LETTER TO THE EDITOR
Psychotomimetic effects at initiation of cannabis use are associated with cannabinoid receptor 1 (CNR1) variants in healthy students

Cannabis induces a diverse range of subjective experiences including relaxation, anxiety, euphoria, sadness, cognitive difficulties and psychotic-like symptoms. In addition, although cannabis use is associated with an overall twofold increased risk of subsequent psychotic disorders, all individuals are not at equal risk of developing psychosis when exposed to cannabis.1 Inconsistent results have been reported regarding the influence of variants in the catechol-O-methyltransferase (COMT) gene2–3 or in other genes of interest, such as brain-derived neurotrophic factor (BDNF), cannabinoid receptor 1 (CNR1)4–5 and more convincingly, v-Akt murine thymoma viral oncogene homolog 1 (AKT1).6 Individual variability in subjective experience is already present at initiation of cannabis use and could reflect intrinsic personal characteristics. From a gene × environment perspective, we hypothesized that the propensity to experience psychotomimetic effects when first using cannabis (PEFU) is influenced by genetic factors. We conducted a large community-based survey profiling the subjective response to cannabis in unrelated healthy young students. We investigated the association of polymorphisms of interest in CNR1, AKT1, BDNF and COMT genes with PEFU and found a significant association with a functional haplotype block in CNR1.

We interviewed 3807 students (age = 19.8 ± 2.5 years) during their mandatory preventive health visit in a university medical center. 44% of them had used cannabis at least once in their lifetime. Subjective experiences during first use were assessed using a 17-item self-report questionnaire (5-level rating), which has a confirmed factorial structure including one grouping psychotic-like experiences: hallucinations (visual and/or auditory) experienced as the experience of at least one 'strongly' or 'very strongly' when first consuming cannabis. A psychologist directly screened a subsample of 1196 students for Caucasian origins and no personal or family psychiatric history (age = 19.8 ± 2 years). After giving their informed written consent, these subjects provided DNA using cheek swabs. Among this group, 45.5% had used cannabis at least once in their lifetime and described their first subjective experiences (n = 544; age at initiation: 15.9 ± 1.8 years) (see socio-demographic characteristics in Supplementary Table 1).

We genotyped 10 variants of interest (CNR1: rs806379, rs1535255, rs2023239, rs1049353, rs12720071, AAT repeat; COMT: rs4680, also known as Val158Met; AKT1: rs2494732, rs1130233; BDNF: rs6265, also known as Val66Met). Allele and genotype frequencies of single nucleotide polymorphisms (SNPs) are shown in Supplementary Table 2. Testing associations with high PEFU was conducted using different genetic models (Supplementary Methods). We found no significant association between any of these markers and high PEFU after Bonferroni correction for multiple testing.

Interestingly, using multimarker analysis, we identified one block of linkage disequilibrium in CNR1 (rs202329, rs1535255, rs806379) (Supplementary Figure). The haplotype omnibus test was significant (kh12 = 14.02, df = 5, P = 0.015). The haplotype trend regression with gender as a covariate showed a significant association with high PEFU (P-Bonferroni = 0.023) (Table 1). Carrying the ‘AAA’ haplotype (21.6% in cases, 34.3% in controls) was significantly protective (Odds ratio, OR = 0.26, confidence interval, CI 95%: 0.1–0.67), whereas carrying the other haplotypes conferred a high risk of PEFU.

Of note, previous reports suggest that this CNR1 haplotype block influences CNR1 functions. The ‘TAG’ haplotype was found to be associated with reduced CNR1 mRNA levels in 39 postmortem brains,7 and its distribution was significantly different between multiple-substance abusers and healthy controls in several independent samples (n = 1536).8 The ‘ATT’ haplotype was associated with agreeableness, neuroticism and depression scores in the general population (n = 1269).9 Moreover, rs202329 was reported to be associated with a variation in CNR1 binding in the prefrontal cortex10 and is in strong linkage disequilibrium with rs9444584, a highly evolutionarily conserved marker strongly involved in MAPK activation.11 In conclusion, our results support the influence of CNR1 genetic variants on the propensity to experience psychotic symptoms when exposed to cannabis in healthy young adults, but do not support the influence of COMT, BDNF and/or AKT1 gene variants, which had previously been reported to be associated with risk of psychosis onset.2–6 Differences in the outcome variable could explain the fact that we did not replicate previous results. The literature has shown that cannabinoid receptors are involved in neurodevelopment, brain maturation, and could also be involved in psychosis and addiction.

Table 1. Haplotypic trend regression results for high psychotomimetic effects with gender as a covariate (SNP & Variation Suite v7.6.2)

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Freq cases (%)</th>
<th>Freq controls (%)</th>
<th>Beta</th>
<th>OR</th>
<th>95% CI</th>
<th>Nominal P</th>
<th>Bonferroni P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAA</td>
<td>52.68</td>
<td>49.56</td>
<td>−2.26</td>
<td>1.33</td>
<td>0.62–2.86</td>
<td>0.461</td>
<td>1</td>
</tr>
<tr>
<td>AAA</td>
<td>21.64</td>
<td>34.35</td>
<td>−1.75</td>
<td>0.26</td>
<td>0.10–0.67</td>
<td>0.003</td>
<td>0.023</td>
</tr>
<tr>
<td>ACT</td>
<td>12.96</td>
<td>9.79</td>
<td>−2.18</td>
<td>1.84</td>
<td>0.51–6.62</td>
<td>0.363</td>
<td>1</td>
</tr>
<tr>
<td>AAT</td>
<td>5.98</td>
<td>3.67</td>
<td>−2.16</td>
<td>3.03</td>
<td>0.45–18.84</td>
<td>0.257</td>
<td>1</td>
</tr>
<tr>
<td>ACA</td>
<td>3.53</td>
<td>1.05</td>
<td>−2.17</td>
<td>15.63</td>
<td>1.34–182.80</td>
<td>0.046</td>
<td>0.326</td>
</tr>
<tr>
<td>GCT</td>
<td>3.20</td>
<td>1.57</td>
<td>−2.16</td>
<td>6.52</td>
<td>0.45–85.75</td>
<td>0.188</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio. Bold and italic: significant P-value (P < 0.05).
By studying the psychotomimetic effects at initiation of cannabis use (i.e. at 15.9 ± 1.8 years of age) in healthy young students with no psychosis or addiction, our study focused on the influence of CNR1 gene variants in the expression of psychosis through the role of CNR1 in the brain maturation occurring during adolescence. In these young students, the association of CNR1 variants with psychotomimetic effects induced by cannabis at first use could accurately reflect the genetic risk of psychosis when exposed to cannabis, by avoiding the confounding factors of preexisting psychosis or the association of these genetic variants to psychosis or addiction. Our results should be replicated in representative samples of the general population. In addition, the predictive value of this genetic marker for developing psychotic disorders when exposed to cannabis has to be determined in prospective studies in the general population as well as in individuals who are at risk for psychosis.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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