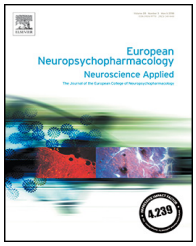
European Neuropsychopharmacology (2019) 29, 643–652

www.elsevier.com/locate/euroneuro



Liprin alfa 2 gene expression is increased by cannabis use and associated with neuropsychological function

Yujie He[a,b], Lot D. de Witte[a,c], Chris D. Schubart[d], Willemijn A. Van Gastel[e], Bobby P.C. Koeleman[f], Simone de Jong[g], Roel A. Ophoff[h], Elly M. Hol[b,i], Marco P. Boks[a,*]

[a] *Department of Psychiatry, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University, The Netherlands*
[b] *Department of Translational Neuroscience, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University, The Netherlands*
[c] *Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, USA*
[d] *Ter Gooi Hospital, Department of Psychiatry, Blaricum, The Netherlands*
[e] *Department of Psychology, Utrecht University, Utrecht, The Netherlands*
[f] *Department of Medical Genetics, University Medical Centre Utrecht, Utrecht University, The Netherlands*
[g] *MRC Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK*
[h] *UCLA Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA, USA*
[i] *Neuroimmunology, Netherlands Institute for Neuroscience, An institute of the royal academy of arts and sciences, Amsterdam, The Netherlands*

Received 4 January 2018; received in revised form 13 January 2019; accepted 2 March 2019

KEYWORDS
Cannabis;
Gene expression;
PPFIA2;
Cognition;
THC;
CBD

Abstract
The relation of heavy cannabis use with decreased neuropsychological function has frequently been described but the underlying biological mechanisms are still largely unknown. This study investigates the relation of cannabis use with genome wide gene expression and subsequently examines the relations with neuropsychological function. Genome-wide gene expression in whole blood was compared between heavy cannabis users ($N = 90$) and cannabis naïve participants ($N = 100$) that were matched for psychotic like experiences. The results were validated

* Corresponding author: MPM Boks, MD PhD, UMC Utrecht Brain Center, Department of Psychiatry, University Medical Centre Utrecht, A01.468, PO box 85500, 3508 GA Utrecht, The Netherlands Tel: +31 88 7556370 Fax: +31 88 7555509.
E-mail address: m.p.m.boks@umcutrecht.nl (M.P. Boks).

https://doi.org/10.1016/j.euroneuro.2019.03.004
0924-977X/

using quantitative real-time PCR. Psychotic like experiences were assessed using the Comprehensive Assessment of Psychotic Experiences (CAPE). Neuropsychological function was estimated using four subtasks of the Wechsler Adult Intelligence Scale (WAIS). Subsequent *in vitro* studies in monocytes and a neuroblastoma cell line investigated expression changes in response to two major psychotropic components of cannabis; tetrahydrocannabinol (THC) and cannabidiol (CBD). mRNA expression of Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting-Protein Alpha-2 (*PPFIA2*) was significantly higher in cannabis users (LogFold Change 0.17) and confirmed by qPCR analysis. *PPFIA2* expression level was negatively correlated with estimated intelligence ($B=-22.9$, $p=0.002$) also in the 100 non-users ($B=-28.5$, $p=0.037$). In vitro exposure of monocytes to CBD led to significant increase in *PPFIA2* expression. However, exposure of monocytes to THC and neuroblastoma cells to THC or CBD did not change *PPFIA2* expression. Change in *PPFIA2* gene expression in response to cannabinoids is a putative mechanism by which cannabis could influence neuropsychological functions. The findings warrant further exploration of the role of *PPFIA2* in cannabis induced changes of neuropsychological function, particularly in relation to CBD.

1. Introduction

Global cannabis consumption is rising and has increased to 182.5 million users in 2016 (Bussink et al., 2016). This has raised concern about potentially detrimental effects of cannabis on mental health and neuropsychological function. Compelling evidence suggests that cannabis increases the risk to psychosis (Vaucher et al., 2017) and psychotic like experiences (Van Gastel et al., 2013). Also neuropsychological function may be affected by cannabis use (Broyd et al., 2016; Grant et al., 2003; Schnakenberg Martin et al., 2016). Acute cannabis use impairs neuropsychological functioning (Morrison et al., 2009), especially short-term episodic and working memory (Ranganathan and D'Souza, 2006) and persistent cannabis use from adolescent age onwards is associated with a decline in intelligence (Castellanos-Ryan et al., 2016; Meier et al., 2012) although the role of potential confounding factors, particularly social economic status is debated (Meier et al., 2017; Moffitt et al., 2013). The mechanisms underlying the relationship between cannabis use, psychotic experiences and decreased neuropsychological function have remained largely unknown.

One of the most prevailing hypothesis on the relation between cannabis and neuropsychological function involves the dopamine system (Bloomfield et al., 2016; Freund et al., 2003). It is thought that one of the most active neuropsychiatric compounds, tetrahydrocannabinol (THC) (Dinis-Oliveira, 2016; Wachtel et al., 2002), dysregulates the dopamine system via the endocannabinoid type 1 receptor (CB1R). Acute THC consumption increases dopamine release (Bloomfield et al., 2016; Bossong et al., 2015, 2009; Stokes et al., 2010), whereas long term THC consumption blunts dopamine synthesis and release capacity (Freund et al., 2003; van de Giessen et al., 2016). Since the dopamine system is closely related to neuropsychological function (Hélie et al., 2012; Nakajima et al., 2013), dysregulation by cannabis is thought to contribute to this impairment in cannabis users. An alternative hypothesis comes from neuroimaging studies that suggest that white matter changes in cannabis users (Jakabek et al., 2016; Weinstein et al., 2011), may account for reduced neuropsychological functioning (Filley and Fields, 2016; O'Muircheartaigh et al., 2014; Ohtani et al., 2017).

However, evidence so far has been inconclusive and further genetic studies may improve our understanding of the effects of cannabis in the brain. A range of studies identified the potential role of a few candidate genes that likely play a role in the mental health effects of cannabis including AKT Serine/Threonine Kinase 1 (*AKT1*) (Boks, 2012; Bossong et al., 2015; Di Forti et al., 2012; Ibarra-Lecue et al., 2018), Catecholamine O-Methyl transferase (*COMT*) (Caspi et al., 2005; Henquet et al., 2009, 2006; Nieman et al., 2016), and the gene that codes for cannabis receptors, cannabinoid receptor type 1 (*CNR1*) (D'Addario et al., 2017; Suarez-Pinilla et al., 2015). However, such a priori selection of specific genes limits the ability to uncover new leads in understanding the effects of cannabis on the brain whereas genome wide studies may facilitate our understanding of the role of cannabis in neuropsychological function. Considering the scarceness of post-mortem human brain samples of cannabis users this study reverts to whole blood of cannabis users and cannabis naïve controls. In order to deal with the potential role of psychotic like experiences, cannabis users and non-users were matched for the level of psychotic like experiences. We examined gene expression rather than the underlying genetic variants in order to directly investigate the biological response of cells to the cannabinoids as a way of informing on the biological mechanisms that are involved in cannabis use, and not genetic vulnerability. The causality of the identified relation between cannabis and gene expression was subsequently examined *in vitro* experiments. To provide insight in potential tissue types differences in the responses to cannabinoids both blood cells and neuronal cell lines were used.

2. Experimental procedures

2.1. Recruitment

The recruitment details are described previously (Schubart et al., 2011a, b). In short, participants were recruited using a website

survey (www.cannabisquest.nl) which launched in 2006 and targeted Dutch young adults and adolescents (18–25 years). The online assessment included several verification questions to protect against random answers, and only those whom completed the survey were eligible for subsequent recruitment. The online survey in the current study was only used to recruit participant. All participants were comprehensively assessed during a hospital visit. From a cohort of 1259 participants that visited the hospital as part of the larger study, 192 participants were selected for the current study. All participants gave written informed consent. This study was approved by Medical Ethical Committee Utrecht. Participants were selected based on absent or extreme cannabis exposure and high or low scores (top or bottom 20%) for psychotic experiences measured by the Community Assessment of Psychic Experiences (CAPE) (Konings et al., 2006). The grandparents of all individuals were all born in the Netherlands to limit genetic heterogeneity. Neuropsychological function (Intelligence Quotient, IQ) of the participants was measured with 4 subtests of the WAIS: symbol substitution, math, block design, and information processing (Hijman et al., 2003).

2.2. Cannabis exposure

In the Netherlands THC-concentration and market value of cannabis are highly correlated (Niesink and Rigter, 2009), therefore weekly amount of Euro's spent on cannabis was used to measure the exposure to THC. Participants that spend more than €10 per week were considered heavy cannabis users and lifetime cannabis naïve participants were considered non-users. Urine samples were obtained to verify their report on recent cannabis use. Alcohol and other drug use were assessed by self-report using the substance abuse module of the Composite International Diagnostic Interview (CIDI) (Robins et al., 1988) and verified by urine screening.

2.3. Whole blood gene expression analysis

Whole blood samples were obtained by venepuncture from all participants at the time of assessment. RNA was isolated and purified from whole blood using the PAXgene extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total mRNA was quantified using Quant-itTM Ribogreen assay (Invitrogen, California, USA). RNA samples were prepared with the Illumina TotalPrep kit (Invitrogen, California, USA) amplification and labelling protocol. The samples were randomly distributed over the different arrays to prevent batch effects. Genome-wide RNA expression profiling was obtained with a HumanHT-12 v3 Beadchip (Illumina, California, USA) that includes over 48,000 probes. Array hybridization was done using 750 ng of amplified and biotinylated labelled cRNA using Illumina's standard protocol at the UCLA Neuroscience Genomics Core facility.

2.4. Expression data preprocessing

GenomeStudio software version 3.2 was used to extract raw data and generate background-corrected gene expression data. Background correction was performed by subtracting the average value of negative control beads present on the array. Further preprocessing was done using the Lumi package in R. A variance stabilizing transformation (VST) and robust spline normalization (RSN) were applied to the data according to the Lumi procedure (Du et al., 2008). Genes were then filtered based on detection values. Expression probes had to reach the detection p-value threshold <0.01 in at least one sample. Array quality and outlier detection was performed by assessing quality statistics and plots before and after transformation and normalization.

2.5. Bead chip array expression data analysis

Expression values were taken as dependent variables and tested for association with cannabis status as the independent variable using the Limma software package (Smyth, 2005). Cigarette smoking (present or absent), the use of other drugs besides cannabis (present or absent), age, gender and psychotic experiences were taken along as covariates. Significance threshold was set at a False-Discovery-Rate (FDR) corrected $P < 0.05$.

2.6. Quantitative real time PCR data analysis

Expression levels of the genome wide significant transcripts was analysed by Quantitative Real Time PCR (qPCR) (Applied Biosystems, California, USA). Absolute quantities were obtained by running all samples in quadruplicates. First-strand cDNA was synthesized according to the manufacturer's instructions. Quantitative PCR was carried out using a TaqMan assay from Applied Biosystems. The following TaqMan gene expression assays were used: *PPFIA2* (Hs01548846), *PPFIA2* (Hs01548855), *PPFIA2* (Hs01548860). *GUSB* gene was selected as reference gene and Ct values were normalized against *GUSB* expression. Log-fold changes were calculated using a and delta-delta method and significance analysed using the non-parametric Mann-Whitney test.

2.7. cis expression quantitative trait loci (eQTL)

Whole-genome Single Nucleotide polymorphisms (SNP) data of all included subjects were available from a previous study. Expression quantitative trait loci (eQTL) analysis was performed to identify SNP's that regulate mRNA expression levels of the identified loci. Genetic association were calculated using PLINK (Purcell et al., 2007), with a linear regression analysis, using gene expression as a quantitative trait. The p-value significance threshold was set for a Bonferroni correction for the number of SNP's in *cis* (within 100 base pairs of the gene).

2.8. Regulation of PPFIA2 after in vitro cannabinoid exposure

2.8.1. Cannabis exposure in monocytes

Changes in *PPFIA2* expression in response to cannabis were investigated in blood cells. Baseline expression in monocytes ($N = 5$) relative to reference genes Ribosomal protein S28 (*RPS28*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was measured in monocytes. Based on a relative expression of 8.20 (arbitrary units (AU)) monocytes were identified as a suitable cell type. Therefore, monocytes from 12 donors were isolated with CD14 microbeads (MACS sorting) (Miltenyl Biotec, Bergisch Gladbach, Germany) from anonymous buffy coat blood donors. Cells were plated in 96 well plate with a density of 100,000 cells/ well in 100ul RPMI 1640 (Life Technology, CA, USA) medium with 10% fetal bovine serum (FBS) (Life Technology, CA, USA). After inspection of dose-response curves for 1, 10 and 100 uM of CBD and THC experiments were conducted using 10 uM based on the fact that no responses were obtained for 1 uM and at the dose of 100uM most of the cells died. Monocytes from 12 donors were treated with 10 uM of CBD (Sigma-Aldrich, Missouri, USA) and 10 uM THC (Farmalyse, Zaandam, NL). Since ethanol was used to dissolve both THC and CBD, it was used as

Table 1 Sample characteristics.

	Total group	Cannabis naïve	Heavy Cannabis users	Statistic, *p*-value
n	190	100	90	
Mean age (sd)	23.1 (2.0)	22.8 (1.8)	23.3 (2.2)	$B=0.533$, $p=0.07$
Gender (%male)	59.5	39.6	81.1	$B=0.411$, $p<0.001$
Other drugs	52 (27%)	0 (0%)	52 (58%)	$B=0.578$, $p<0.001$
Cigarette smoking	80 (42%)	8 (8%)	71 (79%)	$B=0.709$, $p<0.001$
Alcohol use lifetime	170 (89%)	90 (90%)	80 (89%)	$B=-0.011$, $p=0.805$
Medication use	46 (24%)	26 (26%)	20 (22%)	$B=-0.038$, $p=0.550$
Psychotic experiences	93 (49%)	50 (50%)	43 (48%)	$X=5.9$, $p=0.864$
IQ score (sd)	107.69 (13.99)	113.19 (13.31)	101.33 (11.98)	$B=-11.860$, $p<0.001$

Psychotic experiences measured by the Community Assessment of Psychic Experiences (CAPE). IQ score of the participants was calculated with measurement of four subtests of the WAIS: symbol substitution, math, block design, and information processing.

control condition. After 6 h, medium was removed and Phosphate-buffered saline (PBS) was used to wash the cells. After TRIzol (Invitrogen, California, USA) addition for RNA isolation the cells were stored at a temperature of −80 °C until cDNA synthesis.

2.8.2. Cannabis exposure in neuroblastoma cell lines

PPFIA2 is expressed in neurons and oligodendrocytes and plays an important function in neurons (Zhang et al., 2014). We therefore assessed whether the expression of *PPFIA2* in the neuroblastoma cell line SH-SY5Y was affected by exposure to cannabinoids despite the low baseline level of expression that we found in this cell line. A human neuroblastoma cell line, SH-SY5Y was used as an in-vitro model of neuronal response to cannabinoids even though the a priory suitability was not clear. We found that the baseline *PPFIA2* mRNA expression level in SH-SY5Y cell line (normalized to our reference genes) was low (0.51 AU). Nevertheless, cells were plated in 96-well plate with a density of 70,000 cells/well in DMEM high glucose medium (Life Technology, CA, USA) with 10% of FBS. Dose finding for THC and CBD stimulation of neuroblastoma cell line identified a similar optimal concentration of 10 uM as in the monocytes. Ethanol was used as control condition and the protocol was the same as the monocytes (6 h stimulation, PBS washout, TRIzol for RNA isolation, storage at −80 °C until cDNA synthesis).

2.8.3. RNA isolation and qPCR

RNA was isolated using the TRIzol method. Complementary DNA (cDNA) was synthesized by using the Reverse Transcription Kit (Qiagen, Hilden, Germany. Quantitative polymerase chain reaction (qPCR) was performed to quantify mRNA expression level as described before (Melief et al., 2016). Ribosomal protein S28 (*RPS28*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were selected as reference genes. The following primers were used: *PPFIA2* forward (5'- GGCCCAGTGTCCGGTTTTTC −3') and reverse (5'- GGTAACCCCAAGCTGGGAAG −3'), *RPS28* forward (5'- GACACGAGCCGATCCATCATC −3') and reverse (5'- TGACTCCAAAAGGGTGAGCAC −3'), and *GAPDH* forward (5'- TGCACCACCAACTGCTTAGC −3') and reverse (5'- GGCATGGACTGTGGTCATGA −3'). The response to different cannabinoids in gene expression was calculated as fold change relative to its ethanol exposed control.

2.9. Statistical analyses of qPCR PPFIA2 expression data and estimated IQ

Linear regression was used to analyse the association of *PPFIA2* expression as outcome with cannabis use as main indicator. Age, gender, smoking, other drug consumption (yes/no) and psychotic experiences were added as covariate. A separate linear regression model was used to analyse the relation of *PPFIA2* expression with an estimate of IQ, using IQ as outcome and cannabis use as well as *PPFIA2* expression as indicators and age, gender, smoking, other drug consumption and psychotic experiences were added as covariate. Multivariate Analysis Of Covariance (MANCOVA) was used to analyse the relation of *PPFIA2* expression with 4 subtests of the Wechsler Adult Intelligence Scale (WAIS) simultaneously (Hijman et al., 2003). Age, gender, smoking, drug consumption, psychotic experiences and cannabis use were added as covariates in the model. *PPFIA2* expression differences after cannabinoids stimulation in monocytes and neuroblastoma cells were analysed using the nonparametric Wilcoxon paired test. *P*-values smaller than 0.05 were considered to be statistically significant.

3. Results

3.1. Sample

Data on a total of 100 cannabis naïve subjects and 90 heavy cannabis users was analysed after exclusion of two participants with failed expression data due to technical outliers. No significant difference in psychotic experiences between cannabis naïve group and heavy cannabis group was present (due to the selective sampling). The IQ score in the heavy cannabis users was significantly lower than cannabis naïve participants. IQ heavy cannabis users: Mean=101.33, sd=11.98; IQ cannabis naïve participants: Mean=113.19, sd=13.31 ($B=-11.86$, $p < 0.001$). Also, overall performance on all four cognitive test was significantly different in the group of heavy cannabis users ($F(171,4)=3.9$, $p=0.004$). Posthoc analysis showed that the differences were pronounced for all subtests: symbol substitution ($F(174)=22.0$, $p<0.001$); block design ($F(174)=8.2$, $p<0.0035$); math ($F(174)=9.2$, $p<0.005$) and information processing ($F(174)=8.1$, $p<0.005$). Table 1 presents the sample characteristics.

3.2. Whole genome expression analysis

After filtering and quality control, 20,765 probes (42.5%) remained for further analysis. Linear regression analysis yielded 2131 probes with a nominal significance between users and non-users. Supplementary Table 1 gives the full results of the nominal significant probes. After FDR-correction at the 0.05 level, the expression of two

Table 2 Gene transcripts with genome wide association to cannabis and validation.

Gene	Transcript	Probe_ID	Array fold change	Array *p*-value	FDR *p*-value	qPCR fold change	qPCR *p*-value
PPFIA2	ILMN_7280	ILMN_1803318	3.2	3.27×10^{-08}	0.04	1.4	0.05
CX3CR1	ILMN_8593	ILMN_1745788	0.74	3.59×10^{-08}	0.04	1.0	0.50

transcripts, Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting Protein Alpha-2 (*PPFIA2*) (probe ID: ILMN_1,803,318) (LogFold Change 0.17, $p = 3.27 \times 10^{-08}$, FDR adjusted $p = 0.038$) and C-X3-C Motif Chemokine Receptor 1 (*CX3CR1*) (probe ID: ILMN_1,745,788) (LogFold Change −0.42, $p = 3.59 \times 10^{-08}$, FDR adjusted $p = 0.038$) were significantly associated with cannabis use, see Table 2.

3.3. qPCR validation

Due to depletion of RNA quantity, only 65 heavy users and 78 samples from cannabis naïve participants were available for qPCR validation. These participants did not differ from the original sample with respect to the distribution of covariates. We confirmed the upregulation of one of the *PPFIA2* transcript (log Fold Change 1.40, $p = 0.046$), the other *PPFIA2* transcripts were undetectable. The downregulation of *CX3CR1* could not be validated (Fold Change 1.04, $p = 0.516$), see Table 2.

3.4. cis expression quantitative trait loci (eQTL)

The cis eQTL analysis of 262 SNP's in the *PPFIA2* gene region from 190 participants shows no significant association with the expression of the *PPFIA2* gene at a Bonferroni multiple testing threshold of 1.9×10^{-04}. In the GTEx database also no significant eQTLs are reported in blood, but in the hippocampus an eQTL SNP is identified; rs35137985 (GRCh38, Chr12:80,919,319) that is located in an intron of the LIN7A gene that is associated with learning disabilities (Matsumoto et al., 2014).

3.5. PPFIA2 expression and estimated IQ

Neuropsychological function (IQ score) was estimated from four subtests of the WAIS: symbol substitution, math, block design, and information processing. A significant negative association between IQ estimate and *PPFIA2* expression was present in the entire group (unadjusted for cannabis use) ($B = -22.95$, $p = 0.002$), and in the non-cannabis using group ($N = 100$, $B = -28.5$, $p = 0.04$) but not in the entire group while adjusting for cannabis use (Fig. 1). The MANOVA overall test indicated that the scores on the four subtests were significantly related to *PPFIA2* expression ($F(171,4) = 3.9$, $p = 0.004$) over and above the role of cannabis use ($F(171,4) = 7.2$, $p < 0.001$)). Posthoc analysis showed that the differences are most pronounced for the subtests: symbol substitution ($F(174) = 9.5$, $p = 0.002$) and block design ($F(174) = 8.8$, $p = 0.003$) and to a lesser extent for math ($F(174) = 3.5$, $p = 0.064$) and information processing ($F(174) = 3.3$, $p = 0.072$). The results indicate that the relationship between *PPFIA2* expression and cognitive function is specifically strong for particular sub domains of cognitive function.

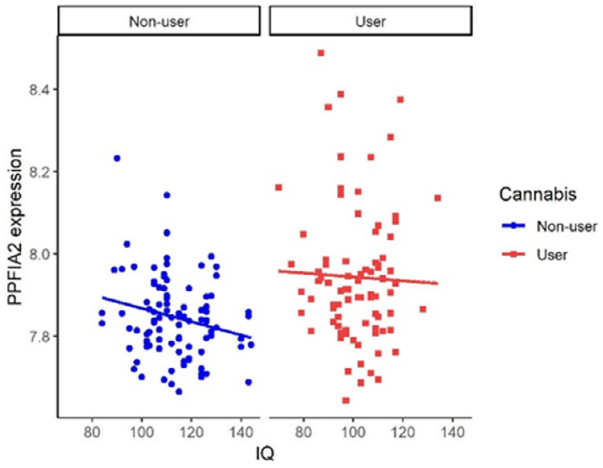


Fig. 1 The association between *PPFIA2* expression and IQ in cannabis non-users and heavy cannabis users.
The y-axis is the *PPFIA2* expression level after adjust age, gender, smoking, other drug consumption and psychotic experiences. The *x*-axis is the IQ score. Significant negative association between IQ estimate and *PPFIA2* expression was present in the non-cannabis using group ($N = 100$, $B = -28.5$, $p = 0.04$) but not in the heavy-cannabis using group.

3.6. Regulation of PPFIA2 gene expression in response to cannabinoids

The response of monocytes to cannabinoids showed a large variability in *PPFIA2* expression: Eight of 12 donors (66.7%) showed increase *PPFIA2* expression in response to CBD 10uM (median fold change = 2.92, Wilcoxon paired rank test, $p = 0.04$). One expression level of *PPFIA2* in CBD exposed monocytes was an outlier (3 standard deviations from the mean) and censoring this observation reduced the significance to ($p = 0.075$). Gene expression in monocytes after THC stimulation, showed an increase in 6 of 9 (66.7%), (median fold change = 1.6, Wilcoxon paired rank test, $p = 0.26$). See Fig. 2.

PPFIA2 gene expression in SH-SY5Y slightly increased after 6 h stimulation by CBD (mean fold change=1.56) and THC (mean fold change=1.37) but the differences did not reach statistical significance. See Fig. 3.

4. Discussion

Comparison of gene expression in whole blood of heavy cannabis users ($N = 90$) and cannabis naïve participants

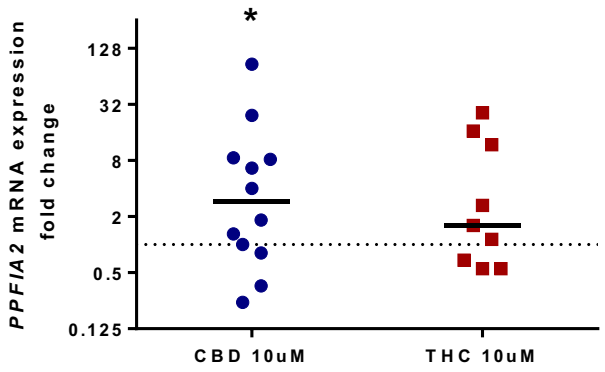


Fig. 2 *PPFIA2* expression fold change after cannabinoids stimulation in monocytes.
The horizontal bar represents the median fold change. Monocytes were treated with 10 uM of cannabidiol (CBD) ($N = 12$), or THC for 6 h. *PPFIA2* expression fold change was calculated by cannabinoid stimulation divided by their ethanol control. Three data points in the THC group that were undetectable were omitted. $^{*}p<0.05$.

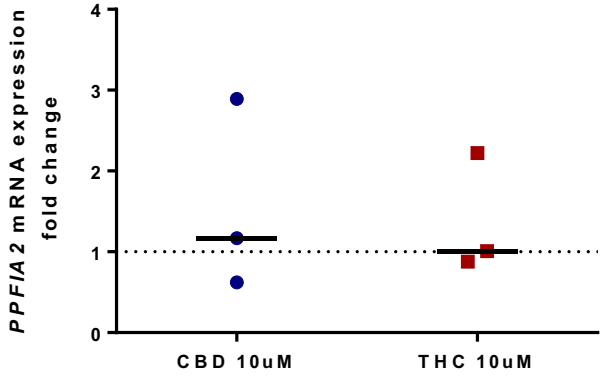


Fig. 3 *PPFIA2* expression fold change after cannabinoids stimulation in SH-SY5Y.
The horizontal bar represents the median fold change. SH-SY5Y cells were treated with 10 uM of CBD or THC for 6 h. *PPFIA2* expression fold change was calculated by cannabinoid stimulation divided by ethanol control. Plotted values are based on triplicates.

($N = 100$), showed that *PPFIA2* and *CX3CR1* expression were significantly higher in heavy cannabis users. Upregulated expression of *PPFIA2* in heavy cannabis users was confirmed with qPCR. *PPFIA2* expression was also significantly negatively related with estimated IQ in the cannabis abstinent group. Supportive evidence for a direct relation between cannabinoid and *PPFIA2* expression comes from data from monocytes that also show *PPFIA2* upregulation in response to cannabinoid stimulation, particularly cannabidiol (CBD).

Cannabis consumption is significantly positively associated with *PPFIA2* expression and negatively associated with IQ. Since *PPFIA2* expression is also significantly associated with IQ in the entire sample, the association between cannabis and *PPFIA2* expression is potentially driven by the different IQ level between the cannabis using and non-using groups instead of cannabis use itself. However, as the association between *PPFIA2* expression and IQ is strongest in the cannabis naïve participants, this demonstrates a relation of *PPFIA2* expression with IQ independent from cannabis use.

The identified gene: Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting Protein Alpha-2 (*PPFIA2*) encodes the liprin-$\alpha-2$ protein. It is highly expressed in neurons and oligodendrocytes (Zhang et al., 2014). Important functions of liprin-α proteins in neuronal cells are suggested by its localization in mossy fibre endings in the CA3 region of the hippocampus, in synapses, axons, dendrites and the cell body (Spangler et al., 2011). A role of liprin-$\alpha-2$ in axon guidance function was suggested by reports that liprin-$\alpha-2$ contributes to the normal density and growth of excitatory synapses and dendritic spines (Baran and Jin, 2002; Dunah et al., 2005; Pulido et al., 1995; Serra-Pagès et al., 1998) in hippocampal neurons. In the synapse active zone in hippocampal neurons Liprin-α proteins have a role in organizing pre and post synaptic vesicle preparation and neurotransmitter function (Ko et al., 2003; Olsen et al., 2005; Owald et al., 2012; Patel et al., 2006). Liprin-alpha-2 is one of the predominant Liprin isoforms in the hippocampus (Spangler et al., 2011; Zürner et al., 2011) where it is likely involved in dendrite development (Spangler and Hoogenraad, 2007) long term depression (LTD) (Dickinson et al., 2009) and neuropsychological function (Kolkman et al., 2004). By forming a liprin-α-GRIP-GluA2 complex, liprin- α interacts with muscarinic acetylcholine receptors (mAChR) and plays a role in mAChR dependent LTD (mAChR-LTD) in hippocampus (Dickinson et al., 2009). Interestingly, previous study discovered that activation of mAChR could enhance the release of endogenous cannabinoids in the hippocampus (Kim et al., 2002). Such increased endocannabinoids could trigger LTD and mediated the release of neurotransmitters in the hippocampal inhibitory synapses (Chevaleyre and Castillo, 2003; Kano et al., 2009). However, when the exogenous cannabinoids, such as THC for example, are administered they can block the endocannabinoid-mediated LTD in the hippocampus (Mato et al., 2004) and lead to disruption of GABA and glutamate neurotransmitter release (Bossong and Niesink, 2010). Therefore, heavy cannabis consumption could disrupt the balance of endocannabinoids system and might further influence the liprin- α level by involving mAChR-LTD. The result from the current study did not directly support a prevailing hypothesis on the role of the dopamine system (Bloomfield et al., 2016; Freund et al., 2003) and white matter changes (Jakabek et al., 2016; van de Giessen et al., 2016) in the relation between cannabis and neuropsychological function. Nevertheless, this shows the advantage of a genome-wide approach to discover new potential pathways.

To overcome the limitation of our cross-sectional discovery in blood RNA, in vitro experimental studies in monocytes and a neuroblastoma cell line were conducted to support a causal relationship between cannabis and *PPFIA2* expression. In monocytes, *PPFIA2* expression increased after exposure to cannabinoids, particularly in response to CBD. Such a relation in an experiment whereby the unexposed cells from the same donor are used as reference gives strong support for longitudinal changes in response to these cannabinoids. We do not know how other blood cell types respond to cannabinoids. However, the results from exposing the

neuroblastoma cell line to cannabinoids did not show a robust increase in *PPFIA2* expression and this demonstrates that large differences between tissue types exist. Several explanations may account for the differences that were observed between monocytes and the neuroblastoma cell line. One reason may be the differences in baseline expression. The expression of *PPFIA2* is quite low in the SH-SY5Y cell line. *PPFIA2* expression in SH-SY5Y was less than 10% compared to monocytes (0.50 versus 8.20 AU respectively). Such a low expression in SH-SY5Y may lead to a relatively low sensitivity to cannabinoids. Further research is required to investigate whether other cells are also involved in the response to cannabinoids, particularly hippocampal cell lines.

The finding that particularly CBD lead to increased *PPFIA2* expression is noteworthy, since CBD is generally viewed as the less toxic of the cannabinoids overall human studies suggest that CBD could counteract adverse effects from THC consumption when administrate with THC simultaneously (Iseger and Bossong, 2015). CBD is also researched for efficacy in treatment of anxiety and potentially psychosis (Mcguire et al., 2018; Schubart et al., 2011a, b; Schubart et al., 2014). However the effects of CBD on psychosis treatment is not always consistent (Boggs et al., 2018). Our finding hint that particularly CBD may play a role in the adverse cognitive effects of cannabis, but since the observations are based on work in monocytes only, further studies are required.

A finding that was not further investigated in this study is the result of the genome wide gene expression profiling that suggested that cannabis use leads to downregulation of *CX3CR1* in whole blood. *CX3CR1* is the receptor of neuron-produced Fractalkine (*CX3CL1*), exclusively expressed by microglia in the central nerve system. The interaction between *CX3CL1* and *CX3CR1* constitutes the neuron-microglial signalling system (Ransohoff and Khoury, 2016) and deficiency either of them could increase the production of pro-inflammatory molecules (Sheridan and Murphy, 2013). Though the role of *CX3CR1* was not validated by qPCR validation in our study, considering the limited power in our study and its role in the central nerve systems, *CX3CR1* remains a potential candidate for further study.

This is the first human genome wide gene expression study to identify genes that may be involved in a cognitive effect of cannabis smoking. Strongpoints are the exclusive inclusion of heavy cannabis users or cannabis naïve individuals to maximize the potential contrast in gene expression changes (Boks et al., 2007) and matching for psychotic like experiences to remove the influence of psychotic experiences from the study. Previous studies on cannabis induced gene-expression investigated preselected genes of interest, thus limiting the ability to discover new genes that are involved in the biological pathway of the cannabis influence. A limitation of this study is the self-report of cannabis use. Though we applied a urine screen to verify self-report abstinence on the day of blood draw, the screen cannot effectively distinguish cannabis use anywhere in the past few days and the same day. Therefore, there was no way to effectively validate abstinence on the day of testing in the heavy cannabis users and although there is no reason to assume effects of acute cannabis use, it is also not possible to rule these out. Another limitation of this study is the crude measure of neuropsychological function. These four subtests of the WAIS can reliably estimate IQ but do not provide a comprehensive overview of cognitive function. Another limitation of this study is the potential presence of residual confounding including smoking and social economic status. This limitation is inherent to the cross-sectional discovery. Furthermore, selective sampling of non-, or heavy cannabis users and those with low or high psychotic symptoms, limits the generalizability. The current study does not aim to resolve the debate on the relation between cannabis use and neuropsychological functions. Analysis of estimated IQ is merely used to identify a putative role of the identified cannabis associate gene transcript. The data presented from the follow up in vitro studies that show expression changes in response to cannabinoids are an essential step to tie CBD exposure to the *PPFIA2* changes and increases the level of evidence. Questions that remain are how neurons (and particularly hippocampal neurons) respond to cannabinoids and how neuropsychological function is altered in human experimental studies of cannabidiol.

Overall the upregulation of *PPFIA2* in response to cannabidiol and the correlation with estimated IQ suggest a role of this gene in the pathways underlying the adverse effects of cannabis on neuropsychological function.

Contributions

Marco Boks and Lot de Witte designed the study, oversaw the writing and the analysis. Yujie He contribute to the *in vitro* study and wrote the manuscript. Chris Schubart lead the assessment of participants, the blood samples and the expression analysis. Willemijn van Gastel collected the intelligence measures. Bobby Koeleman, Simone de Jong and Roel Ophoff contributed to the acquisition whole blood gene expression analysis. Elly Hol was oversaw the in vitro study. All authors contributed to the writing and have approved the final manuscript.

Conflict of interest

The authors have no potential conflict of interest to report.

Funding source

Funding for this study was provided by NWO, the Dutch council for scientific research (ZonMW TOP grant no. 91207039) and the China Scholarship Council (CSC 201408230054); the NOW and CSC had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Acknowledgements

We are grateful to the participants in our study. We thank Nina Notman for her support with data analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.03.004.

References

Baran, R., Jin, Y., 2002. Getting a GRIP on liprins. Neuron. doi:10.1016/S0896-6273(02)00649-9.

Bloomfield, M.A.P., Ashok, A.H., Volkow, N.D., Howes, O.D., 2016. The effects of Δ9-tetrahydrocannabinol on the dopamine system. Nature doi:10.1038/nature20153.

Boggs, D.L., Surti, T., Gupta, A., Gupta, S., Niciu, M., Pittman, B., 2018. The effects of cannabidiol (CBD) on cognition and symptoms in outpatients with chronic schizophrenia a randomized placebo controlled trial 1923–1932.

Boks, M.P., 2012. Progress in gene environment studies. Biol. Psychiatry doi:10.1016/j.biopsych.2012.08.013.

Boks, M.P.M., Schipper, M., Schubart, C.D., Sommer, I.E., Kahn, R.S., Ophoff, R.A., 2007. Investigating gene environment interaction in complex diseases: increasing power by selective sampling for environmental exposure. Int. J. Epidemiol. doi:10.1093/ije/dym215.

Bossong, M.G., Mehta, M.A., van Berckel, B.N.M., Howes, O.D., Kahn, R.S., Stokes, P.R.A., 2015. Further human evidence for striatal dopamine release induced by administration of 9-tetrahydrocannabinol (THC): selectivity to limbic striatum. Psychopharmacology (Berl) 232, 2723–2729. doi:10.1007/s00213-015-3915-0.

Bossong, M.G., Niesink, R.J.M., 2010. Progress in neurobiology adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia 92, 370–385. doi:10.1016/j.pneurobio.2010.06.010.

Bossong, M.G., van Berckel, B.N.M., Boellaard, R., Zuurman, L., Schuit, R.C., Windhorst, A.D., van Gerven, J.M.A., Ramsey, N.F., Lammertsma, A.A., Kahn, R.S., 2009. Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. Neuropsychopharmacology 34, 759–766. doi:10.1038/npp.2008.138.

Broyd, S.J., van Hell, H.H., Beale, C., Yücel, M., Solowij, N., 2016. Acute and chronic effects of cannabinoids on human cognition – a systematic review. Biol. Psychiatry 79, 557–567. doi:10.1016/j.biopsych.2015.12.002.

Bussink, C., Carpentier, C., Dávalos, L.M., Davis, P., Durán-Martínez, A., Eichinger, N., 2016. World Drug Report 2016. United Nations publication.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H.L., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., Craig, I.W., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol. Psychiatry 57, 1117–1127. doi:10.1016/j.biopsych.2005.01.026.

Castellanos-Ryan, N., Pingault, J.-B., Parent, S., Vitaro, F., Tremblay, R.E., Séguin, J.R., 2016. Adolescent cannabis use, change in neurocognitive function, and high-school graduation: a longitudinal study from early adolescence to young adulthood. Dev. Psychopathol. 1–14. doi:10.1017/S0954579416001280.

Chevaleyre, V., Castillo, P.E., 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38, 461–472.

D'Addario, C., Micale, V., Di Bartolomeo, M., Stark, T., Pucci, M., Sulcova, A., Palazzo, M., Babinska, Z., Cremaschi, L., Drago, F., Carlo Altamura, A., Maccarrone, M., Dell'Osso, B., 2017. A preliminary study of endocannabinoid system regulation in psychosis: distinct alterations of CNR1 promoter DNA methylation in patients with schizophrenia. Schizophr. Res. doi:10.1016/j.schres.2017.01.022.

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. Psychiatry 72, 811–816. doi:10.1016/j.biopsych.2012.06.020.

Dickinson, B.A., Jo, J., Seok, H., Son, G.H., Whitcomb, D.J., Davies, C.H., Sheng, M., Collingridge, G.L., Cho, K., 2009. A novel mechanism of hippocampal LTD involving muscarinic receptor-triggered interactions between AMPARs, GRIP and liprin-alpha. Mol. Brain 2, 18. doi:10.1186/1756-6606-2-18.

Dinis-Oliveira, R.J., 2016. Metabolomics of Δ(9)-tetrahydrocannabinol: implications in toxicity. Drug Metab. Rev. 48, 80–87. doi:10.3109/03602532.2015.1137307.

Du, P., Kibbe, W.A., Lin, S.M., 2008. Lumi: A pipeline for processing Illumina microarray. Bioinf. 24, 1547–1548.

Dunah, A.W., Hueske, E., Wyszynski, M., Hoogenraad, C.C., Jaworski, J., Pak, D.T., Simonetta, A., Liu, G., Sheng, M., 2005. LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses. Nat. Neurosci. 8, 458–467. doi:10.1038/nn1416.

Filley, C.M., Fields, R.D., 2016. White matter and cognition: making the connection. J. Neurophysiol. doi:10.1152/jn.00221.2016, jn.00221.2016.

Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066. doi:10.1152/physrev.00004.2003.

Grant, I., Gonzalez, R., Carey, C.L., Natarajan, L., Wolfson, T., 2003. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. J. Int. Neuropsychol. Soc. 9, 679–689. doi:10.1017/S1355617703950016, [doi]\rS1355617703950016 [pii].

Hélie, S., Paul, E.J., Ashby, F.G., 2012. Simulating the effects of dopamine imbalance on cognition: from positive affect to Parkinson's disease. Neural Netw. 32, 74–85. doi:10.1016/j.neunet.2012.02.033.

Henquet, C., Rosa, A., Delespaul, P., Papiol, S., Fananas, L., van Os, J., Myin-Germeys, I., 2009. COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of "switching on" hallucinations in the flow of daily life. Acta Psychiatr. Scand. 119, 156–160. doi:10.1111/j.1600-0447.2008.01265.x.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., Ramaekers, J.G., van Os, J., 2006. An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. Neuropsychopharmacology 31, 2748–2757. doi:10.1038/sj.npp.1301197.

Hijman, R., Hulshoff Pol, H.E., Sitskoorn, M.M., Kahn, R.S., 2003. Global intellectual impairment does not accelerate with age in patients with schizophrenia: a cross-sectional analysis. Schizophr. Bull. 29, 509–517.

Ibarra-Lecue, I., Mollinedo-Gajate, I., Meana, J.J., Callado, L.F., Diez-Alarcia, R., Uriguen, L., 2018. Chronic cannabis promotes pro-hallucinogenic signaling of 5-HT2A receptors through Akt/mTOR pathway. Neuropsychopharmacology 43, 2028–2035. doi:10.1038/s41386-018-0076-y.

Iseger, T.A., Bossong, M.G., 2015. A systematic review of the antipsychotic properties of cannabidiol in humans. Schizophr. Res. 162, 153–161. doi:10.1016/j.schres.2015.01.033.

Jakabek, D., Yucel, M., Lorenzetti, V., Solowij, N., 2016. An MRI study of white matter tract integrity in regular cannabis users: effects of cannabis use and age. Psychopharmacology (Berl) 233, 3627–3637. doi:10.1007/s00213-016-4398-3.

Kano, M., Ohno-shosaku, T., Hashimotodani, Y., Uchigashima, M., 2009. Endocannabinoid-mediated control of synaptic transmission 309–380. doi:10.1152/physrev.00019.2008.

Kim, J., Isokawa, M., Ledent, C., Alger, B.E., 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J. Neurosci. 22, 10182–10191.

Ko, J., Kim, S., Valtschanoff, J.G., Shin, H., Lee, J.-R., Sheng, M., Premont, R.T., Weinberg, R.J., Kim, E., 2003. Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting. J. Neurosci. 23, 1667–1677 doi:23/5/1667 [pii].

Kolkman, M.J.M., Streijger, F., Linkels, M., Bloemen, M., Heeren, D.J., Hendriks, W.J.A.J., Van Der Zee, C.E.E.M., 2004. Mice lacking leukocyte common antigen-related (LAR) protein tyrosine phosphatase domains demonstrate spatial learning impairment in the two-trial water maze and hyperactivity in multiple behavioural tests. Behav. Brain Res. 154, 171–182. doi:10.1016/j.bbr.2004.02.006.

Konings, M., Bak, M., Hanssen, M., van Os, J., Krabbendam, L., 2006. Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. Acta Psychiatr. Scand. 114, 55–61. doi:10.1111/j.1600-0447.2005.00741.x.

Mato, S., Chevaleyre, V., Robbe, D., Pazos, A., Castillo, P.E., Manzoni, O.J., 2004. A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat. Neurosci. 7, 585–586. doi:10.1038/nn1251.

Matsumoto, A., Mizuno, M., Hamada, N., Nozaki, Y., Jimbo, E.F., Momoi, M.Y., Nagata, K., Yamagata, T., 2014. LIN7A depletion disrupts cerebral cortex development, contributing to intellectual disability in 12q21-deletion syndrome. PLoS One 9, e92695. doi:10.1371/journal.pone.0092695.

Mcguire, P., Psych, F.R.C., Sci, F.M., Robson, P., Psych, F.R.C., Cubala, W.J., Ph, D., 2018. Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: a multicenter randomized controlled trial 225–231. doi:10.1176/appi.ajp.2017.17030325.

Meier, M.H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R.S.E., McDonald, K., Ward, A., Poulton, R., Moffitt, T.E., 2012. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc. Natl. Acad. Sci. 109, E2657–E2664. doi:10.1073/pnas.1206820109.

Meier, M.H., Caspi, A., Danese, A., Fisher, H.L., Houts, R., Arseneault, L., Moffitt, T.E., 2017. Associations between adolescent cannabis use and neuropsychological decline: a longitudinal co-twin control study. Addiction. doi:10.1111/add.13946.

Melief, J., Sneeboer, M.A.M., Litjens, M., Ormel, P.R., Palmen, S.J.M.C., Huitinga, I., Kahn, R.S., Hol, E.M., de Witte, L.D., 2016. Characterizing primary human microglia: a comparative study with myeloid subsets and culture models. Glia 64, 1857–1868. doi:10.1002/glia.23023.

Moffitt, T.E., Meier, M.H., Caspi, A., Poulton, R., 2013. Reply to rogeberg and daly: no evidence that socioeconomic status or personality differences confound the association between cannabis use and IQ decline. Proc. Natl. Acad. Sci. U. S. A. 110, E980–E982. doi:10.1073/PNAS.1300618110.

Morrison, P.D., Zois, V., McKeown, D.A., Lee, T.D., Holt, D.W., Powell, J.F., Kapur, S., Murray, R.M., 2009. The acute effects of synthetic intravenous Δ9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol. Med. 39, 1607–1616. doi:10.1017/S0033291709005522.

Nakajima, S., Gerretsen, P., Takeuchi, H., Caravaggio, F., Chow, T., Le Foll, B., Mulsant, B., Pollock, B., Graff-Guerrero, A., 2013. The potential role of dopamine D3 receptor neurotransmission in cognition. Eur. Neuropsychopharmacol. 23, 799–813. doi:10.1016/j.euroneuro.2013.05.006.

Nieman, D.H., Dragt, S., van Duin, E.D.A., Denneman, N., Overbeek, J.M., de Haan, L., Rietdijk, J., Ising, H.K., Klaassen, R.M.C., van Amelsvoort, T., Wunderink, L., van der Gaag, M., Linszen, D.H., 2016. COMT Val(158)Met genotype and cannabis use in people with an at risk mental state for psychosis: exploring gene x environment interactions. Schizophr. Res. 174, 24–28. doi:10.1016/j.schres.2016.03.015.

Niesink, R., Rigter, S., 2009. THC-concentraties in wiet, nederwiet en hasj in Nederlandse coffeeshops (2008–2009).

O'Muircheartaigh, J., Dean 3rd, D.C., Ginestet, C.E., Walker, L., Waskiewicz, N., Lehman, K., Dirks, H., Piryatinsky, I., Deoni, S.C.L., 2014. White matter development and early cognition in babies and toddlers. Hum. Brain Mapp. 35, 4475–4487. doi:10.1002/hbm.22488.

Ohtani, T., Nestor, P.G., Bouix, S., Newell, D., Melonakos, E.D., McCarley, R.W., Shenton, M.E., Kubicki, M., 2017. Exploring the neural substrates of attentional control and human intelligence: diffusion tensor imaging of prefrontal white matter tractography in healthy cognition. Neuroscience 341, 52–60. doi:10.1016/j.neuroscience.2016.11.002.

Olsen, O., Moore, K.A., Fukata, M., Kazuta, T., Trinidad, J.C., Kauer, F.W., Streuli, M., Misawa, H., Burlingame, A.L., Nicoll, R.A., Bredt, D.S., 2005. Neurotransmitter release regulated by a MALS-liprin-alpha presynaptic complex. J. Cell Biol. 170, 1127–1134. doi:10.1083/jcb.200503011.

Owald, D., Khorramshahi, O., Gupta, V.K., Banovic, D., Depner, H., Fouquet, W., Wichmann, C., Mertel, S., Eimer, S., Reynolds, E., Holt, M., Aberle, H., Sigrist, S.J., 2012. Cooperation of Syd-1 with Neurexin synchronizes pre- with postsynaptic assembly. Nat. Neurosci. 15, 1219–1226. doi:10.1038/nn.3183.

Patel, M.R., Lehrman, E.K., Poon, V.Y., Crump, J.G., Zhen, M., Bargmann, C.I., Shen, K., 2006. Hierarchical assembly of presynaptic components in defined C. elegans synapses. Nat. Neurosci. 9, 1488–1498. doi:10.1038/nn1806.

Pulido, R., Serra-Pages, C., Tang, M., Streuli, M., 1995. The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. Proc. Natl. Acad. Sci. U. S. A 92, 11686–11690.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. doi:10.1086/519795.

Ranganathan, M., D'Souza, D.C., 2006. The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl) 188, 425–444. doi:10.1007/s00213-006-0508-y.

Ransohoff, R.Mh., Khoury, J. El, 2016. Microglia in and disease 1–15.

Robins, L.N., Wing, J., Wittchen, H.U., Helzer, J.E., Babor, T.F., Burke, J., et al., 1988. The composite international diagnostic interview. An epidemiologic instrument suitable for use in conjunction with different diagnostic systems and in different cultures. Arch. Gen. Psychiatry 1069–1077.

Schnakenberg Martin, A.M., Bonfils, K.A., Davis, B.J., Smith, E.A., Schuder, K., Lysaker, P.H., 2016. Compared to high and low cannabis use, moderate use is associated with fewer cognitive deficits in psychosis. Schizophr. Res. Cogn. 6, 15–21. doi:10.1016/j.scog.2016.09.001.

Schubart, C.D., Sommer, I.E.C., Fusar-Poli, P., De Witte, L., Kahn, R.S., Boks, M.P.M., 2014. Cannabidiol as a potential treatment for psychosis. Eur. Neuropsychopharmacol. 24, 51–64. doi:10.1016/j.euroneuro.2013.11.002.

Schubart, C.D., Van Gastel, W.a., Breetvelt, E.J., Beetz, S.L., Ophoff, R.A., Sommer, I.E.C., Kahn, R.S., Boks, M.P.M., 2011a. Cannabis use at a young age is associated with psychotic experiences. Psychol. Med. 41, 1301–1310. doi:10.1017/S003329171000187X.

Schubart, C.D., Sommer, I.E.C., van Gastel, W.A., Goetgebuer, R.L., Kahn, R.S., Boks, M.P.M., 2011b. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophr. Res. 130, 216–221. doi:10.1016/j.schres.2011.04.017.

Serra-Pagès, C., Medley, Q.G., Tang, M., Hart, A., Streuli, M., 1998. Liptins, a family of LAR transmembrane protein-tyrosine phosphatase- interacting proteins. J. Biol. Chem. 273, 15611–15620. doi:10.1074/jbc.273.25.15611.

Sheridan, G.K., Murphy, K.J., 2013. Neuron-glia crosstalk in health and disease: fractalkine and CX3CR1 take centre stage. Open Biol. 3, 130181. doi:10.1098/rsob.130181.

Smyth, G.K., 2005. Limma: linear models for microarray data. In: Gentleman, R., Carey, V., Dudoit, S., Irizarry, R., Huber, W. (Eds.), Bioinformatics and Computational Biology Solutions using R and Bioconductor. Springer, New York, pp. 397–420.

Spangler, S.A., Hoogenraad, C.C., 2007. Liprin-alpha proteins: scaffold molecules for synapse maturation. Biochem. Soc. Trans. 35, 1278–1282. doi:10.1042/BST0351278.

Spangler, S.A., Jaarsma, D., De Graaff, E., Wulf, P.S., Akhmanova, A., Hoogenraad, C.C., 2011. Differential expression of liprin-α family proteins in the brain suggests functional diversification. J. Comp. Neurol. 519, 3040–3060. doi:10.1002/cne.22665.

Stokes, P.R.A., Egerton, A., Watson, B., Reid, A., Breen, G., Lingford-Hughes, A., Nutt, D.J., Mehta, M.A., 2010. Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. Neuroimage 52, 1521–1527. doi:10.1016/j.neuroimage.2010.04.274.

Suarez-Pinilla, P., Roiz-Santianez, R., Ortiz-Garcia de la Foz, V., Guest, P.C., Ayesa-Arriola, R., Cordova-Palomera, A., Tordesillas-Gutierrez, D., Crespo-Facorro, B., 2015. Brain structural and clinical changes after first episode psychosis: focus on cannabinoid receptor 1 polymorphisms. Psychiatry Res. 233, 112–119. doi:10.1016/j.pscychresns.2015.05.005.

van de Giessen, E., Weinstein, J.J., Cassidy, C.M., Haney, M., Dong, Z., Ghazzaoui, R., Ojeil, N., Kegeles, L.S., Xu, X., Vadhan, N.P., Volkow, N.D., Slifstein, M., Abi-Dargham, A., 2016. Deficits in striatal dopamine release in cannabis dependence. Mol. Psychiatry 1–8. doi:10.1038/mp.2016.21.

Van Gastel, W.A., MacCabe, J.H., Schubart, C.D., Vreeker, A., Tempelaar, W., Kahn, R.S., Boks, M.P.M., 2013. Cigarette smoking and cannabis use are equally strongly associated with psychotic-like experiences: a cross-sectional study in 1929 young adults. Psychol. Med. 43, 2393–2401. doi:10.1017/S0033291713000202.

Vaucher, J., Keating, B.J., Lasserre, A.M., Gan, W., Lyall, D.M., Ward, J., Smith, D.J., Pell, J.P., Sattar, N., Pare, G., Holmes, M.V, 2017. Cannabis use and risk of schizophrenia: a Mendelian randomization study. Mol. Psychiatry doi:10.1038/mp.2016.252.

Wachtel, S.R., ElSohly, M.A., Ross, S.A., Ambre, J., de Wit, H., 2002. Comparison of the subjective effects of delta(9)-tetrahydrocannabinol and marijuana in humans. Psychopharmacology (Berl) 161, 331–339. doi:10.1007/s00213-002-1033-2.

Weinstein, M., Ben-Sira, L., Levy, Y., Zachor, D.A., Itzhak, E.Ben, Artzi, M., Tarrasch, R., Eksteine, P.M., Hendler, T., Bashat, D.Ben, 2011. Abnormal white matter integrity in young children with autism. Hum. Brain Mapp. 32, 534–543. doi:10.1002/hbm.21042.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S.A., Zhang, C., Daneman, R., Maniatis, T., Barres, B.A., Wu, J.Q., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947. doi:10.1523/JNEUROSCI.1860-14.2014.

Zürner, M., Mittelstaedt, T., tom Dieck, S., Becker, A., Schoch, S., 2011. Analyses of the spatiotemporal expression and subcellular localization of liprin-α proteins. J. Comp. Neurol. 519, 3019–3039. doi:10.1002/cne.22664.