Understanding and mitigating adverse health outcomes in mental illness
by

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Abstract

Patients who have a severe mental illness such as psychosis experience a greater burden in terms of poor physical and mental health outcomes or adverse health outcome/s (AHO). The etiological factors for AHO include the illness itself, medication side effects, poor diet and lifestyle, substance use, and co-morbid medical conditions. AHO have a significant impact on the quality of life of those experiencing psychosis and efforts should be made to mitigate them. Poor bone health and metabolic syndrome are two physical AHO that have a close relationship with antipsychotic and other medications. Almost half of those who have psychosis also have a lifetime history of substance misuse, and substances such as cocaine and cannabis can independently produce a range of physical and psychological AHO. Cannabis has been associated with the AHO of a reduction in the age of onset of psychosis. The goal of my thesis is to understand and examine interventions to mitigate selected AHO in those with a mental illness. The selected AHO examined by me are poor bone health, excessive body weight, substance use and earlier age of onset of psychosis.

In Chapter 2, I investigated the effect of adding and/or switching to aripiprazole on markers of bone turnover and gonadal hormones. A number of studies have now reported that antipsychotic medications are associated with an increased risk of reduced bone mass, osteoporosis and metabolic syndrome. Aripiprazole is a partial agonist at the D_2 receptor and hence does not elevate levels of the hormone prolactin. The work presented in Chapter 2 demonstrates that switching to and/or addition of aripiprazole in patients who have been on other antipsychotic medications significantly reduces the AHO of high prolactin. In terms of the effects of the same intervention on the AHO of poor bone health, I also observed that markers of bone turnover changed in a beneficial manner, only in those switching to aripiprazole.

Chapter 3 has three subsections on the AHO of metabolic syndrome and excessive weight/ high BMI (body mass index). In 3.1, I compared groups of patients treated at either of two metabolic treatment clinics and a control group. My main outcome measure was weight loss over time. The population treated at a metabolic clinic with a dietician was the only one with significant weight loss. The work in Chapter 3 highlights the importance of access to a dietician in reversing the AHO of excessive weight in those with a severe mental illness. I also present two reviews in Chapters 3.2 and 3.3 highlighting the relationship between cannabis, alcohol, and measures of metabolic dysfunction.

In Chapter 4, I examine a genetic variant and lifetime cocaine use in psychiatric patients. In a group of Canadian Caucasian patients who were referred for psychosis, I observed that the likelihood of having a history of cocaine use was significantly related to the G/G genotype of *COMT* rs4680 [G>A], even after adjustment for relevant covariates. *COMT* rs4680 [G/G] had a stronger association if the number of other lifetime substances was used in generating a new categorical variable for those with a history lifetime cocaine use.

Lastly, in Chapter 5, I examined the potential contributions from interaction of two single nucleotide polymorphisms, *BDNF* rs6265 [G>A] and *AKT1* rs2474732 [T>C], with gender and/or regular cannabis use for an effect on age of onset of psychosis in Canadian Caucasian patients. I observed a trend for *BDNF* rs6265 [A/A] by gender interaction on the age of onset of psychosis when adjusted for age of regular cannabis use.

Preface

This thesis is an original work by Rohit J. Lodhi. One of the research projects in this thesis received research ethics approval from the University of Alberta Research Ethics Board:. Project Name "Efficacy of a metabolic syndrome clinic within psychiatry: A retrospective chart review", No. Pro00066992, 11 August 2016.

Chapter 2 of this thesis, has been published as: Lodhi R.J., Masand S., Mir A., Shivakumar K., McAllister V.D.M., Young L.C., Heald A.H. and Aitchison K.J. Change in bone turnover markers in an aripiprazole add-on or switching study. *Schizophrenia Research*, vol. 170, issue 2-3, 245-251. I was responsible for the data analysis as well as the manuscript drafting, revising and final submission. Dr. Katherine J. Aitchison was the supervisory author and responsible for study design, project management, data collection, data analysis and manuscript drafting, revising and approval of final submission. Dr. David Broadhurst provided advice for the statistical analysis involved in this paper. Bristol-Myers Squibb Pharmaceuticals Limited funded this study; however, no grant funding was used for antipsychotic prescriptions and the company played no role in the analysis or interpretation of the data and manuscript preparation. Dr. Katherine J. Aitchison was on the UK Core Steering Group for Abilify® (Aripiprazole) until 2011 and prior to 2011 received consultancy fees from Bristol-Myers Squibb and Otsuka Pharmaceuticals Limited.

A version of Chapter 3.1 of this thesis has been presented as a conference poster as Lodhi R.J., Lee D., Roper L., Chiu J., Aitchison K.J. Efficacy of a metabolic management program within a psychiatric service in Alberta. 67th Annual Conference of the Canadian Psychiatric Association, Ottawa, 14-16 September 2017. Interim results from the same chapter were

presented by me as a keynote speaker at the 2017 Alberta Psychiatric Association Scientific Conference, 30th March – 2nd April 2017, Banff. Dr. John Lind was consulted for the statistical analysis conducted in Chapter 3.1.

Relevant to the methods section of Chapters 4 and 5, is the publication attached as Appendix 3. The publication is: Lodhi R.J., Wang Y., Rossolatos D., Macintryre G., Crocker C., Ren H., Dimitrijevic A., Bugbee D.A., Loverock A., Majeau B., Sivapalan S., Newton V.M., Tibbo P., Purdon S.E., Aitchison K.J. Investigation of the *COMT* Val158Met variant association with age of onset of psychosis, adjusting for cannabis use. Brain and Behavior, Volume 7, Issue 11, e00850 (open access). The study sample, data and biosample collection methods, and methods for genetic analyses presented in these chapters of my thesis, overlap or are similar to the relevant sections of this paper as finalized by me. The Appendix 3 publication was based on the laboratory work and MSc thesis of David Rossolatos.

Chapters 4 and 5 include results from a selected group of patients that participated in a larger genetic study of schizophrenia. Clinical data collection for these studies was funded through the following: A Canadian Institutes of Health Research grant entitled 'NPAS3 variants in schizophrenia and other psychoses'. The principal investigator for this grant was Dr. Diane W. Cox, Co-Applicants Dr. Gina Mcintryre, Dr. Scot E. Purdon, and Dr. Phil Tibbo, which after Dr. D. Cox's retirement was administered by Dr. K. G. Todd. A Nova Scotia Health Research Foundation Establishment grant to Dr. Phil Tibbo (with Dr. Scot Purdon and Dr. Katherine J. Aitchison amongst others), and the Bebensee Schizophrenia Research Unit based at the University of Alberta administered by Dr. Scot E. Purdon also contributed to clinical data

collection costs. Clinical evaluations and sample collection in Edmonton were undertaken with the support of the Alberta Hospital Edmonton Neuropsychology Department. Dr. Russ Greiner was consulted for survival analyses performed in the Appendix 3 publication.

For the studies presented in Chapters 4 and 5, genotyping of *COMT* was conducted by The Applied Genomics Core (TAGC; University of Alberta), funded by the above, and in addition by the Aitchison laboratory. All other genotyping and data analysis were conducted by the Aitchison laboratory. The Aitchison laboratory work was funded by an Alberta Centennial Addiction and Mental Health Research Chair fund (Government of Alberta, Canada) held by Dr. Katherine J. Aitchison and infrastructure grants (Canada Foundation for Innovation and Alberta Innovation and Advanced Education to Dr. Katherine J. Aitchison).

Dedication

I would like to dedicate this thesis to my family, who has been very supportive of my graduate studies over the last four years. I would also like to dedicate this to patients everywhere who participate in research so that the scientific community can improve health and quality of life for everyone, now and in the future.

Acknowledgement

This thesis would not have been possible without the help and guidance of many individuals. First, I would like to acknowledge Dr. Katherine J. Aitchison, my principal supervisor, for her steadfast support, guidance and mentorship throughout my graduate studies. I believe I have acquired a broad set of research skills through the work that I have done under her supervision. The most important lesson that I learned from my co-supervisor, Dr. Scot E. Purdon is the importance of structured clinical measurements and hypothesis generation in research. I thank Dr. Esther Fujiwara for being a part of my supervisory committee and guiding me through the steps required for completion of my PhD program. I thank Dr. David Broadhurst for introducing me to and teaching me how to use STATA statistical analysis software. Dr. John Lind and Dr. Russ Greiner have kindly provided time to me from their busy schedules to support me in statistical analyses related to my thesis. My metabolic research would not have been possible without the support of Drs. John Chiu, Pierre Chue and Jan Banasch. I also thank Dr. Purdon and Dr. Aitchison for guidance on an earlier metabolic syndrome project, not included in my thesis. I would also like to thank other members of the Aitchison Laboratory, in particular Leslie Roper, Dawon Lee, Dr. Sudhakar Sivapalan, Yabing Wang, Hongyan Ren, Rita Whitford and David Rossolatos, the Neuropsychology department at Alberta Hospital Edmonton, the Alberta Centennial Addiction and Mental Health Research Chair in Mental Illness and Addictions held by Dr. Katherine J. Aitchison, Alberta Centennial Addiction and Mental Health Research Chair Research Partnership Committee, the University of Alberta Department of Psychiatry, Tara Checknita, the University of Alberta Doctoral Recruitment Scholarship awarded to me, the Edmonton Zone Medical Staff Association, Dr. Phil Tibbo, Dr. Candice Crocker, Dr. Gina Mcintyre, nursing and administrative staff at the University of Alberta Hospital Department of Psychiatry and at the Edmonton Mental Health Clinic.

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Glossary of Terms

AHO – Adverse health outcome or outcomes

AHS – Alberta Health Services

AKT1 – RAC-alpha serine/threonine protein kinase or Protein Kinase B

AoP – age of onset of psychosis

ARCU – age at regular cannabis use

BDNF – Brain-derived neurotrophic factor

BMD – bone mineral density

BMI – body mass index

BSAP – bone specific alkaline phosphatase

CB_1 – Cannabinoid receptor 1

CB_2 – Cannabinoid receptor 2

CBT – cognitive behavior therapy

CCSA – Canadian Centre on Substance Use and Addictions

COMT – catechol-*O*-methyltransferase

CPZE – chlorpromazine equivalents

CVD – Cardiovascular Disease

DAT – Dopamine transporter

DEXA – dual energy X-ray absorptiometry

ECS – endogenous cannabinoid system

EMH – Edmonton Mental Health Clinic

EPSE – Extrapyramidal side effects

FBG – fasting blood glucose

FEP – first episode psychosis

GAF – Global Assessment of Functioning

GLMM – generalized linear mixed models

HDL – high density lipoproteins

HOMA- IR – Homeostatic model assessment for insulin resistance

HR – Hazard ratio

hs-CRP – high sensitivity C-reactive protein

IDF – International Diabetes Federation

IL-1Ra – interleukin 1 receptor antagonist

LDL – low density lipoproteins

LR – Log-rank test

MD – metabolic dysfunction

MetS – Metabolic syndrome

MMP – Metabolic management program

NCEP – National Cholesterol Education Program

NTX – Type 1 collagen cross linked N-telopeptide

OR – Odds ratio

SMI – Severe Mental Illness

TAU – Treatment as usual

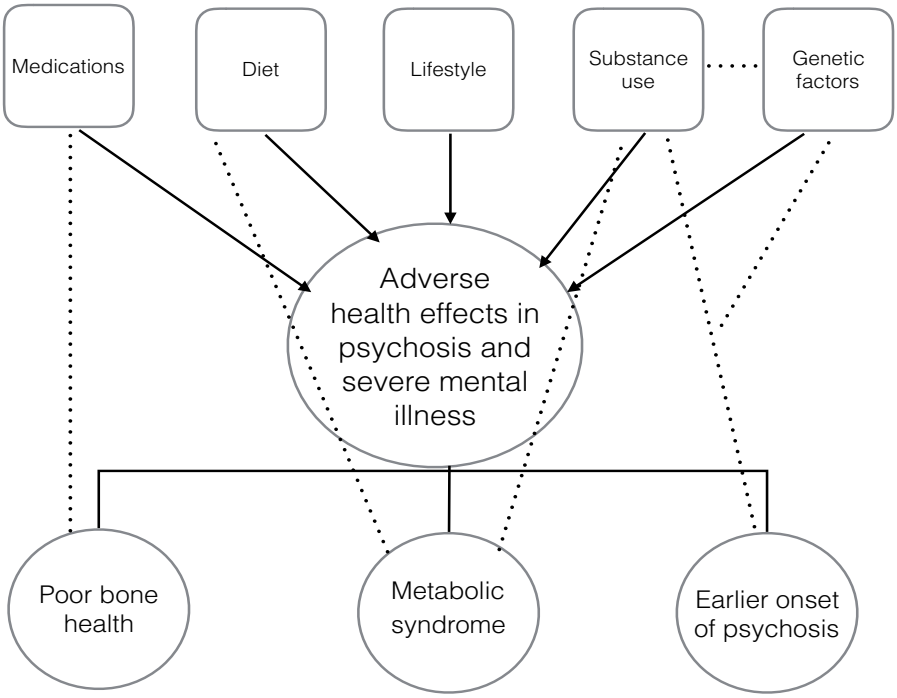
THC – Tetrahydrocannabinol

UAH – University of Alberta MMP site

CHAPTER 1. INTRODUCTION

1.1 OBJECTIVES OF MY THESIS

Figure 1.1 Diagrammatic representation of the components and focus of my thesis



Black arrows connect predictors to adverse health outcomes (physical and psychological) in psychosis and severe mental illness and black lines indicate the outcomes studied in my thesis

The dotted lines represent the relationships studied in my thesis using a clinical study or review of literature

The goal of my dissertation is to understand and treat physical or psychological adverse health outcomes (AHO) occurring in those with severe mental illness (predominantly psychosis) in association with medication or substance use. Two specific examples of antipsychotic AHO on physical health are poor bone health and metabolic syndrome (MetS), and I present studies that examine interventions mitigating these. Considering that substance use is independently associated with AHO, I have examined the role of a genetic factor in lifetime cocaine use, in a group of psychiatric patients, most of whom have psychosis. Lastly, cannabis is associated with an AHO of reducing the age of onset of psychosis (AoP), and I examine the role of genetic variants in this relationship.

1.1.1 Objective 1

Chapter 2 of my thesis explores the effect of antipsychotic change on markers of bone turnover. The goal was to see how aripiprazole, a drug wit D_2 receptor partial agonism, changes markers of bone turnover and gonadal hormones. My role in this scientific project began after clinical data collection was complete. Specifically, I edited and re-organized the dataset. Data were in the form of repeated measures and this required me to learn statistical methods such as repeated measures ANOVA and a generalized linear mixed model. After analyzing the data, I then drafted the manuscript and took it to completion for publication (Lodhi R.J, Masand S, Mir A, Shivakumar K, McAllister V.D.M, Young L.C, Heald A.H and Aitchison K.J. Change in bone turnover markers in an aripiprazole add-on or switching study. *Schizophrenia Research*, vol. 170, issue 2-3, 245-251). The same manuscript is the first study of my thesis (Chapter 2).

1.1.2 Objective 2

MetS is a significant AHO seen in psychosis and requires adequate treatment. In the Alberta Health Services (AHS), Edmonton Zone, there is a dedicated metabolic treatment program that provides treatment for MetS. In Chapter 3.1 of my thesis, I examine the efficacy of this program in reducing weight, one important indicator of the severity of MetS. This study highlighted an important question: Would intervention from a MetS treatment clinic have efficacy and would input from a dietician provide an advantage in weight loss treatment? Weight gain, a marker of metabolic dysfunction, is usually at the top of the list of AHO's considered most troublesome by patients and psychiatrists alike (Llorca et al., 2017). Strategies to reduce this AHO include switching to another antipsychotic (Weiden et al., 2007). My role in this project included: study design and conception, working with other team members to obtain ethics and other operational approvals, data collection, data analysis and drafting the manuscript in Chapter 3.1. Chapters 3.2 and 3.3 contain reviews that I conducted of the roles of cannabis and alcohol in MetS/dysfunction, respectively.

1.1.3 Objective 3

Substance use in the general population can lead to significant health problems and the same effects are seen in psychiatric patients. For example, cocaine use is associated with strokes, ischemia, myocardial infarction, etc. In Chapter 4, I examine the factors associated with the likelihood of having a history of cocaine use, in a patient group, the majority of whom had psychosis as their main diagnosis. The primary variable of interest amongst the associated factors was the single nucleotide polymorphism *COMT* rs4680 [G>A].

1.1.4 Objective 4

In Chapter 5 of my thesis, I present a study of a clinical side effect of cannabis i.e., an earlier onset of psychosis. I explore if certain gene variants (*BDNF* rs6265 [G>A]and *AKT1* rs2494732 [T>C]) moderate the association between onset of psychosis, cannabis and gender. My role in this project was similar to the role I played in the project in Chapter 2.0. The statistical methods that I learned to use for this chapter were linear regression, ANOVA and survival analysis.

1.2 Severe mental illness

The term ‘severe mental illness’ (SMI) usually refers to psychological conditions that result in significant disability and cause impairment in functional and occupational activities. SMI is often used interchangeably with other terms such as chronic mental illness, serious mental illness, persistent mental illness and severe and persistent mental illness. Persons with lived experience of these conditions and advocacy groups have raised objections to the use of such terms because they imply that these conditions are always chronic or untreatable in nature (Goldman et al., 2006). In 1987, the National Institute of Mental Health defined severe mental illness based on: diagnostic criteria – non-organic psychosis and personality disorder, duration – long history of hospitalizations or outpatient treatment, disability – including dangerous or disturbing behavior and impairment – moderate in work and mild in basic needs (National Institute of Mental Health, 1987; Parabiaghi et al., 2006). Numerous definitions of SMI and related terms have been created by other groups and generated inconsistency. Various operational definitions of SMI have also been suggested. For example, one group defined SMI based on a diagnosis of psychosis over two years of service use and a Global Assessment of Functioning (GAF) score of less than or equal to 50. Another definition was the same except that it excluded diagnosis (Ruggeri et al., 2000). Comparing 17 definitions of ‘severe and persistently mentally ill’ in the same group of patients, the authors found estimates of the condition varying from 4% to 88% (Schinnar et al., 1990). The U.S. Congress preferred and defined the term ‘serious mental illness’ in the Alcohol, Drug Abuse, and Mental Health Administration Reorganization Act, 1992. According to the Act, SMI adults are aged 18 and over, who currently or at any time in the past year suffer from a condition defined in the DSM-III-R and resulting functional impairment that substantially interferes with or limits

major life activities. Schizophrenia (psychosis) and bipolar disorder are commonly cited conditions under most definitions of SMI.

1.3 Psychosis

The exact origin of the term 'psychosis' is unclear; however, its earliest use may have been by Cansatt in 1841 (Burgy, 2012) and later by an Austrian psychiatrist Ernst von Feuchtersleben in 1845 (Beer, 1995; Beer, 1996). The concept of psychosis is sometimes difficult to define. Frequently, psychosis is described as 'being out of touch with reality'. Using this description, types of symptoms of psychosis can include normal perceptual distortions. Another characteristic of psychosis is 'lack of insight'. An example of this can be in obsessive compulsive disorder when patients lose insight into the nature of their symptoms. Psychotic symptoms can therefore be defined widely or narrowly, but the contemporary definition of psychosis typically includes delusions, hallucinations, disorganized speech, disorganized behavior and negative symptoms. Psychosis, therefore, occurs mainly within the following DSM 5 diagnoses: schizophrenia, schizoaffective disorder, delusional disorder and mood disorders with psychotic symptoms (American Psychiatric Association, 2013). Schizophrenia is associated with significant disability. It is in the top 25 causes of disability and has been estimated to be associated with an economic burden of at least 0.2 to 1.65% of a country's Gross Domestic Product (Chong et al., 2016). Substance-induced psychosis can present with symptoms that are similar to acute schizophrenia but is characterized by resolution of the symptoms after use of the substance in question is ceased. In Chapters 4 and 5 of my thesis, psychosis includes the diagnosis of substance-induced psychosis.

1.4 Adverse health outcomes in mental illness and psychosis

Severe mental illness (SMI) can affect the risk of mortality. Mortality rates have been increasing over the last few decades in patients with severe mental illness (Saha et al., 2007). According to a meta-analysis, having SMI increases the relative risk of mortality by 2.2 compared to a matched group without such an illness (Walker et al., 2015). Within various psychiatric diagnoses, patients with psychosis are affected most, and have a significantly elevated mortality risk compared to other diagnoses such as depression, bipolar disorder and anxiety (Walker et al., 2015).

The median reduction in life expectancy due to severe mental illness was 10.1 years in the meta-analysis by Walker et al. (2015). Such a reduction translates into a 20% reduced life expectancy in schizophrenia compared to the general population (Hennekens et al., 2005). The causes of excess mortality rates and reduced life expectancy include physical illness and suicide (Kredentser et al., 2014). When compared to the general population, patients with psychosis fare poorly in these physical symptoms: obesity, diabetes, MetS and cardiovascular disease (Heald, 2010). Heart disease is one of the leading causes of premature death in schizophrenia (Hennekens et al., 2005). If a patient with SMI has a myocardial infarction, he or she is more likely to die than someone without an SMI (Boden et al., 2014).

Causes of adverse health outcomes (AHO) in psychosis are many, and they include lifestyle factors such as diet, exercise and substance use, psychiatric and non-psychiatric medications, underutilization of treatment programs by psychiatric patients and issues with medication compliance. Substance use is, therefore, a modifiable risk factor for many health outcomes and should remain a key focus.

1.5 Adverse health outcomes associated with antipsychotics in psychosis

While psychosis itself significantly affects physical and psychological health, antipsychotic medications also contribute to adverse health effects (Young et al., 2015), which can impact the quality of life of an individual (Ritsner et al., 2002). Antipsychotic treatment-emergent adverse effects impose a significant burden on health (Bhavnani et al., 1996), and the prevalence of such side effects increases with the degree of polypharmacy (Westaway et al., 2016). The list of side effects that can occur with antipsychotics is long. Some selected side effects that can commonly or significantly affect the quality of life and health of individuals are listed in the following.

Cardiovascular system

Lin et al. (2014) reported transiently increased risk (OR = 2.52) of acute myocardial infarction in those who are taking antipsychotic medications compared to none. The authors hypothesized that this is mediated by D_3 receptor occupancy (Lin et al., 2014). Other cardiovascular effects of antipsychotics include QT_c prolongation (Takeuchi et al., 2015), torsades de pointes (Takeuchi et al., 2015) and clozapine-related cardiomyopathy or myocarditis (Alawami et al., 2014). Patients on antipsychotics with a high binding affinity of M_1 muscarinic and $\alpha 2$ adrenergic receptors are at an elevated risk of cerebrovascular accidents, i.e., "stroke" (OR = 1.60) (Wu et al., 2013a). The risk of cerebrovascular accidents is higher with second generation antipsychotics (SGA) in elderly patients (Mehta et al., 2010). This increased risk has led to restrictions in use of antipsychotics in the elderly, although some have questioned the need of a 'blanket ban' and the strength of the evidence for association with stroke (Barak et al., 2007).

Reproductive system

In the reproductive system, a common effect of most antipsychotic medications is an elevation of the levels of the hormone prolactin. There is good evidence that antipsychotic-related elevated prolactin levels can lead to reduced libido, sexual function, and fertility (Gonzalez-Blanco et al.,

2016). Sexual dysfunction occurs in those on antipsychotics for a number of reasons, and the prevalence of the same varies between 16 to 60%, depending on the medication (Serretti et al., 2011).

Skeletal system

Poor bone health is associated with antipsychotic use and hyperprolactinemia, and I shall discuss this more in detail in section 1.6

Central Nervous System

In the central nervous system, extrapyramidal side effects (EPSE) of antipsychotics can cause long-term difficulties. However, EPSE in the form of dyskinesias (OR=3.59) and Parkinsonism (OR=5.32) have also been seen at higher rates in antipsychotic-naïve subjects compared to controls (Koning et al., 2010). EPSE may be more common in those who are on older antipsychotics or first-generation antipsychotics compared to the newer medications. Tardive dyskinesia is a movement disorder that is difficult to treat and is often persistent (Carbon et al., 2017). The prevalence of other EPSE at some point in treatment with antipsychotics varies, approximate values being 65% for Parkinsonian symptoms, 31% for akathisia and 2% for acute dystonia (Muscettola et al., 1999). Factors affecting the risk of EPSE include age (Jabs et al., 2003), gender (Barbui et al., 2005), dementia, dose of antipsychotic, genetic variants (Koola et al., 2014; Koning et al., 2012) and comorbid substance use (Maat et al., 2008).

Some studies have reported an increased risk of mortality with antipsychotics in certain situations. In a study of 5391 Parkinsonism patients who died between 2002 and 2008, atypical antipsychotic exposure increased the odds of death (OR=2.8) (Marras et al., 2012). Other studies have reported an increase in the risk of mortality associated with antipsychotic use in Parkinson's

disease (hazard ratio=2.35) (Weintraub et al., 2016). Inpatients admitted for neuroleptic malignant syndrome, an EPSE, can have mortality rates of 3.3% for typical and 2.4% for atypical antipsychotics (Nakamura et al., 2012).

1.6 Altered bone metabolism in patients on treatment for psychosis

1.6.1 Bone structure

Bone structure varies in different parts of the body but is typically described in terms of the parts of a long bone: diaphysis, metaphysis, and epiphysis. The diaphysis is a tube-shaped structure between the ends of a bone. The central part is hollow and called the medullary cavity. This central part consists of yellow bone marrow. The inner membranous lining of the medullary cavity is called the endosteum. The outer section of the diaphysis is comprised of the dense and hard cortical bone. The metaphysis refers to the ends of the diaphysis that broadens before the epiphysis and contains spongy cancellous bone. The end of the bone is called the epiphysis, which also consists of spongy bone and in young people this is separated from the metaphysis by the growth plate or physis. The growth plate, also called the epiphyseal plate, is made of cartilage during the growth period, and this section becomes the epiphyseal line after maturation. Red marrow is seen mainly in the flat bones. The outermost layer of bone is called the periosteum. The periosteum is a fibrous membrane that contains the vascular and lymphatic structures that nourish the bone. Histologically, bone predominantly consists of bone cells surrounded by a mineralized extracellular bone matrix (osteoid). The bone matrix is roughly 25% water, 25% collagen and 50% crystallized minerals. Inorganic salts in the bone matrix, including hydroxyapatite and calcium carbonate, provide hardness to the bone. The organic component of bone consists of type 1 collagen, glycosaminoglycans and proteoglycans. Type 1 collagen is embedded in the glycosaminoglycan gel that bonds calcium, and it consists of two α1 chains and one α2 chain.

Bone cells consist of osteoprogenitor cells, osteoblasts, osteoclasts and osteocytes (Lowe et al., 2015). Osteoprogenitor cells are primitive mesenchymal or stem cells that produce osteoblasts. Osteoblasts synthesize the organic part of the extracellular bone matrix, and most become inactive spindle cells in the mature bone. Osteoblasts trapped in the osteoid differentiate into osteocytes. Osteoclast progenitor cells using the RANK ligand (receptor activator of nuclear factor κ B) and macrophage colony stimulating factor (M-CSF) combine to form osteoclasts, the cells responsible for bone resorption.

1.6.2 Bone metabolism

Bone is a very active organ and continues to 'remodel' throughout life (Frost, 1990). Every year two to five percent of cortical bone will remodel. Remodeling is the result of a balance of osteoblastic and osteoclastic activity. It is described as consisting of three phases – resorption, reversal, and formation. At the bone surface, osteoclasts utilize processes such as acidification and proteolysis to effect resorption of the bone matrix. Osteoblasts produce the matrix by depositing collagen, and this is mineralized at a rate similar to that of the matrix synthesis. Regulation of osteoblastic activity is partly through growth factors such as insulin-like growth factor (Canalis et al., 1993a) and transforming growth factor-beta (Canalis et al., 1993b). Hormones such as parathyroid hormone, thyroid hormone, growth hormone, insulin, progesterone, and prolactin also influence the activity of osteoblasts (Clement-Lacroix et al., 1999; Hadjidakis et al., 2006). The formation phase includes a period of prolonged rest before going back to resorption.

1.6.3 Markers of bone metabolism

Bone mineral density

Dual-energy X-ray absorptiometry (DEXA) is the gold standard for measuring bone density, although its usefulness to predict fractures has been questioned (Wainwright et al., 2005). DEXA scans provide a 'T-score' after measuring the bone mineral density (BMD), defined by the equation ((measured BMD – young adult population mean BMD) / young adult population standard deviation).

Bone turnover markers – formation and resorption

Formation markers

1. Serum bone-specific alkaline phosphatase (BSAP) is a specific marker of bone forming activity and it is synthesized by osteoblasts. BSAP is presumed to be involved in the calcification of the bone matrix.
2. Serum alkaline phosphatase can also be useful as indicator of bone formation. Alkaline phosphatase is the total amount from bone and other tissues such as liver. Serum alkaline phosphatase has been observed to be an acceptable alternative marker to BSAP for monitoring treatment response to bisphosphonates (Mukaiyama et al., 2015).
3. Osteocalcin is an osteoblast specific hormone, produced by the osteoblasts and released in the process of bone formation (Wei et al., 2015)
4. Procollagen Type 1 N-terminal propeptide (PINP) is a peptide derived from type 1 procollagen and it originates mostly from osteoblasts and fibroblasts. PINP has been useful as a bone formation marker (Vasikaran et al., 2011).

Resorption markers

1. Type 1 collagen C-terminal and N-terminal telopeptides are used as markers of bone destruction. Osteoclastic process cleaves these peptides from the telopeptide region of type 1 collagen. Type 1 collagen is a helical protein cross linked at the N-terminal and C-terminal

molecular ends. Type 1 collagen C- terminal telopeptide (CTX) is produced when resorption is mediated through a cathepsin K. When resorption at the same place is mediated by matrix metalloproteinase the marker C-terminal cross-linked telopeptide of type 1 collagen is formed. The peptide sequence of CTX is specific to the bone and created by oseteoclastic activity. The cross-linked N-telopeptide of type 1 collagen (NTX) is the peptide product of destruction at the N-terminal of type 1 collagen. CTX and NTX are small molecules that are secreted in the urine.

2. Hydroxyproline is an important part of collagen, providing stability to the bone and its levels in the urine have been used as marker of bone destruction.
3. Tartrate-resistant acid phosphatase (TRACP) is an enzyme that occurs in two isoforms, 5a and 5b. TRACP 5a is produced in macrophages and dendritic cells, while 5b is from osteoclasts. TRACP 5b is used a marker of bone destruction and osteoclastic activity (Halleen et al., 2006).

1.6.4 Osteoporosis, osteopenia, and low bone mass

Osteoporosis is a consequence of a higher amount of bone resorption compared to bone formation and is a complex systemic skeletal disease. There are multiple causes of osteoporosis. Estrogen deficiency in females following menopause is associated with increased activity of osteoclast compared to osteoblasts, causing osteoporosis. Low bone mineral density itself can have a significant impact on the risk of mortality. Men who have an accelerated form of bone mineral density (BMD) loss compared to those who maintain BMD at the hip have a higher risk of mortality that is not explained by factors such as comorbid conditions, weight change, or level of physical activity (Cawthon et al., 2017). The thirty-day mortality rate in elderly patients who have surgery for a hip fracture ranges from 5.3% to 6.3% (Tsang et al., 2017).

1.6.5 Bone health in psychosis

Psychiatric patients have reduced bone mass and higher rates of osteoporosis than matched subjects in the general population (Stubbs et al., 2014). In patients with a diagnosis of schizophrenia, high rates of osteoporosis are reported (Leucht et al., 2007). Psychiatric patients also suffer from higher rates of fractures and this can be interpreted as a consequence of poor bone health (Stubbs et al., 2015). Antipsychotic medications have been hypothesized to reduce bone density and cause osteoporosis (Kishimoto et al., 2012). The mechanism suggested is that blocking D_2 receptors causes increased prolactin levels and prolactin in turn causes hypogonadism. The consequence of hypogonadism is low estrogen, progesterone, and testosterone, leading to osteoporosis. Another hypothesis is that antipsychotics can directly affect bone cells, with or without hypogonadism (Motyl et al., 2017).

1.7 Metabolic syndrome in psychosis and SMI

1.7.1 Metabolic syndrome: definition

The World Health Organization (WHO), in 1998, was the first to formalize the definition of MetS (Alberti et al., 1998), followed by The National Cholesterol Education Programs Adult Treatment Panel III (NCEP-ATP III) in 2001. The NCEP-ATP III report identified the components of MetS as abdominal obesity, atherogenic dyslipidemia, high blood pressure, insulin resistance, and a proinflammatory and prothrombin states. The NCEP-ATP III MetS criteria are: waist circumference of > 102 cm for men or > 88 cm for women, ≥150 mg/dL (1.7mmol/L) triglycerides, high density lipoproteins (HDL) <40 mg/dL (<1.0 mmol/L) for men or <50 mg/dL (<1.3 mmol/L) for women, blood pressure (BP) of ≥130/≥85 mm Hg, and fasting glucose ≥110 mg/dL (≥ 6.1 mmol/L) (Expert Panel on Detection Evaluation and Treatment of High Blood

Cholesterol in Adults, 2001). The International Diabetes Foundation (IDF) differed in their definition of MetS from the NCEP-ATP III and mandated that out of the five criteria, ethnicity-adjusted abdominal obesity was necessary for a diagnosis of MetS (Alberti et al., 2005; International Diabetes Federation, 2005). The new harmonized criteria for MetS include ethnicity-adjusted elevated waist circumference, ≥1.7 mmol/L for triglycerides, HDL of <1.0 mmol/L for men or <1.3 mmol/L for women, BP of ≥130/≥85 mm Hg, and a fasting glucose ≥ 5.6 mmol/L (Alberti et al., 2009).

1.7.2 Effects of metabolic syndrome

MetS increases the risk of developing cardiovascular illness and diabetes (Bos et al., 2007). MetS increases the risk for a variety of other conditions or outcomes including subclinical hypothyroidism, snoring, poor sleep, polycystic ovary syndrome, colorectal cancer, mortality, post-surgical complications, chronic kidney disease, non-alcoholic fatty liver disease, vascular stiffness, osteoarthritis and accelerated cognitive and functional decline in the elderly (Viscogliosi et al., 2017; Lee et al., 2017; Chang et al., 2017; Thomas et al., 2007; Colicchia et al., 2018; Pan et al., 2018).

1.7.3 Metabolic syndrome and antipsychotics

Antipsychotics have been associated with metabolic dysfunction. The most rapid change in metabolic parameters usually occurs in drug naïve subjects at first antipsychotic usage. A number of studies have examined the evolution of metabolic difficulties soon after medication initiation.

The following are some cross-sectional studies that have studied the prevalence of MetS in first-episode psychosis (FEP). One investigation examined MetS in FEP in children and adolescents (O'Donoghue et al., 2014). One-third of this group had elevated cholesterol and

triglycerides, without a dose-response relationship. In this study, olanzapine and quetiapine had a higher triglyceride elevation than risperidone. In a separate small study of FEP, there was a 25% increase in MetS over six months, with significant abnormalities noted in abdominal circumference, cholesterol and fasting glucose (Martin Otano et al., 2013). There are geographical and cultural variations in the prevalence of MetS in FEP. A Brazilian study (Bensenor et al., 2012) found that in their cohort, 20.7% were obese, 29.3% had hypertension, 39.0% had dyslipidemia, 19.5% had MetS, and 1.2% had a >20% 10-year risk of coronary heart disease based on Framingham scores.

1.7.4 Mechanisms for psychotropic medication related metabolic dysfunction

The following describes some of the suggested mechanisms and risk factors for antipsychotic-associated metabolic dysfunction that includes weight gain.

Psychosis

It is likely that psychosis itself increases the risk of metabolic dysfunction since studies before the introduction of antipsychotic medications indicate that patients with schizophrenia had a higher incidence of abnormal glucose metabolism (Henneman et al., 1954). In fact, in studies as far back as 1922, abnormal glucose tolerance in 'dementia praecox' was reported (Lorenz, 1922), and in 1926 a higher prevalence of diabetes was reported for patients with this diagnosis in comparison to the general population (Kasanin, 1926). More recent studies have similarly described insulin resistance and increased leptin concentrations in antipsychotic-naïve patients with schizophrenia (Arranz et al., 2004).

Adiponectin

Adiponectin deficiency has been associated with metabolic dysfunction, atherosclerosis and coronary heart disease (Kato et al., 2006; Esfahani et al., 2015). GSK-3 site phosphorylation is related to induction of adiponectin gene expression (Park et al., 2004) and GSK-3 stimulation can increase the secretion of adiponectin (Chen et al., 2016). Lithium is an inhibitor of GSK-3 and can, therefore, affect adiponectin levels (Chen et al., 2016). In a human participant study, serum adiponectin levels were reduced in patients after six weeks of treatment with lithium (Soeiro-de-Souza et al., 2014). Another relevant medication that may be influence metabolic dysfunction by lowering adiponectin levels is sodium valproate (Akgun et al., 2017).

Appetite

Antipsychotic medications are known to increase appetite and this is more pronounced for second generation antipsychotic drugs. Mechanisms behind increased appetite can include H_1 receptor blockade. However, there are other pathways too. In an animal study, risperidone treatment resulted in a reduction in mRNA expression levels of appetite regulatory neurohormones such as proopiomelanocortin, agouti-related peptide and neuropeptide Y (Kursungoz et al., 2015). In a longitudinal double-blind parallel groups investigation, the likelihood of food craving and binge eating was close to 50% and 16.75%, respectively, with olanzapine, compared to 23.3% and 8.9% with clozapine (Kluge et al., 2007).

Inflammation

Elevated interleukin 1 receptor antagonist (IL-1Ra) levels are associated with insulin resistance, obesity, and hypertriglyceridemia. Levels of IL-1Ra and high sensitivity C-reactive protein (hs-CRP) were associated with obesity, and the levels of interleukin 6 (IL-6) were associated with obesity in women patients receiving clozapine (Klemettila et al., 2014). Other studies have

observed that patients with schizophrenia who are antipsychotic-naïve have higher rates of abnormal glucose tolerance and an associated increase in a related inflammatory marker IL-6 (Fernandez-Egea et al., 2009).

Leptin and leptin receptor

Leptin is a protein produced by fat cells and provides feedback to the appetite centers, suppressing food intake. Obese subjects have high leptin levels and it has been hypothesized that their suppressive feedback mechanism may be leptin resistant (Klok et al., 2007). Polymorphisms inconsistently associated with antipsychotic-related MetS or weight gain in the *LEP* promoter and *LEPR* include rs1137101[G>A], rs7799039 [G>A], rs1137100 [A>G] and rs8179183 [G>C]. For example, rs1137101 [G>A] was associated with MetS in schizophrenia (Roffeei et al., 2014). The rs77990309 (*LEP* -2548A/G) polymorphism in the promoter region of the *LEP* gene has been associated with clozapine and other antipsychotic-associated weight gain in adults and children (Kang et al., 2014; Mou et al., 2008; Calarge et al., 2009). However, there are studies that have reported no such association (Brandl et al., 2012). A meta-analytic study has implicated ethnic differences that may account for the inconsistent results (Shen et al., 2014). Appendix 2 of my thesis, includes a manuscript currently under review that I co-authored, that reports an association between baseline BMI standard deviation scores and change in the same in Arab children who were prescribed risperidone (Almandil N.B et al., 2017).

Serotonin

The 5-HT_{2C} receptor plays a role in the metabolic effects of antipsychotic-associated weight gain. A blunted olanzapine-induced hyperphagia and obesity response was seen in mice lacking $5HT_{2C}$ receptors (Lord et al., 2017). *HTR2C,* a gene encoding for the 5-HT_{2C} receptor is one of the few genes with single nucleotide polymorphisms of significant effect size for antipsychotic-associated

weight gain, according to one meta-analysis (Zhang et al., 2016b). Variants of the gene *HTR2C* have been associated with metabolic dysfunction (Bai et al., 2011; Mulder et al., 2009). No association between antipsychotic-related weight gain and *HTR3A* and *HTR3B* polymorphisms has been observed (Zai et al., 2016).

Histamine

H_1 receptor affinity has been positively correlated with weight gain across first and second generation antipsychotics (Kroeze et al., 2003). This observation has been replicated and is hypothesized to be the primary cause for weight gain (Matsui-Sakata et al., 2005). The effect of H_1 blockade on appetite is via stimulation of hypothalamic adenosine monophosphate-activated protein kinase (AMP kinase) (Kim et al., 2007).

Dopamine and metabolic dysfunction

The dopamine system has a role in energy metabolism. Obese subjects have been observed to have reduced D_2 receptor density and dopamine is associated with regulation of physical activity. D_2 receptor knockdown models show significantly lower physical activity than wild-type mice (Beeler et al., 2016). The deleterious metabolic effect of antipsychotics may be related to D_2 receptors, perhaps related to their effect on the insulin secreting pancreatic cells (Nash, 2017) and effects on the circadian clock (Freyberg et al., 2017).

Insulin

Excess weight is usually, but not always, related to insulin resistance. Cardiovascular risk factors are more likely to be present in a subset of obese individuals with insulin resistance (Keinanen et al., 2015). Early insulin resistance has been associated with the risk of weight gain in first-episode

psychosis patients (Keinanen et al., 2015). Amongst the antipsychotics studied in the Recovery After an Initial Schizophrenia Episode (RAISE) study, in first-episode schizophrenia spectrum disorders, olanzapine was significantly associated with high insulin and insulin resistance (Correll et al., 2014).

Fat to carbohydrate oxidation ratio

Substrate utilization patterns are indicative of the way our bodies utilize energy stores and can be estimated through measures such as a 24-hour respiratory quotient (RQ). RQ is the ratio of the volume of carbon dioxide produced to the volume of oxygen used (range is 0.7 to 1.0). A ratio of 0.7 indicates mixed fat use and 1.0 exclusive carbohydrate use (Gropper S.S. et al., 2009). A higher RQ (90^{th} percentile) increases the risk of 5 kg weight gain by 2.5 times. (Zurlo et al., 1990). In one FEP study, olanzapine treatment was associated with an increase in RQ by an average of 0.12, and was higher in those with weight gain of more than 5 kg (Graham et al., 2005). The authors indicate that they were not sure if the increase in RQ was primary or secondary to the weight gain.

Other risk factors

Additional risk factors for antipsychotic-associated weight gain/metabolic dysfunction include: female gender, younger age, non-white ethnicity, lower baseline body mass index, higher negative symptoms at baseline in psychosis, lack of smoking and early and rapid weight gain (Basson et al., 2001; Gebhardt et al., 2009; Kinon et al., 2005; Strassnig et al., 2007).

1.8 Cannabis, cocaine use, and adverse health outcomes

Substance use can independently cause or increase the risk of physical and psychological adverse health outcomes, and therefore reduction or cessation of substance use can potentially produce significant health benefits. Studying factors that affect the use of illicit substances may, therefore, provide some guidance to clinicians for targeting treatments or providing preventative strategies. Two substances of relevance to psychosis are cannabis and cocaine.

1.8.1 Adverse health outcomes of cocaine

Cocaine is a naturally occurring plant alkaloid present in the leaves of the coca bush that has a tropane ester chemical structure (Biondich et al., 2016). It is a highly addictive substance that inhibits dopamine internalization when it binds to the dopamine transporter (DAT) (Ritz et al., 1987; Boja et al., 1989). In the DAT, the binding pocket for cocaine is not the same as the binding pocket of dopamine (Merchant et al., 2012). Human and animal studies have contributed to our understanding of the detrimental effects of cocaine. Cocaine sympathomimetic effects in the form of elevated levels of peripheral adrenaline and noradrenaline are responsible for many of its adverse health outcomes. Alpha and β-adrenergic receptor stimulation can cause coronary ischemia due to arterial vasospasm. Mesenteric ischemia and stroke can result from vasospasm in the mesenteric and brain vascular supply. Other cardiovascular complications of cocaine use, in the short and long term, include tachycardia, cardiac arrhythmias, hypertension, myocarditis, infectious endocarditis, ventricular dysfunction, cardiomyopathy, *abruptio placentae*, increased arterial pressure and coronary vascular resistance, reduced coronary sinus blood flow and coronary arterial diameters (Perper et al., 1992; Lange et al., 1990). Increased intracellular calcium intake causes dysthermogenesis or hyperthermia and contraction band necrosis (Laposata, 1991). Cocaine can increase the risk of an acute myocardial infarction in healthy individuals within 60

minutes of use (Mittleman et al., 1999). The effects of cocaine can also be subacute too. Benzoylecgonine, the main metabolite of cocaine, has been related to a biomarker ('ST2', part of the interleukin 1 receptor family) for heart failure and cardiac stress (van Wijk et al., 2017). Cocaine users are more than twice as likely (OR=2.81) to show elevated cardiac troponin levels, a marker for cardiac injury compared to other drugs (Riley et al., 2017). Cocaine use during pregnancy can cause cardiac malformations, preterm delivery, low birth weight and small for gestational age babies (Offidani et al., 1995; Gouin et al., 2011; Hulse et al., 1997). Levamisole, an anthelminthic, is often seen as an adulterant in cocaine and this can cause leukopenia, agranulocytosis, and vasculitis (Larocque et al., 2012). Neuropsychological adverse effects include insomnia, reduced appetite, panic attacks, irritability, grandiosity, increased sociability, euphoria, tardive dyskinesia, seizures and psychotic symptoms. The prevalence of psychotic experiences at some point in cocaine-dependent people varies from 12%, as seen in clinical studies, to 34% or more in experimental studies (Roncero et al., 2012). In the long term, cocaine reduces the availability of D_2 like receptors by about 20% compared to before cocaine use (Nader et al., 2006). Greater duration of cocaine use is associated with increasing reduction in fractional anisotropy and gray matter volume (Lim et al., 2008).

1.8.2 Genetic factors in cocaine use/misuse

Like most addictions, cocaine misuse is likely to be multifactorial with interactions between genetic and environmental factors playing an important part (Kendler et al., 2000). Cocaine misuse has a relatively high heritability. In fact, twin studies that have examined cocaine use and environmental and genetic contributions have concluded that genetic factors could be an important factor (Kendler et al., 2000). This is somewhat in contrast to the importance of genetic factors relative to social and familial factors in other addictions (i.e., nicotine, alcohol, and cannabis),

where genetic factors are less important for initiation or early use (Kendler et al., 2008). In my thesis, the focus is on the association between lifetime cocaine use and the *COMT* rs4680 [G>A], and there are no prior studies available for this in psychiatric patients. *COMT* rs4680 [G>A] is a single nucleotide substitution (G to A) that results in a valine to methionine substitution at codon 158 for the catechol-*O*-methyltransferase (COMT) protein. *COMT* rs4680 [G>A] is relevant to other addictions too. Therefore, I reviewed the literature for associations between *COMT* rs4680 [G>A] with dependence on other substances as well. The results are presented in Table 1.1. The search was done only for dependence and some closely related phenotypes such as personality traits (novelty seeking). It highlights the variation in prior findings to date.

Table 1.1 Review of results for association of rs4680 with various addiction phenotypes

First Author	Year	Ethnicity/Country	Phenotype	Result
Lodhi	2017	CAU (Canadian)	Lifetime cannabis use	Positive association G/G
Malhotra	2016	S/E Asian	Alcohol dependence	Positive association A/A
Celorrio	2016	CAU (Spain)	Excessive alcohol consumption	No association
Van Breda	2015		Novelty seeking	Positive association A/A
Levran	2015	African	Opioid dependence	Negative association A/A
Ermis	2015	Turkey	Cannabis use, psychiatric patients	Positive association G/G
Nedic Erjavec	2014	CAU(Croatia)	Alcohol dependence	Positive association G/G
Van der Knaap	2014	CAU(Dutch)	High cannabis use, MB-COMT methylation	Negative association A/A
Hori	2014		Novelty seeking	Positive association A/A
Schellekens	2013	CAU(Dutch)	Alcohol dependence, ACE	Positive association A/A
Nedic	2011	CAU	Suicidal ideation, Alcohol dependence	Positive association A/A, men
Kibitov	2010	CAU (Russian)	Alcohol dependence in opioid users	Positive association A/A
Salo J	2010	CAU (Finish)	Reward dependence	Negative association A/A
Demetrovics	2010	CAU (Hungary)	Novelty seeking, in opioid users	Positive association A/A
Bousman	2010	CAU	Stimulant dependence	No association
Oosterhuis	2008	Mixed	Opioid dependence, women	Positive association A/A
Lohoff	2008	African	Cocaine dependence	Positive association A/A
Baransel Isir	2008	Turkey	Cannabis dependence	Positive association G/G, A/G
Foroud	2007	EUR	Alcohol dependence	No association
Samochowiec	2006	CAU(Polish)	Alcohol dependence	No association
Sery	2006	CAU(Czech)	Alcohol dependence	Positive association G/G
Hosak	2006	CAU (Czech)	Novelty seeking	Positive association A/A
Kweon	2005	Asian (Korea)	Alcohol dependence	No association
Cao	2003	Asian (Chinese)	Opioid dependence	No association
Hallikainen	2000	CAU(Finnish)	Alcohol dependence, antisocial personality	No association
Kauhanen	2000	CAU(Finnish)	Social drinking	Positive association A/A
Horowitz	2000	Israel	Opioid dependence	Positive association G/G
Tihonen	1999	CAU(Finnish)	Alcohol dependence, late onset	Positive association A/A
Vandenbergh	1997	CAU (US)	Polysubstance misuse	Positive association G/G

CAU- Caucasian

ACE – Adverse childhood experiences

S/E – South-East

1.8.3 Cannabis

Cannabis is a plant that was known to the ancient Indian and Chinese civilizations (Touw, 1981). *Cannabis sativa* is one of the oldest plants to be cultivated by human beings and has more than 60 phytocannabinoids which are low molecular weight lipophilic compounds (Russo, 2007). The effect of cannabis on metabolic function is likely to be mediated through the endogenous cannabinoid system (ECS). The ECS consists of endogenous cannabinoids, a catabolizing enzyme, and two G-protein coupled receptors, CB_1 and CB_2 (Munro et al., 1993; Mouslech et al., 2009). CB_1 receptors have a role predominantly in neurotransmitter release in the central nervous system, and CB_2 receptors participate mainly in peripheral cytokine release and function (Galiegue et al., 1995). The ECS is highly relevant to the cardiovascular, immune, reproductive, gastrointestinal and skeletal systems. It is not surprising then that the ECS also has a role in energy metabolism (Matias et al., 2006). CB_1 receptors are distributed widely in the brain, and some are strategically placed near structures relevant to appetite, and their stimulation by endocannabinoids increases appetite (Kirkham, 2005; Jamshidi et al., 2001). In fact, the role of CB_1 receptors appears to be integral to the vital function of feeding, with evidence that in newborn mice CB_1 receptor blockade results in a devastating effect on milk ingestion and subsequent growth (Fride et al., 2001). Based on such findings, a drug called ‘rimonabant' was manufactured that blocked CB_1 receptors and caused weight loss in humans. It was withdrawn from the market due to significant side effects such as depression, anxiety, suicidal ideations and nausea (Despres et al., 2006; Topol et al., 2010). Additionally, the ECS communicates with leptin and ghrelin, which are important hormones associated with appetite (Viveros et al., 2008; Jo et al., 2005; Kola et al., 2008). ECS has a role in energy metabolism (Simon et al., 2017). Since there is an endogenous pathway relevant to metabolic effects of cannabinoids, we can hypothesize that exogenous cannabinoids will also have metabolic effects.

1.8.4 Adverse health outcomes and cannabis

In Canada in 2012 there were 287 deaths and 55,813 years of life lost to disability caused by cannabis use (Imtiaz et al., 2016). Young people and men account for a higher proportion of the burden attributed to cannabis. Adverse health outcomes associated with cannabis are discussed in a limited manner in Chapter 3.2 of my thesis. Adverse health outcomes of cannabis can include: increased risk of motor vehicular accidents, stroke in younger users, reduced serum testosterone, reduced sperm motility and count, poor fetal development, and low birth weight (Whan et al., 2006; Pardo et al., 1985; Fischer et al., 2016; Gomez Ochoa, 2017). Synthetic cannabis has been associated with myocardial infarction, other complications and mortality (Mir et al., 2011; White, 2017).

One clinical adverse effect of cannabis use discussed and debated often is the increased risk of developing psychosis. Epidemiological data from Canada indicates that only a small number of subjects who use cannabis will develop psychosis (Fischer et al., 2016). At a population level, the increased risk of psychosis with cannabis use is weak (Hamilton, 2017). Here it is important to note that the relationship between psychosis and cannabis use could be bi-directional. A large study across 18 countries observed that psychotic experiences increase the odds of subsequent onset of tobacco (OR=1.5, 05% CI-1.2-1.9), alcohol (OR=1.3, 95% CI=1.1-1.6), and cannabis use (1.3, 95% CI=1.0-1.5) (Degenhardt et al., 2017). In the psychiatric literature, longitudinal studies suggest an association between adolescent cannabis use and psychosis (Volkow et al., 2016; Gage et al., 2016). However, there is a need for clarity on the magnitude of this effect and the factors that increase the strength of the cannabis-psychosis association. High-potency cannabis triples the risk of experiencing a psychotic illness in comparison to those without cannabis use (Di Forti et al., 2015). Variants in *COMT*, *BDNF*, *AKT1*, and *DRD2* have been suggested as moderators of the risk of transitioning to psychosis after cannabis use, however these

results have not been consistent (Colizzi et al., 2015; Caspi et al., 2005; Di Forti et al., 2012; Pelayo-Teran et al., 2012).

The association of cannabis with psychosis is not just about the risk of developing psychosis. Cannabis may reduce the age of onset of psychosis by about three years (Di Forti et al., 2014). The heritability estimate for the age of onset of psychosis is 0.33, indicating a contribution from genetic factors (Hare et al., 2010). Gene polymorphisms have been associated with the age of onset of psychosis. Some of the polymorphisms associated with the age of onset of psychosis are in the following genes: *DBH* (Barlas et al., 2012), *DRD2* (Voisey et al., 2012), *DRD3* (Renou et al., 2007), *PLA2G4A* (Nadalin et al., 2008), *EGF* (Hanninen et al., 2007), *PARK2* (Woolston et al., 2017), *NRN1* (Fatjo-Vilas et al., 2016), *MSI2* (Luan et al., 2017), *TGFB1* (Frydecka et al., 2015), *miRNA137* (Wang et al., 2014), *ITGB3* (Wang et al., 2013) *BDNF* (Suchanek et al., 2013), *UFD1L* (Ota et al., 2010), *ZNF4* (Takase et al., 2001), and *TBP* (Ohi et al., 2009). When cannabis and genetic variant interactions were studied regarding the age of onset of psychosis, a slightly different set of genes were seen to be relevant. In Chapter 5 of my thesis, I have explored the role of two single nucleotide polymorphisms *BDNF* rs6265 [G>A] and *AKT1* rs2494732 [T>C] in the age of onset of psychosis, taking into account the effect of gender and regular cannabis. An rs6265 by gender interaction has been associated with the age of onset of psychosis in a previous study (Decoster et al., 2011).

Age of onset has significant implications on clinical characteristics of psychosis. Typically, schizophrenia starts early in life (Barlas et al., 2012) and those who have a later onset may be a distinct group. Late or very late onset psychosis (after age 65) is perhaps more common in women than men (Stafford et al., 2017; Ramasamy et al., 2017), unlike psychosis that starts at a younger age. Age of onset of psychosis has been reported to be related to prognosis of illness. Adult onset

psychosis subjects are more likely to achieve early positive symptoms remission compared to those with adolescent-onset psychosis (Veru et al., 2016). Younger age of onset of psychosis is one of the factors that can increase the risk of treatment resistance (Demjaha et al., 2017). Chapter 5 of my thesis is an investigation of genetic factors, gender, and cannabis in the age of onset of psychosis.

CHAPTER 2. CHANGE IN BONE TURNOVER MARKERS IN AN ARIPIPRAZOLE ADD-ON OR SWITCHING STUDY

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Keywords

Bone markers, antipsychotics, NTXC, aripiprazole,

2.1 Abstract

Background: The association between mental illness and osteoporosis and fractures is particularly pronounced in psychotic disorders. Antipsychotic use has previously been described to affect bone density.

Method: A 52-week follow-up of patients switched to aripiprazole or with aripiprazole added on, conducting a specific analysis of markers of bone turnover: urinary NTX (a biomarker of bone resorption) and serum BSAP (a biomarker of bone formation). Baseline and serial measurements of bone markers NTX, BSAP and of hormones prolactin, oestrogen and testosterone were done at weeks 0 and 1, 2, 6, 12, 26 and 52, respectively.

Results: NTX concentration reduced over time but this did not reach significance in the whole group (log-NTX: β = -0.0012, p = 0.142). For BSAP the addition of or replacement with aripiprazole produced a significant reduction (log-BSAP: β = -0.00039, p = 0.002). Analysis with prolactin similarly showed a significant reduction (log-prolactin: β = -0.0024, $p < 0.001$); other hormones did not change significantly. Sensitivity analysis to compare the switchers to aripiprazole versus the "add-on" showed that the former group had a significant reduction in NTX.

Conclusions: We found that switching to aripiprazole was associated with changes in molecular biomarkers of bone resorption, indicating a more favourable profile for bone health

2.2 Introduction

Severe mental illness is associated with an increased risk of comorbid physical illness (De Hert et al., 2011b). The basic aetiology of these associated conditions is often three-fold: less than ideal lifestyle management, physiological or endocrine dysfunction related to the pathology underlying the specific mental illness, and physiological disruption induced by psychotropic medication.

Osteoporosis is one of the physical illnesses associated with severe mental illness, with schizophrenia being associated with particularly high rates of this condition and reduced bone density in both women and men (Hummer et al., 2005; Leucht et al., 2007; Wu et al., 2013b; Bolton et al., 2011; Kinon et al., 2013; Kishimoto et al., 2012). A meta-analysis found that rates of osteoporosis were two and half times more in patients with schizophrenia than controls, while rates of reduced bone density were twice as much (Stubbs et al., 2014). Incidence of fractures can be used as an indirect indicator of the reduced bone mineral density (BMD) that defines osteoporosis. In a large cohort of patients, Abel et al. (2008) observed a significant increase in relative fracture risk associated with psychotic disorders (RR 5.12 and 6.41 for females and males aged 45-74 years, respectively) that was not observed for the combined psychiatric disorder cohort (RR 1.90 and 1.4, respectively) (Abel et al., 2008). Stubbs et al. (2015) in a recently published meta-analysis confirmed higher occurrence of fractures in patients with schizophrenia compared to controls (Stubbs et al., 2015).

One major contributing factor to the accelerated trajectory towards osteoporosis observed in schizophrenia may be the use of prolactin-raising antipsychotics, which are independently associated with the incidence of hip fractures (Howard et al., 2007), and higher rates of bone pathology (O'Keane et al., 2005). Drug-induced hyperprolactinemia and associated dysfunction of the hypothalamo-pituitary-gonadal axis results in diminished concentrations of oestrogen and

testosterone (Halbreich et al., 2003; Meaney et al., 2004; Misra et al., 2004; Wyszogrodzka-Kucharska et al., 2006; Hummer et al., 2005). This can accelerate BMD decline. In this regard, O'Keane et al. (2005) observed that elevated prolactin concentrations were related to hypogonadism and low BMD (O'Keane et al., 2005) in young women with schizophrenia.

Mir et al. (2008) previously described a 26-week, open-label, intention-to-treat study of patients with schizophrenia who either switched from or had aripiprazole added on to their treatment in which they observed that switching/adding-on aripiprazole resulted in significantly reduced prolactin concentrations at 12 weeks ($p = 0.003$) and the decreased concentrations persisted to the end of the 26-week study period ($p < 0.001$) (Mir et al., 2008). In the data presented here, we report on the 52-week follow-up of the same patients switched to aripiprazole or with aripiprazole added on, conducting a specific analysis of markers of bone turnover. Our aim was to determine if the addition of and/or switch to aripiprazole resulted in maintenance of the reduction in prolactin concentration to the 52-week time point, favourably affected the biomarkers of bone resorption/formation, and effected any changes in oestrogen and testosterone concentration. In this study, urinary type I collagen cross-linked N-telopeptide (NTX), a marker of bone resorption, and serum bone-specific alkaline phosphatase (BSAP), a marker of bone formation (Singer et al., 2008; Wheater et al., 2013) were used. NTX and BSAP concentration reflect the degree of bone turnover at the whole body level.

To our knowledge, this is the first report of a prospective or longitudinal analysis of these biomarkers in patients with schizophrenia who had a change in antipsychotic treatment regime. We hypothesized that the change to medication profile would be associated with a reduction in prolactin and urinary NTX, and that there would be a change in BSAP.

2.3 Methods

Individuals were recruited and followed up as described in Mir et al. (2008) and Aitchison et al. (2011) (Aitchison et al., 2011; Mir et al., 2008) from the COAST Team (Croydon Early Intervention in Psychosis Service), the Croydon Rehabilitation Team, and Croydon Community Mental Health Teams. In brief, the inclusion criteria were subjects (male or female) in the age range 18 to 65 years, who had a psychotic illness and lived in the community (outpatients), and failed to respond adequately to another antipsychotic (either inadequate therapeutic response or intolerance). There were no restrictions in terms of other factors such as ethnicity. The exclusion criteria were pregnancy and breast-feeding.

An open-label add-on or switch to aripiprazole was offered to eligible participants. The starting dose at the commencement of the study was 10 mg for all subjects, but was reduced to 5 mg once the 5 mg tablets became available in the UK (January 2005). Details of the flexible dose titration schedule, cross taper or concomitant administration of other antipsychotic with aripiprazole and study group are detailed by Mir et al. (2008) and Aitchison et al. (2011).

Thirty-six subjects were referred and deemed eligible for participation. Of these, eight subjects refused participation and of the 28 included, one withdrew prior to the commencement of medication because of non-compliance with any of the medications that were offered. Therefore, 27 were included; however, analysable bone marker or hormone data were available for 26 patients. Eighteen subjects reached the end-point of the study at 52 weeks, while nine dropped out prior to 52 weeks (Aitchison et al., 2011, Figure 1). Four patients dropped out due to lack of improvement (at weeks 8, 18, 27, and 27), four patients dropped out due to non-adherence (at weeks 2, 5, 35, and 48) and one dropped out due to deportation from the country. Patients who were non-adherent were refusing to take any pharmacological treatment at the time of dropout and had a history of non-adherence.

Laboratory analyses

Serial measurements of prolactin, testosterone, and 17-β oestradiol were performed and reported to 26 weeks in the initial study by Mir et al. (2008), using the methods on the ADVIA Centaur immunoassay analyser (Siemens Diagnostics, Frimley, Surrey, UK) (Mir et al., 2008). Serum concentrations of BSAP, albumin, cholesterol, calcium (for which a corrected value was produced), urinary creatinine, and urinary NTX were also measured. The time points for all biological markers were: baseline (0 weeks), 1, 2, 6, 12, 26, and 52 weeks. In addition, for some patients who had been unable to attend the predetermined follow up time points, concentrations of biological markers were present at weeks 18, 22, 30 and 35. The blood draws and urine collection were done between approximately 12 p.m. and 3 p.m., providing relative consistency in timing of sample collection. All available data on the biological markers were used in the analysis.

NTX was measured in the samples using the osteomark assay, which is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA). NTX concentration was quantified spectophotometrically and calculated using a standard calibration curve. A urinary creatinine analysis of the NTX assay values was then used to correct for urinary dilution, producing values labelled 'NTX', expressed in nanomoles of bone collagen equivalents (nmol BCE) per millimole creatinine (mmol creatinine). BSAP was measured in the serum samples using an enzyme-linked immunosorbent assay (ELISA, Ostase, IDS, Boldon, UK).

Statistical analyses

Data were analysed using STATA version 13.1. Outliers for the outcome variables NTX, BSAP, prolactin, testosterone and oestrogen were analysed by creating simple box plots and those that were outside three times the interquartile range were excluded from the analysis. The exceptions to this were three patients who had data points for prolactin that although being outliers were

identified as clinically relevant; for example, one patient had prolactin concentrations of 220, 936, 289, 1002, 1322 and 106, with 1322 mIU/L being an outlier on the box plot. However, this outlier was included due to the fact that it was following a reasonable pattern in this patient.

Analyses of bone markers (NTX and BSAP) and hormones (prolactin, testosterone and oestrogen) were then conducted using the generalized linear mixed model analysis (GLMM) (Breslow et al., 1993), using the 'meglm' command. GLMM analysis is a good tool to analyse repeatedly measured data that accommodates for a variety of distributions of the dependent variable and for random effects in the analysis. By using GLMM, we were able to include 26 patients on whom information was available, despite some follow-up data being missing. GLMM analyses on the bone markers and hormones were done using the following settings: All dependent variables were log transformed and time (measured in weeks) was the within subject variable. For NTX and BSAP, the GLMM assessed fixed effects of the following factors; 'switch status' (comparison based on patients switching to aripiprazole versus patients on another antipsychotic together with aripiprazole), 'baseline medication' (categorized according to olanzapine, amisulpiride, risperidone, quetiapine, clozapine or zuclopenthixol), ethnicity (Caucasian, Black, Asian or Mixed), antidepressant status (no use versus use) and gender (male versus female), and the following covariates; study time in weeks ('week'), age and prolactin concentration. Individuals were treated as a random factor in the model. For the hormones, we used the same predictors used for the analysis of NTX and BSAP, but without prolactin. Predictive variables were checked for collinearity and post-estimation residuals were checked for normality using the pnorm, qnorm and histogram plots.

Sensitivity analysis was conducted for NTX and BSAP by repeating the analysis in the whole group after adding total dose of antipsychotics in terms of chlorpromazine equivalents

(TCPZE) as a predictor to the model. CPZE doses of atypical antipsychotics can be derived from different methods that can produce varying values for the same medication (Patel et al., 2013). For the CPZE of aripiprazole (7.5 mg), risperidone (2 mg), olanzapine (5 mg), quetiapine (75 mg) and clozapine (50 mg), we relied on the information provided by the reference commonly cited for such comparisons by Woods et al. (Woods, 2003). It is difficult to assess the CPZE for amisulpride, but we used 100 mg as equivalent, derived from relatively recent literature (Andreasen et al., 2010; Leucht et al., 2015; Patel et al., 2013). A chlorpromazine equivalent for the zuclopenthixol depot was 100 mg/week (Taylor, 2015). In addition, we ran the primary NTX and BSAP analyses in subgroups based on predictors of interest, i.e., gender and switch status.

2.4 Results

The clinical characteristics and demographics of the 26 patients who were included in the study are detailed in Table 2.1, with their medications (including non-antipsychotics) being listed in Table 2.2 Thirteen males (mean age 26.39, SD 6.67) and 13 females (mean age 28.69, SD 8.87) participated in the study.

Table 2.1 Demographic and clinical characteristics of patients where sufficient data were available and those not included or dropped out prior to two weeks of the study, from 28 patients, over 52 weeks of the study.

	Included N=26 (92.1%)	**'Drop-outs' N=2(7.9%)**
Sex		
Male, N (%)	13 (50)	1 (50)
Female, N (%)	13 (50)	1 (50)
Mean Age, years (SE)	27.53 (7.7)	23 (2.08)
Age range, years	18 - 45	18 - 45
Ethnicity		
White, N (%)	13 (57.7)	2 (100)
Black, N (%)	6 (15.4)	0 (0)
Asian, N (%)	3 (19.2)	0 (0)
Mixed, N (%)	1 (4.3)	0 (0)
Diagnosis		
Schizophrenia, N (%)	15 (57.7)	1 (50)
Schizoaffective, N (%)	6 (15.4)	0 (0)
Bipolar Affective Disorder, N (%)	1 (3.8)	0 (0)
Psychotic Depression, N (%)	6 (23)	1 (50)
Years of psychotic illness		
<1 year, N (%)	0	0 (0)
1-3 years, N (%)	7 (26.9)	0 (0)
3-5 years, N (%)	7 (26.9)	1 (50)
5-7 years, N (%)	5 (19.3)	1 (50)
7-9 years, N (%)	2 (7.7)	0 (0)
9-11 years, N (%)	2 (7.7)	0 (0)
>11 years, N (%)	3 (11.5)	0 (0)
Antipsychotic prior to switching (Baseline medication)		
Amisulpride, N (%)	3 (10.7)	1 (50)
Risperidone, N (%)	8 (28.5)	1 (50)
Quetiapine, N (%)	3 (10.7)	0 (0)
Olanzapine, N (%)	10 (35.7)	0 (0)
Clozapine, N (%)	1 (3.5)	0 (0)
Zuclopenthixol, N (%)	1 (3.5)	0 (0)

Table 2.2 Details of antipsychotics prescribed for subjects included in the study (with Bone Markers data at baseline), at baseline, week 12, week 26 and week 52 of the study, with concomitant medications (one subject per row of the table)

Week 0		Week 12		Week 26		Week 52	
Antipsychotics	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**
Amisulpride 150 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg
Risperidone 2 mg	Clonazepam 1 mg	Aripiprazole 15 mg		Aripiprazole 15 mg, risperidone 1 mg		Aripiprazole 15 mg	
Quetiapine 150 mg	Lorazepam 0.5 mg	Aripiprazole 5 mg, quetiapine 400 mg		Aripiprazole 5 mg, quetiapine 500 mg		Aripiprazole 5 mg, quetiapine 500 mg	
Risperidone 6 mg	Procyclidine 5 mg	Drop out		Drop out		Drop out	
Olanzapine 15 mg	Mirtazapine 30 mg	Aripiprazole 20 mg Olanzapine 10 mg	Mirtazapine 30 mg	Aripiprazole 20 mg, olanzapine 10 mg	Mirtazapine 45 mg	Aripiprazole 20 mg	Mirtazapine
Risperidone 4 mg	Citalopram 40 mg	Aripiprazole 15 mg, risperidone 2 mg	Citalopram 40 mg	Aripiprazole 20 mg	Citalopram40mg	Aripiprazole 20 mg	Citalopram40mg
Clozapine 50 mg		Aripiprazole 15 mg, clozapine 50 mg		Aripiprazole 15 mg, clozapine 25 mg		Aripiprazole 15 mg, clozapine 50 mg	
Amisulpride 400 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 20 mg	Aripiprazole 15 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 20 mg	Aripiprazole 30 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 30 mg	Aripiprazole 20 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 30 mg
Risperidone 2 mg	Fluoxetine 20 mg	Aripiprazole 20 mg, risperidone 1 mg	Fluoxetine 40 mg	Aripiprazole 20 mg	Fluoxetine 40 mg	Aripiprazole 20 mg	Fluoxetine 40 mg
Olanzapine 20 mg		Aripiprazole 20 mg, Olanzapine 10 mg		Aripiprazole 20 mg, Olanzapine 10 mg		Drop out	
Olanzapine 10 mg		Aripiprazole 20 mg, olanzapine 5 mg		Aripiprazole 20 mg Olanzapine 10 mg		Drop out	
Risperidone 4 mg		Aripiprazole 10 mg,		Aripiprazole 15 mg,		Aripiprazole 10 mg,	

Week 0		Week 12		Week 26		Week 52	
Risperidal – Consta Injection 25 mg	Paroxetine 40 mg, Procyclidine 5 mg	risperidone 4 mg Drop out		risperidone 3 mg Drop out		risperidone 2 mg Drop out	
Quetiapine 600 mg	Citalopram 40 mg, zopiclone 7.5 mg	Aripiprazole 15 mg, quetiapine 400 mg	Citalopram 40 mg, clonazepam 1 mg	Aripiprazole 15 mg, quetiapine 400 mg	Citalopram 40 mg	Aripiprazole 20 mg, quetiapine 200 mg	Citalopram 40 mg
Risperidone 3 mg		Aripiprazole 15 mg	Citalopram 20 mg	Aripiprazole 15 mg	Citalopram 20 mg	Aripiprazole 15 mg	
Olanzapine 20 mg		Aripiprazole 20 mg, olanzapine 5 mg		Drop out		Drop out	
Olanzapine 10 mg		Aripiprazole 10 mg, olanzapine 5 mg		Aripiprazole 15 mg		Aripiprazole 15 mg	
Olanzapine 5 mg		Aripiprazole 10 mg		Aripiprazole 10 mg		Aripiprazole 10 mg	
Olanzapine 10 mg		Aripiprazole 15 mg, olanzapine 5 mg	Citalopram 20 mg, procyclidine 5 mg	Aripiprazole 15 mg,	Citalopram 30 mg	Aripiprazole 15 mg,	Mirtazapine 15 mg
Quetiapine 400 mg		Aripiprazole 15 mg, quetiapine 300 mg		Aripiprazole 15 mg, quetiapine 100 mg		Drop out	
Amisulpride 400 mg		Aripiprazole 15 mg, amisulpride 200 mg		Aripiprazole 10 mg, amisulpride 300 mg		Aripiprazole 10 mg, amisulpride 200 mg	
Risperidone 6 mg	Lithium 600 mg	Aripiprazole 5 mg, risperidone 6 mg	Lithium 600 mg	Aripiprazole 5 mg, risperidone 6 mg	Lithium 600 mg, orlistat 120 mg	Drop out	
Olanzapine 15 mg		Aripiprazole 15 mg, olanzapine 10 mg,	Sertraline 50 mg	Aripiprazole 15 mg, olanzapine 5 mg	Sertraline 100 mg	Aripiprazole 20 mg, olanzapine 5 mg	
Olanzapine 7.5 mg		Aripiprazole 5 mg, olanzapine 5 mg		Aripiprazole 5 mg		Aripiprazole 5 mg	
Olanzapine 22.5 mg	Citalopram 60 mg	Aripiprazole 2.5 mg, olanzapine	Citalopram 40 mg	Aripiprazole 2.5 mg, olanzapine 20	Citalopram 40 mg	Aripiprazole 2.5 mg, olanzapine 20	Citalopram 40 mg

Week 0	Week 12	Week 26	Week 52
Zuclopenthixol 250 mg	Citalopram 20 mg + Aripiprazole 5 mg	Citalopram 20 mg + Aripiprazole 5 mg	Drop out

On an initial review of the NTX data using gender-specific cut-off values (66 nmol BCE/mmol creatinine and 65 nmol BCE/mmol creatinine for males and premenopausal females respectively) at study intake, three out of the 26 (11.5 %) had a high NTX concentration. On the other hand, at their last follow-up point in the study, only one out of the 26 (3.8%) had an NTX concentration outside the reference range, and all of the three had a reduction in NTX. The mean NTX at baseline and week 52, after adjusting for outliers, fell from 47.83 to 31.38 nmol BCE/mmol creatinine respectively (Figure 2.1). For those patients who had a reduction in NTX, the average reduction in the concentration was 32.47%. The change in NTX in males and females followed a similar pattern over time (Figure 2.2). Looking at BSAP, none of the patients had a concentration outside the normal range at study intake or at last follow up. The mean BSAP at baseline and week 52 after adjusting for outliers was 74.46 and 70.56 respectively.

Figure 2.1 NTX concentration over the 52-week study period (means with SEMs)

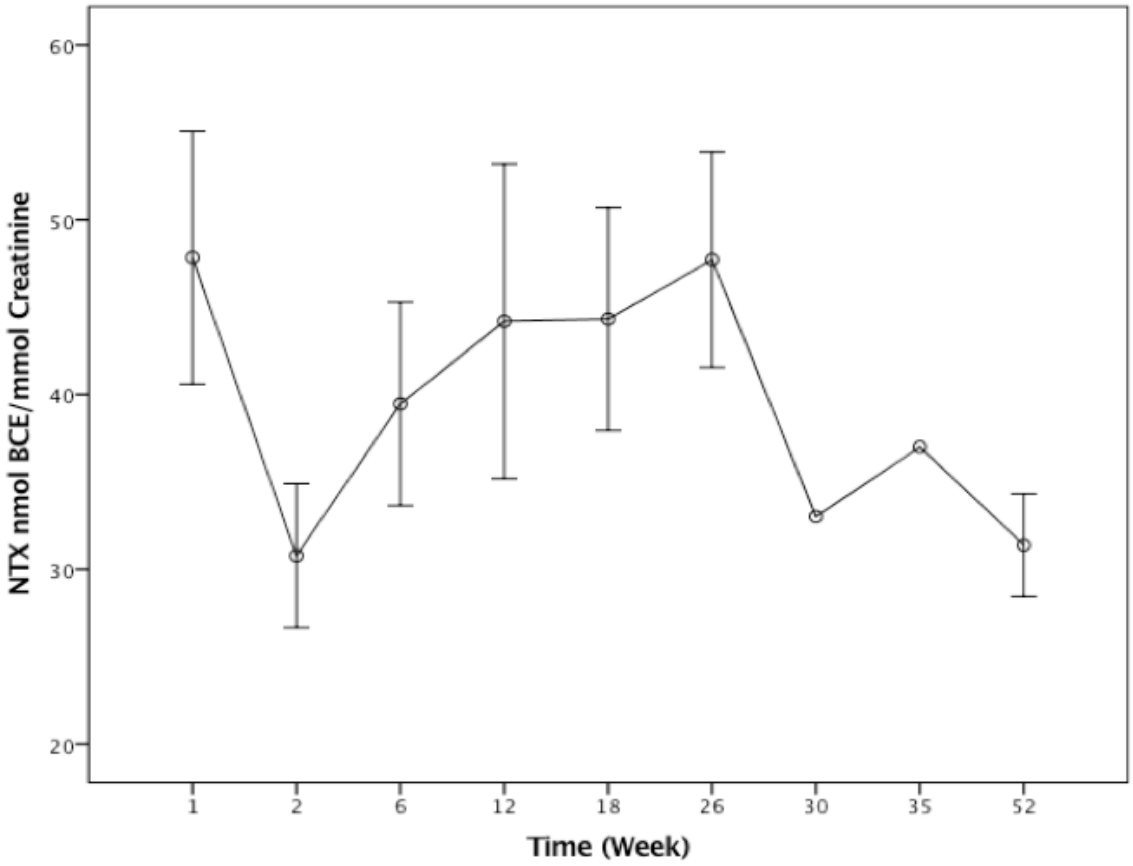
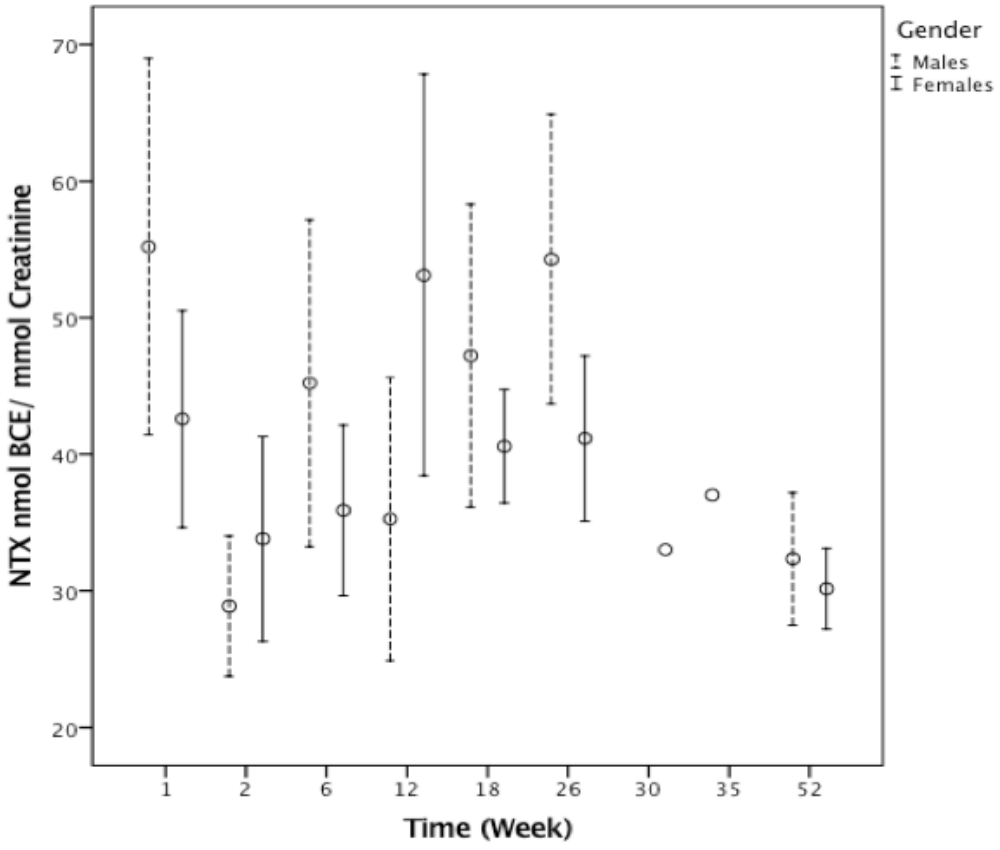


Figure 2.2 NTX concentration over the 52-week study period by gender.



The results of GLMM analyses of bone markers are as follows. NTX showed a reduction over 52 weeks in the whole group, but this did not reach statistical significance (log-NTX: β = -0.0012, p = 0.142). Amongst the predictive factors for NTX, only 'switch status' (p = 0.028) had a significant effect on NTX, while the rest did not (Table 2.3). For BSAP, using the same predictor variables for analysis as NTX over the 52 weeks, the addition of or replacement with aripiprazole produced a significant reduction in BSAP (log-BSAP: β = -0.00039, p = 0.002). Out of the predictor variables, none had significant impact on BSAP.

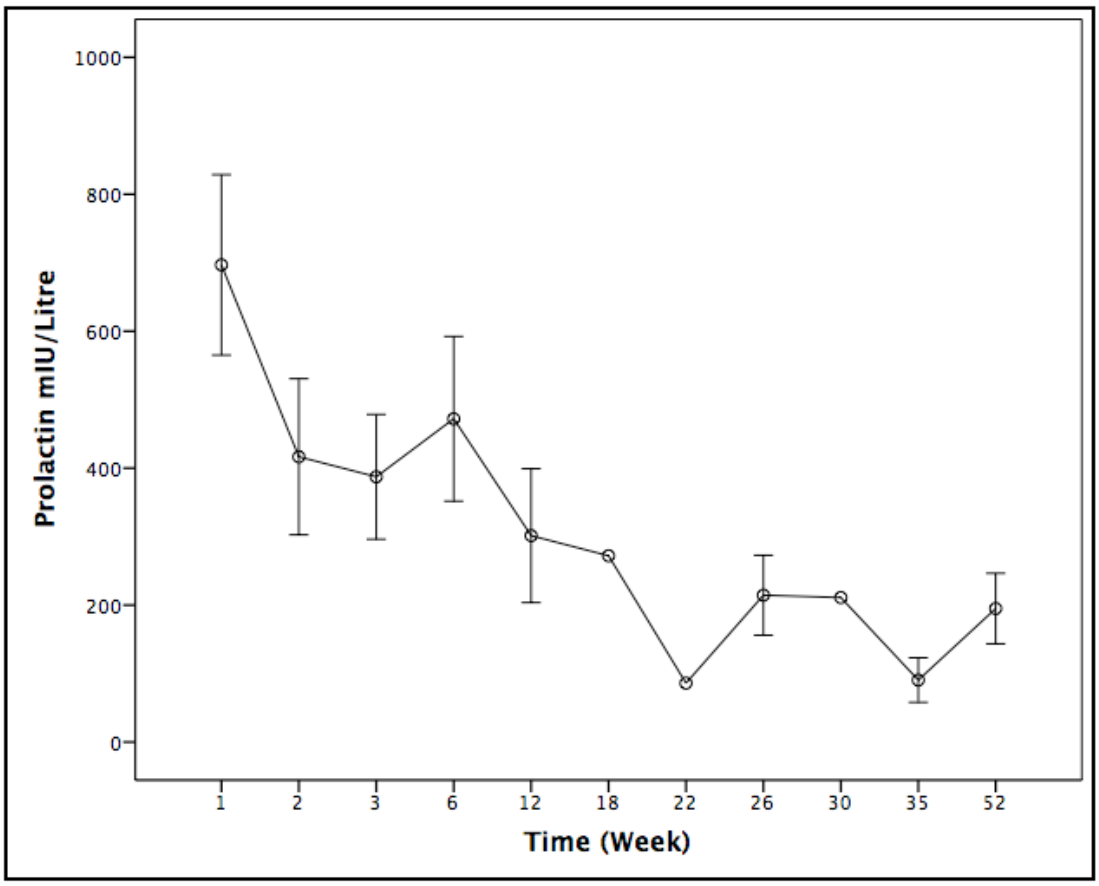
Table 2.3 Test of model effects for the GLMM analysis of log-NTX

Predictor	Coeff	Std Err	Sig
Baseline medication	-0.0239	0.0192	0.214
Ethnicity	0.0149	0.030	0.625
Week	-0.0012	0.0008	0.142
Switch status	-0.0873	0.039	0.028
Age	-0.0031	0.0032	0.329
Gender	-0.0047	0.057	0.934
Antidepressant status	-0.0189	0.0548	0.729
Prolactin	0.00004	0.0004	0.306

Dependent variable- log-NTX

GLMM analyses of hormones were as follows. Prolactin analysis with 'switch status,' 'baseline medication,' gender, ethnicity, antidepressant status, age, and 'week' as predictors, demonstrated a significant reduction in prolactin (log-prolactin: $\beta = -0.0024$, $p < 0.001$) (Figure 3). Gender ($p < 0.001$) and 'switch status' ($p = 0.010$) were significant predictors of change in prolactin. A similar analysis with testosterone and oestrogen as dependent variables showed a non-significant increase in both testosterone (log-testosterone: $\beta = 0.0003$, $p = 0.812$) and oestrogen (log-oestrogen: $\beta = 0.0010$, $p = 0.090$).

Figure 2.3: Prolactin concentration over the 52-week study period (means with SEMs)



Sensitivity analyses were then conducted. With TCPZE as an additional predictor for NTX and BSAP, the reductions in NTX (log-NTX: β = -0.0012, p = 0.134) and in BSAP (log-BSAP: β = -0.00042, p = 0.001) remained similar to those seen in the primary analyses. Additional sensitivity analyses were conducted by repeating the primary NTX and BSAP analyses in sub-groups. When we compared those who had switched to aripiprazole versus those who remained on another antipsychotic in addition to aripiprazole, we saw that the reduction in NTX was significant in the aripiprazole only group (log NTX: β = -0.0033, $p < 0.001$) and not significant

for the rest (log-NTX: β = -0.077, p = 0.510), while BSAP reduced significantly in both subgroups (i.e., for aripriprazole only, log-BSAP: β = -0.0005, p = 0.033 and for the add on group, log-BSAP: β = -0.0033, p = 0.016). NTX analyses in males (log-NTX: β = -0.0013, p = 0.282) and females (log-NTX: β = -0.0009, p = 0.398) separately did not show a significant change in NTX. BSAP reduction was significant in males (log-BSAP: β = -0.0004, p = 0.022) and females (log-BSAP: β = -0.0003, p = 0.041).

2.5 Discussion

Our results support our hypothesis that a change to aripiprazole would be associated with a reduction in NTX, a urinary biomarker of bone resorption, but this effect was observed only in those who switched to aripiprazole. Interestingly there was also a decrease in serum BSAP, a biomarker of bone formation. This implies that bone resorption was in phase with bone formation, *i.e.*, a healthy process of bone turnover was occurring. The decrease in NTX was greater than that of in the switchers group (34.6% versus 5.23% reductions in mean concentration by week 52), suggesting a greater reduction in osteoclast than osteoblast activity. This implies that the BMD and bone stability increased over the one-year follow-up period. In regard to effects specific to the prior antipsychotic, due to the small subsample sizes, it is difficult to draw any definitive conclusions. Association between bone turnover marker NTX and initiation of risperidone in those who are relatively medication naïve patients has been previously reported (Bishop et al., 2012). In our study of patients who were all on antipsychotics at baseline, there was an increase in mean NTX at approximately 26 weeks. It is possible that this reflects a delayed response to the increased mean prolactin that was seen in the sample at six weeks (refer to Figure 2.1).

The median age of the subjects used in these analyses was 25.5 years, with a median duration of illness of five years (Table 2.1), indicating that the majority of the participants had reached their peak BMD prior to the initiation of prolactin-raising antipsychotics. Moreover, they had not yet reached ages associated with age-related normal decreases in BMD.

Aripiprazole is a widely prescribed atypical antipsychotic, with a mechanism distinct from other atypical antipsychotics: in addition to being an antagonist at $5HT_{2A}$ serotonin receptors, it is a partial agonist at $5HT_{1A}$ serotonin receptors and D_2-receptors (Croxtall, 2012; Lawler et al., 1999). Aripiprazole is associated with a reduction in antipsychotic-induced hyperprolactinemia in schizophrenia. For example, Casey et al. (2008) carried out a multicentre, randomized, open-label, eight-week outpatient study that followed patients switched to aripiprazole who were earlier on other antipsychotics (including olanzapine, 55%; and risperidone, 37%) (Casey et al., 2003). They observed a decrease in the mean prolactin concentration, with the most marked change being observed when switching off risperidone to aripiprazole ($p < 0.01$). Patients offered this treatment option had demonstrated intolerance, or inadequate response to, other antipsychotic medication(s). The overall reduction in prolactin by week 52 in our study is consistent with a prior analysis in the same group at week 26 (Mir et al., 2008).

Results of previous studies regarding associations between prolactin-raising antipsychotics and measures of BMD such as osteoporosis and fracture risk are variable and summarized in a meta-analysis by Kishimoto et al. (2012) (Kishimoto et al., 2012). O'Keane et. al (2005) identified an association between the use of prolactin-raising antipsychotic drugs, and both hypogonadism and decreased BMD values (O'Keane et al., 2005); whereas, others have found no association between prolactin and BMD (Lee et al., 2010; Renn et al., 2010; Sugawara

et al., 2011). Prolactin has been found to be a mediating factor for change in NTX in previous studies (Bishop et al., 2012). Concomitant antidepressant medications can also affect bone turnover (Diem et al., 2014). Nine out of 26 patients in our study were on antidepressants. We controlled for prolactin concentration and antidepressant use by including them as predictors in our analyses and they` did not affect NTX and BSAP significantly.

The other confounding factors in studies investigating the association between prolactin-raising antipsychotics and bone mineral density include sample size, age range of participants and the differences in antipsychotics (Rey-Sanchez et al., 2009). Lifestyle factors have been shown to influence the onset of osteoporosis (Body et al., 2011; Kishimoto et al., 2012; Stransky et al., 2009). Patients with psychotic disorders generally exhibit poor lifestyle choices (De Hert et al., 2011b). For young patients in particular, the overall global improvement seen our cohort as indicated by the increase in clinical global impression scale (Aitchison et al., 2011) might well be associated with additional lifestyle modifications (e.g., exercise) that impacted positively on NTX. Abel et al. (2008) illustrate the importance of understanding the full range of factors contributing to reduced BMD and accelerated osteoporosis in psychosis, with an emphasis on identifying underlying physiological mechanisms and any medication-specific effects.

Limitations

As previously discussed (Mir et al. 2008; Aitchison et al. 2011), this is a pilot study in which the sample size means that both type I and type II errors may occur. However, mitigating against this is the repeated measures design, which renders the analysis more powerful. Moreover, GLMM analysis minimises problems associated with missing data and prediction by multiple regression (which can inflate observed correlations).

A further potential consideration is analytical consistency. Although NTX and BSAP correlate significantly with BMD, there has been some debate over their analytical variability between testing centres (Schafer et al., 2010; Seibel et al., 2001). Bone turnover marker concentrations can vary with factors that we were not able to control for, such as the level of activity, smoking, circadian rhythm, dietary factors including alcohol consumption, medications such as oral contraceptive pills, stage of menstrual cycle and comorbid medical illnesses such as Crohn's disease (Ju et al., 1997; Glover et al., 2008; Di Giovanni et al., 2008). However, NTX does have advantages over other markers in that it is not as sensitive to food intake (Schafer et al., 2010; Seibel et al., 2001; Singer et al., 2008). Future studies will benefit from including such factors.

Summary

In conclusion, we found that switching to aripiprazole from an existing antipsychotic was associated with changes in molecular biomarkers of bone turnover, indicating a more favourable profile for bone health. Serum prolactin concentration also decreased with addition or switch to aripiprazole. Of note, to analyse such biomarkers, from a patient's perspective this entails no more than the equivalent of routine clinical approaches (taking urine and blood samples). Moreover, bone turnover markers are being evaluated for routine use in the prediction of fracture risk and management of osteoporosis (Vasikaran et al., 2011). We suggest that our methodology merits replication and further exploration in larger studies, adequately powered to answer related questions such as the effect of prior medication, ethnicity, and lifestyle factors.

Overall, we found that switching to aripiprazole was associated with changes in molecular biomarkers of bone turnover, indicating a more favorable profile for bone health.

2.6 Acknowledgements

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CHAPTER 3. METABOLIC SYNDROME

3.1 Efficacy of a metabolic management program in the treatment of metabolic syndrome

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Keywords:

metabolic syndrome, weight loss, antipsychotics, dietician, psychosis

3.1.1 Abstract

Introduction. Patients with a severe mental illness are likely to die early, and metabolic dysfunction (MD) is a risk factor for conditions causing early mortality. Treatment of MD is relevant for reducing morbidity and mortality in psychosis. Lifestyle changes, such as dietary modification, are helpful in lowering MD. However, no study has compared metabolic outcomes in psychiatric treatment settings offering dietary consultations within services that do not.

Methods. We compared weight change in patients enrolled in a psychiatric metabolic management program (MMP) with patients on treatment as usual (TAU). The MMP consists of one clinic at the University of Alberta Hospital (UAH) and one at the Edmonton Mental Health Clinic (EMH). The clinics are similar except that a dietician provides input only at UAH. 128 clinical records (TAU=31, UAH=64, and EMH=33) were examined retrospectively for weight change between an intake baseline and the 15th visit to the clinic. Linear mixed model analyses were conducted.

Results. Patients in the MMP lost weight (β=-0.007, P=0.007)) compared to TAU controls. Interestingly, the UAH patients lost a significant amount of weight relative to TAU (β=-0.010, P<0.001) but the EMH subjects patients did not (β=-0.002, P=0.393).. Within MMP patients, baseline body mass index (BMI) affects weight loss; patients with class 1 obesity exhibited the most significant change (class 1 obese compared to normal: β=-0.009, P=0.020),

Conclusions. Psychiatric patients referred to a specialized MMP lost weight if the program included consultation with a dietician and this was particularly noteworthy for patients with class 1 obesity.

3.1.2 Introduction

The relative risk of mortality is 2.2 times higher in those with a mental illness than age and gender matched control subjects (Walker et al., 2015) and cardiovascular disease (CVD) is an important cause of mortality in psychiatric patients (Hansen et al., 2001). Metabolic syndrome (MetS) is a risk factor for CVD (Galassi et al., 2006) and one-third of patients with schizophrenia, unipolar depression, and bipolar disorders may fit criteria for MetS (Vancampfort et al., 2015). MetS is multifactorial in origin (Park et al., 2003), and for psychiatric patients, medications such as second-generation antipsychotics(Ballon et al., 2014; Miron et al., 2014), mood stabilizers (Carmona-Vazquez et al., 2015) and antidepressants (Azevedo Da Silva et al., 2016; Crichton et al., 2016) contribute significantly to the emergence of MetS. Treating MetS can therefore potentially reduce the risk of or delay future CVD morbidity and mortality.

Pharmacological interventions for the prevention or treatment of metabolic dysfunction in psychosis include metformin (Zheng et al., 2015), topiramate (Liang et al., 2016; Zheng et al., 2016), orlistat (Joffe et al., 2008), amantadine (Deberdt et al., 2005) and reboxetine with betahistine (Poyurovsky et al., 2013). Medications examined for treatment/prevention of metabolic dysfunction in psychosis also include sibutramine, nizatidine, and rosiglitazone (Maayan et al., 2010). However, some of these medications can cause significant adverse effects. For example, there is a higher incidence of paraesthesia (relative risk=2.67) and attention deficit (relative risk=8.97) compared to controls, according to a meta-analysis of topiramate as an augmenting agent in schizophrenia (Okuyama et al., 2016).

Non-pharmacological interventions for treatment or prevention of metabolic dysfunction in mental illness are available that are relatively free of adverse effects. They may incorporate diverse strategies that could include exercise, dietary counseling, cognitive behavioral therapy

(CBT), and psychoeducation and nutritional education (Papanastasiou, 2012). It has been noted that patients with schizophrenia have increased consumption of fat and sugar while lower consumption of fiber (McCreadie, 2003; Henderson et al., 2006). Adhering to a Mediterranean-style diet, which is rich in whole grain, fruits, vegetables, nuts, and olive oil, has demonstrated efficacy in reducing CVD risks in MetS patients (Esposito et al., 2004). In randomized controlled trials, it has been reported that a combination of nutritional counseling and exercise reduces antipsychotic-induced weight gain in patients with first episode or chronic schizophrenia (Alvarez-Jimenez et al., 2008). In the just referenced study, the interventions were especially effective in preventing MetS during the early phases of antipsychotic treatment.

However, there are many barriers to managing MetS in our population of interest (De Hert et al., 2011a). Psychiatric patients generally have a suboptimal lifestyle with poor nutritional intake and lack of exercise compared to the general population (Cabassa et al., 2010). Secondly, patients sometimes seek less help than required because they may be less capable of comprehending their somatic symptoms due to cognitive deficits (De Hert et al., 2011a). Some individuals may be less aware of their physical illnesses owing to reduced sensitivity to pain. Earlier studies proposed altered pain perception as a possible marker of schizophrenia (Singh et al., 2006). Physicians often make the mistake of regarding physical complaints as psychosomatic symptoms during a medical consultation and thus do not deliver appropriate care (Lawrence et al., 2010). Furthermore, psychiatric patients often are poorly compliant to treatment regimens (Tschoner et al., 2007). In many jurisdictions patients do not have a primary care practitioner to treat their MetS. One solution for treating metabolic dysfunction in psychiatric patients is to provide a metabolic management program (MMP) as a part of the psychiatric service that can offer advice on dietary interventions, exercise, and other specific treatment. Alberta Health

Services, Edmonton zone, offers such a service to patients attending a couple of psychiatric clinics. Our study examined the efficacy of this metabolic management program (MMP) for weight loss.

Although MetS is described as a single entity made up of the five components described above, each of these components can be treated individually or in parallel with each other. For example, with lipid lowering drugs for dyslipidemia, treatment of hypertension will usually only reduce blood pressure. Weight loss can be one useful marker to assess the efficacy of a metabolic management program. Achieving weight loss through treatment can affect all five criteria of MetS. 5% weight loss can cause significant improvement in insulin sensitivity and beta cell function (Magkos et al., 2016).

3.1.3 Method

Study design

We performed a retrospective chart review for weight change in patients attending an MMP at two locations, i.e. the University of Alberta Hospital (UAH) and Edmonton Mental Health Clinic (EMHC), using paper and electronic records. Similar data were collected on the same measures on patients who were being treated as usual (TAU) at the same locations but were not a part of the MMP.

MMP clinics

The two MMP clinics had the same family physician (JC) who assessed and treated metabolic dysfunction, a different nurse at each site and use of the same treatment guidelines for metabolic

dysfunction. The guidelines followed are the Canadian Diabetes Association and Canadian Cardiovascular Society (Canadian Diabetes Association, 2013; Canadian Cardiovascular Society, 2016). The main difference between the two was that the UAH MMP clinic had a dietician on-site within the team and the EMHC MMP clinic provided information to patients on how to access a dietician elsewhere. Visits to the clinic are usually at monthly intervals for the first three months and then once every three months thereafter, but some variability was necessary as clinically indicated.

Sample and data collection

A list of patients who had attended the MMP and were in the TAU category was collected. Records were evaluated, and the first visit to the MMP was identified. Chart review was done by RL who manually went through the paper records, followed by a search on an electronic database 'e-Clinician' for any additional information on the parameters of interest. Baseline information extracted from the record included gender, age, clinical diagnosis, number of visits, medication at intake, height and weight. Progress through the MMP was assessed by recording the change in weight and medications at each visit for up to a maximum of 12 visits.

Inclusion and exclusion criteria

All files of patients, irrespective of diagnosis, who had attended the clinics were eligible for inclusion in the study. However, the extraction was limited to patients that had a minimum of three visits, and for whom most of the study parameters were available. There were no exclusion criteria.

Statistical analysis

STATA 15.1 was used to conduct analyses. Weight was evaluated for normality using graphs with the STATA commands histogram, pnorm, qnorm, and hangroot. Baseline differences between the UAH and EMH patients were assessed using ANOVA and chi-squared tests as appropriate for parametric and categorical variables, respectively. Since the timing of visits varied amongst patients, we created a new visit variable that re-categorized the original visit. The new visit variable was in increments of 10 or 50 days, up to a maximum of 1400 days. Linear mixed model (LMM) analyses was used to analyze the change in weight (in kg). The fixed predictors for the mixed models were age at intake in years ('age'), gender, diagnosis ('diagnosis': psychosis, major depressive disorder and/or anxiety disorder, and bipolar disorder), treatment group ('group': Treatment as usual group (TAU), metabolic management group without a dietitian at the Edmonton Mental Health Clinic (EMH) and metabolic management group with a dietitian (UAH)), baseline BMI category ('BMI': normal, overweight, obese1, obese2 and obese3) and visit number as a continuous variable ('visit'). BMI category ranges were: normal 18.5 to 24.9, overweight 25.0 to 29.9, class 1 obesity 30 to 34.9, class 2 obesity 35 to 39.9, and class 3 obesity equal to or greater than 40 (World Health Organization, 2009). None of the patients had a BMI of less than 18.5. The random part of the model was at one level, i.e., visit by patient. The following LMM models were run: first for the whole group: group by visit interaction was examined as a predictor to assess change over time by location, adjusted for baseline age, gender, baseline diagnosis and baseline BMI, with weight as the dependent variable. In this model, we tested group in two ways: as a two-level variable (TAU and MMP, MMP combining the EMH and UAH groups) and as a three-level variable (TAU, EMH, and UAH). Second, in groups that demonstrated significant weight change, we tested separate interactions of visit: with baseline age, baseline diagnosis, baseline BMI and gender. This was

done to assess the importance of these predictors for any significant weight change. Post-regression for each analysis was in the form of graphs of the residual distribution to ensure that the assumption of normality for linear variables had not been violated.

3.1.4 Results

Sample characteristics

128 subjects were included in the analysis. A summary of the sample characteristics is presented in Table 3.1-1, categorized by group: UAH, EMH or TAU. From this, we can infer that the TAU group was younger than the MMP groups, and more patients had a diagnosis of psychosis, however these differences were not statistically significant..

Table 3.1-1 Description and comparison of patients in the three study groups

Criteria	**TAU**	**UAH**	**EMH**	***p****
Number of patients	31	64	33	
Gender (%)				0.180
Men	74.19	54.69	63.64	
Female	25.81	45.31	36.36	
Mean weight (kg)	93.67	94.86	97.60	0.751
Mean Age (years)	38.79	44.76	44.67	0.083
BMI (%)				0.278
Normal	19.35	7.81	15.15	
Overweight	35.48	28.12	18.18	
Class 1 obesity	22.58	39.06	24.24	
Class 2 obesity	12.90	12.5	24.24	
Class 3 obesity	9.68	12.5	18.18	
Diagnosis (%)				0.053
Psychosis	80.65	56.25	75.76	
Depression and/or anxiety	6.45	23.44	6.06	
Bipolar Disorder	12.90	20.31	18.81	

**p* value from the chi-square test for proportions, a ANOVA for continuous variables

Mixed model analysis results

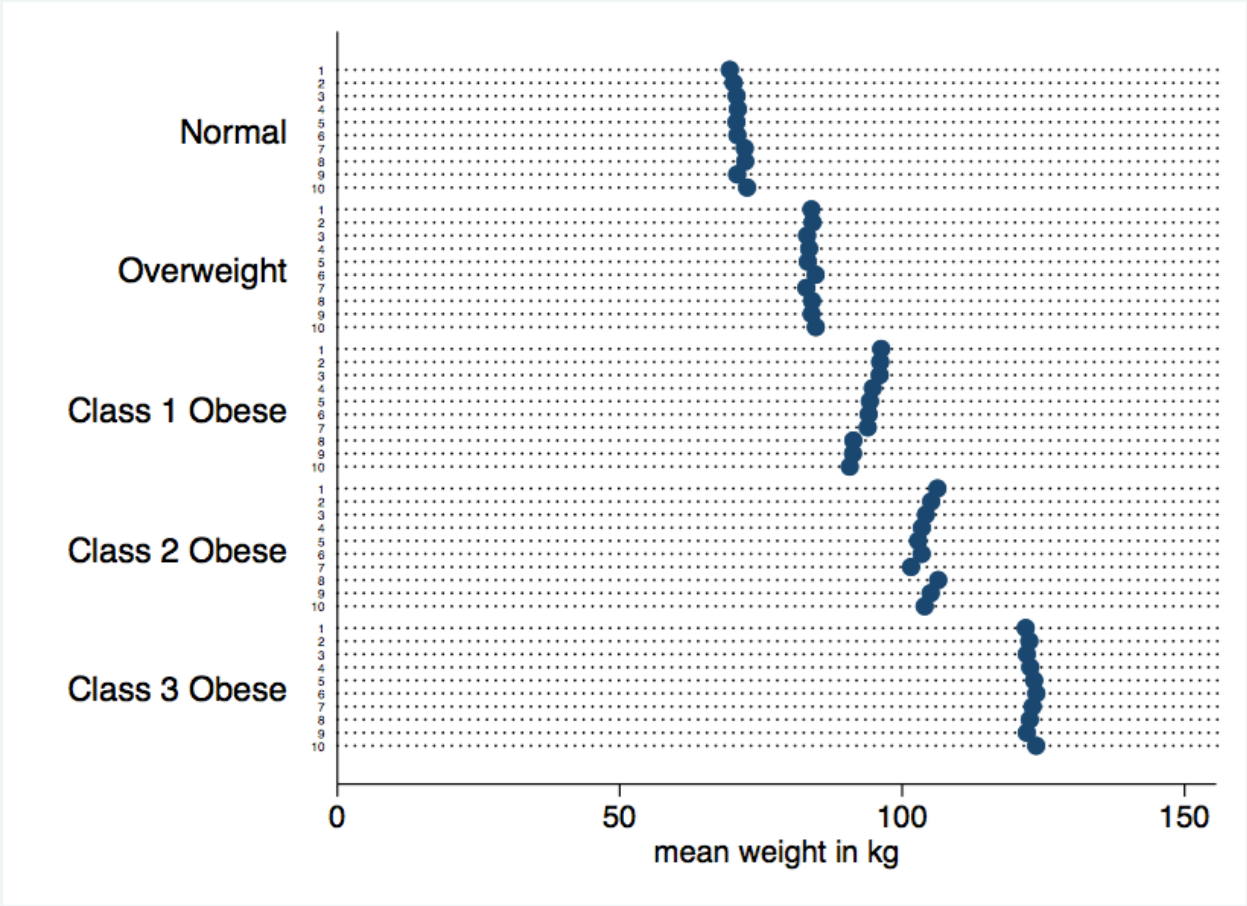
Results for the visit x group interaction analyses for the whole sample were as follows. When we compared TAU receiving patients with those in the metabolic management program (MMP), patients in the MMP show significant weight loss over time (β=-0.007, P=0.007). However, when group was used as a three-level variable (TAU, EMH, and UAH, see Table 3.1-2) it was evident that the weight loss was due to the change in weight in the UAH patients (β=-0.010, P<0.001) since there was no difference in the outcome between the TAU and EMH patients (β=-0.002, P=0.393).

Since the MMP cohort experienced significant weight loss over time, we examined this group of 97 subjects to look at any factors that may have associations with the weight change. In the analyses, the predictive factors were the same as the initial LMM analysis except for the interaction of visit with the variable of interest. Separate interaction analyses for baseline age x visit (β=6.57, P=0.951), gender x visit (β=0.003, P=0.212) and diagnosis x time (for mood/anxiety versus psychosis: β=0.003, P=0.3; for bipolar disorder versus psychosis: β=-0.002, P=0.435) revealed no effect of these on weight change. Baseline BMI x time interaction analysis indicated that the degree of weight loss was significant in the class 1 obese patients compared to those with a normal BMI (β=-0.009, P=0.020), and there was a trend for the class 2 obese patients (β=-0.007, P=0.094). Or in summary, the weight reduction in the class 1 obese patients was significant for those who were in the MMP (see Figure 3.1-1).

Table 3.1-2 Linear mixed model results for weight change, with group by visit interaction, adjusted for the other variables listed in the table

Criteria	Coeff	*p*	95% CI	
Age	-0.18	0.015	-0.34	0.035
Gender	-16.65	<0.001	-20.73	-12.57
Baseline BMI category	15.23	<0.001	13.56	16.89
Diagnosis				
Mood/Anxiety disorders	0.69	0.808	-4.91	6.31
Bipolar disorder	4.54	0.018	0.76	8.36
Group				
EMH	-1.20	0.667	-6.67	4.27
UAH	-0.51	0.837	-5.40	4.37
Visit	0.004	0.066	-0.001	0.008
Group#Visit				
EMH	-0.002	0.393	-0.008	0.003
UAH	-0.010	**<0.001**	-0.015	-0.004

Figure 3.1-1 Weight change over the first 10 visits by baseline BMI



3.1.5 Discussion

Our study confirms that a comprehensive MMP offered to psychiatric patients can lead to weight loss. A recent systematic review and meta-analysis concluded that nutritional interventions to reduce weight are effective in those suffering from a severe mental illness (Teasdale et al., 2016). The interventions were most effective if they occurred soon after initiation of the antipsychotic medication, and they were led by a dietician. Based on the available evidence, the authors concluded that those with a severe mental illness should be offered nutritional interventions as standard care for weight gain. Our study confirms the importance of the dietician in a metabolic management program. Weight loss in our MMP was driven by the UAH group, and the key difference between the UAH and EMH was the availability of the dietician. This indicates that having a dietician within the psychiatric service may be better than referral to one outside the service. Patients should be offered behavioral or non-pharmacological interventions for weight loss. This can be done in a number of ways, such as wellbeing programs, cognitive-behavior therapy, nutritional education, weight management and psychoeducation; as all have proven efficacy (Papanastasiou, 2012). Innovative ways of enhancing behavioral weight loss treatment programs, such as with mobile health technology, may be useful (Aschbrenner et al., 2016). The mean weight loss in the UAH group at six months was around four kg more than the TAU group, similar to previous studies (Carmona-Vazquez et al., 2015). Reduction in weight loss can impact other aspects of well-being, too. Weight loss seen in patients on olanzapine in a psychoeducational program translated into significant increases in functioning and quality of life (Mauri et al., 2008). Similarly, in a study of patients with schizophrenia, exercise therapy was associated with reductions in symptoms of depression as well as of schizophrenia (Scheewe et al., 2013). Some general barriers in reducing metabolic dysfunction have been noted in previous

studies and include difficulty in dietary tracking in those with a severe mental illness and the impact of depression on physical activity (Aschbrenner et al., 2016).

Strengths and limitations

A strength of our study is that it is naturalistic, and if replicated the results will be applicable to patients seen in routine clinical care. We tracked the progress of patients in the program for up to 12 visits, providing us a reasonable duration of follow up for a study phenotype (weight). Weight has been investigated as a useful marker for examining non-pharmacological interventions. To the best of our knowledge, there are no other studies that have examined these questions in a naturalistic setting with this duration of follow up. In addition, the use of linear mixed models increases the power of our study. However, there are also several limitations resulting from a naturalistic retrospective review of patient medical records that does not allow for several potentially important factors. For example, medications are a key factor in weight change, but baseline medications in this study were too variable for entry in our analyses. Other reasons for not including the medication details were incomplete recording of change in doses and lack of indicators of compliance. We were also unable to hold constant either the psychiatric consultants involved in prescribing, or the nursing staff involved with TAU, EMH, or UAH between the different clinics or services. The diet and exercise advice provided by the dietician is individualized and that may have led to some variations in management plans. However, the guidelines that informed care of all patients were based on the nutritional care process. We also did not have detailed information on when and the number of times the patient met the dietician.

To conclude, our study indicates that a metabolic management team can promote significant weight loss, and having a dietician in the team is probably the most important factor. Baseline BMI can influence the chance of achieving weight loss with those in the class 1 obesity

benefitting more than those in other categories of obesity. Replication of our results will be required to confirm the benefits of a similar model of MMP intervention. Future studies should consider the importance of the need for follow up beyond six months, tracking medication changes accurately, and measuring food intake.

3.1.6 Acknowledgements

Dr. Rohit J. Lodhi's graduate studies have been supported by the Alberta Centennial Addiction and Mental Health and Addiction Research Chair Fund (held by Dr. Katherine J. Aitchison) and an Edmonton Zone Medical Staff Association Quality Improvement Grant (PI: Dr. Katherine J. Aitchison, Co-PI: Dr. Avininder Aulakh). Dr. Lodhi received a University of Alberta Doctoral Recruitment Scholarship.

3.2 Cannabis and metabolic syndrome: A review

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3.2.1 Introduction

Cardiovascular disease is one of the leading causes of early and excess mortality in schizophrenia (Olfson et al., 2015), and metabolic syndrome (MetS) is a risk factor for the development of cardiovascular disease and diabetes mellitus (McNeill et al., 2005). MetS is highly prevalent in psychiatric patients (De Hert et al., 2009). The increase in risk of developing MetS in psychosis subsequent to the use of antipsychotics continues to be investigated (De Hert et al., 2012; Raedler, 2010; Mori et al., 2015); however, most such studies tend not to incorporate or adjust for the effect of substance use on this relationship, even though almost half of patients with a psychotic illness also have a diagnosis of lifetime alcohol or cannabis use disorders (Morgan et al., 2013).

In this review, we present a hypothesis about the nature of the relationship between cannabis and MetS in the general population and in psychosis. We then present literature pertaining to the same. A PubMed and EMBASE search was conducted to identify appropriate studies and reviews using the following search strings: “cannabis” AND “tetrahydocannabinol” AND (“metabolic syndrome” OR “weight” OR “abdominal circumference” OR “body mass index” OR “blood pressure” OR “glucose” OR “diabetes” OR “insulin” OR “insulin resistance” OR “lipid” OR “triglycerides” OR “HDL”) AND (“psychosis” OR “schizophrenia”). This search allowed us to identify evidence exploring the relationship between cannabis, psychosis and MetS.

3.2.2 Metabolic syndrome

(Refer to Introduction, Chapter 1.6.1 of this thesis)

3.2.3 Cannabis, the endogenous cannabinoid system (ECS) and energy metabolism

(Refer to Introduction, Chapter 1.7.3 of this thesis)

3.2.4 Hypotheses

We hypothesize that cannabis use will be associated with measures of MetS in the general population, and that the frequency of cannabis use will modulate this relationship. We further hypothesize that in patients with psychosis, cannabis use will be associated with an increase in metabolic dysfunction

3.2.5 Cannabis and waist circumference, weight, and body mass index

The effect of cannabis on abdominal obesity has been directly investigated using the measurement of waist circumference and indirectly using the body mass index (BMI). Cannabis is known to stimulate appetite (Foltin et al., 1988), and individuals who use cannabis appear to consume a higher amount of calories per day than those who do not use cannabis (Rodondi et al., 2006). One would therefore hypothesize that cannabis use should result in an increase in weight and hence, in abdominal circumference and BMI. However, interestingly, some studies support this inference in cannabis users, but larger studies suggest an opposite effect (Hayatbakhsh et al., 2010; Ngueta et al., 2015; Le Strat et al., 2011; Penner et al., 2013). In a 21-year follow-up study in Australia, cannabis users compared to non-users were less likely to be overweight or obese (Hayatbakhsh et al., 2010). A lower rate of obesity in cannabis users was reported from US national surveys such as the National Epidemiologic Survey on Alcohol and Related Conditions

(NESARC; 2001–2002) and the National Comorbidity Survey–Replication (NCS-R; 2001–2003) (Le Strat et al., 2011). Canadian Inuit cannabis users had lower obesity rates and BMI than non-users (Ngueta et al., 2015). At death, cannabis users are least likely to be overweight amongst patients who had an illicit substance addiction (Rajs et al., 2004).

It is possible that the dose of cannabis may differentially affect the risk of obesity. For example, adolescents with higher cannabis use are more likely to develop obesity as adults, while adolescents who used cannabis sporadically have a lower chance of being obese as adults when compared to non-users (Huang et al., 2013). A subsequent investigation described a U-shaped relationship between cannabis use frequency and change in adiposity in men who smoked 0.5 cigarettes/day (Dube et al., 2015). By contrast, for male subjects who smoked more than 15 cigarettes, there was an inverted U relationship between cannabis use and change in adiposity..

In summary, cannabis use, in general, appears to be related to a lower prevalence of obesity, with the dose relationship studies indicating that relatively infrequent cannabis users may drive this effect.

3.2.6 Cannabis and blood pressure

Early evidence of the effects of cannabis on blood pressure came from studies investigating the beneficial effect of cannabis on glaucoma (Merritt et al., 1980). It was observed that a reduction in blood pressure preceded the anticipated decrease in intra-ocular pressure. The cardiovascular effects appear to vary by the type of cannabinoid compound (i.e., from the cannabis plant, an endogenous compound, or a synthetic product). Tetrahydrocannbinol (THC), a plant derivative, has biphasic effects on blood pressure while anandamide, an endogenous compound, may have

triphasic effects (Malinowska et al., 2012). The most consistent cardiovascular effect of THC is a dose-dependent increase in heart rate (Zuurman et al., 2009). There is an acute dose-dependent increase in blood pressure and heart rate, along with orthostatic hypotension, after cannabis use (Jones, 2002; Heishman et al., 1989). For the orthostatic hypotensive effect, patients that have higher baseline blood pressure may be more affected (Crawford et al., 1979). With repeated use of cannabis, tolerance to the acute cardiovascular effects, such as the increased heart rate and orthostatic hypotension, develops after a day or two (Jones, 2002). In fact, with tolerance, blood pressure appears to return to approximately baseline values (Benowitz et al., 1975; Jones, 2002). Upon cessation of repeated cannabis use, the tolerance to acute cardiovascular effects is rapidly lost (Benowitz et al., 1981). A study of patients who had psychosis reported lower odds (OR=0.71) of having a diagnosis of high blood pressure if cannabis misuse was present than when it was not (Moore et al., 2012). Therefore, chronic cannabis use may not lead to long-term or persistent elevation of blood pressure.

3.2.7 Cannabis and glucose metabolism

There are some interesting studies looking at cannabis use and glucose metabolism. At least one study has reported elevated insulin resistance in cannabis users (Muniyappa et al., 2013), but within this area too, several studies have produced contrary results. In 4657 men and women, self-reported cannabis use was associated with a 16% lower fasting insulin level and 17% lower insulin resistance (measured by the Homeostatic Model Assessment (HOMA-IR)) (Penner et al., 2013). Such findings have also been demonstrated in Canadian Inuit subjects (Ngueta et al., 2015). Similarly, a nationwide French study of cannabis users with HIV and hepatitis coinfections reported a lower risk (OR = 0.4) of high HOMA-IR (Carrieri et al., 2015). A US

study, involving more than 10,000 participants, reported that cannabis users had a lower prevalence of diabetes mellitus (Rajavashisth et al., 2012). A more recent meta-analysis of eight independent samples has reported a lower risk of diabetes mellitus among recently active cannabis users (OR=0.7) (Alshaarawy et al., 2015). Although not without exception, the evidence to date may support a beneficial effect of cannabis on glucose metabolism, although without adequate control of relevant covariate information such as dose.

3.2.8 Cannabis and lipid levels

The last two criteria of MetS are serum lipid abnormalities, typically evident in high triglyceride and low HDL values in serum. Few studies have reported lipid levels in relation to the use of cannabis, and the evidence to date remains ambiguous. One report on chronic cannabis use found an association with lower HDL (Muniyappa et al., 2013) levels, and this was replicated in a first episode psychosis sample (Misiak et al., 2014). The authors of the latter investigation suggested that this could be secondary to elevated homocysteine levels. These results were not seen in a larger study that observed no significant difference in triglyceride and HDL levels between cannabis users and non-users (Penner et al., 2013).

3.2.9 Cannabis and metabolic syndrome in psychosis

Surprisingly, there are very few studies that have examined the relationship between cannabis and MetS (i.e., by including all five criteria) in psychosis or in the context of antipsychotic use. A study conducted in 2005 investigated the relationship between cannabis, MetS and psychosis (Isaac et al., 2005). This investigation was done on inpatients and was not representative of a

community sample. In 2012, the second Australian National Survey of Psychosis (SANSP) findings on the impact of alcohol and cannabis use in 1825 psychosis subjects were published (Moore et al., 2012). In this survey, BMI in patients with psychosis was lower in those who used cannabis (Moore et al., 2012). In addition, cannabis users had lower odds of having high blood pressure but similar cholesterol levels compared to non-users (Moore et al., 2012). Data derived from the SANSP were used for a poster presentation in 2014, demonstrating the effect of cannabis on MetS in psychosis (N = 7995). The odds of having MetS after adjusting for age, sex and atypical antipsychotic medication in current cannabis users were lower than for those who did not use cannabis (OR= 0.62) (Waterreus et al., 2014).

3.2.10 Discussion

In summary, clearly cannabis influences energy metabolism and metabolic function. A major limitation in drawing any firm conclusions about the interactions between cannabis use, MetS, and psychosis is the paucity of research investigating all five criteria of MetS in cannabis users. This is a particularly important current research gap given not only the high rates of MetS associated with psychosis but also the high rates of cannabis use within this patient group (Morgan et al., 2013). However, based on the evidence in the general population for the individual criteria of MetS, we can advance the following tentative inferences. First, the average BMI is lower and obesity is less prevalent in cannabis users, although modulation of this relationship by the frequency or amount of cannabis use needs more investigation. Secondly, chronic cannabis use appears not to increase blood pressure, but again the delineation of the association will require further research on contributions from cannabis dose, frequency of use, and duration of use. The research on cannabis and diabetes, insulin, and insulin resistance has

been investigated with larger studies observing a lower prevalence of diabetes in cannabis users, albeit also with limitations regarding information such as frequency of cannabis use. Lastly, details of the nature of the relationship between cannabis use and serum lipid levels are yet to be established.

We should exercise caution in extrapolating any of the findings regarding metabolic dysfunction from patients without psychosis who use cannabis to patients who have psychosis, because there is a probability that the ECS has altered function in patients with psychosis (Leweke et al., 1999; Bioque et al., 2013). This may be illustrated by cannabis having different effect on the ECS in patients with and without psychosis (Leweke et al., 2007): it has been observed that frequent use of cannabis was associated with down-regulation of the endogenous cannabinoid, anandamide, in cerebrospinal fluid, but only in patients with schizophrenia and not in healthy subjects.

If there are any benefits of cannabis on MetS, perhaps they might well be negated by the significant additional cardiovascular problems associated with cannabis use. For example, the risk of myocardial infarction is increased by a factor of five within an hour of smoking THC, and there is a higher rate of mortality after a stroke or myocardial infarction for cannabis smokers (Malinowska et al., 2012). Reports of deleterious cardiac conduction changes after cannabis include sinus tachycardia (Jones, 2002), atrial tachyarrythmia (Fisher et al., 2005), paroxysmal atrial fibrillation (Kosior et al., 2000; Kosior et al., 2001; Lehavi et al., 2005), ectopic atrial rhythm (Fernandez-Fernandez et al., 2011), premature ventricular contractions, brugada electrocardiogram pattern (Romero-Puche et al., 2012), cardiac asystole (Menahem, 2013) and second degree A-V block (Akins et al., 1981). In young people, cannabis use has been associated

with an increased occurrence of intracranial arterial stenosis, a condition that can cause stroke (Wolff et al., 2014).

We suggest that, where possible, naturalistic and prospective studies assessing the impact of psychotropic medications on metabolic dysfunction should adjust more thoroughly for substance use, so that we can learn more about the contribution of substance use to metabolic dysfunction in the context of mental illness and its treatment.

3.2.11 Acknowledgements

Dr. Rohit J. Lodhi's graduate studies have been supported by the Alberta Centennial Addiction and Mental Health and Addiction Research Chair Fund (held by Dr. Katherine J. Aitchison) and the Edmonton Zone Medical Staff Association Quality Improvement Grant (PI: Dr. Katherine J. Aitchison, Co-PI: Dr. Avininder Aulakh). Dr. Lodhi received a University of Alberta Doctoral Recruitment Scholarship.

3.3 Alcohol and metabolic syndrome: A review

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Keywords:

metabolic syndrome, weight loss, antipsychotics, dietician, psychosis

3.3.1 Alcohol

Human beings have been making fermented drinks such as alcohol since at least the seventh millennium B.C. (McGovern et al., 2004), and today they are very widely consumed. However, alcohol consumption has resulted in many deleterious effects. According to the Independent Scientific Committee on Drugs, U.K., ethyl alcohol or alcohol may perhaps be the most harmful substance amongst many licit and illicit substances of misuse (Nutt et al., 2010). In this essay, we shall examine the literature for the effect of alcohol on metabolic syndrome (MetS), a risk factor for cardiovascular disease and diabetes mellitus. The new harmonized criteria for MetS are abdominal obesity with adjustment for ethnicity, ≥1.7 mmol/L of triglycerides, HDL of <1.04 mmol/L for men or <1.30 mmol/L for women, ≥130/≥85 mm Hg of BP and fasting glucose of more ≥ 5.6 mmol/L (Alberti et al., 2009). Pubmed and EMBASE databases were searched using the following search strings: “alcohol” AND “ethyl alcohol” AND (“metabolic syndrome” OR “abdominal circumference” OR “blood pressure” OR “glucose” OR “diabetes” OR “lipid profile” OR “triglycerides” OR “HDL”). The literature includes meta-analytic outcomes as the highest level of finding.

3.3.2 Alcohol and blood pressure (BP)

Alcohol intake is associated with an increase in systolic and in diastolic BP (Wakabayashi, 2011a; Dakeishi et al., 2006; Okubo et al., 2001; Nakanishi et al., 2001). The evidence regarding the effect of the amount of alcohol consumed and BP is inconsistent, with some even suggesting that there may be no relationship between the two (Klatsky et al., 2006) while others have observed a stronger effect beyond a certain cutoff, e.g., 60 g of ethyl alcohol a day (Dakeishi et

al., 2006). Other factors that mediate the effect of alcohol on BP include frequency and severity of alcohol use, age, and use of nicotine. Habitual alcohol use causes an increase in BP, and this effect becomes stronger with advancing age (Rossouw et al., 1992; Wakabayashi, 2007). Smokers who drink alcohol have a higher BP than those who don't (Wakabayashi, 2008). High BP, is the most frequent metabolic abnormality after elevated triglycerides, in alcohol dependent subjects (Mattoo et al., 2013; Kahl et al., 2010). In meta-analyses, alcohol intake along with genetic factors has been shown to elevate BP (Chen et al., 2008) and reduction of alcohol intake was associated with a reduction in high BP (Xin et al., 2001; Dickinson et al., 2006).

3.3.3 Alcohol and triglycerides

Animal studies have shown that alcohol intake increases triglyceride levels (Chang et al., 2007). A similar amount of calories consumed in diets with and without alcohol leads to an increase in triglycerides over two weeks (Contaldo et al., 1989). In animal studies, the effect of alcohol on triglycerides is seen within 12-16 hours after administration (Mallov et al., 1956). Alcohol consumed with fatty food increases triglyceride levels at a faster rate compared to without (Van de Wiel, 2012). A temporary increase in triglyceride levels of up to 15.3% occurs after alcohol intake and this effect is more pronounced in middle-aged compared to younger men (Veenstra et al., 1990). In a meta-analysis, triglycerides increased by 5.69 mg/dl if 30 g of alcohol/day is regularly taken (Rimm et al., 1999). The change in triglycerides with alcohol can follow very different trajectories in men and women (Onat et al., 2008). The amount of alcohol consumed and gender can therefore impact the effect on triglycerides. Heavy alcohol users such as alcohol dependent patients have high triglycerides compared to other addictions (Mattoo et al., 2013). Habitual alcohol use causes an increase in triglycerides, with the effect getting stronger with age

(Rossouw et al., 1992). Conversely, if one consumes alcohol in smaller amounts, it can reduce triglycerides. For example, light drinking was sufficient to significantly lower serum non-high density lipoprotein cholesterol and this effect was more pronounced in women than in men in a Japanese study (Wakabayashi et al., 2009).

3.3.4 Alcohol and high density lipoproteins (HDL)

Alcohol is known to cause an increase in serum HDL consistently (Rossouw et al., 1992; Gupta et al., 1994; Goude et al., 2002; Nanchahal et al., 2000; De Oliveira et al., 2000; Szegedi et al., 2000; Sillanaukee et al., 1993; Andrade et al., 1990; Valimaki et al., 1988; Angelico et al., 1982; Lindner et al., 1981; Sierksma et al., 2002). The increase in HDL occurs with moderate, heavy and binge drinking (Galan et al., 2014). Intake of alcohol leads to an increase in HDL compared to food intake with similar amount of calories consumed, and this suggests the increase in HDL is perhaps not a consequence of any additional calories that alcohol provides (Contaldo et al., 1989). The increase in HDL can be by 3.99 mg/dL with 30 g of alcohol intake per day, according to a meta-analytic study (Rimm et al., 1999). The time taken for alcohol to increase HDL level can be between 10 days or three weeks (Hartung et al., 1990; Sierksma et al., 2002). The increase is usually temporary if alcohol use is stopped and occurs by a margin of up to 11.5% in middle aged compared to younger men (Veenstra et al., 1990). Acute increases in HDL after alcohol consumption are due to hepatic lipase inhibition (Goldberg et al., 1984). Genetic variation in the lipoprotein lipase gene and dietary unsaturated fat interact to increase HDL in moderate alcohol consumers (Baik et al., 2013). Other genes that impact the effect of alcohol consumption on HDL include polymorphisms in the apolipoprotein E (*APOE*) gene (Djousse et al., 2004). Forty to 60% of the atheroprotective effect of alcohol is mediated by the HDL increase through HDL's

role in the reverse cholesterol efflux (RCE). RCE is a process by which HDL protects body cells from the harmful effects of cholesterol by inducing free cholesterol efflux from these cells, which is then transported to the liver and excreted in the feces. The effect of alcohol on RCE via HDL is significant, but the degree of change may not be large (Kralova Lesna et al., 2010). Nonetheless, alcohol-induced elevation in HDL levels is related to a reduced risk of myocardial infarction and CVD (Gaziano et al., 1993; Nanchahal et al., 2000).

3.3.5 Alcohol and total lipid profile

If we look at the change in total lipid profile (LDL, HDL, triglycerides) after alcohol use, an increase in HDL with a reduction in LDL is noticed in light drinkers (Choudhury et al., 1994). The combination of a higher HDL and lower LDL or triglycerides is a reason for the reduction in the risk of developing coronary artery disease. Women may benefit more than men in terms of a favorable change in the lipid ratios, such as LDL/HDL and triglycerides/HDL. Light alcohol intake reduces non-HDL cholesterol in a more pronounced manner in women than men (Wakabayashi et al., 2009). In women, alcohol drinking is inversely associated with atherogenic indices irrespective of smoking status, and the inverse association of alcohol drinking is stronger with the LDL/HDL ratio than with the triglyceride/HDL ratio (Wakabayashi, 2013).

3.3.6 Alcohol and fasting blood glucose/diabetes

Alcohol consumption in high quantities (more than 30 g of ethyl alcohol) has been related to impaired fasting blood sugar (Roh et al., 2009). People who are dependent on alcohol have an increased risk of developing elevated fasting blood glucose (FBG) levels (Kahl et al., 2010).

Genes and gender may interact to produce different effects on fasting blood glucose. For example, in one study, the *ADH2*1/1* genotype men had higher FBG, while *ALDH1*1/1* genotype women had lower FBG; and this did not depend on the amount of alcohol intake (Dakeishi et al., 2008). In this relationship, as similar to others described in the previous paragraphs, the amount of alcohol consumed may increase or decrease the risk of high blood sugar. In animal studies, moderate amounts of alcohol had enhanced sensitivity to insulin in the liver based on the HOMA-IR and lipid profile (Tomie Furuya et al., 2005). In women, moderate regular consumption of alcohol causes increased insulin sensitivity (McCarty, 2000). A large systematic review and meta-analysis published in 2015 observed 63 g/day of alcohol (peak risk reduction between 10-14 g/day) to be a cut-off relevant to the risk of having diabetes (Knott et al., 2015). Those using less than 63 g /day were at a lower risk of type 2 diabetes, while those using more were at a higher risk. Gender affects the way alcohol affects the intake of other food substances. A cohort study of 89,538 women and 48,493 men reported that in women drinking 2.5 to 49.4 g of alcohol a day carbohydrate intake reduced by 24 g/day compared to abstainers, indicating alcohol displaced sucrose, since the reduction in carbohydrate was due to a decrease in sugar consumption. For men on the other hand, calories from alcohol were added to calories from other sources (Colditz et al., 1991).

3.3.7 Alcohol and abdominal circumference

The relationship between alcohol and abdominal circumference depends on the amount and type of alcohol consumed. Total alcohol intake and drinking beer is associated with an increased abdominal circumference (Ferreira et al., 2008; Xiao et al., 2015; Bergmann et al., 2011; Bendsen et al., 2013). Another interesting finding is that this increase may be independent of the

BMI (Dallongeville et al., 1998; Sakurai et al., 1997). Gender has an impact on the change in abdominal circumference associated with alcohol consumption. A negative correlation between body fat and alcohol intake has been observed in women (Brandhagen et al., 2012), especially in those who drink rice wine (Xiao et al., 2015).

3.3.8 Alcohol and metabolic syndrome

As summarized in the previous Chapters, there are many factors that can affect the relationship between alcohol and each of the five individual criteria of MetS. When the effect of alcohol on MetS (with all five criteria) is considered, the following observations can be made. If alcohol consumption is excessive, it increases the risk of developing MetS. For example, in those who are dependent on alcohol, there is a clear increase in prevalence of MetS (Mattoo et al., 2013). Up to 21.6% of subjects who have alcohol dependence may also have MetS (Mattoo et al., 2013). Epidemiological studies have found an association between high level drinking and MetS (Vicente-Herrero et al., 2015; Xi et al., 2013; Villegas et al., 2009). Three (Villegas et al., 2009) to five (Barrio-Lopez et al., 2013) standard drinks of alcohol have been suggested as the cut-off for alcohol consumption beyond which the risk of MetS increases significantly. On the other hand, consuming less than 15g/day of ethanol can reduce the chance of developing MetS (Corbaton-Anchuelo et al., 2013). Moderate consumption of alcohol was also associated with reduced chance of having MetS (Sidorenkov et al., 2010; Gigleux et al., 2006). Therefore, there may be a U- or V-shaped relationship between alcohol and MetS as evidenced in a Japanese study that found an inverse and positive association between MetS with the consumption of light and excessive amounts of alcohol, respectively (Wakabayashi, 2011b; Wakabayashi, 2010). A meta-analysis of 28,862 participants reported that very light drinking (0.1 to 5 g/day) reduces the

risk of MetS by 14% and heavy drinking (>35 g/day) increases the risk of MetS by 84% (Sun et al., 2014). Another meta-analysis suggests that <40 g alcohol per day for men and <20 g alcohol per day for women reduces the risk of developing MetS (Alkerwi et al., 2009).

3.3.9 Discussion

From the above literature, the following conclusions can be drawn: Alcohol use is related to an increase in abdominal circumference, blood pressure, HDL, triglycerides, blood glucose and therefore MetS. However, this relationship is non-linear and evidence points to a benefit of low level or light and even moderate amounts of alcohol intake. In addition, there are other factors such as gender, genetics and other substance use that can impact the relationship between alcohol and MetS. One limitation in using this information for clinical practice is to decide at what amount of alcohol intake harmful metabolic effects emerge. The limit at which the risk of MetS increases in the studies described above can vary between 15 to 40 g of alcohol/day. Many countries have different measures of a standard drink (e.g., UK=8 g and Canada=13.6 g). The current Canadian guidelines for low risk drinking recommend less than two standard drinks per day (27.1 g/day) and less than 10 standard drinks per week (19.4 g/day) for women and, less than three standard drinks per day (40.8 g/day) and less than 15 standard drinks per week (29.1 g/day) for men (Butt et al., 2011). Psychiatric patients consume more alcohol than the general population. For example, in those who have psychosis, up to 50% have a lifetime history of alcohol or cannabis dependence (Morgan et al., 2013). The treatment of psychosis (antipsychotic medications) is known to elevate the risk of developing a MetS (Vancampfort et al., 2015) and psychosis patients are likely to have a sub-optimal lifestyle. For people with mental health

problems, the guideline does recommend no alcohol use, but this is not routinely advised or followed by patients in clinical practice.

Conclusion

The Canadian Centre on Substance Use and Addiction (CCSA) Low-Risk Alcohol Drinking Guidelines recommend that psychiatric patients do not consume any alcohol. This is likely to create difficulties in advising them about the safe amount of alcohol permissible, as no use may not be practically possible. I believe there is need for better guidance from the CCSA on alcohol focused harm reduction strategies, in those with a mental illness.

If we are to lower the risk of developing MetS in women with psychiatric disorders, the definition of 'low risk' daily drinking should be reviewed, since these limits may well be exceeding the cut-off for daily drinking beyond which there is more harm than benefit in terms of MetS.

3.3.10 Acknowledgements

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CHAPTER 4. *COMT* rs4680 AND LIFETIME COCAINE USE IN CAUCASIAN PATIENTS WITH A PSYCHIATRIC ILLNESS

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Keywords:

Catechol *O*-Methyltransferase, rs4680, cocaine, psychiatry, substance-related disorder

4.1 Abstract

Background. Altered functioning of dopamine and related systems is an important factor in the development of addiction. Variations in the gene coding for the dopamine catabolizing enzyme catechol-*O*-methyltransferase have been related to substance use. It has been reported in an African-American sample that A/A homozygotes of the *COMT* rs4680 (Val158Met) variation have a greater risk of cocaine addiction compared to G/G. We examined the association between lifetime cocaine use (LCU) and rs4680 in a Canadian Caucasian sample with a psychiatric diagnosis.

Methods.

200 self-reported Canadian Caucasian psychiatric patients (75% psychosis) were selected from a larger study examining genetic factors in psychosis. Self reported LCU was evaluated using logistic regression with rs4680 and other clinically relevant predictors. A 'sum-score' from 0-7, representing a degree of polysubstance use in lifetime cocaine users, was also examined using ordered logistic regression with the same predictors. Odd ratios (OR) and *p* values are reported.

Results.

The G/G genotype of rs4680 (encoding Val/Val) was associated with LCU (G/G versus A/A: OR=2.58, p=0.032) and there was trend level association with A/G (A/G versus A/A: OR=2.03, p=0.063). The strength of the association increased when comorbid substance use in cocaine users was considered (G/G versus A/A: OR=3.13, p=0.002, G/A versus A/A: OR=1.97, p=0.033)

Conclusions. The *COMT* rs4680 polymorphism is associated with lifetime cocaine use in psychiatric subjects even after adjustment for important co-variates.

4.2 Introduction

Over 5000 years ago, in the Inca Empire of Peru, coca leaves were used for a variety of purposes (Fairley, 2007; Goerig et al., 2012). Modern local anesthesia was preceded by Carl Koller's experiment in 1884, that used cocaine to anesthetize the cornea (Brain et al., 1989; Goerig et al., 2012). Cocaine misuse is a significant problem and according to the 2013 Canadian Tobacco Drug and Alcohol survey, 7.2% of the Canadian population reported a history of lifetime cocaine use (Health Canada, 2013). Risk factors for cocaine use include environmental factors such as cannabis use as a gateway drug (Secades-Villa et al., 2015). Equally important is the genetic contribution to cocaine use disorders, as evidenced by the fact that the estimate of heritability for the same varies between 42 to 79% (Kendler et al., 2000; Tsuang et al., 2001; van den Bree et al., 1998). Candidate genes investigated for cocaine misuse include a variable number tandem repeat polymorphism in a gene encoding the dopamine transporter (*SLC6A3*) (Guindalini et al., 2006), dopamine receptor polymorphisms (the A1 allele in *DRD*2 and the *Msc*I/*Bal*I polymorphism in *DRD3*) (Noble et al., 1993; Comings et al., 1999), *CNR1* SNPs rs6454674[G] and rs806368[C] (Clarke et al., 2013) and the *COMT* rs4680 polymorphism (Lohoff et al., 2008; Levran et al., 2015).

Catechol-*O*-methyltransferase (COMT) is an enzyme involved in catecholamine degradation and occurs in two isoforms: membrane bound COMT (MB-COMT), that is anchored to intracellular membranes, and soluble COMT (S-COMT) (Weinshilboum et al., 1999). In most studies of the two isoforms, MB-COMT is more relevant to behavior (Tammimaki et al., 2016; Walton et al., 2014). The *COMT* rs4680 polymorphism, is a single nucleotide substitution (G>A) which results in a valine to methionine substitution at codon 158 of MB-COMT, hence known as

Val158Met. This is associated with a significant variation in the activity of the COMT enzyme, with the G/G genotype coding for high activity and the A/A genotype for an enzyme with a three- to four-fold lower activity level (Chen et al., 2004; Lachman et al., 1996). Cocaine binds to the outward or extracellular facing confirmation of the dopamine transporter and blocks dopamine reuptake, increasing synaptic dopamine (Ritz et al., 1987; Jean et al., 2017). Dopamine is a key neurotransmitter in reward-related behaviors (Mikhailova et al., 2016), and it can be therefore hypothesized that variability in dopamine metabolism, at least partly mediated by rs4680, could then influence the risk of cocaine use. In an animal study, though, the COMT enzyme activity was not related to the reinforcing effects of cocaine (Mus et al., 2012). On literature review, we came across only one study on rs4680 and cocaine misuse. Lohoff *et al.* (2008), studied subjects of African descent with cocaine dependence and observed an increase in cocaine dependence for those who had the A/A genotype and a two-marker haplotype (Lohoff et al., 2008). Other studies on rs4680 and cocaine examined cocaine-induced paranoia (Ittiwut et al., 2011; Kalayasiri et al., 2010) and prediction of response to computerized CBT treatment (Carroll et al., 2015). Interestingly, rs4680 has been associated with other addictions such as alcohol; however, this is inconsistent (Foroud et al., 2007; Hallikainen et al., 2000; Kweon et al., 2005), and there is variation in the genotype (G/G or A/A) associated (Kauhanen et al., 2000; Nedic Erjavec et al., 2014; Sery et al., 2006). An association with the rs4680 G/G genotype has been reported when a number of substances are studied together, i.e. in polysubstance misuse (Vandenbergh et al., 1997).

Hence, to our knowledge, there is no study that has previously examined rs4680 and lifetime cocaine use with or without consideration of other substances, in a group of subjects most of whom have a diagnosis of psychosis. While results of studies in the general population

may be applicable to subjects with a mental illness, some significant differences are seen in those with a psychiatric illness. For example, patients with a mood disorder may have a different level of cocaine-induced dopamine elevation. In human subjects, low serotonin transmission has been associated with a greater dopaminergic response to cocaine (Cox et al., 2011). Also, there is some evidence that *COMT* can affect dorsolateral prefrontal cortex molecular composition differently in schizophrenia compared to normal subjects (Shukla et al., 2016). We present the results of our study, examining the association between rs4680 and lifetime cocaine use in a group of Canadian Caucasian patients with a psychiatric disorder (predominantly psychosis).

4.3 Methods

Study objective

The first objective of our study was to examine the relationship between rs4680 and lifetime cocaine use in those with a mental illness. The second objective was to assess the influence of rs4680 on lifetime cocaine use when additional comorbid substances were taken into consideration.

Study population

We conducted our investigation in a group of 200 patients with a mental illness from a larger group recruited for a genetic study from the Edmonton Early Psychosis Intervention Clinic, the Nova Scotia Early Psychosis Program and the Alberta Hospital Edmonton. The key criteria for inclusion for this study was having a psychiatric diagnosis. The study description, method of

collecting genetic samples and laboratory methods have been previously described (Lodhi et al., 2017).

Substance use history

Data on substance use was collected using a computerized self-report survey (DRUGS survey) developed by Dr. Purdon, based on the DSM-5 (Purdon, 2007). The DRUGS survey collected information on lifetime cocaine use. Lifetime cocaine use was defined in our study as having *any* history of cocaine use. Additional data on lifetime use of the following substances was also available: tobacco, alcohol, cannabis, stimulants, opioids and hallucinogens.

Psychiatric symptom severity

Two measures of psychiatric symptom severity were available: the Beck Depression Inventory - II (BDI-II), a self-report instrument for the presence and severity of depression (Beck et al., 1996) and the Peters Delusion Inventory (PDI), a 21-item measure of unusual beliefs (Peters et al., 2004).

Genotyping

Please refer to an earlier publication describing our genotyping methods (Lodhi et al., 2017)

Statistical analysis

STATA version 3.1 was used to perform the statistical analysis. Chi-square and t-tests were utilized to examine baseline differences between those with and without a history of lifetime cocaine use. Using logistic regression, clinically relevant individual factors were assessed for association with the categorical outcome of lifetime cocaine use history (absent or present). Only those that had associations at the level of $p < 0.20$ were then included in a multivariate logistic

regression. Since some patients use multiple other substances, a novel category for polysubstance use was created. Lifetime history of other substances used with cocaine were assigned to a new ordinal variable, 'sum score'. Sum score (0-7) was created as follows: 0=No lifetime cocaine use, 1=lifetime cocaine use, 2=1+lifetime nicotine, 3=2+lifetime alcohol, 4=3+lifetime cannabis, similarly for 5,6 and 7, the substances added were opioids, stimulants and hallucinogens. Subjects were categorized as 0-7 based on the highest number of substances they had reported using in their lifetime, after the appearance of that substance in the order. Ordered logistic regression was used to assess the impact of rs4680 on the sum score. The following covariates were examined for inclusion in the logistic and ordered logistic regressions: age in years, gender, psychiatric diagnosis, BDI-II and PDI score, and education level. Regression results are reported using odds ratio (OR) and significance level (p).

4.4 Results

Table 4.1 provides a summary of the sample studied and the distribution of the predictors used in regression analyses based on the history of lifetime cocaine use. Our sample predominantly comprised individuals with a history of psychosis (75%). The genotypic distributions were in Hardy-Weinberg equilibrium (X^2= 1.2073, df=1, p=0.272). There was a trend for more G/G and A/G subjects in those with a history of lifetime cocaine use. The lifetime cocaine use history variable was also associated with education, age and median PDI scores.

Table 4.1 Details of the study sample by lifetime cocaine use

	Lifetime cocaine use present	Lifetime cocaine use absent	*p*
Number of patients	88	112	
Gender (%)			0.053
Men	76.14	63.39	
Women	36.61	36.61	
Diagnosis (%)			0.198
Psychosis	70.45	79.46	
Affective/Anxiety/Other	13.64	12.50	
Substance use disorder	15.91	8.04	
rs4680 (%)			0.053
A/A	21.59	35.71	
A/G	47.73	44.64	
G/G	30.68	19.64	
Education			**0.002**
Grade 12 or less	44.32	27.68	
Graduate	29.55	21.43	
Post-secondary	26.14	50.89	
Median PDI[##] score	7.5	4	**0.032**
Mean BDI-II score	14.5	11.5	0.094

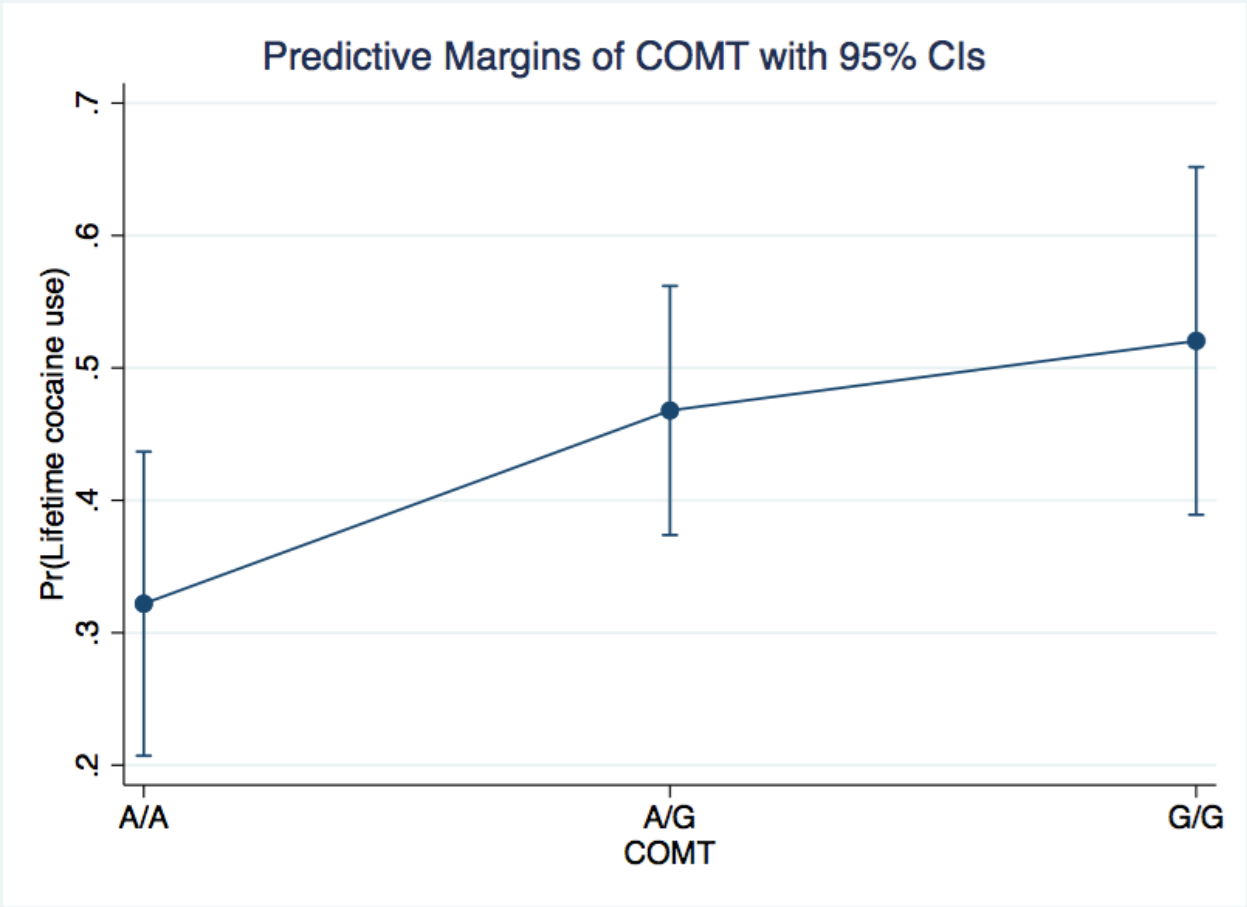
*****Using a chi-square test for categorical data, *t*-test for normally distributed data, or a Kruskal-Wallis *k*-samples test for non-parametric data

[#] BDI-II – Beck Depression Inventory II

[##] PDI – Peters Delusion Inventory

On logistic regression analysis adjusting for the other variables, there was a significant effect for rs4680 G/G versus A/A (OR=2.59, p=0.031) and a trend level effect for A/G versus A/A (OR=2.03, p=0.064) on the risk of lifetime cocaine use. Other significant factors in the logistic regression associated with lifetime cocaine use were: education (OR=0.63, p=0.022) and PDI score (OR=1.10, p=0.006). Margins were generated post- regression and a plot of the predictive margins for rs4680 and the risk of lifetime cocaine use is shown below in Figure 4.1.

Figure 4.1 Post logistic regression predictive margins of rs4680 with 95% confidence intervals for the risk of lifetime cocaine use



Ordered logistic regression with the same predictors was also used to assess the impact of rs4680 on the sum score. The association of rs4680 with the sum score (representing polysubstance use including cocaine) was greater than the association with lifetime cocaine use alone (G/G versus A/A: OR=3.14, p=0.002, A/G versus A/A: OR=1.97, p=0.033,). Additional variables of significance in the ordered logistic regression analysis were age (OR=0.96, p=0.029) and PDI score (OR=1.09, p=0.003) with a trend for education (OR=0.72, p=0.053).

4.5 Discussion

We herein report an association between *COMT* rs4680 and lifetime cocaine use in Canadian Caucasians with a history of psychiatric illness. In a general population study, A/A carriers were at risk of cocaine misuse and in the present study G/G is the at-risk group (Lohoff et al., 2008). This variation could be due to differences between the studies. Subjects were of Caucasian ethnicity in the current study and the prior sample was of African-American descent. The other significant difference is that the current study examined lifetime cocaine use, whereas the previous study investigated cocaine misuse.

Our results are concordant with the hypothesis that, in Caucasians, due to lower experience of reward from daily life, G/G subjects may be more vulnerable to use substances (Wichers et al., 2008). Our results are also consistent with a previous rs4680-polysubstance use study, implicating G/G as the at risk genotype (Vandenbergh et al., 1997).

The observation that the strength of the rs4680 association with the cocaine phenotypes increased when additional substances were considered is open to several interpretations: Additional substances may be indicative of increased severity of cocaine use. Alternately rs4680 may be associated with a general increase in substance use, not specifically associated with

cocaine. Our dataset was too small to assess the rs4680-lifetime cannabis/alcohol use owing to the distribution of users and non-users for these substances, hence we tested other substances using the ordered logistic regression, and excluded cocaine use data from their sum scores. As a sensitivity analysis, none of the sum scores from other substances were significantly associated with rs4680 if cocaine is excluded from their sum score. This perhaps means that lifetime cocaine use is the main driver for the association with rs4680. The contribution to the variance in the general vulnerability to substance use by a single nucleotide polymorphism may be around 20%, and can increase with the addition of polymorphisms (Palmer et al., 2015). The use of additional polymorphisms relevant to cocaine and/or polysubstance use may have improved variance explained by our regression models.

To conclude, we observed a significant association between rs4680 with lifetime cocaine use in Canadian Caucasians with a psychiatric diagnosis, after adjustment for clinically relevant covariates. Future studies of this relationship should note that our study had a mixture of psychiatric diagnoses evaluated using the SCID, used genetic markers to confirm Caucasian ethnicity (Lodhi et al., 2017), and included information on other substances used while evaluating lifetime cocaine use.

4.6 Acknowledgements

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CHAPTER 5. EFFECT OF *BDNF* AND *AKT1* ON THE AGE OF ONSET OF PSYCHOSIS

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Key words: Brain derived neurotrophic factor, cannabis, psychosis, gene, *AKT1*

5.1 Abstract

Aims. *BDNF* rs6265 [A/A] interacts with cannabis use to influence age of onset of psychosis (AoP) and gender may affect this relationship. An *AKT1* rs2494732-cannabis interaction on AoP has not been previously investigated. We examined the gender and cannabis use-adjusted role *of BDNF* rs6265 [G>A] and *AKT1* rs2494732 [T>C] on AoP in Canadian Caucasians.

Methods. 167 subjects with psychosis were recruited as previously described. Data on AoP, lifetime and age of regular cannabis use were collected. Kaplan Meier, Cox regression analyses and ANOVA were conducted.

Results. A trend effect for a *BDNF* rs6265 [A/A] – gender interaction (HR = 2.08, $p = 0.067$) on AoP, controlling for regular cannabis use was observed. No association was observed between *AKT1* rs2494732 [T>C], or a *BDNF* rs6265 [G>A] - *AKT1* rs2494732 [T>C] interaction and AoP.

Conclusion. Larger collaborative research projects are required to investigate the important role gender plays in mediating the effect of *BDNF* rs6265 [G>A] – cannabis interaction on AoP.

Keywords. Brain-Derived Neurotrophic Factor, cannabis, gender, psychotic disorders, polymorphism

5.2 Introduction

Premorbid cannabis use is a risk factor for psychosis (Marconi et al., 2016) and it has also been associated with an earlier age of onset of psychosis (AoP) (Di Forti et al., 2014). Besides cannabis use, other factors independently associated with the AoP are gender (Castle et al., 1998) and genetic variants (Wang et al., 2011; Lett et al., 2013; Vares et al., 2010; Takase et al., 2001). However, in terms of prior investigations of the interactions of genes and cannabis on AoP, only a limited number of genes have been reported to be of significance (Caspi et al., 2005; Decoster et al., 2011). Investigating the independent and interaction effects of genetic factors mediating the effect of cannabis on phenotypes of psychosis such as AoP may add to the understanding of underlying biological mechanisms.

The *BDNF* rs6265 variant results in a valine to methionine substitution at codon 66 (rs6265), and it has been associated with psychosis (Numata et al., 2006; Mezquida et al., 2016). Environmental effects via epigenetic mechanisms, e.g. methylation in response to hypoxia, of the CpG dinucleotide, which is created or abolished by the single nucleotide polymorphism (SNP) rs6265, can affect the risk of psychosis too. Such methylation effects are associated with intermediate schizophrenia phenotypes and affected by genotype, with G/G experiencing more methylation compared to A/G and methylation not possible in A/A (Ursini et al., 2016). To our knowledge, there are two publications that evaluated the rs6265 – cannabis interaction effect (interaction χ^2 (1) = 4.99, p=0.026 in women) on AoP (Decoster et al., 2011; Mané et al., 2017a). A three-way rs6265 - cannabis use - gender interaction was related to earlier AoP in one study (Decoster et al., 2011). In this study, rs6265 Met allele female cannabis users had an earlier AoP.

The other study reported an independent but no interaction effect of rs6265 Met-carriers and early cannabis use on AoP (Mané et al., 2017b); however, the effect of gender was not reported.

The enzyme AKT also known as protein kinase B (encoded by the gene *AKT1*) is important for dopamine signaling via D_2 receptor stimulation and the AKT-GSK3 signaling cascade (Beaulieu et al., 2007). AKT/GSK3 signaling pathway dysfunction is seen in schizophrenia (Emamian, 2012), along with low AKT levels (Szamosi et al., 2012). The GABA hypothesis of schizophrenia involves AKT and *AKT1* gene variants (Chang et al., 2016). Cannabis can influence phosphorylation of AKT by stimulation of the cannabinoid receptor (CB_1) (Ozaita et al., 2007). The C/C genotype of rs2494732, an *AKT1* SNP, is reported to influence the risk of developing psychosis in cannabis users (Di Forti et al., 2012). To the best of my knowledge, there are no data available relevant to the rs2494732 – cannabis interaction on AoP; furthermore, there was only one AoP study of single nucleotide polymorphisms in *AKT1* (Chow et al., 2016).

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

5.3 Methods

Patients with psychosis were recruited from Edmonton and Halifax, and cannabis use information was collected using a self-reported electronic questionnaire. Details of the sample,

cannabis use data collection, diagnosis and laboratory methods for DNA extraction have been previously described (Lodhi et al., 2017).

Genotyping

The rs6265 marker was genotyped using the TaqMan® SNP Genotyping Assay C__11592758_10 on a ViiA7 real-time PCR system (Thermo Fisher Scientific, Canada), with polymerase chain reaction (PCR)-restriction fragment length based polymorphism (RFLP) analysis for confirmation. For the latter, the PCR forward and reverse primers were 5′-AAA GAA GCA AAC ATC CGA GGA CAA G-3′and 5′-ATT CCT CCA GCA GAA AGA GAA GAG-3′, respectively, with reaction conditions of 94 °C for 5 min; 35 cycles of 1 min at 94 °C, 2 min at 55 °C, and 2 min at 72 °C; followed by a final elongation at 72 °C for 4 min. PCR was carried out using FroggaMix (FroggBio, Toronto, Canada), with 0.125U/µl Taq DNA polymerase, 0.2mM dNTPs, 1.6 mM $MgCl_2$ followed by digestion with *Nla* III (New England Biolabs, USA). Repeats were conducted for any calls not readily resolved; the concordance rates between duplicates was 100%. In this manner, 99.5% of the samples were genotyped.

Statistical analyses

STATA 13.1 was used to analyze the study data. The rs6265 A/A and A/G genotypes were combined to create an rs6265 'A carriers' group because of the low frequency of the A/A genotype patients and for consistency with a previous similar study (Decoster et al., 2011). Predictors for AoP included: genotypes (rs6265: Val and Met carriers; rs2494732: C/C, C/T and T/T), gender (female and male) and age at regular cannabis use (ARCU): no regular use, regular use before age 20, and at or after age 20). Kaplan-Meier analyses (results reported using the log-rank test (LR)) followed by Cox-regression analyses were performed. The latter using three

models: main effect of gender, genotypes and cannabis use variables; interaction of gender and genotypes controlling for cannabis use variables; interaction of genotypes and cannabis use variables controlling for gender; and lastly a gene x gene interaction controlling for gender and cannabis use. We also replicated key survival analysis results using ANOVA.

5.4 Results

One hundred and sixty-seven subjects were included: see Table 5.1 for sample details. The mean age of the study sample was 27.83 years (SD=9.77), with a mean age of onset of psychosis of 22. 93 years (SD=6.38). The duration of illness (psychosis, DUI) based on the DSM criteria varied in our sample. Approximately 48% (47.90%) had a chronic psychosis (mean DUI: 9.37 years), 41.32% were first episode (mean DUI: 0.37 years) and 10.78% were in the early stage of illness (mean DUI: 2.32 years). The frequency of rs6265 genotypes was 126 (75.45%), 40 (23.95%) and 1 (0.6%) G/G, A/G and A/A respectively, and for rs2494732 it was 49 (29.34%), 85 (50.90%) and 33 (19.76%) for T/T, C/T, and C/C, respectively.

In the Kaplan-Meier analyses, gender (p = 0.010) and ARCU (p = 0.0029) significantly affected AoP, while rs6265 (p = 0.38), rs2494732 (p = 0.91) and LCU (p = 0.63) did not. The mean AoP for men and women were 22.51 and 24.85 years respectively, and for those with ARCU before 20 and at or after 20 years were 21.29 and 24.53 years respectively.

On Cox regression analysis of AoP for the main effects of gender, ARCU, and rs6265, there was a main effect of ARCU (hazard ratio (HR) = 1.51, p = 0.028). Interaction analysis revealed no significant effects, with a trend being seen for an rs6265 by gender interaction when

controlling for ARCU. From Table 2, we infer that in the men, rs6265 Met-carriers had a trend for an earlier age of onset of psychosis (the mean AOP for rs6265 A carriers and G/G genotype was 21.62 and 22.26 years for men, and 23.82 and 27.5 years for women, respectively).

Table 5.1 Description of study sample based on age at *regular cannabis use* (RU) using number and percentage N(%) in each category

Category	Never RU	RU before 20 years	RU at of after age 20	p
Gender				**0.001**
Men	33 (53.23)	67 (81.71)	17 (73.91)	
Women	29 (46.77)	15 (18.29)	50 (29.95)	
Education				
less than Grade 12	15 (24.19)	39 (47.56	5 (21.74)	**0.005**
Marital Status				**0.007**
Single	56 (90.32)	80 (97.56)	18 (78.26)	
Married/Partner/Separated etc	6 (9.68)	2 (2.44)	5 (21.74)	

There was no significant gender controlled effect of the rs6265 by ARCU interaction term. For rs2494732, similar to rs6265, only ARCU (hazard ratio = 1.52, $p = 0.027$, for ARCU before 20 years versus no regular use) was significantly associated with AoP (Cox regression analysis for the main effect). No rs2494732 interactions with gender or cannabis use were significant for AoP. Lastly, the epistatic effect of rs6265 x rs2494732 was not significant for AoP in the Cox regression models.

We performed a confirmatory analysis using ANOVA of results with the same predictors as shown in Table 5.2. The rs6265 by gender interaction term similarly indicated a trend level effect on AoP (p=0.0521) when adjusted for the age at regular cannabis use category variable.

Table 5.2 Interaction analysis of rs6265 with gender, controlling for age at regular cannabis use (ARCU) using Cox regression for age of onset of psychosis

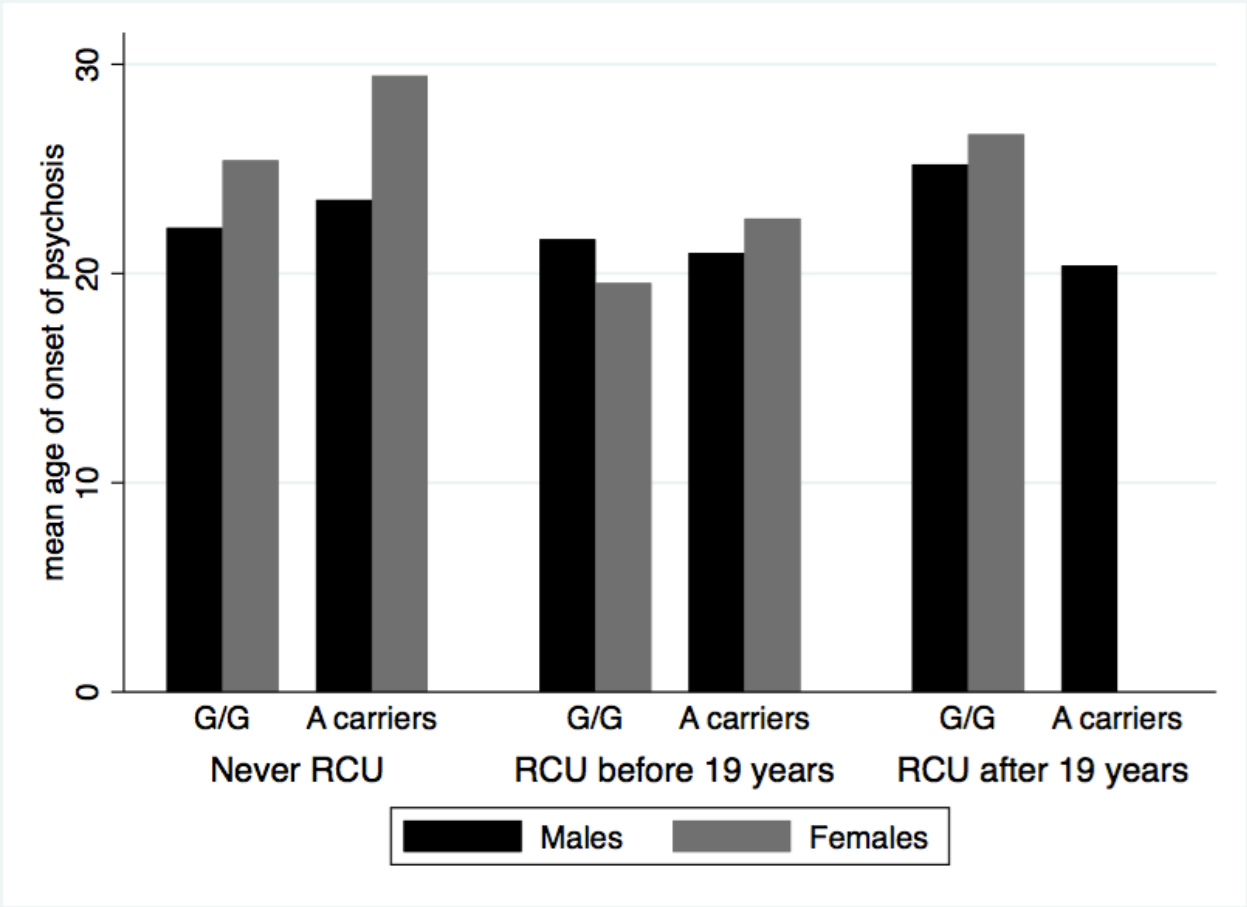
	Hazard Ratio	Std. Err.	*p*	95% CI
rs6265				
Met carriers	0.57	0.19	0.095	0.29 to 1.1
Gender				
Men	1.07	0.23	0.719	0.71 to 1.63
rs6265 # Gender				
Met carriers # Men	2.08	0.83	**0.067**	0.94 to 4.58
ARCU				
Before 19 years	1.51	0.28	**0.028**	1.04 to 2.2
After 19 years	0.89	0.22	0.65	0.542 to 1.47

5.5 Discussion

We observed a trend for an rs6265 x gender interaction; however, this was with men Met–allele carriers, unlike the previous study (Decoster et al., 2011). Figure 5.1 provides information on the mean ages of onset of psychosis for various groups categorized by age at regular cannabis use, genotype and gender. In the Decoster *et al*. (2011) sample, cannabis use was associated with reduced AoP in women who were A carriers. The contrast between their findings and ours could be at least partly due to the small sample and sub-sample sizes in our study.

Of note, there are other differences between ours and other studies such as our AoP definition being based on age of diagnosis by structured clinical interview instead of the age of first admission/first contact with a psychiatrist (Decoster et al., 2011; Mané et al., 2017a). In the present study, our sample has a lower mean age of 27.83 years, compared to 36.1 years in Decoster et al. (2011). The mean duration of illness was shorter for rs6265 G/G and A-carriers was 4.24 and 6.92 years in the present study, compared to 11.6 and 11.0 years, respectively, in Decoster et al. (2011)The signal in our study for the rs6265 – gender interaction on AoP was from *regular* cannabis use. Although "regular" cannabis use was subjectively interpreted by our participants, of note, regular cannabis use is an important factor for the transition to psychosis (Compton et al., 2009; Myles et al., 2016), and could reasonably be hypothesized to be particularly relevant to any effect mediated by a neurotrophin such as BDNF. By contrast, Decoster et al. (2011) used a cannabis usage variable defined simply as at least five times in the person's life.

Figure 5.1 Mean ages of onset of psychosis (AOP) for rs6265 genotypes by age at regular cannabis use category and gender



RCU – regular cannabis use

The limitations include a small sample size and self-reported nature of cannabis use and have been described in an earlier publication co-authored by me (Lodhi et al., 2017). In addition, if we correct for the number of genes tested (two in this analysis), the trend level signal from the rs6265-gender adjusted for regular cannabis use on AoP disappears. Our results provide some support for the inclusion of gender while examining the rs6265-cannabis interaction in psychosis, as first noted by Decoster et al. (2011). This is also consistent with prior data; for example, a gender effect on the regulation of *BDNF* gene expression in the developing hippocampus in animal models (Kight et al., 2017), an association between male gender and lower serum BDNF in schizophrenia (Zhang et al., 2014), and a gender-based effect of *BDNF* in another psychiatric disorder (i.e., Alzheimer's disease; (Li et al., 2017). As our analysis, especially the gene-gender interaction part, was limited by sample size, we suggest that future studyies of a gene-gender interaction that is adjusted for cannabis use or a three-way interaction of gene-gender-cannabis on AoP should be undertaken, and could be used for better understanding sub-groups that are most at risk of developing psychosis earlier. This and other prior work indicates that larger collaborative research projects incorporating consistent methodology are required to further delineate the effects not only of the two genes herein examined but also that of others (e.g., *CNR1*, encoding the cannabis CB_1 receptor) on age of onset of psychosis.

3.6 Acknowledgements

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CHAPTER 6. CONCLUSION

The burden of adverse health outcomes caused by medications and substances in those with a mental illness is significant. Mitigating these adverse health outcomes should improve the quality of life of our patients. In Chapters 2 and 3.1, we observed that different interventions, a medication in Chapter 2.0 and diet and lifestyle in Chapter 3.1, were useful in reducing the burden of side effects. Since bone metabolism and energy metabolism have overlapping aetiologies, it is possible that interventions in one may benefit the other. It has been reported that the addition of aripiprazole can lead to weight loss (Whitney et al., 2015) and lifestyle changes can improve bone function (Willems et al., 2017). This highlights the breadth of effect of such interventions.

Chapter 2.0 is a pilot study with a small sample size and therefore its results should be interpreted with caution. Our duration of follow-up was one year and one clinically relevant interpretation is that a long time may be required to improve bone health. In our dataset, the reduction in NTX becomes a bit more pronounced only after six months (see Figure 1.1 in Chapter 2.0). NTX is a dynamic bone turnover marker, and changes in its levels should be seen faster than on investigations such as DEXA (Dual energy X-ray absorptiometry) scans. Change in NTX levels may be seen as early as four weeks after antipsychotic medication initiation, depending on the type of medication (Liang et al., 2015). Since the change in NTX was after 26 weeks or so, it may imply that in our study if we had used DEXA scans, the improvement in bone health in might not have been detectable in many patients. Of note, DEXA scans are currently the standard method of assessing bone health, while our data and that of others regarding bone turnover markers indicates that such markers are useful in assessing bone health in psychiatry. Elevated levels of other markers of accelerated bone resorption, such as

osteocalcin, are seen in patients on long term antipsychotic medications in comparison to drug-naïve patients (Zhang et al., 2016a). Currently, bone turnover markers such as NTX are not used widely in routine clinical practice; guidelines for their use in clinical practice are evolving. The overall effect of antipsychotics on bone health, can, however, be complex. For example, the weight gain caused by antipsychotic medications can be beneficial for bone health and could be one reason for a delay in the appearance of bone pathology (Doknic et al., 2011). Additional studies are required to examine the role of antipsychotic medications and prolactin in bone health (Akinlade et al., 2017).

Other interventions that may also improve bone health and should be examined in future projects include smoking cessation and exercise. Nicotine directly affects bone resorption and osteoclastic activity, increasing the risk of osteoporosis, and hence reduction in its use may benefit bone health (Tanaka et al., 2013). Vitamin D is important in bone metabolism and its levels are likely to be lower in those diagnosed with psychosis compared to controls (Doknic et al., 2011). A comprehensive approach is required to improve bone health in psychiatric patients that includes dietary, lifestyle and pharmacological interventions.

A reduction in hyperprolactinemia had been demonstrated in an earlier study of the same sample at 26-week follow up (Mir et al., 2008). Chapter 2 demonstrates that the reduction in prolactin levels persisted at 52 weeks. While the best treatment would be to cease the antipsychotic responsible for hyperprolactinemia, it may not be possible to do that clinically. There is enough evidence to suggest a trial augmentation with aripiprazole in patients with antipsychotic related hyperprolactinemia (Montejo et al., 2017; Madhusoodanan et al., 2010; Mir et al., 2008; Lodhi et al., 2016).

Chapter 3.1 highlights that in patients who have a severe mental illness and are at risk of metabolic dysfunction, monitoring metabolic markers and providing access to a dietician should be standard care. What Chapter 3.1 also demonstrates is the more integrated dietary counselling is with the psychiatric service, the better the outcome. It will be interesting to know if components of the dietary counselling/intervention vary in effectiveness by gender, psychiatric diagnosis, comorbid substance use, genetic profile and type of psychiatric medication. Although Chapter 3.1 indicates that baseline BMI is an important predictor of future weight loss, this needs to be cautiously interpreted and assessed using a prospective study. The type and number of medications that the subjects were on at baseline were known to us, but we did not use that in Chapter 3.1 for reasons described in the discussion of that chapter. If a prospective study is conducted, I suggest that patients should be on one type or group of medications to make it easier to assess the effect of an intervention. At the UAH site, the dietician met the patient each time they visited the physician and she also arranged follow up appointments in conjunction with the physician. We could not obtain the number of times each patient saw a dietician but that would be an interesting variable to study as a 'dose-response' relationship between the number of times a dietician sees a patient and the amount of weight loss. The counselling provided by the dietician covered not just the type of food consumed and rough caloric intake, but also included looking at behaviour such as buying unhealthy food, eating larger portions, meal restriction etc.

Sections 3.2 and 3.3 of Chapter 3 highlight the difficulties in assessing a complex phenotype such as obesity. These reviews demonstrate the extent to which substances affect energy metabolism and influence markers of metabolic dysfunction. I found the U-shaped relationship between some markers of metabolic dysfunction and alcohol to be very interesting, and was surprised by the possibility that a similar relationship could exist with cannabis. Clinical

implications from 3.2 and 3.3 are that a thorough history is essential for all substances used by psychiatric patients while assessing weight, BMI and other variables related to energy metabolism.

Appendices 3 and 4 led to the hypothesis that the *COMT* rs4680 [G>A] variant is related to addiction or substance use, and this hypothesis has been tested in Chapter 4 of my thesis. Appendix 4 is a paper co-authored by me investigating the role of rs4680 in age of onset of psychosis, in which we observed a trend for earlier onset of psychosis in those who first used cannabis before age 20 (Lodhi et al., 2017). Although not included in the paper, this investigation indicated that *COMT* rs4680 [G/G] may be related to lifetime cannabis use, but since the number of subjects without cannabis use was small, we could not make firm conclusions. A reasonable hypothesis could then be that *COMT* rs4680 [G/G] influences the risk of using a substance, which in turn reduces the age of onset of psychosis. I was the first author of a book chapter that reviewed the role of genetic variants in various addictions (Appendix 3). Cocaine is one of the 13 substances we had information on in the clinical sample, and cocaine and cannabis were the only substances that had an association with *COMT* rs4680 [G/G]. As mentioned in the discussion of this chapter, since comorbid lifetime substance use is present in all cocaine users in our dataset, it is difficult to conclude that *COMT* rs4680 [G/G] is related to cocaine alone. A group of 40 patients who endorsed using all seven substances analysed in the chapter were the principal drivers for the signal in the ordered logistic regression. Hence it is quite likely that our result indicates an association between *COMT* rs4680 [G/G] and lifetime 'polysubstance use', with cocaine use being the most important substance in the model since without it there is no association with *COMT* rs4680 [G/G].

Chapter 5 (similar to Appendix 4) confirms previous associations observed between gender and age of onset of psychosis. The strength of the association between the age at regular cannabis use variable and AoP was stronger than the age at first cannabis use variable in the Appendix 4 publication. Chapter 5 also highlights the importance of having larger sample sizes and adequate sub-sample sizes in conducting a study of genetic factors, especially when interactions are being investigated. The women in our study sample are interesting and the difference in the age of onset of psychosis by rs6265 was wider in this group compared to men. When cannabis use was added to the rs6265-gender interaction, this resulted in small sub-sample sizes; hence the interaction analysis should be interpreted with caution. Previous studies indicate that *BDNF* rs6265 [A/A] patients are at risk of early psychosis, in interaction with gender (Decoster et al., 2011). If this experiment were to be repeated, it would be useful to consider the following in addition to sample size: epigenetic analysis for methylation in the promoter and at the rs6265 site of the *BDNF* gene, examine if the rs6265 by gender interaction affected other features of psychosis (for example, test if the *BDNF* rs6265 [G>A] were also associated with the onset of prodromal symptoms of psychosis), develop a way of calculating the approximate dose of cannabis used by someone before they developed psychosis analogous to cigarette 'pack-years', perhaps exclude those with substance-induced psychosis, and obtain as close an estimation as possible of the age of onset of cannabis use.

In summary, I have investigated three specific AHO: poor bone health, metabolic syndrome and early age of onset of psychosis. I have examined the effect of psychotropic agents (prescribed medication, substance use), genetic vulnerability and other clinical and demographic variables on these AHO. I have attempted to highlight that contribution to these AHO, that are complex phenotypes, is multifactorial and there may be a relationship between predictors

themselves. I demonstrate pharmacological and non-pharmacological interventions can partially reverse the AHO. The process of completing the studies with individual strengths and limitations have helped in shaping some suggestions for future research into these specific AHO. I hope that the studies and reviews described here are helpful in informing clinical management of AHO.

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Appendix

Appendix 1:

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CHANGE IN BONE TURNOVER MARKERS IN AN ARIPIPRAZOLE ADD-ON OR SWITCHING STUDY

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Keywords

Bone markers, antipsychotics, NTXC, aripiprazole,

Abstract

Background: The association between mental illness and osteoporosis and fractures is particularly pronounced in psychotic disorders. Antipsychotic use has previously been described to affect bone density.

Method: A 52-week follow-up of patients switched to aripiprazole or with aripiprazole added on, conducting a specific analysis of markers of bone turnover: urinary NTX (a biomarker of bone resorption) and serum BSAP (a biomarker of bone formation). Baseline and serial measurements of bone markers NTX, BSAP and of hormones prolactin, oestrogen and testosterone were done at weeks 0 and 1, 2, 6, 12, 26 and 52, respectively.

Results: NTX concentration reduced over time but this did not reach significance in the whole group (log-NTX: β = -0.0012, p = 0.142). For BSAP the addition of or replacement with aripiprazole produced a significant reduction (log-BSAP: β = -0.00039, p = 0.002). Analysis with prolactin similarly showed a significant reduction (log-prolactin: β = -0.0024, $p < 0.001$); other hormones did not change significantly. Sensitivity analysis to compare the switchers to aripiprazole versus the "add-on" showed that the former group had a significant reduction in NTX.

Conclusions: We found that switching to aripiprazole was associated with changes in molecular biomarkers of bone resorption, indicating a more favourable profile for bone health.

Introduction

Severe mental illness is associated with an increased risk of comorbid physical illness (De Hert et al., 2011). The basic aetiology of these associated conditions is often three-fold: less than ideal lifestyle management, physiological or endocrine dysfunction related to the pathology underlying the specific mental illness, and physiological disruption induced by psychotropic medication.

Osteoporosis is one of the physical illnesses associated with severe mental illness, with schizophrenia being associated with particularly high rates of this condition and reduced bone density in both women and men (Hummer et al., 2005; Leucht et al., 2007; Bolton et al., 2011; Kishimoto et al., 2012; Kinon et al., 2013; Wu et al., 2013). A meta-analysis found that rates of osteoporosis were two and half times more in patients with schizophrenia than controls, while rates of reduced bone density were twice as much (Stubbs et al., 2014). Incidence of fractures can be used as an indirect indicator of the reduced bone mineral density (BMD) that defines osteoporosis. In a large cohort of patients, Abel et al. (2008) observed a significant increase in relative fracture risk associated with psychotic disorders (RR 5.12 and 6.41 for females and males aged 45-74 years, respectively) that was not observed for the combined psychiatric disorder cohort (RR 1.90 and 1.4, respectively) (Abel et al., 2008). Stubbs et al. (2015) in a recently published meta-analysis confirmed higher occurrence of fractures in patients with schizophrenia compared to controls (Stubbs et al., 2015).

One major contributing factor to the accelerated trajectory towards osteoporosis observed in schizophrenia may be the use of prolactin-raising antipsychotics, which are independently associated with the incidence of hip fractures (Howard et al., 2007), and higher rates of bone

pathology (O'Keane and Meaney, 2005). Drug-induced hyperprolactinemia and associated dysfunction of the hypothalamo-pituitary-gonadal axis results in diminished concentrations of oestrogen and testosterone (Halbreich et al., 2003; Meaney et al., 2004; Misra et al., 2004; Hummer et al., 2005; Wyszogrodzka-Kucharska and Rabe-Jablonska, 2006). This can accelerate BMD decline. In this regard, O'Keane et al. (2005) observed that elevated prolactin concentrations were related to hypogonadism and low BMD (O'Keane and Meaney, 2005) in young women with schizophrenia.

We have previously described a 26-week, open-label, intention-to-treat study of patients with schizophrenia who either switched from or had aripiprazole added on to their treatment in which we observed that switching/adding-on aripiprazole resulted in significantly reduced prolactin concentrations at 12 weeks ($p = 0.003$) and the decreased concentrations persisted to the end of the 26-week study period ($p < 0.001$) (Mir et al., 2008). In the data presented here, we report on the 52-week follow-up of the same patients switched to aripiprazole or with aripiprazole added on, conducting a specific analysis of markers of bone turnover. Our aim was to determine if the addition of and/or switch to aripiprazole resulted in maintenance of the reduction in prolactin concentration to the 52-week time point, favourably affected the biomarkers of bone resorption/formation, and effected any changes in oestrogen and testosterone concentration. In this study, urinary type I collagen cross-linked N-telopeptide (NTX), a marker of bone resorption, and serum bone-specific alkaline phosphatase (BSAP), a marker of bone formation (Singer and Eyre, 2008; Wheater et al., 2013) were used. NTX and BSAP concentration reflect the degree of bone turnover at the whole body level.

To our knowledge, this is the first report of a prospective or longitudinal analysis of these biomarkers in patients with schizophrenia who had a change in antipsychotic treatment regime.

We hypothesized that the change to medication profile would be associated with a reduction in prolactin and urinary NTX, and that there would be a change in BSAP.

Methods

Individuals were recruited and followed up as described in Mir et al. (2008) and Aitchison et al. (2011) (Aitchison et al., 2011) from the COAST Team (Croydon Early Intervention in Psychosis Service), the Croydon Rehabilitation Team, and Croydon Community Mental Health Teams. In brief, the inclusion criteria were subjects (male or female) in the age range 18 to 65 years, who had a psychotic illness and lived in the community (outpatients), and failed to respond adequately to another antipsychotic (either inadequate therapeutic response or intolerance). There were no restrictions in terms of other factors such as ethnicity. The exclusion criteria were pregnancy and breast-feeding.

An open-label add-on or switch to aripiprazole was offered to eligible participants. The starting dose at the commencement of the study was 10 mg for all subjects, but was reduced to 5 mg once the 5 mg tablets became available in the UK (January 2005). Details of the flexible dose titration schedule, cross taper or concomitant administration of other antipsychotic with aripiprazole and study group are detailed by Mir et al. (2008) and Aitchison et al. (2011).

Thirty-six subjects were referred and deemed eligible for participation. Of these, eight subjects refused participation and of the 28 included, one withdrew prior to the commencement of medication because of non-compliance with any of the medications that were offered. Therefore, 27 were included; however, analysable bone marker or hormone data were available for 26 patients. Eighteen subjects reached the end-point of the study at 52 weeks, while nine dropped out prior to 52 weeks (Aitchison et al., 2011, Figure 1). Four patients dropped out due to lack of

improvement (at weeks 8,18, 27 and 27), four patients dropped out due to non-adherence (at weeks 2, 5, 35 and 48) and one dropped out due to deportation from the country. Patients who were non-adherent were refusing to take any pharmacological treatment at the time of dropout and had a history of non-adherence.

Laboratory analyses

Serial measurements of prolactin, testosterone, and 17-β oestradiol were performed and reported to 26 weeks in the initial study by Mir et al. (2008), using the methods on the ADVIA Centaur immunoassay analyser (Siemens Diagnostics, Frimley, Surrey, UK) (Mir et al., 2008). Serum concentrations of BSAP, albumin, cholesterol, calcium (for which a corrected value was produced), urinary creatinine, and urinary NTX were also measured. The time points for all biological markers were: baseline (0 weeks), 1, 2, 6, 12, 26, and 52 weeks. In addition, for some patients who had been unable to attend the predetermined follow up time points, concentrations of biological markers were present at weeks 18, 22, 30 and 35. The blood draws and urine collection were done between approximately 12 p.m. and 3 p.m., providing relative consistency in timing of sample collection. All available data on the biological markers were used in the analysis.

NTX was measured in the samples using the osteomark assay, which is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA). NTX concentration was quantified spectophotometrically and calculated using a standard calibration curve. A urinary creatinine analysis of the NTX assay values was then used to correct for urinary dilution, producing values labelled 'NTX', expressed in nanomoles of bone collagen equivalents (nmol BCE) per millimole

creatinine (mmol creatinine). BSAP was measured in the serum samples using an enzyme-linked immunosorbent assay (ELISA, Ostase, IDS, Boldon, UK).

Statistical analyses

Data were analysed using STATA version 13.1. Outliers for the outcome variables NTX, BSAP, prolactin, testosterone and oestrogen were analysed by creating simple box plots and those that were outside three times the interquartile range were excluded from the analysis. The exceptions to this were three patients who had data points for prolactin that although being outliers were identified as clinically relevant; for example, one patient had prolactin concentrations of 220, 936, 289, 1002, 1322 and 106, with 1322 mIU/L being an outlier on the box plot. However, this outlier was included due to the fact that it was following a reasonable pattern in this patient.

Analyses of bone markers (NTX and BSAP) and hormones (prolactin, testosterone and oestrogen) were then conducted using the generalized linear mixed model analysis (GLMM) (Breslow and Clayton, 1993), using the 'meglm' command. GLMM analysis is a good tool to analyse repeatedly measured data that accommodates for a variety of distributions of the dependent variable and for random effects in the analysis. By using GLMM, we were able to include 26 patients on whom information was available, despite some follow-up data being missing. GLMM analyses on the bone markers and hormones were done using the following settings: All dependent variables were log transformed and time (measured in weeks) was the within subject variable. For NTX and BSAP, the GLMM assessed fixed effects of the following factors; 'switch status' (comparison based on patients switching to aripiprazole versus patients on another antipsychotic together with aripiprazole), 'baseline medication' (categorized according to olanzapine, amisulpiride, risperidone, quetiapine, clozapine or zuclopenthixol),

ethnicity (caucasian, black, asian or mixed), antidepressant status (no use versus use) and gender (male versus female), and the following covariates; study time in weeks ('week'), age and prolactin concentration. Individuals were treated as a random factor in the model. For the hormones, we used the same predictors used for the analysis of NTX and BSAP, but without prolactin. Predictive variables were checked for collinearity and post-estimation residuals were checked for normality using the pnorm, qnorm and histogram plots.

Sensitivity analysis was conducted for NTX and BSAP by repeating the analysis in the whole group after adding total dose of antipsychotics in terms of chlorpromazine equivalents (TCPZE) as a predictor to the model. CPZE doses of atypical antipsychotics can be derived from different methods that can produce varying values for the same medication (Patel et al., 2013). For the CPZE of aripiprazole (7.5 mg), risperidone (2 mg), olanzapine (5 mg), quetiapine (75 mg) and clozapine (50 mg), we relied on the information provided by the reference commonly cited for such comparisons, Woods (2003) (Woods, 2003). It is difficult to assess the CPZE for amisulpride, but we used 100 mg as equivalent, derived from relatively recent literature (Andreasen et al., 2010; Patel et al., 2013; Leucht et al., 2015). Chlorpromazine equivalents for the zuclopenthixol depot was 100 mg/week (Taylor, 2015). In addition, we ran the primary NTX and BSAP analyses in subgroups based on predictors of interest, i.e., gender and switch status.

Results

The clinical characteristics and demographics of the 26 patients who were included in the study are detailed in Table 1, with their medications (including non-antipsychotics) being listed in

Table 2. Thirteen males (mean age 26.385, SD 6.6651) and 13 females (mean age 28.692, SD 8.8730) participated in the study.

On an initial review of the NTX data using gender specific cut off values (66 nmol BCE/mmol creatinine and 65 nmol BCE/mmol creatinine for males and premenopausal females respectively) at study intake, three out of the 26 (11.5 %) had a high NTX concentration. On the other hand, at their last follow-up point in the study, only one out of the 26 (3.8%) had an NTX concentration outside the reference range, and all of the three had a reduction in NTX. The mean NTX at baseline and week 52, after adjusting for outliers, fell from 47.83 to 31.38 nmol BCE/mmol creatinine respectively (Figure 1 near here). For those patients who had a reduction in NTX, the average reduction in the concentration was 32.47%. The change in NTX in males and females followed a similar pattern over time (Figure 2 near here). Looking at BSAP, none of the patients had a concentration outside the normal range at study intake or at last follow up. The mean BSAP at baseline and week 52 after adjusting for outliers was 74.46 and 70.56 respectively.

The results of GLMM analyses of bone markers are as follows. NTX showed a reduction over 52 weeks in the whole group, but this did not reach statistical significance (log-NTX: β = -0.0012, p = 0.142). Amongst the predictive factors for NTX, only 'switch status' (p = 0.028) had a significant effect on NTX, while the rest did not (Table 3 near here). For BSAP, using the same predictor variables for analysis as NTX over the 52 weeks, the addition of or replacement with aripiprazole produced a significant reduction in BSAP (log-BSAP: β = -0.00039, p = 0.002). Out of the predictor variables, none had significant impact on BSAP.

GLMM analyses of hormones were as follows. Prolactin analysis with 'switch status,' 'baseline medication,' gender, ethnicity, antidepressant status, age, and 'week' as predictors, demonstrated a significant reduction in prolactin (log-prolactin: $\beta = -0.0024$, $p < 0.001$) (Figure 3 near here). Gender ($p < 0.001$) and 'switch status' ($p = 0.010$) were significant predictors of change in prolactin. A similar analysis with testosterone and oestrogen as dependent variables showed a non-significant increase in both testosterone (log-testosterone: $\beta = 0.0003$, $p = 0.812$) and oestrogen (log-oestrogen: $\beta = 0.0010$, $p = 0.090$).

Sensitivity analyses were then conducted. With TCPZE as an additional predictor for NTX and BSAP, the reductions in NTX (log-NTX: $\beta = -0.0012$, $p = 0.134$) and in BSAP (log-BSAP: $\beta = -0.00042$, $p = 0.001$) remained similar to those seen in the primary analyses. Additional sensitivity analyses were conducted by repeating the primary NTX and BSAP analyses in sub-groups. When we compared those who had switched to aripiprazole versus those who remained on another antipsychotic in addition to aripiprazole, we saw that the reduction in NTX was significant in the aripiprazole only group (log NTX: $\beta = -0.0033$, $p < 0.001$) and not significant for the rest (log-NTX: $\beta = -0.077$, $p = 0.510$), while BSAP reduced significantly in both subgroups (i.e., for aripriprazole only, log-BSAP: $\beta = -0.0005$, $p = 0.033$ and for the add on group, log-BSAP: $\beta = -0.0033$, $p = 0.016$). NTX analyses in males (log-NTX: $\beta = -0.0013$, $p = 0.282$) and females (log-NTX: $\beta = -0.0009$, $p = 0.398$) separately did not show a significant change in NTX. BSAP reduction was significant in males (log-BSAP: $\beta = -0.0004$, $p = 0.022$) and females (log-BSAP: $\beta = -0.0003$, $p = 0.041$).

Discussion

Our results support our hypothesis that a change to aripiprazole would be associated with a reduction in NTX, a urinary biomarker of bone resorption, but this effect was observed only in those who switched to aripiprazole. Interestingly there was also a decrease in serum BSAP, a biomarker of bone formation. This implies that bone resorption was in phase with bone formation, *i.e.*, a healthy process of bone turnover was occurring. The decrease in NTX was greater than that of in the switchers group (34.6% versus 5.23% reductions in mean concentration by week 52), suggesting a greater reduction in osteoclast than osteoblast activity. This implies that the BMD and bone stability increased over the one-year follow-up period. In regard to effects specific to the prior antipsychotic, due to the small subsample sizes, it is difficult to draw any definitive conclusions. Association between bone turnover marker NTX and initiation of risperidone in those who are relatively medication naïve patients has been previously reported (Bishop et al., 2012). In our study of patients who are all on an antipsychotic at baseline, there was an increase in mean NTX at approximately 26 weeks. It is possible that this reflects a delayed response to the increased mean prolactin that was seen in the sample at six weeks (refer to Figure 3).

The median age of the subjects used in these analyses was 25.5 years, with a median duration of illness of five years (Table 1), indicating that the majority of the participants had reached their peak BMD prior to the initiation of prolactin-raising antipsychotics. Moreover, they had not yet reached ages associated with age-related normal decreases in BMD.

Aripiprazole is a widely prescribed atypical antipsychotic, with a mechanism distinct from other atypical antipsychotics: in addition to being an antagonist at $5HT_{2A}$ serotonin receptors, it is a partial agonist at $5HT_{1A}$ serotonin receptors and D_2-receptors (Lawler et al., 1999; Croxtall, 2012). Aripiprazole is associated with a reduction in antipsychotic-induced

hyperprolactinemia in schizophrenia. For example, Casey et al. (2008) carried out a multicentre, randomized, open-label, eight-week outpatient study that followed patients switched to aripiprazole who were earlier on other antipsychotics (including olanzapine, 55%; and risperidone, 37%) (Casey et al., 2003). They observed a decrease in the mean prolactin concentration, with the most marked change being observed when switching off risperidone to aripiprazole ($p < 0.01$). Patients offered this treatment option had demonstrated intolerance, or inadequate response to, other antipsychotic medication(s). The overall reduction in prolactin by week 52 in our study is consistent with a prior analysis in the same group at week 26 (Mir et al., 2008).

Results of previous studies regarding associations between prolactin-raising antipsychotics and measures of BMD such as osteoporosis and fracture risk are variable and summarized in a meta-analysis by Kishimoto et al. (2012) (Kishimoto et al., 2012). O'Keane and Meaney identified an association between the use of prolactin-raising antipsychotic drugs, and both hypogonadism and decreased BMD values (O'Keane and Meaney, 2005); whereas, others have found no association between prolactin and BMD (Lee et al., 2010; Renn et al., 2010; Sugawara et al., 2011). Prolactin has been found to be a mediating factor for change NTX in previous studies (Bishop et al., 2012). Concomitant antidepressant medications can also affect bone turnover (Diem et al., 2014). Nine out of 26 patients in our study were on antidepressants. We controlled for prolactin concentration and antidepressant use by including them as predictors in our analyses and they` did not affect NTX and BSAP significantly.

The other confounding factors in studies investigating the association between prolactin-raising antipsychotics and bone mineral density include sample size, age range of participants and the differences in antipsychotics (Rey-Sanchez et al., 2009). Lifestyle factors have been shown to

influence the onset of osteoporosis (Stransky and Rysava, 2009; Body et al., 2011; Kishimoto et al., 2012). Patients with psychotic disorders generally exhibit poor lifestyle choices (De Hert et al., 2011). For young patients in particular, the overall global improvement seen our cohort as indicated by the increase in clinical global impression scale (Aitchison et al., 2011) might well be associated with additional lifestyle modifications (e.g., exercise) that impacted positively on NTX. Abel et al. (2008) illustrate the importance of understanding the full range of factors contributing to reduced BMD and accelerated osteoporosis in psychosis, with an emphasis on identifying underlying physiological mechanisms and any medication-specific effects.

Limitations

As previously discussed (Mir et al. 2008; Aitchison et al. 2011), this is a pilot study in which the sample size means that both type I and type II errors may occur. However, mitigating against this is the repeated measures design, which renders the analysis more powerful. Moreover, GLMM analysis minimises problems associated with missing data and prediction by multiple regression (which can inflate observed correlations).

A further potential consideration is analytical consistency. Although NTX and BSAP correlate significantly with BMD, there has been some debate over their analytical variability between testing centres (Seibel et al., 2001; Schafer et al., 2010). Bone turnover marker concentrations can vary with factors that we were not able to control for, such as the level of activity, smoking, circadian rhythm, dietary factors including alcohol consumption, medications such as oral contraceptive pills, stage of menstrual cycle and comorbid medical illnesses such as Crohn's disease (Ju et al., 1997; Di Giovanni et al., 2008; Glover et al., 2008). However, NTX does have advantages over other markers in that it is not as sensitive to food intake (Seibel et al., 2001;

Singer and Eyre, 2008; Schafer et al., 2010). Future studies will benefit from including such factors.

Summary

In conclusion, we found that switching to aripiprazole from an existing antipsychotic was associated with changes in molecular biomarkers of bone turnover, indicating a more favourable profile for bone health. Serum prolactin concentration also decreased with addition or switch to aripiprazole. Of note, to analyse such biomarkers, from a patient's perspective this entails no more than the equivalent of routine clinical approaches (taking urine and blood samples). Moreover, bone turnover markers are being evaluated for routine use in the prediction of fracture risk and management of osteoporosis (Vasikaran et al., 2011). We suggest that our methodology merits replication and further exploration in larger studies, adequately powered to answer related questions such as the effect of prior medication, ethnicity, and lifestyle factors.

Overall we found that switching to aripiprazole was associated with changes in molecular biomarkers of bone turnover, indicating a more favourable profile for bone health.

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Table 1. Demographic and clinical characteristics of patients where sufficient data was available and those not included or dropped out prior within two weeks of the study, from 28 patients, over 52 weeks of the study.

	Included	**'Drop-outs'**
	N=26 (92.1%)	**N=2(7.9%)**
Sex		
Male, N (%)	13 (50)	1 (50)
Female, N (%)	13 (50)	1 (50)
Mean Age, years (SE)	27.53 (7.7)	23 (2.08)
Age range, years	18 - 45	18 - 45
Ethnicity		
White, N (%)	13 (57.7)	2 (100)
Black, N (%)	6 (15.4)	0 (0)
Asian, N (%)	3 (19.2)	0 (0)
Mixed, N (%)	1 (4.3)	0 (0)
Diagnosis		
Schizophrenia, N (%)	15 (57.7)	1 (50)
Schizoaffective, N (%)	6 (15.4)	0 (0)
Bipolar Affective Disorder, N (%)	1 (3.8)	0 (0)
Psychotic Depression, N (%)	6 (23)	1 (50)
Years of psychotic illness		

<1 year, N (%)	0	0 (0)
1-3 years, N (%)	7 (26.9)	0 (0)
3-5 years, N (%)	7 (26.9)	1 (50)
5-7 years, N (%)	5 (19.3)	1 (50)
7-9 years, N (%)	2 (7.7)	0 (0)
9-11 years, N (%)	2 (7.7)	0 (0)
>11 years, N (%)	3 (11.5)	0 (0)
Antipsychotic prior to switching		
(Baseline medication)		
Amisulpride, N (%)	3 (10.7)	1 (50)
Risperidone, N (%)	8 (28.5)	1 (50)
Quetiapine, N (%)	3 (10.7)	0 (0)
Olanzapine, N (%)	10 (35.7)	0 (0)
Clozapine, N (%)	1 (3.5)	0 (0)
Zuclopenthixol, N (%)	1 (3.5)	0 (0)

Table 2. Details of antipsychotics prescribed for subjects included in the study (with Bone Markers data at baseline), at baseline, week 12, week 26 and week 52 of the study, with concomitant medications (one subject per row of the table)

Week 0		**Week 12**		**Week 26**		**Week 52**	
Antipsychotics	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**	**Antipsychotics**	**Concomitant Medication**
Amisulpride 150 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg	Aripiprazole 10 mg	Citalopram 40 mg
Risperidone 2 mg	Clonazepam 1 mg	Aripiprazole 15 mg		Aripiprazole 15 mg, risperidone 1 mg		Aripiprazole 15 mg	
Quetiapine 150 mg	Lorazepam 0.5 mg	Aripiprazole 5 mg, quetiapine 400 mg		Aripiprazole 5 mg, quetiapine 500 mg		Aripiprazole 5 mg, quetiapine 500 mg	
Risperidone 6 mg	Procyclidine 5 mg	Drop out		Drop out		Drop out	
Olanzapine 15 mg	Mirtazapine 30 mg	Aripiprazole 20 mg	Mirtazapine 30 mg	Aripiprazole 20 mg, olanzapine	Mirtazapine 45 mg	Aripiprazole 20 mg	Mirtazapine

		Olanzapine 10 mg		10 mg			
Risperidone 4 mg	Citalopram 40 mg	Aripiprazole 15 mg, risperidone 2 mg	Citalopram 40 mg	Aripiprazole 20 mg	Citalopram40mg	Aripiprazole 20 mg	Citalopram40mg
Clozapine 50 mg		Aripiprazole 15 mg, clozapine 50 mg		Aripiprazole 15 mg, clozapine 25 mg		Aripiprazole 15 mg, clozapine 50 mg	
Amisulpride 400 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 20 mg	Aripiprazole 15 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 20 mg	Aripiprazole 30 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 30 mg	Aripiprazole 20 mg	Lithium 1000 mg, reboxetine 8 mg, citalopram 30 mg
Risperidone 2 mg	Fluoxetine 20 mg	Aripiprazole 20 mg, risperidone 1 mg	Fluoxetine 40 mg	Aripiprazole 20 mg	Fluoxetine 40 mg	Aripiprazole 20 mg	Fluoxetine 40 mg

Olanzapine 20 mg		Aripiprazole 20 mg, Olanzapine 10 mg		Aripiprazole 20 mg, Olanzapine 10 mg		Drop out	
Olanzapine 10 mg		Aripiprazole 20 mg, olanzapine 5 mg		Aripiprazole 20 mg	Olanzapine 10 mg	Drop out	
Risperidone 4 mg		Aripiprazole 10 mg, risperidone 4 mg		Aripiprazole 15 mg, risperidone 3 mg		Aripiprazole 10 mg, risperidone 2 mg	
Risperidal – Consta Injection 25 mg	Paroxetine 40 mg, Procyclidine 5 mg	Drop out		Drop out		Drop out	
Quetiapine 600 mg	Citalopram 40 mg, zopiclone 7.5 mg	Aripiprazole 15 mg, quetiapine 400 mg	Citalopram 40 mg, clonazepam 1 mg	Aripiprazole 15 mg, quetiapine 400 mg	Citalopram 40 mg	Aripiprazole 20 mg, quetiapine 200 mg	Citalopram 40 mg
Risperidone 3		Aripiprazole 15	Citalopram 20	Aripiprazole 15	Citalopram 20	Aripiprazole 15	

mg	mg	mg	mg	mg	mg	
Olanzapine 20 mg	Aripiprazole 20 mg, olanzapine 5 mg		Drop out		Drop out	
Olanzapine 10 mg	Aripiprazole 10 mg, olanzapine 5 mg		Aripiprazole 15 mg		Aripiprazole 15 mg	
Olanzapine 5 mg	Aripiprazole 10 mg		Aripiprazole 10 mg		Aripiprazole 10 mg	
Olanzapine 10 mg	Aripiprazole 15 mg, olanzapine 5 mg	Citalopram 20 mg, procyclidine 5 mg	Aripiprazole 15 mg,	Citalopram 30 mg	Aripiprazole 15 mg,	Mirtazapine 15 mg
Quetiapine 400 mg	Aripiprazole 15 mg, quetiapine 300 mg		Aripiprazole 15 mg, quetiapine 100 mg		Drop out	

Amisulpride 400 mg		Aripiprazole 15 mg, amisulpride 200 mg		Aripiprazole 10 mg, amisulpride 300 mg		Aripiprazole 10 mg, amisulpride 200 mg	
Risperidone 6 mg	Lithium 600 mg	Aripiprazole 5 mg, risperidone 6 mg	Lithium 600 mg	Aripiprazole 5 mg, risperidone 6 mg	Lithium 600 mg, orlistat 120 mg	Drop out	
Olanzapine 15 mg		Aripiprazole 15 mg, olanzapine 10 mg,	Sertraline 50 mg	Aripiprazole 15 mg, olanzapine 5 mg	Sertraline 100 mg	Aripiprazole 20 mg, olanzapine 5 mg	
Olanzapine 7.5 mg		Aripiprazole 5 mg, olanzapine 5 mg		Aripiprazole 5 mg		Aripiprazole 5 mg	
Olanzapine 22.5 mg	Citalopram 60 mg	Aripiprazole 2.5 mg, olanzapine 22.5	Citalopram 40 mg	Aripiprazole 2.5 mg, olanzapine 20	Citalopram 40 mg	Aripiprazole 2.5 mg, olanzapine 20	Citalopram 40 mg

		mg		mg		mg
Zuclopenthixol 250	Citalopram 20 mg	Aripiprazole 5 mg	Citalopram 20 mg	Aripiprazole 5 mg	Citalopram 20 mg	Drop out

Table 3. Test of model effects for the GLMM analysis of log-NTX

Predictor	Coeff	Std Err	Sig
Baseline medication	-0.0239	0.0192	0.214
Ethnicity	0.0149	0.030	0.625
Week	-0.0012	0.0008	0.142
Switch status	-0.0873	0.039	0.028
Age	-0.0031	0.0032	0.329
Gender	-0.0047	0.057	0.934
Antidepressant status	-0.0189	0.0548	0.729
Prolactin	0.00004	0.0004	0.306

Dependent variable- log-NTX

Appendix 2:

Almandil N., **Lodhi R.J.**, Ren H.Y., Besag F., Rossolatos D., Ohlsen R., Slomp C., Lapetina D., Plazzotta G., Murray M., Al-Sulaiman A., Gringras P., Wong I.C.K, Aitchison K.J. (2017). The LEP promotor SNP *rs7799309* variant is associated with baseline weight and weight gain in a group of Arab children and adolescents treated with risperidone. *Molecular Neuropsychiatry*, under review.

Effect of *LEP* promoter and *LEPR* SNP's on baseline weight and change in BMI z, in a group of Arab children and adolescents treated with risperidone

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Abstract

The most frequently prescribed atypical antipsychotic in children and adolescents with various psychiatric diagnoses is risperidone, which may result in weight gain. The leptin (*LEP*), leptin receptor (*LEPR*), and serotonin 5-HT2C receptor (*HTR2C*), genes are amongst those with the strongest evidence for association with antipsychotic-induced weight gain. Data on baseline weight and BMI z, and change in BMI z categories was collected from 181 risperidone-treated children and adolescents attending neurological and psychiatric clinics in the Kingdom of Saudi Arabia (KSA). Owing to the differences in genotype distribution in Black and White Arabs, we herein report the results from available data from the White Arab KSA sample (144). Weight and BMI z were measured at baseline when medication-free and at three follow-up time points. Linear regression was performed for baseline weight and BMI z, while change in BMI z was assessed using random effects ordered logistic regression, after BMI z was converted to an ordinal variable. The following SNP's were genotyped: rs7799039 (in the *LEP* promoter), rs1414334 (in the *HTR2C*), and rs8179183, rs1137100, and rs1137101 (in the *LEPR*). We found a nominally significant association between rs7799309 and baseline weight, adjusting for height, age, gender and diagnosis (A/G genotype, P=0.035, β=-3.62, compared to G/G). rs1137101 (G/G genotype, P=0.018, OR=4.13 compared to A/A) and rs8179183 (C/G genotype, P=0.042, OR=0.54 compared to G/G) were nominally significant for change in BMI z categories, while rs7799309 demonstrated a trend for the same outcome (A/G genotype, P=0.068, OR=1.71 compared to G/G). Our data therefore provide supportive evidence for previous associations between these SNPs and antipsychotic-associated weight gain, especially in children and adolescents, whilst being the first to report such an association in those of Arab ethnicity.

Key words (MeSH terms): Antipsychotic Agents; Weight Gain; Child; Adolescent; Serotonin; 5-HT2C

Abbreviations:

Attention deficit hyperactivity disorder (ADHD) body mass index (BMI)

high density cholesterol (HDL-C) Kingdom of Saudi Arabia (KSA) leptin gene (*LEP*)

leptin receptor gene (*LEPR*) linear mixed model (LMM)

multiple analysis of variance (MANOVA)

odds ration (OR)

polymerase chain reaction (PCR)

root mean squared error of approximation, RMSEA serotonin5-HT2C receptor gene (*HTR2C*)

single nucleotide polymorphism (SNP) Structural equation modeling (SEM) United Kingdom (UK)

Introduction

Antipsychotics are now relatively commonly used in children and adolescents for a variety of indications, including mood disorders, disruptive behavior disorders, developmental disorders, and psychosis [1-4]. While weight gain on antipsychotics has received greater attention in adults, it is also a relatively common adverse effect in children and adolescents [1]. The distribution of such weight gain tends to be central (abdominal), which is associated with dysregulation of adipokines, including leptin, ghrelin, and adiponectin. Such dysregulation may be associated with cognitive impairment, as well as with long-term adverse health outcomes (diabetes, cardiovascular disease, and some forms of cancer) [5-7]. Risperidone is the most frequently prescribed atypical antipsychotic in children and adolescents [1-4].

There are clear inter-individual differences in the magnitude of weight gain in patients treated with antipsychotics. Underlying such inter-individual differences are both genetic and environmental factors such as diet and sedentary lifestyle, and the interaction between these [8, 9]. The serotonin5-HT2C receptor (*HTR2C*, *e.g.*, rs1414334), leptin (*LEP*), and leptin receptor (*LEPR*) genes are among those with the strongest evidence for association with antipsychotic-induced weight gain [10-16], including specifically in risperidone-treated patients [17-20].

Markers rs7799039 A/G, rs1137101 A/G, rs1137100 A/G and rs8179183 C/G are single nucleotide polymorphisms (SNPs) in the *LEP* promoter and *LEPR* with some previously identified relevant associations [21, 22], although results have been inconsistent [23-26]. SNP rs7799309 (-2548G/A) is a SNP located on chromosome 7 (position 128238480-128238980), in the promoter region of the *LEP* gene. It has been associated with a measure of metabolic

dysfunction, cholesterol/high density cholesterol (HDL-C) ratio, in adult male patients using atypical antipsychotics who were at a relatively early phase (less than a year) in treatment [24]. Other studies did not find any association between rs7799309, and obesity after three months of antipsychotic use [27] and weight gain after antipsychotic use [28], in adult patients. To our knowledge, there is only one study that examined rs7799309 for antipsychotic related weight gain in children and adolescents, and this reported that A allele carriers had a steeper weight gain [17]. This study was done in a mainly Arab sample with attention deficit hyperactivity disorder (ADHD) as the main diagnosis. We herein report genetic association analysis of above SNPs in the *LEP* and *LEPR* as well as of the *HTR2C* rs1414334 in children and adolescents treated with risperidone, most of whom were of Arab ethnicity, with a variety of diagnoses such as ADHD, psychosis, and autism.

Materials and methods

Sample

The inclusion criteria were children and adolescents aged 18 years or under who were taking risperidone. The following patients were excluded: those with anorexia or bulimia nervosa, those taking more than one antipsychotic drug, those taking other medications that could affect weight gain (e.g., corticosteroids, valproic acid, or methylphenidate), and those with concurrent medical conditions that could affect weight gain (e.g., diabetes, Cushing's syndrome, or renal disease).

Ethical approval was obtained from the Department of Neurology, King Fahd Hospital of the University of Damman in the Kingdom of Saudi Arabia (KSA). All procedures were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study information sheets and consent forms were provided in multiple versions: for parents or guardians, and in age-appropriate versions so that participants could also understand (children under 8 years, 8-11 years, and 12-15 years; and adolescents aged 16-18 years; with assent forms additionally for those over 8 years of age). Eligible participants were identified from a pediatric neurology clinic at the Department of Neurology, King Fahd Hospital of the University of Damman. All were seen at the hospital by the researcher (NBA) with the responsible physician. NBA or the physician provided verbal translations for the study information sheets and consent forms as required. Informed consent was obtained from the parents or guardians for all participants included and, in children with capacity, assent was additionally obtained. Data on the following were extracted from patient clinical records: age, gender, date of birth, ethnicity, diagnosis, risperidone dose, dates of patient visits from baseline until the last visit recorded, weight and height. Clinic visits were recorded from baseline (first date prescribed risperidone, all patients being antipsychotic naïve at baseline, visit 0) to the third visit (visit 3); the time between visits was 3 to 6 months depending on the patient's condition.

Genetic analysis

DNA was extracted from buccal swabs at the Institute of Psychiatry, Psychology and Neuroscience, London, UK as previously described [29]. Genotyping for five single nucleotide polymorphisms (SNPs), the *HTR2C* rs1414334 C/G intronic polymorphism, the -2548G/A

promoter SNP (rs7799039) for *LEP* and 3 SNPs in *LEPR,* Q223R (rs1137101), K656N (rs8179183, now merged into rs1805094 in the *LEPR*), and K109R (rs1137100) was performed using TaqMan SNP Genotyping Assays on a ViiA™ 7 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems/Life Technologies/Thermofisher, Canada) at the University of Alberta, Canada.

Statistical analysis

STATA 15.1 was used to conduct the analyses. One-way Analysis of variance (ANOVA) was used to initially examine the effect of potential covariates to include in the model on baseline weight (in kilograms). Linear regression was performed for baseline outcome variables – weight (kg) and BMI z, to explore which one of the two should be used for the repeated measures analysis. The linear regression model was as follows: Baseline weight as the dependent variable and the following as independent variables: age in years, gender, diagnosis (psychosis versus the rest), height and genotype. The genotypes analyzed were rs1414334, rs7799039, rs1137101, and rs8179183, and separate regressions were run for each. Association of genotypes with baseline BMI z (without gender, age and height, since BMI z is adjusted for these variables) was also tested. We analyzed the regression model regression results for weight and BMI z using post-estimation residual distribution and r^2 statistic. We concluded that BMI z was best used for assessing change over time, however, this required converting BMI z into an ordinal variable for the assessing change in BMI z over time. The ordinal numbers for the BMI z groups were 0, 1, 2 and 3 for BMI z scores <-2, -2 to 1.99, 2 to 2.99, and >/= 3, respectively. This classification of BMI z is based on previous reviews [30]. Analysis of BMI z categorical change over visits was

conducted using random effects ordered logistic regression (REOLR). REOLR in STATA is an efficient method to test longitudinal trends in an ordered variable [31]. REOLR predictors in all analyses were: genotype, baseline BMI z and diagnosis. To assess the effect of genotype over visits on BMI z, an interaction term between genotype as a factorial predictor and visit as a continuous predictor was employed, adjusted for baseline BMI z, with each genotypic analysis being run separately. In the REOLR STATA analysis settings; Subjects were used for the 'Panel ID variable' and visit was the 'time variable'. REOLR results have been reported using odds ratio (OR) and *p* value. All results are not adjusted for multiple testing and are therefore reported as nominally significant.

Results

Of the 181 Arab patients approached, all provided consent and usable data were available for 162 (144 white Arabs, 18 black Arabs). As the minor allele frequencies were significantly different in the black Arabs than in the white Arabs (data not shown), and, given the relatively small number of black Arabs, these were excluded from further analyses. The diagnostic distribution in the 144 white Arabs (98 boys, 46 girls) was as follows: 23 (15.97%) had autism, 65 (45.14%) had ADHD, 50 (34.72%) had a psychotic disorder (schizophrenia/schizoaffective disorder/bipolar disorder/psychosis not otherwise specified), and the number of subjects with a diagnosis of disruptive behavioral disorder or aggression and delayed developmental disorder were 3 (2.08%) and 3 (2.08%), respectively. The mean age was 12.58 years (SD 4.99), and there was a significant difference in age between the different diagnostic groups ($P<0.001$). The mean ages

of those with autism, ADHD, psychotic disorder, disruptive behavior disorder or aggression, disruptive/behavioral disorder and delayed/developmental disorder were 12.01, 10.26, 15.92, 16.43 and 7.69 years, respectively. The mean number of days between baseline, and first, second and third follow-up visits was 106.7 (95% CI 102.1-111.4), 209.7 (CI 202.9-216.5), and 313.6 (CI 303.5-323.7), respectively.

The genotyping call rate was 100%. All SNPs were in Hardy-Weinberg equilibrium (*P*=0.58, 0.82, 0.21, 0.18, and 0.42 for rs7799039, rs1137101, rs8179183, rs1137100, and rs1414334 respectively, with the *P* value for the latter being calculated from the subgroup of girls). Consistent with prior findings, in our data, rs1137101 was in high linkage disequilibrium with rs1137100 ($r^2 = 0.98$); it was therefore not necessary to take forward both of these into the analysis, and rs1137100 (arbitrarily this one rather than rs1137101) was excluded. As rs1414334 is an X- linked SNP, genotypes for boys were coded as 0 and 1 and for girls as 0, 1, or 2.

On examination by ANOVA of potential covariates (height, age, gender, and diagnosis) to include in the linear regression model of *baseline* weight, there was a significant effect of height (F= 598.04, *P*<0.0001), and diagnosis (F=9.53, *P*<0.0001), age (F=357.8, *P*<0.001) and gender (F=4.27, *P*=0.04) on baseline weight. Risperidone dose was not included in this baseline analysis as all patients were *drug-naïve* at baseline. Owing to the significant difference in age between the different diagnostic groups, graphical visual inspection of weight by the diagnostic group was performed, which showed that those with a psychotic disorder had a higher baseline weight. The mean weight of those with a psychotic disorder was 63.21 (95% CI 58.78 to 67.64) while that of the rest was ranged between 43.96 (95% CI 39.63 to 48.30).

Linear regression analyses of baseline weight by genotype showed a nominally significant effect of rs7799039 A/G genotype (*P*=0.035, β=-3.62) with a trend for A/A genotype (*P*=0.097, β=-4.34), both being associated with lower baseline weight compared to G/G (Table 1). There was no effect of the other genotypes: for rs1414334, no difference between C/G (*P*=0.666, β=1.25) and C/C genotypes (*P*=0.216, β=2.55) versus the G/G genotype; for rs1137101, no difference in baseline weight for A/G (*P*=0.625, β=-0.848) and G/G (*P*=0.614, β=-1.41) genotypes compared to A/A; and for rs8179183, no difference in baseline weight for C/G (*P*=0.898, β=-0.228) and C/C (*P*=0.918, β=0.417) genotypes compared to G/G.

In linear regression for BMI z the results were as follows. For rs7799039: Significant effect of A/G genotype (*P*=0.049, β=-0.60) versus G/G, and a trend for A/A genotype (*P*=0.097, β=-4.34); for rs1414334: no difference between C/G (*P*=0.387, β=0.36) and significant effect of C/C genotypes (*P*=0.022, β=0.82) versus the G/G genotype; for rs1137101: no difference in baseline BMI z for A/G (*P*=0.864, β=-0.05) and G/G (*P*=0.225, β=-0.59) genotypes compared to A/A; and for rs8179183: no difference in baseline BMI z for C/G (*P*=0.911, β=-0.03) and C/C (*P*=0.603, β=0.381) genotypes compared to G/G. The proportion of the variance accounted for weight as outcome model (adjusted R^2) was high e.g., for rs7799039 it was 81%, and the residual distribution good (Figure 1).

[Insert Table 1 and Figure 1 about here]

In the REOLR analyses of change in the ordinal BMI z variable over time, we observed the following: No significant effect of rs1137101 A/G (*P*=0.826, OR=1.06) but a significant one for

G/G (*P*=0.018, OR=4.13) genotypes compared to A/A. A significant effect of rs8179183 C/G (*P*=0.042, OR=0.547) and a trend level effect of G/G (*P*=0.083, OR=0.268) genotypes compared to G/G was seen. In the rs7799039 analysis, a trend level effect, with individuals of A/G genotype (*P*=0.068, OR=1.71) than G/G and no such trend being seen for the A/A genotypic group (*P*=0.678, OR=0.84). For rs1414334 C/G (*P*=0.431, OR=0.724) and C/C genotypes (*P*=0.431, OR=0.78) compared to G/G genotype. All results are for genotype by visit interaction for ordinal BMI z change over time, with analyses being performed as described in the statistical methods above.

[Insert Tables 2 and 3 about here]

Discussion

We observed a nominally significant association between *LEP* rs7799039 and baseline weight, with the A/G genotype being nominally associated (*P*=0.035) with lower baseline weight and this also shows a trend level signal (*P*=0.068) for association with increase in BMI z group level over the three follow-ups. At least partially consistent with the latter finding, in a previous study of risperidone in this age group by Calarge and colleagues [17], rs7799039 genotypes containing the A allele gained more weight. Although Calarge et al. did not find an association with baseline weight, of note, the ethnicity of their sample was different to ours (74 patients, 84% non-Hispanic Caucasian, 12% African American, 3% Hispanic, 1% Other). In the linear regression

with baseline BMI z as the dependent variable and genotype and diagnosis as independent variables, the *P* value for the association between rs7799039 A/G genotype was slightly lower (0.049) than in the analysis of weight, the adjusted R^2 was much less than in the model with weight (3.5% versus 81%) and the plot of residuals indicated poor model fit (data not shown), while the residuals plot for weight was good (Figure 1).

[Add summary and discussion of results of ordinal logistic regression from Tables 2 and 3]

In the REOLR we also observed nominally significant effects for polymorphisms in the *LEPR* gene i.e., rs1137101 and rs8179183. The rs1137101 G/G (*P*=0.018, OR=4.13) genotype increased odds of BMI z increase compared to A/A, a result consistent with previous literature. Meta-analytic evidence suggests that rs1137101 [G/G] increases the odds of type 2 diabetes mellitus, and other studies suggest a role for the same in obesity [32, 33]. rs8179183 C/G (*P*=0.042, OR=0.547) genotype reduced odds of BMI z increase compared to G/G, and this again is consistent with earlier studies. rs8179183 G-allele carriers were relatively protected against weight gain compared to rs8179183 [C/C], in a risperidone treatment related weight study [26].

We included children treated with only one antipsychotic medication (risperidone), all of which were antipsychotic naïve at baseline, unlike other studies where adults treated with a variety of antipsychotics were pooled into a single analysis [26, 27, 34, 35]. This is first report of associations of genetic marker with weight gain in Arab children and adolescents treated with risperidone. Of note, there is a relative paucity of publicly available data on DNA sequence variation in Arabs and therefore this study makes a particularly valuable contribution.

The limitations of this study include sample size, gender distribution (low proportion of girls), lack of a measure of compliance, and lack of availability of a measure of activity level. Limited sample sizes increase the risk not only of finding a spurious positive association (a type I error) but also of missing genetic associations with small effect sizes (a type II error). However, analysis of the power of the sample size of 144 using Quanto 1.2.4 [36] with a SNP minor allele frequency of 0.25 revealed there was more than 80% power to detect a mean difference of 2.0% in an outcome variable between carriers and non-carriers of the risk genotype in an additive model; our sample was therefore sufficiently powered to generate a result of nominal significance, without adjustment for multiple testing. We have not adjusted for multiple testing and hence report our findings as of nominal significance. An adjustment for multiple testing such as Bonferroni would be too conservative, as a Bonferroni correction assumes independence of tests conducted. In fact, the variants are significantly correlated with each other in terms of r^2. Moreover, the three genes investigated are also functionally related (including *HTR2C* polymorphism being associated with circulating levels of leptin, Reynolds et al., 2006). Another potential criticism could be the use of baseline weight in children in addition to the baseline BMI z score in the linear regression analyses, however, we felt that since the efficacy of the model for baseline weight was better, it was important to present both the results. It is important to note that as the Centers for Disease Control and Prevention 2000 growth charts to not specifically include Arab ethnicity, it may be that the use of the weight variable, adjusting for age, gender, height and diagnosis, is more appropriate for the baseline analysis on such charts. The distribution of the residuals after a preliminary linear mixed model analyses for change in BMI z was not normal, hence the need to categorize BMI z and arrange it in an ordinal manner.

Conclusion

Our investigation of weight gain and BMI z, and relevant genetic variants in Arab children and adolescents treated with risperidone revealed that both baseline weight was associated with the *LEP* promoter SNP rs7799039. Change in BMI z categories was associated with *LEPR* SNP's rs1137101and rs8179183. Replication is desirable. We recommend further studies of this and other variants in the *LEP* promoter and *LEPR* versus weight gain and BMI z on treatment with risperidone and other antipsychotics in individuals of Arab and other ethnicities in this age group and in adults. Given the association between markers in the leptin pathway and relevant Mendelian genetic disorders, it would also be interesting to see if markers in the leptin pathway are associated with persistent weight gain on psychotropics despite interventions aimed at reducing weight gain, and, in a larger sample, whether such markers are associated with weight gain to an unhealthy extent (to an at least obese level). More thorough analysis of this gene including sequencing and haplotype analysis might result in the identification of other functionally relevant variants in this ethnic group. Although often difficult in practice, replication studies should ideally use protocols with antipsychotic monotherapy, especially for children and adolescents in their first psychotic episode. Additionally, extending the analysis to other antipsychotics and to more genes, as well as conducting more complex analyses including consideration of gene–environment interactions including epigenetics and gene–gene interactions models could shed further light on relevant biological mechanisms. Patients and their caregivers are certainly interested in preventing and ameliorating antipsychotic-associated weight gain and predicting who will respond well to interventions for this and who will not, which should encourage further, coordinated, research efforts in this area.

Conflict of Interest

KJA reports consultancy services for Otsuka Canada Pharmaceutical Inc., and Lundbeck Canada and a research grant from Jannsen Inc., Canada.

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Table 1 Linear regression showing the nominally significant association between baseline weight and rs7799039 genotype (with G/G as the reference genotype)

	β	t	*P*	95% CI	
rs7799039					
A/G	-3.62	-2.13	**0.035**	-6.97	-0.266
A/A	-4.34	-1.67	0.097	-9.48	0.801
Age (years)	0.001	0.90	0.372	-0.001	0.003
Gender	0.383	0.21	0.833	-3.19	3.96
Diagnosis	-2.50	-1.32	0.188	-6.24	1.24
Baseline height	0.71	7.90	**<0.001**	0.533	0.888

Table 2 Ordered logistic regression analysis of BMI z category, showing a nominally significant rs1137101 by time interaction

	Odds Ratio	*z*	*P*	95% CI	
rs1137101					
A/G	0.857	-0.19	0.852	0.169	4.34
G/G	0.163	-1.16	0.245	0.008	3.48
Time	2.00	3.61	<0.001	1.37	2.92
rs1137101*Time					
A/G	1.06	0.22	0.826	0.612	1.85
G/G	4.13	2.37	**0.018**	1.28	13.38
Diagnosis	2.95	1.53	0.127	0.734	11.82
Baseline BMI z	21.15	7.54	<0.001	9.57	46.75

Table 3 Ordered logistic regression analysis of BMI z category, showing a nominally significant rs8179183 by time interaction

	Odds Ratio	*z*	*P*	95% CI	
rs8179183					
C/G	1.63	0.57	0.568	0.304	8.71
C/C	1.58	0.22	0.824	0.028	90.43
Time	2.86	5.47	<0.001	1.96	4.17
rs8179183*Time					
C/G	0.55	-2.04	**0.042**	0.307	0.977
C/C	0.269	-1.74	**0.083**	0.061	1.19
Diagnosis	2.80	1.46	0.145	0.70	11.21
Baseline BMI z	19.75	7.63	<0.001	9.17	42.51

Appendix 3:

Lodhi R.J., Rossolatos D., Aitchison K.J. Genetics and genomics in addiction research (2016) In: Wolff K, White J, Karch S (eds), The SAGE Handbook of Drug & Alcohol Studies Volume 2, Section 1, Chapter 1.

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1

Genetics and Genomics in Addiction Research

Rohit Jaibhagwan Lodhi, David Rossolatos and Katherine Jean Aitchison

Introduction

Substance use disorders are complex, multifactorial disorders that are typically characterized by the repeated use of a psychoactive substance that continues despite harmful consequences or varying degrees of impairment in function (DSM-5, 2013). Internet addiction, gambling disorders and sexual addiction are examples of a broader concept of addictions that is not simply limited to psychoactive substance use. The current approach towards studying genetic influences in such addictions involves much more than looking at the dichotomy of a disorder being present or absent. Agrawal *et al.* (2012) highlighted the importance of looking at addiction in stages, with early stages being less affected by genetic factors than the later stages. Many factors mediate the transition from initial exposure to a substance to abuse or dependence, and these include those that are specific to the individual (e.g. family history of addiction or mental illness, gender, psychiatric comorbidity, etc.) or environmental (such as access to or availability of drugs, and quality of the social network). Key questions of both research and clinical importance include to what extent the various substance use conditions and behavioural addictions have shared versus distinct genetic and epigenetic mechanisms.

Phenotypes for genetic studies of addictions

Genetic studies of addiction investigate associations of clinical phenotypes with genotypes. Such a method of investigation works best for clearly defined and observable phenotypes,for example, cardiomyopathy. However, in psychiatry it is sometimes difficult to achieve diagnostic concordance between clinicians looking at the same patient. The methods to diagnose substance use disorders vary in the available genetic studies and they include physician interviews using a DSM checklist (Bart et al., 2004) or structured interviews such as the Structured Clinical Interview for DSM disorders (SCID) (Clarke et al., 2013) and Mini International Neuropsychiatric Interview (MINI) (Benyamina et al., 2009). In addition, changes to diagnostic criteria have an impact on what studies will define as a diagnostic phenotype. For example, the fifth version of the DSM (APA, 2013), released in 2013, combined the DSM-IV categories of abuse and dependence in a single diagnosis. Many genetic studies have instead used addiction behavioural phenotypes, such as the number of cigarettes (Rice et al., 2012), time to first cigarette (Haberstick et al., 2007) and number of sexual partners (Cherkas et al., 2004) to study association. Similarly, animal studies that research genetic influences involve different behavioural phenotypes. Therefore, there can be a lot of variation in what genetic studies are measuring, and this should be taken into consideration when comparing studies. It has been suggested that without a major move towards defining phenotypes, the progress in genomic medicine will be very slow (MacRae, 2015). We shall now go on to have a closer look at the different methods used to research genetic influences in addiction and review the relevant literature.

Heritability of substance use disorders

Initial evidence of the role of heritable influences in the risk of developing these conditions came from twin, adoption and family studies. The twin study methodology is used to estimate the degree to which genes may be contributing towards a particular phenotype. The difference in prevalence of that phenotype between monozygotic and dizygotic twins can then point towards a genetic contribution to the phenotype by estimating its heritability and separating the effects of shared versus unique environments. Drawbacks of twin studies include that they may be neither genetically nor environmentally representative of the general population (e.g. owing to the differential likelihood of risk of exposure to relevant factors), which leads to difficulties in generalizability. Adoption studies look at individuals adopted into families other than their family of origin; this design is also useful for examining genetic versus environmental influences. Family studies examine the risk of relatives developing a phenotype of interest.

Twin, adoption and family study findings in addictions

Addictions have a relatively high heritability, with estimates ranging from 39% to 72%; cocaine and opiate addictions are the most heritable (Goldman et al., 2005). Merikangas *et al*. (1998) found that having family history of drug abuse increased the risk of having a drug disorder by eight times. Tsuang *et al.* 1996 (Vietnam Era Twin Registry study on drug use and dependence) found a higher concordance among monozygotic twins than dizygotic twins. They also concluded that 34% of the variance of the risk of developing a drug use disorder was explained by genetic factors (Tsuang et al., 1996). *et al.* (2000) studied heavy substance use, abuse and dependence in 1198 male twins and found a higher heritability, of up to 60–80% for most substances (Kendler et al., 2000).

Carmelli *et al*. (1990) published one of the earliest large male twin studies in nicotine addiction from the National Academy of Sciences-National Research Council (NAS-NRC) twin

registry; they found that heritable influences explained up to 53% of the variation in risk of addiction. The contribution of genetic influences to the risk of nicotine addiction varies from 11% to 75% in other studies (Haberstick et al., 2007; Han et al., 1999; Kendler et al., 1999; True et al., 1997). Twin studies of nicotine addiction have demonstrated that not just dependence but also other aspects of the addiction, such as withdrawal and failed smoking cessation, are also heritable (Xian et al., 2003). Adult adoption studies have found a correlation between adoptees' smoking behaviour and smoking behaviour of biological relatives in the same generation (Osler et al., 2001).

The estimates of heritable influences on alcoholism range from 45% to 65% in twin and family studies (Pickens et al., 1991; Prescott and Kendler, 1999). Bierut *et al.* (1998) reported that rates of alcohol dependence in males and females were 50% and 25% respectively in the siblings of alcohol-dependent subjects. The risk of alcoholism in adoptees is more similar to that of their biological parents than that of their adoptive parents (Sigvardsson et al., 1996).

Twin studies have confirmed that genetic influences also play a role in cannabis dependence as well (Agrawal et al., 2007; Lynskey et al., 2012). Initial estimates of heritability for cannabis dependence varied from 30% to 80% (Agrawal and Lynskey, 2009). A meta-analysis of twin studies of the initiation of cannabis use reported heritability estimates of 45% in males and 39% in females (Verweij et al., 2010). For cannabis use disorders, the study reported heritability estimates of 50–60%.

Kendler *et al*. (1999) found that opiate addiction was heritable in a population based female twin study and replicated the finding a year later in a male twin study (Kendler et al., 2000).

Heritability estimates for cocaine use, abuse and dependence were 39%, 79% and 65% in a telephone interview study of female twins (Kendler and Prescott, 1998). A twin and sibling Swedish study of cannabis, cocaine, other stimulants and sedatives found heritability ranged from 64% to 70% for these substances (Kendler et al., 2015). Other twin studies of cocaine use have found evidence of a genetic contribution to the risk of cocaine use, abuse or dependence (Kendler et al., 2006).

Small family studies of probands with gambling disorder (Black et al., 2006), kleptomania (Grant, 2003) or compulsive buying (Black et al., 1998) each found that first-degree relatives of those probands had significantly higher lifetime rates of alcohol and other substance use disorders. Heritability estimates for problematic or compulsive internet use are about 48% in both genders (Vink et al., 2015). Another twin study found the heritability estimates for internet addiction to be even higher at 58–66%.

Compulsive sexual behaviour that is not part of another psychiatric disorder such as bipolar disorder is another example of a behavioural addiction that has been difficult to categorize in mental health, due to issues such as phenotypic heterogeneity (Derbyshire and Grant, 2015). It can be conceptualized as repeated and intrusive thoughts and/or actions that are of a sexual nature but cause distress and impairment in an individual's life. Hypersexual behaviour has been researched and its inclusion in DSM-5 been discussed (Kor et al., 2013). Family studies of patients with Tourette's syndrome or attention deficit hyperactivity disorder found that a variety of sexual behaviours in relatives of the patients were correlated with the degree of genetic loading, suggesting a genetic contribution to each (Comings, 1994). Familial influences on sexual behaviour such as age at first intercourse have been seen in twin and family studies (Carlson et al., 2014; Donahue et al., 2013; Guo and Tong, 2006; Harden and Mendle, 2011;

Rodgers et al., 2008; Waldron et al., 2008). Siblings and daughters of teenage mothers have an elevated risk of teenage pregnancy (East and Jacobson, 2001; Meade et al., 2008).

Candidate gene studies

Candidate gene studies look at gene variants or polymorphisms within candidate genes, or in regions adjacent or in linkage disequilibrium, using *a priori* hypotheses about their relationship with the addiction phenotype. Subjects with an addiction phenotype are compared with those that do not have the addiction. Limitations of candidate gene studies in addiction include the requirement of prior knowledge of the gene and its function(s) and the fact that many addictions are polygenic in nature. In the following sections, the risk alleles are shown in square brackets after each single nucleotide polymorphism (SNP) rs number.

Nicotine

Candidate gene studies investigating the gene cluster *CHRNA5-CHRNA3-CHRNB4* located on 15q25.1 that encodes for subunits of the nicotinic acetylcholine receptors (nAChRs) and smoking have found significant associations consistent between European (Bierut et al., 2008), African (Li et al., 2010) and Asian (Li et al., 2010) ancestry populations. Saccone *et al*. (2007) were the first group to report an association between the *CHRNA5* non-synonymous SNP rs16969968[A] and nicotine dependence, although in addition to the signals from the *CHRNA5-CHRNA3-CHRNB4* gene cluster*,* they found that *CHRNB3* polymorphisms were also significant. Within the dense *CHRNA5-CHRNA3-CHRNB4* locus, the *CHRNA5* SNP rs16969968[A] and *CHRNA3* SNP rs578776[G] may represent two groups of risk variants (Wen et al., 2014). Gene variants in the *CHRNA5-A3-B4* gene cluster are important for the treatment of nicotine dependence too. The number of cigarettes smoked per day and response to treatment with smoking cessation therapy, such as bupropion and transdermal nicotine patches, can be predicted

by SNPs rs8192475[T], rs680244[A] and rs12914008[A] in the *CHRNA5-CHRNA3-CHRNB4* gene cluster (Sarginson et al., 2011). Neuronal signaling pathway genes such as neuregulin 1 (*NRG1*) and Erb-B2 receptor tyrosine kinase 4 (*ERBB4*) that have been associated with psychosis (Bakker et al., 2004; Bramon et al., 2008; Douet et al., 2014; Wang et al., 2009a) have also been investigated in nicotine dependence. Animal studies provide support for the role of neuregulin 3 (*NRG3*) in nicotine dependence and a clinical trial demonstrated that a SNP rs1896505[A] in this gene might play a role in smoking cessation (Turner et al., 2014). Within the category of genes expressing metabolizing enzymes for nicotine, specific *CYP2A6* alleles (*CYP2A6*9*, *CYP2A6*12*, *CYP2A6*2* and *CYP2A6*4*) provide some protection against nicotine addiction and increase chances of smoking cessation (Gold and Lerman, 2012; Iwahashi et al., 2004; Mwenifumbo et al., 2007).

Alcohol

Alcohol addiction has been studied in much more detail than other addictions. Genetic studies of alcohol addiction were the first amongst such studies in addiction and early candidate gene studies of alcoholism focused on genes that express enzymes involved in the metabolism of ethanol. The *ALDH* genes for the aldehyde dehydrogenase enzymes that catabolize acetaldehyde to acetic acid have been studied extensively in alcohol addiction. Variants in *ALDH2* have been well known to confer a protective effect against alcohol dependence in northeast Asians and this finding is highly replicated (Higuchi, 1994; Samochowiec et al., 2014; Whitfield, 1994). Individuals homozygous for the *ALDH2*2* variant will experience severe nausea and vomiting with small amounts of alcohol intake and a have lower risk of developing alcohol dependence. This relates to the finding that individuals who are homozygous for the *ALDH2*2* variant have a flushing syndrome (Thomasson et al., 1993) and experience a greater degree of flushing than

heterozygotes. Alcohol dehydrogenase (*ADH*) polymorphisms contribute towards the risk of developing alcoholism (Higuchi, 1994; Thomasson et al., 1991). In a Chinese study, the *ADH1*B* and *ADH1*C* alleles were found to protect against alcohol dependence, but this effect is smaller than that of *ALDH2* variants (Thomasson et al., 1991). Similarly, in British and Irish populations, a SNP rs12229984[G] in the *ADH1B* gene conferred protection against alcohol dependence (Way et al., 2015). Individuals who have alcohol addiction also tend to use nicotine, and there is some evidence linking the aforementioned nicotinic receptor gene cluster (*CHRNA5-CHRNA3-CHRNB4,* SNPs rs1979906A/G, rs3841324L/S, rs601079A/T, rs680244A/G, rs621849A/G, rs692780C/G, rs6495307C/T, rs1051730C/T) to alcohol dependence (Edenberg and Foroud, 2014; Wang et al., 2009b). Among its many effects, alcohol affects gamma-aminobutyric acid (GABA). The chromosomal region of 4p12 has a cluster of four genes that encode for GABA-A receptors and chromosome 5q contains another cluster of GABA-A receptor genes, and both are relevant to alcohol problems (Grzywacz et al., 2012). *GABRA2* encodes for the GABA-A alpha2 receptor. Edenberg *et al*. (2004) found multiple SNPs in the *GABRA2* gene were associated with alcohol dependence and the beta frequency of the electroencephalogram in patients who had alcohol dependence and their relatives (Edenberg et al., 2004). In this study, the region from intron 3 up to past the 3′ end of GABRA2 gene, with a three-SNP haplotype, had a strong correlation with alcohol dependence. Covault et al. (2008) extended the markers studied by Edenberg et al. 2004 by genotyping into the 5′ region of *GABRAG1* and found that these variations better explained the association with alcohol dependence (Covault et al., 2008; Villafuerte et al., 2012). There are many other genes that have been linked to alcohol dependence such as *ACN9* (Hill et al., 2015), X-ray repair complementing defective repair in Chinese hamster cells 5 (*XRCC5*) (Juraeva et al., 2015), dopamine receptor

type 2 (*DRD2*) (Buhler et al., 2015), ankyrin repeat and kinase domain containing 1 (*ANKK1*) that is 10 Kb upstream of the *DRD2* in the complementary strand (Buhler et al., 2015), serine incorporator 2 (*SERINC2*) (Zuo et al., 2013a), *KIAA0040* (Hill et al., 2013; Wang et al., 2011) and *NRD1* (Wang et al., 2011). The chromosome 7q region is of interest in alcoholism as evidenced in a genome-wide linkage study by Hill *et al*. (2004) (Hill et al., 2004). Six SNPs (three upstream of the gene, two within intron 1 and one in exon 4) in the *ACN9* gene (involved in gluconeogenesis) located on chromosome 7q were associated with alcohol dependence in a family-based association study (Hill et al., 2015). Gene variation in *XRCC5* can affect the maximum blood alcohol concentration in an allele-dose-dependent manner (Juraeva et al., 2015). The Taq1A polymorphism located downstream of the DRD2 gene in *ANKK1* on the complementary strand has been associated with alcohol dependence in a large-scale meta-analysis (Wang et al., 2013) and suicidal behaviour in alcohol dependence may also be associated with haplotypes in the *ANKK1* and *DRD2* genes (Jasiewicz et al., 2014). Among these two genes, the *ANKK1* gene may have a stronger association with alcohol dependence than *DRD2* (Ma et al., 2015). Zuo et al. (2013a) found that a rare variant constellation was *NKAIN1-SERINC2* was correlated with alcohol dependence in a European-American population (Zuo et al., 2013a). Six SNPs in the *KIAA00040* gene were significantly associated with alcohol dependence in a family-based association analysis (Hill et al., 2013). Gene variants such as the *COMT*Val158Met substitution (rs4680) may moderate the effect of adverse childhood experiences on the risk of having alcohol dependence (Schellekens et al., 2013), with Met carriers being more at risk than Val/Val homozygotes. In a study of genomic losses in copy number (CNV) in alcohol dependence, an excess of losses was found at 16q12.2, which would

affect the genes *CES1p1* and *CES1*, involved in the generation of alcohol from chemicals including esters (Ulloa et al., 2014).

Cannabis

A few candidate genes have been investigated for association with cannabis dependence. SNPs rs806368[C] and rs806380[A] in the cannabis receptor 1 gene (*CNR1*) were associated with cannabis dependence in a study that consisted largely of alcohol-dependent subjects (Agrawal and Lynskey, 2009; Agrawal et al., 2009). The *CNR1* SNP rs2023239[G] mediated the effect of heavy cannabis use on reduced hippocampal volume (Schacht et al., 2012). The *NRG1* SNP rs17664708[T] was associated with cannabis dependence in patients of African-American ethnicity (Han et al., 2012). Met/Met or Met/Val genotypes of the *COMT* Val158Met polymorphism have been associated with cannabis dependence (Baransel Isir et al., 2008). Other genes implicated in cannabis use disorders are ATP-binding cassette, sub-family B (MDR/TAP), member 1 (*ABCB1*) (Benyamina et al., 2009) and monoglyceride lipase (*MGLL*) (Agrawal and Lynskey, 2009). In addition, genotypic and haplotypic variations at or near the *GABRA2* gene are associated with vulnerability to cannabis, alcohol and nicotine dependence (Philibert et al., 2009). Genotypes can influence withdrawal from cannabis too, and evidence to support this includes the fact that the *CNR1* SNP rs2023239[G] exerts an influence on cannabis withdrawal and the fatty acid amide hydrolase(*FAAH*) SNP rs324420[C] affects craving during abstinence (Haughey et al., 2008). These SNPs may have an additive effect on cannabis withdrawal.

The *COMT* Val158Met polymorphism has been investigated for moderating the effect of cannabis use on the development of psychotic symptoms, and it has been found that the Val/Val genotype has a significant association in many studies (Caspi et al., 2005; Ermis et al., 2015; Estrada et al., 2011; Henquet et al., 2006, 2009) but not all (Zammit et al., 2011). Van Winkel *et*

al. (2008) observed that the Met allele of *COMT*Val158Met was an important mediator of the effect of stress on psychotic symptoms (van Winkel et al., 2008). *COMT*Val158Met variants can mediate more complex relationships such as those between adverse childhood experiences, cannabis use and psychosis, with Vinkers *et al.* (2013) finding that childhood trauma moderated the effect of cannabis use on psychotic experiences in Val carriers. Later, this was confirmed by Alemany *et al*. (2014), who in addition noted that having the Met allele affected psychotic experiences in individuals who suffered childhood abuse but did not use cannabis.

Opioids

The opioid receptors mu, kappa and delta are intricately involved in the pharmacodynamic effects of opioids. The mu receptor gene (*OPRM1*) SNP rs1799971 (118A/G, Asn40Asp) has been associated with opioid dependence in Indian (Kapur et al., 2007) and European Caucasian (Bart et al., 2004; Drakenberg et al., 2006) study populations. Prior to this, Bond *et al.* (1998) had sequenced DNA from opioid addicts to identify five SNPs in the same gene and the 118A/G was the most prevalent SNP (Bond et al., 1998). Endorphin binds the 118A/G receptor three times more tightly than the Asn (i.e. aspargine) form of the receptor (Bond et al., 1998). Postmortem brain analysis of 118G heroin users has shown significant alterations in the opioid neuropeptide system, such as reduced preproenkephalin transcription (Drakenberg et al., 2006). However, the 118A/G SNP association with opioid addiction was not replicated in Han Chinese (Glatt et al., 2007). Yuferov *et al.* (2004) reported that the 36G>T variation in the kappa receptor gene (*OPRK1*) was associated with opioid addiction in a Hispanic population (Yuferov et al., 2004) and this finding was replicated in a West European Caucasian study sample (Gerra et al., 2007). G alleles of the SNP rs6265 and rs13306221 in the gene encoding brain-derived neurotropic factor (*BDNF*) are more frequently found in subjects with heroin addiction (Jia et al., 2011).

Melanocortin receptor type 2 (*MC2R*) gene polymorphisms are also associated with heroin addiction (Proudnikov et al., 2008). Specifically, rs2186944[A] may protect against and rs4797824[T] may increase the risk of developing heroin addiction in Hispanics. Haplotype analysis in the same group found the haplotype GACT (rs2186944, -179A>G, rs28926182 and rs4797824) to be a risk factor for heroin addiction, while the AACT haplotype from the same variants was protective against heroin addiction.

Methadone is a treatment for opiate addiction. The *ABCB1* gene SNP rs1128503[C>T] differentiated between patients who required high and low dose methadone maintenance treatment (Levran et al., 2008). In the same study, patients with the three-locus genotype pattern TT-TT-TT (rs1045642, rs2032582 and rs 1128503 in the *ABCB1* gene) were five times more likely to require high methadone maintenance dose and those who were heterozygous for the SNPs were three times as likely to require a low methadone maintenance dose.

Amphetamines

Genetic polymorphisms in the opioid system genes have been assessed for a role in amphetamine addiction (Ide et al., 2004; Levran et al., 2012). Within these genes, the *OPRM1* SNP rs2075572 in intron 2 has been associated with methamphetamine dependence, and methamphetamine-induced psychosis (Ide et al., 2006). Methamphetamine-induced euphoria was moderated by intronic SNPs rs510769 (A/A genotype) and rs2281617 (C/C genotype), a two-SNP (AA) haplotype of rs1799971 and rs510769 and a three-SNP haplotype (ATA) of rs1918760, rs2281617 and rs1998220 (Dlugos et al., 2011). *OPRD1* variants were not a risk factor for methamphetamine dependence and induced psychosis (Kobayashi et al., 2006). Methamphetamines promote the release of dopamine in the synaptic cleft, and genetic variants rs509707[C] and rs4709426[C] and haplotypes of these in the monoamine transporter *SLC22A3*

gene may have a role in the development of polysubstance use in patients with methamphetamine dependence (Aoyama et al., 2006). Jugurnauth *et al*. (2011) observed an association of a *COMT* gene haplotype, including A alleles of rs4680 and rs165599, with methamphetamine abuse. Hosak *et al.* (2006) found that having the Met allele compared to Val of the *COMT Val158Met* polymorphism was related to novelty seeking in a Czech methamphetamine use population but not to methamphetamine dependence (Hosak et al., 2011). Other variants that are associated with methamphetamine addiction are prokineticin 2 receptor gene (*PROKR2)* SNPs rs6085086(G>A), rs3746682(G>C) and rs4815787(G>A) (Kishi et al., 2010), ghrelin signaling system gene polymorphisms (*GHRL* SNP rs4684677[T] and *GHSR* SNP rs2948694[G]) (Suchankova et al., 2013), V-Akt murine thymoma viral oncogene homolog 1 (AKT1) SNP rs3730358 (C>T) (Ikeda et al., 2006) and the adenosine receptor gene (*ADORA2A*) SNP rs5751876[C] (Kobayashi et al., 2010).

Cocaine

The 'dopamine hypothesis' has often been used to explain the reinforcing properties of cocaine (Kuhar et al., 1991). Genetic polymorphisms within the dopamine system are therefore logical targets for research in cocaine addiction. Associations with certain types of cocaine use with dopamine system gene variants include the 30-bp variable number tandem repeat polymorphism in intron 8 of a dopamine transporter gene (*SLC6A3*) (Guindalini et al., 2006), minor alleles (A1 and B1) of *DRD2* polymorphisms (Noble et al., 1993), the *Msc*I/*Bal*l polymorphism of *DRD3* (Comings et al., 1999) and the Met allele of the *COMT*Val158Met polymorphism and a two-marker haplotype of this with rs737865 (Levran et al., 2015; Lohoff et al., 2008). However, other studies looking at the same genes did not find similar results (Fernandez-Castillo et al., 2010; Gelernter et al., 1999; Lohoff et al., 2010). A study in European-Americans found that a

minor[A] allele of the *CHRNA5* SNP rs16969968 was associated with an increased risk of nicotine dependence and reduction in risk of cocaine dependence (Grucza et al., 2008), while a more recent investigation concluded that multiple variants in the *CHRNB3-A6* gene locus were associated with an increased risk of developing nicotine and cocaine dependence (Sadler et al., 2014). Interestingly, *CNR1* SNPs rs6454674[G] and rs806368[C] were associated with cocaine addiction (Clarke et al., 2013).

Genome-wide association studies

The human genome project, HapMap and other collaborative efforts generating such data have improved understanding of the variability of the human genome, and advances in array technology to facilitate high-throughput multiplex genotyping have rendered genome-wide association studies (GWAS) feasible. Arrays or gene chips enable the genotyping of tens of thousands to millions of gene markers per individual. GWAS can identify common SNPs (minor allele frequencies of greater than 1%) associated with a disorder or a particular phenotype. However, the testing of up to 1 million SNPs for association with disease may generate false positives. For genome-wide significance, correction for multiple testing gives a P-value between 5×10^{-7} and 10^{-8} (660,000–1 million SNPs). Each SNP may be considered to be independent (if for example they are haplotype tagging SNPs with a recombination fraction, r^2 less than or equal to 0.5); however, as there may be functional connection between them, this approach is conservative. Although such conservative correction reduces the risk of false-positive findings, a drawback is that true association signals with small effect sizes may be overlooked. The National Institutes of Health compiled a catalog of SNP-trait associations from published GWAS, which was available online at the National Human Genome Research Institute (http://www.genome.gov/gwastudies/), and detailed findings at a significance level of $P<1 \times$

10^{-5}. The main GWAS database is http://www.gwascentral.org/. In addition, there is a specific addiction GWAS resource (http://addictiongwas.com/AAGR/), hosted by the psychiatry department of Amsterdam Academic Medical Centre (AMC). GWAS look at all markers tested without *a priori* hypotheses regarding the relationship between the markers and phenotypes.

A limitation of GWAS is that this approach can only detect associations with variants that are relatively common in the general population; thus, rare variants with larger effect sizes will be missed. However, GWAS are designed to identify genes involved in common aetiological mechanisms for clinical conditions of interest including underlying pathways that may interact with each other and with the environment. It is hoped that this will feed into the discovery of drugs that may be relevant for many people. Currently, at least some of the gene polymorphisms that are used to predict disorder or risk of disorder are, in fact, those with relatively large effect size that do not account for the majority of the disease (for example, breast cancer genes). It is possible that prediction of the development of polygenic disorders might become more feasible once enough common risk alleles and clinical factors including environmental interactions have been identified. By analogy with *ALDH2,* it is envisaged that genes related to metabolism may be relevant not only to response to treatment for addiction, but also to susceptibility to disorder.

GWAS in addiction

The following section describes the progress in genetic association for substance abuse disorders that has been achieved through the GWAS approach. The majority of GWAS in addiction to date have focused on drinking behaviours; the next most common phenotype studied is smoking.

[TS: Insert Table 1.1 here]

Table 1.1

GWAS have confirmed that the nAChRs genes are associated with nicotine dependence. In fact, meta-analyses of tobacco GWAS have confirmed the importance of genes encoding the nAChRs in susceptibility to nicotine addiction (Liu et al., 2010; Thorgeirsson et al., 2010; Tobacco and Genetics, 2010). The Tobacco and Genetics Consortium found SNPs in genes *CHRNA3* [rs1051730A], *EGLN2* [rs3733829G], and in the 10q25 [rs1329650G, rs1028936A] were associated with the number of cigarettes per day, and a SNP in *BDNF* [rs6265C] was associated with smoking initiation (Tobacco and Genetics, 2010). Loukola *et al.* (2014) provided tentative evidence of *ERBB4* [rs7562566G] and nicotine-dependence association in a GWAS study (Loukola et al., 2014). Nicotine-metabolizing enzyme *CYP2A6* and *CYP2B6* [rs4105144C] and *CHRNB3-CHRNA6* [rs6474412T] genes were also relevant to smoking behaviour in meta-analyses of GWAS (Thorgeirsson et al., 2010).

GWAS in other addictions to date are limited by issues such as variability of phenotype identification and sample size. Interestingly, the role of the *ADH* gene cluster in alcohol dependence was confirmed by a study that investigated associations using a polygenic risk model (Frank et al., 2012). Other GWAS of alcohol addiction found that rs2066702[T] and rs1229984[A] in *ADH1B* affect the risk of developing alcohol dependence in African-American and European-American patients respectively (Hart et al., 2015). A recent GWAS found that genes involved in signal transduction and neurogenesis are possibly involved in 'alcohol problems' in young adults (Edwards et al., 2015). Variants in *PTP4A1-PHF3-EYS* were associated with alcohol dependence in a GWAS conducted by Zuo *et al.* (2014) (Zuo et al., 2014).

GWASs of cannabis dependence (Agrawal et al., 2011; Verweij et al., 2012) and cannabis use initiation (Verweij et al., 2013) have to date not identified any associations significant at the genome-wide level (Minica et al., 2015).

A GWAS found that rs2377339[G] in the *NCK2* gene (NCK is a family of adaptor proteins) was associated with opioid dependence in men of African origin (Liu et al., 2013). Nielsen *et al.* (2010) conducted a pooled GWAS using a relatively low density array of 100,000 markers in a comparatively small sample consisting of 325 former heroin addicts (200 Caucasians, 125 African-Americans) and 250 controls (150 Caucasians, 100 African-Americans) (Nielsen et al., 2010). An apparent association was nonetheless detected in Caucasians for the variant rs10494334[A] (located at chr1q23.3), and in people of African-American ethnicity, the variant most significantly associated was rs950302[T], located in the cytosolic dual specificity phosphatase 27 gene (*DUSP27*), which may be involved in energy metabolism.

The *CDH13* (cadherin 13) gene SNP rs3784943[G] in the eighth intron was associated with response to d-amphetamine in healthy volunteers in a GWAS (Hart et al., 2012).

Functional genomics

Assessing the functional implications of any genetic variant that has been found to be associated with a particular phenotype is key to further understanding. For some variants, such as some repeat regions and non-synonymous coding SNPs, functional biological consequences have been identified. However for the majority of the SNPs identified by GWASs, the functional correlates are, as yet, unknown. Functional genomics includes the identification of expression quantitative loci (eQTLs), *in vitro* studies, bioinformatics, the establishment of relevant databases (such as ENCODE), forward genomics, reverse genomics and convergent genomics.

In forward genomics, candidate genes are identified by animal studies. An example of this is gene expression and other molecular studies in alcohol-preferring and non-preferring rats that led to the identification of neuropeptide Y (NPY), alpha-synuclein and corticotrophin-releasing factor receptor 2 as being associated with the linkage signal for alcohol consumption on rat chromosome 4 (Spence et al., 2005). The role of NPY in regulating alcohol consumption and other alcohol-related behaviour has been convincingly demonstrated by the use of NPY knockout and NPY overexpressing mice (Hayes, et al. 2012; Thiele et al., 1998). Alpha-synuclein is expressed throughout the central nervous system (especially in presynaptic nerve terminals; Iwai et al., 1995; Maroteaux et al., 1988; Mori et al., 2002), and may inhibit dopamine synthesis by tyrosine hydroxylase inhibition (Perez et al., 2002). Moreoever, alpha-synuclein has been shown to reduce dopamine transporter activity *in vitro* (Wersinger and Sidhu, 2003).

In reverse genetics, attempts are made to delineate gene function by manipulating the gene in animal models (such as knockouts and knockin with transgenes). The hypothesis that the expression of networks of genes is disrupted in alcohol dependence has been tested using postmortem pre-frontal cortex RNA profiling from alcohol-dependent patients (Farris et al., 2015a, 2015b), which revealed sustained pairwise differential expression profiles related to alcohol use disorder. Convergent functional genomics, a term coined by Alexander Niculescu, approaches gene identification by looking at different lines of evidence (Niculescu et al., 2000). The aim is to create an overall ranking using Bayesian scoring based on the multiple sources of evidence (e.g. from human and animal studies). For example, a convergent functional genomics (CFG) score can be obtained from combining animal genomic, transcriptomic and proteomic data, which is then added to lines of evidence obtained from human studies (linkage, GWAS, genomic, gene expression and proteomic data). In this manner, candidate genes can be ranked

according to their CFG score. The potential applicability of this methodology to addictions has been reviewed by Spanagel *et al* (2013).

The Encyclopedia of DNA Elements (ENCODE) database (The ENCODE Project Consortium, see nature.com/ENCODE) aims to facilitate predictions of the functional effects of SNPs by answering the following questions: 1) is the nucleotide transcribed? 2) is the nucleotide part of a transcription factor binding site (TFBS)? 3) is the nucleotide part of a DNase I hypersensitive site (DHS)? 4) is the nucleotide part of a region with altered chromatin marks (histone modifications or DNA methylation): and 5) does the nucleotide physically interact with DNA at great distance from it on the chromosome?. The potential role of ENCODE in nicotine addiction research has been reviewed by Vandenbergh and Schlomer (2014). For example, ENCODE shows that the glucocorticoid receptor (transcribed from *NR3C1*) is the transcription factor that binds to the DNA around SNP rs4105144 (relevant to CYP2A6, and associated with number of cigarettes smoked per day) (Onica et al., 2008; Vandenbergh and Schlomer, 2014).

Epigenetics

Epigenetics is defined as all meiotically and mitotically heritable changes in gene expression that are not coded by the DNA sequence itself (Egger et al., 2004). Such changes may be affected by environmental factors, with gene silencing being effected by mechanisms including changes in chromatin structure (Toyokawa et al., 2012). Recent data indicates that the three different types of mechanisms involved in such silencing (DNA methylation, histone modification, RNA-associated silencing) may interact with and stabilize each other (Egger et al., 2004). Disruption of one or more of these may lead to inappropriate expression as well as to silencing of genes.

Methylation has long been recognized as an epigenetic silencing mechanism of fundamental importance (Holliday and Pugh, 1975; Riggs, 1975), relevant to transcriptional repression of genes, silencing of transposable elements such as Alu repeat sequences and defence against viral sequences. In DNA methylation, a family of DNA methyltransferases are responsible for adding a methyl group to DNA at a CpG site (Egger et al., 2004), i.e. where a cytosine is linked to a guanine nucleotide by its usual phosphate bridge on a strand of DNA, with methylation occurring at the C^5 position of the cytosine, resulting in 5-methylcytosine. 5-methylcytosine is readily converted to thymine (by spontaneous deamination), resulting in loss of the CpG site. Residual CpG islands (regions of more than 500 base pairs with a GC content of greater than 55%) are conserved in areas of the genome such as promoter regions owing to relative hypomethylation of these areas. Such regions may then be subject to differential methylation, e.g. in response to early environmental insults, leading to differential susceptibility to the effects of subsequent exposures including addictive substances and behaviours.

In chromatin, DNA coiled around a core group of eight histone proteins is known as a nucleosome, with this level of structure acting as a regulatory site for subsequent higher levels of coiling and looping of DNA, which render the DNA more (in euchromatin) or less (in heterochromatin) accessible for transcription. The eight core histone proteins comprise two each of four types of histones (H2A, H2B, H3 and H4), with each nucleosome also containing one linker histone (H1). Post-translational modifications of histone proteins (acetylation, methylation, phosphorylation or ubiquitylation) play essential roles in regulating the dynamic structure of chromatin. The particular combination of histone modifications found in a cell has been termed a ‘histone code, and is one epigenetic mechanism whereby the information potential of the genetic code is extended. Histone acetylation or methylation occurs at conserved lysine

residues in histone amino acid tail domains, with acetylation (by histone acetyl transferase) in most cases enhancing transcription and deacetylation (by histone deacetylases, otherwise known as HDACs) being associated with inactive chromatin. Histone methylation, by contrast, can be a marker for both active and inactive regions of chromatin, with H3 lysine 9 methylation (H3-K9) occurring in gene promoters that have been 'silenced' and H3 lysine 4 (H3-K4) methylation occurring in promoters of active genes. Interactions between histone deacetylases, histone methyltransferases and methylcytosine-binding proteins may lead to the recruitment of DNA methyltransferases (Egger et al, 2004), and hence methylation of susceptible regions of DNA.

Mechanisms of RNA-associated silencing include antisense transcripts, noncoding RNAs and RNA interference (RNAi). In a case of alpha-thalassemia, it was shown that antisense transcription could lead to DNA methylation and stable silencing of a globin gene (Egger et al., 2004; Tufarelli et al., 2003). Noncoding RNAs (biologically functional RNAs that do not encode proteins) include microRNAs (miRNAs, approximately 22 nucleotides long), which bind to mRNAs resulting in post-transcriptional silencing and may be particularly relevant to the regulation of gene expression in the brain (Miska et al., 2004).

Epigenetics and substance use

In drug addiction, epigenetics has been used to explain phenomena relevant to addictions, such as the formation of memories, drug-seeking behaviour (Malvaez et al., 2009), toxicity (Kovatsi et al., 2011) and withdrawal and other behavioural changes (Pizzimenti and Lattal, 2015). The effects on gene expression can be seen after acute administration of substances as well as with chronic exposure. An example of an acute epigenetic effect is the decrease in HDAC activity that occurs in the amygdala of rats after an acute injection of alcohol (Pandey et al., 2008). Repeated exposure to drugs of addiction can lead to persistent alterations in dendritic structure and

dendritic spines, in motivation and reward-related neurons (Robinson and Kolb, 2004) that is mediated through epigenetic mechanisms. Patients with addiction have a higher incidence of adverse childhood experiences (Felitti, 2003). Such experiences lead to adaptational changes in and around the hypothalamo-pituitary-adrenal axis, including via methylation changes in genes that are related to the stress response (Brockie et al., 2013). Weaver *et al*. (2004) conducted an elegant animal study to demonstrate the effects of behaviour on the epigenome. They observed that rat pups that experienced high levels of licking, grooming or nursing had reversible differences in DNA methylation at the glucocorticoid receptor gene promoter in the hippocampus, compared to pups that experienced low levels of similar activity(Weaver et al., 2004). Epigenetic changes in response to stressful events such as adverse childhood experiences may therefore be pivotal in the predisposition to drug addiction (Brockie et al., 2013).

Methylation of two CpG islands in the monoamineoxidase-A (MAOA) gene is associated with alcohol and nicotine dependence in women (Philibert et al., 2008). Chronic alcohol consumption increases NMDA receptor *N2RB* gene expression through demethylation (Marutha Ravindran and Ticku, 2004). Alcohol-mediated anxiolysis has been associated with reduced HDAC activity, while anxiety-like behaviour in alcohol withdrawal has been associated with increased HDAC activity in the rat amygdala (Pandey et al., 2008). Elevated homocysteine levels may be seen in those who are alcohol dependent and this has been related to increased homocysteine-induced endoplasmic reticulum protein (*HERP*) gene promoter DNA hypermethylation, which has been shown to reduce *HERP* mRNA expression (Bleich et al., 2006). Hypermethylation in the promoter of the alpha synuclein (*SNCA*) gene has also been correlated with elevated homocysteine levels in alcohol dependence (Bonsch et al., 2005). Nicotine use has been found to cause differential DNA methylation at loci near the *F2EL3*,

AHPR, *GPR12*, *IE3*, *ALPP*, *RARA*, *GNG12*, *ZNF385D*, *PRSS23*, *AVPR1B*, *PSEN2*, *LINC00299*, *RPS6KA2*, *KIAA0087* and *LRP5* genes (Tsaprouni et al., 2014). Nielsen *et al*. (2009) analysed methylation at 16 CpG sites in the *OPRM1* promoter region and found that two sites had higher methylation in patients who were former heroin addicts compared to the controls. In an animal study, methamphetamine reduced mRNA and protein levels of GluA1 and GluA2 AMPA receptor subunits through epigenetic mechanisms (Cadet and Jayanthi, 2013). An epigenome-wide association study for smoking found an association with 30 probes in 15 loci in a discovery cohort. Twenty-nine of these probes were significant in the replication cohort of this study (Tsaprouni et al., 2014), and 9 of the 15 loci had previously been found to be significantly associated with to smoking.

The Schaefer *et al.* (2010) study is a good example of how miRNA-mediated change in gene and protein expression is related to addiction. They demonstrated substantial alterations in multiple miRNA expression after cocaine exposure, some of which affect genes related to motivation, such as bdnf, fosB (FBJ murine osteosarcoma viral oncogene homolog B) and cdk5r1 (cyclin-dependent kinase 5 activator 1). Argonaute proteins bind miRNA, and are a vital component of the multiprotein RNA-induced silencing complex (RISC) that executes miRNA functions (Hutvagner et al., 2001; Lingel et al., 2003). Schaefer *et al.* (2010) found that deficiency of the Argonaute 2 (Ago2) in mouse brain D2-receptor expressing neurons was related to a reduction in certain miRNA subtypes and a motivation to self-administer cocaine. In addition, increases in the expression of Ago2 and specific miRNA increases have been seen after cocaine exposure (Eipper-Mains et al., 2011).

Genetics of Behavioural Addictions

Internet addiction and internet video game addiction

Although problematic internet use is not yet included in DSM-5, this may be of a nature and severity fitting the term behavioural addiction and may pose significant problems for an individual or those with whom they interface. The Young's Internet Addiction Scale can be used to identify problematic internet use (Young, 1999). Montag et al. (2012) linked a marker in the nicotinic receptor *CHRNA4* gene, rs1044396 SNP, to internet addiction, which appeared to be particularly relevant in females. The serotonin system also appears to be relevant, specifically the low expression (SS or short-short) variant *5-HTTLPR* has associated with excessive internet use (Lee et al., 2008). This variant has also been associated with depression in individuals exposed to childhood maltreatment (Cerda et al., 2010; Uher et al., 2011).

In regard to the dopamine system, individuals with excessive internet video game addiction had higher frequencies of the Met variant of the *COMT*Val(158)Met, and *DRD2* Taq1A1 alleles (Han et al., 2007).

Gambling

Approximately 50% (range 43–60%) of the variance in gambling behaviour (e.g. buying a lottery ticket, time or funds spent gambling, etc.) is attributable to genetic factors (Eisen et al., 1998; Lobo and Kennedy, 2009; Slutske et al., 2009) with problem gambling showing some common genetic loading with alcohol dependence. The first GWAS of disordered gambling was conducted on 1312 twins from 894 Australian families (Lind et al., 2013). Although no single genetic marker reached genome-wide significance, this may be owing to the moderate heritability of the trait, more than 2 million markers being used, and phenotypic variation. Suggestive evidence of association was found for *MT1X* (metallothionein), *ATXN1* (ataxin1) and *VLDLR* (encoding the very low density lipoprotein receptor), the latter confirmed in secondary case–control analyses as being associated with pathological gambling. The *VLDLR* is a receptor

for reelin, and the reelin-VLDLR/ApoER2 signalling pathway controls cortical neuronal migration in early development and modulates synaptic plasticity, memory and learning in the adult brain (Herz and Chen, 2006). This signalling pathway has also been implicated in a variety of mental illnesses, including depression, bipolar disorder, and schizophrenia (Barr et al., 2007; Suzuki et al., 2008). Other pathways that have previously been associated with addictions (including dopamine agonist-induced problem gambling in the context of Parkinson's disease) also appeared to be over-represented in terms of marker associations. A study conducted in Alberta and Ontario combined information from rat models and gamblers. In this study, tagSNPs in the genes *DRD3* (rs167771) and CAMK2D (rs3815072) were associated with disordered gambling (Lobo et al., 2015). Further genetic research in gambling is warranted, paying attention to the particular gambling phenotypes being measured and comorbidity.

Sexual addiction

Repeat regions in genes include variable number tandem repeats (VNTRs) and short tandem repeats (STRs). Sexual addiction phenotypes may have an association with these types of repeats in genes such as those encoding the dopamine-4 receptor (*DRD4*), the dopamine transporter (*SLC6A3*), the arginine vasopressin 1A receptor (*AVPR1A*), the oxytocin receptor (*OXTR*) and the serotonin transporter (*HTT* or *SLC6A4).* In addition, some SNPs in the above genes have been associated with relevant phenotypes, together with the above markers in haplotypic association analysis.

Ben Zion *et al*. (2006) reported such an association between a 5-marker *DRD4* haplotype comprising the functional exon 3 VNTR and promoter SNPs with self-reported measures of human sexual behaviour in a group of university students. Possession of at least one *DRD4* exon 3 'long' allele (defined as 7–11 repeats) was found to be associated with early sexual onset in an

adverse environment when compared to *DRD4* 'short' allele (defined as 6 or fewer repeats) homozygosity in African-American youths (Kogan et al., 2014). Guo and Tong (2006) found that the exon 3 *DRD4* polymorphism was associated with age at first intercourse. Men with least one 10-repeat (10R) of 40-bp in the dopamine transporter gene *DAT1* have an 80–100% increase in number of partners compared to those with two 9-repeat alleles (Guo et al., 2007), while the Taq1A SNP polymorphism in the same gene has also been associated with relevant phenotypes. In *Drosophila*, a subset of dopamine neurons regulates age-associated male courtship activity (Kuo et al., 2015). Prichard *et al.* (2007) reported an association between *AVPR1A* and *OXTR* gene polymorphisms and behavioural phenotypes in sexual and reproductive domains. Walum *et al.* (2008) subsequently reported an association between the polymorphic repeat in the 5′ flanking region of the *AVPR1A* (RS3 variant) and traits reflecting pair-bonding behaviour in men, including partner bonding, perceived marital problems and marital quality as perceived by spouses, and marital status. Kogan *et al*. (2010) found that 5-*HTTLPR* moderated the effect of early adolescent substance use and risky sexual behaviour in African-American youths 2 years later, with the 'at risk' group being those with at least one copy of the short ('s') allele.

Future Directions

Many of the associations summarized above have not yet been replicated. The first step forward must be replication and validation. Next comes understanding of the functional, or biological consequences, of any genetic or epigenetic markers. Subsequently, and, in some cases appropriately in parallel, translation to clinical application may occur. This may range from identification of those at above average risk of developing a disorder, to those more likely to respond better or worse to specific treatment (psychological or pharmacological). Where a robust functional association has been identified with a single biomarker, this is readily translated to an

assay that may be efficiently deployed. With larger sets of biomarkers, technologies such as high density microarrays render large-scale testing feasible.

Appendix 4:

Lodhi R.J., Wang Y., MacIntyre G., Bowker A., Crocker C., Ren H., Dimitrijevic A., Bugbee D.A., Loverock A., Rossolatos D., Granger B., Sivapalan S., Newton V.M., Tibbo P., Purdon S.E., Aitchison K.J. (2017)**.** Investigation of the *COMT* Val158Met variant association with age of onset of psychosis, adjusting for cannabis use. *Brain and Behavior* http://onlinelibrary.wiley.com/doi/10.1002/brb3.850/abstract

Investigation of the *COMT* Val158Met variant association with age of onset of psychosis, adjusting for cannabis use

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Abstract

Objective. *COMT* rs4680 (Val158Met) genotype moderates the effect of cannabis on the age of onset of psychosis (AoP). We investigated the association between rs4680 and AoP, after adjusting for relevant covariates, in a Canadian Caucasian sample.

Methods. 169 subjects with psychosis were recruited. AoP, defined as age of DSM-IV diagnosis was established using the Structured Clinical Interview for DSM-IV. Cannabis use data were collected using a self-report computerized questionnaire. DNA was extracted from saliva and genotyping of the *COMT* Val158Met polymorphism was done by SNaPshot and TaqMan assays. Logistic regression and Kaplan-Meier analysis results are reported.

Results. In those who had used cannabis before 20 years of age, rs4680 had a trend level effect on AoP (median AoP: Val/Val < Val/Met < Met/Met 19.37, 20.95, 21.24 years respectively; log rank test p=0.051).

Conclusion. Our data are indicative of the need to further investigate the association between the *COMT* rs4680 variant and age of onset of psychosis in the context of adolescent cannabis use.

Keywords**:** cannabis, psychotic disorders, catechol-*O*-methyltransferase, genes, sex

Introduction

Cannabis use is associated with an increased risk of psychosis (1). An earlier meta-analysis reported a 40% increase in risk (95% confidence interval [CI] 20-65%) of any psychotic outcome in cannabis users compared with never users (2). In a birth cohort study, cannabis use by age 15 years was associated with increased risk of schizophreniform disorder at age 26 years (3). In this study, the effect size (OR = 1.65, CI 0.65 to 4.18) in those first using cannabis by age 18 years was less than that for those first using cannabis by age 15 years (OR = 4.50, CI 1.11 to 18.21). Although the odds ratios are different, the wide and largely overlapping confidence intervals for these two odds ratios indicate that the risk of psychosis is similar in the two age groups. It has been recommended that further studies be done to identify high-risk groups particularly susceptible to the effects of cannabis on psychosis (1), such as those who are genetically susceptible.

Caspi et al. (2005) reported that a functional variant (rs4680, a G>A substitution that results in a valine to methionine substitution at amino acid codon 158) in the *COMT* gene encoding the enzyme catechol-*O*-methyltransferase (COMT) was associated with increased risk of schizophreniform disorder at age 26 years for those with a history of adolescent cannabis use (4). The risk genotype was Val/Val (OR 10.9, CI 2.2-54.1), with the Val/Met being associated with a lesser degree of risk (OR 2.5, CI 0.78-8.2). Consistent with this, others reported a similar gene-environment (*COMT* rs4680 - cannabis) effect on the age of onset of psychosis (AoP) among individuals within a schizophrenia spectrum disorder (5). In individuals with

schizophrenia spectrum disorders, Val/Val genotype had the earliest AoP, followed by Val/Met, followed by Met/Met. Subjects with a first episode of a non-affective psychosis also exhibit a significant cannabis - rs4680 interaction on AoP as well as on duration of untreated psychosis (6). In a recent publication, however, rs4680 was not associated with AoP, neither independently nor in interaction with another genetic variant that has been previously studied in relation to psychosis and cannabis consumption, namely the *BDNF* Val66Met (7). The exact mechanism behind the association between rs4680 and AoP is not clear but cannabis is an important factor affecting AoP (8-10). Other significant factors affecting AoP are gender (7) and family history of psychosis/schizophrenia (11, 12). There have also been reports of associations between rs4680 and other relevant phenotypes e.g., healthy individuals of Val/Val genotype were at increased risk of experiencing hallucinations on cannabis consumption if they had high levels of psychosis vulnerability (13), rs4680 moderating the effect of Δ-9-tetrahydrocannbinol on psychos.is and cognition (14) and those at risk of transitioning to psychosis showing an increased risk of positive symptoms in the case of Val/Val individuals with a history of cannabis use at least weekly, with a lesser degree of effect for those of Val/Met genotype (15).

To understand the *COMT*-cannabis-psychosis/AoP relationship, it is important to consider the following. Dopamine plays a role in psychosis (16) and in cannabis use (17). A genome-wide association study reported the D_2 receptor gene (*DRD2*) as one of the 108 loci associated with schizophrenia (18). Dopaminergic agonists and stimulants worsen psychosis (19) while dopamine receptor (D_2/D_3) antagonism is key to reducing symptoms of psychosis (20, 21). Substance misuse, including cannabis, is linked to reward mechanisms, in which dopamine plays a central role (17, 22). There is significant interaction between dopamine and the endogenous

cannabinoid system (ECS) in substance misuse. It has been suggested that the ECS has a significant role to play in the core reward system (23). The ECS promotes midbrain dopamine cell activation and dopamine release in the nucleus accumbens, thereby facilitating reward behaviors (24). Cannabis is known to increase dopamine levels in the cortex (25), striatum (26) and the mesolimbic pathway (27). Given its key role in dopamine metabolism, especially in the prefrontal cortex, COMT has been suggested to be a good candidate for gene-environment interaction effects in psychosis, such as with cannabis (28).

The association of rs4680 with psychosis phenotypes in the context of cannabis use has been difficult to replicate (29-31). This may be due to heterogeneity between studies, including differences in age of assessment of psychotic symptoms and variability in the allelic frequency of rs4680 between different ethnic groups including apparently similar ethnic groups with differing degrees of admixture. An important factor could be that the relationship between rs4680, cannabis and psychosis is complicated by rs4680 being associated not only with psychosis phenotypes but also with cannabis use. A study reported higher frequency of the rs4680 Val/Val genotype in individuals with schizophrenia who had used cannabis premorbidly (88.9%) compared to those who had not (68.4%)(32). However, the allelic effect is not consistent in the literature: An earlier study had observedhigher cannabis use in *COMT* Met/Met homozygotes compared to Val/Val homozygotes (33). Indeed, in a study of genetic aetiology more broadly, there is evidence of common genetic predisposition to schizophrenia and to the risk of cannabis use (34). Owing to this, schizophrenia and cannabis use may be both associated and correlated with eachother. In our study, we sought to re-examine the effect of *COMT* rs4680 genotype on onset of psychosis, adjusting for relevant covariates such as cannabis use, in a Canadian

Caucasian sample. Our hypothesis was that rs4680 Val/Val genotype would confer an earlier AoP, adjusting for relevant covariates including cannabis use.

Methods

Sample

Recruitment was done from a variety of locations. These included two first episode psychosis teams, one in Edmonton, Alberta (the Edmonton Early Psychosis Intervention Clinic, EEPIC), and one in Halifax, Nova Scotia (the Nova Scotia Early Psychosis Program, NSEPP). In addition, patients were recruited from Alberta Hospital Edmonton (Neuropsychology Department), and from various Halifax community mental health teams. 52.66% were minimally medicated or in the early stages of their illness and 47.34% were medicated for more than three months. The mean duration of illness for the minimally medicated and medicated for more than three month groups were 0.78 (95% CI 0.507 to 1.054) and 9.37 (95% CI 7.213 TO 11.545) years, respectively. Research ethics committee approval was obtained at both sites as part of a study investigating genetic associations with psychotic disorders, and all patients provided informed consent. Patients with the following DSM-IV diagnoses (made using the Structured Clinical Interview for DSM-IV, or SCID-I) were included: schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic episode, psychosis not otherwise specified, and substance-induced psychosis. AoP in our study was defined as age in years when DSM diagnostic criteria were met. This was determined retrospectively from the time of intake through interviews and medical records. A self-rated drug screen in the form of a computerized questionnaire was used to gather information on substance use including lifetime

cannabis use and age at first usage (35). The latter was collected using the following categories: less than 11 years, 11 to 15 years, 16 to 19 years, 20 to 29 years, 30 to 39 years, and above 40 years. These were collapsed into never used cannabis, first use of cannabis before 20 years (the first three categories, equal to 19 years and under), and first use of cannabis at or after 20 years (the last three categories, which will be referred to as first cannabis use after 20 years).

DNA was extracted from saliva collected using Oragene DNA collection kits (DNA Genotek Inc., Ottawa, Canada). The earlier part of the collection was extracted in the University of Alberta Applied Genomics Core (TAGC) on a Beckman Biomek NX automated workstation, with the latter part of the sample being extracted manually according to the manufacturer's instructions with a minor modification. For the former, 0.6 ml of saliva/Oragene buffer was processed using the Agencourt GenFind v2 kit, without initial lysis (as a 50^0C incubation of sample with the lysis buffer preceded loading on to the instrument), and in the initial step, two washes with 75% ethanol were performed prior to elution of the genomic DNA in 55 µl of elution buffer. For the latter, the Invitrogen PureLink Genomic DNA mini kit protocol was used with minor modifications. In brief, 2ml of the saliva/buffer mixture was processed. Genomic DNA was precipitated, re-suspended in Phosphate Buffered Saline and then bound to a filter on a spin column. The column was washed twice with 60% to 70% ethanol, and the DNA was eluted twice; the first time with 100 µl of pre-warmed elution buffer, the second time with 50 µl pre-warmed elution buffer. Both elutions were then transferred to sterile cryovials for storage at -80°C, in which the samples were encoded using laboratory numbers with no personal identifiers.

Genotyping

Genotyping was initially conducted using a SNaPshot assay in The Applied Genomics Centre (TAGC) at the University of Alberta, with this being continued in the Aitchison laboratory by a TaqMan® SNP Genotyping Assay (ID: C__25746809_50) on an Applied Biosystems ViiA^{TM}7 Real-Time PCR System (ThermoFisher Scientific, Canada, formerly Applied Biosystems by Life Technologies, Canada). DNA fragments for use in the SNaPshot reaction were generated by polymerase chain reaction (PCR) using the following conditions: 95^{0}C for 5 minutes; 32 cycles of 95^{0}C for 30 seconds, 65^{0}C for 90 seconds, and 72^{0}C for 30 seconds; with a final extension at 68^{0}C for 10 minutes. PCR template (0.056 pmols) was added to 2 µL of SNaPshot multiplex ready reaction mix and 3 µl of 2.5x BigDye (ABI, USA) sequencing buffer. The SNaPshot conditions for primer extension were as follows: 25 cycles of 96^{0}C for 10 seconds, 50^{0}C for 5 seconds and 60^{0}C for 30 seconds. Primer extended products were treated with shrimp alkaline phosphatase (SAP), denatured at 98^{0}C for 3 minutes, and chilled on ice for 3 minutes before processing on an AB3130 Genetic Analyzer (ThermoFisher Scientific, Canada, formerly Applied Biosystems by Life Technologies, Canada). Data were analyzed using Applied Biosystems GeneMapper v 4.0. For the TaqMan assay, all samples were genotyped in duplicate, with an in-house automated data comparison to compare genotypes between duplicates. Repeats were conducted for any calls not readily resolved.

Population stratification analysis

Given the differential allelic frequency of rs4680 by ethnic group, we restricted the analysis to Caucasians (strictly defined using all available data from the grandparent level). We ensured that were no ethnic variations by genotyping twenty-four markers with known allele frequencies in

Caucasians (36). Data for one of these did not pass quality control; for the remaining twenty-three, the minor allele frequencies did not significantly differ from those expected in Caucasians (p=0.35).

Statistical analysis

Statistical analysis was conducted using STATA 13.1. The distribution of demographic variables and other results were compared by chi-square or t-tests for categorical and linear variables respectively. Kaplan-Meier time-to-event analyses were performed using the log-rank 'sts test' with AoP as a continuous variable, entering gender, age at first cannabis use categories (before versus on or after 20 years) and rs4680 genotype (Val/Val, Val/Met, and Met/Met) as predictors. Kaplan-Meier analysis was repeated in subgroups of interest.

Results

One hundred and sixty-nine individuals met diagnostic criteria and had data available on age of onset of psychosis, cannabis use and *COMT* rs4680 genotype. There was 100% concordance between the data from TAGC and the Aitchison laboratory, and all samples except one were genotyped by both methods. The genotypes were in Hardy-Weinberg equilibrium (X^2=0.4095, p=0.52), with 0.47 and 0.53 being the allele frequencies for the Val and Met respectively. Lifetime cannabis use was more common among males (p=0.004), and first cannabis use under 20 years of age was more common in males (p=0.01).

[Table 1 about here]

On Kaplan-Meier time-to-event analysis, male subjects had an earlier age of onset of psychosis than females (median AoP: males=20.61 years, females=21.66 years, log rank test p=0.0086, Figure 1A). The main effect of rs4680 in the time-to-event analysis was not significant (although the pattern of the median AoPs was Val/Val < Val/Met < Met/Met, 19.70, 20.96 and 21.90 years respectively; log rank test p=0.251). In those who had used cannabis, first use of cannabis prior to 20 years of age was associated with earlier AoP (p=0.005).

Given previous findings, we explored the effect of rs4680 genotype in those who had first used cannabis at less than 20 years of age; in these, the association between rs4680 genotype and AoP was close to significant, with patients of Val/Val genotype developing psychosis earliest (median AoP: Val/Val < Val/Met < Met/Met 19.37, 20.95, 21.24 years respectively; log rank test p=0.051). On repeating this stratifying by gender, a trend level association remained (log rank test p=0.079).

[Figure 1 about here]

Discussion

In summary, although our data did not indicate a significant effect, there was a trend level signal in the same direction as some previous studies (5) that examined rs4680, specifically that

Val/Val genotype may be associated with an earlier age of onset of psychosis, in those who had first used cannabis relatively early in life (prior to 20 years). STATA provides the option of statistically testing for the trend of survivor functions and when we used the test for the trend of rs4680 on AoP in first cannabis users before 20 years of age, it was significant (p=0.029). The pattern of the trend is consistent with an additive pattern of genotype effect (Val/Val > Val/Met > Met/Met), consistent with the co-dominant pattern described for these alleles.

These results echo the investigation of rs4680 in the Dunedin birth cohort study, with the Val/Val genotype being the at risk genotype for a psychotic disorder in Caucasians (4). Our study had similarities to the Dunedin study, for example, the majority of our patients had a schizophrenia spectrum disorder, and the association described in the former was with schizophreniform disorder. Another likely similarity is the ethnicity of Canadians and the New Zealanders, and therefore the genetic background upon which the *COMT* rs4680 variant sits and with which it may interact to determine overall expression of the COMT enzyme (37). As described above, we have also confirmed ethnic homogeneity.

The usefulness and contributions of studies that the examine effect of genetic variants in the context of environmental factors in psychiatry has been questioned (38) and part of the reason for this is non-replication of earlier results. In the case of the moderation by rs4680 of the association between cannabis and psychosis, although a number of studies have been done, these vary in: the population in which studies were conducted (e.g., normal subjects or those with psychosis), the definition of psychotic disorder or of psychotic phenomena, the use of DSM for diagnostic criteria, the length of follow-up of patients, the definition of cannabis use or misuse, and the ethnicity of participants. We included substance induced psychosis in our definition of psychosis and while this may theoretically introduce some genetic heterogeneity, there are

studies that indicate that primary and drug induced psychosis may be genetically linked (39). In our opinion, our study has many similarities with that of Dunedin study and hence the consistency in findings. *COMT* remains an interesting candidate gene for gene-environment interactions in psychosis since it modulates dopamine function, is dynamically regulated and its expression alters with environmental stimuli (37). In psychosis, the Val/Val rs4680 genotype may be predispose individuals to stress related mesolimbic hyperactivity (40) and is known to increase the vulnerability to psychosis after cannabis use in those exposed to childhood abuse (41). rs4680 has also been reported to moderate the effect of stress in induction of psychotic symptoms in a study of army recruits (42).

In our sample, the effect of age at first use of cannabis before 20 years on the age of onset of psychosis was significant. This is consistent with earlier examinations of the cannabis-psychosis relationship (2, 3, 10, 43-48) (for a meta-analysis, see (49)). The effect of gender on age of onset of psychosis was significant in our study, with males being at risk of developing psychosis earlier than females. This is consistent with prior studies (50, 51), especially for schizophrenia spectrum disorders. In a review of pertinent data, it has been suggested that where such an effect has not been found, factors such as atypical marital status and pre-morbid personality may contribute (52). Of note, 92% of our patients were single and only 2.86% were married, which is as expected for schizophrenia spectrum disorders (the majority of our sample). It is therefore not surprising that our sample demonstrated the same effect of gender on age of onset as the majority of schizophrenia spectrum samples that have been studied to date.

Strengths and limitations

Strengths of this study include defining psychotic disorder by structured clinical interview for DSM-IV diagnosis and careful definition of Caucasian ethnicity. The main limitations of the study were the relatively small sample size for a genetic association analysis, especially that of the controls, the self-report nature of the cannabis data and collection of first cannabis use age data in the form of age ranges. It is worth noting, however, that the time to event analysis for rs4680 was sufficiently powered to detect significant effects. We calculated the power of our sample using the 'stpower logrank' command in STATA, which uses the Freedman method (53) to estimate sample sizes for a two sample comparison of survivor functions. Based on a one sided 0.05 significance level log-rank test, a ratio of 1:3 for the frequency of the Val/Val genotype versus the rest (as seen in our data), a 50% reduction in hazard ratio (as seen in our sample for Val/Val group compared to the rest), to achieve power of 0.8, a sample size of 104 patients with only 73 with onset of psychosis was required. Hence with our sample of 175 subjects, we were sufficiently powered to detect the effect of the genotype. Forty-nine volunteers without psychosis, of a similar age but with different gender and cannabis use patterns to the psychosis subjects and with cannabis data were recruited and genotyped in parallel as controls for this genetic study. Of note, when these controls were added to the time-to-event analysis, we observed that the association between rs4680 and age of onset of psychosis in early cannabis users now reached significance even after adjustment for gender (median AoP: Val/Val < Val/Met < Met/Met 19.37, 21.48, 22.34 years respectively; log rank test p=0.0243). The self-report nature of the cannabis use data could also be viewed as a limitation; it would be useful to supplement this with additional material from interviews and medical record documentation in the future. It is unlikely, however, that rs4680 genotype would have exerted a biasing effect on an individual's self-reported cannabis use history.

Future directions

Independent replication in another Caucasian sample is clearly desirable and intended. In addition, extension of this to include childhood trauma data is desirable (54). This could be combined with epigenetic analysis of, for example, the *COMT* promoter, methylation of which has been shown to be associated with frequency of cannabis use in adolescents and young adults (55). We suggest that future studies should examine the relationship between age of onset of prodromal symptoms, duration of untreated psychosis, age at first presentation and age of onset of DSM symptoms, with *COMT* genotyping and thorough cannabis data collection, preferably in the same sample and in controls in parallel, to enhance the understanding of these effects.

In summary, our study shows that, in those who used cannabis before age 20 years, having the rs4680 Val/Val genotype could be associated with the earliest age of diagnosis of psychosis, with the Val/Met having a slightly later age of diagnosis and the Met/Met still later. The earlier the illness commences, the more crucial social transitions (such as completing educational and vocational training, independent living, and partner selection) may be interrupted. With some genetically vulnerable individuals, cannabis use may be associated with a lasting schizophrenia type of psychotic illness.

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Declaration of interest

KJA reports consultancy services for Otsuka Canada Pharmaceutical Inc., and Lundbeck Canada SEP has received honoraria for speaking, advisory board consultation, and contracted services from Merck Pharma and Lundbeck Canada, and an investigator initiated operating grant from the Zyprexa Research Foundation of Eli Lilly Canada, plus royalties from sales of the Screen for Cognitive Impairment in Psychiatry - Spanish language version (SCIP-S). PT has been in

Advisory Boards and/or received speaker fees over the last 12 months from Janssen Inc., Otsuka Canada Pharmaceutical Inc. and Roche (Canada).

Table 1. Sample characteristics by cannabis use categories (p values by chi-squared analysis or t-test)

	Sample	**First cannabis use**			***p***
		Never users	Before 20 years	After 20 years	
N	169	24	127	24	
Gender (N(%))					**0.01**
Male	119	11 (45.83)	95 (76.00)	15 (65.0)	
Female	50	13 (54.17)	30 (24.00)	7 (35.0)	
Diagnosis N (%)					0.282
Schizophrenia spectrum disorder	109	18 (75)	80 (64.00)	11 (55.0)	
Psychosis NOS	37	6 (25)	26 (20.80)	5 (25.00)	
Substance induced psychosis	23	0 (0)	19 (15.20)	4 (20.00)	
Education (N(%))					**0.009**
Grade 12 or less	61	6 (25)	53 (42.00)	2 (10.0)	
Relationship status (N(%))					0.404
Single	156	22 (91.67)	117 (93.60)	17 (85.0)	
Married/been with partner	13	2 (8.33)	8 (6.40)	3 (15.0)	
Mean AoP* (years, SD)	22.86 (6.38)	23.33	22.12	26.92	**0.006**
Mean age (years)	27.71	32.58	26.25	31.01	**0.003**

*AoP – Age of onset of psychosis