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Cannabinoids and psychotic symptoms: A potential role for a genetic variant in the P2X purinoceptor 7 (*P2RX7*) gene

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ABSTRACT

To investigate the biological mechanisms underlying the higher risk for psychosis in those that use cannabis, we conducted a genome-wide environment-interaction study (GWEIS). In a sample of individuals without a psychiatric disorder ($N = 1262$), we analyzed the interactions between regular cannabis use and genotype with psychotic-like experiences (PLE) as outcome. PLE were measured using the Community Assessment of Psychic Experiences (CAPE). The sample was enriched for those at the extremes of both cannabis use and PLE to increase power. A single nucleotide polymorphism in the *P2RX7* gene (rs7958311) was associated with risk for a high level of psychotic experiences in regular cannabis users ($p = 1.10 \times 10^{-7}$) and in those with high levels of lifetime cannabis use ($p = 4.5 \times 10^{-6}$). This interaction was replicated in individuals with high levels of lifetime cannabis use in the IMAGEN cohort ($N = 1217$, $p = 0.020$). Functional relevance of *P2RX7* in cannabis users was suggested by *in vitro* experiments on activated monocytes. Exposure of these cells to tetrahydrocannabinol (THC) or cannabidiol (CBD) reduced the immunological response of the P2X7 receptor, which was dependent on the identified genetic variant. *P2RX7* variants have been implicated in psychiatric disorders before and the P2X7 receptor is involved in pathways relevant to psychosis, such as neurotransmission, synaptic plasticity and immune regulation. We conclude that *P2RX7* plays a role in vulnerability to develop psychotic symptoms when using cannabis and point to a new pathway that can potentially be targeted by newly developed P2X7 antagonists.

1. Introduction

Cannabis use has been consistently associated with psychotic experiences (Van Gastel, 2013) and psychotic disorders such as schizophrenia (Di Forti, 2019); (Large et al., 2011). The nature of this relationship is subject to continuous debate. Several studies suggest that genetic predisposition to schizophrenia is associated with higher levels of cannabis use (Verweij, 2017); (Power, 2014), but there is also

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compelling evidence that cannabis is causally associated with psychosis (Di Forti, 2019); (Murray, 2017; Marconi et al., 2016), such as from studies using Mendelian randomization (Gage, 2017); (Vaucher, 2017). Irrespective of the nature of the relation between cannabis use and psychosis, the majority of cannabis users do not develop a psychotic disorder (Van Gastel, 2013). It is therefore plausible that the association between cannabis use and psychotic disorders depends on individual biological vulnerability. A genetic component of cannabis vulnerability is supported by several family studies showing that the mental-health impact of cannabis exposure is higher in first grade relatives of schizophrenia patients (McGuire et al., 1995) and is related to polygenic risk scores of schizophrenia (Guloksuz, 2019). Identification of genes that modify the risk between cannabis use and psychosis is important for understanding the underlying biology of psychosis, modes of action of cannabis and has the potential to inform new targets for treatment and prevention.

The aim of the present study was therefore to discover genetic variants associated with the higher risk of psychotic symptoms when using cannabis. To focus on vulnerability to cannabis in the general population and discovery beyond genes for schizophreni, we investigate the relation in the general population using Psychotic Like Experiences (PLE) as an outcome. PLE are not the full-blown psychotic symptoms that lead to a diagnosis of schizophrenia, but a proxy for psychosis vulnerability in the general population (Sullivan, 2020). We conducted the first genome wide interaction study (GWEIS) of this interaction. Our previously described extreme sampling method was used to increase power (Boks, 2007) and replication was sought in the IMAGEN cohort (Schumann, 2010). To further explore the functional relevance of the genetic findings we subsequently conducted *in vitro* cannabis exposure experiments.

2. Methods

2.1. Utrecht cannabis cohort (CannabisQuest)

Participants were recruited using a project website launched in 2006 targeted at Dutch young adults from 18 to 25 years (Schubart, 2011). Strategies to generate traffic on the project website included collaboration with over a hundred colleges, universities, and youth centres, as well as the use of online commercial advertisement products (i.e. banners and text links) (Schubart, 2011). The chance to win an Apple iPod™ or a Nintendo Wii™ was used as an incentive. Double entries were prevented by exclusion of individuals with an identical e-mail address, surname and date of birth. Anonymous submission of data was not possible. The online assessment included verification questions to protect against random answers. Participants failing to complete the verification questions correctly were excluded. From the online data (N = 17,698), 1259 participants were included for subsequent genetic assessment at our hospital in two waves. Weekly amount of euros spent on cannabis was used as a proxy measure of exposure to THC, as THC-concentration and market value of cannabis are highly correlated in the Netherlands (Schubart, 2011). We gathered this information by online questionnaires to assess the amount in euros (€) that individuals spent on cannabis per week. THC exposure was categorized into five classes: cannabis naïve, cannabis use equivalent of less than €3 per week, €3 to €10 per week, €10 to €25 per week and more than €25 per week. We also assessed the relationship with lifetime cannabis exposure, categorized as never, 1, 2, 5–9 and > 10 times. Urine drugs screens were performed to confirm recent cannabis use or abstinence.

In order to increase power for gene × environment interactions we used our previous reported extreme sampling method (Boks, 2007). We combined an unselected sample of 719 individuals from the general population with a sample of 540 participants selected from the top or bottom quintile of total scores of PLE as measured by the Community Assessment of Psychic Experiences (CAPE) score who were either non-users (defined by a lifetime cannabis exposure frequency < 2 times) or heavy cannabis users (i.e. current expenditure for personal cannabis use exceeded €10 weekly).

To reduce genetic heterogeneity and the change for population stratification, we confined inclusion to individuals with four grandparents born in The Netherlands. In individuals with a high levels of PLE and high cannabis use, mental health was assessed with the validated Dutch version of either the Structured Clinical Interview (SCID) (Spitzer et al., 1992) or the MINI International Neuropsychiatric Interview (Sheehan, 1998). Participants with a history of any psychotic disorder (Bipolar disorder and schizophrenia spectrum) were excluded, other disorders were not assessed. . The possible concomitant use of recreational drugs was assessed with the substance-abuse module of the Composite International Diagnostic Interview (CIDI) (Robins, 1988). The study was approved by the Ethical Review Board of the University Medical Center Utrecht and all participants gave written informed consent.

2.2. Genotyping

DNA was extracted from whole blood of two 10 ml EDTA tubes obtained using venipuncture. For logistic reasons, genotype data for individuals of Dutch ancestry were generated on two different array platforms; 576 individuals on Illumina® HumanOmniExpress (733,202 SNPs) and 768 individuals on the Illumina® Human610-Quad Beadchip (620,901 SNPs). For validation of the genetic findings, we genotyped 1211 samples of which DNA was not exhausted from the original discovery cohort using Taqman probes for *P2RX7* rs7958311 (GGTTCCA TCACTGCC[A/G]TCCCAAATACAGTT).

2.3. Pre-processing genotype data, quality control and imputation

For both SNP platforms, initial quality-control procedures were performed using PLINK (Purcell, 2007). Participants were excluded based on errors in sex and on > 5% missing genotypes. We used linkage disequilibrium (LD) based SNP pruning to select the most informative SNPs ($R^2 < 0.2$), only for the subsequent quality control step. Datasets were merged with Hapmap Phase 3 to check ethnicity. Ethnic outliers were detected by visual inspection. After these QC procedures, we performed quality control at a SNP level. All SNPs were filtered on missingness (> 2%), Minor Allele Frequency (MAF) > 5%, and Hardy Weinberg ($p > 1e^{-6}$) before merging the two datasets. We imputed the merged dataset with Hapmap3 release 24 using Beagle (Browning and Browning, 2009). SNPs with an imputation score > 0.8 and SNPs that were present originally in both datasets were extracted.

2.4. P2RX7 genotyping in monocytes

To determine *P2RX7* genotype of the monocyte donors we extracted genomic DNA using a commercial kit (QIAamp DNA Mini Kit, Qiagen, Germany). A PCR was performed to amplify the target DNA fragment containing SNP rs7958311 using the following primers: Forward CTT CAGGGCGGAATAATGGG; Reverse TTGGAGTTACCTGAAGTTGTAG. Sanger sequencing was subsequently used to determine *P2RX7* genotype by sequencing the PCR products using the BigDye sequencing kit (BigDye Teminator version 3.1, Applied Biosystems, USA), a 3730xl Capillary sequencer (Applied Biosystems, USA) and analysis with ChromasLite 2.1.1 software.

2.5. IMAGEN cohort

The IMAGEN cohort comprises of 2087 adolescents recruited from the general population. Personality measures and cognitive/neuropsychological tests were collected through an online platform ("Psytools"; Delosis, London, UK). Participants were assessed for cannabis and other drug outcomes at 14, 16 and 18 years of age through the European School Survey Project on Alcohol and Other Drugs

(ESPAD). Lifetime cannabis use at the age of 18 was categorized based on the frequency of reported lifetime cannabis use (as never, '1–2', '3–5','6–9','10-19','20 or more times). The CAPE questionnaire was administered once to assess PLE at 18 years of age. Detailed descriptions of this study, genotyping procedure and data collection methods were previously published (Schumann, 2010). Genotypes and CAPE scores were available for a total of (N = 1217) participants from European descent.

2.6. IMAGEN genotyping and quality control

Method and quality control of genotyping in the IMAGEN study were similar as previously published (Schumann, 2010); (Huguet, 2018) . DNA was extracted from blood and genotyped with the Illumina® Human610-Quad Beadchip (620,901 SNPs; N = 708) and Illumina® Human660W-Quad Beadchip (657,366 SNPs; N = 1,385) by the Centre National de Genotypage (CNG; Paris, France). Only common SNP present on both arrays with a call rate of > 0.99 were used. As the first step of filtering, we used PLINK to remove 24 participants missing > 2% SNPs genotype and 6 more for being duplicate samples. Through PLINK, SNPs were then filtered for MAF > 5%, Hardy-Weinberg ($p > 1e^{-6}$), and missing genotype rates of < 5%. Afterwards, we used PLINK to remove 15 other individuals for gender mismatch and 22 for relatedness based on the identity by state (IBS). We defined ancestry by multidimensional scaling based on the IBS matrices of genetic distances with reference populations from HapMap3 (International HapMap Consortium, 2003) using PLINK (Purcell, 2007) and 4 ancestry components (African, Asian, European and Indian) using Admixture (Alexander et al., 2009). The ancestry estimation identified European ancestry components (> 80% European ancestry), in 1826 individuals, who were selected to imputation. We saved autosomic SNPs and applied corrections with bcftools (Li, 2011) of references alleles and forward sense of genotype based on "human_g1k_v37.fasta" (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/). Imputation was done with the tool developed by Sanger Institute (https://imputation.sanger.ac.uk/). The references cohort for imputation was the UK10K + 1000 Genomes Phase3 (n = 6,285) with recommended pipeline (each chromosome was phased against using EAGLE2 (Loh, 2016) (v2.0.5) and imputated with PBWT) (Durbin, 2014). After imputation, SNPs with imputation score < 0.7 were removed.

2.7. Monocyte isolation

To determine the impact of cannabinoids on the function of P2X7 we analyzed one of the key and well-known functions of this receptor *in vitro*: the release of IL-1β from activated monocytes after binding adenosine triphosphate (ATP) (Wewers and Sarkar, 2009). We used monocytes for this study, since these cells can be easily isolated from blood and express *P2RX7*, as well as cannabinoid receptors *CB1*, *CB2*, and *GPR18* as confirmed by qPCR (data not shown). We used peripheral blood samples of 42 individuals obtained from Sanquin (www.sanquin.nl) and the University Medical Center Utrecht. All donors provided informed consent approved by the local institutional review boards prior to participation in the study. Monocytes were isolated by density gradient separation using Ficoll (Ficoll Paque plus, GE Healthcare, Sweden), followed by magnetic isolation using CD14-microbeads according to manufacturer's protocol (Miltenyi Biotech, Germany).

2.8. P2X7 function in vitro

$1x10^5$ monocytes were plated in 96-well plates and stimulated with 10 ng/ml lipopolysaccharide (LPS) from E. Coli (Sigma, USA) for four hours. Monocytes were then incubated with 1 μM or 10 μM CBD (Sigma, USA), THC (Farmalyse, the Netherlands), or their ethanol carrier as control for 10 min, followed by different concentrations of ATP (Sigma, USA) for one hour. These concentrations were selected based on previous studies that analyzed the impact of cannabinoids on monocytes (Williams, 2014); (Shivers et al., 1994) . It is, however, important to note that only the higher concentration (10 μM) reflects physiological plasma concentrations of the peak concentration of cannabinoids after smoking marihuana (Ujváry and Hanuš, 2016); (Grotenhermen, 2003) . Thereafter, the cells were centrifuged, the supernatant was harvested and the concentration of IL-1β was quantified using a commercial IL-1β enzyme-linked immunosorbent assay (ELISA) kit (Ready-SET-go, eBioscience, USA), according to the manufacturer's protocol.

2.9. Statistical analysis

To calculate the effect of gene by environment interaction on PLE, we performed a logistic regression, using genotype, cannabis use, a GxE interaction term and sex as predictors. Dichotomized PLE scores (CAPE scores by median split) were used as the dependent variable. All genome-wide association analyses were performed using the open-source whole genome association analysis toolset PLINK (Purcell, 2007). In order to investigate the dose–response relation between cannabis use, PLE, and the identified SNP we subsequently studied four categories of lifetime cannabis use in a stepwise manner, whereby the lower use frequency categories served as a reference. This procedure avoids repetitive testing against one single group while allowing analyzing interaction with a dichotomous outcome. Replication in the IMAGEN sample was investigated in a similar way but due to a low frequency of homozygote variants (AA) we merged this group with heterozygotes and analyzed A-carrier status.

To analyze the functional interaction between cannabinoids and *P2RX7* genotype *in vitro,* we compared the effect of cannabinoids on the ATP response between different genotypes. Due to the low minor allele frequency rate of the rs7968311 only four homozygote variants (AA) were present in the 42 monocyte donors. We therefore compared the impact of cannabinoids on P2X7 function between A and non-A carrier status using a mixed model for repeated measures (MMRM) whereby pre- and post-cannabinoid stimulation in IL-1β response was related to carrier status.

3. Results

3.1. Sample description

The mean age of the included participants was 20.5 years. The mean CAPE score in the total sample was 67.3. Sex as a covariate was significantly associated with PLE in both datasets, B = 2.45, t = 2.92, $p = 0.00353$) as well as in the replication (B = 0.41, t = 3.45, p = 0.000561), underscoring previous studies that show that men have higher levels of PLE[1]. For further details on characteristics of the discovery sample as well as the replication sample, see Table 1.

3.2. Pre-processing, quality control and imputation

Linkage disequilibrium (LD) based SNP pruning resulted in ~ 78 k SNPs for the sets to assess heterozygosity (F < 3SD), homozygosity (F > 3SD) and relatedness by pairwise identical by descent (IBD) values (pihat > 0.1). Quality control resulted in the exclusion of 101 individuals. Four duplicates and three related sample-pairs were detected in the merged datasets (according to criteria described in methods section) and one outlier after clustering the merged dataset. Imputation resulted in 2,504,766 SNPs for analysis.

3.3. GWEIS analysis

Fig. 1 shows a Manhattan plot of *p*-values for the GxE term and the regional association plot. Table 2 lists the top 10 results of the GxE

Table 1
Characteristics of the subjects used in this GxE study. Psychotic-like experiences (PLE) were measured using the Community Assessment of Psychic Experiences (CAPE).

Discovery Characteristic	All (N = 1259)	Users (N = 401)	Non users (N = 858)
Age mean in years (sd)	20.5 (2.5)	20.7 (2.5)	20.4 (2.5)
Male %	47%	73%	35%
PLE mean (sd)	67.3 (13.9)	70.5 (15.5)	65.9 (12.8)
Replication Characteristic	All (N = 1217)	Users (N = 401)	Non users (N = 589)
Age mean in years (sd)	18.9 (0.76)	19.1 (0.80)	18.8 (0.71)
Male %	52%	45%	60%
PLE mean (sd)	19.8 (11.6)	18.8 (11.0)	20.5 (11.9)

logistic regression analysis with high PLE score as a binary outcome. A variant in the *P2RX7* gene (rs7958311, Chr12:121167552) was ranked highest with a higher risk for experiencing psychotic symptoms in heavy cannabis users (OR: 1.746, p = 1.10 x10^{-07}). The identified SNP was imputed (imputation score of 0.885) and has a minor allele frequency (MAF) of 0.289. Fig. 2 shows the Q-Q plot for the interaction in the imputed data set (2.5 M SNPs), λ = 0.993. Inspection of the genetic principle components (PC) show that ancestry was very homogeneous (see Supplementary Fig. 1) and adjustment for ancestry was not required. Adjustment of the primary finding using two genetic PC retained effect size (from B = 0.936 to B = 0.927), the p-value reflects a reduction of power (p = 1.21 × 10^{-5}). Six SNPs that are in LD in the *CADM2* gene (p = 1.05 x10^{-6}) ranked second in our GWEIS. We genotyped 1211 samples from the original discovery cohort to validate the imputation. Interaction between cannabis and *P2RX7* remained significant (B = 0.936, t = 4.420, p = 9.86 x10^{-6}). Results of analyzing the interaction between *P2RX7* and lifetime cannabis exposure categories with PLE as outcome are presented in Table 3.

3.4. Replication

A significant interaction was observed between regular lifetime cannabis use and rs7958311 in the discovery cohort as well as in the IMAGEN cohort. In the IMAGEN cohort there was a dose dependent increase for this interaction. Only for the strongest cannabis use category (20 + lifetime exposures) the interaction with rs7958311 was significant, consistent with the strongest effects for regular users in the discovery sample. Joint analysis of the two cohorts (total N = 3088) comparing heavy users (> 10 times) to lower frequency users showed a strong overall interaction (B = 0.817 t = 3.916, p = 8.99x 10^{-5}).

3.5. Other substances

In this sample, substance use was as follows: Nicotine N = 285, Alcohol N = 746, Benzodiazepines (off-license) N = 59, Opiates N = 83, Cocaine N = 90, Psychedelics N = 138. Overall illicit drug use (substances excluding alcohol and nicotine) was significantly more frequent in the cannabis users (Chi-square = 220, p < 0.001). Adjustment of the model for illicit drug use showed that illicit drug use was not associated with PLE (B = 0.06, t = 0.12, p = 0.7) and slightly reduced the strength of the interaction with *P2RX7* (mainly reflecting loss of power): B = 0.93, t = 4.43, p = 9.7 × 10^{-6}. For alcohol a similar role was present: Alcohol use was not associated with PLE (B = 0.196, t = 1.64, p = 0.10) and the interaction between *P2RX7* and cannabis use retained (B = 0.938, t = 4.42, p = 9.76 x10^{-6}). Consistent with previous studies[1], nicotine use did impact on PLE (B = 0.379, t = 2.42, p = 0.015) and therefore reduces the interaction between cannabis use and *P2RX7* (B = 0.927, t = 4.36, p = 1.31 x10^{-5})

3.6. The interaction between P2RX7 and cannabinoids in vitro

The P2X purinoceptor 7 (P2X7) protein is a purinergic receptor. Triggering of the receptor with low concentrations of adenosine triphosphate (ATP) leads to formation of a cation-specific channel, whereas a higher concentration of ATP results in the formation of a non-selective large pore in the cell membrane (Li et al., 2005). This receptor is ubiquitously expressed, including brain and immune cells. ATP activation of P2X7 results in a variety of downstream effects, including the secretion of IL1-related cytokines. To investigate the role of rs7958311 we looked up the eQTL in the GTEx database https://gtexportal.org/home/snp/rs7958311) and found a modest negative associations between rs7598311 and *P2RX7* expression (around r = -0.2) in blood and nerve tissue. To further investigate whether cannabinoids could have an impact on the function of P2X7, we analyzed whether the cannabinoids tetrahydrocannabinol (THC) and cannabidiol (CBD) affect one of the well-known functions of P2X7: secretion of IL-1β after triggering activated monocytes with ATP. ATP-induced secretion of IL-1β was confirmed in our experiments (Fig. 3A-D). Treating the cells with 10 μM cannabidiol before the cells were exposed to ATP resulted in a decrease of the concentration-dependent IL-1β release in all donors (Fig. 3B). THC and lower concentrations of CBD pretreatment resulted in a decrease of P2X7-induced Il-β release in some, but not all donors (Fig. 3A, C, D). We hypothesized that *P2RX7* genotype may be involved in the interaction between cannabinoids and P2X7. We therefore investigated the impact of cannabinoids on P2X7 function in relation to the associated *P2RX7* SNP in a larger sample. We performed the same experiment using monocytes from 42 donors that were genotyped for *P2RX7* rs7958311 (Fig. 3E and F). Mixed model repeated measure (MMRM) analysis showed that the inhibition of the ATP response by cannabinoids was stronger in A-allele carriers compared to non-A allele carriers (CBD: t = 4.27, p = 1.17 x10^{-4}, THC: t = 2.93, p = 0.006).

4. Discussion

We performed a genome wide environment interaction study (GWEIS) in a sample of 1261 participants, enriched for individuals at the extremes of psychotic experiences and cannabis use. We found a SNP (rs7958311, 12: 121167552) in the *P2RX7* gene that moderates the relationship between regular cannabis use and psychotic experiences at borderline genome-wide significance level (p = 1.10 × 10^{-7}). A similar relationship was present in the discovery sample with lifetime cannabis use (10 + times) as determinant (t = 4.377, p = 1.2x 10^{-5}). This was replicated in the IMAGEN cohort (N = 1217) in a dose response fashion whereby the strongest lifetime cannabis use category (20 + times) showed a significant interaction with genotype for the risk to experience psychotic like symptoms (t = 2.329, p = 0.020). To further explore the interaction between cannabinoids and *P2RX7* we performed *in vitro* experiments in monocytes and show that THC and CBD reduced the immunological response of the P2X7 receptor to ATP dependent on the identified genetic variant.

4.1. rs7958311

The *P2RX7* gene is located on chromosome 12 at 12q24.31 and encodes for the P2X7 receptor. The identified SNP, rs7958311, is common and has a minor allele frequency (MAF) of 0.289. This non-synonymous coding SNP results in an amino acid change from Arginine to Histidine (Arg270 > His) in an extracellular subunit of the P2X7 transmembrane receptor. Two studies have shown that the arginine variant leads to a gain of function (Stokes, 2010); (Sorge, 2012) but one study did not find an alteration of P2X7 function for this SNP (Sun et al., 2010). The monocyte experiments showed that this genetic variant

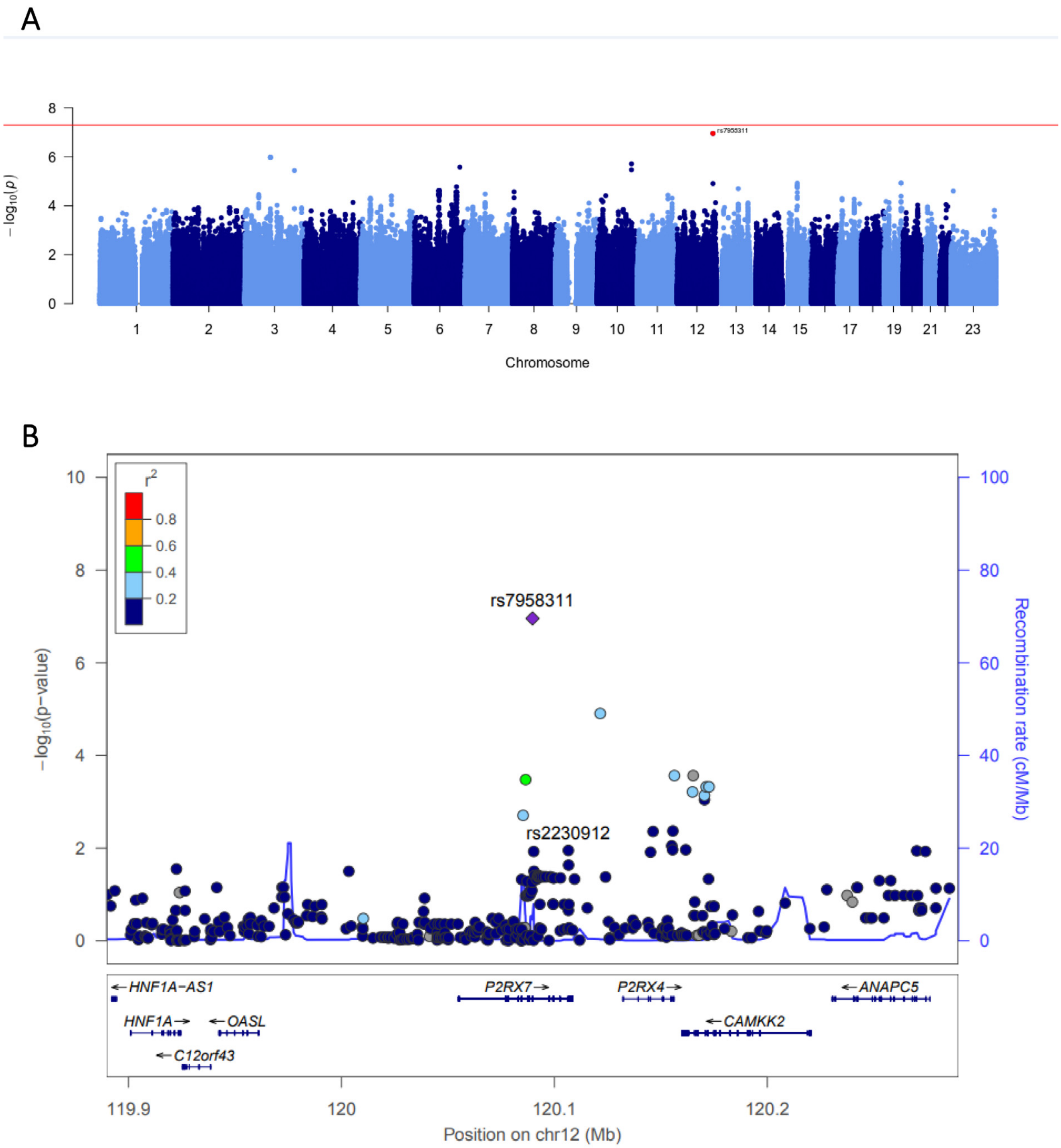

Fig. 1. Genome-wide environment-interaction study (GWEIS) between cannabis use and genotype with psychotic-like experiences as outcome. In a sample of 1262 individuals without a psychiatric disorder, part of the Utrecht Cannabis Cohort (CannabisQuest), we analyzed the interactions between cannabis use and genotype with psychotic-like experiences (PLE) as measured using the Community Assessment of Psychic Experiences (CAPE) as outcome. The sample was enriched for those at the extremes of both cannabis use and PLE to increase power. A) shows the Manhattan plot for interaction *p*-values and B) the regional association plot.

influences the inhibition of the immunological response by cannabinoids, but no differences in general immunological response of the cells to ATP.

4.2. *P2RX7* and psychiatric disorders

Variation in the *P2RX7* gene has previously been associated with a higher risk for several psychiatric disorders. Linkage scans in bipolar disorder and depression have firstly implied P2X7 as a candidate gene (McQuillin, 2009); (Barden, 2006). The 12q24 area, in which the *P2RX7* gene is located, is also linked to unipolar depression in two genome-wide linkage scans (Abkevich, 2003); (McGuffin, 2005). Several follow up studies corroborated these findings and a recent *meta*-analysis showed that one particular SNP in the *P2RX7* gene (rs2230912; Gln460Arg) is associated with both bipolar disorder and unipolar depression (Czamara et al., 2017). The 12q24 region has also been implied in schizophrenia (Bailer, 2000); (Bailer, 2002), but in the single candidate gene study on *P2RX7* and schizophrenia, no association was found for nine different *P2RX7* SNPs. The most recent GWAS *meta*-analysis of schizophrenia did not show an genome wide association with *P2RX7* variants either (Ripke et al., 2011); (Steinberg, 2011) but rs7958311 was nominal associated to schizophrenia ([47]); (Ruderfer, 2018) and in two studies with the difference between schizophrenia and bipolar disorder (Ruderfer, 2018); (Ruderfer, 2014). Rs7958311

Table 2
Top 10 genome-wide GxE association results.

CHR	SNP	BP	Allele	MAF	OR	T	*p*-value	GENE
12	rs7958311	120,089,738	G > A	0.2889	1.746	5.309	$1.10x10^{-07}$	*P2RX7*
3	rs1003984	85,952,889	T > C	0.2587	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
3	rs1003985	85,952,993	A > T	0.2170	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
3	rs1003986	85,953,053	T > C	0.2582	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
3	rs12487728	85,953,621	T > C	0.2212	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
3	rs9968137	85,953,797	G > A	0.2995	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
3	rs12488483	85,954,040	A > G	0.2212	1.981	4.881	$1.05\ x10^{-06}$	*CADM2*
10	rs4342983	115,440,504	C > T	0.0421	2.031	4.76	$1.93\ x10^{-06}$	*CASP7*
6	rs17710848	153,108,495	A > G	0.0696	2.469	4.696	$2.65\ x10^{-06}$	Not annotated
10	rs3121454	115,365,937	C > T	0.0481	2.029	4.645	$3.39\ x10^{-06}$	*NRAP*

CHR, Chromosome; SNP, Single Nucleotide Polymorphism; A1, Allele; MAF, minor allele frequency; OR, Odds Ratio; T, t statistic; P, p-value.

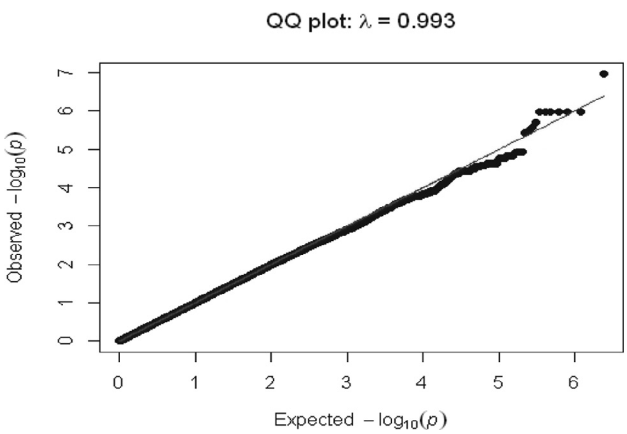

Fig. 2. QQ plot for interaction term in the imputed set (2.5 M) using a full model.

Table 3
Interaction between lifetime cannabis use and the *P2XR7* SNP on the frequency of psychosis like experiences.

Utrecht Cannabis Cohort	N = 1262			
Lifetime use	N	B	t	*p*-value
Never *	597	–	–	–
1	60	1.089	0.237	4.5×10^{-6}
2	56	0.926	3.909	9.3×10^{-5}
5–9	29	0.945	3.942	8.1×10^{-5}
10+	481	1.023	4.230	2.3×10^{-5}
IMAGEN	N = 1217			
Lifetime use		B	t	*p*-value
Never *	589	–	–	–
1–2	155	0.282	1.203	0.229
3–5	99	0.307	1.275	0.202
6–9	61	0.438	1.718	0.086
10–19	71	0.481	1.784	0.074
20+	234	0.695	2.329	0.020

* Each level of lifetime use relative to all lower levels of frequencies of lifetime use
N = sample size, B = regression coefficient of interaction term, t = t-statistic
P-value = significance of the interaction term

has been previously been related to chronic pain (Sorge, 2012), neurotic sensitivity (Nagel et al., 2018), anxiety (Otowa, 2016) and cigarette smoking (Tobacco and Genetics Consortium, 2010). A previous GxE candidate gene study on cannabis use with schizophrenia as outcome investigated another *P2RX7* SNP (rs2230912) that did not present any evidence for GxE interaction (Van Winkel, 2011). This SNP (rs2230912) is in linkage disequilibrium (D'= 0.756, p = 0.007) with the rs7958311 SNP and was nominally significant in the current study (p = 0.01) (Stokes, 2010; Fuller et al., 2009). Further fine mapping of this locus is needed to pinpoint the most relevant genetic variant.

4.3. An immunological role for P2RX7

The abundant expression of the *P2RX7* gene in the central nervous system is strongest in microglia (BioGPS gene expression database). Downstream effects include the regulation of neurotransmitter release and long term potentiation (LTP) as well as immune functions such as secretion of cytokines (Sperlágh et al., 2006). Triggering of the P2X7 receptor by ATP modulates the production of endocannabinoids in microglia (Lu, 2012) and astrocytes (Gao, 2017). Changes in P2X7 function by rs7958311 may therefore modulate the vulnerability to cannabinoids at multiple levels; by altering production of endocannabinoids as well as altering the response to THC and CBD. Because of the predominant expression of *P2RX7* in immune cells, we explored the interaction between cannabinoids and P2X7 receptor functioning in monocytes, their peripheral myeloid equivalents.

Both THC and CBD decreased the ATP-dependent IL-1β release in monocytes particularly in A-carriers of the rs7958311. These experiments indicate that cannabinoids can alter the immunological function of P2X7 receptor. How the similar effect that is induced by THC and CBD can be reconciled with the fact that THC but not CBD is linked to inducing psychosis is not clear (Schubart, 2014). A potential explanation may lie in the high concentrations of cannabinoids that were used in the culture experiments. High concentrations will obscure differences between the affinities of the two compounds for different cannabinoid receptors. In addition, it has been shown for both THC and CBD that they can inhibit inflammatory pathways through mechanisms related to CB2 (Gu, 2019), which is highly expressed on immune cells (Cabral, 2009). The interaction of those cannabinoids and P2X7 may be different on cells primarily expressing CB1, such as neurons. Further pharmacodynamic studies are therefore necessary to understand the interaction between different cannabinoids and P2X7 function.

4.4. CADM2 gene identified from this study

Although we focused in this study on validation of the most significant GxE association *P2RX7*, our genome-wide analysis also identified six SNPs in *CADM2*. *CADM2* codes for the protein synaptic cell adhesion molecule 1, which belongs to the immunoglobulin (Ig) superfamily. Interestingly, previous studies found that variants in this gene were associated with autism spectrum disorders (Casey, 2012), cognitive functioning (Davies, 2016); (Ibrahim-Verbaas, 2016) and alcohol consumption (Clarke, et al., 2017) among others. Large GWAS have also identified strong associations of this gene with lifetime cannabis use (Stringer, 2016) which strengthens the results of our GWEIS.

4.5. Strengths and limitations

Strengths of this study are the sampling from a homogeneous population of Dutch adolescents, enhanced power by the enrichment for individuals at the extremes of cannabis use and PLE, replication in an independent cohort, as well as the functional validation of the

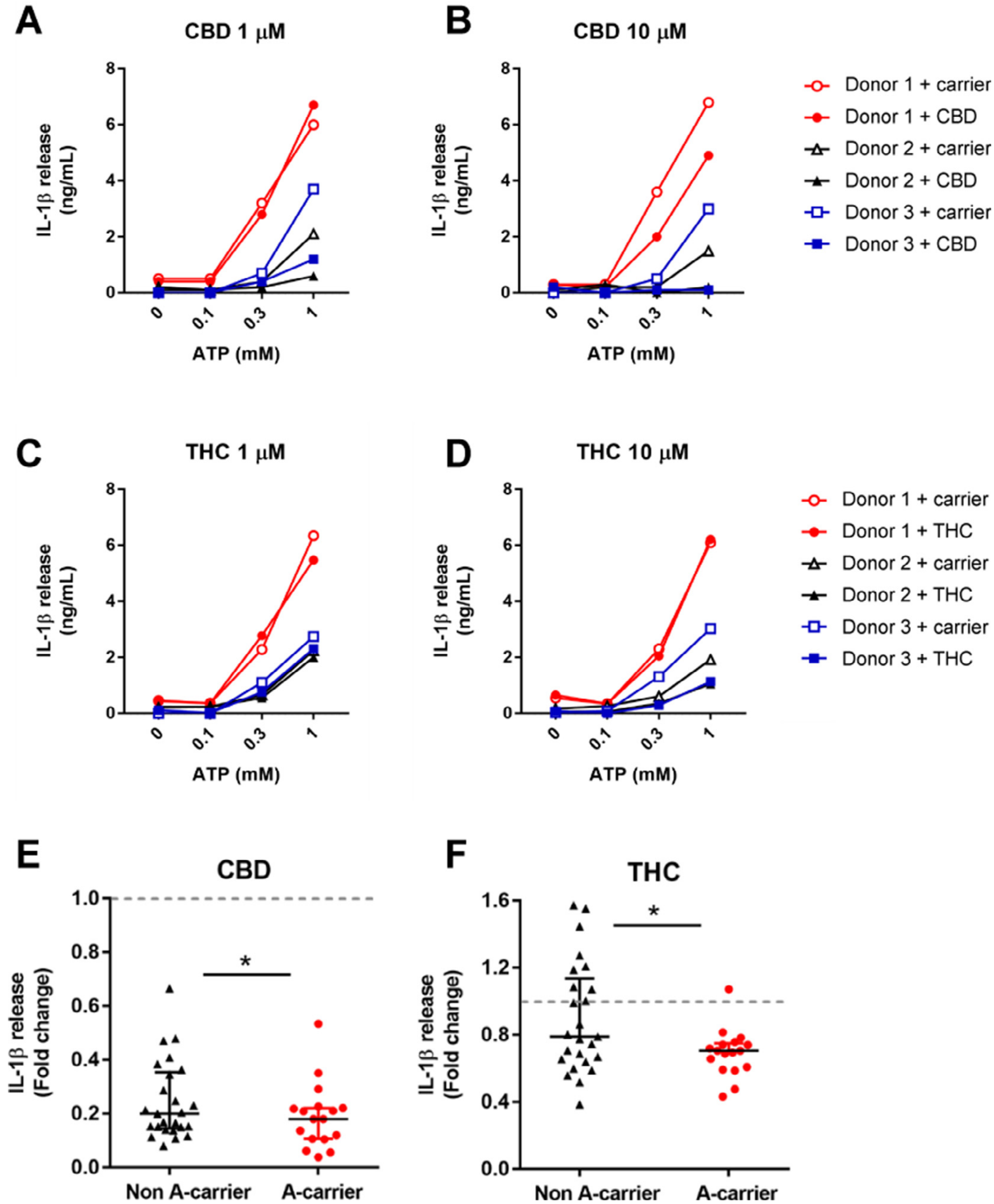

Fig. 3. The impact of the cannabinoids THC and CBD on P2X7 function *in vitro* and the role of SNP rs7958311 genotype. A-D) Monocytes were stimulated with 10 ng/ml LPS for 4 h and subsequently exposed for 30 min to 1 or 10 μM CBD, THC or the ethanol carrier as control. The cells were subsequently stimulated with different concentrations of ATP for 1 h to measure the release of IL-1β: one of the key inflammatory functions of the P2X7 receptor. E, F) The impact of rs7958311 genotype on the effect of 10 μM cannabinoids on P2X7 function is shown comparing A-carriers (AA or GA; N = 17) and non-A-carriers (GG; N = 25). The fold change is depicted, representing [IL-1β release with cannabinoids]/[IL-1β release with carrier control]. Grey dashed line represents no change in IL-1β release. The differences between genotype groups were analyzed using a mixed model for repeated measures. * = significantly different as measured by a mixed model for repeated measures.

identified GxE interaction in cell models. Nevertheless, a number of limitations have to be considered when interpreting the data. One such limitation is the use of a dichotomized outcome measure for PLE instead of treating the PLE as a quantitative trait. As the PLE were not normally distributed regression analysis with a GxE interaction term lead to differences in distribution, heteroscedasticity and violation of assumptions that varied per SNP. In our sample, this resulted in inflation of type I error reflected by a high lambda. Robust error correction did not resolve this issue. We therefore decided to dichotomize the dependent (CAPE score) and performed a binary trait analysis. Another limitation is the fact that the discovery was performed using an imputed SNP call ($R^2 = 0.885$). PCR validation could only be performed in a subgroup of the discovery cohort and yielded a lower significance level under the strict genome wide threshold. Moreover, with this study we cannot exclude that the index SNP is in linkage disequilibrium with a causal variant that is not covered by this approach. The presented replication in the IMAGEN study was therefore essential to strengthen the evidence. We also reiterate that this study does not investigate schizophrenia vulnerability per se. The CAPE questionnaire is a well validated instrument to measure psychotic like experiences as reflection of psychosis vulnerability. However, the majority of participants that have high PLE have not and will never develop a psychotic disorder. Another potential limitation is the inevitable reliance on self-report data of cannabis use even though we measured the presence of cannabis metabolites in urine in the majority of our sample and found no indication of over- or underreporting of cannabis exposure. A final limitation pertains to the fact that using a selected sample of participants is useful for increasing power but limits the generalizability of the findings.

Overall, we identified a putative role for the P2X7 receptor in the biological mechanisms underlying the relationship between cannabis and psychosis. These results warrant further mechanistic study on the role of P2X7 receptor and its crosstalk with (endo) cannabinoids in causing psychosis. Considering the evidence for a role of P2X7 receptor in neurologic and psychiatric disorders and the recent interest in treatment of these conditions through P2X7 antagonists (Pevarello et al., 2017); (Timmers, 2018), this seems highly relevant.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.04.051.

References

Van Gastel, W.A., et al., 2013. Cigarette smoking and cannabis use are equally strongly associated with psychotic-like experiences: A cross-sectional study in 1929 young adults. Psychol. Med. https://doi.org/10.1017/S0033291713000202.
Di Forti, M., et al., 2019. The contribution of cannabis use to variation in the incidence of psychotic disorder across Europe (EU-GEI): a multicentre case-control study. The Lancet Psychiatry. https://doi.org/10.1016/S2215-0366(19)30048-3.
Large, M., Sharma, S., Compton, M.T., Slade, T., Nielssen, O., 2011. Cannabis Use and Earlier Onset of Psychosis. Arch. Gen. Psychiatry 68, 555.
Verweij, K.J.H., et al., 2017. Short communication: Genetic association between schizophrenia and cannabis use. Drug Alcohol Depend. 171, 117–121.
Power, R.A., et al., 2014. Genetic predisposition to schizophrenia associated with increased use of cannabis. Mol. Psychiatry 19, 1201–1204.
Murray, R.M., et al., 2017. Cannabis-associated psychosis: Neural substrate and clinical impact. Neuropharmacology 124, 89–104.
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. 42, 1262–1269.
Gage, S.H., et al., 2017. Assessing causality in associations between cannabis use and schizophrenia risk: a two-sample Mendelian randomization study. Psychol. Med. 47, 971–980.
Vaucher, J., et al., 2017. Cannabis use and risk of schizophrenia: a Mendelian randomization study. Mol. Psychiatry. https://doi.org/10.1038/mp.2016.252.
McGuire, P.K., Jones, P., Harvey, I., Williams, M., McGuffin, P., Murray, R.M., 1995. Morbid risk of schizophrenia for relatives of patients with cannabis-associated psychosis. Schizophrenia Res. 15 (3), 277–281. https://doi.org/10.1016/0920-9964(94)00053-B.
Guloksuz, S., et al., 2019. Examining the independent and joint effects of molecular genetic liability and environmental exposures in schizophrenia: results from the EUGEI study. World Psychiatry 18, 173–182.
Sullivan, S.A., et al., 2020. A Population-Based Cohort Study Examining the Incidence and Impact of Psychotic Experiences From Childhood to Adulthood, and Prediction of Psychotic Disorder. Am. J. Psychiatry. https://doi.org/10.1176/appi.ajp.2019.19060654.
Boks, M.P.M., et al., 2007. Investigating gene-environment interaction in complex diseases: Increasing power by selective sampling for environmental exposure. Int. J. Epidemiol. https://doi.org/10.1093/ije/dym215.
Schumann, G., et al., 2010. The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. Mol. Psychiatry 15, 1128–1139.
Schubart, C.D.D., et al., 2011. Cannabis use at a young age is associated with psychotic experiences. Psychol. Med. 41, 1301–1310.
Schubart, C.D., et al., 2011. Cannabis with high cannabidiol content is associated with fewer psychotic experiences. Schizophr. Res. 130, 216–221.
Spitzer, R.L., Williams, J.B., Gibbon, M., First, M.B., 1992. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch. Gen. Psychiatry 49, 624–629.
Sheehan, D.V., et al., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin Psychiatry 59 (Suppl 2), 22–33.
Robins, L.N., 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 45, 1069–1077.
Purcell, S., et al., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.
Browning, B.L., Browning, S.R., 2009. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. Am. J. Hum. Genet. 84, 210–223.
Huguet, G., et al., 2018. Measuring and Estimating the Effect Sizes of Copy Number Variants on General Intelligence in Community-Based Samples. JAMA psychiatry 75, 447–457.
International HapMap Consortium, 2003. The International HapMap Project. Nature 426, 789–796.
Purcell, S., et al., 2007. PLINK: A Tool Set for Whole Genome Association and Population Based Linkage Analyses. Am. J. Hum. Genet. epub ahead.
Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19, 1655–1664.
Li, H., 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27, 2987–2993.
Loh, P.-R., et al., 2016. Reference-based phasing using the Haplotype Reference Consortium panel. Nat. Genet. 48, 1443–1448.
Durbin, R., 2014. Efficient haplotype matching and storage using the positional Burrows-Wheeler transform (PBWT). Bioinformatics 30, 1266–1272.
Wewers, M.D., Sarkar, A., 2009. P2X(7) receptor and macrophage function. Purinergic Signal. 5, 189–195.
Williams, J.C., et al., 2014. Δ9-tetrahydrocannabinol treatment during human monocyte differentiation reduces macrophage susceptibility to HIV-1 infection. J. Neuroimmune Pharmacol. 9, 369–379.
Shivers, S.C., Newton, C., Friedman, H., Klein, T.W., 1994. delta 9-Tetrahydrocannabinol (THC) modulates IL-1 bioactivity in human monocyte/macrophage cell lines. Life Sci. 54, 1281–1289.
Ujváry, I., Hanuš, L., 2016. Human metabolites of cannabidiol: a review on their formation, biological activity, and relevance in therapy. Cannabis Cannabinoid Res. 1, 90–101.
Grotenhermen, F. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42: 327-360. Clin. Pharmacokinet. 42, 327–360 (2003).
Li, Q., Luo, X., Muallem, S., 2005. Regulation of the P2X7 receptor permeability to large molecules by extracellular Cl- and Na+. J. Biol. Chem. 280, 26922–26927.
Stokes, L., et al., 2010. Two haplotypes of the P2X(7) receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function effect and enhanced interleukin-1beta secretion. FASEB J. 24, 2916–2927.
Sorge, R.E., et al., 2012. Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. Nat. Med. 18, 595–599.
Sun, C., Chu, J., Singh, S., Salter, R.D., 2010. Identification and characterization of a novel variant of the human P2X(7) receptor resulting in gain of function. Purinergic Signal. 6, 31–45.
McQuillin, A., et al., 2009. Case-control studies show that a non-conservative amino-acid change from a glutamine to arginine in the P2RX7 purinergic receptor protein is associated with both bipolar- and unipolar-affective disorders. Mol. Psychiatry 14, 614–620.
Barden, N., et al., 2006. Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141, 374–382.
Abkevich, V., et al., 2003. Predisposition locus for major depression at chromosome 12q22-12q23.2. Am. J. Hum. Genet. 73, 1271–1281.
McGuffin, P., et al., 2005. Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum. Mol. Genet. 14, 3337–3345.
Czamara, D., Müller-Myhsok, B., Lucae, S., 2017. The P2RX7 polymorphism rs2230912 is associated with depression: A meta-analysis. Prog. Neuropsychopharmacol. Biol. Psychiatry. https://doi.org/10.1016/j.pnpbp.2017.11.003.
Bailer, U., et al., 2000. Genome scan for susceptibility loci for schizophrenia. Neuropsychobiology 42, 175–182.
Bailer, U., et al., 2002. Genome scan for susceptibility loci for schizophrenia and bipolar disorder. Biol. Psychiatry 52, 40–52.
Ripke, S., Sanders, A., Kendler, K., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat. 43, 969–976.
Steinberg, S., et al., 2011. Common variants at VRK2 and TCF4 conferring risk of schizophrenia. Hum. Mol. Genet. 20, 4076–4081.
Nature 511, 421–427.
Ruderfer, D.M., et al., 2018. Genomic Dissection of Bipolar Disorder and Schizophrenia, Including 28 Subphenotypes. Cell 173, 1705–1715.e16.
Ruderfer, D.M., et al., 2014. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Mol. Psychiatry 19, 1017–1024.
Nagel, M., Watanabe, K., Stringer, S., Posthuma, D., van der Sluis, S., 2018. Item-level analyses reveal genetic heterogeneity in neuroticism. Nat. Commun. 9, 905.
Otowa, T., et al., 2016. Meta-analysis of genome-wide association studies of anxiety disorders. Mol. Psychiatry 21, 1391–1399.
Tobacco and Genetics Consortium, 2010. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat. Genet. 42, 441–447.
Van Winkel, R., et al., 2011. Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: Sibling analysis and proband follow-up. Arch. Gen. Psychiatry 68, 148–157.

Fuller, S.J., Stokes, L., Skarratt, K.K., Gu, B.J., Wiley, J.S., 2009. Genetics of the P2X7 receptor and human disease. Purinergic Signal. 5, 257–262.
Sperlágh, B., Vizi, E.S., Wirkner, K., Illes, P., 2006. P2X7 receptors in the nervous system. Progress in Neurobiology 78, 327–346.
Lu, Y.M., et al., 2012. P2X7 signaling promotes microsphere embolism-triggered microglia activation by maintaining elevation of Fas ligand. J. Neuroinflammation. 9, 172-.
Gao, P., et al., 2017. P2X7 receptor-sensitivity of astrocytes and neurons in the substantia gelatinosa of organotypic spinal cord slices of the mouse depend on the length of the culture period. Neuroscience. https://doi.org/10.1016/j.neuroscience.2017.02.030.
Schubart, C.D., et al., 2014. Cannabidiol as a potential treatment for psychosis. Eur. Neuropsychopharmacol. 24.
Gu, Z., et al., 2019. Marijuana-Derived Cannabinoids Trigger a CB2/PI3K Axis of Suppression of the Innate Response to Oral Pathogens. Front. Immunol. 10, 2288.
Cabral, G.A., 2009. G.-T. L. Emerging Role of the CB2 Cannabinoid Receptor in Immune Regulation and Therapeutic Prospects. Expert Rev. Mol. Med. https://doi.org/10.1017/S1462399409000957.Emerging.
Casey, J.P., et al., 2012. A novel approach of homozygous haplotype sharing identifies candidate genes in autism spectrum disorder. Hum. Genet. 131, 565–579.
Davies, G., et al., 2016. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N = 112 151). Mol. Psychiatry 21, 758–767.
Ibrahim-Verbaas, C.A., et al., 2016. GWAS for executive function and processing speed suggests involvement of the CADM2 gene. Mol. Psychiatry 21, 189–197.
Clarke, T.-K. et al. Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N = 112,117). bioRxiv 1–9 (2017). doi:10.1101/116707.
Stringer, S., et al., 2016. Genome-wide association study of lifetime cannabis use based on a large meta-analytic sample of 32 330 subjects from the International Cannabis Consortium. Transl. Psychiatry 6, e769.
Pevarello, Paolo, Bovolenta, Silvia, Tarroni, Paola, Za, Lorena, Severi, Elda, Torino, Domenica, Vitalone, Rocco, 2017. P2X7 antagonists for CNS indications: recent patent disclosures. Pharmaceutical Patent Analyst 6 (2), 61–76. https://doi.org/10.4155/ppa-2016-0044.
Timmers, M., et al., 2018. Clinical pharmacokinetics, pharmacodynamics, safety, and tolerability of JNJ-54175446, a brain permeable P2X7 antagonist, in a randomised single-ascending dose study in healthy participants. J. Psychopharmacol. 32, 1341–1350.