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Short communication

Trend level gene-gender interaction effect for the BDNF rs6265 variant on age of onset of psychosis

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ABSTRACT

A BDNF rs6265 [A/A] by gender by cannabis use interaction has been associated with age of onset of psychosis (AoP). We examined the gender and cannabis use-adjusted association between BDNF rs6265 [G > A] and AKT1 rs2494732 [T > C] and AoP. Data from 167 Caucasians on AoP and age at first regular cannabis use were collected. Kaplan-Meier and Cox regression analyses were conducted. A trend level gene-gender interaction effect was observed for the BDNF rs6265 A/A genotype, controlling for age at first regular cannabis use. Larger collaborative research projects are required to further investigate this effect.

1. Introduction

Premorbid cannabis use is a risk factor for psychosis (Marconi et al., 2016), while gender (Castle et al., 1998) and genetic variants (Lett et al., 2011; Takase et al., 2001; Vares et al., 2010; Wang et al., 2013) have been independently associated with age of onset of psychosis (AoP). However, in terms of prior investigations of the interactions of genes and cannabis on AoP, a limited number of genes have been reported to be of significance (Decoster et al., 2011; Estrada et al., 2011; Lodhi et al., 2017; Mané et al., 2017a; Pelayo-Teran et al., 2010). Investigating independent and interaction effects of genetic factors moderating the effect of cannabis on phenotypes of psychosis such as AoP may add to the understanding of underlying biological mechanisms in psychosis.

The *BDNF* rs6265 variant results in a valine to methionine substitution at codon 66, and has been associated with psychosis (Mezquida et al., 2016; Numata et al., 2006). Two publications have evaluated the rs6265-cannabis interaction effect on AoP (Decoster et al., 2011; Mané et al., 2017a). A three-way rs6265-cannabis use-gender interaction was associated with earlier AoP in one study, with rs6265 Met allele female cannabis users having an earlier AoP (Decoster et al., 2011). Another study reported an independent but no interaction effect of rs6265 Met-carriers and early cannabis use on AoP (Mané et al., 2017b).

The enzyme AKT (protein kinase B, encoded by the gene *AKT1*), is important for dopamine signaling via D_2 receptor stimulation and the AKT-GSK3 signaling cascade (Beaulieu et al., 2007). The C/C genotype of rs2494732 in *AKT1* has been associated with the risk of developing psychosis in cannabis users (Di Forti et al., 2012). To the best of our knowledge, there are no available data on an rs2494732–cannabis interaction and AoP, with only one study examining the main effect of polymorphisms in *AKT1* on AoP (Chow et al., 2016).

The objectives of our study were: to assess the main effects of rs6265 and rs2494732 on AoP, adjusted for gender and age at regular cannabis use, and to evaluate the influence of gene by gender or age at regular cannabis use interactions on AoP. We hypothesized that gender, age at regular cannabis use, rs6265 Met allele status and rs2494732[C] would be associated with reduced AoP, independently or in interaction with each other.

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2. Methods

2.1. Study population, data collection and DNA extraction

Patients with psychosis were recruited from Edmonton (54.49%) and Halifax (45.51%), and cannabis use information was collected using a self-reported electronic questionnaire (Purdon, 2007). Details of the questionnaire, definition of psychosis (using SCID) and laboratory methods for DNA extraction have been previously described (Lodhi et al., 2017).

2.2. Genotyping

The rs6265 and rs2494732 markers were genotyped in duplicate using TaqMan® SNP Genotyping Assays C_11,592,758_10 and C_16,191,608_10, respectively, on a ViiA7 real-time PCR system (Thermo Fisher Scientific, Canada) in duplicate, with polymerase chain reaction (PCR)-restriction fragment length based polymorphism (RFLP) analysis for confirmation for rs6265. PCR forward and reverse primers were 5′-AAAGAAGCAAACAT CCGAGGACAAG-3′ and 5′-ATTCCTCCA GCAGAAAGAGAAGAG-3′, respectively. PCR was carried out using FroggaMix (FroggBio, Toronto, Canada) and 0.125 U/μl Taq DNA polymerase, 0.2 mM dNTPs, 1.6 mM $MgCl_2$ with reaction conditions of 94 °C for 5 min; 35 cycles of 1 min at 94 °C, 2 min at 55 °C, and 2 min at 72 °C; followed by a final elongation at 72 °C for 4 min. Digestion with *Nla* III (New England Biolabs, USA) at 37 °C for 16 h was then conducted, with product resolution on a 3.0% agarose gel. Repeats were conducted for calls not readily resolved. In this manner, 99.5% of the samples were genotyped.

2.3. Statistical analyses

STATA-13.1 was used for data analysis. We combined the rs6265 A/A and A/G genotypes into an rs6265 `A carriers' group due the low frequency of the A/A genotype, consistent with a similar study (Decoster et al., 2011). AoP predictors included: genotypes (rs6265: G/G and A carriers; rs2494732: C/C, C/T and T/T), gender (women and men) and age at regular cannabis use (ARCU): no regular use, regular use before age 20, and at or after age 20. Kaplan-Meier analyses (results reported using the log-rank test (LR)) followed by Cox-regression (CR) analyses were performed. The variance inflation factor (VIF) was used to assess collinearity among predictors before CR. CR models included: main effect of gender, genotypes and ARCU; gender-genotype interactions controlling for ARCU; and genotype-ARCU interactions controlling for gender.

3. Results

One hundred and sixty-seven subjects were included (Table 1). The mean age of the study sample was 27.83 years (SD = 9.77). The mean AoP was 22.93 years (SD = 6.38) with a right skew distribution (STATA skewness and kurtosis test: $p < 0.001$). The duration of illness, defined as current age minus age of onset of psychosis, varied in our sample. Approximately 48% had a chronic psychosis (mean DUI: 9.37 years), 41.32% were first episode (mean DUI: 0.37 years) and 10.78% were in an early stage of illness (mean DUI: 2.32 years). The frequency of rs6265 genotypes was 126 (75.45%), 40 (23.95%) and 1 (0.6%) for G/G, A/G and A/A respectively, and for rs2494732 it was 49 (29.34%), 85 (50.90%) and 33 (19.76%) for T/T, C/T, and C/C, respectively.

In the Kaplan-Meier analyses, gender ($p = 0.010$) and ARCU ($p = 0.0029$) significantly affected AoP, while rs6265 ($p = 0.39$) and rs2494732 ($p = 0.91$) did not. The mean AoPs for men and women were 22.51 and 24.85 years respectively, and for those with ARCU before 20 and at or after 20 years were 21.29 and 24.53 years respectively. The VIF value was 1.04 (should be less than 10) (Hair et al., 1998). ARCU (Hazard ratio HR = 1.51, $p = 0.031$ for ARCU before 20 versus no regular use) significantly affected AoP in the main effect CR analysis. Amongst CR interaction analyses, there was a trend for an rs6265-gender interaction (HR = 2.01, $p = 0.067$), adjusted for ARCU. Met-carriers had a trend for an earlier AoP (the mean AoP for rs6265 A carriers and those of G/G genotype was 21.62 and 22.26 years for men, and 23.82 and 27.5 years for women, respectively). Lastly, no significant effects were observed in the genotypes by cannabis interaction analyses.

Table 1
Description of study sample based on age at *regular* cannabis use (RU) using number and percentage N (%) in each category.

Category	Never RU	RU before 20 years	RU at or after age 20	P*
Gender				**0.001**
Men	33 (53.23)	67 (81.71)	17 (73.91)	
Women	29 (46.77)	15 (18.29)	6 (26.09)	
Education				
less than Grade 12	15 (24.19)	39 (47.56	5 (21.74)	**0.005**
Marital Status				**0.007**
Single	56 (90.32)	80 (97.56)	18 (78.26)	
Married/Partner/ Separated etc.	6 (9.68)	2 (2.44)	5 (21.74)	
Age at interview (years)	30.74 (1.49)	24.45 (0.88)	28.21 (1.34)	**<0.001**

* Calculated using chi-squared test for categorical variables and ANOVA for age at interview.

4. Discussion

We observed a trend signal for an rs6265 x gender interaction, adjusted for age at regular cannabis use, on AoP. However, unlike the previous study (Decoster et al., 2011), our trend for the rs6265-gender effect was in men. It is possible that both men and women have an earlier AoP if they have the rs6265 A (Met) allele, with the contrasting findings between our study and that of Decoster et al. (2011) relating to by gender subsample size differences. Our sample was particularly limited in terms of the women (Fig. 1), while theirs appears to have been more limited in terms of the men (Fig. 2, Decoster et al., 2011). Both of these figures are not inconsistent with an earlier age of onset of psychosis for Met allele carriers in both men and women.

It is noteworthy that the two studies are approximately consistent with each other despite other – likely less important - contrasts between them, such as AoP definition being age of diagnosis by structured clinical interview versus age of first admission/first contact with a psychiatrist (Decoster et al., 2011; Mané et al., 2017a). Our sample has

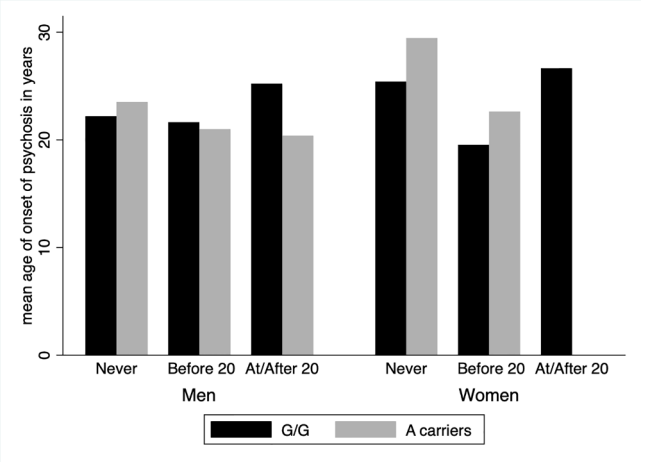

Fig. 1. Mean age of onset of psychosis by age at regular cannabis use (never, before 20 and at or after 20) and gender.

a lower mean age of 27.83 years, compared to 36.1 years in Decoster et al. (2011). The rs6265–gender interaction signal for AoP in our study was from age at *regular* cannabis use, a subjectively interpreted variable by our participants. Regular cannabis use is an important factor for the transition to psychosis (Compton et al., 2009; Myles et al., 2016), and could reasonably be hypothesized to be particularly relevant to any effect mediated by a neurotrophin such as BDNF. By contrast, Decoster et al. (2011) used a lifetime cannabis usage variable defined as use at least five times in the person's life.

The limitations of small sample size and the self-reported nature of cannabis use have been described (Lodhi et al., 2017). Correcting for the number of genes tested (two in this analysis), weakens the signal from the rs6265-gender adjusted for regular cannabis use on AoP. Our analysis therefore provides suggestive support for including gender while examining the rs6265-cannabis interaction effect on AoP, first noted by Decoster et al. (2011). This is consistent with a gender effect on the regulation of *BDNF* gene expression in the developing hippocampus in animal models (Kight and McCarthy, 2017), and an association between male gender and lower serum BDNF in schizophrenia (Zhang et al., 2014). As our analysis, especially the gene-gender interaction part, was limited by sample size, we suggest that future studies of a gene-gender interaction adjusting for cannabis use or a three-way interaction of gene-gender-cannabis on AoP should be undertaken. This and other prior work indicates that larger collaborative research projects incorporating consistent methodology are required to further delineate the effects not only of the two genes herein examined but also that of others.

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References

Beaulieu, J.M., Gainetdinov, R.R., Caron, M.G., 2007. The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol. Sci. 28 (4), 166–172.

Castle, D., Sham, P., Murray, R., 1998. Differences in distribution of ages of onset in males and females with schizophrenia. Schizophr. Res. 33 (3), 179–183.

Chow, T.J., Tee, S.F., Yong, H.S., Tang, P.Y., 2016. Genetic association of TCF4 and AKT1 gene variants with the age at onset of schizophrenia. Neuropsychobiology 73 (4), 233–240.

Compton, M.T., Kelley, M.E., Ramsay, C.E., Pringle, M., Goulding, S.M., Esterberg, M.L., Stewart, T., Walker, E.F., 2009. Association of pre-onset cannabis, alcohol, and tobacco use with age at onset of prodrome and age at onset of psychosis in first-episode patients. Am J. Psychiatry 166 (11), 1251–1257.

Decoster, J., van Os, J., Kenis, G., Henquet, C., Peuskens, J., De Hert, M., van Winkel, R., 2011. Age at onset of psychotic disorder: cannabis, BDNF Val66Met, and sex-specific models of gene-environment interaction. Am J. Med. Genet B Neuropsychiatr. Genet. 156B (3), 363–369.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M.A., Paparelli, A., Sirianni, M., La Cascia, C., Stilo, S.A., Marques, T.R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A.S., Morgan, C., Powell, J., Murray, R.M., 2012. Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. Biol. Psychiastry 72 (10), 811–816.

Estrada, G., Fatjo-Vilas, M., Munoz, M.J., Pulido, G., Minano, M.J., Toledo, E., Illa, J.M., Martin, M., Miralles, M.L., Miret, S., Campanera, S., Bernabeu, C., Navarro, M.E., Fananas, L., 2011. Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr. Scand. 123 (6), 485–492.

Hair, J.F., Tatham, R.L., Anderson, R.E., Black, W.C., 1998. Multivariate Data Analysis, fifth ed. Prentice-Hall, NJ.

Kight, K.E., McCarthy, M.M., 2017. Sex differences and estrogen regulation of BDNF gene expression, but not propeptide content, in the developing hippocampus. J. Neurosci. Res. 95 (1–2), 345–354.

Lett, T.A., Zai, C.C., Tiwari, A.K., Shaikh, S.A., Likhodi, O., Kennedy, J.L., Muller, D.J., 2011. ANK3, CACNA1C and ZNF804A gene variants in bipolar disorders and psychosis subphenotype. World J. Biol. Psychiatry 12 (5), 392–397.

Lodhi, R.J., Wang, Y., Rossolatos, D., MacIntyre, G., Bowker, A., Crocker, C., Ren, H.Y., Dimitrijevic, A., Bugbee, D.A., Loverock, A., Majeau, B., Sivapalan, S., Newton, V.M., Tibbo, P., Purdon, S.E., Aitchison, K.J., 2017. Investigation of the COMT VAL158MET variant association with age of onset of psychosis, adjusting for cannabis use. Brain Behav. 7 (11), e00850.

Mané, A., Bergé, D., Penzol, M., Parellada, M., Bioque, M., Lobo, A., Gonzalez-Pinto, A., Corripio, I., Cabrera, B., Sanchez-Torres, A., Saiz, J., Bernardo, M., 2017a. Cannabis use, COMT, BDNF and age at firstepisode psychosis. Psychiatry Res.

Mané, A., Bergé, D., Penzol, M., Parellada, M., Bioque, M., Lobo, A., Gonzalez-Pinto, A., Corripio, I., Cabrera, B., Sanchez-Torres, A., Saiz, J., Bernardo, M., 2017b. Cannabis use, COMT, BDNF and age at firstepisode psychosis. Psychiatry Res. 250, 38–43.

Marconi, A., Di Forti, M., Lewis, C.M., Murray, R.M., Vassos, E., 2016. Meta-analysis of the association between the level of cannabis use and risk of psychosis. Schizophr. Bull.

Mezquida, G., Penades, R., Cabrera, B., Savulich, G., Lobo, A., Gonzalez-Pinto, A., Penzol, M.J., Corripio, I., Fernandez-Egea, E., Gasso, P., Cuesta, M.J., Bernardo, M., group, P.E., 2016. Association of the brain-derived neurotrophic factor VAL66MET polymorphism with negative symptoms severity, but not cognitive function, in first-episode schizophrenia spectrum disorders. Eur. Psychiatry 38, 61–69.

Myles, H., Myles, N., Large, M., 2016. Cannabis use in first episode psychosis: meta-analysis of prevalence, and the time course of initiation and continued use. Aust. N. Z. J. Psychiatry 50 (3), 208–219.

Numata, S., Ueno, S., Iga, J., Yamauchi, K., Hongwei, S., Ohta, K., Kinouchi, S., Shibuya-Tayoshi, S., Tayoshi, S., Aono, M., Kameoka, N., Sumitani, S., Tomotake, M., Kaneda, Y., Taniguchi, T., Ishimoto, Y., Ohmori, T., 2006. Brain-derived neurotrophic factor (BDNF) VAL66MET polymorphism in schizophrenia is associated with age at onset and symptoms. Neurosci. Lett 401 (1–2), 1–5.

Pelayo-Teran, J.M., Perez-Iglesias, R., Mata, I., Carrasco-Marin, E., Vazquez-Barquero, J.L., Crespo-Facorro, B., 2010. Catechol-O-Methyltransferase (COMT) VAL158MET variations and cannabis use in first-episode non-affective psychosis: clinical-onset implications. Psychiatry Res. 179 (3), 291–296.

Purdon, S.E., 2007. Self-rated Computerized Drug Survey. PNL Inc., Edmonton, AB.

Takase, K., Ohtsuki, T., Migita, O., Toru, M., Inada, T., Yamakawa-Kobayashi, K., Arinami, T., 2001. Association of ZNF74 gene genotypes with age-at-onset of schizophrenia. Schizophr Res. 52 (3), 161–165.

Vares, M., Saetre, P., Deng, H., Cai, G., Liu, X., Hansen, T., Rasmussen, H.B., Werge, T., Melle, I., Djurovic, S., Andreassen, O.A., Agartz, I., Hall, H., Terenius, L., Jonsson, E.G., 2010. Association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and age of onset in schizophrenia. Am J. Med. Genet B Neuropsychiatr. Genet. 153B (2), 610–618.

Wang, K.S., Liu, X., Arana, T.B., Thompson, N., Weisman, H., Devargas, C., Mao, C., Su, B.B., Camarillo, C., Escamilla, M.A., Xu, C., 2013. Genetic association analysis of ITGB3 polymorphisms with age at onset of schizophrenia. J. Mol. Neurosci. 51 (2), 446–453.

Zhang, X.Y., Chen, D.C., Tan, Y.L., Tan, S.P., Wang, Z.R., Yang, F.D., Xiu, M.H., Hui, L., Lv, M.H., Zunta-Soares, G.B., Soares, J.C., 2014. Gender difference in association of cognition with BDNF in chronic schizophrenia. Psychoneuroendocrinology 48, 136–146.