A variant in the COMT gene and psychosis in youth who have used cannabis

by

David Rossolatos

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Psychopharmacology

Department of Psychiatry
University of Alberta

Abstract

Cannabis use is a known risk factor for the development of psychotic disorders. Clinical and preclinical genetic studies provide growing evidence that genes related to dopamine signalling and neuroprotection, like *COMT*, are implicated in the cannabis-psychosis association. Considering the burden of psychosis on society, as well as increasing use of cannabis among the teenage population due to perceived safety regarding its use, it is of considerable current interest to determine some of the reasons why some teens who do use cannabis frequently grow up into adults unaffected by psychosis while others do not.

Using TaqMan genotyping technology, the role of the *COMT* marker rs4680 (Val158Met) and cannabis use was explored in the development of psychosis in a sample of 231 patients recruited in Edmonton and Halifax, Canada. Data on cannabis use and other relevant variables were collected.

Using Cox-regression survival analysis it was determined that cannabis use commencing during teenage years was associated with an earlier age of onset of psychosis in adulthood. Stratifying by users versus non-users of cannabis during adolescence, a Cox-regression survival analysis using *COMT* genotype as a factor showed that homozygous Val/Val *COMT* genotype was associated with earlier age of onset of psychosis in the presence of cannabis use under 20 years of age.

The results are in line with previous study findings (Caspi et al., 2005; Decoster et al., 2011; Estrada et al., 2011) regarding cannabis use during the critical prefrontal cortex development period, and the interaction between cannabis and *COMT*.

Preface

The research project, of which this thesis formed a part, received research ethics approval from the University of Alberta Research Ethics Board, project name "NPAS3 in psychoses (CIHR)", number Pro00010593, on Dec 11, 2009.

«Ουκ ένι ιατρικήν είδέναι, όστις μη οίδεν ότι εστίν άνθρωπος»
-Ιπποκράτης

Acknowledgements

Thank you to Dr Purdon for letting me into this collaborative project and allowing me to use the database for my thesis project. Thank you to Dr Greenshaw for his encouragement, and for always providing me the outside view with constructive criticism to get me thinking outside the box. Thank you to Dr McIntyre for helping me untangle the lab history of the samples used. Thank you to my fellow lab colleagues throughout the years: Dr Rohit Lodhi for helping me with SPSS and survival analyses, Dr Sudhakar Sivapalan for co-authoring the excel sheet macros, Dr Yabing Wang for conducting genotyping, Caitlin Slomp especially for stepping in for me, and Brodie, Rita, and Beatriz for your friendship, and support.

I also wanted to thank the Department of Psychiatry and Dr Fujiwara for giving me the opportunity to complete my MSc within the department and supporting my success as well as the Alberta Centennial Addiction and Mental Health Research Chair grant for funding me and providing some of the most exciting scientific instruments I have had the privilege to get to use in my project.

Much gratitude goes to my supervisor Dr Aitchison for her excellent mentorship, her patience and kindness, for believing in my abilities, and for providing me the inspiration of what a physician researcher should aspire to be. I feel blessed and fortunate to have been part of her team.

I also want to thank my mother and my fiancée, for their continuous love and care as well as my stepfather Dr Brian Bishop for sparking my scientific curiosity, challenging me to push the boundaries of my abilities, and introducing me to the local psychiatric community to show me first-hand the importance of medicine in mental health.

Contents

List of Tables

List of Figures

Abbreviations and Symbols

AHE	Alberta Hospital Edmonton
AHS	Alberta Health Services
AIDS	Acquired immune deficiency syndrome
AISH	Assured Income for the Severely Handicapped
AOP	Age of onset for diagnosis of psychosis
APA	American Psychiatric Association
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CIHR	Canadian Institutes of Health Research
COMT	Catechol-O-methyltransferase
D_1	Dopamine receptor 1
D_2	Dopamine receptor 2
D2L	D_2 dopamine receptors, long form
D2S	D_2 dopamine receptors, short form
D_4	Dopamine receptor 4
DA	Dopamine
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revised
EEPIC	Edmonton Early Psychosis Intervention Clinic
GWAS	Genome-Wide Association Studies
GxE	Gene-Environment
HPA	Hypothalamus-Pituitary-Axis
HWE	Hardy-Weinberg equilibrium
IQ	Intelligence Quotient
MGB	Minor Groove Binder
MHCC	Mental Health Commission of Canada
NAc	Nucleus Accumbens
NFQ	Non-fluorescent Quencher
NPAS3	Neuronal PAS Domain Protein 3
NSEPP	Nova Scotia Early Psychosis Program
PANSS	Positive and Negative Syndrome Scale
PCR	Polymerase Chain Reaction
PFC	Prefrontal cortex
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SCID	Structured Clinical Interview for DSM Disorders
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SUD	Substance use disorder
TAGC	Applied Genomics Core
THC	Delta-tetrahydrocannabinol

1.0 INTRODUCTION

1.1 SCHIZOPHRENIA AND PSYCHOSIS

Schizophrenia affects 1% of the population worldwide yet it contributes to 2.74% of years lived with disability globally (Institute for Health Metrics and Evaluation (IHME), 2016). In Canada alone schizophrenia has been estimated to cost about 1.4% of direct health care costs (Goeree et al., 2005) but the societal and economical effects may be far more reaching than originally calculated as outlined in the Mental Health Commission of Canada (MHCC) latest reports (Mental Health Commission of Canada, 2010, 2011, 2012). Even at the height of the AIDS epidemic in 1988, the editor of Nature said "schizophrenia is arguably the worst disease affecting mankind, even AIDS not excepted" ("Where next with psychiatric illness?" 1988) likely due to the illness' ability to disrupt associations between an individual and reality, and even worse disconnecting them from their most intimate connections to the world, unless they carefully manage their illness with antipsychotics.

Schizophrenia is briefly defined by the Mayo Clinic as a "severe brain disorder in which people interpret reality abnormally" (The Mayo Clinic, 2014). This separation between cognition and perception was what led Eugen Bleuler in 1908 to name the disease from the combination of the Greek words schizein (σχίζειν, "to split") and phrēn, phren- (φρήν, φρεν-, "mind") (Bleuler & Bleuler, 1986). Since the 2013 release of DSM-5 by the APA, schizophrenia has been diagnosed by the presence of at least two symptoms for the majority of a month, with evident impact on social or occupational functioning for a minimum of six months.

Symptoms in schizophrenia are generally divided into positive symptoms (feelings and behaviours that are not seen in individuals who are well) and negative symptoms (a lack of feelings and behaviours that are present in people who are well). Positive symptoms can present as delusions (beliefs that are not based in reality), hallucinations (either auditory, olfactory, gustatory, visual, somatosensory, or defined in general as having a sensory experience without the presence of a physical stimulus), or disorganized speech (which is secondary to thought disorder and may vary in severity). Negative symptoms include loss of interest in everyday activities such as grooming and dressing (reduced self-care), feeling out of touch with people around one, apathy (lack of motivation), and anhedonia (loss of ability to experience pleasure in previously pleasurable activities). A primary symptom for diagnosis must be of the positive type yet

secondary symptoms for diagnosis can include either negative symptoms or other related features, such as catatonia, which usually occurs in those severely affected.

Since Bleuler's coining of the term schizophrenia, there have been many theories that have attempted to explain its cause. Such theories have included psychological/family interpretations like the "refrigerator mother" theory, as evident in American psychiatrist Silvano Arieti's *Interpretation of Schizophrenia* (1955), and more modern neuropathological explanations, such as the glutaminergic and dopaminergic theories of schizophrenia. For the purposes of this study, focus will be given to the dopamine hypothesis, as it relates to the cannabinoid processing pathways.

The dopamine hypothesis, which proposes that the biological basis for schizophrenia is due to disturbed and hyperactive dopaminergic signal transduction, specifically in the mesolimbic pathway of the brain, has been held as the most prominent explanation since the 1960s. The more recent "attribution of salience model" (Kapur, 2003) attempts to provide an explanation of the relationship between aberrant dopaminergic activity in the mesolimbic pathway and schizophrenia. The model suggests that the mesolimbic system mediates the assignment of either an attractive or aversive value to an otherwise neutral external stimulus. Therefore, dysregulation of transmission leads to stimulus-independent release, which results in the aberrant assignment of salience to external objects and representations. Salience in this sense is defined as an unconscious subjective value that an individual places upon a stimulus, which may be based on previous experiences. Consequently, delusions are the effort of the patient's brain to make sense of the aberrantly salient experiences, whereas the content of hallucinations may reflect an attempt to resolve aberrantly salient internal representations. Through this paradigm, antipsychotics not only adjust the neurotransmitter imbalance that results in phenomena such as hallucinations but also reduce the aberrant salience that contributes to delusions to provide a platform upon which psychological resolution may take place.

Specifically, dysregulation of D_2 dopamine receptors is what is thought to result in the aberrant activation of the mesolimbic system. D_2 receptors come in two main isoforms with different properties: the long form (D2L) acts as a 'canonical' postsynaptic receptor, propagating an electric potential once activated, whereas the short form (D2S) is a presynaptic receptor that regulates dopamine in the synaptic cleft by stopping further dopamine release once activated. A gene knock-out study in mice has shown that these two forms differentially mediate the effect that antipsychotic and psychomimetic agents have on mouse behaviour (R. Xu, Hranilovic, Fetsko,

Bucan, & Wang, 2002). Further research, however, is needed to provide a deeper understanding of the role the two isoforms play in humans and schizophrenia and antipsychotic mechanisms of action.

1.2 CANNABIS

Cannabis has long been used as a recreational drug mainly due to its psychoactive effect of relaxation and to a lesser degree to its euphoria (Karila et al., 2014). Exogenous cannabinoids include the well-known substances delta-9-tetrahydrocannabinol (THC), cannabidiol, and cannabinol. THC is the most famous of these, as the main psychotogenic ingredient in cannabis, the plant that gave the cannabinoid system its name. THC resembles endocannabinoids and so mediates its effects by acting as a partial agonist (a substrate that when bonded to a given receptor has partial efficacy of activation relative to the full agonist) at the cannabinoid receptor CB_1. This receptor is expressed mainly in the central nervous system, and THC also acts at the related CB_2 receptor, expressed more widely across the body, for example, in cells of the immune and gastrointestinal systems (Iversen, 2003).

The endocannabinoid system is a complex interface of multiple body systems including the immune and nervous systems. Most importantly, however, the endocannabinoid system coordinates the response to pain and promotes homeostasis after inflammation due to injury. In the nervous system, endocannabinoids and their externally produced homologues, the cannabinoids, are often found stabilizing excessive nerve cell firing to ameliorate the release of pro-inflammatory substances to prevent further damage. However, there have been studies to suggest that endocannabinoids may not only limit the effects of pro-inflammatory substances on the "cellular behaviour" needed to "survive" an adverse environment, but also the organism's behaviour to promote the capacity to learn to move on from the adverse environment through learning, stress reduction, and openness. The two best understood endocannabinoids that interact with CB_1 and CB_2 receptors are anandamide and 2-arachidonoylglycerol, which are synthesized 'on demand' at the cell membrane as they have a short half-life.

As CB_1 receptors are found on the pre-synaptic neuron, cannabinoids act on them to modulate the amount of neurotransmitter released by that cell. Being widely dispersed within the central nervous system, the effects of cannabinoids on these by brain region vary. For example,

although in the spinal cord cannabinoids inhibit pain, in the basal ganglia they slow down reaction time, whereas in the hypothalamus they increase appetite. Cannabis through the effect of THC on CB_1 receptors in the mesolimbic "reward" pathway in the brain mediates its effects at least partly through reducing the GABAergic inhibition of dopaminergic neurons giving the user the sense of euphoria – which is known as the "high" that recreational users consume cannabis for.

Apart from cannabis' desired recreational effects, increased cancer risk through smoking (Jouanjus, Lapeyre-Mestre, Micallef, French Association of the Regional, & Dependence Monitoring Centres Working Group on Cannabis, 2014), and cardiac conduction changes (Jones, 2002; Sidney, 2002), cannabis is also known to have numerous undesired neurological effects on cognition (including executive functioning, and memory), coordination, and other motor effects reviewed in Moore et al. (2007). A 20 year longitudinal study by Meier et al. (2012) of 1,000 cannabis users noticed that the greatest impairments were in the domains of executive function and processing speed. THC also has been observed to suppress long-term potentiation in the hippocampus, which is the likely cause for the observation that cannabis users have significantly smaller hippocampi than non-users (Ashtari et al., 2011), associated with memory loss. Meier also observed that serious deleterious effects were evident in the group of individuals in the study who had an adolescent onset of cannabis use. Those individuals experienced IQ decline as a function of cannabis use when compared with an adult onset group. Furthermore, the within group cannabis decline was apparent in the adolescent onset of cannabis use group regardless of frequent or infrequent use being reported within the year prior to testing, which meant that adolescent onset former persistent users did not show restoration of neuropsychological functioning after the cessation of cannabis.

Despite this, cannabis use among high school students has been steadily increasing (Substance Abuse and Mental Health Services Administration, 2014), due to popularized beliefs that cannabis is a healthier alternative compared to any other substance that may be consumed including tobacco. This has significant implications, as almost a third of patients with first episode psychosis have cannabis substance use disorder (SUD) (Wisdom, Manuel, & Drake, 2011). Furthermore, cannabis SUD is associated with more positive psychotic symptoms in this patient group (Wisdom et al., 2011). In most studies young men tend to consume more cannabis than women (Wisdom et al., 2011). In addition, the onset of schizophrenia is younger in males than females, and such behaviour only compounds the risk in males (Ochoa, Usall, Cobo, Labad, &

Kulkarni, 2012). Today there are several studies associating cannabis use with the first episode of a psychotic illness (Andreasson, Allebeck, Engstrom, & Rydberg, 1987; Arseneault et al., 2002; Zammit, Allebeck, Andreasson, Lundberg, & Lewis, 2002; Decoster et al., 2011; Gage, Hickman, & Zammit, 2016; Mane et al., 2017). Decoster et al. (2011) showed that the earlier the use of cannabis, the earlier the onset of psychosis was in his sample of 585 patients with schizophrenia. The study design was such that patients were considered cannabis users if they reported using at least 5 times in the lives and their use preceded their first admission for symptoms of psychosis. Patients who didn't meet the criteria were still used in the analysis but treated as non-users. Of interest was the study classification of age of first use of cannabis into age groups (younger than 13, between 13 and 18, and older than 18) to take into consideration of critical periods in adolescent brain development, which facilitated the comparison of onset of psychosis between groups. Despite this research, the perception of risk coming from regular use of marijuana has been steadily decreasing annually among high schoolers (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2016).

1.3 COMT

Catechol-*O*-methyltransferase (COMT) is one of several enzymes that degrade catecholamines such as dopamine (DA), epinephrine, and norepinephrine. In humans, the catechol-*O*-methyltransferase protein is encoded by the *COMT* gene. A number of genetic variants, known as single nucleotide polymorphisms, such as rs4680, rs737865 and rs165599, have been described in the *COMT* gene, and together define a haplotype (or series of genetic markers on a chromosome that are usually found together). The most studied variant is Val158Met (rs4680), a SNP that does not lie within the substrate binding site of the enzyme. For a detailed description of genetic variants of *COMT*, see Tunbridge (2010). This variant is associated with differential enzyme activity due to the single nucleotide change from guanine (G) to adenine (A) in the DNA code which when later translated by the ribosomes results to a valine to methionine change at position 158 of the COMT protein, leading to a conformational change (Lachman et al., 1996). Homozygosity in the Val allele is associated with a three to four-fold increase in activity of the COMT enzyme relative to Met homozygotes (Lachman et al., 1996), whereas heterozygotes are intermediate (Weinshilboum & Raymond, 1977; Spielman & Weinshilboum, 1981; Syvanen, Tilgmann, Rinne, & Ulmanen, 1997). However, it should be noted that the *in vitro* assay from which Lachman et al.

(1996) deduced the fold change in activity did not use dopamine as a substrate, rather 3,4-dihydroxybenzoic acid, and enzymes may have different activity for different substrates. Nonetheless, *in vivo* studies have yielded results consistent with the Val/ Met variant being associated with different enzyme activity (Egan et al., 2001; Tunbridge, Farrell, Harrison, & Mackay, 2013).

1.3.1 Psychosis

Several family based studies have provided evidence towards the hypothesis that the *COMT* Val allele regulates the development of psychosis beyond the underlying familial genetic risk inherent in families with a history of schizophrenia (Li et al., 1996; Glatt, Faraone, & Tsuang, 2003; McIntosh et al., 2007). This association between the *COMT* Val allele and schizophrenia may explain why some studies find that the Val allele seems to be preferentially transmitted with schizophrenia (Li et al., 1996; Kunugi et al., 1997), while other studies do not report such a finding (Karayiorgou et al., 1998; Wei & Hemmings, 1999).

More recently, however, a GWAS by the Schizophrenia Working Group of the Psychiatric Genomics (2014) shed light on more than a hundred genomic loci that could provide a better understanding of the genetic contributions to schizophrenia.

1.3.1 Cognition

COMT has been associated with cognitive processes. For example, one study has identified the *COMT* low-activity allele (*COMT*(L)/ Met158) as a risk factor that mediates the reduction of prefrontal volume and cognition, as well as the development of psychotic symptoms in children with 22q11 microdeletions (Gothelf et al., 2005). Goldman, Weinberger, Malhotra, and Goldberg (2009) suggest that functional variation of *COMT* serves in the manipulation of information rather than in its storage. Their suggestion perhaps supports the earlier discussed theory of aberrant salience in psychosis, with COMT playing a part in how that salience is interpreted. Goldman mentions in his paper that COMT plays a critical role in tuning the levels of DA in the prefrontal cortex effectively, allowing it to process its target representation despite distracters.

1.3.2 Anhedonia & Addiction in Schizophrenia

Studies have observed dopamine dysregulation in the prefrontal cortex is associated with schizophrenia suggesting that dopamine plays an important role in certain aspects of hedonic experience and reward processing (Braver, Barch, & Cohen, 1999; Gold, Waltz, Prentice, Morris, & Heerey, 2008; Strauss, Waltz, & Gold, 2014). Anhedonia is a main symptom of schizophrenia, which has been observed not only in high-risk individuals who are not using their medications, but also in individuals who have yet to receive medications (Horan, Reise, Subotnik, Ventura, & Nuechterlein, 2008). This observation that schizophrenia patients show reduced reward processing is thought to be due to their inherently reduced ability to process reward due to diminished dopaminergic action (Juckel et al., 2006).

Several studies have implicated *COMT* in the anhedonia experienced by patients with schizophrenia. One such study of a sample of chronic patients with schizophrenia (Wang, Fang, Shen, & Xu, 2010) observed that Val homozygous individuals had greater Positive and Negative Syndrome Scale (PANSS)-rated negative syndrome scores than their homozygous Met counterparts, which were determined to be associated to the patients' anhedonia and social withdrawal symptoms. Pelayo-Teran et al. (2011) showed that the Val/Val genotype is associated with a higher score on the Scale for the Assessment of Negative Symptoms (SANS)-rated negative symptoms of psychosis, but not with scores on the Scale for the Assessment of Positive Symptoms (SAPS)-rated positive or disorganized symptoms. Another study by Molero, Ortuno, Zalacain, and Patino-Garcia (2007) also supported this association in a mixed chronic schizophrenia spectrum sample, but also observed that Val/Val individuals scored higher on the PANSS-rated positive and general symptoms of psychosis.

Some researchers have suggested that the increased anhedonia and abnormal neural reward processing that has been reported is isolated to patients receiving second generation antipsychotics, perhaps because of lower D_2 receptor affinities resulting in a lower degree of blunting of the DA response associated with anhedonia (e.g. Horan et al., 2006b). This is consistent with a model in which anhedonia, reward processing deficits, and other negative symptoms result from diminished DA in the prefrontal cortex, with diminished hedonic experience in Val158Val subjects (Docherty et al. 2008) owing to enhanced metabolism of DA.

The mesolimbic pathway, which carries outputs from prefrontal neural networks to the nucleus accumbens, is important in explaining addiction processes. There are two hypothesized

states that the prefrontal neural networks operate in, using either low affinity dopamine D_1 or high affinity dopamine D_2 receptor mediated signalling. State 1 is observed under D_2 activation while state 2 is present during D_1 activation. State 1 operates with a kind of "open gate" policy by reducing inhibition and allowing multiple excitatory outputs from the PFC to the nucleus accumbens. Whereas, state 2 uses "loudest only" to select for only the strongest signals to pass its relatively inhibitory filter to the nucleus accumbens. In addicts, although the PFC is relatively suppressed in terms of DA function, its excitatory projections are enhanced (Avery & Krichmar, 2015). Such modifications in the intracellular signaling pathways result in a state 2 environment whereby the addict will preferentially respond to strong drug-induced stimulation but not to weaker natural reinforcers. COMT variation could affect the above described process in the PFC whereby *COMT* Met/Met individuals (slow dopamine metabolizers) would be hypothesized to be relatively protected due to excess dopamine in the PFC, while *COMT* Val/Val individuals (fast metabolizers) would be more at risk due to the low levels of dopamine in the PFC.

The Val/Val also appears to mediate higher levels of self-reported physical anhedonia, similar to the diminished response to natural reinforcers seen in addicts, within first degree relatives of patients with schizophrenia. The study by Vandenbergh, Rodriguez, Miller, Uhl, and Lachman (1997) linked the Val *COMT* allele with Caucasian polysubstance abuse, giving initial evidence of an association between *COMT* and drug use. Costas et al. (2011) described an association between *COMT* genotype and lifetime prevalence of cannabis use in a sample of patients with schizophrenia. Val homozygous individuals had double the probability of lifetime cannabis use than individuals homozygous for the Met allele.

1.3.3 Associations between cannabis and age of onset of psychosis

COMT has been linked specifically to the development of psychosis after cannabis use. Alemany et al. (2014) showed that in a cohort of 533 individuals with cannabis use after exposure to childhood trauma, that their vulnerability to cannabis induced psychotic episodes was mediated by their *COMT* genotype, with Val carriers being the most vulnerable. That may suggest a more complex gene-environment interaction than just the one between cannabis and *COMT*. A prospective longitudinal population cohort-based study from which a causal effect may be inferred was published by Caspi et al. (2005). In this study, it was shown that the onset of schizophreniform disorder was earlier for Val/Val homozygotes compared with Met/Met carriers only after they had

consumed cannabis during adolescence. More specifically the effect was graded with Val/Val individuals having the earliest onset followed by Val/Met, and Met/Met having the latest overall onset. The study by Estrada et al. (2011) using 80 inpatients with schizophrenia spectrum disorders and 77 inpatients with other non-psychotic disorders also observed the same affect on age of onset of psychosis (AOP), with order of effect size being Val/Val > Val/Met > Met/Met. Furthermore, the group also found that the distribution of *COMT* Val158Met genotypes did not differ between cannabis users and non-users in their sample, and that age of first use of cannabis correlated with age of onset of psychosis.

There has yet to be a proposed model to describe the reason for the association between *COMT*, cannabis use in adolescence, and psychosis. Perhaps, however, one may consider such a mechanism taking place within the stress diathesis model of psychosis, since cannabis could be regarded as a biological stress factor during adolescent development. Another approach could be from genetic neurodevelopment as coordinated by, for example, a homeobox gene, a gene that orchestrates the activation and deactivation of other genes during an organism's development. Nonetheless, even without a full understanding of the association between the aforementioned factors, further research supporting clinical associations could lead to the development of public health interventions that could potentially reduce the incidence and effects of cannabis induced psychosis in young adults.

In this study, I explore the findings from Decoster et al. (2011), Estrada et al. (2011), and Caspi et al. (2005) by seeking to replicate their genetic associations using a Canadian sample of individuals with first episode psychosis. I hypothesize that: 1) an earlier start of consumption of cannabis will be associated with an earlier onset of psychosis 2) the Val/Val homozygotes will exhibit a significantly earlier onset away from the mean.

2.0 METHODS

2.1 DESIGN AND SAMPLE

The clinical data used in the current study were collected as part of a Canadian Institutes of Health Research (CIHR) funded study, *NPAS3* variants in schizophrenia and other psychoses, which also part-funded the genotyping. The remainder of the genotyping and data analysis were funded by an Alberta Centennial Addiction and Mental Health Chair and infrastructure grants (from the Canada Foundation for Innovation, John R. Evans Leaders Fund, and Alberta Innovation and Advanced Education, Small Equipment Grants Program). This was a two-centre genetic association study designed to investigate the following: genetic/molecular mechanisms that are hypothesized to lead to onset of schizophrenia, psychological markers that may provide an endophenotype for schizophrenia, lifestyle and environmental factors that may mediate the association, and molecular mechanisms that may underlie the contribution from one particular gene (*NPAS3*) which had been previously associated with schizophrenia (Macintyre et al., 2010).

Consecutive eligible participants (n=231) were recruited from two treatment centres in Canada: Edmonton, Alberta (Edmonton Early Psychosis Intervention Clinic, otherwise known as EEPIC, and Alberta Hospital Edmonton also known as AHE), and Halifax, Nova Scotia (Nova Scotia (NS) Early Psychosis Program (NSEPP and surrounding community mental health teams). Eligibility was predefined as patients referred with a diagnosis of psychotic disorder (diagnosis in Edmonton was made by a neuropsychologist using the SCID defined by DSM-IV-TR). Given that the COMT Val158Met variant varies in its frequency by ethnicity, to reduce the impact of mixed ethnicity on the genetic analysis, inclusion was restricted to Caucasians of Northern European ancestry.

The Alberta sample (n=140) was recruited between March 2010 and December 2013 and constituted 75 patients from the inpatient population of AHE and 65 from the outpatient population of EEPIC. The participants from those two populations were recruited to the study by a psychometrist in discussion with clinical staff of Alberta Health Services (AHS) due to suspected psychosis. From this sample group, before data analysis, 1 case was excluded due to not providing a DNA sample and not completing the drug survey, 3 for not providing DNA samples (two of which were Aboriginal), 4 for not completing the drug survey, and a further 10 who were not of

European Caucasian ethnicity (7 aboriginal, 1 Asian, 1 African, and 1 mixed). This brought the total number of cases used for analysis of this group to 122.

The NS patient sample was recruited between July 2010 and October 2014 from NSEPP and consisted of 92 patients. All patients in this sample were of European Caucasian ethnicity. Five were excluded from data analysis due to not providing DNA and another seven due to not completing the drug survey. This brought the total cases used for analysis for this group to 79.

In summary, the working case group from both sources used for analysis was 201 eligible individuals who had provided a DNA sample and completed the drug survey.

2.2 MEASURES

The diagnostic & symptom severity measures in the Edmonton centres were undertaken by well-trained psychometrists under the supervision of a registered clinical Neuropsychologist (Purdon) or his colleagues such as Dr. Kim Goddard & Dr. Virginia Newton. The assessments are detailed below.

2.2.1 Measuring psychosis symptoms

The Structured Clinical Interview for DSM-IV diagnosis (SCID-IV-TR Part B) was administered by experienced staff in EEPIC (AB) and NSEPP (NS) and the data from these were later reviewed and entered by a research associate in Edmonton under the supervision of Dr. Purdon into a database to ensure quality control. Individual items from the Psychosis section of the SCID (B1 to B25) were entered as 'ever present' or 'never present' which means that they were given one number if there ever were symptoms and another number if the symptoms were ever present. The total of all endorsed SCID B items was calculated (variable name in the database: SCID_SUM).

The SCID was supplemented with data from patient medical records, and (in Alberta) a Neuropsychological Screening Interview. Age of onset of psychosis (AOP) was defined as age of onset of DSM-IV diagnosis of psychotic disorder

2.2.2 Measuring drug use

The Computerized Neuropsychological Drugs Survey, shortened to Drugs Survey (Drugs Survey; Purdon, 2007) was used to collect a detailed history of drug use. This screen was developed from

the substance use disorder criteria of DSM-IV-TR. The survey consists of 15 drug classes listed below, evaluated on up to 47 items each.

Table 1: Drug classes surveyed with corresponding analysis codes and survey prompts

Drug Class	Code	Survey prompts
Tobacco	TOB	*cigarettes, pipe, cigars, or smokeless*
Alcohol	ALC	
Cannabis	CAN	*marijuana, hashish, tetrahydrocannabinol (THC), pot, grass, weed, or reefer*
Sedatives	SED	*anxiolytics (downers), Quaalude (ludes), Seconal (reds), Valium, Xanax, Librium, barbiturates, Ativan, Dalmane, or Halcion (prescribed use or recreational use)*
Stimulants	STI	*amphetamine, speed, crystal methamphetamine, Dexedrine, Ritalin, diet pills, ice (prescribed or recreational)*
Opioids	OPI	*codeine, heroin, morphine, opium, oxycodone, Oxycontin, Methadone, Darvon, Percodan, Demerol, or Dilaudid (prescribed or recreational)*
Cocaine	COC	*snorting, IV, freebase, crack, or speedball*
Hallucinogens (psychedelics)	HAL	*LSD, mescaline, peyote, psilocybin, mushrooms, Ecstasy, or MDMA*
PCP (phencyclidine)	PCP	*angel dust, or Special K*
Steroids	STE	
Inhalants	INH	*glue, paint, gasoline, or other inhalants*
Ethyl Chloride	ETH	
Nitrous Oxide (laughing gas)	NIT	*amyl nitrate, or butyl nitrate (poppers)*
Sleeping pills	SLE	*prescribed or recreational*
Other substance*	OTH	

* The other substance drug responses were not used during analysis

For each drug class listed above the subject was queried about their lifetime use. If the substance was never used, then the program went on to the next drug class. If the subject used the substance one or more times, they were queried on the date of last use, age at first use, age at last use, age at start of regular use, age at stop of regular use, and frequency of use in the past year. If the subjects had indicated use more than 9 times, they were also administered four items related to drug abuse, five items related to drug dependence, and they were queried on a wide range of symptoms relating to tolerance and withdrawal.

2.3 GENETIC ANALYSES

2.3.1 Variants genotyped

The *COMT* variant selected for this study was SNP rs4680, and, in addition to this variant, a panel of 24 SNPs with known frequencies in Europeans were selected in order to determine if the marker frequencies in our sample group were as expected for Caucasians.

2.3.2 Genotyping

Up to two ml of saliva was collected using an Oragene DNA collection kit (OG-250) from consenting participants. Upon vial closure the saliva was automatically mixed with 1.9 ml of lysis buffer located in the vial cap. After thorough mixing, the samples were stored at room temperature at the collection sites until a suitable batch size could be sent for processing at The Applied Genomics Core (TAGC). Prior to storage, the vials were heated to 50°C for two hours, and stored at room temperature until DNA extractions were performed using a modified version of the Agencourt GenFind v2 kit protocol. Briefly, 0.6 ml of the saliva/buffer mixture was processed on a Beckman NX instrument, no lysis step was included in the process, and after binding the DNA to magnetic beads, two washes with 75% ethanol were performed prior to elution of the genomic DNA in 55 µl of elution buffer. Forty-five microliters of the eluate were then transferred to a clean tube for storage at 4°C. A Nanodrop 8000 was used to measure the concentration of the DNA, and also the OD260/280 absorbance ratio, to assess nucleic acid purity. DNA samples were available for 246 of the 271 participants initially recruited in the study. Even though data from only 201 of the participants were analysed owing to missing clinical data, all 246 samples were successfully genotyped for the *COMT* rs4680 SNP.

The *COMT* Val158Met polymorphism was genotyped using the rs4680 TaqMan SNP Genotyping assay (assay ID C__25746809_50, Applied Biosystems, California, USA) in the Aitchison Lab. The assay was run on a ViiA™ 7 Real-Time Polymerase Chain Reaction (PCR) (Applied Biosystems by Life Technologies Thermo Fisher Scientific, Canada). In addition, the 24 SNP markers with known frequency in Caucasians (Table 1) were genotyped in the same manner. During genotyping, there was one marker (rs772262) for which the probes supplied did not give reliable data with the low concentrations of DNA used, and this was hence excluded from the analysis. Each sample was genotyped in duplicate to ensure precision. Furthermore, genotyping

was also done in the Macintyre Lab using a SNaPshot® Multiplex System for SNP genotyping (Thermo Fisher Scientific). There was 100% concordance between the genotyping done using different systems in different labs; so when for one sample, for which genotyping was not initially possible in the Aitchison lab, the result from the Mcintyre lab was used. The sample was subsequently also genotyped in the Aitchison lab, and a concordant result was seen.

The genotyping method performed on the ViiA™ 7 Real-Time PCR System utilized TaqMan® Minor Groove Binder (MGB) probes to anneal specifically to their complementary sequence surrounding the SNP of interest. A fluorescent oligonucleotide probe carries a fluorescent dye on its 5' end. On the 3' end of the probe is attached a non-fluorescent quencher (NFQ). When the probe is intact, the proximity of the quencher to the dye quenches the reporting signal. During PCR, AmpliTaq Gold® DNA polymerase extension starts where the reverse primer or probe is bound to the genomic DNA template, this probe is downstream from the fluorescent annealed (hybridized) probe. When the polymerase reaches the hybridized probe site, it cleaves it, separating the quencher from the dye, resulting in a fluorescence signal which is detected by the ViiA™ 7 machine. The reverse happens with extension from the 3' end of the forward primer, *i.e.*, Taq extends from the 5' to the 3' direction, with the 5' to 3' exonuclease activity of Taq releasing the reporter from the quencher dye when it reaches the 3' end of the annealed fluorescent probe.

2.3.3 Quality control

Data were analysed by the ViiA™ 7 using the Aitchison laboratory data analysis protocol (which included adjustment of thresholds if necessary) then quality control checked by myself or colleagues, including Yabing Wang and Rita Whitford, before being exported using the ViiA™ 7 Software v1.2.4. In this quality control check, we first verified that amplification occurred in all samples. When amplification did not occur, we specifically looked for the reason the sample did not amplify. Cases that did not amplify were manually excluded prior to reanalysis. We also checked the integrity and resolution of the allelic discrimination plot clusters to ensure that they were clearly distinct from each other with minimum tails of called samples trailing towards the graph origin. Finally, we would check that the multicomponent plot that illustrates the change in probe intensity for each probe was consistent with the position of a sample in the allelic discrimination plot.

After data export from the ViiA™ 7 machine, excel code, which I developed with colleague Sudhakar Sivapalan, modified the exported files so that they could be "read" into our datasheet in an automated fashion. In addition, the code checked to see if each member of a sample duplicate pair shared the same genotype and flagged any duplicates for which this was not the case. This introduced a second level of quality control. The excel code named "Transfiguration Vault" was further enhanced by Sudhakar who converted it into an automated macro operated by a 1-click user interface. Because of all of this, genotypic data that is released from the lab (produced by a probe for each sample genotyped) has been duplicated (with concordant calls) at least once. This excel-facilitated data export & quality control method was used not just in the study reported herein but also in other Aitchison lab work.

The call rate is defined as the percentage of samples for which a probe could provide a concordant genotype in duplicate from the total number of cases studied. Our call rate for *COMT* was 99.59% initially and subsequently 100%.

2.3.4 Creation of an automated data sheet

To ensure that the genotyping data from the ViiA™ 7 were categorized and collated in a database, a custom excel spreadsheet integrated with a combination of macro commands and logical formulas (authored by me, and edited by Drs. Aitchison and Sivapalan) was used to efficiently handle the large amount of genotyping data produced. This was a product of my work in the laboratory between November 2012 and November 2015, and was used not only for the project reported in in this thesis, but also for other genotyping projects in the Aitchison laboratory, representing a key innovation to facilitate efficient and effective data quality check and extraction for onward processing. Code within the spreadsheet mediated the transfer of exported data from the modified exported data files coming from the ViiA™ 7 and organized each data point per experiment run date, SNP, sample ID, and sample batch. This allowed for further checking that genotypes remained consistent between each run for samples that were repeatedly genotyped on different dates. The final Excel spreadsheet automatically calculates allele frequencies for each SNP as well as their genotypic Hardy-Weinberg equilibrium (HWE) values, as data comes in, to provide indications of possible probe dysfunction. Also, the spreadsheet allows for immediate allelic frequency and HWE calculations to be made per sample batch, case/ control status, or even gender. This was further modified at a later stage to allow for these calculations to take place per

any customizable attribute a user chooses to introduce to the database and associate with each sample (such as gender, ethnicity, or other). Finally, a version of the spreadsheet is able to transpose in real-time, as it receives data, all genotyping data in a separate sheet in the format of a pedigree file (a format used by more advanced genetic stratification analysis programs, such as PLINK).

2.3.5 Variable creation

As our dataset contained similar data to the Decoster et al. (2011) study in terms of cannabis use, some variables needed to be created to replicate Decoster's analyses using our dataset. Decoster et al. (2011) used only subjects who had started using cannabis prior to their age of onset of psychosis. Since our study did not exclude individuals who started using cannabis after the age of onset of their diagnosis, a new variable was necessary, CAN_use_class. This new variable allowed for the categorization of our subjects according to their reported approximated age range of first use of cannabis. Three categories resulted: 1) those who certainly used cannabis before their age of onset of psychosis 2) those who may have used for the first time cannabis shortly before or after age of onset of psychosis, and 3) those who used cannabis for the first time after their age of onset of psychosis. This tri-option grouping was necessary as data on each subject's reported age of first use was ascertained in the form of an estimated age range, rather than a specific year as was done in the Decoster study.

To separate the subject pool into two groups (*i.e.* binarize the *CAN_use_class* variable), two extra variables were created, *CAN_bin_str* (strict form) and *CAN_bin_lof* (soft form). The '*strict form*' variable was assigned only to subjects who would appear to have definitely used cannabis before their age of onset (*i.e.*, their reported period of first use preceded and did not overlap the period of the AOP, equating to group 1 of the *CAN_use_class* variable) whereas the '*soft*' form variable was assigned to subjects for which there was ambiguity about whether their use of cannabis preceded their diagnosis (*i.e.*, their reported period of first use overlapped the period of the AOP, that is, groups 1 and 2 of the *CAN_use_class* variable).

Using the 'strict' method of assessing cannabis use before age of onset of psychosis yielded 103 eligible subjects (68.2% of total cannabis users), whereas, the 'soft' method yielded 144 eligible subjects (95.4%). This exercise resulted in identifying 7 cannabis users who using the

'soft' method reported their first use to be after the age of onset of their psychosis, who were removed from the analysis.

3.4 STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS Statistics 23 (IBM), with the syntax used for analysis being provided in the Appendix.

3.4.1 Association of cannabis use with age of onset for psychosis

As this is a case only analysis, non-users were composed of those who reported no cannabis use prior to their onset of psychosis and users were composed of those who reported starting cannabis use before their diagnosis was made. This strategy was consistent with that taken by Decoster et al. (2011).

A survival or time-to-event analysis can be employed to affirm the significance of the difference between two groups as well as to visualise and quantify the difference in AOP associated with use of cannabis. Survival models take into consideration that the independent variable, here cannabis use, may affect the time it takes until the dependent event occurs, in this case, onset of psychosis. Additionally, they are advantageous as they can be performed on either parametric or non-parametric data, unlike other methods such as linear regression, for which normality is required, and without which errors may arise.

3.4.2 Association of COMT genotype with age of onset for psychosis

To analyse the association between *COMT* genotype and age at diagnosis of psychosis (AOP), a model was created using survival analysis. The survival analysis used individuals who reported cannabis use likely before AOP as per Decoster et al. (2011) and compared the three *COMT* groups with respect to their AOP timeline.

To assess the effect of the presence of history of cannabis use by *COMT* genotype, the same analysis was conducted including individuals who did not use cannabis prior to their onset of psychosis, and stratifying the analysis by cannabis use.

4.0 RESULTS

Genotypic and clinical data from the 201 European Caucasian subjects who were patients recruited either from Nova Scotia or Alberta were used in the following analyses. Genotypic data for *COMT* were available for each subject. In addition to the Drug Survey data, the following clinical data were also utilized: gender, age of individual at testing, age of onset of DSM-IV-TR disorder, DSM IV Axis I diagnosis, and ethnicity.

4.1 Diagnostic clustering and filtering

Although the subjects recruited needed to exhibit a psychotic disorder of moderate severity to be eligible for the study, the diagnosis assigned to each varied, and was not limited to schizophrenia, psychosis, or substance-induced psychosis. Table 3 shows the comprehensive distribution of diagnoses across all 201 subjects before commencing further analyses.

Table 2: Frequencies of diagnoses among selected participants

Diagnosis	Frequency
Bipolar II Disorder	1 (0.5%)
Substance-Induced Psychotic (SIP) Disorder	8 (4%)
SIP with Delusions	12 (6%)
SIP with Hallucinations	3 (1.5%)
SC - Schizophrenia (SC) - Not Otherwise Specified (NOS)	2 (1%)
SC - Disorganized	2 (1%)
SC - Paranoid	66 (32.8%)
SC - Schizophreniform	10 (5%)
SC - Residual	1 (0.5%)
SC - Schizoaffective	15 (7.5%)
SC - Affective BP Type	5 (2.5%)
SC - Undifferentiated	7 (3.5%)
Bipolar I Disorder (BPDI) - Manic episode unspecified	3 (1.5%)
Bipolar I Disorder, Single Manic Episode, Severe with Psychotic Features	1 (0.5%)
Major Depressive Disorder - Single Episode	2 (1%)
MDD – First episode (1EP) severe w Psychosis	1 (0.5%)
MDD - Recurrent - Severe with psychosis	2 (1%)
MDD Recurrent in partial remission	1 (0.5%)
BPD I - Recent Manic	8 (4%)
BPD I - Recent depression/ depressive disorder (DEP) no psychosis	3 (1.5%)
BPD I - Recent DEP w psychotic features	1 (0.5%)
BPD I Recent Episode Mixed Severe with Psychotic Features	1 (0.5%)
Delusional Disorder	3 (1.5%)
Brief Psychotic Disorder	3 (1.5%)
Psychotic Disorder NOS	38 (18.9%)
Adjustment Disorder Unspecified	2 (1%)
Total	201

The above diagnostic categories were further grouped by Dr. Aitchison working with students Brodie Heywood and Beatriz Henriques into the following seven diagnostic clusters according to their DSM-IV TR codes with minor edits by Dr. Purdon (who reports also creating diagnostic categories seperatly) to the SUD and other categories: SC (Schizophrenia) [295.000 to 295.900, 297.100, 298.800], Psy NOS (Psychosis NOS) [298.900], SUD (Substance Use Disorder) [292.000 to 292.120, 305.200], MDD (Major Depressive Disorder) [296.200 to 296.360, 300.400, 311.000], BPD (Bipolar Disorder) [296.100, 296.040, 296.400 to 296.890, 296.000, 301.130], Anx (Anxiety Disorder) [300.00 to 300.3, 309.000 to 309.400, 309.810], and Other [293.81, 292.820, 294.80, 299.80, 300.020, 301.20, 301.22, 314.010, 799.900, 292.84, 292.900]. I later assigned two cases with diagnostic code [309.90] to the Other category and one case with diagnostic code [269.89] to the Bipolar Disorder category as they were not assigned within the above parameters. The redistribution of all subjects among these clusters can be seen in Table 4. As the focus of this study was to strictly assess the contribution of cannabis use and *COMT* genotype to age of onset of psychosis, only the 175 individuals that belonged to the diagnostic clusters of schizophrenia, psychosis NOS, or SUD were utilized going forward.

Table 3: Frequencies of diagnostic clusters among selected participants

Diagnostic category cluster	Frequency
Schizophrenia	114 (56.7%)
Psychosis NOS	38 (18.9%)
Substance Use Disorder	23 (11.4%)
Major Depressive Disorder	6 (3.0%)
Bipolar Disorder	18 (9.0%)
Anxiety Disorder	0 (0%)
Other	2 (1.0%)
Total	201

4.2 Descriptive statistics

Out of the 175 participants selected 122, (69.7%) were male and 53 (30.3%) were female. Most subjects had used cannabis at least once (n=151, 86.6%) with a relatively small proportion having reported no use in their lifetime (n=24, 13.7%). Most of our users also reported first use of cannabis in the critical period of before age 20 (n=124, 84%). Age of onset of psychosis values were then grouped into specific age ranges to match those used in the age ranges for the first use of cannabis variable (CAN4), with the majority of our cannabis user subjects' AOP values falling in age ranges 16 to 19 (n=44, 29.1%) and 20 to 29 (n=85, 56.3%). As mentioned above, 7 were excluded owing

to having used cannabis apparently after their age of onset of psychosis, resulting in a sample size of 168, of characteristics shown in Table 4.

Before I commenced with further analyses, I wanted to determine whether there was a significant difference in the age of onset of psychosis (AOP) between the non-user and user groups, and compare the distributions of variables across these two groups (Table 4).

Table 4: Sample characteristic comparisons between cannabis user and non-user groups excluding the seven who used cannabis after onset of psychosis

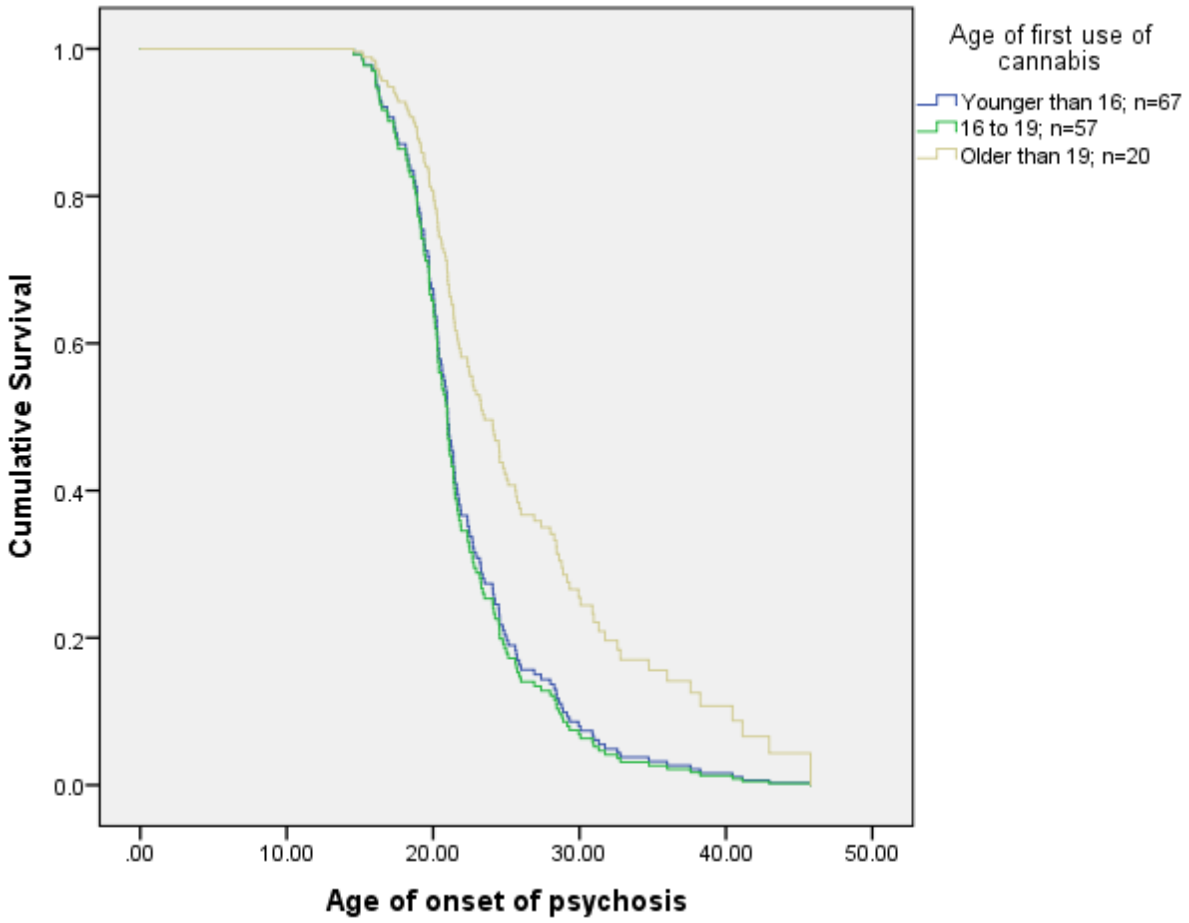
Sample Characteristic	**Users** (n= 144, 85.7%)	**Non-users** (n= 24, 14.3%)	**Probability**
Age of onset of psychosis	Median = 21.1 ± 5.7 (SD)	Median = 18.9.1 ± 9.6 (SD)	P = 0.165*
Gender	Males = 107, 74.3% Females = 37, 25.7%	Males = 11, 45.8% Females = 13, 54.2%	P = 0.005**
DSM diagnostic category	SC = 90, 62.5% NOS = 31, 21.5% SUD = 23, 16%	SC = 18, 75% NOS = 6, 25% SUD = 0, 0%	P = 0.108**
COMT genotype	G/G = 37, 25.7% G/A = 67, 46.5% A/A = 40, 27.8%	G/G = 1, 4.2% G/A = 13, 54.2% A/A = 10, 41.7%	P = 0.0.55**
* Mann-Whitney U test ** Chi-square test			

4.3 Association of age of first use of cannabis with age of onset of psychosis

Like Decoster et al. (2011), statistical evaluation of our model was performed using Cox-regression survival analysis. This statistical model allows evaluation of the effects of different factors on the response variable (which is time to event) without having to make the assumption that the underlying distribution is normal (Bewick, Cheek, & Ball, 2004). To replicate Decoster's model, the age of onset (at diagnosis) was used as the event variable and age of first use of cannabis as the factor, split into 3 classes under the variable *CAN_age_class*: i) younger than 16 ii) 16 to 19 years, and iii) older than 19. Gender was included as a co-factor to be controlled for, as studies have pointed towards gender's involvement in earlier AOP (van Os et al., 2002; Ochoa et al., 2012). Only subjects who could possibly have consumed cannabis before age of onset of diagnosis were included using the 'soft' method described above. Note that in our case the teenage period upper limit is 19 and not 18 as per Decoster et al. (2011) due to the construction of the preset age ranges in the Drug Survey questionnaire.

Our analysis replicated Decoster et al. (2011) by yielding moderate significance (P=0.021) for age of first use of cannabis affecting age of onset of psychosis when grouping the later variable in a similar manner to Decoster et al. As can be seen from Figure 1, individuals who first used cannabis at an age younger than 19 were more likely to develop psychosis earlier than those consuming cannabis after their teen years. Gender (P=0.158) did not have any significant contribution to an earlier onset of psychosis.

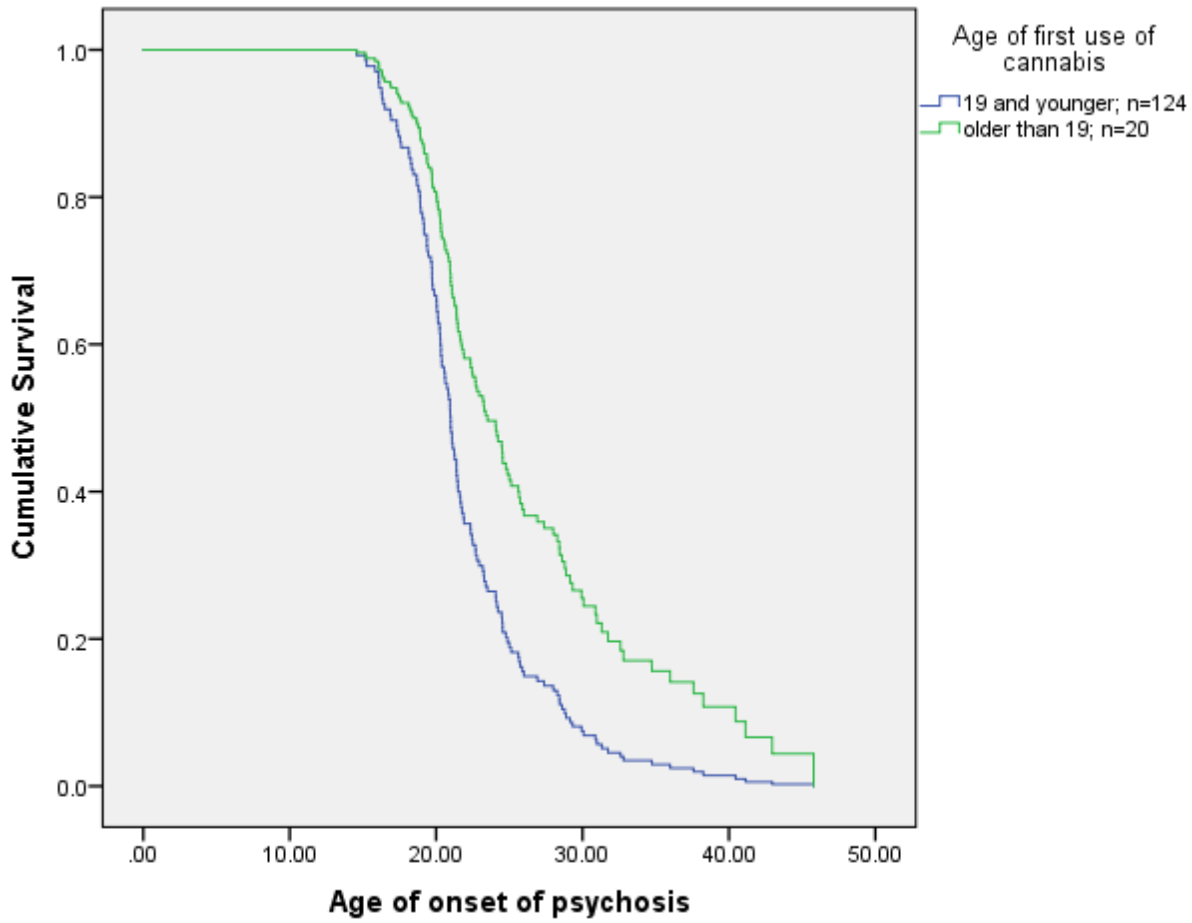
Figure 1a. Cox-regression survival analysis of AOP by age of first use of cannabis (P=0.021)



The same survival analysis model was rerun selecting cannabis using the 'strict' method which showed no significance for any of the factors likely due to the severely minimized sample size of the 'older than 19' subgroup from 20 to 3.

To further investigate the association between the age of first use of cannabis and age of onset of psychosis, the analysis was rerun by collapsing the 'younger than 16' and '16 to 19' groups together into one '19 and younger' group which was compared to the 'older than 19' group. This model resulted in a more significant result at (P=0.008) and a more pronounced graphical separation of the teenage group from the older than 19 group. Again, gender was not calculated to be a significant contributor in the variance in this model (P=0.168).

Figure 1b. Cox-regression survival analysis of AOP by age of first use of cannabis (P=0.008)



4.4 Association of COMT genotype on age of onset of psychosis

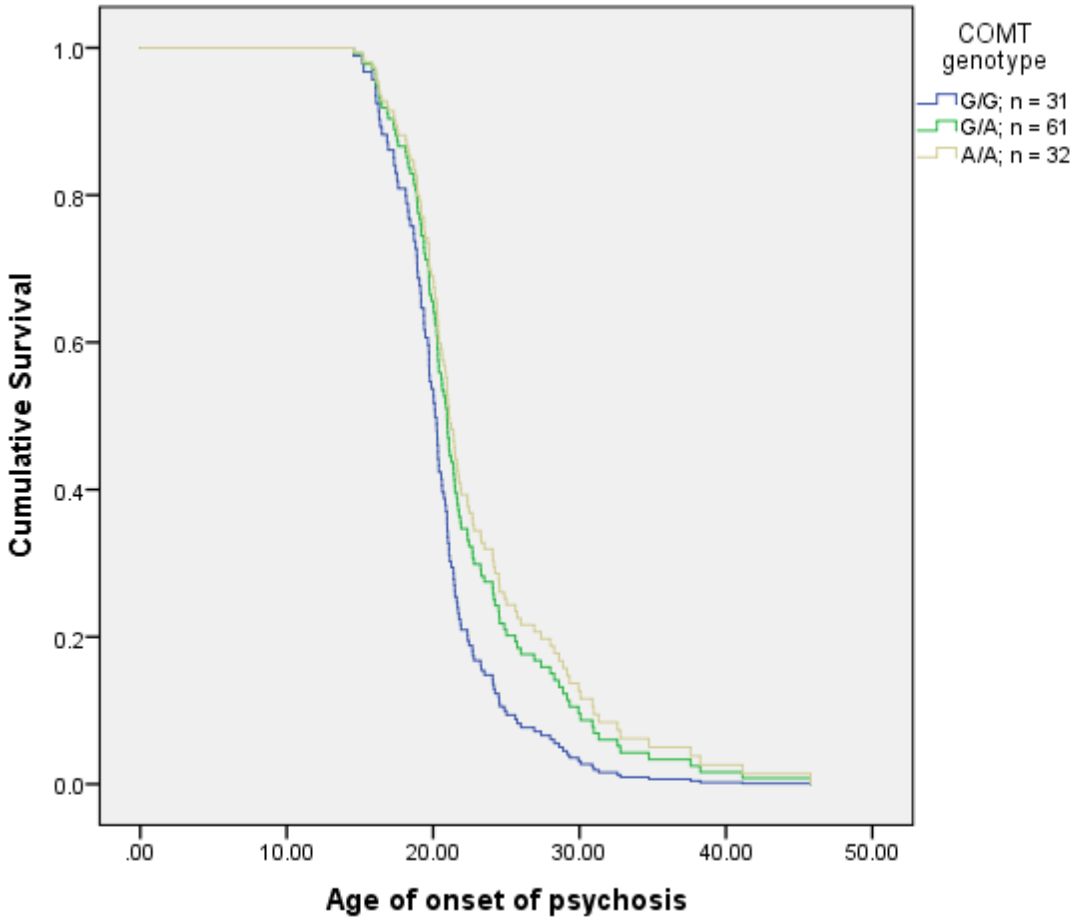
To investigate the association between *COMT* genotype and age of onset of psychosis, another Cox-regression survival analysis was conducted. Gender was used as a co-factor. The subjects

were restricted to only those who first used cannabis in their teenage years or younger using the 'soft' method.

The analysis yielded a trend (P=0.097) that *COMT* genotype influences age of onset of psychosis. Gender was not determined to be a significant factor at P=0.285. However, as can be seen from Figure 2 below, the trend supports the findings of Caspi et al. (2005) that the G/G *COMT* genotype is associated with an earlier age of onset of psychosis for those who used cannabis in their teens or younger.

The analysis was rerun using subjects selected using the 'strict' method, but all factors were determined insignificant (likely due to the marked shrinkage in sample size).

Figure 2. Cox-regression survival analysis for AOP separated by *COMT* genotype (P=0.097)

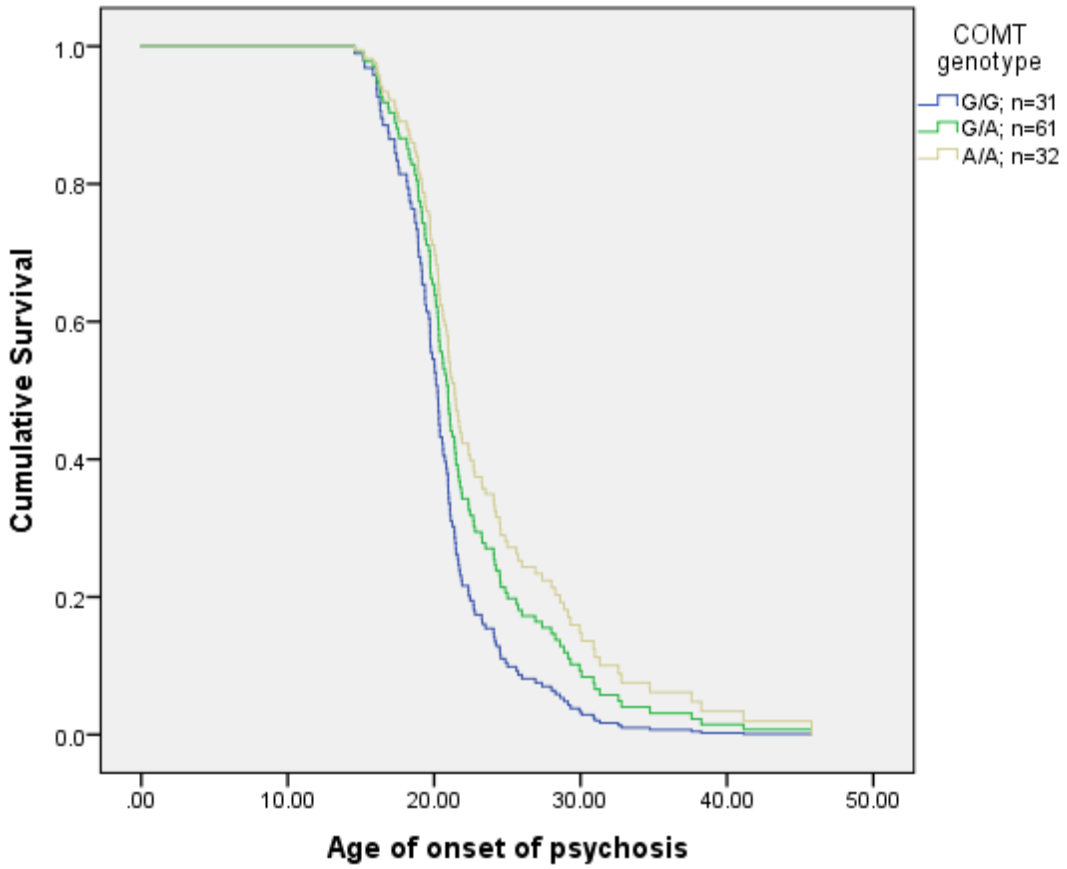


4.5 Association of COMT genotype on age of onset of psychosis as a function of cannabis use.

To elucidate the role of cannabis in the association between *COMT* genotype and age of onset of psychosis as in Caspi et al. (2005), another Cox-regression survival analysis model was employed, similar to the previous one, using *COMT* genotype and gender as factors, and selecting only subjects who either reported no cannabis use or reported first cannabis use in their teens or younger while stratifying the user and non-user groups.

This method of analysis yielded *COMT* as a significant factor (P=0.019) in affecting the age of onset of psychosis, supporting the data of Caspi et al. (2005), and Estrada et al. (2011) that *COMT* does appear to play a role in the onset of psychosis depending on whether or not an individual consumed cannabis in their critical years of neuronal development.

Figure 3. Cox-regression survival analysis for AOP separated by *COMT* genotype after stratifying for cannabis use before AOP (P=0.019), showing users only



5.0 DISCUSSION

5.1 Cannabis use during prefrontal cortex development

The analysis showed that consumption of cannabis during adolescence was associated with an earlier onset of psychosis. This finding replicates the conclusion of the Decoster et al. (2011) study that there is a period of adolescent prefrontal cortex development that is vulnerable to cannabis. Support for this explanation may come from postnatal rat brain studies. It has been observed that during the rat development period equivalent to human adolescence, the levels of dopamine D_1,D_2, and D_4 receptors rise several fold in the frontal cortex, entorhinal cortex, and hippocampus (Tarazi & Baldessarini, 2000), areas that have been associated with memory processing including recall, and with schizophrenia, and in which *COMT* may play a role (Krach et al., 2010). In the same period, in the rat striatum and nucleus accumbens (NAc), the dopamine pathway undergoes synaptic pruning as the motor and reward pathways mature (Teicher, Andersen, & Hostetter, 1995; Krach et al., 2010). Moreover, cannabis has been shown to have particularly marked effects in the early adolescent period in the rat (Harte & Dow-Edwards, 2010). Potentially this process might be affected by *COMT* genotype with the NAc being an important part of the mesolimbic pathway (highly relevant to both psychosis and cannabis use).

I therefore suggest that the connections between the role of/ interface between *COMT* genotype and the development of the adolescent brain and its interaction with cannabis are further investigated, especially in adolescent humans.

5.2 Cannabis and COMT interaction

A gene x environment interaction that was observed between cannabis use, *COMT* genotype, and onset of psychosis has been previously reported by Estrada et al. (2011). Perhaps, cannabis interacts differentially with the three genotypic groups of *COMT*, furthermore, the reason why psychosis may endure after cannabis use has ceased is also unknown. Perhaps a good place to start is by viewing the stress diathesis model of schizophrenia first introduced by E. F. Walker and Diforio (1997), later updated by E. Walker, Mittal, and Tessner (2008), and reviewed in Roper, Purdon, and Aitchison (2015). The model proposes HPA axis involvement in the regulation of dopamine and ultimately in the development of psychosis. This model explains the association with adverse childhood experiences as stressors but it may be a bit more complex to explain how

cannabis is involved. After all, cannabis is reportedly taken mainly for stress alleviation and enjoyment. The key could lie in the hippocampus, which plays an important role in managing the HPA axis (McEwen & Gianaros, 2010). As mentioned in the introduction, Ashtari et al. (2011) observed that cannabis affects long term potentiation and is associated with hippocampal volume reduction. The effect of cannabis on hippocampal volume and hence on HPA axis function may be related to *COMT* genotype. A study is available that does observe the involvement of the COMT enzyme in the hippocampus as an important modulator of dopamine metabolism and hence a possible site of therapeutics (COMT inhibitors) to improve cognition (Laatikainen, Sharp, Bannerman, Harrison, & Tunbridge, 2012).

Perhaps looking at the relationship of *COMT* with a homeobox gene might shed some light on how *COMT* might be contributing to psychosis if activated during adolescent neurodevelopment by cannabis. Homeobox genes are a family of genes responsible for the sequential activation of other genes during the development of any multicellular organism (Krumlauf, 2016). Of interest is the *HOX* subset of homeobox genes that coordinate the development of an organism along the posterior-anterior axis (Alonso, 2002). Although *HOX* genes are most active during the embryonic phase of an organism's development, their activity continues into adolescence, especially in the development of the brain (W. Xu et al., 2014). *HOXC8* is one of those genes that amongst all its functions also regulates nerve growth and it has been found that its dysregulation is associated with disorders such as neurofibromatosis type 1 (Liu et al., 2014). One of the many genes *HOXC8* targets is *ZNF804A*, a gene expressed throughout the brain and associated with schizophrenia through GWAS studies (O'Donovan et al., 2008; Schizophrenia Working Group of the Psychiatric Genomics, 2014). Specifically, a SNP of *ZNF804A* (rs1344706) has been quite controversial as it has been correlated with slightly disturbed coupling in brain regions of healthy individuals, resembling those observed in schizophrenia (Esslinger et al., 2009; Hill, Jeffries, Dobson, Price, & Bray, 2012), while another study has failed to link rs1344706 to schizophrenia (Walters et al., 2010). Nonetheless, it is currently accepted that *ZNF804A* plays a role in the neurodevelopment of the human brain and its shaping towards psychosis either in schizophrenia, bipolar disorder, or depressive disorder (Tao et al., 2014). In a study by Girgenti, LoTurco, and Maher (2012) using immunochemistry techniques on rat progenitor cells they confirm that *ZNF804A* controls the expression of *COMT* among other genes

associated with schizophrenia such as *PRSS16*. Effectively they demonstrated that the hypothesized dysregulation of *ZNF804A* may be associated with increased expression of *COMT*.

Homeobox genes are known to operate like many other genes in feedback loops. For example, as discussed in Tzchori et al. (2009), limb development is coordinated by signal loops back to the homeobox gene *LIM* to signal growth completion along an axis. Perhaps such a feedback loop could play part in the neurodevelopmental expression of *COMT* in the brain as modulated by *ZNF804A* along the mesolimbic pathway and the hippocampus. It could be possible that such a mechanism exists that normally signals back that *COMT* can handle the tuning of dopamine in the brain as discussed earlier, and therefore no more *COMT* expression is needed. There are many other factors that could affect this relationship, and the effect may not be the same in all people with a specific genetic variant. This mechanism could work sufficiently for both slow and fast *COMT* metabolizer variants through a simple negative feedback loop inhibition. When cannabis, however, is consumed and excess dopamine is found along the mesolimbic pathway, this could trigger extra *COMT* expression via *ZNF804A*. I hypothesize that slow *COMT* metabolizers expressing more COMT via, for example, *ZNF804A*, would have a lesser effect on the speed by which dopamine is cleared by the synapse, while *COMT* Val/Val fast metabolizers may be more sensitive to changes in DA levels, with a lower pre-existing DA level triggering compensatory behaviour such as cannabis consumption. Of interest, Di Forti et al. (2009); Di Forti et al. (2014) have reported associations between psychosis and potency (skunk) of cannabis and regularity of consumption. Chronic regular use may alter the 'set point' of the DA system, leading to chronic and enduring dysregulation.

The above specific description of a mechanism for a neuron x gene x environment interaction has not been proposed to my knowledge before, and serves as a suggestion of how cannabis might interact with a specific genetic variant and trigger psychosis enduring into early adulthood. There is a precedent for a GxE interactive effect that is counterintuitive in work reported by Heils et al. (1996) and Caspi et al. (2003), on the role that the serotonin transporter promoter polymorphism short/short (s/s) genotype plays in depression. A s/s genotype would be expected to contribute to an excess of serotonin due to reduced transporter activity and therefore result in an elevated mood (as depression is commonly thought to be a disorder associated with deficient serotonergic neurotransmission). However, it was determined that it was in fact the s/s genotype

that was associated with the greatest probability of a major depressive episode when the environmental stimulus of stressful life events had been present.

5.3 Limitations

As we did not adjust for multiple testing, all results with a p-value less of 0.05 should be regarded as nominally significant. One could rigorously adjust for such, using a method such as Bonferroni correction, but this would be too stringent as this method assumes independence of all variables, and variables tested herein were not independent.

This was a case-only analysis, with limited negative controls (individuals with schizophrenia who had not used cannabis before the onset of their illness). A more robust representation of psychosis affected individuals within our sample who had not used cannabis could yield more statistical power in the analysis.

Another variable that could not be controlled for was the distinction between inpatients and outpatients as a factor. Whether a patient is hospitalized has several consequences to their immediate environment such as access to drugs, stimulation in terms of sensory queues, and access to care. Generally, inpatients tend to be sicker than outpatients which influences the severity of psychosis (as measured, for example, using the PANSS) between subjects. Also, inpatients tend to have a greater polygenic risk score and therefore might have more genes interacting with *COMT* in effects on any dependent variables, which could alter the *COMT* effect size. Income levels, access to Assured Income for the Severely Handicapped (AISH), and sociogenic drive could also be included in future larger studies.

Another limitation of this study is its reliance on recall to assess cannabis use variables including first use. Although there is no alternative way that is more reliable for collecting information on age at first use without resorting to a more comprehensive longitudinal study design, one can assess more reliably last use and frequency if individuals are selected by their admittance to treatment for substance-induced psychosis. A limitation with the current study is the lack of power to differentiate between associations in substance induced psychosis and associations in schizophrenia. Following admission to hospital, individuals may be more likely to correctly recall their last use, especially as it may have precipitated their admission to care (providing their state of acute psychosis does not negatively impact recall, which may in fact be observed in patients with schizophrenia). Furthermore, using urinalysis one can categorize more

effectively users according to their frequency of use (occasional, frequent, heavy) as per, for example, the National Drug Court Institute guidelines for cannabis drug testing that have been used many times for litigation purposes (Goodwin et al., 2008) – with cannabis being detectable within urine to at least three weeks after cessation following regular, heavy use. It is important to control for cannabis use across samples used for *COMT*-psychosis association studies and in this study this was accomplished by asking each patient to estimate, for example, within a predetermined selection of lifetime use which one best represented their use.

Another important step in future research is the separation of the effects of different substances in cannabis-induced psychosis association studies to eliminate or significantly limit cross substance interference. Controlling for alcohol usage as well as limiting the number of subjects using other psychoactive substances would be preferential although quite difficult to robustly accomplish without a much larger dataset.

5.4 Future of cannabis x schizophrenia research

The current of study could be translated into educational resources in efforts to combat the rising consumption of cannabis among adolescents and contribute to policy recommendations in the way marijuana is marketed and sold with the coming changes in legislation. Additionally, as part of precision health and with our ability to genotype more cheaply with better accuracy and efficiency, one could use the findings reported herein to predict risk of psychosis on cannabis consumption, which could be used in health promotion strategies. For example, individuals genotyped as Val/Val carriers could be educated at a young age about the increased risks of cannabis use to promote abstinence from the substance. This could be welcomed by members of the general public. For example, as one "pot user" put it "marijuana didn't affect him the way it affected most other people. Instead of mellowing him out, it amped him up, fuelling his impulses and delusions – though he didn't recognize them for what they were at the time," and was subsequently diagnosed with schizoaffective disorder (Lupkin, 2016). Consequently, monitoring of those Val/ Val carriers who have been using cannabis in the critical age period could be associated with specific early health interventions either to prevent the onset of psychosis or reduce the impact of the disease.

6.0 WORKS CITED

Alemany, S., Arias, B., Fatjo-Vilas, M., Villa, H., Moya, J., Ibanez, M. I., . . . Fananas, L. (2014). Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. *Acta Psychiatr Scand, 129*(1), 54-62. doi:10.1111/acps.12108

Alonso, C. R. (2002). Hox proteins: sculpting body parts by activating localized cell death. *Curr Biol, 12*(22), R776-778.

Andreasson, S., Allebeck, P., Engstrom, A., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet, 2*(8574), 1483-1486.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ, 325*(7374), 1212-1213.

Ashtari, M., Avants, B., Cyckowski, L., Cervellione, K. L., Roofeh, D., Cook, P., . . . Kumra, S. (2011). Medial temporal structures and memory functions in adolescents with heavy cannabis use. *J Psychiatr Res, 45*(8), 1055-1066.

Avery, M. C., & Krichmar, J. L. (2015). Improper activation of D1 and D2 receptors leads to excess noise in prefrontal cortex. *Front Comput Neurosci, 9*, 31. doi:10.3389/fncom.2015.00031

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 12: survival analysis. *Crit Care, 8*(5), 389-394. doi:10.1186/cc2955

Bleuler, M., & Bleuler, R. (1986). Dementia praecox oder die Gruppe der Schizophrenien: Eugen Bleuler. *Br J Psychiatry, 149*, 661-662.

Braver, T. S., Barch, D. M., & Cohen, J. D. (1999). Cognition and control in schizophrenia: a computational model of dopamine and prefrontal function. *Biol Psychiatry, 46*(3), 312-328.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., . . . 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*(10), 1117-1127. doi:10.1016/j.biopsych.2005.01.026

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., . . . Poulton, R. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science, 301*(5631), 386-389. doi:10.1126/science.1083968

Costas, J., Sanjuan, J., Ramos-Rios, R., Paz, E., Agra, S., Tolosa, A., . . . Arrojo, M. (2011). Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. *Schizophr Res, 127*(1-3), 22-27. doi:10.1016/j.schres.2011.01.014

Decoster, J., van Os, J., Kenis, G., Henquet, C., Peuskens, J., De Hert, M., & van Winkel, R. (2011). Age at onset of psychotic disorder: cannabis, BDNF Val66Met, and sex-specific models of gene-environment interaction. *Am J Med Genet B Neuropsychiatr Genet, 156B*(3), 363-369. doi:10.1002/ajmg.b.31174

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., . . . Murray, R. M. (2009). High-potency cannabis and the risk of psychosis. *Br J Psychiatry, 195*(6), 488-491. doi:10.1192/bjp.bp.109.064220

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., . . . Murray, R. M. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophr Bull, 40*(6), 1509-1517. doi:10.1093/schbul/sbt181

Egan, M. F., Goldberg, T. E., Kolachana, B. S., Callicott, J. H., Mazzanti, C. M., Straub, R. E., . . . Weinberger, D. R. (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A, 98*(12), 6917-6922. doi:10.1073/pnas.111134598

Esslinger, C., Walter, H., Kirsch, P., Erk, S., Schnell, K., Arnold, C., . . . Meyer-Lindenberg, A. (2009). Neural mechanisms of a genome-wide supported psychosis variant. *Science, 324*(5927), 605. doi:10.1126/science.1167768

Estrada, G., Fatjo-Vilas, M., Munoz, M. J., Pulido, G., Minano, M. J., Toledo, E., . . . Fananas, L. (2011). Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. *Acta Psychiatr Scand, 123*(6), 485-492. doi:10.1111/j.1600-0447.2010.01665.x

Gage, S. H., Hickman, M., & Zammit, S. (2016). Association Between Cannabis and Psychosis: Epidemiologic Evidence. *Biol Psychiatry, 79*(7), 549-556. doi:10.1016/j.biopsych.2015.08.001

Girgenti, M. J., LoTurco, J. J., & Maher, B. J. (2012). ZNF804a regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2. *PLoS One, 7*(2), e32404. doi:10.1371/journal.pone.0032404

Glatt, S. J., Faraone, S. V., & Tsuang, M. T. (2003). Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case-control and family-based studies. *Am J Psychiatry, 160*(3), 469-476. doi:10.1176/appi.ajp.160.3.469

Goeree, R., Farahati, F., Burke, N., Blackhouse, G., O'Reilly, D., Pyne, J., & Tarride, J. E. (2005). The economic burden of schizophrenia in Canada in 2004. *Curr Med Res Opin, 21*(12), 2017-2028. doi:10.1185/030079905X75087

Gold, J. M., Waltz, J. A., Prentice, K. J., Morris, S. E., & Heerey, E. A. (2008). Reward processing in schizophrenia: a deficit in the representation of value. *Schizophr Bull, 34*(5), 835-847. doi:10.1093/schbul/sbn068

Goldman, D., Weinberger, D. R., Malhotra, A. K., & Goldberg, T. E. (2009). The role of COMT Val158Met in cognition. *Biol Psychiatry, 65*(1), e1-2; author reply e3-4. doi:10.1016/j.biopsych.2008.07.032

Goodwin, R. S., Darwin, W. D., Chiang, C. N., Shih, M., Li, S. H., & Huestis, M. A. (2008). Urinary elimination of 11-nor-9-carboxy-delta9-tetrahydrocannnabinol in cannabis users during continuously monitored abstinence. *J Anal Toxicol, 32*(8), 562-569.

Gothelf, D., Eliez, S., Thompson, T., Hinard, C., Penniman, L., Feinstein, C., . . . Reiss, A. L. (2005). COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci, 8*(11), 1500-1502. doi:10.1038/nn1572

Harte, L. C., & Dow-Edwards, D. (2010). Sexually dimorphic alterations in locomotion and reversal learning after adolescent tetrahydrocannabinol exposure in the rat. *Neurotoxicol Teratol, 32*(5), 515-524. doi:10.1016/j.ntt.2010.05.001

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *J Neurochem, 66*(6), 2621-2624.

Hill, M. J., Jeffries, A. R., Dobson, R. J., Price, J., & Bray, N. J. (2012). Knockdown of the psychosis susceptibility gene ZNF804A alters expression of genes involved in cell adhesion. *Hum Mol Genet, 21*(5), 1018-1024. doi:10.1093/hmg/ddr532

Horan, W. P., Reise, S. P., Subotnik, K. L., Ventura, J., & Nuechterlein, K. H. (2008). The validity of Psychosis Proneness Scales as vulnerability indicators in recent-onset schizophrenia patients. *Schizophr Res, 100*(1-3), 224-236. doi:10.1016/j.schres.2007.12.469

Institute for Health Metrics and Evaluation (IHME). (2016). GBD Compare Data Visualization. Retrieved 2017-02-01, from IHME, University of Washington http://vizhub.healthdata.org/gbd-compare

Iversen, L. (2003). Cannabis and the brain. *Brain, 126*(Pt 6), 1252-1270.

Johnston, L. D., O'Malley, P. M., Miech, R. A., Bachman, J. G., & Schulenberg, J. E. (2016). *Monitoring the Future national survey results on drug use, 1975-2015: Overview, key findings on adolescent drug use.* Retrieved from Ann Arbor:

Jones, R. T. (2002). Cardiovascular system effects of marijuana. *J Clin Pharmacol, 42*(11 Suppl), 58S-63S.

Jouanjus, E., Lapeyre-Mestre, M., Micallef, J., French Association of the Regional, A., & Dependence Monitoring Centres Working Group on Cannabis, C. (2014). Cannabis use: signal of increasing risk of serious cardiovascular disorders. *J Am Heart Assoc, 3*(2), e000638. doi:10.1161/JAHA.113.000638

Juckel, G., Schlagenhauf, F., Koslowski, M., Filonov, D., Wustenberg, T., Villringer, A., . . . Heinz, A. (2006). Dysfunction of ventral striatal reward prediction in schizophrenic patients treated with typical, not atypical, neuroleptics. *Psychopharmacology (Berl), 187*(2), 222-228. doi:10.1007/s00213-006-0405-4

Kapur, S. (2003). Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry, 160*(1), 13-23. doi:10.1176/appi.ajp.160.1.13

Karayiorgou, M., Gogos, J. A., Galke, B. L., Wolyniec, P. S., Nestadt, G., Antonarakis, S. E., . . . Pulver, A. E. (1998). Identification of sequence variants and analysis of the role of the catechol-O-methyl-transferase gene in schizophrenia susceptibility. *Biol Psychiatry, 43*(6), 425-431.

Karila, L., Roux, P., Rolland, B., Benyamina, A., Reynaud, M., Aubin, H. J., & Lancon, C. (2014). Acute and long-term effects of cannabis use: a review. *Curr Pharm Des, 20*(25), 4112-4118.

Krach, S., Jansen, A., Krug, A., Markov, V., Thimm, M., Sheldrick, A. J., . . . Kircher, T. (2010). COMT genotype and its role on hippocampal-prefrontal regions in declarative memory. *Neuroimage, 53*(3), 978-984. doi:10.1016/j.neuroimage.2009.12.090

Krumlauf, R. (2016). Hox Genes and the Hindbrain: A Study in Segments. *Curr Top Dev Biol, 116*, 581-596. doi:10.1016/bs.ctdb.2015.12.011

Kunugi, H., Vallada, H. P., Sham, P. C., Hoda, F., Arranz, M. J., Li, T., . . . Collier, D. A. (1997). Catechol-O-methyltransferase polymorphisms and schizophrenia: a transmission disequilibrium study in multiply affected families. *Psychiatr Genet, 7*(3), 97-101.

Laatikainen, L. M., Sharp, T., Bannerman, D. M., Harrison, P. J., & Tunbridge, E. M. (2012). Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase inhibition. *J Psychopharmacol, 26*(12), 1561-1568. doi:10.1177/0269881112454228

Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y. M., Szumlanski, C. L., & Weinshilboum, R. M. (1996). Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics, 6*(3), 243-250.

Li, T., Sham, P. C., Vallada, H., Xie, T., Tang, X., Murray, R. M., . . . Collier, D. A. (1996). Preferential transmission of the high activity allele of COMT in schizophrenia. *Psychiatr Genet, 6*(3), 131-133.

Liu, Y., Kang, D., Li, C., Xu, G., Tan, Y., & Wang, J. (2014). Microarraybased identification of nerve growthpromoting genes in neurofibromatosis type I. *Mol Med Rep, 9*(1), 192-196. doi:10.3892/mmr.2013.1785

Lupkin, S. (2016). This Is How One Pot Smoker Learned That Weed Plays a Mysterious Role in Psychosis. *Health.* Retrieved from https://news.vice.com/article/this-is-how-one-pot-smoker-learned-that-weed-plays-a-mysterious-role-in-psychosis

Macintyre, G., Alford, T., Xiong, L., Rouleau, G. A., Tibbo, P. G., & Cox, D. W. (2010). Association of NPAS3 exonic variation with schizophrenia. *Schizophr Res, 120*(1-3), 143-149. doi:10.1016/j.schres.2010.04.002

Mane, A., Berge, D., Penzol, M. J., Parellada, M., Bioque, M., Lobo, A., . . . Group, P. (2017). Cannabis use, COMT, BDNF and age at first-episode psychosis. *Psychiatry Res, 250*, 38-43. doi:10.1016/j.psychres.2017.01.045

McEwen, B. S., & Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci, 1186*, 190-222. doi:10.1111/j.1749-6632.2009.05331.x

McIntosh, A. M., Baig, B. J., Hall, J., Job, D., Whalley, H. C., Lymer, G. K., . . . Johnstone, E. C. (2007). Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. *Biol Psychiatry, 61*(10), 1127-1134. doi:10.1016/j.biopsych.2006.05.020

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S. E., . . . Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences, 109*(40), E2657–E2664. doi:10.1073/pnas.1206820109

Mental Health Commission of Canada. (2010). *Life at Risk® Mental Health Application: Data Review and Modeling Strategy*. Retrieved from Toronto, ON:

Mental Health Commission of Canada. (2011). *The Life and Economic Impact of Major Mental Illnesses in Canada*. Retrieved from Toronto, ON:

Mental Health Commission of Canada. (2012). *Life and Economic Impact of Hypothetical Intervention Scenarios on Major Mental Illnesses in Canada: 2011-2041*. Retrieved from Toronto, ON:

Molero, P., Ortuno, F., Zalacain, M., & Patino-Garcia, A. (2007). Clinical involvement of catechol-O-methyltransferase polymorphisms in schizophrenia spectrum disorders: influence on the severity of psychotic symptoms and on the response to neuroleptic treatment. *Pharmacogenomics J, 7*(6), 418-426. doi:10.1038/sj.tpj.6500441

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *The Lancet, 370*(9584), 319-328.

O'Donovan, M. C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., . . . Molecular Genetics of Schizophrenia, C. (2008). Identification of loci associated with

schizophrenia by genome-wide association and follow-up. *Nat Genet, 40*(9), 1053-1055. doi:10.1038/ng.201

Ochoa, S., Usall, J., Cobo, J., Labad, X., & Kulkarni, J. (2012). Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review. *Schizophr Res Treatment, 2012*, 916198. doi:10.1155/2012/916198

Pelayo-Teran, J. M., Perez-Iglesias, R., Vazquez-Bourgon, J., Mata, I., Carrasco-Marin, E., Vazquez-Barquero, J. L., & Crespo-Facorro, B. (2011). Catechol-O-methyltransferase Val158Met polymorphism and negative symptoms after acute antipsychotic treatment in first-episode non-affective psychosis. *Psychiatry Res, 185*(1-2), 286-289. doi:10.1016/j.psychres.2010.06.006

Roper, L., Purdon, S., & Aitchison, K. J. (2015). Childhood and later life stressors and Psychosis. *Clinical Neuropsychiatry, J Treatment Evaluation, 12*(6), 148-156.

Schizophrenia Working Group of the Psychiatric Genomics, C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature, 511*(7510), 421-427. doi:10.1038/nature13595

Sidney, S. (2002). Cardiovascular consequences of marijuana use. *J Clin Pharmacol, 42*(11 Suppl), 64S-70S.

Spielman, R. S., & Weinshilboum, R. M. (1981). Genetics of red cell COMT activity: analysis of thermal stability and family data. *Am J Med Genet, 10*(3), 279-290. doi:10.1002/ajmg.1320100311

Strauss, G. P., Waltz, J. A., & Gold, J. M. (2014). A review of reward processing and motivational impairment in schizophrenia. *Schizophr Bull, 40 Suppl 2*, S107-116. doi:10.1093/schbul/sbt197

Substance Abuse and Mental Health Services Administration. (2014). *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings*. Retrieved from Rockville, MD:

Syvanen, A. C., Tilgmann, C., Rinne, J., & Ulmanen, I. (1997). Genetic polymorphism of catechol-O-methyltransferase (COMT): correlation of genotype with individual variation of S-COMT activity and comparison of the allele frequencies in the normal population and parkinsonian patients in Finland. *Pharmacogenetics, 7*(1), 65-71.

Tao, R., Cousijn, H., Jaffe, A. E., Burnet, P. W., Edwards, F., Eastwood, S. L., . . . Kleinman, J. E. (2014). Expression of ZNF804A in human brain and alterations in schizophrenia, bipolar disorder, and major depressive disorder: a novel transcript fetally regulated by the psychosis risk variant rs1344706. *JAMA Psychiatry, 71*(10), 1112-1120. doi:10.1001/jamapsychiatry.2014.1079

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. *Int J Dev Neurosci, 18*(1), 29-37.

Teicher, M. H., Andersen, S. L., & Hostetter, J. C., Jr. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Brain Res Dev Brain Res, 89*(2), 167-172.

The Mayo Clinic. (2014). Definition. *Schizophrenia.* Retrieved from http://www.mayoclinic.org/diseases-conditions/schizophrenia/basics/definition/con-20021077

Tunbridge, E. M. (2010). The catechol-O-methyltransferase gene: its regulation and polymorphisms. *Int Rev Neurobiol, 95*, 7-27. doi:10.1016/B978-0-12-381326-8.00002-8

Tunbridge, E. M., Farrell, S. M., Harrison, P. J., & Mackay, C. E. (2013). Catechol-O-methyltransferase (COMT) influences the connectivity of the prefrontal cortex at rest. *Neuroimage, 68*, 49-54. doi:10.1016/j.neuroimage.2012.11.059

Tzchori, I., Day, T. F., Carolan, P. J., Zhao, Y., Wassif, C. A., Li, L., . . . Yang, Y. (2009). LIM homeobox transcription factors integrate signaling events that control three-dimensional limb patterning and growth. *Development, 136*(8), 1375-1385. doi:10.1242/dev.026476

van Os, J., Bak, M., Hanssen, M., Bijl, R. V., de Graaf, R., & Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol, 156*(4), 319-327.

Vandenbergh, D. J., Rodriguez, L. A., Miller, I. T., Uhl, G. R., & Lachman, H. M. (1997). High-activity catechol-O-methyltransferase allele is more prevalent in polysubstance abusers. *Am J Med Genet, 74*(4), 439-442.

Walker, E., Mittal, V., & Tessner, K. (2008). Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu Rev Clin Psychol, 4*, 189-216. doi:10.1146/annurev.clinpsy.4.022007.141248

Walker, E. F., & Diforio, D. (1997). Schizophrenia: a neural diathesis-stress model. *Psychol Rev, 104*(4), 667-685.

Walters, J. T., Corvin, A., Owen, M. J., Williams, H., Dragovic, M., Quinn, E. M., . . . Donohoe, G. (2010). Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia. *Arch Gen Psychiatry, 67*(7), 692-700. doi:10.1001/archgenpsychiatry.2010.81

Wang, Y., Fang, Y., Shen, Y., & Xu, Q. (2010). Analysis of association between the catechol-O-methyltransferase (COMT) gene and negative symptoms in chronic schizophrenia. *Psychiatry Res, 179*(2), 147-150. doi:10.1016/j.psychres.2009.03.029

Wei, J., & Hemmings, G. P. (1999). Lack of evidence for association between the COMT locus and schizophrenia. *Psychiatr Genet, 9*(4), 183-186.

Weinshilboum, R. M., & Raymond, F. A. (1977). Inheritance of low erythrocyte catechol-o-methyltransferase activity in man. *Am J Hum Genet, 29*(2), 125-135.

Where next with psychiatric illness? (1988). *Nature, 336*(6195), 95-96. doi:10.1038/336095a0

Wisdom, J. P., Manuel, J. I., & Drake, R. E. (2011). Substance use disorder among people with first-episode psychosis: a systematic review of course and treatment. *Psychiatr Serv, 62*(9), 1007-1012. doi:10.1176/appi.ps.62.9.1007

Xu, R., Hranilovic, D., Fetsko, L. A., Bucan, M., & Wang, Y. (2002). Dopamine D2S and D2L receptors may differentially contribute to the actions of antipsychotic and psychotic agents in mice. *Mol Psychiatry, 7*(10), 1075-1082. doi:10.1038/sj.mp.4001145

Xu, W., Xin, C., Lin, Q., Ding, F., Gong, W., Zhou, Y., . . . Hu, S. (2014). Adolescent mouse takes on an active transcriptomic expression during postnatal cerebral development. *Genomics Proteomics Bioinformatics, 12*(3), 111-119. doi:10.1016/j.gpb.2014.04.004

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I., & Lewis, G. (2002). Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ, 325*(7374), 1199.

APPENDIX

```
COMMENT Extract from original only sources from AHE, EEPIC, and NS collected up to 2014.
DATASET ACTIVATE DataSet1.
DATASET COPY  COMT20170127NPAS3WorkingfileDR.
DATASET ACTIVATE  COMT20170127NPAS3WorkingfileDR.
FILTER OFF.
USE ALL.
SELECT IF (source  < 4).
EXECUTE.
DATASET ACTIVATE  DataSet1.

COMMENT Group cases according to diagnoses.
RECODE DSM_AXIS_I
(295.000 thru 295.900=1) (297.100=1) (298.800=1)
(298.900=2)
(292.000 thru 292.120=3) (305.200=3)
(296.200 thru 296.360=4) (300.40=4) (311=4)
(296.100=5) (296.040=5) (296.400 thru 296.890=5) (296.000=5) (301.130=5)
(300.00 thru 300.3=6) (309.000 thru 309.400=6)  (309.810=6)
(293.81=7) (292.820=7) (294.80=7) (299.80=7) (300.020=7) (301.20=7) (301.22=7)
(314.010=7) (799.900=7) (292.84=7) (292.900=7) (269.89=5) (309.90=7)
INTO diag_type1.
VARIABLE LABELS diag_type1 "New diagnosis labelling".
VALUE LABELS diag_type1 1 'SC' 2 'Psy NOS' 3 'SUD' 4 'MDD' 5 'BPD' 6 'Anx' 7 'Other'.
EXECUTE.

COMMENT Filter out individuals without DNA data.
DATASET ACTIVATE COMT20170127NPAS3WorkingfileDR.
FILTER OFF.
USE ALL.
SELECT IF (ID_DNA > 0).
```

```
EXECUTE.

COMMENT Filter out individuals without cannabis data.
FILTER OFF.
USE ALL.
SELECT IF (CAN2 > 0).
EXECUTE.

COMMENT Filter out individuals of non Europid ethnicity.
FILTER OFF.
USE ALL.
SELECT IF (ethnicity=1).
EXECUTE.

COMMENT Check frequencies of samples from each source.
FREQUENCIES VARIABLES=source
  /ORDER=ANALYSIS.

COMMENT Check frequencies of samples from each DSM diagnosis.
FREQUENCIES VARIABLES=DSM_AXIS_I
  /ORDER=ANALYSIS.

COMMENT Select only individuals with SC, PSY NOS, or SUD.
FILTER OFF.
USE ALL.
SELECT IF (diag_type1 < 4).
EXECUTE.

COMMENT Check frequencies of sexes from those selected.
FREQUENCIES VARIABLES=sex
  /ORDER=ANALYSIS.
```

```
COMMENT Check frequencies of cannabis use from those selected.
FREQUENCIES VARIABLES=CAN2
  /ORDER=ANALYSIS.

COMMENT Confirm earlier onset of psychosis for males vs females.
EXAMINE VARIABLES=onset_age_dsm_years_derived BY sex
  /PLOT NONE
  /STATISTICS DESCRIPTIVES
  /CINTERVAL 95
  /MISSING LISTWISE
  /NOTOTAL.

COMMENT Create new variable for age group for onset of psychosis.
IF (onset_age_dsm_years_derived < 11) onset_age_dsm_years_derived_group=1.
IF (onset_age_dsm_years_derived >= 11) onset_age_dsm_years_derived_group=2.
IF (onset_age_dsm_years_derived >= 16) onset_age_dsm_years_derived_group=3.
IF (onset_age_dsm_years_derived >= 20) onset_age_dsm_years_derived_group=4.
IF (onset_age_dsm_years_derived >= 30) onset_age_dsm_years_derived_group=5.
IF (onset_age_dsm_years_derived >= 40) onset_age_dsm_years_derived_group=6.
IF (onset_age_dsm_years_derived >= 55) onset_age_dsm_years_derived_group=7.
IF (onset_age_dsm_years_derived > 74 ) onset_age_dsm_years_derived_group=8.
VARIABLE LABELS onset_age_dsm_years_derived_group 'Age of onset time group'.
VALUE LABELS
onset_age_dsm_years_derived_group
1 '<11 years'
2 '11 to 15'
3 '16 to 19'
4 '20 to 29'
5 '30 to 39'
6 '40 to 54'
```

```
7 '55 to 74'
8 '>74'.
EXECUTE.

COMMENT Create new variable comparing age for use of cannabis for 1st time relative to their age of onset for DSM disorder.
IF (CAN4 < onset_age_dsm_years_derived_group) CAN_use_class=1.
IF (CAN4 = onset_age_dsm_years_derived_group) CAN_use_class=2.
IF (CAN4 > onset_age_dsm_years_derived_group) CAN_use_class=3.
VARIABLE LABELS CAN_use_class 'When first use of cannabis was in relation to onset of DSM disorder'.
VALUE LABELS.
CAN_use_class
1 'Definitely used cannabis before age of onset'
2 'Used cannabis around age of onset'
3 'Used cannabis after age of onset'.
EXECUTE.

IF (CAN_use_class = 1) CAN_usebin_str=1.
IF (CAN_use_class <= 2) CAN_usebin_lof=1.
VARIABLE LABELS CAN_usebin_str 'Did first use of CAN occur before age of onset'.
VARIABLE LABELS CAN_usebin_lof 'Did first use of CAN occur before and/or around age of onset'.
VALUE LABELS
CAN_usebin_str
1 'Yes'.
EXECUTE.
VALUE LABELS
CAN_usebin_lof
1 'Yes'.
EXECUTE.
```

```
COMMENT Check frequencies of users before psychosis per str or lof method.
FREQUENCIES VARIABLES=CAN_usebin_str
  /ORDER=ANALYSIS.
FREQUENCIES VARIABLES=CAN_usebin_lof
  /ORDER=ANALYSIS.


COMMENT Filter only cannabis users.
DATASET ACTIVATE COMT20170127NPAS3WorkingfileDR.
USE ALL.
COMPUTE filter_$=(CAN2>1).
VARIABLE LABELS filter_$ 'CAN2>1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.

COMMENT Check frequencies of age of onset of psychosis age groups.
FREQUENCIES VARIABLES=onset_age_dsm_years_derived_group
  /ORDER=ANALYSIS.

COMMENT Check frequencies of age of first use of cannabis.
FREQUENCIES VARIABLES=CAN4
  /ORDER=ANALYSIS.

COMMENT Setup age of first use of cannabis classification according to decoster.
IF (CAN4 < 3) CAN_age_class=1.
IF (CAN4 = 3) CAN_age_class=2.
IF (CAN4 > 3) CAN_age_class=3.
VARIABLE LABELS CAN_age_class 'Decoster classification of age of first use'.
```

```
VALUE LABELS
CAN_age_class
1 'Younger than 16'
2 '16 to 19'
3 'Older than 19'.
EXECUTE.

COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use in
different age periods (lof method).
USE ALL.
COMPUTE filter_$=(CAN_usebin_lof=1).
VARIABLE LABELS filter_$ 'CAN_usebin_lof=1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.

COXREG onset_age_dsm_years_derived
  /STATUS=CAN_usebin_lof(1)
  /PATTERN BY CAN_age_class  /CONTRAST(CAN_age_class)=Indicator
  /METHOD=ENTER sex CAN_age_class
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use in
different age periods (str method).
USE ALL.
COMPUTE filter_$=(CAN_usebin_str=1).
VARIABLE LABELS filter_$ 'CAN_usebin_lof=1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
```

```
COXREG onset_age_dsm_years_derived
  /STATUS=CAN_usebin_str(1)
  /PATTERN BY CAN_age_class  /CONTRAST(CAN_age_class)=Indicator
  /METHOD=ENTER sex CAN_age_class
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use either
in their teens or after their teens (lof method).
USE ALL.
COMPUTE filter_$=(CAN_usebin_lof=1).
VARIABLE LABELS filter_$ 'CAN_usebin_lof=1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.

RECODE CAN_age_class (3=2) (Lowest thru 2=1) INTO CAN_age_class_newvold.
VARIABLE LABELS  CAN_age_class_newvold 'Age of first use'.
VALUE LABELS
CAN_age_class_newvold
1 '19 and younger'
2 'older than 19'.
EXECUTE.

COXREG onset_age_dsm_years_derived
  /STATUS=CAN_usebin_lof(1)
  /PATTERN BY CAN_age_class_newvold  /CONTRAST(CAN_age_class_newvold)=Indicator
  /METHOD=ENTER sex CAN_age_class_newvold
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

```
COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use in
their teens (lof method).
USE ALL.
COMPUTE filter_$=(CAN_usebin_lof = 1  &  CAN4 < 4).
VARIABLE LABELS filter_$ 'CAN_usebin_lof = 1  &  CAN4 < 4 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

COXREG onset_age_dsm_years_derived
  /STATUS=CAN_usebin_lof(1)
  /PATTERN BY COMT_TYPE3  /CONTRAST(COMT_TYPE3)=Indicator
  /METHOD=ENTER sex COMT_TYPE3
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).


COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use in
their teens (str method).
USE ALL.
COMPUTE filter_$=(CAN_usebin_str = 1  &  CAN4 < 4).
VARIABLE LABELS filter_$ 'CAN_usebin_str = 1  &  CAN4 < 4 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

COXREG onset_age_dsm_years_derived
  /STATUS=CAN_usebin_str(1)
```

```
  /PATTERN BY COMT_TYPE3  /CONTRAST(COMT_TYPE3)=Indicator
  /METHOD=ENTER sex COMT_TYPE3
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).

COMMENT Binarize users and non users.

RECODE CAN_use_class (Lowest thru 2=1) (MISSING=2) INTO CAN_usebin_lofv2.
VARIABLE LABELS CAN_usebin_lofv2 'Did first use of CAN occur before and/or around age
of onset (without the 7)'.
VALUE LABELS
CAN_usebin_lofv2
1 'Users'
2 'Non-users'.
EXECUTE.

COMMENT Comparison of users vs non users.

USE ALL.
COMPUTE filter_$=(CAN_usebin_lofv2  > 0).
VARIABLE LABELS filter_$ 'CAN_usebin_lofv2  > 0 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=CAN_usebin_lofv2
  /ORDER=ANALYSIS.

COMMENT Split users from non-users.
DATASET ACTIVATE DataSet1.
```

```
SORT CASES  BY CAN_usebin_lofv2.
SPLIT FILE SEPARATE BY CAN_usebin_lofv2.


COMMENT Descriptive statistics and comparisons of user vs non user groups.
EXAMINE VARIABLES=age_derived
  /PLOT NPPLOT
  /STATISTICS DESCRIPTIVES
  /CINTERVAL 95
  /MISSING LISTWISE
  /NOTOTAL.

FREQUENCIES VARIABLES=sex
  /ORDER=ANALYSIS.

EXAMINE VARIABLES=onset_age_dsm_years_derived
  /PLOT NPPLOT
  /STATISTICS DESCRIPTIVES
  /CINTERVAL 95
  /MISSING LISTWISE
  /NOTOTAL.

EXAMINE VARIABLES=CAN4
  /PLOT NPPLOT
  /STATISTICS DESCRIPTIVES
  /CINTERVAL 95
  /MISSING LISTWISE
  /NOTOTAL.

FREQUENCIES VARIABLES=COMT_TYPE3
  /ORDER=ANALYSIS.
```

```
SPLIT FILE OFF.

NPAR TESTS
  /CHISQUARE=COMT_TYPE3 CAN_usebin_lofv2
  /EXPECTED=EQUAL
  /MISSING ANALYSIS.

CROSSTABS
  /TABLES=COMT_TYPE3 BY CAN_usebin_lofv2
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.

CROSSTABS
  /TABLES=diag_type BY CAN_usebin_lofv2
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.

CROSSTABS
  /TABLES=sex BY CAN_usebin_lofv2
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI
  /CELLS=COUNT EXPECTED
  /COUNT ROUND CELL.

NPAR TESTS
  /M-W= onset_age_dsm_years_derived BY CAN_usebin_lofv2(1 2)
```

```
  /STATISTICS=DESCRIPTIVES
  /MISSING ANALYSIS.

COMMENT Survival analysis of age of onset of psychosis for users starting cannabis use in
their teens stratifying over cannabis use.

USE ALL.
COMPUTE filter_$=(CAN4new < 3 ).
VARIABLE LABELS filter_$ 'CAN4new < 3  (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMATS filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

COXREG onset_age_dsm_years_derived
  /STATUS=Inclusion_Exclusion(1)
  /STRATA= CAN_usebin_lofv2
  /PATTERN BY COMT_TYPE3  /CONTRAST(COMT_TYPE3)=Indicator
  /METHOD=ENTER sex COMT_TYPE3
  /PLOT SURVIVAL
  /CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```