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Cannabis and a lower BMI in psychosis: What is the role of AKT1?

Edith J. Liemburg [a,b,*], Jojanneke Bruins [a], Nico van Beveren [c,d], Md. Atiqul Islam [a,e], Behrooz Z. Alizadeh [f], GROUP investigators

GROUP investigators: R. Bruggeman, D. Wiersma, W. Cahn, R.S. Kahn, L. de Haan, C.J. Meijer, I. Myin-Germeys, J. van Os:

[a] Rob Giel Research Center, University of Groningen, University Medical Center Groningen, CC72, Hanzeplein 1, 9713 GZ Groningen, The Netherlands
[b] Department of Neuroscience, and BCN Neuroimaging Center, University of Groningen, University Medical Center Groningen, FA32, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands
[c] Department of Psychiatry, Erasmus Medical Center, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands
[d] Department 'Nieuwe Kennis', Delta Center for Mental Health Care, Boerhaavestraat 10,3083 DA Rotterdam, The Netherlands
[e] Department of Statistics, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh
[f] Department of Epidemiology, University of Groningen, University Medical Center Groningen, FA40, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

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ABSTRACT

Cannabis use has been associated with favorable outcomes on metabolic risk factors. The cause of this relation is still unknown. In this study we investigated whether this effect is mediated by the *AKT1* gene, as activation of the related enzyme by cannabis may cause metabolic changes.

Six Single Nucleotide Polymorphisms (SNPs) of the AKT1 gene (*rs*1130214, *rs*1130233, *rs*2494732, *rs*2498784, *rs*3730358, and *rs*3803300) of patients with psychotic disorders ($n = 623$) were related to Body Mass Index (BMI), levels of glycosylated hemoglobin (HBA_{1c}) and total metabolic risk. Next, mediation analysis was performed with BMI as outcome, cannabis as predictor, and AKT1 as mediator.

Cannabis use was inversely related to BMI but not with levels of HBA_{1c} and total metabolic risk. Moreover, out of 6 *AKT1* SNPs, *rs*2494732 was associated with cannabis use, but *AKT1* did not mediate the effect of cannabis on BMI. In conclusion, cannabis use is likely to be associated with a lower BMI in patients with a psychotic disorder. Moreover, *AKT1* risk alleles may increase the incidence of cannabis use in patients with a psychotic disorder, but *AKT1* does not appear to mediate the effect of cannabis on BMI.

1. Introduction

Patients with a psychotic disorder have on average a life expectancy 13–30 years shorter than the general population (De Hert et al., 2011; Hennekens et al., 2005). Metabolic disorders combined with an unhealthy lifestyle (Scott and Happell, 2011), which develop quickly after onset of psychosis (Britvic et al., 2013), are the main medical conditions that lead to a shortened life expectancy (Casey et al., 2011; Laursen et al., 2013; O'Connor et al., 2014). Interestingly, while many psychotic patients use cannabis (Green et al., 2005), which can trigger psychotic symptoms (Henquet et al., 2010), cannabis has been associated with a lower body mass, levels of glucose and cholesterol and a smaller waist circumference in both healthy individuals (Le Strat and Le Foll, 2011; Penner et al., 2013; Smit and Crespo, 2001; Warren et al., 2005) and patients with a psychotic disorder (Bruins et al., 2016). On the other hand, cannabis use has also been related to an unhealthy lifestyle (Rodondi et al., 2006) and heightened glucose levels and stronger increases in bodyweight in patients with a psychotic disorder (Isaac et al., 2005). These inconsistent effects of cannabis may be mediated by the endocannabinoid system. The main psychoactive constituent of cannabis, (−)-trans-Δ9-tetrahydrocannabinol (THC) increases appetite and food intake and stimulates the storage of body fat (Di Marzo and Matias, 2005; Hillig and Mahlberg, 2004) by activating the endocannabinoid system (Matias and Di Marzo, 2007). At the same time, cannabinoids (Hillig and Mahlberg, 2004) decrease appetite and food intake and therefore lead to metabolic improvements (Christopoulou and Kiortsis, 2011).

Thus far no study has been performed to uncover the underlying cause for the effect of cannabis use on metabolic indices. While studies showed differential effects of cannabis on metabolic parameters, the reasons for these differential findings remain undetermined. It is

* Corresponding author at: Rob Giel Research Center, University of Groningen, University Medical Center Groningen, CC72, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail addresses: e.j.liemburg@umcg.nl (E.J. Liemburg), j.bruins@umcg.nl (J. Bruins), N.Beveren@antesgroep.nl (N. van Beveren), a.islam@umcg.nl (M.A. Islam), b.z.alizadeh@umcg.nl (B.Z. Alizadeh), r.bruggeman@umcg.nl (R. Bruggeman).

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plausible that genetic susceptibility may mediate the effect of cannabis use on metabolic syndrome and leads to differential effects, i.e. both increased and decreased body weight. Notably, the serine–threonine protein kinase *AKT1,* which encodes for the enzyme RAC-alpha serine/threonine-protein kinase, has been implicated in schizophrenia (Meijer et al., 2012; Steiner et al., 2014). *AKT1* represents a good candidate gene to explain differential effects of cannabis on metabolic outcomes. While *AKT1* risk allele (i.e. *rs*2494732*C) carriers have an increased risk to develop schizophrenia by using cannabis (Di Forti et al., 2012; Van Winkel et al., 2011), carriers (i.e. *AKT1-rs*1130214*T) are also likely to develop metabolic syndrome (Devaney et al., 2011; Harmon et al., 2010). As THC and cannabinoids are able to activate *AKT1* (Ozaita et al., 2007; Sanchez et al., 2003), the use of cannabis may well reduce the risk of diabetes and metabolic syndrome by activating *AKT1* in carriers of non-risk alleles. While AKT1 influences insulin pathways by augmenting glucose uptake (Elghazi et al., 2006; Zdychová and Komers, 2005) and affecting adipocyte differentiation (Baudry et al., 2006), and insulin resistance and obesity are often inter-related, we expect the strongest effects to be found on glucose levels and body mass (BMI) (Devaney et al., 2011). We therefore hypothesize that cannabis users have a lower BMI and lower glucose levels compared to non-users, given cannabis may activate the transcription of the *AKT1*, and that this effect is weaker in carriers of *AKT1* risk alleles that may have impaired function of the enzyme. Moreover, we will also investigate other indices of metabolic syndrome, including waist circumference, triglycerides and HDL cholesterol.

2. Materials and methods

2.1. Study population

We included 623 patients (aged 15–50 years old) from an ongoing longitudinal study (Genetic Risk and OUtcome after Psychosis; GROUP, version 3.02) in selected representative geographical areas in the Netherlands and partly in Belgium. The study outline has been described in detail elsewhere (Korver et al., 2012). Body Mass Index (BMI) was measured as body weight/height^2 (kg/m^2) and glycosylated hemoglobin (HBA_{1c}) as %. Cannabis use was defined as a positive answer in the Composite International Diagnostic Interview (CIDI) and cannabis urine screening (immunoassay with a cutoff of 50 ng/ml). Metabolic syndrome was established for referential purposes according to NCEP-ATP-III criteria (Grundy et al., 2005), without the criteria for medication use. The individual metabolic components were standardized and combined to create a continuous variable for the metabolic syndrome as marker for metabolic risk (MetRisk). For blood values, the means and standard deviations of the patients having values within healthy range were used: HDL-C (1.1–2.0 mmol/l in female and 0.9–1.7 mmol/l in male patients), triglycerides (≤2.2 mmol/l) and HbA1c (<8.0%), creating Z-scores for the five components of the metabolic syndrome. A combined metabolic syndrome Z-score was created by taking the mean of the five components (Pekelharing et al., 2016). Haloperidol equivalents were calculated according to Gardner et al. (2010). To investigate the metabolic effect of antipsychotics, antipsychotic medication was ordinally classified as high metabolic side effects (olanzapine and clozapine), medium metabolic side effects (risperidone, quetiapine, chlorprothixene, paliperidone, pipamperone and levomepromazine) and low metabolic side effects (aripiprazole, haloperidol, bromperidol, flupentixol, pimozide, sulpiride, zuclopenthixol) (Leucht et al., 2013).

Six Single Nucleotide Polymorphisms (SNPs) of the *AKT1* gene on chromosome 14 were genotyped in patients: *rs*1130214 (104793397, G/**T**; $MAF_{EUR} = 0.28$), *rs*1130233 (104773557, G/**A**, $MAF_{EUR} = 0.24$), *rs*2494732 (104772855, T/**C**, $MAF_{EUR} = 0.44$), *rs*2498784 (104798626, C/**T**, $MAF_{EUR} = 0.9$), *rs*3730358 (104780070, C/**T**, $MAF_{EUR} = 0.16$), and *rs*3803300 (104803442, A/**G**, $MAF_{EUR} = 0.10$). Genotype distribution of the polymorphisms in our sample did not deviate significantly from the Hardy Weinberg equilibrium and MAF of these SNPs was in accordance with their corresponding MAF of European population reported in 1000 genome project (see web link). Genetic data of good quality on all SNPs were available for 452 patients. We examined the linkage disequilibrium (LD) between these SNPs as presented in a regional LD plot in Supplementary Fig. 1. Per each of the SNPs the corresponding genotypes were coded as additive, with 0 for carriers of no risk allele (i.e. wild type), 1 for heterozygotes for risk allele (1 copy), and 2 for carriers of 2 risk alleles, and modeled as co-variables in a linear effect (Cordell and Clayton, 2005). Per subject, we calculated an unweighted genetic risk score (GRS_{AKT1}) for *AKT1* by summing up the number of risk allele yielding a range between 0 (i.e. a person with no risk allele in any of the SNPs) and a maximum of 12 (i.e. a person with 2 risk alleles per each SNP). Both an unweighted GRS_{AKT1} and the separate SNPs were investigated. Cannabis use (yes or no) was analyzed as a linear effect (van Winkel and Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011).

2.2. Data analysis

BMI, HBA_{1c} and MetRisk were normalized using the Box-Cox Power Transformation procedure (AID package of RStudio 098 (Inc.)). BMI was transformed by taking the inverse square root and HBA_{1c} by taking the fourth root. MetRisk did not have to be transformed. Haploview 4.2 was used to determine whether the SNPs could be combined into haploblocks ($R^2 > 70$). The linear association between transformed BMI or HBA_{1c} and cannabis use was investigated adjusted first for metabolic risk antipsychotics (no, low, high), gender and age, and next with, smoking status (yes/no), and alcohol use (yes/no) as additional covariates (Scott and Happell, 2011). In case of a significant association between outcome variable and cannabis use, mediation analysis was performed per SNP, and per *AKT1* risk score with BMI as outcome, cannabis as predictor, AKT1 as mediator (because cannabis induces *AKT1*), and age and gender as confounders. Three models for both the GRS_{AKT1} and the separate SNPs were used, including Model I: Cannabis = β_0 + SNP + age + gender + metabolic risk antipsychotics, model II: BMI = β + gene + age + gender + metabolic risk antipsychotics, and model III: β0 + SNP + cannabis + age + gender + metabolic risk antipsychotics. If Model I and II were both significant, a Sobel test was conducted to test whether cannabis had a significantly mediating effect. Correction for multiple comparison in subsequent regression analyses was applied by dividing $\alpha = 0.05$ by the number of haploblocks and independent SNPs. There were no SNPs with a strong LD ($r^2 > 0.70$), thus we used an α of 0.05/6 = 0.008. SPSS 20.0 (IBM Inc. New York, USA) was used for statistical analyses.

3. Results

Characteristics of the study population is presented in Table 1. Regression analysis revealed that the mean BMI was lower in cannabis-users (25.0 ± SE 0.37) compared to non-users (26.7 ± SE 0.24) (β = 0.179, SE = 0.002, $p < 0.0005$; Supplementary Table 1). This effect remained significant after adding the covariates (β = 0.198, SE = 0.002, $p < 0.0005$). There was no significant difference in mean levels of HBA_{1c} between user and non/user of cannabis (Supplementary Table 2) or a significant effect of total metabolic risk (MetRisk).

The distribution of the GRS_{AKT1} ranged from 0–8. We observed that the mean of *AKT1* GRS is significantly higher in users (3.1) compared to non-user (2.5) of cannabis. As Fig. 1 presents, the percent of cannabis users (i.e. grey bars) increased as the dosage of GRS_{AKT1} increased, yielding a significant relation between the *AKT1* GRS and cannabis use (β = 0.19, SE = 0.33, $p = 0.004$).

We also found a significant relation between *rs2494732* genotype and cannabis users as proportional cannabis use was significantly higher in CT and TT genotypes compared to CC yielding a β of 0.20 (SE = 0.110, $p = 0.001$); there was also a significant effect of *rs1130233* (β = 0.16, SE = 0.090, $p = 0.013$), where A gave an

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