C/C: 19.0	0.20	0.07	.007	C
rs673871	*LRRTM1*	A/A: 78.4 A/T: 20.0 T/T: 1.6	1.17	0.57	.0001	T	A/A: 78.1 A/T: 21.3 T/T: 0.6	–0.31	0.17	.084	

Abbreviations: CIDI, Composite International Diagnostic Interview; G × E, gene × environment; SIS-R, Structured Interview for Schizotypy–Revised; SNP, single-nucleotide polymorphism.
[a] Outcome is CIDI lifetime use; both SNP (coded as 0, 1, or 2) and CIDI lifetime use (coded as 0, 1, 2, or 3) were analyzed as continuous variables, thus examining the hypothesis that increased risk allele loading was associated with increasing levels of lifetime use (ie, examination of linear trend). One hundred twelve patients were excluded from the analysis because the most intensive period of use occurred after illness onset.
[b] Outcome is SIS-R positive schizotypy; SNPs (coded as 0, 1, or 2) were analyzed for linear trend in interaction with recent cannabis use (yes/no).
[c] Significant and directionally similar evidence for SNP × cannabis interaction in both the at-risk and the case-only G × E paradigm.

patients who did or did not provide DNA. Cannabis use was highly prevalent: only 38.0% of the patients reported never having used cannabis, and 42.7% had used cannabis daily in the lifetime period of heaviest use; 11.4%, weekly; and 8.0%, less than weekly. In the cannabis-using patients, the most intense period of use preceded onset of psychosis in 77.3%. In addition, 16.9% tested positive for recent cannabis use by urinalysis.

In the patients, 1 of the SNPs in *AKT1* (rs2494732) showed a robust and consistent association with CIDI lifetime use, restricted to use preceding onset of psychosis, whereas rs1130233 in *AKT1* and rs673871 in *LRRTM1* did not (**Table 3**). Post hoc multinomial logistic regression analysis showed that individuals with rs2494732 C/C genotypes had a relative risk of 1.90 for daily cannabis use compared with those with T/T genotypes (**Table 4**) (**Figure**). No evidence for association with CIDI lifetime use was found in siblings or controls for either rs673871 in *LRRTM1* (siblings: β = –0.06; SE, 0.14; $P = .650$; controls: β = –0.06; SE, 0.15; $P = .670$) or rs1130233 (siblings: β = 0.02; SE, 0.07; $P = .686$; controls: β = –0.05; SE, 0.08; $P = .561$) or rs2494732 (siblings: β = –0.01; SE 0.06; $P = .912$; controls: β = –0.05; SE, 0.07; $P = .464$) in *AKT1*, supporting the assumption of independence of the implicated genetic variants and population exposure to cannabis.

CASE-SIBLING AND CASE-CONTROL FOLLOW-UP

Genetic data were available in 419 of the 593 controls (46.3% male; mean [SD] age, 27.4 [8.0] years). No large or significant differences in sex, CIDI lifetime use, or recent use of cannabis, cocaine, or amphetamines were found for controls who did or did not provide DNA, although controls who provided DNA were somewhat

Table 4. Lifetime Frequency of Cannabis Use and Relative Risks, Determined by Multinomial Logistic Regression Analysis, According to *AKT1* rs2494732 Genotype in 679 Patients[a] With a Psychotic Disorder

	CIDI Lifetime Use, %			RR		
	T/T (n=237)	C/T (n=313)	C/C (n=129)	T/T (n=237)	C/T (n=313)	C/C (n=129)
No use	48.1	44.7	35.7	[b]	[b]	[b]
Less than weekly use	9.7	6.4	6.2	1 [Reference]	0.71	0.86
Weekly use	9.3	11.8	12.4	1 [Reference]	1.31	1.72
Daily use	32.5	37.1	45.7	1 [Reference]	1.23	1.90[c]

Abbreviations: CIDI, Composite International Diagnostic Interview; RR, relative risk.
[a] From the original sample of 801 patients with DNA samples; 112 individuals were excluded from the analysis because the most intensive period of use occurred after illness onset and genotyping was unsuccessful in a further 10 patients.
[b] Base outcome to which the different outcomes are compared in a multinomial logistic regression model.
[c] $P < .01$.

younger (29.7 vs 32.1 years; SE, 0.95; $P = .016$). The case-sibling and case-control analysis similarly provided support for an *AKT1* rs2494732 × cannabis interaction, albeit trend-significant in the case-control follow-up (**Table 5**). No evidence was found for interaction between cannabis and rs1130233 in *AKT1* or rs673871 in *LRRTM1* (Table 5). Effect sizes were comparable in the case-sibling and case-control paradigm, with individuals with C/C genotypes displaying approximately 2-fold odds of being diagnosed with a psychotic disorder when having used cannabis (**Table 6**). In the unaffected siblings, the *AKT1* rs2494732 × cannabis interaction, compared with the model without the interaction, explained 2.2% additional variance in schizotypy in unaffected siblings and 19.0% additional variance in the sample restricted to the cannabis-using siblings.

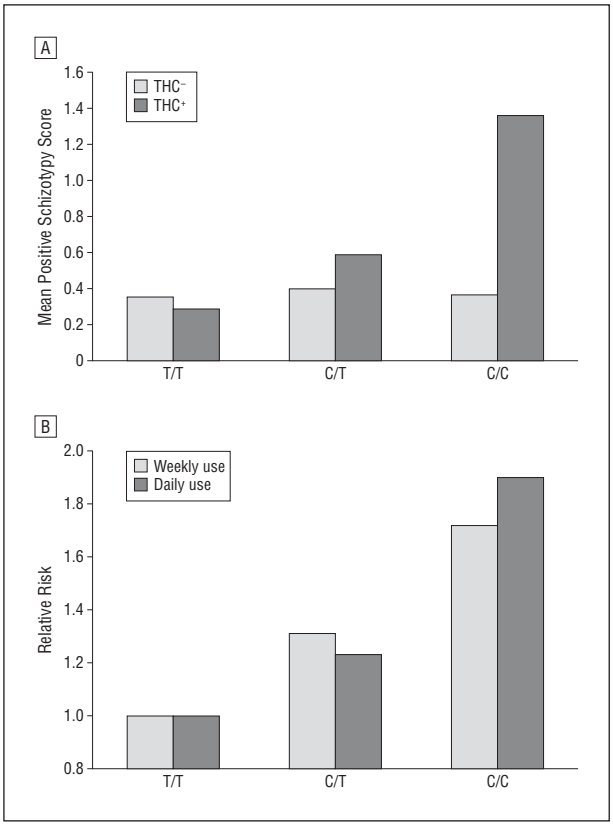

Figure. *AKT1* rs2494732 × cannabis interaction in the at-risk and case-only paradigm. A, Mean positive schizotypy scores according to *AKT1* rs2494732 genotype in 728 unaffected siblings with (n=55) and without (n=673) recent cannabis use. Genotyping was unsuccessful in 12 unaffected siblings. THC indicates tetrahydrocannabinol. B, Relative risks for weekly and daily lifetime cannabis use in the patients according to *AKT1* rs2494732 genotype.

COMMENT

An at-risk strategy was adopted to investigate whether genetic variation moderates the association between recent cannabis use and psychosis in a large family-based sample, using interview-based measures. The application of this strategy allowed for the examination of gene × cannabis interactions without possible confounds of illness duration, illness severity, phase of the illness (acute or stable), and treatment. The method also allowed for proximity between exposure to the environmental factor and outcome, which was put forward as an important, but difficult to carry out aspect of studies of G × E interaction.[37]

A range of gene × cannabis interactions was identified at the $P < .05$ level, many of which have considerable biological plausibility. Three gene × cannabis interactions for SNPs in *AKT1* and *LRRTM1* survived stringent correction for multiple testing, ie, Bonferroni correction for 152 SNP × cannabis interactions tested. The robustness of these associations is illustrated by the fact that 2 of these 3 SNPs, including rs2494732 in *AKT1*, would have survived stringent Bonferroni correction for up to 500 SNPs. Using different epidemiological designs, evidence was found that rs2494732 SNP in *AKT1* may also moderate possible long-term developmental effects of cannabis on psychotic disorder. *COMT* Val158Met, previously implicated as a candidate moderator of psychotic response to cannabis,[2-4] did not show evidence for gene × cannabis interaction in the unaffected siblings.

CANNABINOIDS, *AKT1*, AND PSYCHOSIS

Arguably, the most important finding is the observation of an *AKT1* × cannabis interaction. This interaction impacted the short-term psychotomimetic effects of cannabis use in an at-risk population and, in addition, also

Table 5. Case-Sibling and Case-Control Follow-up of the SNPs Surviving Bonferroni Correction in the At-Risk Paradigm

		Case-Sibling (n=689 Cases, 813 Siblings)[a]			Case-Control (n=689 Cases, 419 Controls)[a]		
SNP	Gene	Effect Size, β	SE	Interaction *P* Value	Effect Size, β	SE	Interaction *P* Value
rs1130233	*AKT1*	0.06	0.17	.714	0.36	0.22	.111
rs2494732	*AKT1*	0.30	0.14	.040	0.36	0.19	.057
rs673871	*LRRTM1*	–0.32	0.35	.358	–0.21	0.41	.603

Abbreviation: SNP, single-nucleotide polymorphism.
[a] One hundred twelve patients of the original 801 were excluded from the analysis because the most intensive period of cannabis use occurred after illness onset.

Table 6. Case-Sibling and Case-Control ORs of Being Diagnosed With a Psychotic Disorder as a Function of *AKT1* rs2494732 Genotype in Cannabis Users

	Case-Sibling (n=689 Cases, 813 Siblings)[a]		Case-Control (n=689 Cases, 419 Controls)[a]	
rs2494732	OR (95% CI)	*P* Value[b]	OR (95% CI)	*P* Value[c]
T/T	1 [Reference] (0.17)	.714	1 [Reference]	
C/T	1.05 (0.67-1.64)	.815	1.35 (0.76-2.41)	.303
C/C	1.96 (1.09-3.53)	.026	2.08 (0.92-4.67)	.077

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] One hundred twelve patients of the original 801 were excluded from the analysis because the most intensive period of cannabis use occurred after illness onset.
[b] Overall significance of the *AKT1* rs2494732 × cannabis interaction, *P*=.040.
[c] Overall significance of the *AKT1* rs2494732 × cannabis interaction, *P*=.057.

influenced long-term developmental effects on psychotic disorder. AKT1 is a serine/threonine kinase that is activated by phosphatidylinositol-3-kinase (PI3K).[38] One of the essential functions of AKT is the phosphorylation of glycogen synthase kinase (GSK-3) at Ser21 in GSK-3α and Ser9 in GSK-3β, causing its inactivation.[39] AKT and GSK-3 have emerged as the focal point for many signal-transduction pathways, regulating multiple cellular processes including transcription, apoptosis, endoplasmic reticulum stress response, cell proliferation, and cell survival.[38] Importantly, cannabinoids are able to activate the AKT1/PI3K pathway by acting on CB1 and CB2 receptors in vitro.[40] Moreover, immediate administration of THC in mice activates AKT1 in vivo (through AKT1 phosphorylation) in several brain areas, including the striatum, independent of dopaminergic D_1 and D_2 receptor blockade.[41]

Decreased AKT1 levels have been observed in lymphoblasts and the postmortem prefrontal cortex of patients with schizophrenia,[42,43] and several studies have shown evidence for genetic association with schizophrenia,[43-47] although not all studies were able to confirm this.[48,49] Furthering the biological plausibility of *AKT1* moderating environmental influences on psychotic disorder is the observation of G × E interaction between obstetric complications and multiple SNPs in *AKT1*,[50] including rs1130233. This particular SNP also demonstrated significant Bonferroni-adjusted interaction with recent cannabis use in the at-risk paradigm (but not in the follow-up paradigms) and is known to be in very high linkage disequilibrium with rs2494732. Moreover, a recent study supported the involvement of both SNPs in the gene × obstetric complications interaction in schizophrenia, although this was only observed in female patients.[51] Pertinent to its possible involvement in psychosis, dopamine D_2 receptors may signal through an AKT1/GSK-3 signaling pathway via β-arrestin 2, and multiple lines of evidence support the involvement of the β-arrestin-2/AKT/GSK-3 pathway in the regulation of dopamine-associated behaviors and the response to antipsychotic treatment.[52] If psychotomimetic effects of THC are indeed modulated by the AKT1/GSK-3 signaling cascade, this could potentially explain why dopamine D_2 receptor blockade is ineffective in reducing psychotomimetic effects of THC in healthy individuals[53] and why substance-using patients with schizophrenia respond more poorly to antipsychotic treatment,[54] because the hypothesized cannabinoid-regulated AKT1/GSK modulation would occur downstream of the dopamine D_2 receptor, rendering its blockade inefficient. Thus, the data reported herein do not only suggest a robust and directionally consistent effect of genetic variation in *AKT1* on the psychotic response to cannabis; the involvement of *AKT1* in moderating psychotic responses to THC is also substantiated by multiple lines of evidence that suggest important links between environmental influences including cannabis on the one hand and AKT1 signaling, dopaminergic neurotransmission, and psychotic disorder on the other.

The present study, in contrast to previous studies, found no evidence that SNPs in *COMT* interact with cannabis use to influence positive schizotypy in unaffected

Genetic Risk and Outcome of Psychosis (GROUP) Investigators

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siblings. Although this does not exclude the possibility of *COMT* × cannabis interaction, previous positive findings were based on smaller samples[2-4] and inconclusive findings have also been reported.[5] On the other hand, some studies have suggested epistatic interactions between rs1130233 in *AKT1* and *COMT* Val158Met on prefrontal functioning[55] and, perhaps of even greater interest, on AKT1 phosphorylation in a cultured cell model.[56] In addition, NRG1-induced AKT1 phosphorylation was significantly diminished in *COMT* Val carriers in both normal subjects and in patients with schizophrenia in this cell model, as was NRG1-induced translocation of AKT1 to the plasma membrane.[56] These findings suggest that the previously reported *COMT* × cannabis findings and the present finding of *AKT1* × cannabis interaction may represent different genetic signals pointing to the same, or related, underlying molecular mechanism and that more complex models of interaction, including gene × gene and gene × gene × cannabis interactions, may need to be considered in future studies.

CANNABIS INTERACTION STUDIES

In addition to the observed *AKT1* rs2494732 × cannabis interaction, we also observed a significant (Bonferroni-adjusted) interaction with a SNP in *LRRTM1*. The importance of this finding is unclear and requires further replication. In the absence of robust support from the case-only, case-sibling, and case-control analyses, we would tentatively interpret the *LRRTM1* finding as a false-positive finding, or at least a finding that is limited to the short-term effects of cannabis use.

The present study found a range of SNP × cannabis interactions, only 3 of which were robust against Bonferroni correction. Of those 3, only 1 SNP showed consistent evidence for G × E in all applied paradigms. This once again demonstrates the complexity of pinpointing the genetic architecture of schizophrenia and suggests that it is only by combining different paradigms, such as (genome-wide) association studies, animal studies, imaging genetics, epigenetic approaches, and G × E interaction, that the underlying complexity of psychosis may be unraveled. However, the findings regarding the marginal effects of the investigated candidate schizophrenia genes were even more modest and actually quite close to null expectation. This suggests that underlying genetic liability to psychosis may often only become expressed in the context of exposure to relevant environmental risk factors, as put forward in recent developmental models of psychosis and other mental illness.[57-60]

STRENGTHS AND LIMITATIONS

The current study is unique in that it assessed a large sample of extensively phenotyped patients with psychosis and their unaffected siblings, using a comprehensive list of a priori candidate SNPs, examining both short-term as well as developmental effects of gene × cannabis interactions. Nevertheless, some limitations need to be taken into account. The prevalence of recent cannabis use was relatively low, which could have impacted the statistical power to detect gene × cannabis interactions; despite this, a range of significant interactions were identified, 3 of which surpassed Bonferroni correction. In participants screening positive for recent cannabis use, underlying heterogeneity in the degree of previous exposure may be expected. Although we tried to statistically control for previous cannabis exposure, exposure heterogeneity is difficult to overcome in the current design and it is possible that this has influenced the results to a degree. The adopted approach, with an emphasis on short-term moderation of cannabis response, could have missed genetic variation, gradually impacting developmental changes associated with psychotic disorder, such as neuroanatomical changes. These types of interactions, however, may be better studied in neuroimaging studies of G × E interaction. Lastly, gene selection was based on published literature prior to the major genome-wide association studies of schizophrenia. Nevertheless, the selected SNPs are a fair and comprehensive representation of the most widely studied candidate genes for schizophrenia.

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