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**Obstetric Complications, Genetic Liability and Psychosis
A study of Gene X Environment Interaction**

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**Obstetric Complications,
Genetic Liability and Psychosis;
A study of Gene X Environment Interaction**

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Thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

There is no doubt a strong genetic component to psychosis, but family and twin studies have shown that simple genetic transmission is far from the whole story. Furthermore, a number of environmental factors have also been shown to increase risk of psychosis. Among these non-genetic causes, Obstetric Complications (OCs) are among the best replicated.

In order to get a better insight into the mechanisms by which OCs impact on brain development to increase the risk of psychosis, I employed a Gene X Environment causation model.

A total of 377 psychotic patients, 65 controls and 103 unaffected siblings were available for my project. I obtained data concerning clinical and socio-demographic status, obstetric history, together with samples of blood/cheek swabs for genetic analysis from these subjects. I also genotyped most of the subjects (N=399) for selected genetic variants that might have functional significance in relation to the individual's exposure to OCs (namely AKT1 rs 2494753, rs1130233, rs3803300; BDNF rs2049046, rs56164415; DNMBP1 rs875462; GRM3 rs7808623; AK573765-TWIST2 rs9751357; CACNA1C rs4765905; CEACAM21 rs4803480; CNNM2 rs7914558; CSMD1 rs10503253; Erbb4 rs1851196; ITIH3/4 rs2239547; LOC645434-NMBR rs2066036; LRRFIP1 rs12052937; MIR137 rs1625579; MMP16 rs7004633; NKAPL rs1635; NRG rs12807809; NT5C2 rs11191580; PCLO rs6979348; PLXNA2 rs752016; PGBD1 rs2142731; PCGEM1 rs17662626; RELN

rs7341475; SDCCAG8 rs6703335; STT3A rs548181; TCF4 rs17512836; UGT1A1 HJURP rs741160; rs10489202; rs16887244).

In a case-control design, I investigated how exposure to OCs influenced the risk of psychotic disorder.

Then, I tested, under a multiplicative model, the hypothesis that a range of genetic variants interacted with OCs in increasing the risk of psychotic disorder.

Lastly I examined whether rats that had experienced perinatal asphyxia during birth show abnormalities in gene expression and methylation status at various developmental periods.

My findings didn't show any interaction between genes and OCs in increasing the risk of psychosis. On the other hand, in rats following hypoxic insult many of the genes had heterogeneous pattern of expression, suggesting an important role for genes in mediating the reactions of the CNS to environmental stimuli such hypoxia. In general, at post neonatal day CNNM2 was down regulated, whereas CSMD1 and TCF4 were up regulated; at 5 weeks CNNM2, CSMD1, MMP16, STT3a were down regulated, whereas TRIM26 was overexpressed. Hypoxia in the prenatal and perinatal period could regulate the expression of specific genes contributing to the neurodevelopmental alterations later found in schizophrenic patient.

STATEMENT OF CONTRIBUTION TO THE INVESTIGATIONS

All my work has been guided and reviewed by Sir Professor Robin M. Murray, my first supervisor and Dr John Powell, my second supervisor. I was responsible in formulating the study hypotheses and selecting the research methods. I contributed to defining the assessments used in the project and in my Thesis.

All studies involving human subjects were approved by the South London and Maudsley National Health System Trust and Institute of Psychiatry Ethical Committee. My measures didn't fall within GAP study's ethics (REC reference number 05/Q0706/158) so I was responsible for amending the ethics (amendments ETHICS N 8, N9 and N11, respectively necessary to assess relatives, and administer the Maternal Interview Schedule, MIS).

Assessment: Obstetric history from all participants was elicited using a standard questionnaire the Maternal Interview Schedule (MIS) personally developed from other published reports under the supervision of Professor Robin M. Murray, Dr John Powell, Dr Jane Boydell and Dr Muriel Walshe. I arranged regular training sessions with the Italian Collaborator researchers to implement a correct and reliable use of the MIS.

Recruitment: In the first 24 months my activity was mostly focused on recruitment of both cases and controls, as well as assessment of participants and their mothers, and in the blood/cheek swab collection together with the GAP research team. In London I was the only individual responsible for approaching mothers and conducting the interview on obstetric complications.

Genotyping: I worked in the genetic lab for 7 months and under the supervision of Dr John Powell, Dr Iyegbe Conrad and Dr Rebecca Smith I conducted the genotyping of all our participants.

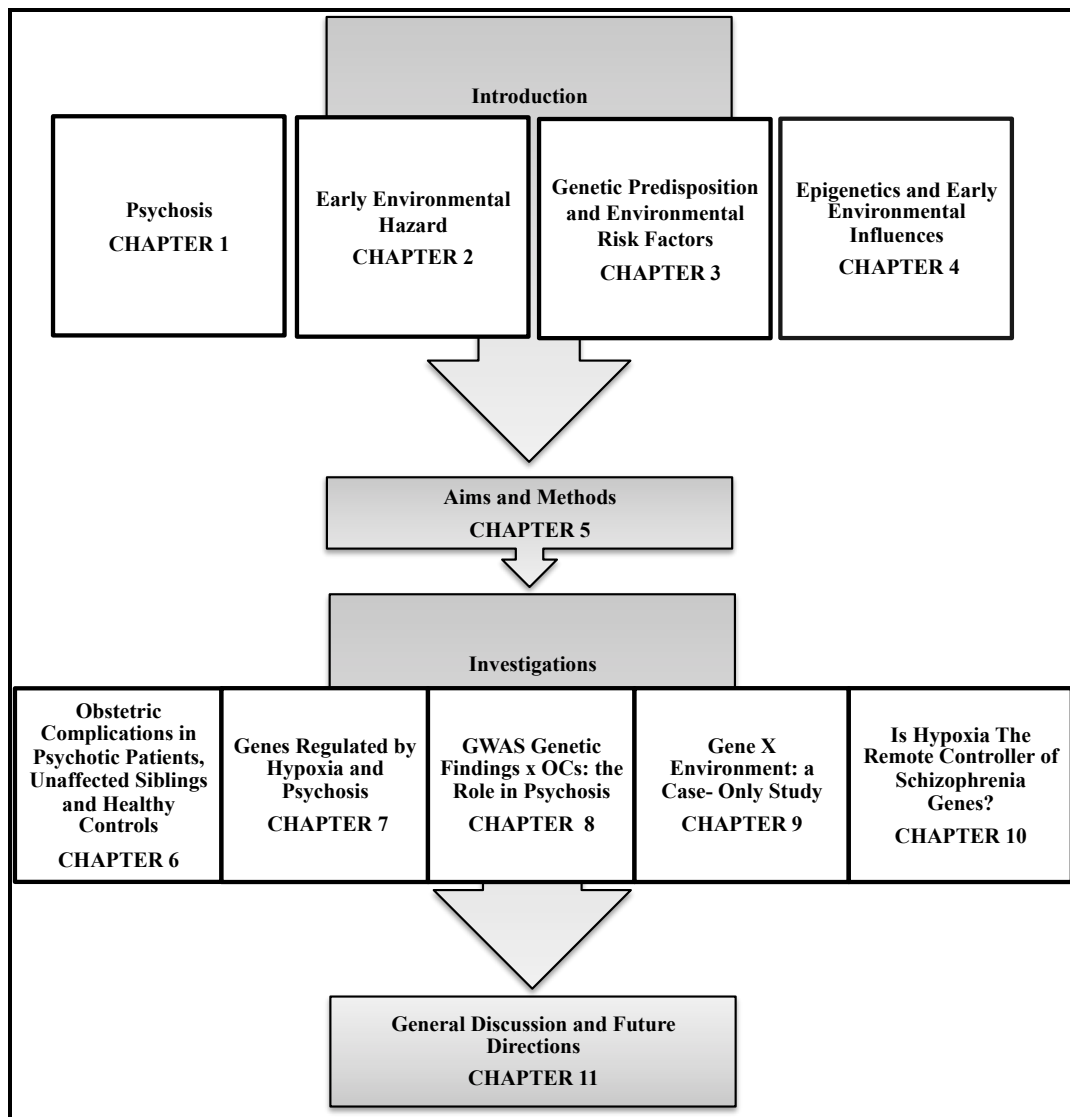
Database: I extracted all suitable data from the MIS, established the necessary database (SPSS 20) and conduct all statistical analyses and interpretation and report writing. I also re-scored and entered part of previously collected data onto an electronic database.

Animal Study: I applied for a Grant to the Japan Society for the Promotion of Science (JSPS) and was awarded a Fellowship 2011- 2012 to conduct an animal study at the Hamamatsu University School of Medicine for 6 months under supervision of Professor Nori Takei and Dr Keiko Iwata.

Importantly, I could have never reached an adequate sample size to test the hypothesis in my Thesis without the support of the Maudsley Family Study, the Psychosis Incidence Cohort Study in Verona, and my colleagues in the Genetic and Psychosis study.

ORGANIZATION OF THE THESIS

The thesis comprises of a total of 11 Chapters. **CHAPTERS 1, 2, 3 and 4** are introductory chapters to psychosis, obstetric complications, research on genetic predisposition to psychosis and epigenetics. General aims, methods and statistical analyses are presented in **CHAPTER 5**. The experimental part of the thesis is presented in **CHAPTERS 6, 7, 8, 9 and 10**. Each of these chapters has a brief introduction, followed by the specific methods, results, summary of results, discussions and limits. In **CHAPTER 11** a general discussion about the findings is presented. A chart describing the organization of the thesis is shown in the next page.



All'aperitivo di Negroni

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LIST OF ABBREVIATIONS

ACTB = B-Actin

AESOP = Aetiology and Ethnicity of Schizophrenia and Other Psychoses

AKT1 = V-Akt Murine Thymoma Viral Oncogene Homolog 1

APS = Average Pain Sensitivity

B3GAT2 = Beta-1,3-Glucuronyltransferase 2

BDNF = Brain-Derived Neurotrophic Factor

C-Section = Caesarean Section

CACNA1C = Calcium Channel, Voltage-Dependent, L Type, Alpha 1C Subunit

CB = Cannabinoid

CCDC68 = Coiled-Coil Domain Containing 68

CHCl₃ = Chloroform

CI = Confidence Interval

CNNM2 = Cyclin M2

CNR1 = Cannabinoid Receptor Gene 1

CNS = Central Nervous System

CNVs = Copy Number Variants

COMT = Catecol-O-Metil-Trasferasi

CSMD1 = CUB And Sushi Multiple Domains 1

DAT1 = Dopamine Transporter

DEPC = Diethylpyrocarbonate

DGKI = Diacylglycerol Kinase

DISC1 = Disrupted in Schizophrenia 1

DNA = Deoxyribonucleic Acid

DNMT = DNA Methyltransferases

DRD1 = Dopamine Receptor D1

DSM-IV TR = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision

DTNBP1 = Dystrobrevin-Binding Protein 1

DZ = Dizygotic (twins)

EDTA = Ethylenediaminetetraacetic Acid

GxE = Gene x Environment Interaction

GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase

GRM3 = Glutamate Receptor Metabotropic 3

HDAC4 = Histone Deacetylase 4

HiP = Hippocampus

HPS = High Pain Sensitivity

HR = High Risk

HWE = Hardy-Weinberg Equilibrium

H₂O = Water

ICD-10 = International Classification of Diseases, Tenth Edition

JSPS = Japan Society for Promotion of Science

LBW = Low Birth Weight

LPS = Low Pain Sensitivity

LSD = Lysergic Acid Diethylamide

LR = Low Risk

GAP = Genetic and Psychosis

GWAS = Genome-Wide Association Study

MAOA = Neurotransmitter-Metabolizing Enzyme Monoamine Oxidase A

MHC = Major Histocompatibility Complex

Met = Methionine

MFS = Maudsley Family Study

MMP = Matrix Metalloproteinase

MMP16 = Matrix Metalloproteinase 16

miRNAs = microRNAs

MIR137 = microRNA 137

MIS = Maternal Interview Schedule

MRC = Medical Research Council

MRI = Magnetic Resonance Imaging

MTHFR = Methylene tetrahydrofolate Reductase

MZ = Monozygotic (twins)

NCBI RefSeq = National Center for Biotechnology Information Reference Sequence

NMDA = N-Methyl-D-Aspartate

NRG1 = Neuregulin 1

NRXN1 = Neurexin 1

OC = Obstetric Complication

OR = Odds Ratio

PCP = Phencyclidine

PCR = Polymerase Chain Reaction

PET = Positron Emission Tomography

PFC = Prefrontal Cortex

PKB = Protein Kinase B

PICOS = Psychosis Incident Cohort Outcome Study

PND = Post Natal Day

RNA = Ribonucleic Acid

RR = Relative Risk

RT-PCR = Reverse Transcription-Polymerase Chain Reaction

SCAN = Schedules for Clinical Assessment in Neuropsychiatry

SE = Standard Error

SEM = Standard Error of the Mean

SGDP = Social, Genetic and Developmental Psychiatry Centre

SLAM = South London and Maudsley

SNPs = Single-Nucleotide Polymorphisms

SPET = Single Photon Emission Tomography

SPSS = Statistical Package for the Social Sciences

SST3A = Subunit of the Oligosaccharyl-Transferase Complex, Homolog A

STR = Striatum

ST6GALNAC1 = Alpha-N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 1

TCF4 = Transcription Factor 4

TPH = Tryptophan Hydroxylase

TRIM = Tripartite Motif

TRIM26 = Tripartite Motif Containing 26

Val = Valine

WHO = World Health Organization

5-HT = 5-hydroxytryptamine

5-HTTLPR = Serotonin Transporter Gene Promoter Region

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CHAPTER 1

Psychosis

1.1 Introduction

The term psychosis - from the Greek “psyche”, for mind/soul, and “-osis” for abnormality- was introduced in 1845 by Von Feuchtersleben with the meaning of “mental illness or madness” (Feuchtersleben, 1985). It now refers to a severe alteration of mental balance, characterised by a set of “psychotic” symptoms in which thoughts, feelings, affective response, ability to recognize reality and ability to communicate and relate to others are sufficiently impaired to interfere grossly with the individual’s capacity to deal with reality (Sadock and Sadock, 2003).

The “psychotic” symptoms are classified as positive and negative symptoms (Crow, 1980).

Positive symptoms include: *Hallucinations* are defined as sensory perceptions in the absence of external stimuli and may occur in any of the five senses. They have qualities of normal perceptions and are independent from the person’s will; they can be auditory, visual, olfactory, gustatory, tactile or somatic in nature. Auditory hallucinations are a common and often prominent feature of psychosis. A *Delusion* “is a false, unshakeable idea or belief, which is out of keeping with the patient’s educational, cultural and social background; it is held with extraordinary conviction and subjective certainty” (Sims, 2003). Delusions can be classified according to their content (e.g. persecutory, grandiose, depressive, reference, religious, jealous, erotomaniac, of misidentification, somatic, nihilistic). *Disorders of thought* are

disturbances in the thought process characterized by disorganized speech, and loosening of association.

Negative symptoms are deficits of normal emotional responses or of other thought processes. They commonly include flat or blunted affect and emotion, poverty of speech (alogia), inability to experience pleasure (anhedonia), lack of desire to form relationships (asociality), and lack of motivation (avolition).

It is still controversial whether negative symptoms are primary or in part secondary to either severe positive symptoms or treatment side effects. Depending upon severity, all psychotic symptoms can lead to impaired social interaction and poor self-care, and result in an overall decline in level of functioning.

1.2 Classification of Psychotic Disorders

Psychosis is generally subdivided into organic and functional: an organic psychosis is a condition due to a physical illness (for instance a consequence of dementia or of a brain injury) while a functional psychosis is a primary disorder not related to other physical disease. In current diagnostic systems, Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR) and the International Classification of Diseases (ICD-10), psychotic disorders are classified following a categorical model. According to the DSM IV-TR there are nine types (codes in bracket) of functional psychotic disorders: Schizophrenia (that has five subtypes: paranoid type - 295.30; disorganized type - 295.10; catatonic type - 295.20; undifferentiated type - 295.90; residual type - 295.60), schizophreniform disorder (295.40), schizoaffective disorder (295.70), delusional disorder (297.1), brief psychotic episode (298.8), shared psychotic disorder

(297.30), substance-induced psychotic disorder (291.3 and 291.5 or 292.11 and 291.12 according to the presence of hallucinations and substance of abuse), psychotic disorder not otherwise specified (298.9) and psychosis due to a general medical condition (293.81 and 293.82 according to the presence of hallucinations).

Psychotic symptoms can also occur in mood disorders, such as major depressive episodes and manic episodes (296.04, 296.24, 296.34, 296.44, 296.54, 296.64 according to the mood disorder). In the case of mood disorders, psychotic features generally occur only when the mood disorder is at a severe level and psychotic features can be either congruent or incongruent with the mood.

The above classification systems are currently under revision in preparation for the general release and implementation of the DSM-5 (<http://www.dsm5.org>) and ICD-11.

The classification depends upon type of symptoms, their duration and the presence or absence of prominent affective symptoms.

1.3 Historical Views and Current Position

Schizophrenia is the major psychotic disorder and is the eighth cause of disability worldwide in adolescents and adults (World Health Report, 2011). The first to describe it was the German psychiatrist Kraepelin (1896) with his famous concept of “dementia praecox” (Kraepelin, 1986); since then the description of the disease, its causes and treatment has undergone a very considerable evolution; in 1911 the Swiss psychiatrist Eugene Bleuler used the current term of “schizophrenia” (Bleuler, 1911).

The term schizophrenia is derived from the Greek “split mind” σχίζω (schizo, split) and φρενός (phrenos, brain). While Kraepelin considered as key elements of the syndrome, an early onset and inevitable progress towards the mental deterioration, Bleuler put the emphasis on mental dissociation, with the splitting of the unity of mind as an essential aspect of psychopathology of schizophrenia.

A few decades later, another German psychiatrist, Kurt Schneider (1957), proposed the basis of the definition and symptoms of schizophrenia that we are using today (Schneider, 1957). Schneider’s main concern was to improve diagnosis in psychiatry. He was also interested in differentiating schizophrenia from other forms of psychosis by listing the psychotic symptoms that are particularly characteristic of schizophrenia. These have become known as Schneider’s First-Rank Symptoms or simply, first-rank symptoms.

A further turning point came with Crow in 1980; he proposed different syndromes which integrated type I schizophrenia presenting clinically with positive symptoms such as delusions and hallucinations and type II schizophrenia, presenting with symptoms such as affective flattening and poverty of speech, negative symptoms (Crow, 1980).

However, further investigations suggested the positive-negative dichotomy to be an oversimplification and provided evidence for the existence of a third core syndrome, called the disorganized syndrome, which is characterised by symptoms such as formal thought disorder and inappropriate affect (Bilder et al., 1985; Liddle, 1987; Andreasen et al., 1995; Mass et al., 2000).

The prevalent view among most researchers today is that schizophrenia is a syndrome rather than a specific disease and it may include several conditions. In this I will

briefly review the Epidemiology (PARAGRAPH 1.4) and Aetiological Theories (PARAGRAPH 1.5) of the condition before going on in the next chapters to discuss Early Environmental Hazards (CHAPTER 2), Gene X Environment Interactions (CHAPTER 3) and Epigenetics (CHAPTER 4).

1.4 Epidemiology

Schizophrenia occurs most frequently towards the end of adolescence or at the start of adult life (Almeida et al., 1995) and affects approximately 0.7% of the world population (Mueser and McGurk, 2004). The lifetime prevalence rates for psychotic disorders according to DSM IV- TR are: 0.87% for schizophrenia, 0.32% for schizoaffective disorders, 0.24% for bipolar I disorders, 0.35% for major depressive disorders with psychotic features (Perälä et al., 2007) (FIGURE 1.1).

Diagnosis	No. of Subjects	All Subjects	Men	Women	All Subjects, Including Nonresponder†
Nonaffective psychotic disorders	153	1.94 (1.63-2.29)	1.64 (1.24-2.17)	2.19 (1.78-2.70)	2.29 (1.95-2.69)
Schizophrenia	67	0.87 (0.68-1.11)	0.82 (0.56-1.19)	0.91 (0.65-1.27)	1.00 (0.79-1.25)
Schizoaffective disorder	24	0.32 (0.21-0.46)	0.14 (0.06-0.34)‡	0.47 (0.30-0.72)	
Schizophreniform disorder	5	0.07 (0.03-0.16)	0.11 (0.04-0.30)	0.02 (0.00-0.17)	
Delusional disorder	15	0.18 (0.11-0.30)	0.16 (0.07-0.34)	0.21 (0.11-0.40)	
Brief psychotic disorder	4	0.05 (0.02-0.14)	0.08 (0.03-0.26)	0.02 (0.00-0.17)	
Psychotic disorder NOS	38	0.45 (0.33-0.62)	0.33 (0.19-0.56)	0.56 (0.39-0.82)	
Affective psychoses	49	0.59 (0.45-0.77)	0.72 (0.50-1.04)	0.49 (0.32-0.72)	0.62 (0.47-0.80)
BPI disorder§	20	0.24 (0.16-0.37)	0.31 (0.18-0.55)	0.18 (0.09-0.36)	
With psychotic features	10	0.12 (0.06-0.23)	0.14 (0.06-0.34)	0.10 (0.04-0.24)	
Without psychotic features	10	0.12 (0.07-0.23)	0.17 (0.08-0.37)	0.09 (0.03-0.23)	
MDD with psychotic features	29	0.35 (0.24-0.51)	0.41 (0.24-0.69)	0.29 (0.17-0.50)	
Substance-induced psychotic disorder	32	0.42 (0.30-0.59)	0.82 (0.58-1.17)‡	0.07 (0.02-0.23)	0.43 (0.31-0.60)
Alcohol-induced	31	0.41 (0.29-0.57)	0.79 (0.55-1.14)‡	0.07 (0.02-0.23)	
Other substance-induced	2	0.03 (0.01-0.11)	0.06 (0.01-0.23)	0	
Psychotic disorder due to a GMC	23	0.21 (0.14-0.32)	0.04 (0.01-0.18)‡	0.36 (0.23-0.55)	0.22 (0.15-0.34)
Any psychotic disorder	249	3.06 (2.66-3.51)	3.11 (2.53-3.82)	3.01 (2.54-3.57)	3.48 (3.06-3.96)

Abbreviations: BPI, bipolar I; GMC, general medical condition; LTP, lifetime prevalence; MDD, major depressive disorder; NOS, not otherwise specified.
 *Data are given as percentages and are presented as lifetime prevalence (95% confidence interval), unless otherwise indicated.
 †Calculated for diagnostic groups, not for specific diagnoses, except for schizophrenia.
 ‡Difference between sexes was statistically significant at $P < .05$.

FIGURE 1.1 Lifetime Prevalence Estimates of DSM-IV Psychotic and BPI Disorders. Taken from Perälä et al., 2007.

The risk of morbidity, that is the probability that a person born in a particular population/group develop the disease if he/she survives during the entire period of risk for this disease (ranging from 15 to 54 years in schizophrenia), is estimated to be in the range of 0.5/100 to 1.6/100 persons (World Health Organization (WHO), 1992).

Contrary to previous interpretations, the incidence of schizophrenia shows prominent variation between sites with rates for incidence from 7.7 to 43 per 100000 with a worldwide variation up to fivefold and a male to female ratio of 1.4 (McGrath et al., 2008). In particular, it is higher among migrants (Cantor-Graae and Selten, 2005) and people living in urban areas (Mortensen et al., 1999). Moreover the incidence of schizophrenia can also vary over time (Boydell, et al., 2003).

1.5 Aetiological Theories

“It is easy enough to speculate about an illness which has been the subject of so much investigation and remains so barren in established aetiology, but the first essential for any directed programme of research is a hypothesis based on fact, otherwise observation hardly progresses beyond the stage of cataloguing” (Osmond and Smythies, 1952).

The aetiology of schizophrenia has been the subject of much research, and a single common cause can be excluded. Rather epidemiological studies suggest that biological risk, such as genetic factors, early environmental hazards, substance abuse and social factors, such as urbanicity, migration, social adversity are important contributory factors (reviewed by Stilo and Murray, 2011). Thus, it results from a

combination of both brain vulnerabilities (either inherited or acquired) and life events (Murray et al., 2002). From a biological point of view, the symptoms of psychosis have a possible mechanistic cause in organic changes at various levels, from genetic predisposition to the altered functioning of neurotransmitters such as dopamine, serotonin, glutamate, gamma-aminobutyric acid, N-Methyl-D-Aspartate (NMDA), peptides, and others (Sadock and Sadock, 2003).

1.5.1 Neurochemistry

1.5.1a Model Psychosis and The Serotonin Hypothesis

In 1938 Hofmann synthesized the Lysergic Acid Diethylamide (LSD), but only 5 years later did he discover its hallucinogenic properties when, after accidentally spilling some on his skin, he experienced “its unforeseeable effects in my own body – or rather, in my own mind,” seeing uninterrupted streams of “fantastic pictures, extraordinary shapes with intense kaleidoscopic play of colours” (Hofmann, 1980). In the 1960s, the use of LSD and other recreational drugs spread among young people across the Western world. Neuropharmacologists began to ask whether schizophrenia might be caused by chemical alterations similar to those produced by LSD. Shaw and Woolley (1956) hypothesized that LSD might act on 5-hydroxytryptamine (5-HT) receptors, and later Anden et al. (1968) suggested a direct agonist effect at 5-HT receptors in the Central Nervous System (CNS) (Shaw and Woolley, 1956; Anden et al., 1968). The idea that the psychotic state induced by LSD resulted from actions at serotonin receptors led to the suggestion that LSD provides a “model psychosis” and to the serotonin hypothesis of schizophrenia. Glennon et al. (1983) proposed the 5-HT₂ receptor subtypes as the specific target of hallucinogenic drugs such as LSD, later supported by studies in rodents (Nichols, 2004), and in humans (Vollenweider et

al., 1998; Carter et al., 2007). The serotonin hypothesis received further support from the finding of 5-HT₂-receptor abnormalities in post-mortem schizophrenic brains, but in vivo studies of 5-HT₂-receptor abnormalities reported conflicting results (Mita et al., 1986; Gurevich and Joyce, 1997). The evidence that clozapine is particularly efficacious in the care of treatment resistant schizophrenia provoked further interest because of the 5-HT₂ receptor antagonism induced by this drug and other atypical antipsychotics (Lieberman et al., 1998; Meltzer, 1999; Tamminga and Holcomb, 2005).

1.5.1b The Dopamine Hypothesis

The “Dopamine Hypothesis” is still the best-known theory of schizophrenia (Kapur et al., 2003). This hypothesis was developed following the discovery, in the 1950s, of drugs that improved the acute psychotic phase of the disease (Carlsson, 1957). The first of these drugs was chlorpromazine, followed by other molecules later called “typical antipsychotics”, such as haloperidol. The first useful key to understand the mechanism of antipsychotics came from the analysis of their major side effects. In fact, these molecules frequently cause Parkinsonian-like syndrome. Carlsson and Lindqvist (1963) identified that antipsychotic drugs increased the metabolism of dopamine when administered to animals (Carlsson and Lindqvist, 1963). Following the intuition of Arvid Carlsson, several studies showed that, despite differences in chemical structure, all clinically effective antipsychotic drugs block dopamine receptors. From there the idea developed that an excess of dopaminergic transmission could play an important role in the pathogenesis of schizophrenia.

Additional support for the dopamine theory came from the evidence that certain drugs lead to schizophrenia-like psychosis. The similarity of amphetamine psychosis to schizophrenia was first clearly described in the 1950s by Tatetsu et al. (1956) and was substantiated by a similar report in Britain from Connell (1958) (Tatetsu et al., 1956; Connel, 1958). Subsequently, Angrist et al. (1974) found that amphetamine administered experimentally produced a picture similar to paranoid psychosis in healthy individuals and exacerbated psychotic symptoms in approximately one-third of schizophrenic patients (Lieberman et al., 1987); moreover antipsychotic drugs blocked these psychotogenic effects of amphetamines (Espelin and Done, 1968). Amphetamine was found to stimulate dopamine outflow, while, in contrast, antipsychotics were found to block dopamine receptors in the brain (Carlsson and Lindqvist, 1963; Van Rossum, 1967). Subsequently Single Photon Emission Tomography (SPET) and Positron Emission Tomography (PET) studies demonstrated that acute administration of amphetamine induced greater striatal dopamine release in first-episode schizophrenia patients compared to healthy controls (Laruelle et al., 1996; Breier et al., 1997). Together these observations provided the basis for the dopamine hypothesis of schizophrenia (Snyder, 1972; Kapur, 2003).

In 1991 Davis et al. showed a different brain distributions of D1 and D2 dopamine receptors (respectively predominantly cortical and subcortical), suggesting that the effects of abnormalities in dopamine function could vary by brain region (Davis et al., 1991). Subsequent PET and animal studies provided the best evidence of regional brain dysfunction in schizophrenia showing a regionally specific prefrontal hypodopaminergia and a subcortical hyperdopaminergia (Pycock et al., 1980; Scatton et al., 1982; Howes and Kapur, 2009).

This provided a mechanism to propose that schizophrenia is characterized by frontal hypodopaminergia and striatal hyperdopaminergia (Howes and Kapur, 2009). In particular, negative symptoms of schizophrenia seemed to be related to frontal hypodopaminergia while positive symptoms were hypothesized to result from striatal hyperdopaminergia (Davis et al., 1991). Therefore it would be more correct to talk about an imbalance of dopaminergic tone (Abi-Dargham et al., 2000; Kapur et al., 2005).

In rodents, repeated administration of amphetamine leads to reversed tolerance and an increased neurochemical and behavioural reaction to each dose. Boileau et al. (2006) tested whether this might happen also in humans (Boileau et al., 2006). They administered dextroamphetamine by mouth on days 1, 3, and 5 to 10 healthy volunteers, and measured the effect on striatal dopamine release before exposure, then the day of first exposure, then 2 weeks later after the third dose, using the PET/[11C] raclopride technique. Each dose of amphetamine caused greater dopamine release in the ventral striatum together with greater behavioural responses. Indeed, 1 year later there was a greater psychomotor response and greater increase dopamine release compared to the initial dose, in the ventral striatum, progressively extending to the dorsal caudate and putamen (Boileau et al., 2006). Such findings have led to the “dopamine sensitization” hypothesis of schizophrenia, which postulates that a sensitized dopamine system is responsible for the genesis of psychotic symptoms (Peleg-Raibstein et al., 2009).

Since then many articles have been published on the dopamine hypothesis of schizophrenia but it was not until recent years that we were able to develop the “Dopamine Hypothesis -version III”, that incorporates the most comprehensive

understanding of the role of dopamine in schizophrenia and psychosis in particular taking in account the multiple environmental and genetic risk factors for the illness finally leading to a final common pathway of presynaptic striatal hyperdopaminergia (Howes and Kapur, 2009). These authors hypothesized that the interaction of multiple “hits” results in dopamine dysregulation and that this dysregulation was at presynaptic dopaminergic control level (Di Forti et al, 2007; Howes and Kapur, 2009). Furthermore, the dopamine dysregulation is hypothesized to alter the valuation of stimuli through a process of aberrant salience (Howes and Kapur, 2009). Thus dopamine abnormalities may represent an end state for multiple environmental and genetic factors that operate together to push a vulnerable individual to symptomatic onset (Eyles et al., 2012).

1.5.1c The Glutamate Theory

The “dopamine theory” of schizophrenia has dominated attempts to explain the positive symptoms seen in schizophrenic patients (Kapur and Mamo, 2003) but is less able to account for negative symptoms. Drugs acting on the glutamate system have been proposed to model these. Kotz and Merkel (1926) discovered the compound, 1-piperidinocyclohexanecarbonitrile, and thus laid the groundwork for the preparation of Phencyclidine (PCP) which was developed as an intravenous general anesthetic in the 1940s by Parke, Davis & Co. Unfortunately, up to 50% of adult patients given PCP developed agitation and hallucinations (Greifenstein et al., 1958; Johnstone et al., 1958; Collins et al., 1960), so the focus of investigation of PCP shifted from its possible use as an anesthetic to its capacity to produce model psychoses (Luby et al., 1962). Subsequently, ketamine, another glutamate antagonist was introduced as an anesthetic, and later reports of ketamine induced psychosis started to appear. In 1983

Anis et al. (1983) showed that ketamine and PCP selectively reduce responses of central neurons acting at the NMDA receptor (Anis et al., 1983; Seeman and Tallerico, 2005). The observation that PCP and ketamine are NMDA antagonists together with the findings of lower levels of glutamate (by about 50%) in cerebrospinal fluid samples from schizophrenic patients compared to controls gave rise to the “Glutamate Hypothesis of Schizophrenia” (Kim et al., 1980). Although, this latter finding was not consistently replicated, the idea of hypoglutamatergic transmission in schizophrenia has continued to attract great interest as has the effects of NMDA antagonists (Tamminga and Holcomb, 2005). A dissenting view comes from Pommerol-Clotet et al. (2006) who gave ketamine to 10 healthy volunteers (Pommerol-Clotet et al., 2006). The commonest feature was referential thinking of a delusional nature, together with a range of perceptual abnormalities perhaps best described as dissociative. However, it did not induce hallucinations, and the authors were doubtful about its ability to cause thought disorder; furthermore, although negative-like symptoms resulted they could not exclude the possibility that this was simply due to its anesthetic effects (Pommerol-Clotet et al., 2006).

1.5.2 The Neurodevelopment Model of Schizophrenia

The theory that psychosis might be, in part, a developmental disorder was first proposed in the late 1800s by Thomas Clouston who identified a “developmental insanity” (O’Connell et al., 1997). However, this was subsequently forgotten. It is now more than two decades since Weinberger, Murray and Lewis re-formulated the neurodevelopmental hypothesis of schizophrenia as opposed to the degenerative hypotheses (Weinberger, 1986; Murray and Lewis, 1987). They suggested that subtle brain lesions acquired during intrauterine life initiate a process that could cause

dysfunction of the mature brain, predisposing to schizophrenia later in life

(Weinberger, 1986; Murray and Lewis, 1987).

Major supports to the formulation of the neurodevelopmental hypothesis came from the evidence that pregnancy and delivery complications (collectively termed obstetric complications (OCs)) increase risk of schizophrenia (Murray et al., 1985)

(**PARAGRAPH 2.2**). Moreover children who later develop schizophrenia tend to display early neurological and cognitive problems such as delayed milestones, speech problems, poor motor co-ordination, attention and information processing deficits (Jones et al., 1994; Erlenmeyer-Kimling et al., 2000; Cannon et al., 2002).

Since then it has been the dominant paradigm for schizophrenia research and received support from epidemiological, developmental and neuroimaging studies (Marengo and Weinberger, 2000; McDonald and Murray, 2000).

Schizophrenic subjects present morphological abnormalities in the brain such as enlargement of the lateral and third ventricles and a specific volume reduction in the medial temporal lobe, superior temporal gyrus, prefrontal cortex, cingulate gyrus, thalamus and insula (Wright et al., 2000). Many of these abnormalities are detectable in patients at the onset of psychosis suggesting pathophysiologic processes affecting the early developing brain (De Lisi et al., 1991; Hyrasyu et al., 1998; Fannon et al., 2000). In adult schizophrenic patients some of the brain abnormalities identified such as aqueduct stenosis, arachnoid and septal cysts, and agenesis of the corpus callosum cannot be wholly secondary to disease progression or due to treatment, they are likely to result from impairment of normal neurodevelopment (Lewis, 1990; Murray et al., 2002).

Some of the brain abnormalities identified in adult schizophrenic patients are also found in the unaffected relatives of patients suggesting that such abnormalities are a manifestation of familial risk factors, the most likely candidates being genes influencing neurodevelopment. (Murray et al., 2003).

It has been suggested schizophrenia may result from an interplay between genes involved in the development of the CNS and exposure to environmental factors capable of alternating gene expression (Basset et al., 2001). A series of correlations have been reported between early environmental risk factors for schizophrenia and structural deviations in the adult brain (reviewed by McGrath and Murray, 1995).

Some evidence points to an interaction between genetic risk for schizophrenia and hypoxic birth events upon structural abnormalities (Staal et al., 2000; Stefanis et al., 1999; McNeil et al., 2000).

Recent advances in molecular technologies have made mouse genetic models the first choice for most human genetic diseases; so it is with schizophrenia (reviewed by Chen et al., 2006). These models suggest that animals with mutations in the genes involved in brain development show abnormalities of cortical development that remain silent mostly until early adulthood, providing evidence in support of the abnormal neurodevelopment hypothesis (Chen et al., 2006).

“Static” neurodevelopmental models implicate events occurring around the time of birth, while “progressive” or “cumulative” neurodevelopmental models include insults that may occur until the final stages of brain development such as childhood maltreatment, head trauma, or adolescent cannabis use (King et al., 2010). Genetic and environmental risk factors may combine in additive or multiplicative ways to increase an individual’s risk (Malaspina et al., 1999). The neurodevelopmental model

suggests that different varieties of environmental insults can act early, during the most critical phase of the formation of the CNS, but also later in childhood or adolescence, in an individual genetically vulnerable (Murray et al., 2002) (FIGURE 1.2). The neurodevelopmental impairment resulting would determine the onset of schizophrenia later in life (Murray, 1994).

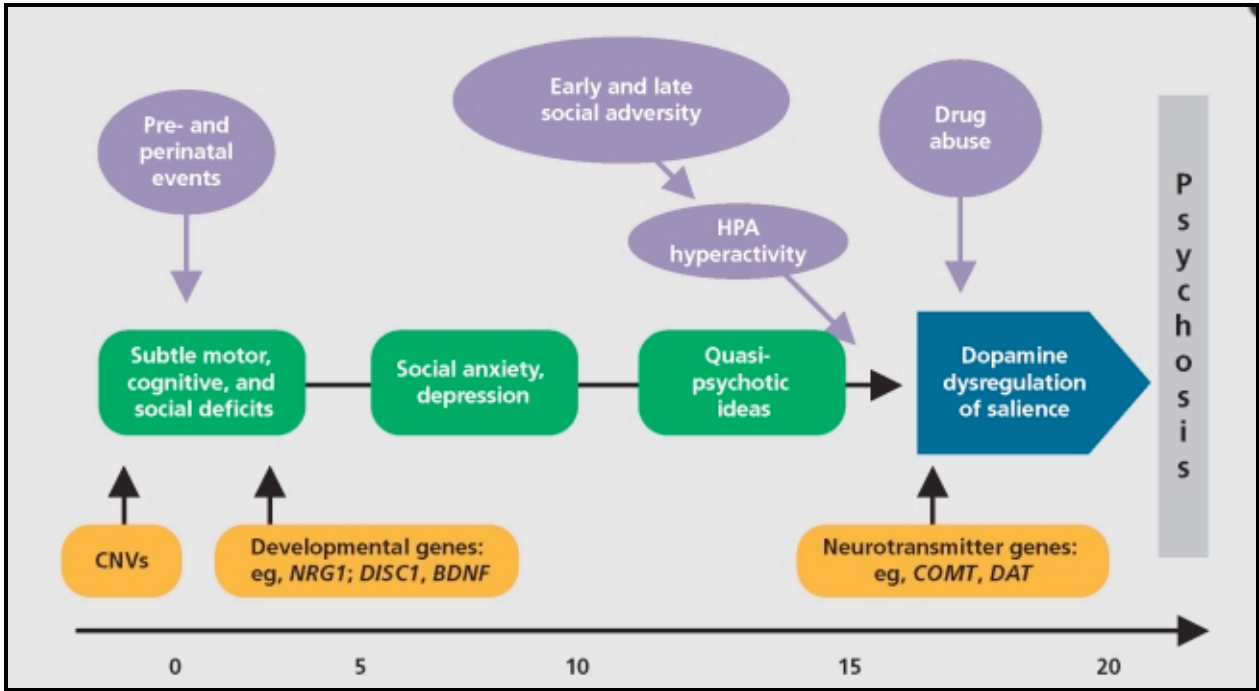


FIGURE 1.2 Developmental Cascade Towards Schizophrenia. Taken from Stilo and Murray, 2010.

1.5.3 Childhood Adversity and Social Disadvantage

Compatible with the Neurodevelopmental Theory of Schizophrenia, childhood represents a critical period of exposure to adversities that might increase the risk of developing psychosis later in life. Why and how childhood trauma leads to psychosis is still debated. One suggestion is that exposure to traumatic experiences early in life

might alter the function of the hypothalamus-pituitary-adrenal axis, impairing its healthy response to stress across the life span (Read et al., 2005).

A recent meta-analysis including 41 studies examined the association between childhood adversity and trauma (sexual abuse, physical abuse, emotional/psychological abuse, neglect, parental death, and bullying) and psychotic outcome (Varese et al., 2012). They found evidence for an interaction between childhood adversity and psychosis with an overall effect of OR 2.78 (95% CI = 2.34–3.31) (Varese et al., 2012) (FIGURE 1.3).

Source	Project	Sample Size	Age (y)	No. of Cases	No. of Controls	Cases Age	Controls Age
Case-control studies ^a							
Friedman and Harrison ¹¹ (United States)		35		20	15	30.8	31.9
Convoy et al ¹² (Czech Republic)		200		100	100	33.3(m); 43.4 (f)	36.4 (m); 38.1 (f)
Furukawa et al ¹³ (Japan)	GLADS	337		225	112		
Agid et al ¹⁴ (Israel)		152		76	76	42.5	41.7
Dell' Erba et al ¹⁵ (Italy)		114		54	60	32.7	33.4
Giblin et al ¹⁶ (UK)		32		14	18	77.7	73.4
Fennig et al ¹⁷ (Israel)		60		40	20	18.1	18.1
Morgan et al ¹⁸ (UK)	AESOP	781		390	391	30.5	37.3
Weber et al ¹⁹ (Germany)		63		42	31	32.6	40.3
Rubino et al ²⁰ (Italy)		484		174	310	43.1	37.4
Cohen et al ²¹ (United States)		302		198	113	61.5	63.0
Fisher et al ²² (UK)	AESOP	428		182	246	31	39
Husted et al ²³ (Canada)		147		79	68	51.8	49.6
Bartels-Velthuis et al ²⁴ (The Netherlands)		212		60	152		
Evans ²⁵ (UK)		60		29	31	27.7	23.7
Heins et al ²⁶ (The Netherlands)	GROUP	499		272	227	28.1	32.3
Varese et al ²⁷ (UK)		65		45	20	42.7	39.5
Daalman et al ²⁸ (personal communication)				100	124		
McCabe et al ²⁹ (Australia)	ASRB	675		408	267	40.7	39.27
Prospective cohort studies ^a							
Mäkiyö et al ³⁰ (Finland)	NFBC	11017					
Janssen et al ³¹ (The Netherlands)	NEMESIS	4045	41.4				
Spauwen et al ³² (Germany)	EDSP	2524	21.7				
De Loore et al ³³ (The Netherlands)	YHCSL	1129	15.1				
Schreier et al ³⁴ (UK)	ALSPAC	6437	12.9				
Arseneault et al ³⁵ (UK)	E-RISK	2127					
Cutajar et al ³⁶ (Australia)	VPCR	5436	33.7	2759	2677		
Wigman et al ³⁷ (The Netherlands)	TRAILS	2149	13.6	217	1834		
Cross-sectional studies ^a							
Murphy et al ³⁸ (United States)		391					
Ross and Joshi ³⁹ (United States)		502	45.2				
Whitfield et al ⁴⁰ (United States)	ACE	17337	57				
Kim and Kim ⁴¹ (Republic of Korea)		1672	15.7				
Shevlin et al ⁴² (United States)	NCS	5877	32.2				
Shevlin et al ⁴³ (United States)	NCS	5782					
Houston et al ⁴⁴ (United States)	NCS	5877	32.02				
Kelleher et al ⁴⁵ (Ireland)	Challenging Times	211		14	197		
Nishida et al ⁴⁶ (Japan)	ESPA	4894	13.3				
Shevlin et al ⁴⁷ (United States)	NCS-repl	2353	44.35				
Harley et al ⁴⁸ (Ireland)	Challenging Times	211					
Bebbington et al ⁴⁹ (UK)	APMS	7298					
Van Nierop et al ⁵⁰ (The Netherlands)	NEMESIS-II	6250		384	5866		

FIGURE 1.3 Studies Suggesting an Association Between Childhood Adversity and Psychosis.

Taken from Varese et al., 2012.

These findings combined with the literature on the impact of childhood adversity in the general population and other mental illnesses (Kessler et al., 2010) point out the importance of these disruptive experiences early in development on subsequent abnormal functioning in adult (Varese et al., 2012). Moreover, they also suggest that future studies should focus on differentiating adversity type, as well as consider the possible interaction between trauma and other risk factors (eg, cannabis, genetic risk), the developmental stage of exposure to trauma, and mechanisms linking adversity to specific positive and negative symptoms (Varese et al., 2012).

It has been consistently reported that an association between loss of a parent in childhood either by death or separation increases risk of psychosis in adulthood of about 3 fold (Agid et al., 1999; Morgan et al., 2007). Parental separation is often associated with a range of adverse early experiences, including family conflict, socioeconomic disadvantage, neglect and abuse (Rutter, 2006). Therefore, it is difficult to separate the impact of each of these social factors from their cumulative effect on the risk of psychosis (Rutter, 2006). Moreover individuals with established psychotic disorders often experience marked social disadvantage in adult life such as being unemployed, living alone, lack of a long-term relationships, with consequent social isolation and exclusion (Stilo and Murray, 2010). It is uncertain whether the association between social disadvantage and psychosis is a consequence of the developing disorder itself, or a contributory cause of the illness.

In this regards Stilo et al. (2012), using as a marker of social disadvantage in childhood long-term separation from, or death of, one or both parents, explored, in a sample of 278 first episode psychotic patients and 226 healthy controls, social disadvantage (1) in childhood, (2) at 5 years and 1 year prior to first presentation to

psychiatric services, (3) at first presentation with psychosis and (4) their association with psychosis while controlling for a wider range of potential confounders (Stilo et al. , 2012).

They found that exposure to social disadvantage prior to onset increases the risk of psychosis, both during childhood and adulthood. Consistent with published studies long-term, separation from and death of a parent before the age of 17 were both strongly associated with approximately 2- to 3-fold-increased odds of psychosis independent of a number of potential confounders. Secondly, social disadvantage in adult life (living alone, being single, and unemployment) was also strongly associated with psychosis. Third, their results show an association between early disadvantage and adult social disadvantage (OR 1.73, 95% CI 1.00–2.99, $P = .04$) explaining in part how adulthood social disadvantage in first-episode psychosis patients could be a possible a consequence of childhood disadvantage. Finally when they looked at the cumulative impact of social disadvantage, they found that the odds of being a case increased in line with increasing number of indicators of disadvantage present.

Furthermore, social disadvantage was not simply a consequence of the patients having a long history of untreated psychosis, suggesting that disadvantage is already well established before onset of first symptoms (Stilo et al., 2012).

1.5.4 Drug Use

Certain drugs can contribute to the onset of schizophrenia-like psychosis. As shown previously, the major pharmacological theories of schizophrenia have their origins in the effects of drugs of abuse; in chronological order, the effects of LSD initiated the serotonergic model; amphetamines the dopamine hypothesis, PCP, and ketamine the

glutamatergic model (PARAGRAPH 1.5.1). Most recently, the effects of cannabis have provoked interest in the role of endocannabinoids (Paparelli et al., 2011).

1.5.4a Cannabis and Psychosis

Cannabis, the most widely used recreational drug, has also been consistently associated with psychosis in both experimental and epidemiological studies (Paparelli et al., 2011). Cannabis has been linked to the development of psychotic symptoms for a very long time. The world's oldest pharmacopeia, the Pen-Ts'ao Chin, traditionally attributed to the Chinese Emperor Shen Nung (2737 a.c.), suggests cannabis use as a remedy for rheumatic pain, intestinal constipation, disorders of the female reproductive system, and malaria, but adds that "if taken in excess will produce visions of devils ... over a long term, it makes one communicate with spirits and lightens one's body..." (Zuardi, 2006). In the mid-nineteenth century, the French psychiatrist Moreau de Tour noted the phenomenological similarities between drug-induced states and psychosis. Indeed, he proposed the use of hashish to study madness a full century before the LSD model of psychosis (Beringer, 1927; Hofmann, 1980).

The first convincing evidence that cannabis might be a risk factor for psychosis came from the Swedish Conscripts Study in which 45,570 inductees into the military were followed-up for 15 years. This study reported a risk for schizophrenia 2.4 times higher among those who had used cannabis by 18 years than among non-users. Moreover, there was a dose-response relationship in that risk for schizophrenia rose to 6.0 times in heavy cannabis smokers (use of cannabis more than 50 times at initial interview; Andreasson et al., 1987). After 15 years of silence, other epidemiological studies on cannabis use and schizophrenia started to be published (Arseneault et al.,

2002; Van Os et al., 2002; Zammit et al., 2002; Fergusson et al., 2003). Today consistent evidence supports an association between heavy cannabis use and the risk of psychotic symptoms and illness (Green et al., 2005; Henquet et al., 2005; Fergusson et al., 2006; Barnett et al., 2007; Di Forti et al., 2007b; Moore et al., 2007; Mc Grath et al., 2010) even if the strength and nature of that association is still an object of discussion (D’Souza et al., 2004) (**FIGURE 1.4**).

Study	N	Design	Prevalence/use of cannabis	Outcome	Adjusted risk
Andreasson et al. (1987)	45,570	Longitudinal conscript cohort (Sweden)	>50 times	Hospitalization	2.3
Arseneault et al. (2002)	759	Prospective birth cohort (Dunedin)	Lifetime	Any schizophreniform Disorders	3.1
Van Os et al. (2002)	4095	Longitudinal population-based (Netherlands)	Lifetime	Any psychotic symptom	2.8
Fergusson et al. (2003)	1011	Birth cohort (Christchurch)	DSM-IV dependence	Any psychotic symptom	1.8
Zammit et al. (2002)	50,087	Longitudinal conscript cohort (Sweden)	>50 times	Hospitalization	3.1
Henquet et al. (2005)	3467	Prospective cohort (Germany)	Lifetime	Any psychotic symptom	1.7
Di Forti et al. (2009)	280	Case-control (UK)	Lifetime	First-episode psychosis	6.8 (skunk users)
Mc Grath et al. (2010)	3801	Prospective cohort (Australia)	≥6 years duration	Non-affective psychosis	2.1

FIGURE 1.4 Studies Suggesting an Association Between Cannabis Use and Psychosis. Taken from Paparelli et al., 2011.

Recent meta-analyses reported an increase in risk between 1.4 and 1.9 times in people using cannabis that might account for 8–14% of cases of schizophrenia in different countries (Moore et al., 2007). Furthermore, Di Forti et al. (2009) showed a positive association between the potency and frequency of cannabis use and the risk of psychotic illness (Di Forti et al., 2009).

1.5.5 The Contribution of Genes to Schizophrenia

1.5.5a Family Studies

A family history of psychotic disorders in first-degree relatives is the strongest and most replicated risk factor for schizophrenia and for psychosis in general (Gottesman, 1991).

Back in 1916 Ernst Rüdin, who later became involved with Nazi eugenics, conducted the first systematic family study of what we call now schizophrenia (Rüdin, 1916).

He found that the rate of illness was higher in the biological relatives of his cases and the mode of transmission didn't follow simple Mendelian patterns. He then postulated a possible two-gene recessive model (Rüdin, 1916).

Since then more sophisticated studies have been conducted in order to examine the morbid risk of schizophrenia within the same family. Those studies show a higher morbid risk of psychosis in the relatives of patients than the relatives of general population controls (Gottesman, 1991). In addition the risk increases with the degree of biological relatedness and if more than one relative was affected (Gottesman, 1991) (**FIGURE 1.5**).

Kendler et al. (1993) carried out a large family study on three proband groups, schizophrenic patients, affective psychosis patients and matched randomized healthy controls, revealing a risk of schizophrenia among first-degree relatives of 6.5% and among siblings about 9.2% (Kendler et al., 1993).

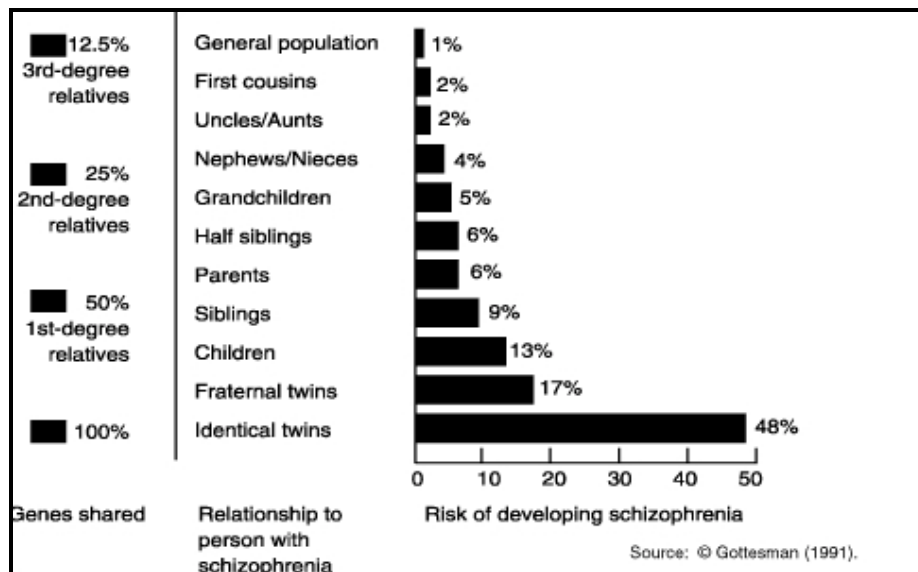


FIGURE 1.5 Risk of Developing Schizophrenia. Taken from Gottesman 1991.

1.5.5b Twin Studies

Heritability estimates are derived mostly from twin studies (Gottesman, 1991). The first twin study dates from 1928 and was carried out by Luxenberger (Luxenberger, 1928). He found a high concordance in monozygotic twins (MZ) and none in dizygotic twins (DZ) (Luxenberger, 1928).

Subsequent studies reporting probandwise rates for schizophrenia, confirmed that concordance among MZ was higher than DZ pairs (Kallmann, 1946; Slater, 1953; Kendler, 1983; McGuffin et al., 1984; Onstad et al., 1991; Cardno and Gottesman, 2000). In this regard, the concordance rates between MZ twins (almost 50%) provides strong evidence for the involvement of genes but also raises the question of what other factors may be involved in the aetiology of schizophrenia (Cardno et al., 1999). A large twin studies carried out at the Maudsley Hospital to estimate the variance for genetic and environmental contribution to liability to schizophrenia, demonstrated

that 83% of the variance in the liability to schizophrenia was due to genetic effects (Cannon et al., 1998; Cardno et al., 1999).

In other studies, the heritability for schizophrenia has been variously calculated as between 66% and 85% (Cardno, et al. 1999). Its mode of transmission is still unclear but most likely to be polygenic and non-Mendelian (Owen and Cardno, 1999).

1.5.5c Adoption Studies

In order to further examine the contribution of genes and environment in the transmission of schizophrenia, Kety et al. (1976) carried out a study among biological and adoptive children (Kety et al., 1976). Almost 20% of adoptees with a biological parent with schizophrenia developed a schizophrenia spectrum illness themselves, compared to 5.8% of adoptees with healthy biological fathers (Kety et al., 1976). In 1981 Kendler et al. confirmed this finding using operational criteria, providing a strong evidence for a genetic component to schizophrenia (Kendler et al., 1981). Thus, shared genes rather than shared environments seem to explain the increased risk in biological relatives of schizophrenia probands (Owen et al., 2004).

1.5.5d Molecular Genetic Studies

Hypothesis-driven candidate gene studies have been a key approach to the genetics of schizophrenia; since 1965 more than 1700 studies, 150 published each year, and 1008 different genes have been tested so far for association (Allen et al., 2008; Collins et al., 2011).

Those studies target genes selected on the basis of plausible pathophysiology (i.e. schizophrenia as a synaptic or a neurodevelopmental disorder) or suggested by a linkage study (Cichon et al., 2009). In the last decade, they have contributed to a list of putative susceptibility genes for psychotic disorders (**TABLE 1.1**).

RANKING BASED ON HUGENET INTERIM GUIDELINES FOR THE ASSESSMENT OF GENETIC ASSOCIATION STUDIES							
#	Gene	Polymorphism	Ethnicity	N minor (Grade)	I ² (Grade)	Bias Reason (Grade)	Overall Grade
1	PRSS16	rs8932590	All	13934 (A)	n.a. (A)	(A)	A
2	PGBD1	rs13211507	All	5075 (A)	0 (A)	(A)	A
3	NRGN	rs12807809	All	12820 (A)	0 (A)	(A)	A
4	NOTCH4	rs3131296	All	7829 (A)	19 (A)	(A)	A
5	HIST1H2BJ	rs8913860	All	10335 (A)	0 (A)	Low OR (C)	C
6	PDE4B	rs910894	All	2393 (A)	2 (A)	(A)	A
7	TCF4	rs9960767	All	4143 (A)	20 (A)	(A)	A
8	GWA_16p13.12	rs7192086	All	10078 (A)	16 (A)	Low OR (C)	C
9	ZNF804A	rs1344706	Caucasian	13839 (A)	55 (C)	Low OR (C)	C
10	DRD4	rs4646984	All	1513 (A)	0 (A)	(A)	A
11	DRD2	rs6277	Caucasian	6721 (A)	79 (C)	Regr (C)	C
12	DAOA	rs778293	Asian	3809 (A)	18 (A)	(A)	A
13	AHI1	rs2084430	All	15114 (A)	0 (A)	Low OR (C)	C
14	GWA_11p14.1	rs1602565	Caucasian	3973 (A)	46 (B)	(A)	B
15	TPH1	rs1800532	All	5411 (A)	20 (A)	(A)	A
16	HTB2A	rs6311	Caucasian	4865 (A)	22 (A)	(A)	A
17	RPP21	rs3130375	All	1392 (A)	63 (C)	(A)	C
18	CCKAR	rs1800857	Caucasian	704 (B)	0 (A)	Regr (C)	C
19	GABRB2	rs1816072	Caucasian	1621 (A)	0 (A)	Regr (C)	C
20	DTNBP1	rs3213207	Caucasian	4086 (A)	0 (A)	Low OR (C)	C
21	C6orf217	rs1475069	All	10686 (A)	19 (A)	Low OR (C)	C
22	RELN	rs7341475	Caucasian	4149 (A)	0 (A)	Low OR (C)	C
23	MDGA1	rs11759115	All	1347 (A)	15 (A)	(A)	A
24	CMYA5	rs10043986	All	4300 (A)	3 (A)	Low OR (C)	C
25	DISC1	rs3737597	Caucasian	102 (B)	0 (A)	(A)	B
26	NRG1	rs10503929	All	2854 (A)	0 (A)	Low OR (C)	C
27	MTHFR	rs1801133	All	6832 (A)	59 (C)	Low OR (C)	C
28	AKT1	rs3803300	Caucasian	574 (B)	18 (A)	(A)	B
29	RGS4	rs2661319	All	17088 (A)	17 (A)	Low OR (C)	C
30	PPP3CC	rs10108011	All	6263 (A)	0 (A)	Low OR (C)	C

TABLE 1.1 Top 30 Genetic Loci With at Least One Nominally Significant Meta-Analysis Result. Adapted from SZGene, last update 2011.

The most well known genetic variants associated with schizophrenia have included genes such as NRG1 (Neuregulin 1), DTNBP1 (Dystrobrevin-Binding Protein 1) and DISC1 (Disrupted in Schizophrenia 1) involved in the regulation of neurodevelopmental processes, or genes such as COMT (Catecol-O-Metil-Trasferasi), DAT1 (Dopamine Transporter), BDNF (Brain-Derived Neurotrophic Factor) and others involved in the regulation of the dopamine system (Harrison and Owen, 2003).

So far only a few studies have shown similar results between each other and according to a meta-analysis done by Allen et al. (2008), four genes, namely Dopamine Receptor D1 (DRD1), DTNBP1, Methylenetetrahydrofolate Reductase (MTHFR) and Tryptophan Hydroxylase (TPH) seemed to be the best candidate genes in increasing the risk for schizophrenia (Allen et al., 2008).

1.5.5e Genome-wide Association Studies

Up to 5 years ago genetic studies could only investigate an extremely small proportion of the genome, but now genotyping and cost improvements permit us to conduct studies as Genome-wide association studies (GWAS), equipped to assess a million genetic variants in huge sample (Stone et al., The International Schizophrenia Consortium, 2008). Since 2008 more than ten GWAS for schizophrenia have been published (TABLE 1.2).

GWAS (GENOME-WIDE ASSOCIATION STUDIES)							
Study	Design	Type	Population	Source	Platform	# of SNPs	Genotype Data Publicly Available
CAUCASIAN							
Athanasou, 2010	CC	GWAS	Norway (TOP), Europe (SGENE-plus)	CL	Affymetrix 6.0	572,888	No
Djurovic, 2010	CC	GWAS	Norway	CL	Affymetrix 6.0	618,860	No
ISC, 2009	CC	GWAS, and meta-analysis (ISC, MGS, SGENE)	Europe (ISC)	CL	Affymetrix 500K, 5.0, 6.0	739,995	No
Jia, 2010		GWAS re-analysis (pathways)	USA (GAIN)	CL	see primary study	see primary study	Yes
Kirov, 2008	FBAT	GWAS	Bulgaria	CL	Illumina HumanHap (550K)	433680	No
Lencz, 2007	CC	GWAS	USA	CL	Affymetrix (500K)	439511	No
Mah, 2006	CC	GWAS	USA	CL	Selected cSNPs	25494	No
Need, 2009	CC	GWAS (multi-stage)	Mixed	CL	Illumina HumanHap (300,550K)	312,565	No
Shifman, 2008	CC	GWAS (multi-stage)	Israel	CL	Affymetrix (500K)	510552	No
Stefansson, 2009	CC	GWAS, and meta-analysis (ISC, MGS, SGENE)	Europe (SGENE-plus)	CL	Illumina HumanHap (300,550K)	314,868	No
ASIAN							
Ikeda, 2010	CC	GWAS	Japan	CL	Affymetrix 5.0	297,645	No
OTHER/MIXED							
O'Donovan, 2008	CC	GWAS (multi-stage)	Mixed	CL	Affymetrix (500K)	362,532	No
Shi, 2009	CC	GWAS, and meta-analysis (ISC, MGS, SGENE)	USA (MGS)	CL,PO	Affymetrix 6.0	696,788-843,798	Yes
Sullivan, 2008	CC	GWAS	USA (CATIE)	CL	Affymetrix (500K) & Perlegen (customized)	492900	Yes

TABLE 1.2 Genome-Wide Association Studies. Adapted from SZGene, last update 2010.

Unfortunately, reports suggesting particular candidate genes as risk factors of schizophrenia have not been consistently replicated or confirmed by GWAS. Collins et al. (2011) compared the top 5% genes listed in SzGene (Allen et al. 2008) with the largest schizophrenia GWAS published to date (Stone et al., The International Schizophrenia Consortium, 2008; Purcell et al. 2009) looking for an overlap between

the two types of studies. They found no evidence for over-representation between the best findings from GWAS and the results driven from the “first generation” candidate genes studies (Collins et al., 2011). They also demonstrated that poor choices of candidate genes or inadequate assessment (i.e. small samples or incomplete coverage of common genetic variation) could possibly play a role for inconsistent or confusing results (Collins et al., 2011).

Nevertheless the large GWAS collaborations have demonstrated that the strength of the association between individual genes and psychiatric disorders is weak and often non-specific.

More recently, in a joint analysis with a bipolar disorder sample, a GWAS identified 8 new schizophrenia loci (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) (FIGURE 1.6).

SNP	Chr.	Mb	Alleles	Frequency	P (GC-adjusted P)	OR (95% CI)	Consistency of direction	Gene	Distance (kb)
rs1625579	1p21.3 ^a	98.3	TG	0.80	5.72×10^{-7} (5.52×10^{-6}) 2.65×10^{-8} (n.a.) 1.59×10^{-11} (6.87×10^{-10})	1.14 (1.08–1.19) 1.11 (1.07–1.16) 1.12 (1.09–1.16)	++++++	MIR137	Intragenic
rs17662626	2q32.3 ^a	193.7	AG	0.91	3.09×10^{-5} (2.60×10^{-5}) 1.70×10^{-3} (n.a.) 4.65×10^{-8} (1.25×10^{-6})	1.22 (1.13–1.30) 1.16 (1.06–1.27) 1.20 (1.13–1.26)	+ - + + +	PCGEM1	343
rs2021722	6p21.3-p22.1	30.3	CT	0.78	4.30×10^{-11} (2.76×10^{-9}) 1.55×10^{-3} (n.a.) 2.18×10^{-12} (2.88×10^{-10})	1.18 (1.13–1.23) 1.10 (1.03–1.17) 1.15 (1.11–1.19)	+ + - + +	TRIM26	Intragenic
rs10503253	8p23.2 ^a	4.2	AC	0.19	3.84×10^{-7} (4.71×10^{-6}) 7.60×10^{-3} (n.a.) 4.14×10^{-8} (8.98×10^{-7})	1.14 (1.09–1.19) 1.08 (1.01–1.14) 1.11 (1.07–1.15)	+ + + + +	CSMD1	Intragenic
rs7004633	8q21.3 ^a	89.8	GA	0.18	1.45×10^{-8} (3.22×10^{-7}) 0.011 (n.a.) 2.75×10^{-8} (7.03×10^{-7})	1.16 (1.11–1.21) 1.05 (1.01–1.10) 1.10 (1.07–1.14)	++ + + + +	MMP16	421
rs7914558	10q24.32 ^a	104.8	GA	0.59	1.58×10^{-7} (2.27×10^{-6}) 1.07×10^{-3} (n.a.) 1.82×10^{-9} (3.11×10^{-8})	1.11 (1.07–1.15) 1.08 (1.03–1.13) 1.10 (1.07–1.13)	+ + + + +	CNNM2	Intragenic
rs11191580	10q24.33 ^a	104.9	TC	0.91	2.23×10^{-8} (4.58×10^{-7}) 5.09×10^{-3} (n.a.) 1.11×10^{-8} (3.72×10^{-7})	1.22 (1.15–1.29) 1.09 (1.02–1.16) 1.15 (1.10–1.20)	++++ + + +	NTSC2	Intragenic
rs548181	11q24.2	125.0	GA	0.88	2.91×10^{-8} (5.69×10^{-7}) 0.068 (n.a.) 8.87×10^{-7} (1.74×10^{-5})	1.20 (1.13–1.26) 1.04 (0.98–1.11) 1.11 (1.07–1.16)	++++ + +	STT3A	1
rs12966547	18q21.2	50.9	GA	0.58	1.00×10^{-6} (1.03×10^{-5}) 2.29×10^{-3} (n.a.) 2.60×10^{-10} (5.99×10^{-9})	1.10 (1.06–1.14) 1.08 (1.04–1.12) 1.09 (1.06–1.12)	+ + + + + + +	CCDC68	126
rs17512836	18q21.2	51.3	CT	0.02	2.35×10^{-8} (4.78×10^{-7}) 0.085 (n.a.) 1.05×10^{-6} (2.86×10^{-5})	1.40 (1.28–1.52) 1.08 (0.96–1.20) 1.23 (1.14–1.31)	- + + + + + +	TCF4	Intragenic

FIGURE 1.6 Top Genome-Wide Association Results for Schizophrenia. Taken from The Schizophrenia Psychiatric GWAS Consortium, 2011

The strongest new finding was with rs1625579 within an intron of a putative primary transcript for MIR137 (microRNA 137). Four other schizophrenia loci (TCF4, CACNA1C, CSMD1 and C10orf26) contained predicted targets of MIR137, suggesting MIR137-mediated dysregulation as a previously unknown etiologic mechanism in schizophrenia (GWAS, 2011).

Moreover these results, together with previously published data, suggest that the genetic susceptibility to bipolar disorder and schizophrenia partially overlaps and is likely to be mediated by several common genetic variants of small effect rather than by a single rare gene mutation (Sullivan et al., 2008; Kirov et al., 2009).

1.5.5f Rare Structural Variants

Another approach to studying the genetic contribution to psychosis is to examine rare structural variants. Recent findings have established that both rare mutations of large effect and common variants of modest effect contribute to genetic risk for schizophrenia (Sebat et al., 2009) (FIGURE 1.7).

Locus ^b	Gene(s) ^{c,d}	Copy number change	Frequency in SCZ (%)	OR	Reference	Other Associated Disorders ^d
Replicated significant associations from case-control studies ^a						
1q21.1	~10 genes	Deletion	0.23-0.32	6.6-14.8	[12,13]	DD, CM [60,70]
15q13.3	~10 genes	Deletion	0.17-0.3	11.5-17.9	[12,13]	GE [71,72], MR [73]
16p11.2	>25 genes	Duplication	0.3	8.3-25.4	[11,53]	ASD, BD, MD, P-NOS [53,62]
22q11.2	>25 genes	Deletion	0.5-2.0	30	[12,13,37]	VCFS [42], Anxiety, Depression, ADHD, OCD [68]
Significant association reported in a single cohort						
2p16.3	NRXN1	Deletion	0.47	9.0	[14,55]	ASD [65,66,109]
15q11.2	~10 genes	Deletion	0.55	2.73	[13]	ASD, DD [110]
17p12	~10 genes	Deletion	0.13	7.8	[15]	HNPP [111]
16p13.1	~14 genes	Duplication	0.30	3.3	[54]	DD [64,112]
1q42.2	DISC1	Balanced translocation	NA	NA	[38]	BD, MDD [41]
Non-significant associations reported in individual studies						
1p31.3	PDE4B	Balanced translocation	NA	NA	[39]	
14q13.1	NPAS3	Balanced translocation	NA	NA	[40]	
7q35	CNTNAP2	Deletion	NA	NA	[56]	ADHD [113], ASD [114-116]
15p13.1	APBA2	Duplication	NA	NA	[55]	

FIGURE 1.7 Genomic Regions Implicated by Rare Structural Variants in Schizophrenia. Taken from Sebat et al., 2009.

Walsh et al. (2008) found that novel deletions and duplications of genes were present in 5% of controls compared with 15% of schizophrenic patients and 25% of subjects with early-onset schizophrenia (Walsh et al., 2008). Moreover subjects with schizophrenia were 1.15 times more likely to have a higher frequency of copy number variants (CNVs) than controls (Stone et al., The International Schizophrenia Consortium, 2008).

The best-supported loci are deletions at 1q21, 2p53, 3q29, 15p11.2, 15q11.3, 17q12, 22q11.2 and Neurexin 1 (NRXN1), and duplications at 7q36.3, 25q11–13, 16p11.2 and 16p13.1 (Rapoport et al., 2012). The majority of genes identified are important for brain development, including synaptic long-term transmission, NRG signaling, axonal guidance, and integrin signalling (Fatemi et al., 2009).

Mutations at 16p11.2 confer high risk for schizophrenia and for other neuropsychiatric disorders, and at the same time, duplication of this region, confers substantial risk to the individuals who carry it (McCarthy et al, 2009). In addition, 16p11.2 microdeletion both predisposes to neuropsychiatric phenotypes as a single event and exacerbates neurodevelopmental phenotypes in association with other large deletions or duplications (Girirajan et al., 2010). Therefore a ‘two-hit’ model has been proposed, where the ‘second hit’ could be another CNV, a disruptive single base pair mutation, or an environmental event influencing the phenotype (Girirajan et al., 2010).

In conclusion, although GWAS have identified a substantial number of polymorphisms associated with schizophrenia, these findings are characterized by small effect sizes (Pidsley and Mill, 2011). It is unlikely that common genetic variants cumulatively account for all the population variance in risk for psychosis

(O'Donovan et al., 2009). CNVs have also been implicated, but these de novo events are extremely rare and only found in a small number of patients (Merikangas et al., 2009). Thus, the genetics of psychosis seems to comprise the cumulative effects of a large number of genes of small effects in the majority of cases plus the effects of CNVs in minority of cases. Moreover, it is likely that the effect of genes on the disease causal path might partly depend on environmental exposures.

1.6 Summary

Characterized by hallucinations, delusions and thought disorder, schizophrenia is the most severe of the functional psychotic disorders. In recent years much evidence has suggested that multiple factors seem to be involved in the development of the illness: liability to psychosis in general, and to schizophrenia in particular, involves genetic predisposition which is then compounded by exposure to a range of environmental factors including neonatal hypoxia, urbanicity, migration, and certain types of social adversity (Stilo and Murray, 2011).

Genetic and environmental factors and their interaction seem to be one of the most likely explanation of the origins of psychosis.

CHAPTER 2

Early Environmental Hazards

2.1 Prenatal Risk Factors for Schizophrenia

Over the past three decades much research effort has focused on the identification of prenatal risk factors for schizophrenia. These factors include winter birth, parental age, prenatal infection, and prenatal maternal stress (reviewed by King et al., 2010) (FIGURE 2.1).

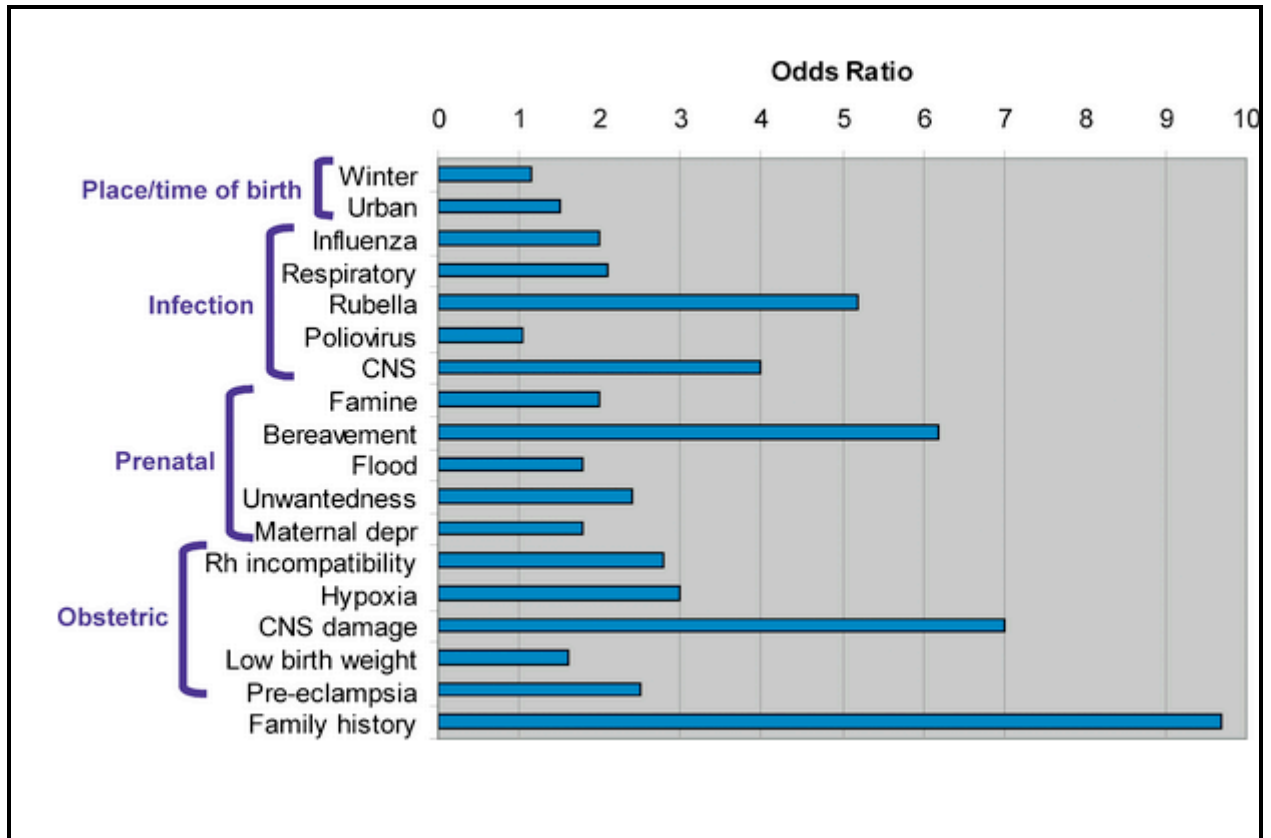


FIGURE 2.1 Pre- and Peri-natal Factors for Schizophrenia. Taken from Sullivan, 2005.

2.1.1 Time and Place of Birth

2.1.1a Season of Birth

Reported for the first time in 1929, winter birth is one of the most firmly established environmental risk factors for schizophrenia (Tramer, 1929). Compared to the general population, schizophrenic patients show an excess of birth in winter and early spring of 5%- 8% (Torrey et al., 1997). This has been observed more consistently in the northern hemisphere (Hare, 1975; Kendell and Adams, 1991; Mortensen et al., 1999) possibly because of little variation in seasonal temperatures found in equatorial regions and because relatively few studies have been carried out in the southern hemisphere (King et al., 2010). There could be differential fertility and seasonal patterns of procreation in the parents of individuals with schizophrenia (Hare, 1976; Suvisaari et al., 2001). Seasonally fluctuating factors have also been suggested including nutrition, hormones, maternal exposure to viral infections (Torrey et al., 1997), and certain meteorological factors (e.g., sunlight exposure and vitamin D, temperature, or severe weather) (Susser et al., 1996; Cannon et al., 2003; reviewed by Tochigi et al., 2004).

2.1.1b Urbanicity

It has been known for many years that living in urban areas increases the risk of psychosis at least 2 fold (Faris and Dunham, 1939; Hare, 1956; Lewis et al., 1992; Marcelis et al., 1998). This has been interpreted in terms of socio -economic and behavioural factors such as stress, anonymous lifestyle (Hare, 1955; Ventura et al., 1989; Malla et al., 1990; Castle et al., 1993; Thornicroft et al., 1993) or due to the greater exposure to chemical factors and infections (Brown et al., 2000; Torrey et al., 2001). Lewis et al. in 1992 and then Pedersen in 2001 showed that larger the town

and longer the individual has lived in the city, the greater the risk (Lewis et al., 1992; Pedersen and Mortensen, 2001). Some have pointed out the possible role played by “neighborhood social capital”, understood as a set of characteristics of the social structure in which the subject lives (McKenzie et al., 2002). McKenzie et al. (2002) claimed that the absence or lack of proper “neighborhood social capital” can participate significantly in the development of psychosis (McKenzie et al., 2002). In 2006, the Aetiology and Ethnicity of Schizophrenia and Other Psychoses (AESOP) study demonstrated that even within one city there were wide variation in rates and the highest rates were found in the area with least social cohesion (Fearon et al., 2006; Kirkbride et al., 2006).

2.1.1c Immigration

Ethnic factors are responsible for discrepancies in incidence inside the same country; some immigrant populations, such as African-Caribbeans in the UK (Harrison et al., 1997) and the Surinamese in the Netherlands (Selten et al., 1997) show higher incidence rates of schizophrenia, with a meta-analysis showing a mean weighted relative risk among migrants (first and second generation) of 2.7 (95% CI 2.3-3.2) (Cantor-Graae and Selten, 2005).

This is true not only in immigrants, but also in the children of immigrants born in these countries (Boydell et al., 2003). Selective migration, obstetric complications, neurological diseases, previous infections, and substance abuse have been hypothesized to be the cause (Boydell et al., 2003). Other studies have also pointed out that the immigration is a possible risk factor for being more often on the margins of social life and more easily exposed to stressful situations and also the subject of

racial discrimination (Hutchinson et al., 1999). This, in susceptible individuals, could result in a greater chance of developing paranoid attitudes (van Os et al., 2002).

2.1.2 Parental Age at Birth

Advanced parental age has been cited as a risk factor for schizophrenia among offspring for more than half a century (Barry, 1945; Johanson, 1958; El-Saadi et al., 2004) (FIGURE 2.2).

Parental age group	Non-F2 cohort		F2 cases		Unadjusted OR (95% CI)		p-value	
	PA	MA	PA	MA	PA	MA	PA	MA
≤25 ^a	1140	2196	19	36	1	1	–	–
25–30	1860	1718	52	56	1.67 (0.98–2.85)	1.98 (1.30–3.03)	0.056	0.001
30–35	1217	954	34	28	1.67 (0.95–2.95)	1.79 (1.08–2.95)	0.074	0.022
35–40	584	476	18	17	1.84 (0.96–3.55)	2.17 (1.21–3.91)	0.064	0.009
40–45	251	157	8	6	1.91 (0.82–4.41)	2.33 (0.96–5.61)	0.130	0.059
45–50	102	19	3	2	1.76 (0.51–6.06)	6.42 (1.44–28.59)	0.360	0.014
>50	24	5	2	1	5.00 (1.10–22.68)	12.20 (1.38–107.08)	0.037	0.024

Abbreviations: CI, confidence interval; OR, odds ratio; PA: paternal age; MA: maternal age.
^a Reference group.

FIGURE 2.2 Odds Ratios for Psychotic Disorders for Parental Age. Taken from Lopez-Castroman et al., 2010.

2.1.2a Paternal Age

Back in 1958 Johanson reported an association between paternal age and schizophrenia (Johanson, 1958). Since then other studies have replicated the same finding (Hare and Moran, 1979; Raschka, 1998; Malaspina et al, 2001; Brown et al, 2002; Dalman and Allebeck, 2002; Zammit et al., 2003). Children of fathers over 45 years of age have a doubled relative risk (RR=2.02; 95% CI, 1.17-3.51) to develop schizophrenia than children of younger fathers (Malaspina, et al. 2001; Sipos and Rasmussen, 2004).

Advanced paternal age has been associated with major congenital malformation syndromes and isolated birth defects known to be caused by autosomal dominant

mutations (Brown et al., 2002). It is still controversial what causes such association. One possibility proposed by Penrose was the “copy error” hypothesis as a mechanism underlying these congenital anomalies (Penrose, 1955). After puberty, spermatocytes divide every 16 days; de novo genetic mutations resulting from replication errors and defective DNA repair mechanisms are believed to propagate in successive clones of spermatocytes; as a result de-novo mutations increasing the risk of schizophrenia are more likely to occur (Malaspina, 2001; Byrne et al., 2003) in aging sperm. However, in paper by Petersen et al. (2011) showed that the association between paternal age and risk of schizophrenia was accounted for by the father’s greater age when his first child was born, and not by the father’s age when later children were conceived; such results do not support the de-novo mutation hypothesis but raise the possibility of fathers marrying late because of some personality characteristic (Petersen et al., 2011).

More recent Miller et al. (2011) found a significant increase in risk of schizophrenia in the offspring of fathers older than 30 years of age, but they also found a significant increase in risk of schizophrenia in the offspring of younger fathers (<25 years of age) (Miller et al., 2011).

2.1.2b Maternal Age

The literature on perinatal complications supports an independent role for maternal age in increasing risk of psychotic disorders (McNeil, 1995; Cantor-Graae et al., 1997; Preti et al., 2000). Recently Lopez- Castroman et al. (2010) found a positive linear increase in risk for psychotic disorders both with maternal and paternal age, but the correlation was only significant for increasing maternal age (Lopez-Castroman et

al., 2010). Late maternal age (>34 years) tends to increase the risk of schizophrenia due to higher maternal morbidity for obstetric complications (Ekeus et al., 2006). Advanced maternal age is a risk factor for antepartum haemorrhage, abnormalities of placentation, and certain types of chromosomal anomalies (Turnbull and Chamberlain, 1989). Obstetric complications are also common in younger mothers (<20 years) (Cantor- Graae et al., 1997); a younger age confers an increased risk of adverse pregnancy outcomes such as delivering low-birth weight, premature, and small-for-gestational-age infants (Fraser et al., 1995; Ip et al., 2010). Moreover the risk of anaemia during pregnancy increases significantly with lower maternal age (Jolly et al., 2000; Menacker et al., 2004; Conde-Agudelo et al., 2005; de Vienne et al., 2009). In 2004 El-Saadi et al. used data from three sources examining psychosis: a population-based cohort study (Denmark), and two case-control studies (Sweden and Australia) (El-Saadi et al., 2004). Looking at the association between maternal age and risk of psychosis, they found that when adjusted for paternal age, the Danish study (cohort study) showed that the offspring of younger mothers had a small but significant increased risk of psychosis (RR = 1.12) (El-Saadi et al., 2004). The two case-control studies both found the highest Odds Ratio for psychosis in younger mothers; however this was not statistically significant (El-Saadi et al., 2004). The offspring of younger mothers are at increased risk of developing psychosis possibly because such women are more likely to suffer obstetric complications, well known to be involved in increasing the risk of schizophrenia (Cannon et al., 2002). The literature on OCs suggests an independent role for maternal age in increasing the risk of psychotic disorders (Preti et al., 2000), and early-onset schizophrenia (Byrne et al., 2007).

2.1.3 Maternal Infection

Epidemiological studies linked influenza epidemics to an increase in population levels of schizophrenia (Mednick, 1988) mainly if occurring during the 2nd trimester of pregnancy, particularly the 5th or 6th month (McGrath et al., 1994; Wright et al., 1999). Late in 2004 Brown et al. found that the risk for schizophrenia increased 7 fold if the infection occurred in the 1st trimester and prenatal exposure to influenza accounted for 14% of all schizophrenia cases (Brown et al, 2004). Prenatal infections that have been repeatedly associated with schizophrenia include toxoplasmosis (2.6-fold increase), influenza (3-fold increase), or genital or reproductive infection (5-fold increase) (Brown and Derkits, 2010). Proposed mechanisms include the direct effects of the pathogen on the fetal brain, maternal immune reaction, fever, stress, and use of analgesics and anti-inflammatory drugs (Boksa, 2008).

2.1.4 Other Risk Factors

2.1.4a Maternal Nutrition

Both under eating and overeating during pregnancy may increase schizophrenia risk in the offspring (King et al., 2010). Epidemiological studies show a twofold increase in risk for schizophrenia among individuals who were exposed to famine in their first trimester of gestation (Susser and Lin, 1992; Susser and Lin, 1994). It has been suggested that nutritional insufficiencies (e.g., folic acid, essential fatty acids, iron, vitamin A) increase the risk of spontaneous genetic mutations and/or disrupt proper neurodevelopment, ultimately resulting in schizophrenia (King et al., 2010).

Additionally, high maternal pre and early pregnancy body mass index (BMI) is associated with a 2.8-fold increase in risk for schizophrenia in offspring (Schaefer et al., 2000). This it may be explained by metabolic problems (e.g., diabetes), dietary

restrictions, or poor maternal care, all of which may affect neurodevelopment and/or increase the risk of obstetric complications (King et al., 2010).

2.1.4b Maternal Stress

Stressful events occurring during pregnancy are claimed to result in increased risk for schizophrenia for the offspring later in adulthood (King et al., 2010). The stressors studied to date have ranged from military invasion (Van Os and Selten, 1998) to tornados (Kinney et al., 1999) to personal loss (Huttenen and Niskanen, 1978), unwanted pregnancy (Myhrman et al., 1996) and maternal depression during pregnancy (Jones et al., 1998). In 1978 Huttenen and Niskanen (1978) showed a significant increase in risk in individuals whose fathers had died while they were in utero compared with those whose father died during their first year of life (Huttenen and Niskanen, 1978). More recently, a study concluded that the death of a close relative during the first trimester of pregnancy increases risk for schizophrenia in the child by 67% (Khashan et al., 2008).

Animal studies demonstrate that externally generated stressors to the pregnant female (i.e. loud noise, social isolation, or pain) result in a surge of stress hormones passing through the placenta to the fetus (Beydoun and Saftlas, 2008). These changes in maternal hormones result in permanent changes to parts of the fetal brain that are associated with schizophrenia (King et al., 2010). Prenatal stress may increase risk for schizophrenia directly, by influencing brain development, or indirectly, by increasing the likelihood of other risk factors such as obstetric complications (Beydoun and Saftlas, 2008).

2.2 Obstetric Complications

Obstetric complications are among the best documented environmental risk factors for schizophrenia. This term is used to cover both pre- and perinatal adverse events. OCs occur in approximately 25-30% of the general population (Cannon et al., 2002) (FIGURE 2.3).

Complication ^b	Number of Studies	Number of Schizophrenic Subjects		Number of Comparison Subjects		Fixed Pooled Estimate (Odds Ratio)	95% CI	z	p
		Total	Exposed to Complication	Total	Exposed to Complication				
Diabetes in pregnancy	2	237	3	1,909	3	7.76	1.37–43.90	2.3	<0.03
Placental abruption	2	308	3	508,352	1,643	4.02	0.89–18.12	1.8	0.07
Birth weight <2000 g	2	504	6	10,926	78	3.89	1.40–10.84	2.6	0.009
Emergency Cesarean section	3	818	20	507,863	1,595	3.24	1.40–7.50	2.7	0.006
Congenital malformations	3	737	10	508,781	6,144	2.35	1.21–4.57	2.5	<0.02
Uterine atony	2	659	27	507,703	16,913	2.29	1.51–3.50	3.8	<0.001
Rhesus variables ^c	3	759	18	17,537	2,911	2.00	1.01–3.96	1.9	<0.05
Threatened premature delivery	2	308	8	508,352	6,498	1.98	0.79–4.90	1.5	0.14
Asphyxia ^d	3	1,109	60	2,297	119	1.74	1.15–2.62	2.6	0.008
Bleeding in pregnancy	6	1,223	34	524,972	9,367	1.69	1.14–2.52	2.6	0.009
Birth weight <2500 g ^e	5	1,294	60	536,045	19,343	1.67	1.22–2.29	3.2	0.002
Head circumference <32 cm	2	758	53	508,315	15,388	1.38	0.97–1.91	1.7	0.08
Smoking in pregnancy	2	105	26	17,886	5,752	1.38	0.88–2.14	1.4	0.16
Preeclampsia	6	1,712	75	510,275	18,286	1.36	0.99–1.85	1.9	0.05
Anemia in pregnancy	3	522	20	1,526	96	1.26	0.69–2.28	0.7	0.45
Gestational age <37 weeks	5	1,290	67	536,051	21,710	1.22	0.90–1.65	1.3	0.20
Small for gestational age	5	1,272	86	519,229	23,485	1.21	0.91–1.61	1.3	0.19
Induction of labor	4	689	186	2,361	232	1.18	0.89–1.56	1.1	0.25
Apgar score <7 at 1 minute after birth	2	390	18	507,434	22,771	1.09	0.62–1.92	0.3	0.76
Gestational age >42 weeks	3	1,187	34	508,747	16,065	1.08	0.69–1.68	0.3	0.72
Child stayed in hospital after mother discharged	3	973	110	1,488	99	1.07	0.79–1.44	0.4	0.65
Forceps delivery or vacuum extraction	7	1,724	124	527,058	29,753	1.07	0.85–1.35	0.6	0.48
Birth length <49 cm	3	761	130	51,320	105,205	1.06	0.86–1.31	0.5	0.59
Cephalopelvic disproportion	2	662	10	2,338	42	1.04	0.28–3.82	<0.1	0.95
Cord around neck	2	893	171	1,345	333	1.03	0.81–1.31	0.2	0.83
Cesarean section	5	1,214	63	526,045	42,947	0.99	0.70–1.41	<0.0	0.98
Birth weight <2500 g and premature	4	954	41	11,376	215	0.96	0.62–1.46	–0.2	0.84
Nonvertex presentation	6	1,667	74	510,208	61,130	0.89	0.67–1.20	–0.7	0.45
Breech delivery	3	464	11	508,508	13,580	0.87	0.38–1.97	–0.3	0.74
Urinary tract infection in pregnancy	3	690	20	507,730	7,115	0.86	0.48–1.55	–0.5	0.63
Nonspontaneous delivery	2	331	46	17,108	1,554	0.63	0.39–1.01	–1.9	<0.06

FIGURE 2.3 Meta-Analysis of Eight Prospective Population-Based Studies of the Association Between Obstetric Complications and Schizophrenia. Taken from Cannon et al., 2002.

An association between OCs and schizophrenia was first mentioned in 1934. Rosanoff et al. (1934) in “The Etiology of So-Called Schizophrenic Psychoses” suggested that schizophrenia could be regarded at least in part as a “decerebration syndrome which may result from birth trauma” (Rosanoff et al., 1934). Since then,

according to Cannon et al. (2002), research into OCs as a risk factor for schizophrenia has gone through different phases (Cannon et al., 2002). Following the time line proposed by Cannon et al. (2002), surprisingly it was not until several decades later that first reports of significant association between prematurity, toxemia, bleeding, and maternal illness were published (Terris et al., 1964). In the early '50s it had been noted that complications of pregnancy and delivery as well as prematurity were associated with brain injury resulting in death of the baby. Depending upon the severity, type, and localization of damage, Pasamanick et al. (1956) proposed that children who were not killed by their traumatic experiences could have developed various sequel of brain injury as a “continuum of reproductive casualty” (Pasamanick et al., 1956).

In 1966 attention shifted to the weight at birth when Lane and Albee reported a significant correlation between low weight at birth (<2500 g) and schizophrenia (Lane et al., 1966). There appeared to be a “shift in distribution” of birth weight within a population of cases compared with controls (Cannon et al., 2002). In 1987, Lewis and Murray did a case-control study finding that patients with schizophrenia were more likely to have a history of OCs than patients with other psychiatric disorders (Lewis and Murray, 1987). They introduced the “Lewis-Murray” scale for rating retrospective information on OCs (Lewis et al., 1989). To examine specific complications, in 1999 Geddes et al. conducted a meta-analysis on 12 case-control studies that had used the Lewis-Murray scale. They found that the following OCs were significantly associated with schizophrenia: premature rupture of membranes, prematurity, use of resuscitation or incubator, birth weight <2500 g, pre-eclampsia, and forceps delivery (Geddes et al., 1999).

Thus, the risk of developing schizophrenia is increased in individuals who were exposed to various complications during pregnancy. Cannon et al. (2002) concluded that there are three major categories of complications: (a) complications of pregnancy (bleeding, preeclampsia, diabetes and rhesus incompatibility), (b) abnormal fetal growth and development (low birth weight, congenital malformations and small head circumference), and (c) delivery complications (asphyxia, uterine atony and emergency caesarean sections) (Cannon et al., 2002). Many obstetric complications seem to compromise neurodevelopment inducing the oxygen deprivation (Hypoxia) in the foetus (McGrath and Murray, 2003).

Recent epidemiological studies further document the relationship between obstetric complications and psychosis-like symptoms or deficit in cognitive function (Zammit et al., 2009) and fetal hypoxia has been proposed as a common denominator for most of these pre- and perinatal events (van Os et al., 2010).

Perinatal hypoxia models in rats and mice have revealed several behavioral, pharmacological, neurochemical and neuroanatomical abnormalities in adulthood with relevance to schizophrenia (Brake et al., 1997; Brake et al., 2000; El-Khodor and Boksa, 2000; Wakuda et al., 2008). Rat models of perinatal hypoxia include (1) unilateral carotid artery ligation combined with exposure to a hypoxic atmosphere in the postnatal rat (Rice et al., 1981) (2) exposure of rat pups to global anoxia during a Caesarean-section (C-Section) birth, by immersion of the isolated intact uterus into a saline bath (Bjelke et al., 1991) and (3) exposure of postnatal rats to anoxic or hypoxic atmospheres (Boksa, 2004). In rats C-section birth alone is sufficient insult to produce long-term enhancement of AMPH-induced locomotor responses, in comparison to vaginal birth (Boksa and El-Khodor, 2003).

2.3 Summary

There is no doubt a strong genetic component to schizophrenia. However it is now clear that neither environmental risk factors nor candidate genes alone play a necessary or sufficient role in the onset of psychosis. Different varieties of environmental insults can act early, during the most critical phase of the formation of the CNS, but also later in childhood or adolescence, in an individual genetically vulnerable (Murray et al., 2002). The neurodevelopmental impairment resulting would determine the onset of schizophrenia later in life (Murray, 1994).

CHAPTER 3

Genetic Predisposition and Environmental Risk Factors

3.1 Gene X Environment Interaction

Back in 1986 Carpenter suggested that schizophrenia is an illness that requires the development of a vulnerable personality resulting from the combination of genetic and environmental influences (Carpenter, 1986). The mode of transmission is most likely complex and non-Mendelian (Owen and Cardno, 1999). We now know that the heritability of schizophrenia is approximately 80% (Cannon et al., 1998; Cardno et al., 1999). However, concordance between MZ twins (almost 50%) not only provides strong evidence for the involvement of genes but also raise the question of what other factors may be involved in the aetiology of schizophrenia (Cardno et al., 1999). The stress-vulnerability model suggests that genetic sensitivity to adverse environmental circumstances explains why some individuals are more likely to develop symptoms when exposed to specific environmental factors than others (Zubin and Spring, 1977). Biological interaction refers to a casual mechanism in which two casual factors (e.g., a genetic variant and environmental exposure) contribute to an outcome (e.g., mental illness) but neither of them is a sufficient cause in itself (Rothman et al., 2008; Uher, 2011). Thus, a genetically transmitted predisposition to schizophrenia is a condition necessary but not sufficient for the development of the disease; for this to happen the individual must be exposed to the action of environmental stimuli. Thus, one does not inherit the disease itself but a greater susceptibility to it.

In statistical terms, as defined by Greenland et al. (2009), a Gene x Environment (GxE) interaction occurs when: “the risk of disease if exposed to both the gene (G) and the environmental exposure (E) is significantly different from that predicted by the statistical model being used” (Greenland, 2009).

In GxE studies, a family history for the outcome illness has been often used as a proxy variable for the genetic risk. Now schizophrenia research is moving toward studies that consider direct molecular genetic information to explain how various risk factors work together to increase risk of the illness.

3.1.1 MAOA x Child Maltreatment

The first important GxE findings in psychiatry came from the Dunedin Multidisciplinary Health and Development Study also known as the Dunedin study (Caspi et al., 2002). The Dunedin Study comprises 1037 babies born in Dunedin, New Zealand between 1 April 1972 and 31 March 1973 at the Queen Mary Maternity Hospital. Numerous measures of exposure to environmental risks factor were collected along with DNA samples at several follow-up points. The babies were first followed up at the age of 3, and then at 5, 7, 9, 11, 13, 15, 18, 21, 26 and 32. The investigators examined if a functional polymorphism in the promoter region of the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (MAOA) would increase the likelihood of violent behavior in maltreated children. The first Dunedin cohort findings showed that maltreated children, carrying the genotype coding for the low-activity MAOA enzyme, were more likely to develop conduct disorder, antisocial personality and adult violent crime than children carrying the genotype for the high-activity MAOA enzyme (Caspi et al., 2002). An important replication of these findings was published in 2006 (Kim-Cohen et al., 2006). In a

cohort of 975 seven year old children they found that among those with a history of physical maltreatment, boys carrying the low-activity MAOA allele had significantly higher mental health problems compared to high- activity MAOA (Kim-Cohen et al., 2006). This finding suggests that maltreatment early in life might increase risk of the development of psychopathology conditionally to the child's MAOA genotype and ultimately to the enzyme functioning. Recently Fergusson et al. (2011) attempted to replicate the GxE interaction effects between MAOA, childhood maltreatment and the development of antisocial behaviour. Their findings add to the evidence suggesting that there is a stable GxE interaction involving MAOA, abuse exposure and antisocial behaviour across the life course (Fergusson et al., 2011).

3.1.2 HTTLPR X Stress

The first report testing the interaction between the serotonin transporter gene promoter region (5- HTTLPR) and stress on the risk of depression came in the 1990's; individuals carrying one or two copies of the 5- HTTLPR short allele had higher personality traits linked to propensity to depression (Lesch, et al., 1996). But it was not until 2003 that the Dunedin study published the first evidence suggesting a GXE interaction between 5- HTTLPR and stress (Caspi et al., 2003). Carriers of the 5- HTTLPR short allele were more likely to develop depression following stressful life events compared to individuals with two copies of the 'long' allele (Caspi et al., 2003).

The Dunedin findings on the 5- HTTLPR generated a huge interest in GXE research and over 50 studies followed attempting to replicate this interaction. Ten of these replication studies were analyzed in a meta-analysis done by Risch et al. (2009). They

concluded there were no convincing evidences of a 5-HTTLPR genotype x Stress interaction on the risk of depression (Risch et al., 2009).

In contrast, a more recent meta-analyses on 54 studies confirmed once again evidence of the 5-HTTLPR genotype x Stress interaction on depression showing how the quality of the studies included in a meta-analysis could influence the direction of its results (Karg et al., 2011). Indeed they investigated whether different measures of exposure to stress across studies could affect the overall result leading to contrasting finding among meta-analysis. Studies using self-report questionnaire ($p=0.042$) showed less striking results when compared to the ones using objective measures ($p=0.000003$) and interview assessment ($p=0.0002$).

This may explain why in the meta- analysis done by Risch et al. (2009), in which most of studies included used self report measures of exposure to stress, the association was not significant (Risch et al., 2009).

3.1.3 COMT x Stress

In line with this strategy, Stefanis et al. (2007) investigated how exposure to stress could lead to psychosis due to different genetic make up (Stefanis et al., 2007).

Dopamine plays an important role in the biological processes that mediate stress responses (Adler et al., 2000). A popular candidate gene in psychiatric genetics is COMT, which codes for the enzyme that catabolizes dopamine, norepinephrine and epinephrine in the prefrontal cortex. The COMT gene contains a G to A missense polymorphism that generates a valine (Val) to methionine (Met) substitution at the codon 158 (Val 158 Met). This results in a different pattern of enzymatic activity with COMT Val-Val carriers having the greater activity with a consequent faster break down of dopamine in the prefrontal cortex.

Stefanis et al. (2007) tested whether exposure to stress was associated with an increased risk of psychotic symptoms in interaction with a particular polymorphism in COMT genotype (Stefanis et al., 2007); carriers of the COMT Val allele were more likely to report psychotic symptoms following a stressful event compared to those with COMT Met/Met genotype (Stefanis et al., 2007). Thus, it is very likely that the effect of environment might be influenced by the interaction with several genetic variants as a risk factor for psychosis.

3.1.4 Genes X Cannabis

3.1.4a COMT x Cannabis

The role of COMT in the breakdown of dopamine and the evidence suggesting that exposure to the cannabis active ingredient, D9-THC, affects striatal dopamine transmission, made COMT and cannabis plausible candidates for a GxE model of liability to psychotic disorders.

Once more the Dunedin Study showed a GxE interaction between environment and genes: in adolescent cannabis users the risk of developing psychosis later in life was moderated by the functional polymorphism in COMT gene (Caspi et al., 2005). This association showed an increased risk of psychosis of around 10-fold in the COMT Val-Val carriers who had used cannabis. Indeed, Cannabis users carrying the COMT Val allele were more likely to develop psychotic symptoms compare to the ones carrying two copies of the COMT Met allele. In line with this finding in 2009 Henquet et al. showed that COMT Val Val genotype were significantly more likely to experience hallucinations than those with COMT Met-Met following cannabis use (Henquet et al., 2009).

However, other studies have failed to support this finding (Zammit et al., 2007; Costas et al., 2011; van Winkel, 2011). One of the first attempts to replicate the above findings came from Zammit et al. in 2007. They investigated if the individual risk of psychotic disorders was conditional on the genotype of COMT and another plausible candidate gene Cannabinoid Receptor Gene 1 (CNR1). They fail to show any evidence for a differential effect of cannabis use on psychosis risk according to variation in either of the genes (Zammit et al., 2007). But it was not until 2011 that Zammit et al. were able to replicate the Dunedin study on the relationship between cannabis and COMT as originally described. Once again they didn't report any association between any of the COMT single-nucleotide polymorphisms (SNPs) or haplotypes examined and cannabis use in the onset of psychosis; Cannabis increased risk of psychosis irrespective of underlying COMT genotypes (Zammit et al., 2011). In conclusion, results from the Dunedin study found no evidence that risk of psychosis was increased by cannabis use in individuals who were methionine homozygotes while Zammit et al. (2011) indicate that cannabis use is likely to increase the risk of individuals developing psychotic experiences irrespective of underlying COMT genotypes (Zammit et al., 2011). It would then be incorrect to state that methionine homozygotes can use cannabis with impunity in relation to risk of psychosis (Zammit et al., 2011).

3.1.4b BDNF x Cannabis

Some studies have also suggested interactions between cannabinoids and BDNF, with increased levels of serum BDNF in humans following injection of Delta-9-TCH (Jockers-Scherubl et al., 2004).

Van Winkel et al. (2009) showed that psychotic patients who were BDNF Met-carriers were significantly more likely to have smoked cannabis in adolescence (van Winkel et al, 2009). They tested for an interaction between BDNF, Cannabis use and seven polymorphisms of the COMT gene on risk of schizophrenia. Four of these polymorphisms constitute its three common functional haplotypes, named after their capacity to influence sensitivity to experimental pain: low pain sensitivity (LPS) haplotype, associated with the highest COMT enzymatic activity, the high pain sensitivity (HPS) haplotype, associated with an 18-fold lower enzyme activity compared to LPS and finally the average pain sensitivity (APS) haplotype, associated with intermediate enzymatic activity.

They found that the effect of BDNF Met was greater for COMT non-LPS homozygotes, and the effect of COMT LPS homozygosity was greater for BDNF Val/Val carriers suggesting that both these genetic variants play a role in the developing brain sensitivity to cannabis (van Winkel et al, 2009). These complex findings require replication.

3.1.4c AKT1 x Cannabis

More recently the V-Akt Murine Thymoma Viral Oncogene Homolog 1 (AKT1) gene has become an attractive candidate to be tested for an interaction with cannabis use on psychotic disorders. AKT1 gene codes for a protein kinase (Protein kinase B, PKB), that forms an integral part of a signaling cascade mediating transmission of striatal dopamine. Moreover, cannabinoids are able to activate the AKT1 pathway by acting on Cannabinoid receptors 1 and 2 (CB1 and CB2).

In a study of 740 unaffected siblings of 801 patients with psychosis and 419 controls, van Winkel et al. (2011) examined the interactions between cannabis use and 152

single-nucleotide polymorphisms in 42 genes, including two polymorphisms of the AKT1 gene (rs2494732 and rs1130233) (van Winkel et al., 2011). Among those who used cannabis, carriers of the AKT1 rs2494732 C/C genotype had a significant two fold increased risk of schizophrenia compared to the AKT1 rs2494732 T/T carriers. Most recently, Di Forti et al. (2012) confirmed that the AKT1 genotype influences the risk of psychosis in cannabis users (Di Forti et al., 2012).

3.2 Genes x Obstetric Complications

The OCs I have discussed in the previous chapter explain a small, but significant, increase in risk for schizophrenia (Cannon et al., 2002).

For several labour and delivery complications associated with increased incidence of schizophrenia, perinatal hypoxia is highly likely to be a common factor and the incidence of schizophrenia increases linearly with the number of hypoxia-associated birth complications (Cannon et al., 2000). Back in 1968 the Copenhagen High-Risk Study, examining the characteristics of a group of offspring of schizophrenic parents, found that the “high-risk” children who were psychiatrically ill by their early 20s were more likely to have suffered one or more serious OCs than the comparison group (Mednick and Schulsinger, 1968). Mednick’s explanation was that people with genetic predisposition would become schizophrenic only if the hippocampus was selectively injured by anoxia at birth (Mednick, 1970). The hippocampus is especially sensitive to hypoxic injury. There have been several findings of hippocampal abnormalities in individuals with schizophrenia particularly in those who experienced OCs (Stefanis et al., 1999; van Erp et al., 2002). It has therefore been hypothesized

that hypoxia in the prenatal and perinatal period contributes to the neurodevelopmental alterations that later in life are involved in the onset of schizophrenia (Buka et al., 1993; Zornberg et al., 2000; Dalman et al., 2001; Van Erp et al., 2002; Murray et al., 2004).

A recent systematic review suggested that more than 50% of genes potentially associated with schizophrenia are subject to regulation by hypoxia and/or are expressed in the vasculature (Schmidt-Kastner et al., 2006; Schmidt-Kastner et al., 2012). It seems that the hypoxic regulation of expression of some of these genes could be a potential key player in the aetiopathogenesis of schizophrenia. Vice-versa, gene variants can also influence the reaction to hypoxia (Schmidt-Kastner et al., 2012).

In a sample of 116 family-trios (affected individuals and their unaffected parents), Nicodemus et al. (2008) examined 13 genes SNPs, known to regulate neurovascular processes (Nicodemus et al., 2008). Three SNPs of the AKT1 gene, two of BDNF gene, one of DTNBP1 and finally one of Glutamate Receptor Metabotropic 3 (GRM3) gene showed evidence for a significant interaction with obstetric complications in increasing the risk of schizophrenia (LRT P-values ranged from 0.011 to 0.037) (**FIGURE 3.1**).

<i>SNP</i>	<i>Gene</i>	<i>OC status</i>	<i>OR</i>	<i>95% CI</i>	<i>OR P-value</i>	<i>N families^a</i>	<i>N minor allele transmissions (%)</i>	<i>LRT P-value</i>
rs2494735	AKT1	Absent	1.0	—	—	59	36 (61.0)	—
		Present	7.18	(0.91, 56.75)	0.062	17	15 (88.2)	0.037
rs3803300	AKT1	Absent	1.0	—	—	78	11 (14.1)	—
		Present	3.89	(0.83, 18.20)	0.085	24	14 (58.3)	0.012
rs1130233	AKT1	Absent	1.0	—	—	71	31 (43.7)	—
		Present	3.97	(1.13, 13.92)	0.031	24	16 (66.7)	0.02
rs2049046	BDNF	Absent	1.0	—	—	73	23 (31.5)	—
		Present	0.15	(0.032, 0.73)	0.019	21	3 (14.3)	0.011
ss76882600	BDNF	Absent	1.0	—	—	74	39 (52.7)	—
		Present	12.45	(1.63, 94.60)	0.015	23	17 (73.9)	0.028
rs875462	DTNBP1	Absent	1.0	—	—	61	39 (63.9)	—
		Present	9.49	(1.23, 73.30)	0.031	18	14 (77.8)	0.025
rs7808623	GRM3	Absent	1.0	—	—	72	22 (30.6)	—
		Present	3.39	(0.95, 12.17)	0.061	23	12 (52.2)	0.035

FIGURE 3.1 Odds Ratios for Serious OCs and Minor Allele Carrier Status. Taken from Nicodemus et al., 2008

All of these genes have been shown to play a neuroprotective role in response to hypoxic/ischemic insults during neurodevelopment so they could explain the toxic effect of obstetric complications on brain development (Nicodemus et al., 2008). Two other studies purported to confirm the significance of particular SNPs within the AKT1 locus (Joo et al., 2009); however the analyses in these two studies are obscure. More recent, Forsyth et al. (2012) using familial liability (family history of psychiatric illnesses) as a proxy measure of genetic risk, tested if this mediated the effect of OCs to affect development (Forsyth et al., 2012). They hypothesized that the presence of OCs would differentially predict neurobehavioral outcomes in individuals at high risk (HR) for the illness (ie, having 1 or 2 parents with schizophrenia) vs those at low risk (LR; ie, having parents with no psychiatric illnesses). Early studies reported that OCs predicted motor impairments in HR individuals but not in LR individuals and they also interacted with genetic risk to predict ventricular enlargement (Cannon et al., 1989; Fish et al., 1992; Cannon et al., 1993). Moreover Clarke et al. (2009) reported that siblings exposed to prenatal

infection had a 5-fold increased risk to schizophrenia in HR compared with LR offspring (Clarke et al., 2009).

Forsyth et al. (2012) obtained data on OCs and school records in individuals born in Finland between January 2, 1987 and December 31, 1993 at HR vs LR for schizophrenia. This resulted in the identification of 373 HR offspring and 1070 LR offspring from 107 schools in the Helsinki area. Thus, they examined the interaction of Low Birth Weight (LBW) and hypoxia in relation to school outcome at 15–16 years.

HR offspring performed worse than LR offspring across academic, nonacademic, and physical education domains. LBW predicted poorer academic and physical education performance in HR offspring, but not in LR offspring. Hypoxia predicted poorer physical education score across risk groups. Rates of LBW and hypoxia were similar for LR and HR offspring.

It is very likely that the effect of the OCs confers augmented vulnerability of the developing brain influenced by the interaction with several genetic variants.

Therefore, although this study does not use a specific “biological plausible” polymorphism as a marker of genetic risk, its findings are suggestive of a gene x environment interaction between shared genes (familial liability) and the environment in influencing the risk of psychosis, possibly via epigenetic mechanisms.

Results from several GWAS in schizophrenia show several interesting matches between genes and ischemia–hypoxia or vascular factors but unfortunately differences in design and data presentation in different GWAS studies preclude a quantitative analysis (Schmidt-Kastner et al., 2012).

Epigenetic regulation of gene expression (for example, through histone alterations, DNA methylation or microRNAs (miRNAs)) could possibly explain the effects of pre- and perinatal exposure to hypoxia–ischemia on gene expression and aberrant neurodevelopmental processes (Schmidt-Kastner et al., 2012). In particular a recent GWAS study showed a significant association with miRNA137 and schizophrenia. Elevated levels of miRNAs were found in the prefrontal cortex of patients afflicted by schizophrenia and have been also documented to regulate the response to hypoxia. No studies have yet provided direct evidence for epigenetic mediation of hypoxia–ischemia during pre- and perinatal life in schizophrenia so further research is warranted to identify direct links between hypoxia, epigenetic alterations and schizophrenia (Schmidt-Kastner et al., 2012).

3.3 Summary

There is no doubt a strong genetic component to schizophrenia. However twin studies have shown that simple genetic transmission is far from the whole story. Variability in people's responses to environmental risk factors suggests that genes and environments operate together to produce psychosis. Liability to psychosis in general, and to schizophrenia in particular, is distributed through the general population in a similar continuous way to liability to medical disorders such as hypertension and diabetes. As with these disorders, some people show a high liability to the condition and as blood pressure can be increased by obesity, lack of exercise, and cigarette smoking, so exposure to a range of environmental factors including neonatal hypoxia, urbanicity, migration, and certain types of social adversity, can increase an

individual's likelihood of expressing psychotic symptoms. Thus it appears that multiple genetic and environmental factors operate together to push individuals over a threshold into expressing the characteristic clinical picture (Tsuang et al., 2004; Di Forti et al., 2007b).

CHAPTER 4

Epigenetics and Early Environmental Influences

4.1 Introduction

Despite sharing the same DNA sequence, the concordance rate between MZ twins for schizophrenia is estimated to be less than 50% (Gottesman, 1991). This could reflect environmentally or stochastically mediated epigenetic variation (Dempster et al., 2013). Thus, recent research has raised the notion that epigenetic mechanisms are involved in the pathogenesis of schizophrenia and psychosis in general (Tsankova et al., 2007; Mill et al., 2008).

Conrad Waddington first defined Epigenetics in 1940 as "...the interactions of genes with their environment which bring the phenotype into being" (Waddington, 1940). Epigenetics is the study of the changes in gene expression not encoded by the DNA sequence. These changes comprise molecular modifications to both DNA and chromatin, the most extensively investigated of which are DNA methylation and histone modifications (Jirtle and Skinner, 2007). Other epigenetic mechanisms of gene-expression control include regulation by microRNAs (miRNAs) and mechanisms that control the higher-level organization of chromatin within the nucleus (Jirtle and Skinner, 2007).

Epigenetic modifications are essential for normal development and the maintenance of gene expression patterns in mammalian cells. In particular DNA methylation is involved in normal cellular control of expression, histone modifications control the accessibility of the chromatin and transcriptional activities inside a cell, and miRNAs

are small RNA molecules that can negatively control their target gene expression post-transcriptionally (Jirtle and Skinner, 2007).

Importantly, epigenetic changes can be inherited mitotically in somatic cells, providing a potential mechanism by which environmental effects on the epigenome can have long-term effects on gene expression (Jirtle and Skinner, 2007). In addition increasing evidence suggests that epigenetic alterations might also be inherited transgenerationally, thereby potentially affecting the health of future generations (Jirtle and Skinner, 2007).

4.2 Epigenetic Studies on Psychosis

Epigenetic processes are known to regulate key neurobiological and cognitive processes in the brain and, through regulation of gene expression, have been widely acclaimed as the “missing piece” of the etiological puzzle for complex disease such as schizophrenia (Dempster et al., 2013). Some researchers have therefore suggested that schizophrenia may arise from alterations in how some genes are “turned on or off” due to exposure to nongenetic factors (Rutten and Mill, 2009).

Initial studies were investigating methylation in candidate genes using postmortem brains and peripheral blood samples including genes involved in neurotransmitter systems, synaptic plasticity, oxidative stress and oligodendrocyte viability and myelination (reviewed by Pishva et al., 2014). Although these studies on candidate genes have provided important first insights into possible epigenetic dysregulation in the psychotic disorders were not always replicated (Labrie et al., 2012).

The first epigenome-wide study characterizing DNA methylation in major psychosis surveyed 12,000 GC-rich regions, including CpG islands, in the prefrontal cortex of the brain (Mill et al., 2008). This study identified several sites with significant epigenetic differences between affected individuals and controls, which contained genes involved in brain development and neurotransmitter pathways, particularly genes involved in glutamatergic and GABAergic neurotransmission, and which had previously been associated with major psychosis (Mill et al., 2008).

Dempster et al. (2011) performed a genome-wide analysis of DNA methylation on peripheral blood DNA samples obtained from a MZ twin pairs discordant for major psychosis (Dempster et al., 2011). They found numerous disease-associated differences in DNA methylation, many located in the vicinity of genes previously implicated in psychosis, supporting the hypothesis that epigenetic alterations play an important role in the etiology of psychosis (Dempster et al., 2011). Overall, the top differentially methylated psychosis-associated site was located in the promoter region of the gene encoding alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1 (ST6GALNAC1), which was hypomethylated in affected individuals compared with their unaffected co-twins (mean $\Delta\beta = 0.06$, $P = 4.03E - 04$) (Dempster et al., 2011).

Another methylome-wide association study of patients with schizophrenia and controls showed differentially methylated regions in the promoter region of several genes associated with schizophrenia, such as beta-1,3-glucuronyltransferase 2 (B3GAT2), histone deacetylase 4 (HDAC4) and diacylglycerol kinase (DGKI) (Kinoshita et al., 2013).

Recently a large study on 1479 schizophrenic patients and controls examining DNA from peripheral blood cells found additional evidence for differential methylation in

the loci of several genes previously already been linked to the pathogenesis of schizophrenia (Aberg et al., 2014).

Perturbations of DNA methylation in major psychosis may also result from the abnormal activity of DNA methyltransferases (DNMTs) or changes in the levels of methyl-group donors and co-factors affecting DNA methylation (Labrie et al., 2012). In particular as reviewed by Pishva et al. (2014) schizophrenic patients express higher levels of DNMTs on peripheral lymphocytes (Pishva et al., 2014). On the other hand, investigations on the levels of methyl-group donors and cofactors affecting DNA methylation have detected differences in the levels of S-adenosyl methionine, a methyl donor, and other molecules such as homocysteine and folate in patients with major psychosis (reviewed by Pishva et al., 2014).

Differences in patterns of histone modifications have also been found in major psychosis (Labrie et al., 2012). In particular gene expression studies on histone modifying enzymes have provided further evidence for involvement of histone tail alterations in psychosis (reviewed by Pishva et al., 2014).

Although these findings provide some support for the role of epigenetic dysfunction in the etiology of schizophrenia, they represent a fairly crude initial snapshot of the regulatory changes involved in pathogenesis and indicate that a more systematic investigation across much larger numbers of samples is warranted (Dempster et al., 2013).

4.3 Epigenome and Early Environmental Influences

Increasing evidence from animal studies indicates that both prenatal and early postnatal environmental factors can result in altered epigenetic programming and subsequent changes in the risk of developing disease (Jirtle and Skinner, 2007).

The results of these studies support the hypothesis known as the fetal basis or developmental origins of adult-onset disease (Jirtle and Skinner, 2007). Thus, the evolution of developmental plasticity, which empower an organism to adapt to environment during early life, can also increase the risk of diseases when there is a mismatch between the perceived environment and that which is encountered in adulthood (Jirtle and Skinner, 2007).

Growing evidence seems to suggest that early environmental factors are linked to disease phenotypes through modifications of the epigenome (Jirtle and Skinner, 2007). Particularly in psychosis findings from both animal and human studies together suggest that adversity in early life may lead to persistent epigenetic marks on specific genes associated with psychotic disorders (reviewed by Pishva et al., 2014).

Inherited genetic and epigenetic profiles constitute the molecular basis of an individual; during life course environmental factors seem to interact with genetic and epigenetic profiles leading to long-term alterations of the epigenome (Pishva et al., 2014). The possible alteration of gene expression resulting may influence behavioral and experiential phenotypes of individuals (Pishva et al., 2014). Additional evidence suggests the existence of allele-specific epigenetic differences in genes associated with psychosis constituting a further element in the dynamic interplay between the environment, genome and epigenome (Pishva et al., 2014).

Despite considerable speculation about the role of epigenetics in psychosis this is a relatively nascent area of research and although publications in the field are growing,

they account mainly of non-research articles and animal studies (Dempster et al., 2013). Epigenetic processes are influenced by a spectrum of external environmental factors, including early environmental factors, diet, toxins, drugs, and stress. Moreover polymorphisms can also exert an effect on gene function via epigenetic processes (Gamazon et al., 2013). These suggest a common pathway behind both genetic and environmental effects possibly through gene–environment interaction (Dempster et al., 2013).

4.4 Conclusion

Progress in understanding the pathways through which various etiologic factors disrupt brain development in schizophrenia is necessary. A growing number of studies are attempting to measure both genes and environments to explain why people respond differently to the same environment (Van Os et al., 2008).

GxE research represents an important approach to explain the occurrence of psychosis. Nevertheless exposure to certain environmental factors could also contribute to spontaneous genetic mutations that give rise to vulnerability for the illness.

Despite considerable interest on epigenetic mediation of environmental factors in the development of psychosis, little evidence is currently available. No studies have yet provided direct evidence for epigenetic mediation of hypoxia–ischemia during pre- and perinatal life in schizophrenia so further research is warranted to identify direct links between hypoxia, epigenetic alterations and schizophrenia (Schmidt-Kastner et al., 2012).

In the context of this Thesis, which focuses on obstetric complications and psychosis, the major aim is to address the question of whether or not selected genes interact with the presence of OCs in the aetiology of psychosis. In particular two different approaches will be described, one human and one animal. In humans, firstly I attempted to replicate the findings coming from Nicodemus et al. (2008) looking at different gene polymorphisms of AKT1, BDNF, DTNBP1 and GRM3 (**CHAPTER 7 and 9**); then I looked at 25 SNPs selected from the GWAS findings published so far (**CHAPTER 8**). In animals, I attempted to explore epigenetic mediation of hypoxia–ischemia during pre- and perinatal life in rats (**CHAPTER 10**).

CHAPTER 5

Aims and Methods

5.1 Introduction

Following the description of the aims and hypothesis of my thesis, in this chapter I now outline the study design and the recruitment strategy for participants. This includes the power calculation applied to select the appropriate sample size.

I then describe the main assessment tools used to gather the clinical and socio-demographic data and the history of any Obstetric Complications (OCs) relevant for the analyses.

A separate section of the methods describes the collection and processing of DNA samples.

Finally, I present a summary of the statistical analyses applied to test the Thesis hypotheses and my personal contribution to the collection and processing of the data.

5.2 Aims

In a case-control design, I investigated how exposure to OCs influenced the risk of psychotic disorder, by employing a GxE causation model.

Specifically the principal aims of this project are to: -

1. Investigate obstetric history in psychotic patients, their unaffected siblings and healthy controls (**CHAPTER 6**).

2. Test, under a multiplicative model, the hypothesis that a range of genetic variants interacted with OCs in increasing the risk of psychotic disorder:

a) Examine whether the following genes, selected on the basis of their pathophysiologic mechanism, play a role in the onset of psychotic disorders in interaction with OCs as seen in a previous study conducted by Nicodemus et al (2008): AKT1 rs 2494753, rs1130233, rs3803300; BDNF rs2049046, rs56164415; DNMBP1 rs875462 and GRM3 rs7808623 (**CHAPTER 7 and CHAPTER 9**);

b) Examine whether the following susceptibility genes, deriving from the most recently available GWAS studies, play a role in the onset of psychotic disorders in interaction with OCs: AK573765-TWIST2 (intergenic) rs9751357; CACNA1C rs4765905; CEACAM21 rs4803480; CNNM2 rs7914558; CSMD1 rs10503253; Erbb4 rs1851196; ITIH3/4 rs2239547; LOC645434-NMBR rs2066036; LRRFIP1 rs12052937; MIR137 rs1625579; MMP16 rs7004633; NKAPL rs1635; NRG rs12807809; NT5C2 rs11191580; PCLO rs6979348; PLXNA2 rs752016; PGBD1 rs2142731; PCGEM1 rs17662626; RELN rs7341475; SDCCAG8 rs6703335; STT3A rs548181; TCF4 rs17512836; UGT1A1 HJURP(intergenic)rs741160; rs10489202; rs16887244 (**CHAPTER 8**).

3. Examine whether rats that had experienced perinatal asphyxia during birth show any abnormality in gene expression and consequently in methylation status in Prefrontal Cortex (PFC), Hippocampus (HiP) and Striatum (STR) at

three developmental periods, post natal day (PND) (T1: 0 week), adolescence (T2: 5 weeks old) and adulthood (T3: 12 weeks old) (**CHAPTER 10**).

5.3 Hypotheses

I will test the following hypotheses:

1. Psychotic patients to be more likely than controls to have experienced a definite OC.
2. The effect of OCs on odds of psychotic disorder to be conditional on the individual's genotype.
3. The hypoxic rats to show abnormal gene expression and methylation status in different brain regions compared to controls.

	Aims	Hypotheses	Analytic Approach
1	Investigate obstetric history in psychotic patients, their unaffected siblings and healthy controls	I expect psychotic patients to be more likely than controls to have experienced a definite OC	Logistic Regression χ^2 Tests T-Tests
2	a) Replicate Nicodemus et al. (2008) b) Examine whether genes deriving from the most recently available GWAS play a role in the onset of psychotic disorders in interaction with OCs	I expect the effect of OCs on odds of psychotic disorder to be conditional on the individual's genotype	Logistic Regression χ^2 Tests T-Tests
3	Examine whether rats that had experienced perinatal asphyxia during birth show any abnormality in gene expression and methylation status	I expect the hypoxic rats to show abnormal gene expression and methylation status compared to controls	ANOVA

TABLE 5.1 Summary of Main Investigations and Related Hypotheses and Statistical Analysis

5.4 Study Design

The project is a case-control study design; the majority of subjects have been recruited as part of the Maudsley Family Study (MFS), London. This large study has been running under the overall supervision of Professor Robin Murray since the early 1990's and includes data for over 1000 subjects comprising patients with psychotic illness, their unaffected first-degree relatives and controls. The families were recruited by self-referral in response to advertisements, through voluntary

organizations, or by direct referral from their local psychiatric service. All participants were Caucasian.

The major aim of the study is to identify neurobiological deviations associated with psychosis that are linked to genetic susceptibility (Frangou et al., 1997; Frangou et al., 1997b; Sharma et al., 1997; Crawford et al., 1998; Griffiths et al., 1998; Sharma et al., 1998; Sharma et al. 1999; Birkett et al., 2008).

I increased the available sample of patients as part of the Genetic and Psychosis (GAP) study, London – active since 2004. Under the overall supervision of Professor Robin Murray and Dr Marta Di Forti, the study includes patients (age 18-65 yrs) presenting with their first episode of psychosis to the Lambeth, Southwark, Croydon and Lewisham adult in-patient units of the South London and Maudsley Foundation NHS trust and healthy matched controls (Di Forti et al., 2009).

These projects used similar diagnostic criteria and recruitment strategy to collect OCs data. Although the GAP study is a multiethnic project only Caucasian patients recruited in the South London and Maudsley Hospital Trust were included in the present PhD project that guaranteed homogeneity of the subjects in term of demographic characteristics.

5.5 Ethics

All studies involving human subjects were approved by the South London and Maudsley NHS Trust and the Institute of Psychiatry Ethical Committee. My measures didn't fall within the GAP study's ethical approval (REC reference number

05/Q0706/158) and so I had to amend the ethics application and forms in order to assess the relatives (Amendment numbers 8, 9, and 11). Written informed consent was obtained after a full explanation of the study was given to participants.

5.6 Recruitment

5.6.1 Patients

The sample comprised individuals who are participants in the Maudsley Family Study of Psychosis, comprising patients with psychotic illness, their unaffected first-degree relatives and controls. The families were recruited by self-referral in response to advertisements, through voluntary organizations, or by direct referral from their local psychiatry services. They were chosen on the basis that the index patient had a lifetime DSM-IV diagnosis of schizophrenia or schizoaffective disorder. All participants were Caucasian.

As part of the GAP study, my colleagues and I approached all patients aged 18 to 65 years presenting with their first episode of psychosis to the Lambeth, Southwark, Croydon and Lewisham adult in-patient units of the South London and Maudsley Foundation NHS Trust (SLAM) between December 2005 and October 2011.

A weekly screening through the electronic-Patient Journey System of SLAM was done in order to identify new admissions of first episode of psychosis patients in the psychiatric wards from the catchment area.

All patients who met ICD10 criteria for a diagnosis of nonorganic psychosis (F20-F29 and F30-F33), validated by administering the Schedules for Clinical Assessment in Neuropsychiatry (SCAN), were invited to participate in the study.

Of the total (n=745) approached, 20% (n=149) refused to participate. The two most common reasons for refusal were lack of interest in the research and the length of our study assessment (Woodal et al., 2011). If patients were too unwell to cooperate, they were re-contacted following improvement.

5.6.1a Inclusion and Exclusion Criteria

The following inclusion criteria were applied: contact with psychiatric services for psychosis, Caucasian, fluent speaker of English, aged between 18 and 65 years old.

Exclusion criteria: organic psychosis (as defined by ICD-10, WHO, 1992) and IQ below 70.

5.6.2 Siblings

Siblings were recruited as part of the Maudsley Family Study of Psychosis. They were chosen among first-degree relatives on the basis that the index patient had a lifetime DSM-IV diagnosis of schizophrenia or schizoaffective disorder. All participants were Caucasian.

5.6.2a Inclusion and Exclusion Criteria

Siblings were all Caucasian aged 16-69.

Exclusion criteria: not fluent speaker of English, history of organic brain disease, history of head trauma resulting in loss of consciousness for more than 5 minutes,

fulfilled DSM-IV criteria for substance or alcohol dependence in the 12 months prior to the assessment.

5.6.3 Controls

Over the same time frame, from the area served by the same mental health units, I obtained a healthy control sample, aged 18 to 65 years, which was broadly similar to the local population in terms of ethnicity, educational attainment and employment status ([www.statistics.gov.uk/census 2001](http://www.statistics.gov.uk/census2001)), using internet and newspaper adverts and distribution of leaflets at train stations, shops and job centres.

Volunteers willing to take part in the study were administered the Psychosis Screening Questionnaire, and excluded if they met criteria for a psychotic disorder or if they reported a previous diagnosis of psychotic illness (Bebbington and Nayani 1995).

5.6.3a Inclusion and Exclusion Criteria

Control participants are recruited from the local population living in the area served by SLAM mental health services. All participants were Caucasian.

Exclusion criteria: not fluent speaker of English, current or past diagnosis of psychotic disorders and IQ below 70.

5.6.4 Mothers

The sample comprised firstly 252 individuals who are participants in the MFS. All participants were Caucasian.

Furthermore I contacted all Caucasian patients who had already been assessed as part of the GAP study. Of a total of 596 who agreed to participate in the GAP study since 2005, 196 were Caucasian (35.7%). Among them only 56 individuals supplied informed consent for maternal interview. Of the remaining 140, 69 individuals refused to participate, 46 were ineligible (4 patients died, 17 patients withdrawn from GAP in general, 16 mothers were dead, 9 mother non-English-speaking or living abroad); 25 could not be contacted.

After informed consent was obtained, the mother of each participant was identified wherever possible and invited either to attend for interview or to take part in a telephone interview (depending on their preference). Of the 56 individuals who had given permission to contact their mother and who had completed the proband interview, 23 were not included because their mother subsequently refused or could not be contacted. Finally I collected a total of 33 cases and 28 controls.

5.6.4a Inclusion and Exclusion Criteria

The following inclusion criteria were applied: Caucasian and fluent speaker of English.

Exclusion criteria: women over the age of 70 were not included in the study as evidence suggests that a decline occurs on tests of episodic memory in women after this age (Herlitz et al., 1997).

5.7 Assessment

In the GAP study, subjects were given a wide range of assessments, including blood and/or cheek swabs for genetic analysis, structural and functional MRI, biological measures, wide clinical and socio-demographical interview, substance use, a neuropsychological battery and other questionnaires.

As part of the GAP study, I also contributed to the collection of socio- demographic data (age; gender; ethnicity; level of education attainment and employment status) on both patients and controls using a modified version of the Medical Research Council (MRC) socio-demographic scale (Mallett et al., 2002). Ethnicity was assessed by asking participants to select the ethnic group that most closely described their ethnicity from a list used in the last UK census (www.statistics.gov.uk/census 2001).

5.7.1 Obstetric History

Each participant's history of OCs was assessed in the same way. Consenting mothers of subjects were interviewed using the Maternal Interview Schedule (MIS) elicited using a standard questionnaire developed from other published reports (Lewis and Murray, 1987; McCreadie et al, 1992; McNeil et al., 1995; Cannon et al., 2002) (APPENDIX).

The MIS includes the following questionnaires:

1. Sociodemographic Schedule
2. Obstetric Complications Questionnaire
3. Family History for Genetic Study

In detail the Obstetric Complications Questionnaire includes the following data:

- Maternal health in brief

- Place of birth
- Paternal age at birth
- Maternal age at birth
- Pregnancy complications
- Gestational infections
- Maternal substance use during pregnancy and breast feeding
- Labour and delivery complications
- Neonatal complication

5.7.2 Lewis-Murray Scale

The Lewis-Murray Scale (Lewis and Murray, 1987) was used as the principal measure of OCs as it includes the greatest number of complications of potential relevance to the aetiology of schizophrenia (FIGURE 5.1).

Table 2. Lewis-Murray obstetric complications scale (Lewis et al. 1989)	
Definite	Equivocal
Antepartum	
1. Rubella or syphilis	—
2. Rhesus incompatibility	—
3. Preeclampsia: severe and/or leading to early induction or hospitalization	Preeclampsia NOS
4. Ante partum hemorrhage or threatened abortion	—
Intrapartum	
5. Premature rupture of membranes, > 24 hours	—
6. Labor > 36 hours or < 3 hours	Labor > 24 hours or "long"/difficult/"precipitate" NOS
7. Twin birth, complicated	Twin birth NOS
8. Cord prolapse	Cord knotted or around neck
9. Gestational age < 37 weeks or > 42 weeks	"Premature"/"postmature" NOS
10. Caesarean, complicated or emergency	Caesarean NOS
11. Breech or abnormal presentation	—
12. High or "difficult" forceps	Forceps or other instrumental delivery, NOS
13. Birthweight < 2,000 g	< 2,500 g or "small" NOS
14. Incubator > 4 weeks	Incubator resuscitation/"blue" NOS
15. Gross physical anomaly	—
Rating points: definite = 2; equivocal = 1; absent = 0; insufficient information = 9	
Note.—NOS = not otherwise specified.	

FIGURE 5.1 The Lewis-Murray Scale. Taken from Lewis et al., 1989.

The Lewis-Murray Scale consists of 17 individual items including information about rubella, syphilis, Rh incompatibility, pregnancy-induced hypertension, obstetric bleeding, premature membrane rupture, labour duration, twin birth, cord prolapse, gestational age, Caesarean section, breech or abnormal presentation, instrumental delivery, birth weight, foetal distress, and gross physical anomaly. Lewis-Murray Scale includes several items of potential hypoxic harm to the foetus such as maternal preeclampsia; neonatal cyanosis; emergency caesarean section; umbilical cord wrapped around the neck; requiring incubator care for more than 1 week in the neonatal period; requiring neonatal resuscitation (Lewis et al., 1989).

Each item was evaluated as definite (score 1), or absent (score 0). Subjects were rated as having a “definite” (score 1) complication if they had suffered at least one significant complication (Lewis et al., 1989). I did not rate labour of less than 3 h as a definite complication (O'Callaghan et al., 1990). Other items on the scale were rated as unknown if the record could not be used to determine whether an event had or had not occurred. Maternity records were not used if they could not supply information for at least 10 items from the Lewis-Murray Scale.

5.8 DNA Samples Collection and Storage

A blood sample was also collected from both patients and controls (two 6 mls EDTA tubes) in the GAP study. This was used to obtain DNA samples. Participants who refused venopuncture (25%) were asked to provide a DNA sample using a cheek swab kit, provided by the laboratory of the MRC Social, Genetic and

Developmental Psychiatry Centre (SGDP). The DNA was extracted from both blood samples and cheek swabs, following standard procedures. All samples were bar-coded to preserve confidentiality and blindness to clinical status and appropriately stored in the SGDP -80 ° C degree freezer for later analysis. Similar procedures had been followed in the Maudsley Family Study.

The genotyping of selected genetic polymorphisms was carried out under the overall supervision of my supervisor Dr John Powell. In particular I have been working at the Institute of Psychiatry Neuroscience Department laboratory under the supervision of Dr Conrad Iyedgbe and at the Social, Genetic and Developmental Psychiatry Centre (SGDP) under Dr Rebecca Smith supervision. The procedures followed for the genotyping are described in the **CHAPTER 7 and 8**.

5.9 Sample Size

Overall OCs data was available on 313 subjects. A total of 145 psychotic patients, 65 controls and 103 unaffected siblings were available for my project. Variations on sample size are present across the different statistical analyses carried out in this PhD. For each experiment, sample size is specified in **TABLE 5.2** and in the relevant chapter.

	OCs			GxE			Case Only			
	MFS	GAP	Tot	MFS	GAP	Tot	MFS	GAP	PICOS	Tot
Cases	112	33	145	105	22	127	112	33	232	377
Siblings	103	-	103	95	-	95				
Controls	37	28	65	33	7	40				
Total	252	61	<u>313</u>	233	29	<u>262</u>				<u>377*</u>

TABLE 5.2 Sample Size. Sample size for each experiment * DNA material was obtained from 290 subjects in total.

Assuming an exposure of OCs of 30% among the general population (Cannon et al., 2002), I have the 80% power and a significance level of 0.05% (two-tailed) of detecting an OR of 2.0 with 95% confidence in our population.

For a G×E interaction purpose in a case-control design in order to have 80% power and a significance level of 0.05% (two-tailed) a sample of 355 case-control pairs is required. In a case-only design in order to have 80% power and a significance level of 0.05% (two-tailed) a sample of 140 cases is required. This assumes an exposure of 30% among the general population (Cannon et al., 2002) and with a mean allele frequency of 0.5.

Power Calculations were made using the Quanto 1.2.4 software using the gene-environment module (John Morrison and W. James Gauderman at the University of Southern California).

QUANTO is a program for computing either power or required sample size for association studies of genes, environmental factors, gene-environment (G×E) interaction, or gene-gene (G×G) interaction.

5.10 Statistical Analysis

All the data collected, including the genotyping results, were recorded and analyzed in SPSS version 20.

As presented in more detail in the results chapters, χ^2 tests and t-tests (or Mann-Whitney U tests) were used:

1. To test for associations between the presence of psychotic disorder, exposure to OCs, and genotype.
2. To establish whether OCs were more likely to occur in individual carriers of a particular genotype class (a GxE correlation).

Logistic regression was then used, as illustrated in the Chapters detailing the results:

1. To analyse the association between the exposure to OCs and risk of psychotic disorders.
2. To analyse the association between candidate genotype and presence of a psychotic disorder along with any history of OCs.
3. To test in separate analyses for an interaction between exposure and genotypes. The interaction term was used to identify the effect of genotype on presence of psychosis, conditional on history of OCs.

ANOVA

1. To analyse the abnormality in gene expression in different brain regions at three developmental periods in hypoxic rats.
2. To measure DNA methyltransferase expression in different brain regions at two developmental periods followed in hypoxic rats.

CHAPTER 6

Obstetric Complications in Psychotic Patients, Unaffected Siblings and Healthy Controls

6.1 Introduction

OCs are among the best documented environmental risk factors for schizophrenia with an increased risk of about 2.0 (Cannon et al., 2002). OCs occur in approximately 25-30% of the general population, and individuals with schizophrenia are about twice as likely to have experienced obstetric complications compared with healthy controls (Geddes et al., 1999; Cannon et al., 2002).

6.2 Aim

My aim is to investigate the rate of OCs in Psychotic Patients, Unaffected Siblings and Healthy Controls.

6.3 Hypothesis

I expect psychotic patients to be more likely than controls and siblings to have suffered an OC.

6.4 Methodology

6.4.1 Recruitment and Assessment

As discussed more in detail in **CHAPTER 5**, all data on OCs were retrospectively collected from mother and scored using the Lewis-Murray Scale. In brief each item is evaluated as definite (score 1), or absent (score 0). Subjects were rated as having a “definite” (score 1) complication if they had suffered at least one significant complication (Lewis et al., 1989).

6.4.2 Sample size and Power calculation

Overall OC data was available on 313 Caucasian subjects, comprising 145 psychotic cases, 103 unaffected siblings and 65 healthy controls. Assuming an exposure of OCs of 30% among the general population (Cannon et al., 2002), I have 80% power of detecting an OR of 2.0 with 95% confidence in our population. This calculation has been made using the Quanto 1.2.4 software using the environment module (John Morrison and W. James Gauderman at the University of Southern California).

6.4.3 Statistical Analysis

The one-way Analysis of Variance (ANOVA) and post hoc comparisons with Hochberg’s GT2 (due to the sample size differences among groups) were used to analyze difference in the mean for age, gestational age, weight at birth and labour duration in the three groups, cases, siblings and controls.

Logistic regression was used to analyse the associations between the occurrence of OCs and case–control status, and to test for interaction effects while controlling for potential confounders.

Confounders were selected after testing, using χ^2 tests and t-tests (or Mann-Whitney tests), for their association with the presence of history of OCs and of psychotic disorders.

6.5 Results

6.5.1 Description of the Whole Sample

6.5.1a Clinical Characteristics of the Sample

Subjects included in the study consisted in 145 subjects who met ICD10 criteria for a diagnosis of non-organic psychosis (F20-F29 and F30-F33), 103 of their unaffected siblings and 65 healthy controls.

6.5.1b Demographic Characteristic of the Sample

The demographic characteristics for the three groups are shown in TABLE 6.1.

		Cases n= 145	Siblings n= 103	Controls n= 65	P Value
		Mean (SD)	Mean (SD)	Mean (SD)	
Age		23.11 (4.9)	32.57 (8.9)	29.65 (7.8)	p<0.001
Maternal age at delivery		26.98 (4.5)	27.55 (4.4)	28.35 (4.8)	p=0.13
		n (%)	n (%)	n (%)	
Gender	Male	97 (66.9%)	50 (48.5%)	31 (47.7%)	p=0.04

TABLE 6.1 Demographic Characteristics of the Sample. P values from t-tests and χ^2 tests.

The mean age of the whole sample is 27.68 years (SD 8.33); among the groups, patients were the youngest (23.11 years old, SD 4.86, 95% CI 22.28-23.94, $F(2, 298) = 54.88$; $p < 0.001$). There was a significant group difference for gender with an excess of males in the patients' group ($\chi^2 = 11.09$ $p = 0.004$). Regarding ethnicity, only Caucasian people were included.

6.5.1c Maternal Characteristics of the Sample

The demographic characteristics for the three groups are shown in **TABLE 6.1**. The mean age of the mother at the time of the interview was 59.14 years (SD 8.07). The groups did not significantly differ in the measure of maternal age at delivery (mean age 27.45 years, SD 4.52; 95% CI 26.94-27.95, $F(2-308) = 2.08$, $p=0.127$).

6.5.2 Obstetric Complications

The rate of definite OCs, mean of gestational ages and mean birth weights for each group are displayed in **TABLE 6.2**, and individual complications in **TABLE 6.3**.

	Cases n= 145	Siblings n= 103	Controls n= 65	P Value
	n (%)	n (%)	n (%)	
Definite OCs	56 (38.9%)	22 (21.4%)	22 (34.4%)	p=0.01
	Mean (SD)	Mean (SD)	Mean (SD)	
Gestational age in weeks	40 (1.8)	40 (1.5)	40 (1.7)	p=0.71
Birth weight in gr	3321(519.5)	3364(571.2)	3483(527.1)	p=0.14
Labour duration in hour	12 (11.5)	11 (8.5)	13 (14.4)	p=0.37

TABLE 6.2 Rates of OCs, Mean of Gestational Ages, Mean Birth Weights and Labour Duration in Each Subject Group. P values from t-tests and χ^2 tests.

38.9% of psychotic patients had a positive history for OCs compared to 34.4% of the controls and to 21.4% of siblings ($\chi^2=8.64$, $p=0.013$). There was no significant difference among the three groups for gestational age (mean age 39.9 weeks, SD 1.69, 95% CI 39.71- 40.09, $F(2-302)=0.34$, $p=0.71$) and weight at birth (mean weight 3370 gr, SD 540.4, 95% CI 3308- 3430, $F(2-303)= 2$, $p=0.137$).

Moreover the three groups did not significantly differ on labour duration at time of birth (mean time 11.81 hours, 11.28, 95% CI 10.51- 13.10, F (2-290) = 0.99, p=0.371).

	Cases	Siblings	Controls
	n (%)	n (%)	n (%)
Rubella	-	-	-
Syphilis	-	-	-
Rhesus incompatibility	-	-	-
Antepartum hemorrhage	6 (4.1%)	5 (4.9%)	5 (7.7%)
Severe Pre-eclampsia	16 (11%)	6 (5.8%)	4 (6.2%)
Premature rupture of membrane	-	-	1 (3.6%)
Labour > 36 hours	9 (6.3%)	3 (3%)	4 (6.3%)
Birth weight 2000 gr or less	2 (1.4%)	3 (3%)	-
Complicated twin birth	-	-	-
Cord prolapse	-	1 (1%)	-
Gestational age <37 weeks	9 (6.2%)	3 (3%)	4 (6.5%)
Gestational age >42 weeks	5 (3.5%)	2 (2%)	1 (1.6%)
Emergency Caesarian Section	5 (3.1%)	-	2 (3.5%)
Breech or abnormal presentation	7 (4.9%)	3 (2.9%)	7 (5.2%)
Mid to high forceps	19(13.2%)	14 (13.7%)	14 (22.6%)
Incubator > 4 weeks	3 (2.1%)	1 (1%)	3 (4.7%)

TABLE 6.3 Individual Obstetric Events on Lewis-Murray Scale. I did not rate as a “Definite” OCs labour lasting less than 3 hours.

6.5.3 Case- Control Prediction Analyses

I applied logistic regression to analyse if OCs significantly increased the risk of psychosis, while controlling for potential confounders such as age and gender (FIGURE 5.1). To maximise statistical power siblings and the healthy controls were collapsed into one group of “controls/siblings”

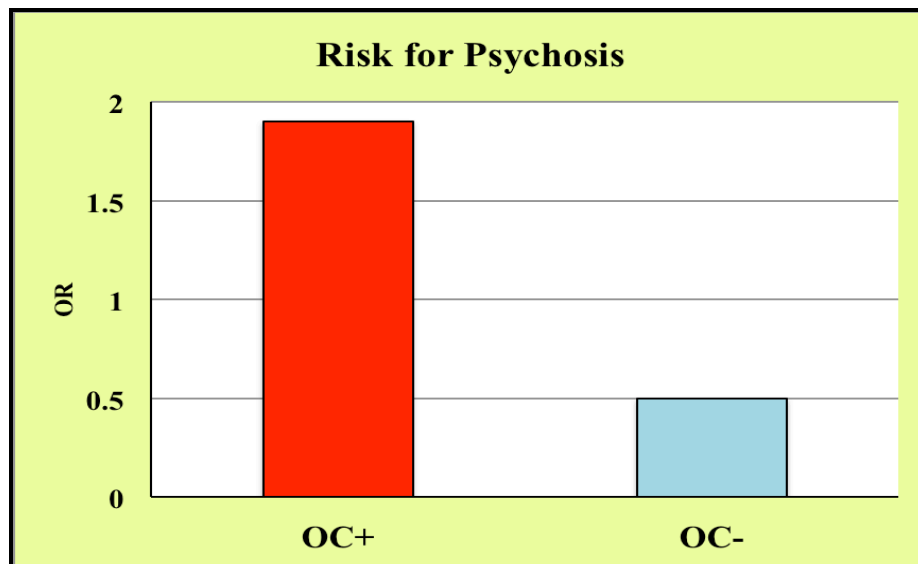


FIGURE 6.1 Adjusted OR of Psychosis for Subjects with OCs.

As expected there was a statistical difference in the rate of definite complications in the patient group when compared to the “controls/siblings” group: patients were 2 times more likely to have had an OC (Adj OR = 1.88; 95% CI 1.36 -3.41; $p=0.038$).

6.6 Summary of Results

In our sample patients were more likely than non- psychotic individuals to report an OC. The case-control logistic regression analysis showed that the people who had experienced OCs had a significantly increased risk of psychotic disorder compared to those who didn't.

This study reports an effect for OCs in increasing the risk for psychosis of about 1.9 similar to the widely reported risk of 2.0 for schizophrenia (Cannon et al., 2002).

6.7 Discussions

It is now established that the risk of developing schizophrenia is increased in individuals who were exposed to various complications during pregnancy (Cannon et al., 2002). Moreover recent epidemiological studies further document the relationship between obstetric complications and psychosis-like symptoms or deficit in cognitive function (Zammit et al., 2009). My study confirms that patients are more likely than non-psychotic individuals to report an OC, with an effect for OCs in increasing the risk for psychosis of about 1.9, similar to the widely reported risk of 2.0 for schizophrenia (Cannon et al., 2002).

OCs increase risk of schizophrenia but not everyone that experienced OCs develops psychosis later in life. It was of interest that in my study the siblings had the lowest prevalence of OCs. It is possible that in families carrying some genetic risk of schizophrenia, to remain well, siblings need to have less exposure to OCs. A difficult challenge is to identify plausible susceptibility genes to investigate how, and if, they moderate the effect of OCs on the risk of psychosis.

In order to get a better insight into the mechanisms by which OCs impact on brain development to increase the risk of psychosis, in the next three chapters (**CHAPTER 7, 8 and 9**) I tested, under a multiplicative model, the hypothesis that a range of genetic variants interacted with OCs in increasing the risk of psychotic disorder. It was also

suggested that OCs themselves alter the function, structure and consequently the expression of genes which could be another route resulting in schizophrenia, thus in **CHAPTER 10** I examined whether rats that had experienced perinatal asphyxia during birth show abnormalities in gene expression and methylation status at various developmental periods.

6.8 Limitations

6.8.1 Sample Size

The sample size is small and many of the individuals obstetric complications are uncommon. Moreover unless a specific complication could be reasonably ruled out, items were left as unknown and records were not used at all if they could not supply information for at least 10 items from the scale. It is possible that a larger sample size may have detected an increased risk for psychosis due to specific complications that the present study did not have sufficient power to assess. In addition, to maximise statistical power siblings and the healthy controls were collapsed in one group of “controls”. It was of interest that the siblings had the lowest prevalence of OCs. This may have lowered the overall prevalence in my combined “controls/siblings” group. However, this should not interfere with the main purpose of this Thesis, which is to examine Gene x Environment Interactions

6.8.2 Retrospective Data

The use of retrospective maternal reporting of obstetric data has been questioned as potentially unreliable. Potential biases include cognitive impairment or schizotypal personality traits often found in relatives of schizophrenic (Buka et al; 2000); a tendency to false positive responses (Cantor- Graae et al., 1998; McIntosh et al., 2002); increased parity, level of education and lower social class (Hoekelman et al., 1976; Sheehan and MacAirt, 1981; Gayle et al., 1988). Moreover mothers of schizophrenic patients tend to underestimate the rate of OCs in their affected offspring compare to controls (Cantor- Graae et al., 1998; Buka et al., 2000).

Studies assessing the validity of maternal recall compared to birth records have shown different results (Walshe et al., 2011). As reviewed by Walshe et al. (2011) two studies both reported good agreement between retrospective maternal recall and contemporaneous birth records using the Lewis–Murray scale (O’ Callaghan et al., 1990; Franzek and Stober, 1995). Other studies have found that mothers were able to recall OCs decades after birth (Buka et al., 2004; Sou et al., 2006) and the sensitivity of maternal recall improves for severe or acute events (Filippi et al., 2000) especially for those occurring in the perinatal period (Yawn et al., 1998). In contrast to these findings, Cantor-Graae et al. (1998) and McIntosh et al., (2002) reported considerable discrepancies between maternal interviews and birth records.

As explained by Walshe et al. (2011), one major methodological difference which may account for this disagreement is the type of obstetric complication scale used such as The Lewis–Murray scale (Lewis and Murray, 1987) and the McNeil-Sjostrom scale (McNeil et al., 1995), used in the studies of Cantor-Graae et al. (1998) and McIntosh et al. (2002).

As explained in **CHAPTER 5**, the Lewis-Murray scale (Lewis and Murray, 1987) consists of 17 individual items and each item is evaluated as present or absent. In contrast, the McNeil-Sjostrom scale (McNeil et al., 1995) rates hundreds of pregnancy, delivery and neonatal events on a range of severity. Using this scale, 60–80% of non-schizophrenic individuals tend to be rated as having experienced a clearly potentially harmful OC (Cantor-Graae et al., 1998; Gunduz et al., 1999; Jablensky et al., 2005; Verdoux et al., 2002), twice the rate of “definite complications” identified using the Lewis–Murray scale (Jones et al., 1998; Walshe et al., 2005; reviewed by Walshe et al., 2011).

While the Lewis-Murray scale scoring system lacks exactitude, it maximizes statistical power in studies that are limited by sample size from examining individual complications. Mothers are much better at remembering some events than others but it is likely that overall are more likely to remember relatively uncommon events requiring medical attention (Walshe et al., 2011).

Clearly, birth records represent the “gold standard” for collecting obstetric history although largely unstandardized and lacking sufficient detail (Hewson and Bennett, 1987; Buka et al., 2004). However, given the difficulty of accurately acquiring such records in most countries, the use of a maternal recall scale such as the Lewis–Murray scale remains valuable in schizophrenia research, for example to explore the association of significant obstetric complications with other risk factors and neurobiological traits in samples for whom prospective data is not available.

CHAPTER 7

Genes Regulated by Hypoxia and Psychosis

7.1 Introduction

It is likely that genes play an important role in mediating the reactions of the CNS to environmental stimuli such hypoxia. A difficult challenge is to identify plausible susceptibility genes to investigate how, and if, they moderate the effect of OCs on the risk of psychosis.

As described in the Introduction, in 2006 a systematic review suggested that more than 50% of genes associated with schizophrenia are subject to regulation by hypoxia and/or are expressed in the vasculature (Schmidt-Kastner et al., 2006). Nicodemus and colleagues (2008) examined 13 of these genes in a sample of 119 family-trios (affected individuals and their unaffected parents). They reported significant evidence for genes involved in neurovascular function or regulated by hypoxia interacting with the presence of serious OCs to increase risk for schizophrenia. Thus, AKT1, BDNF, DTNBP1 and GRM3 showed significant evidence for gene-by-environment interaction (LRT P-values ranged from 0.011 to 0.037) (Nicodemus et al., 2008).

They hypothesized that causal variants that reduced expression levels of the neuroprotective products of AKT1, BDNF, GRM3 and DTNBP1 genes may lead to vulnerability to hypoxic insult during neurodevelopment (Nicodemus et al., 2008).

So far only two small studies have examined the interaction of specific candidate genes with serious OCs in the development of schizophrenia (Nicodemus et al., 2008; Joo et al., 2009). Thus, this interaction model has not yet been fully empirically

tested. In this study I selected genetic variants of 4 genes, which are regulated by hypoxia, namely AKT1, BDNF, DTNMBP1, GRM3, to replicate the interaction with OCs in increasing the risk of psychosis which Nicodemus et al (2008) found.

7.2 V-Akt Murine Thymoma Viral Oncogene Homolog 1 (Akt1): rs1130233, rs2494735 and rs3803300.

The first candidate to test for a gene x OCs interaction is the Akt1 gene, located on chromosome 14q32.32. Akt1 gene variants have been associated with schizophrenia and bipolar disorders (Toyota et al., 2003; Emamian et al., 2004; Schwab et al., 2005; Thiselton et al., 2008; Mathur, et al. 2010; Van Winkel, 2011; Karege et al., 2012), though some authors failed to replicate these results (Ohtsuki et al., 2004; Lee et al., 2010).

The serine-threonine protein kinase encoded by the Akt1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. Akt1 and the related Akt2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of Akt1. The activation occurs through phosphatidylinositol 3-kinase (FIGURE 7.1).

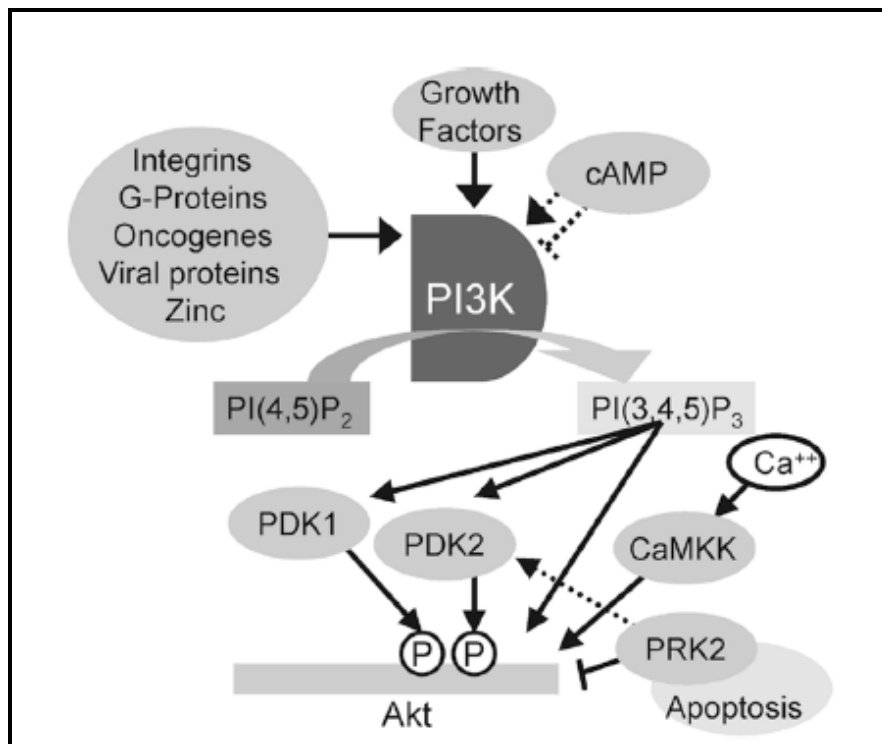


FIGURE 7.1 The Upstream Signaling Mechanisms Regulating Akt Activity. Taken from Kim and Chung, 2002.

In the developing nervous system Akt is a critical mediator of growth factor-induced neuronal survival (Dudek et al., 1997; Kim and Chung, 2002). Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase Akt1, which then phosphorylates and inactivates components of the apoptotic machinery (Kim and Chung, 2002) (**FIGURE 7.2**).

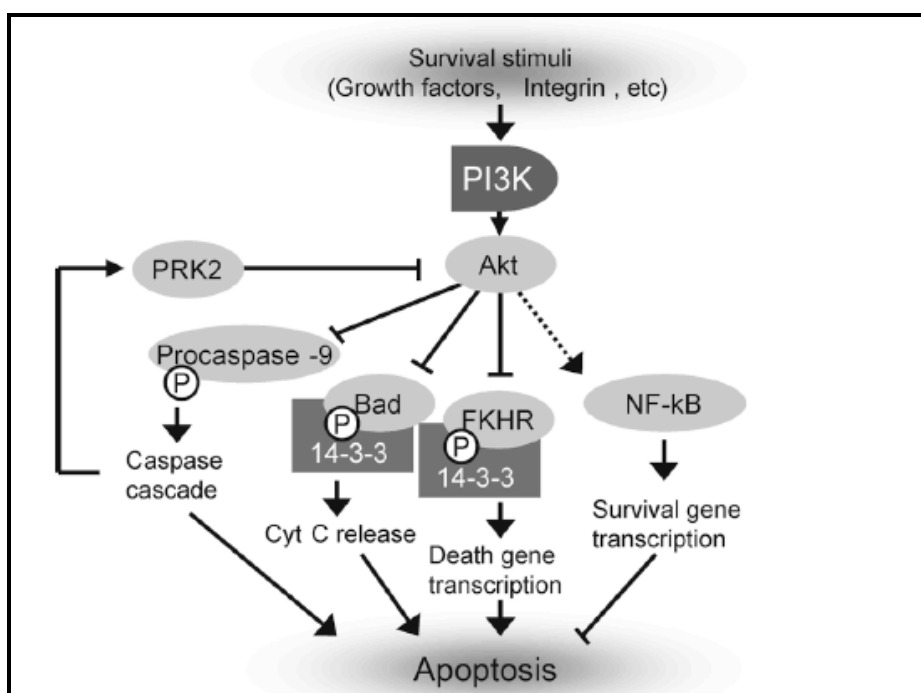


FIGURE 7.2 Inhibition of Apoptosis by Akt. Taken from Kim and Chung, 2002.

Moreover, Akt1 has been shown to facilitate dopamine signaling (Beaulieu et al., 2009) and is an important regulator of glycogen synthase kinase-3 β (GSK- 3 β), a protein known to be involved in both mood disorder and psychosis (Grimes and Jope, 2001).

Increased Akt1 has been reported in focal brain ischemia (Kitagawa et al., 1999), and a neuroprotective role of Akt1 activation has been proposed (Yin et al., 2005). Akt1 also participates in the HIF-1 signaling pathway (Zhong et al., 2000).

In their study, Nicodemus et al. (2008) concluded that those who carried a particular allele of one of three polymorphisms of the Akt1 gene, namely rs1130233, rs3803300 and rs2494735, were especially at risk of schizophrenia when exposed to OCs (Nicodemus et al., 2008).

In light of these findings, I followed a hypothesis driven approach limiting the GXE analyses to rs1130233, rs3803300 and rs2494735 polymorphisms of the Akt1 gene.

7.3 Brain-Derived Neurotrophic Factor (BDNF): rs2049046 and rs56164415.

Brain-derived neurotrophic factor (BDNF), a neurotrophin known to be responsible for development, regeneration, survival and maintenance of neurons (FIGURE 7.3) has been implicated in the pathophysiology of schizophrenia in some but not all studies (Sklar et al., 2002; Neves-Pereira et al., 2002; Hong et al., 2003; Geller et al., 2004; Oswald et al., 2004; Skibinska et al., 2004). In humans this gene is located on chromosome 11p13.

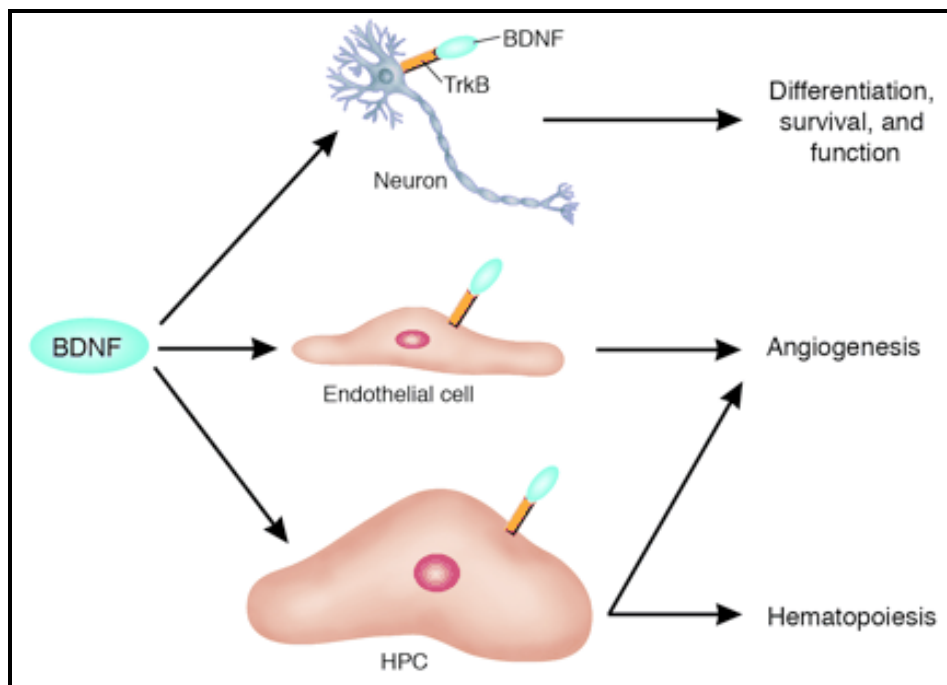


FIGURE 7.3 BDNF Mechanism in Neurons. Taken from Hempstead, 2004.

BDNF is protective against brain ischemia (Schabitz et al., 1997; Schmidt-Kastner et al., 2006). This gene is upregulated by ischemia (Lindvall et al., 1992; Schmidt-Kastner et al., 2001) and is expressed in neurons and endothelial cells during development (Leventhal et al., 1999; Schmidt-Kastner et al., 2006).

Nicodemus and colleagues found that two SNPs in BDNF, rs2049046 and ss76882600 (renamed as rs56164415), showed significant evidence for serious OC by

SNP interaction; probands who suffered a serious obstetric complication were preferentially transmitted the major allele at rs2049046 (OR= 0.15; 95% CI= 0.032, 0.73; LRT P-value = 0.011; OR P-value = 0.019) whereas the opposite was observed at ss76882600 (OR= 12.45; 95% CI= 1.63, 94.6; LRT P-value = 0.028; OR P-value = 0.015) (Nicodemus et al., 2008).

In light of these findings, I attempted to replicate the GXE analyses looking at rs2049046 and rs56164415 polymorphisms of the BDNF gene (Nicodemus et al., 2008).

7.4 Dysbindin, Dystrobrevin-Binding Protein 1 (DTNBP1): rs875462.

Dysbindin, which binds dystrobrevin, is found expressed in the vasculature of muscles by immunolabeling, and a complex containing dysbindin has been discussed for cranial blood vessels (Benson et al., 2001; Schmidt-Katstner, et al., 2006).

DTNBP1 is expressed in the vasculature (Schmidt-Kastner et al., 2006), and is located on chromosome 6p22.3. Dystrobrevin is expressed in endothelial cells and perivascular glia (Schmidt-Kastner et al., 2006). Dysbindin appears to have

neuroprotective functions (Numakawa et al., 2004; Schmidt-Kastner et al., 2006).

Early evidence indicated that dysbindin was one of the most promising candidate genes for schizophrenia though some more recent studies have been less positive (reviewed by Williams et al., 2005) (FIGURE 7.4).

Study	Population	rs760666	rs2619539	rs3213207	rs1011313	rs2619528	rs2005976	rs760761	rs2619522	rs1018381	rs909706	rs2619538
Straub, Jiang, MacLean, et al., 2002; van den Oord, Sullivan, Jiang, et al., 2003	Irish	C	G	G	C	A	A	T	C	C	C	
Schwab et al., 2003 ^a	German and Israeli				C			C				
Van Den Bogaert, Schumacher, Schulze, et al., 2003	Swedish									T		
Funke, Finn, Plocik, et al., 2004 ^b	Caucasian									T		
Kirov, Ivanov, Williams, et al., 2004	Bulgarian			A								
Williams, Preece, Morris, et al., 2004	United Kingdom/Irish		C	A								T
Morris, McGhee, Schwaiger, et al., 2003; Williams, Preece, Morris, et al., 2004	Irish		C	A								T
Tang, Zhou, Fan, et al., 2003	Chinese		G						T	C	A	
Numakawa, Yagasaki, Ishimoto, et al., 2004	Japanese			G	C			T	C			A

FIGURE 7.4 Complex Pattern of Genetic Association at DTNBP1. Taken from Williams et al., 2005.

In particular one SNP in DTNBP, namely rs875462, was found to show preferential transmission of the minor allele to probands who experienced serious OCs (OR= 9.49; 95% CI = 1.23, 73.3; LRT P-value = 0.025; OR P-value = 0.031).

In the present study I attempted further replication of the rs875462 gene polymorphism of DTNBP1 (Nicodemus et al., 2008).

7.5 Metabotropic Glutamate Receptor 3 (GRM3): rs7808623.

The metabotropic glutamate receptors are a family of G protein-coupled receptors located on 7q21.1-q21.2 chromosome. They have been divided into 3 groups on the basis of sequence homology, putative signal transduction mechanisms, and pharmacologic properties. Group I includes GRM1 and GRM5 and these receptors have been shown to activate phospholipase C. Group II includes GRM2 and GRM3 while Group III includes GRM4, GRM6, GRM7 and GRM8. Group II and III receptors are linked to the inhibition of the cyclic AMP cascade but differ in their

agonist selectivities.

Agonists of the metabotropic glutamate receptor mGlu2/3 (encoded by GRM3) are reported to exert neuroprotective effects (Bond et al., 2000; Schmidt-Kastner et al., 2006). GRM3 mRNA is down regulated by brain ischemia (Raghavendra Rao et al., 2002; Lu et al., 2004; Schmidt-Kastner et al., 2006).

Some but not all studies find genetic association of GRM3 polymorphisms with psychosis (reviewed by Harrison et al., 2008) (FIGURE 7.5).

Study	Design	Sample size	Population	SNPs studied (rs number)	Association with schizophrenia
Marti, <i>et al.</i> (2002)	CC	265S, 227C	German	2228595	T allele associated ($P = 0.0022$)
	CC/F	288S, 162C, 128T	German	2228595	None
Fujii, <i>et al.</i> (2003)	CC	100S, 100C	Japanese	274622, 724226, 917071, 1468412, 1989796, 1476455	rs1468412 A allele associated ($P = 0.011$); three-marker haplotype (274622-1468412-1989796) associated ($P = 0.0008$)
Egan, <i>et al.</i> (2004)	F	217S, 311SI, 262P, 136C	Caucasian, USA	187993, 13242038, 917071, 6465084, 228595, 1468412, 7804100	rs6465084 A allele associated ($P = 0.02$); rs917071 C allele, associated ($P = 0.06$). Overtransmission of several haplotypes including rs6465084 (best global $P = 0.006$)
	F	67F	Caucasian, USA	Same seven SNPs	rs917071 C allele associated ($P = 0.07$)
	F	51F	AA	Same seven SNPs	rs1468412 T allele associated ($P = 0.03$).
Chen, <i>et al.</i> (2005)	CC	752S, 752C	Chinese	274622, 2282958, 724226, 757656, 2237562, 1468412, 2299225	rs2299225 C allele associated ($P = 0.03$); three-marker haplotype (2237562-1468412-2299225) associated ($P = 0.002$)
Fallin, <i>et al.</i> (2005)	F	274 trios	Ashkenazi Jew	10 SNPs	No SNP or haplotype associations
Norton, <i>et al.</i> (2005)	CC	674S, 716C	Caucasian, UK	187993, 13242038, 917071, 6465084, 2228595, 1468412, 7804100	No SNP or haplotype associations
Todhigi, <i>et al.</i> (2006)	CC	402S, 468C	Japanese	274622, 2282960, 2299214, 917071, 757656, 6465084, 2237562, 1468412, 2299225, 7781414	No SNP or haplotype associations
Schwab, <i>et al.</i> (2006)	F	Not stated	Not stated	rs2237562, and four others	rs2237562 associated ($P = 0.01$).

^aCC, case-control; F, family; S, schizophrenia cases; C, control subjects; T, trios; SI, siblings of patients; P, parents of patients; AA, African American.

FIGURE 7.5 GRM3 Genetic Associations with Schizophrenia. Taken from Harrison et al., 2008.

Nicodemus et al. (2008) found that one SNP in GRM3, rs7808623, showed preferential transmission of the minor allele to probands who experienced serious OCs (OR= 3.39, 95% CI= 0.95, 12.17; LRT P-value = 0.035; OR P-value = 0.061) (Nicodemus et al., 2008).

For the present study I attempted further replication of the interaction between OCs and rs7808623 gene polymorphism of GRM3 in increasing the risk for schizophrenia (Nicodemus et al., 2008).

7.6 Aims

I aim to:

1. Test if each of the selected genetic variants is associated with an increased risk of psychotic disorders or the presence of OCs.
2. Test if each of the selected genetic variants interacts with exposure to OCs to moderate the risk of psychotic disorders.

7.7 Hypotheses

I expect the following SNPs to interact with exposure to OCs in increasing the risk of psychotic disorders:

1. AKT1 rs1130233 “G” allele
2. AKT1 rs2494735 “C” allele
3. AKT1 rs3803300 “G” allele.
4. BDNF rs2049046 “T” allele.
5. BDNF rs56164415 “A” allele.
6. DTNBP1 rs875462 “G” allele.
7. GRM3 rs7808623 “T” allele .

7.8 Methods

The presence of OCs was assessed through maternal interview on 313 subjects, comprising 145 psychotic cases, 103 unaffected siblings and 65 healthy controls.

7.8.1 Assessment

Subjects were given a wide range of assessments, and donated blood for genetic analysis. OCs was retrospectively collected from mothers of each participant using the Maternal Schedule Interview (MIS) and coded using the Lewis-Murray scale as explained in details in CHAPTER 5.

7.8.2 Genotyping

DNA was obtained from 262 subjects in total (127 cases, 95 siblings, 40 controls) from either blood (75% of subjects) or from cheek swab samples (25% of subjects). AKT1 rs1130233, rs3803300 and rs2494735 alleles, BDNF rs2049046 and rs56164415 alleles, DTNBP1 rs875462 allele and GRM3 rs7808623 allele were chosen as the genetic variants.

DNA was extracted using a standard phenol-chloroform extraction procedure.

TaqMan® Gene Expression Assays Protocol was used to conduct the entire genotyping following the manufacturer's instructions (**FIGURE 7.6**).

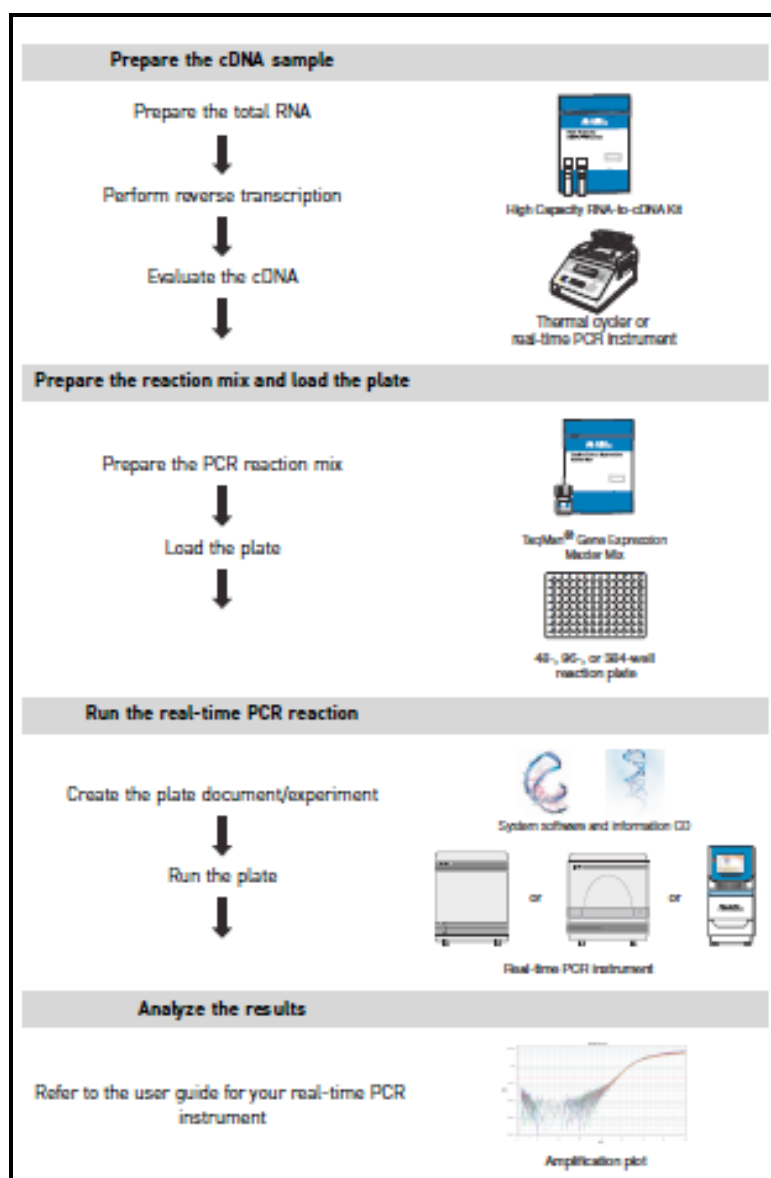


FIGURE 7.6 TaqMan® Gene Expression Assays Protocol.

After an initial Taq polymerase activation/DNA denaturation step, samples were subjected to PCR reaction following standard Applied Biosystems dry DNA protocol. Amplification products were analyzed using the Applied Biosystems 7900HT Fast Real-time PCR System. Genotype calls were made based on a clustering algorithm with quality value of 95%.

Presence of each allele is marked by color and position on a generated plot due to level of fluorescence presented in each allele. Homozygotes are displayed on extremities of the Y and the X axes whereas heterozygotes are displayed in the middle. Allele discrimination can be visualized in a plot showing the two alleles and the distribution of the three genotypes, the two homozygous and the heterozygous one (FIGURE 7.7).

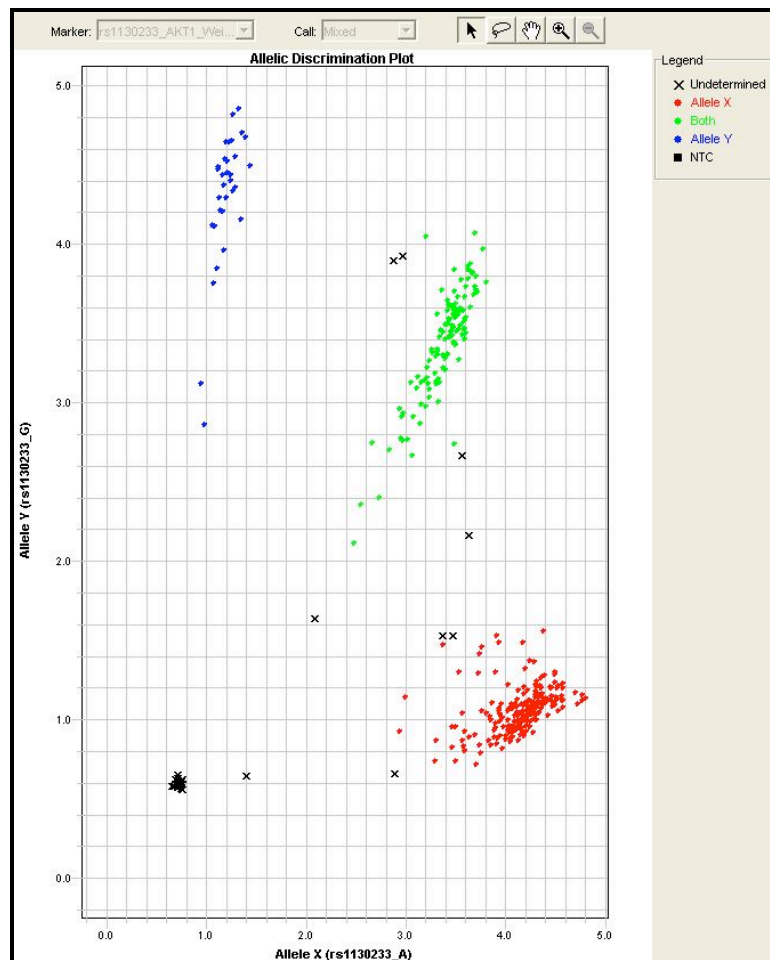


FIGURE 7.7 Allelic Discrimination Plot Resulting from the TaqMan Gene Expression Assay Procedure. The two alleles are shown in the axis and in different colours are shown the three different genotypes: blue colour homozygous -Y allele-, red colour homozygous- X allele-, green colour heterozygous -both alleles-.

7.8.3 Sample Size and Power Calculation

In order to have 80% power and a significance level of 0.05% (two-tailed) a sample of 355 case-control pairs is required to be able to detect an OR= 2. This assumes for an exposure of 30% among the general population (Cannon et al., 2002) and with a mean allele frequency of 0.5. Overall I collected genetic data on 262 subjects of the original 313 (51 refused to give blood or cheek swabs); with a sample of 127 patients and 135 controls (95 unaffected siblings and 40 healthy controls). Thus, I have the 80% power of detecting an interaction of OR>4.5 with 95% confidence in our population. These calculations have been made using the Quanto 1.2.4 software using the gene-environment module (John Morrison and W. James Gauderman at the University of Southern California).

7.8.4 Statistical Analysis

Data was recorded in IBM SPSS version 20.0 and analysed in the software package UNPHASED 3.1.7 (Dudbridge, 2008) as a way of combining related and unrelated individuals.

Based on the existing literature and on the findings reported in the previous chapter, I used history of OCs as the environmental exposures of interest. As explained in **CHAPTER 5**, the Lewis-Murray Scale (Lewis and Murray, 1987) was used as the principal measure of OCs; each item was evaluated as definite (score 1), or absent (score 0). Subjects were rated as having a “definite” (score 1) complication if they had suffered at least one significant complication (Lewis et al., 1989).

As discussed earlier, AKT1 rs1130233, rs3803300 and rs2494735 alleles, BDNF rs2049046 and rs56164415 alleles, DTNBP1 rs875462 allele and GRM3 rs7808623 alleles were chosen as the genetic variants.

Hardy-Weinberg equilibrium (HWE) test of SNP was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (Wigginton and Abecasis, 2005).

χ^2 tests and t-tests (or ANOVA) were used to test for associations between the potential confounding variables and the genotype. Further χ^2 tests were carried out to establish whether OCs were more likely to occur in individual carriers of a particular genotype class (a Gene x Environment correlation).

Each SNPs association was investigated using a likelihood-based association test for nuclear families and unrelated subjects under the assumption of Hardy-Weinberg Equilibrium (HWE), implemented in the software package UNPHASED 3.1.7 (Dudbridge, 2008). Forward stepwise regression within each SNPs was applied to determine independent association signals. Using UNPHASED, for each SNP, one marker at a time was tested for association to case/control status and the presence of OCs. In order to determine whether there was a gene x environment interaction among the SNPs investigated and OCs, effect modification by OCs was explored. Adjustment for multiple testing was performed using Bonferroni and Holm (Holm, 1979) correction, and I considered SNPs with $p \leq 0.007$ to be significantly associated with OCs.

7.9 Results

7.9.1 AKT1 x OCs Interaction and Risk of Psychosis

7.9.1a rs1130233 x OCs

I obtained AKT1 rs1130233 genotyping data on 112/127 cases, 86/95 siblings and 37/40 controls, with an overall call rate of 89.7% (**FIGURE 7.8 and 7.9**). The AKT1 rs1130233 polymorphism allele was in HWE (**TABLE 7.1**).

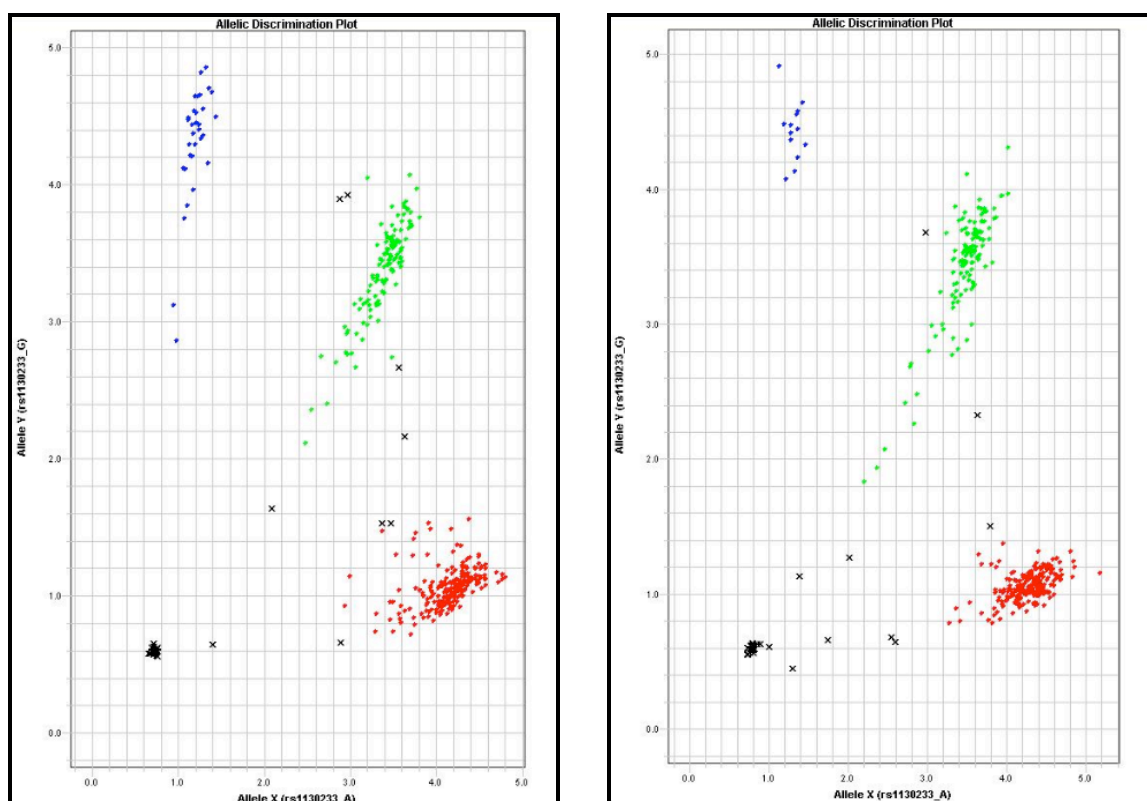


FIGURE 7.8 and 7.9 TaqMan AKT1 rs1130233 Allelic Discrimination Plot. Blue colour homozygous -G allele-, red colour homozygous- A allele-, green colour heterozygous -AG alleles-.

		Cases n= 112	Siblings n= 86	Controls n= 37	HWE
AKT1 rs1130233 Allelic frequency	A/A	61 (54.5%)	46 (53.5%)	25 (67.6%)	p= 1
	A/G	46 (41.4%)	30(34.9%)	11 (29.7%)	
	G/G	5 (4.5%)	10(11.6%)	1 (2.7%)	

TABLE 7.1 AKT11 rs1130233 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in AKT1 rs1130233 allelic distribution by gender ($\chi^2=1.88$; $p=0.39$).

There was no evidence of a correlation between the AKT1 rs1130233 genotype and history of OCs ($\chi^2=1.02$, $p=0.31$) or with increased likelihood of a psychotic disorder ($\chi^2=0.06$, $p=0.80$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs1130233 genotype. Among those who experienced OCs, there was no significant change in individual risk of psychosis according to AKT1 rs1130233 genotype (OR=1.28, 95% CI 0.76-2.16, $p=0.35$).

These findings indicate that AKT1 rs1130233 genotype doesn't modify the effect of OCs on the risk of a psychotic disorder.

7.9.1b rs2494735 x OCs

I obtained AKT1 rs2494735 genotyping data on 110/127 cases, 87/95 siblings and 34/40 controls, with an overall call rate of 88.2% (**FIGURE 7.10 and 7.11**). The AKT1 rs2494735 polymorphism allele was in HWE (**TABLE 7.2**).

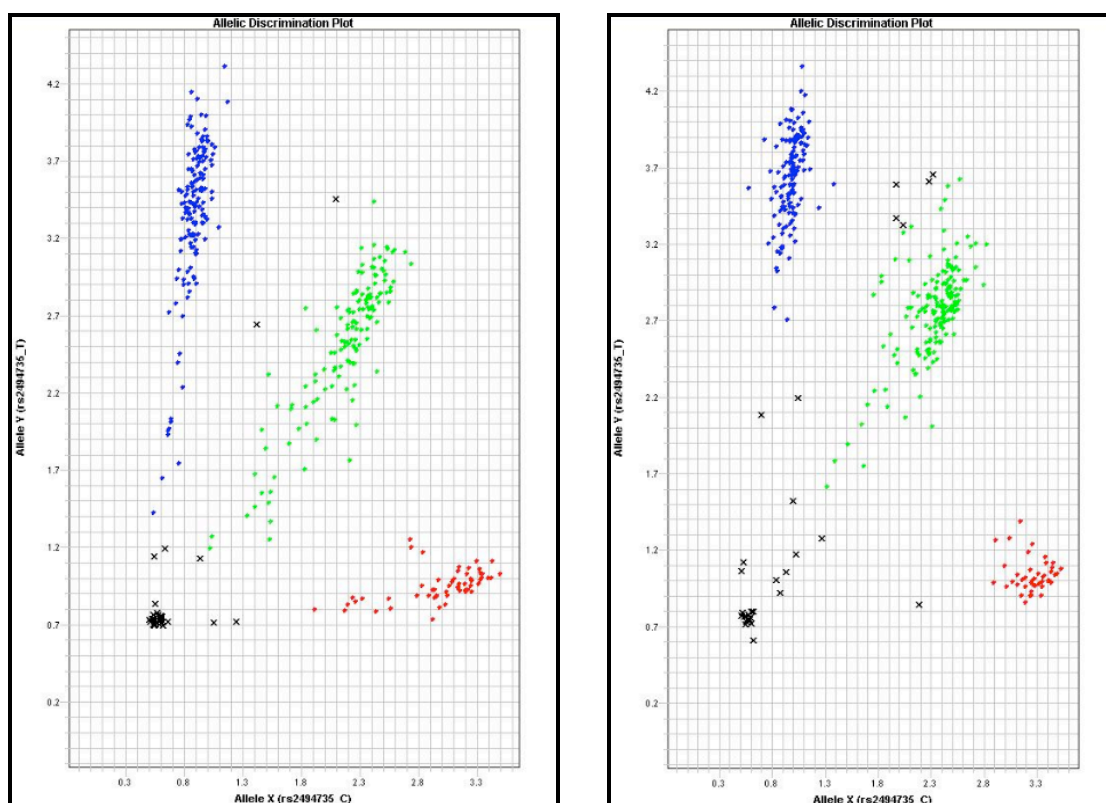


FIGURE 7.10 and 7.11 TaqMan AKT1 rs2494735 Allelic Discrimination Plot. Blue colour homozygous -T allele-, red colour homozygous- C allele-, green colour heterozygous -CT-.

		Cases n= 110	Siblings n= 87	Controls n= 37	HWE
AKT1 rs2494735 Allelic frequency	C/C	16 (14.5%)	17 (19.5%)	25 (67.6%)	p= 0.44
	C/T	55 (50%)	33 (37.9%)	11 (29.7%)	
	T/T	39 (35.5%)	37 (42.5%)	1 (2.7%)	

TABLE 7.2 AKT11 rs2494735 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in AKT1 rs2494735 allelic distribution by gender ($\chi^2=0.94$; $p=0.62$),

There was no evidence of a correlation between the AKT1 rs2494735 genotype and history of OCs ($\chi^2=3.12$, $p=0.08$) or with increased likelihood of a psychotic disorder ($\chi^2=2.48$, $p=0.11$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs2494735 genotype. Among those who experienced OCs, there was no significant change in individual risk of psychosis according to AKT1 rs2494735 genotype (OR=1.40, 95% CI 0.88-2.21, $p=0.15$).

These findings indicate that AKT1 rs2494735 genotype does not modify the effect of OCs on the risk of a psychosis.

7.9.1c rs3803300 x OCs

I obtained AKT1 rs3803300 genotyping data on 103/127 cases, 81/95 siblings and 36/40 controls, with an overall call rate of 84% (**FIGURE 7.12 and 7.13**). The AKT1 rs3803300 polymorphism was in HWE (**TABLE 7.3**).

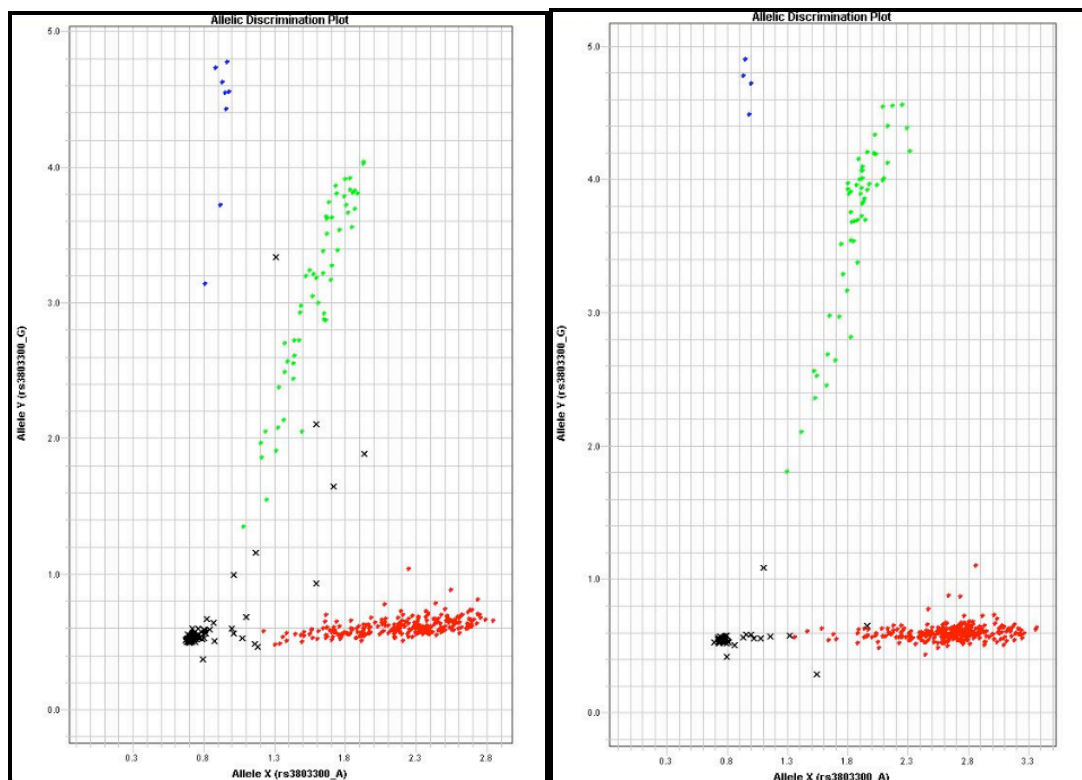


FIGURE 7.12 and 7.13 TaqMan AKT1 rs3803300 Allelic Discrimination Plot. Blue colour homozygous -G allele-, red colour homozygous- A allele-, green colour heterozygous -AG alleles-.

		Cases n= 103	Siblings n= 81	Controls n= 36	HWE
AKT1 rs3803300 Allelic frequency	A/A	86 (83.5%)	62 (76.5%)	32 (88.9%)	p= 0.10
	A/G	17 (16.5%)	16 (19.8%)	3 (8.3%)	
	G/G	0 (0%)	3 (3.7%)	1 (2.8%)	

TABLE 7.3 AKT11 rs3803300 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in AKT1 rs3803300 allelic distribution by gender ($\chi^2=2.69$; $p=0.26$).

There was no evidence of a correlation between the AKT1 rs3803300 genotype and history of OCs ($\chi^2=0.34$, $p=0.56$) or with increased likelihood of a psychotic disorder ($\chi^2=0.05$, $p=0.82$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs3803300 genotype. Among those who experience OCs, there was no significant change in individual risk of psychosis according to AKT1 rs3803300 genotype (OR=1.28, 95% CI 0.63-2.58; $p=0.49$).

These findings indicate that the AKT1 rs3803300 genotype doesn't seem to modify the effect of OCs on the risk of a psychotic disorder.

7.9.2 BDNF x OCs Interaction and Risk of Psychosis

7.9.2a rs2049046 x OCs

I obtained BDNF rs2049046 genotyping data on 100/127 cases, 78/95 siblings and 31/40 controls, with an overall call rate of 79.8% (FIGURE 7.14 and 7.15). The BDNF rs2049046 polymorphism was in HWE (TABLE 7.4).

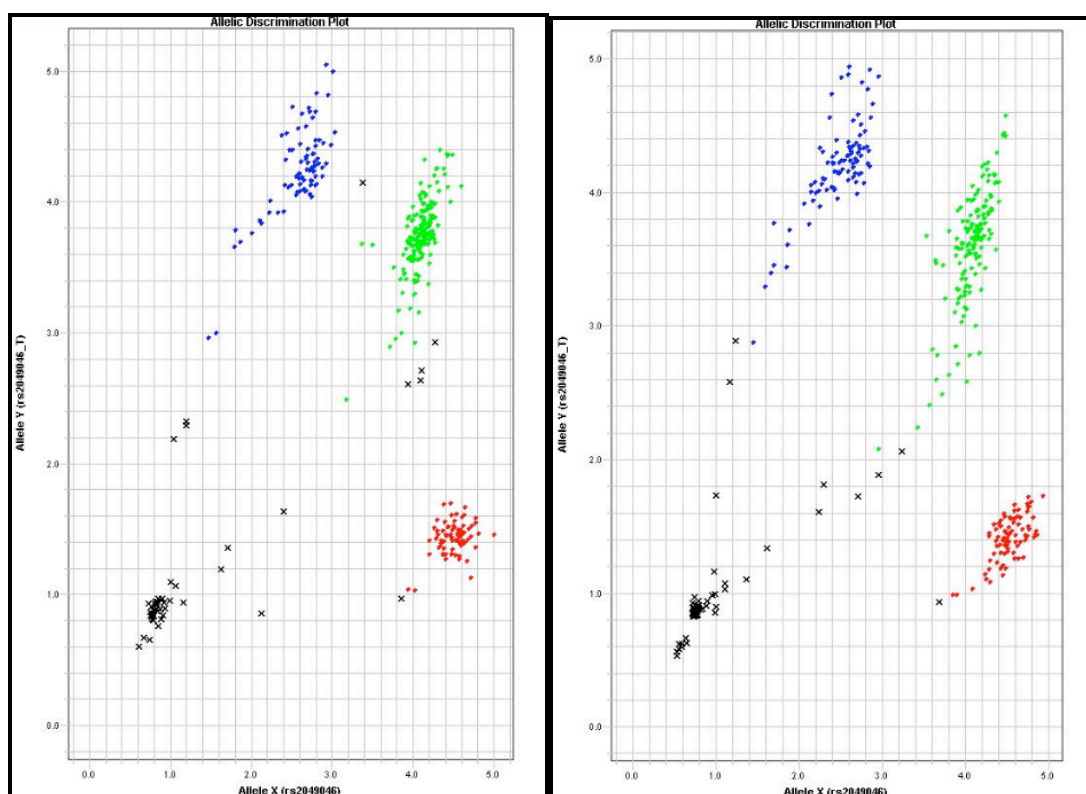


FIGURE 7.14 and 7.15 TaqMan BDNF rs2049046 Allelic Discrimination Plot. Blue colour homozygous -T allele-, red colour homozygous- A allele-, green colour heterozygous -AT alleles-.

		Cases n= 100	Siblings n= 81	Controls n= 36	HWE
BDNF rs2049046 Allelic frequency	A/A	24 (24%)	25 (32.1%)	6 (19.4%)	p= 0.90
	A/T	45 (45%)	35 (44.9%)	20 (64.5%)	
	T/T	31 (31%)	18 (23.1%)	5 (16.1%)	

TABLE 7.4 BDNF rs2049046 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in BDNF rs2049046 allelic distribution by gender ($\chi^2=1.57$; $p=0.46$).

There was no evidence of a correlation between the BDNF rs2049046 genotype and history of OCs ($\chi^2=1.50$, $p=0.22$) or with increased likelihood of a psychotic disorder ($\chi^2=0.08$, $p=0.78$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the BDNF rs2049046 genotype. Among those who experience OCs, there was no significant change in individual risk of psychosis according to BDNF rs2049046 genotype (OR= 1.17 95% CI 0.76-1.79, $p=0.48$).

These findings indicate that the BDNF rs2049046 genotype doesn't seem to modify the effect of OCs on the risk of a psychotic disorder.

7.9.2b rs56164415 x OC

I obtained BDNF rs56164415 genotyping data on 107/127 cases, 83/95 siblings and 38/40 controls, with an overall call rate of 87% (**FIGURE 7.16 and 7.17**). The BDNF rs56164415 polymorphism was in HWE (**TABLE 7.5**).

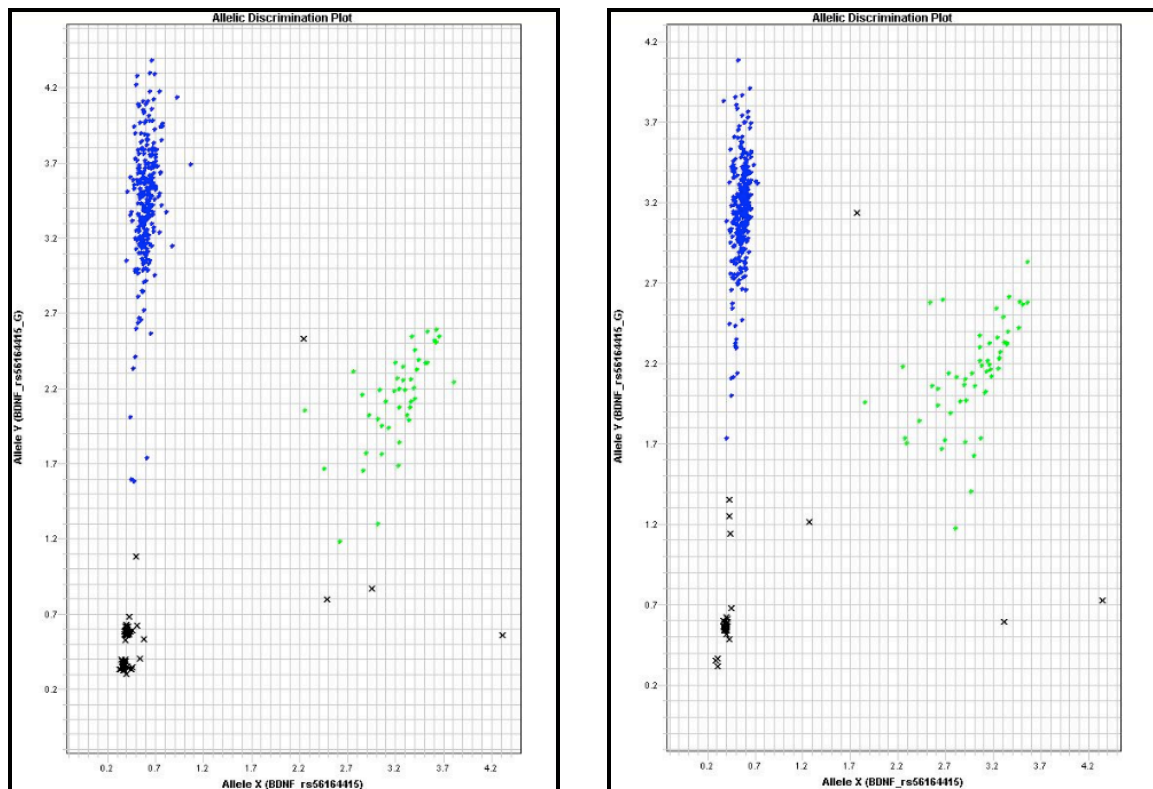


FIGURE 7.16 and 7.17 TaqMan BDNF rs56164415 Allelic Discrimination Plot. Blue colour homozygous –G allele-, red colour homozygous- A allele-, green colour heterozygous -AG alleles-.

		Cases n= 100	Siblings n= 81	Controls n= 36	HWE
BDNF	A/A	1 (0.9%)	0 (0%)	1 (0%)	p= 0.58
rs56164415	A/G	11 (10.3%)	10 (12%)	11 (10.5%)	
Allelic frequency	G/G	95 (88.8%)	73 (88%)	95 (89.5%)	

TABLE 7.5 BDNF rs56164415 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in BDNF rs56164415 allelic distribution by gender ($\chi^2=2.57$; $p=0.28$).

There was no evidence of a correlation between BDNF rs56164415 genotype and history of OCs ($\chi^2=0.97$, $p=0.32$) or with increased likelihood of a psychotic disorder ($\chi^2=0.23$, $p=0.63$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the BDNF rs56164415 genotype. Among those who experience OCs, there was no significant change in individual risk of psychosis according to BDNF rs56164415 genotype (OR= 0.93, 95% CI 0.35-2.52, $p=0.89$).

These findings indicate that BDNF rs56164415 genotype doesn't seem to modify the effect of OCs on the risk of a psychotic disorder.

7.9.3 DTNBP1 rs875462 x OCs Interaction and Risk of Psychosis

I obtained DTNBP1 rs875462 genotyping data on 85/127 cases, 83/95 siblings and 34/40 controls, with an overall call rate of 77% (**FIGURE 7.18 and 7.19**). The DTNBP1 rs875462 polymorphism allele was in HWE (**TABLE 7.6**).

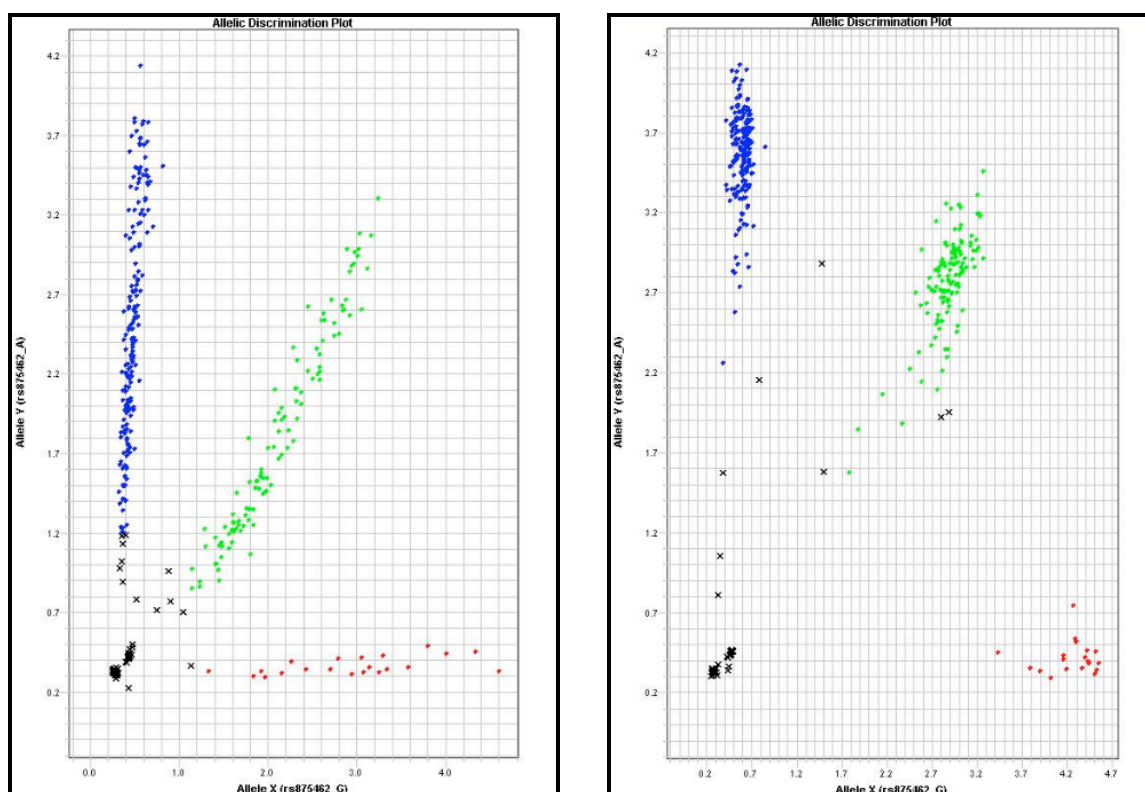


FIGURE 7.18 and 7.19 TaqMan DTNBP1 rs875462 Allelic Discrimination Plot. Blue colour homozygous -A allele-, red colour homozygous- G allele-, green colour heterozygous -AG alleles-.

		Cases n= 100	Siblings n= 81	Controls n= 36	HWE
DTNBP1	A/A	52 (61.2%)	47 (56.6%)	19 (55.9%)	p= 0.36
rs875462	A/G	33 (38.8%)	36 (43.4%)	14 (41.2%)	
Allelic frequency	G/G	0 (0%)	0 (0%)	1 (2.9%)	

TABLE 7.6 DTNBP1 rs875462 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in DTNBP1 rs875462 allelic distribution by gender ($\chi^2=3.33$; $p=0.19$).

There was no evidence of a correlation between the DTNBP1 rs875462 genotype and history of OCs ($\chi^2=1.36$, $p=0.24$) or with increased likelihood of a psychotic disorder ($\chi^2=2.39$, $p=0.12$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the DTNBP1 rs875462 genotype. Among those who experience OCs, there was no significant change in individual risk of psychosis according to DTNBP1 rs875462 genotype (OR=0.71, 95% CI 0.43-1.18, $p=0.19$).

These findings indicate that the DTNBP1 rs875462 genotype doesn't seem to modify the effect of OCs on the risk of a psychotic disorder.

7.9.4 GRM3 rs7808623 x OCs Interaction and Risk of Psychosis

I obtained GRM3 rs7808623 genotyping data on 115/127 cases, 85/95 siblings and 37/40 controls, with an overall call rate of 90.4% (FIGURE 7.20 and 7.21). The GRM3 rs7808623 polymorphism allele was in HWE (TABLE 7.7).

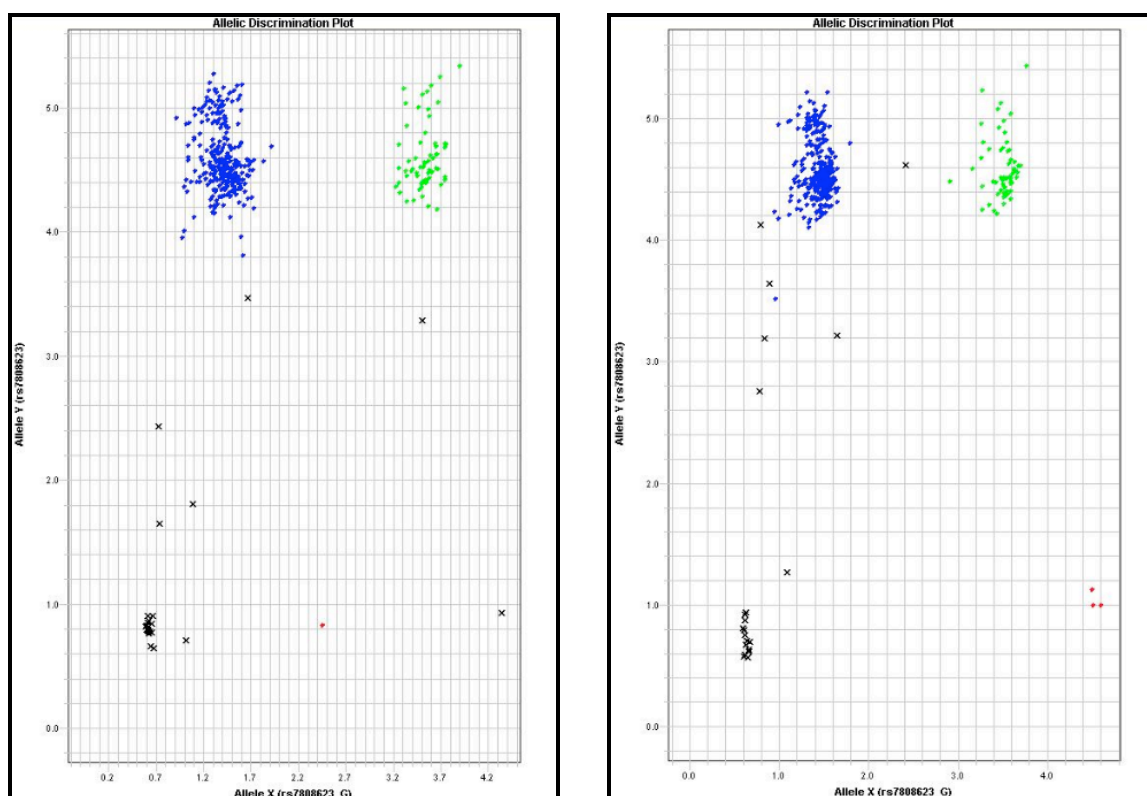


FIGURE 7.20 and 7.21 TaqMan GRM3 rs7808623 Allelic Discrimination Plot. Blue colour homozygous -G allele-, red colour homozygous- T allele-, green colour heterozygous -GT alleles-.

		Cases n= 100	Siblings n= 81	Controls n= 36	HWE
GRM3 rs7808623 Allelic frequency	G/G	96 (83.5%)	71 (83.5%)	32 (86.5%)	p= 1
	G/T	17 (14.8%)	14 (16.5%)	5 (13.5%)	
	T/T	2 (1.7%)	0 (0%)	0 (0%)	

TABLE 7.8 GRM3 rs7808623 Genotype. Hardy-Weinberg equilibrium (HWE) was performed looking at unrelated individuals using Pedigree Statistics - 0.6.12 (c) (1999-2006 Goncalo Abecasis, 2002-2006 Jan Wigginton).

I found no significant difference in GRM3 rs7808623 allelic distribution by gender ($\chi^2=0.28$; $p=0.87$),

There was no evidence of a correlation between the GRM3 rs7808623 genotype and history of OCs ($\chi^2=1.55$, $p=0.21$) or with increased likelihood of a psychotic disorder ($\chi^2=0.10$, $p=0.75$).

The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the GRM3 rs7808623 genotype. Among those who experience OCs, there was no significant change in individual risk of psychosis according to GRM3 rs7808623 genotype (OR=1.58, 95% CI 0.81-3.10, $p=0.18$).

These findings indicate that the GRM3 rs7808623 genotype doesn't seem to modify the effect of OCs on the risk of a psychotic disorder.

7.10 Summary of Results

OCs increase risk of schizophrenia but not everyone that experienced OCs develops psychosis later in life.

In this study I selected known genotype variants of 4 genes, which are regulated by hypoxia, namely AKT1, BDNF, DTNMBP1, GRM3, in an attempt to replicate the findings of Nicodemus et al. (2008). I expected carriers of the above risk alleles to be significant at risk of developing a psychotic disorder in subjects exposed to definite OC.

None of them was associated with psychosis or with likelihood to have had OC.

Moreover my results do not suggest any interaction between the presence of OCs and selected genotype on the risk of a psychotic disorder.

Although I have failed to replicate the findings of Nicodemus et al. (2008), my final sample size (n= 262) was underpowered. Therefore these findings do not provide definite evidence against a possible role of the above genotypes in modifying the effect of OCs on risk of psychotic disorders.

7.11 Discussions

There is no doubt a strong genetic component to psychosis, but family and twin studies have shown that simple genetic transmission is far from the whole story. In addition more than 50% of genes potentially associated with schizophrenia are subject to regulation by hypoxia and/or are expressed in the vasculature (Schmidt-Kastner et al., 2006; Schmidt-Kastner et al., 2012). Numbers of environmental factors have also been shown to increase risk of psychosis, among them OCs are the best replicated (Cannon et al., 2002). Variability in people's responses to environmental risk factors suggests that genes and environments operate together to produce psychosis (Tsuang et al., 2004; Di Forti et al., 2007b).

GxE research represents an important approach to explain the occurrence of psychosis. In the context of this chapter the major aim was to address the question of whether or not selected genes interact with the presence of OCs in the aetiology of psychosis. In particular I selected genotype variants of a total of 4 genes, namely AKT1, BDNF, DTNBP1 and GRM3, which are regulated by hypoxia and/or expressed in the vasculature to investigate the interaction. My study didn't show any significant evidence for gene-by-environment interaction but, because of limitations of power, I am unable to provide definite evidence against a possible role of selected genotypes in modifying the effect of OCs on risk of psychotic disorders.

It is likely that genes play an important role in mediating the reactions of the CNS to environmental stimuli such hypoxia. A difficult challenge is to identify plausible susceptibility genes to investigate how, and if, they moderate the effect of obstetric complications on the risk of psychosis. An answer could be looking at the interaction among genes regulated by hypoxia and the presence of OCs regardless their involvement in schizophrenia.

7.12 Limitations

7.12.1 Recruitment

Case-control designs are often prone to selection bias in both cases and controls recruitment.

7.12.1a Case Recruitment Bias

The cases in this thesis are all individuals presenting with psychotic disorders, therefore includes patients who eventually will receive different categorical diagnoses. To date evidence is stronger for an association between OCs and increased risk of schizophrenia spectrum psychosis rather than for affective psychosis (bipolar, psychotic depression). Therefore, it is possible that because my outcome group includes both affective and non-affective psychosis, both the main effect of exposure to OCS and its interaction with the selected candidate genes on the risk of developing a psychotic disorder is underestimated.

7.12.1b Control Recruitment Bias

Control recruitment is a challenge, as controls need to be a representative sample of the population from which cases are recruited. Controls used in the analyses were similar, according to the last UK census data, on a number of socio-demographic

factors (age, gender, ethnicity, level of education, employment rates) to the population that the cases come from (Census data 2001). Moreover, there was no evidence that the control recruitment under/over sampled those who suffered an OCs. About 25%-30% of births involve at least one Lewis-Murray complication (Jones et al., 1998; Cannon et al., 2002), thus the proportion of controls with a history of OC (21.4%) was comparable to the general population estimate.

To overcome the above limitation siblings are increasingly used as comparison groups in case-control studies. The "within-pair" estimates acquired through these comparisons are free from confounding from all factors that are shared by the siblings, including cultural and social background, parental characteristics and genetics. Although within-pair estimates will not be confounded by factors shared by the siblings, attenuation of associations will be higher in the within-pair estimate, leading within-pair associations to be weaker than corresponding unpaired associations, even in the absence of confounding (Frisell et al., 2012).

Finally to override genetic bias within-pair each SNPs association was investigated using a likelihood-based association test for unrelated subjects using UNPHASED 3.1.7 (Dudbridge, 2008).

7.12.2 Sample Size

The final sample size ($n=262$) was underpowered to estimate whether there was any interaction of $OR < 4.5$ in our population, according to the QUANTO estimate. The recruitment had proven very difficult and time consuming. It had required different steps, which delayed the start of the recruitment resulting in a smaller final number of subjects.

It is possible that a larger sample size may have detected an increased risk for psychosis due to Gene x Environment that the present study did not have sufficient power to assess.

7.12.3 Retrospective Data

One of the most common sources of bias is information bias as the history of exposure to OCs is collected retrospectively. However, as already mentioned in **CHAPTER 6**, studies assessing the validity of maternal recall compared to birth records have shown good agreement between retrospective maternal recall and contemporaneous birth records using the Lewis–Murray scale (reviewed by Walshe et al., 2011).

CHAPTER 8

GWAS Genetic Findings x OCs: the Role in Psychosis

8.1 Introduction

As discussed in the introduction, OCs explain a small, but significant, increase in risk for schizophrenia and psychosis in general possibly due to hypoxic insult to the brain. Different genetic variants can also influence the reaction to hypoxia as previously discussed (CHAPTER 7).

Results from several GWAS in schizophrenia show interesting matches between genes and ischemia– hypoxia or vascular factors but unfortunately differences in design and data presentation in different GWAS studies preclude a quantitative analysis (Schmidt-Kastner et al., 2012). Thus further research is warranted to identify direct links between hypoxia, genetic vulnerability and schizophrenia (Schmidt-Kastner et al., 2012).

No studies have yet provided direct evidence for an interaction between genes identified as genome wide significant in GWAS studies (abbreviated as GWAS genes) and hypoxia during pre- and perinatal life in increasing the risk for psychosis; therefore I aim to contribute with a GxE study in a sample of case controls looking at SNPs selected on the basis of recent GWAS studies.

8.2 GWAS Genes Polymorphisms Selected for the Analysis.

Genes selected for the analysis have been chosen looking at the GWAS published between 2008-2012 conducted on schizophrenia and psychosis in general. 39 SNPs reached the p value of at least $\leq 10^{-5}$ (TABLE 8.1).

Chromosome	Gene region	Symbol	Full name	SNPs	Significance	Reference
1	1p21.3	MIR137	(intron 3 of miRNA transcript)	rs1625579	Significant	Schizophrenia GWAS Consortium, 2011
	1q24.2			rs10489202	Significant	Shi et al., 2011
	1q32.2	PLXNA2	plexin A2	rs752016	Strongly Suggestive	Mah et al., 2006
	1p36.1	CDC42	cell division cycle 42	rs2473277	Strongly Suggestive	Gilks et al., 2012
	1q43	SDCCAG8	serologically defined colon cancer antigen 8	rs6703335	Significant	Hamshire et al., 2013
2	2p16.1	VRK2	vaccinia related kinase 2	rs2312147	Significant	Steinberg et al., 2011
	2q32.1	ZNF804A	zinc finger protein 804A	rs1344706	Strongly Suggestive	O'Donovan et al., 2008
	2q32.3	PCGEM1	prostate-specific transcript 1 (non-protein coding)	rs17662626	Significant	Schizophrenia GWAS Consortium, 2011
	2q33.3-q34	ErbB4	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	rs1851196	Strongly Suggestive	Shi et al., 2009
	2q34-q35	ACSL3-KCNE4	acyl-CoA synthetase long-chain family member 3	rs6739367	Significant	Alkelai et al., 2011
	2q37.1	UGT1A1-HJURP (intergenic)	Holliday junction recognition protein	rs741160	Significant	Alkelai et al., 2011
	2q37.3	AK573765-TWIST2 (intergenic)	twist homolog 2 (Drosophila)	rs9751357	Significant	Alkelai et al., 2011
	2q37.3	LRRFIP1	leucine rich repeat (in FLII)interacting protein 1	rs12052937	Significant	Alkelai et al., 2011
3	3p21.1	ITIH3/4	inter-alpha-trypsin inhibitor heavy chain 3/4	rs2239547	Significant	Schizophrenia GWAS Consortium, 2011
	3q21-q23	RELN	reelin	rs7341475	Strongly Suggestive	Shifman et al., 2008

6	6p21.3	TRIM26	tripartite motif containing 26	rs2021722	Significant	Schizophrenia GWAS Consortium, 2011
	6p21	ZKSCAN4	zinc finger with KRAB and SCAN domains 4	rs1233710	Significant	Yue et al., 2011
	6p21.3	NOTCH4	notch 4	rs2071287	Strongly Suggestive	Stefansson et al., 2009
	6p22.1	MHC region		rs3130375	Significant	Shi et al., 2009; Stefansson et al., 2009; International Schizophrenia Consortium, 2009
	6p22.1	NKAPL	NFKB activating protein-like	rs1635	Significant	Yue et al., 2011
	6p22.1	PGBD1	piggyBac transposable element derived 1	rs2142731	Significant	Stefansson et al., 2009
	6q21-qter	LOC645434 -NMBR	(intergenic) neuromedin B receptor	rs2066036	Significant	Alkelai et al., 2011
7	7q11.23-q21.3	PCLO	piccolo (presynaptic cytomatrix protein)	rs6979348	Strongly Suggestive	Athanasou et al., 2010
	7q31.1	DOCK4	dedicator of cytokinesis 4	rs2074127	Strongly Suggestive	Alkelai et al., 2012
8	8p12			rs16887244	Significant	Shi et al., 2011
	8q21	MMP16		rs7004633	Significant	Schizophrenia GWAS Consortium, 2011
	8p21-p12	NRG1	neuregulin 1	rs4316112	Strongly Suggestive	Shi et al., 2009
	8p23.2	CSMD1	CUB and sushi domain-containing protein 1; protein phosphatase 1, regulatory subunit 24	rs10503253	Significant	Schizophrenia GWAS Consortium, 2011
	8p23.1	MSRA	methionine sulfoxide reductase A	rs7017212	Strongly Suggestive	Ma et al., 2011
10	10q21	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	rs10994359	Significant	Schizophrenia GWAS Consortium, 2011

	10q24.32	CNNM2	cyclin M2	rs7914558	Significant	Schizophrenia GWAS Consortium, 2011
	10q24.32	NT5C2	5'-nucleotidase, cytosolic II	rs11191580	Significant	Schizophrenia GWAS Consortium, 2011
11	11q23.3	STT3A	subunit of the oligosaccharyltransfe rase complex, homolog A	rs548181	Significant	Schizophrenia GWAS Consortium, 2011
11	11q24.2	NRGN	neurogranin (protein kinase C substrate, RC3)	rs12807809	Significant	Stefansson et al., 2009
12	12p13.3	CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	rs4765905	Significant	Schizophrenia GWAS Consortium, 2011
18	18q21	CCDC68	coiled-coil domain containing 68	rs12966547	Significant	Schizophrenia GWAS Consortium, 2011
	18q21.1	TCF4	transcription factor 4	rs17512836	Significant	Schizophrenia GWAS Consortium, 2011
19	19q13.2	CEACAM21	carcinoembryonic antigen-related cell adhesion molecule 21	rs4803480	Significant	Alkelai et al., 2012
X/Y	Xp22.3 & Yp13.3	IL3RA	interleukin 3 receptor, alpha	rs17883192	Strongly Suggestive	Lencx et al., 2007

TABLE 8.1 List of GWAS SNPs Related to Psychosis. p value < 10^{-8} was considered strongly significant; p value comprises between 10^{-6} and 10^{-8} was considered significant; p value = 10^{-5} was considered strongly suggestive.

For the given set of SNPs listed above I designed one MassEXTEND (hME) assay panel using the latest version of the Sequenom MassARRAY Assay Design Software under the supervision of Dr Conrad Iyegbe. Finally, among those listed above, the following 25 SNPs could be simultaneously assayed in one multiplex panel with standardized conditions: AK573765-TWIST2 rs9751357, CACNA1C rs4765905, CEACAM21 rs4803480, CNNM2 rs7914558, CSMD1 rs10503253, Erbb4 rs1851196, ITIH3/4 rs2239547, LOC645434-NMBR rs2066036, LRRFIP1

rs12052937, MIR137 rs1625579, MMP16 rs7004633, NKAPL rs1635, NRGN
rs12807809, NT5C2 rs11191580, PCLO rs6979348, PLXNA2 rs752016, PGBD1
rs2142731, PCGEM1 rs17662626, RELN rs7341475, SDCCAG8 rs6703335, STT3A
rs548181, TCF4 rs17512836, UGT1A1 HJURP rs741160, rs10489202, rs16887244.

8.3 Aims

I aim to:

1. Test if each of the selected genetic variants is associated with an increased risk of psychotic disorders.
2. Test if each of the selected genetic variants is associated with an increased risk of OCs occurrence.
3. Test if each of the selected genetic variants interacts with exposure to OCs to moderate the risk of psychotic disorders.

8.4 Hypothesis

I expect each SNP minor allele to interact with exposure to OCs in increasing the risk of psychotic disorders.

8.5 Methods

The presence of OCs was assessed through maternal interview on 313 subjects, comprising 145 psychotic cases, 103 unaffected siblings and 65 healthy controls. The

sample comprised 252 individuals who are participants in The Maudsley Family Psychosis Study and 61 participants personally recruited from the GAP study.

8.5.1 Assessment

As previously discussed (**CHAPTER 5**) OCs were retrospectively collected from mothers of each participant using the MIS and coded using the Lewis-Murray scale.

8.5.2 Genotyping

DNA material was obtained from 262 subjects in total (127 cases, 95 siblings, 40 controls) from either blood or from cheek swab samples with an overall call rate of 79.4%. It comes almost entirely from the MFS, with the exception of 6 patients recruited in the GAP study. AK573765-TWIST2 rs9751357, CACNA1C rs4765905, CEACAM21 rs4803480, CNNM2 rs7914558, CSMD1 rs10503253, Erbb4 rs1851196, ITIH3/4 rs2239547, LOC645434-NMBR rs2066036, LRRFIP1 rs12052937, MIR137 rs1625579, MMP16 rs7004633, NKAPL rs1635, NRGN rs12807809, NT5C2 rs11191580, PCLO rs6979348, PLXNA2 rs752016, PGBD1 rs2142731, PCGEM1 rs17662626, RELN rs7341475, SDCCAG8 rs6703335, STT3A rs548181, TCF4 rs17512836, UGT1A1 HJURP rs741160, rs10489202, rs16887244 alleles were chosen as the genetic variants.

The DNA was extracted using a standard phenol-chloroform extraction procedure. Sequenom MassARRAY platform was used to conduct the entire genotyping following the manufacturer instructions under the supervision of Dr Rebecca Smith (**FIGURE 8.1**).

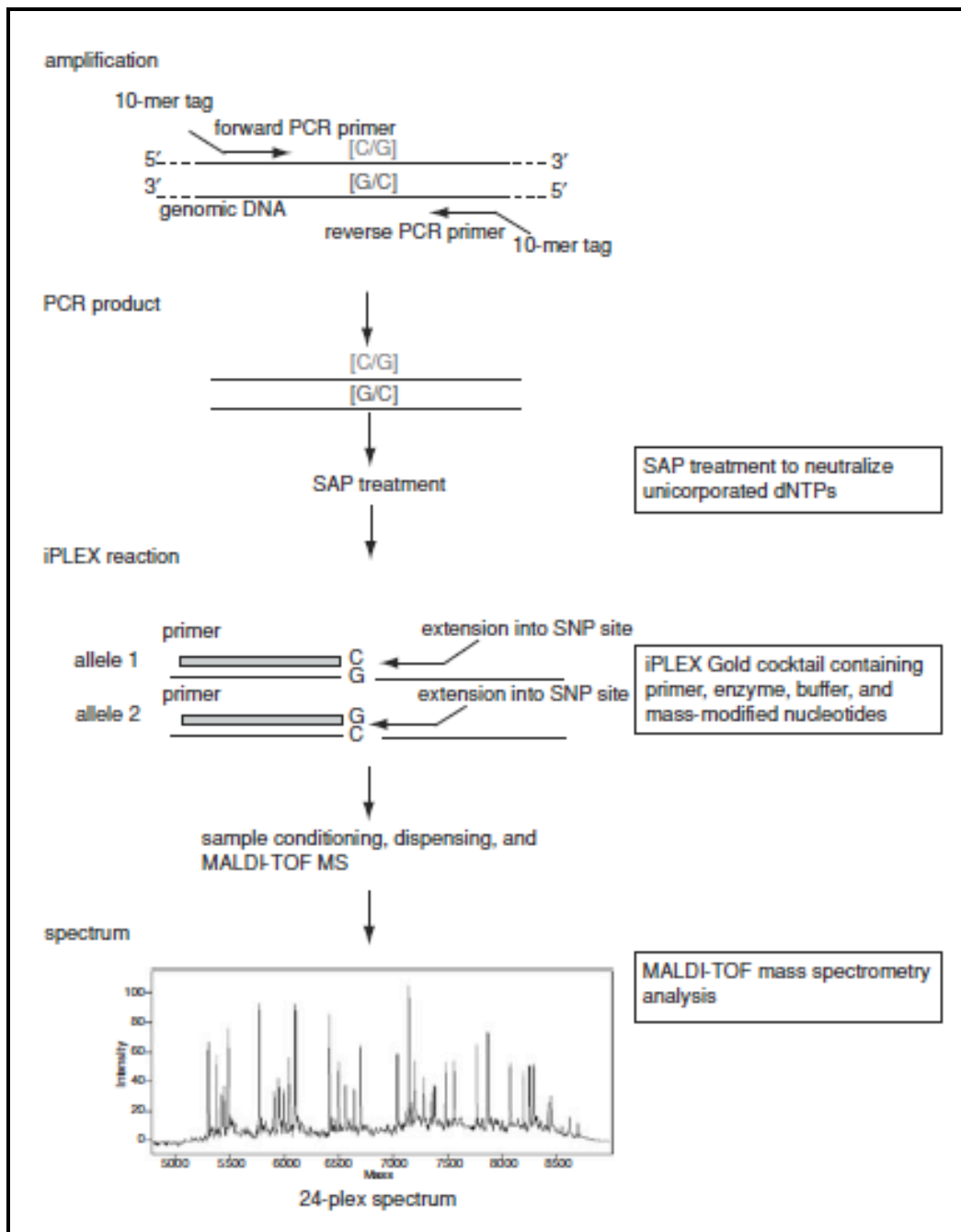


FIGURE 8.1 The MassEXTEND iPLEX Reaction. A schematic of the genotype reaction of a C-to-G SNP. Taken from Gabriel et al., 2009.

The assay consists of an initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using MALDI-TOF mass spectrometry, the distinct mass of the extended primer identifies the SNP allele.

8.5.3 Sample Size and Power Calculation

In order to have 80% power and a significance level of 0.05% (two-tailed) a sample of 355 case-control pairs is required to be able to detect an OR= 2. This assumes for an exposure of 30% among the general population (Cannon et al., 2002) and with a mean allele frequency of 0.5. Overall I collected genetic data on 262 subjects of the original 313 (51 refused to give blood or cheek swabs); with a sample of 127 patients and 135 controls (95 unaffected siblings and 40 healthy controls) I have the 80% power of detecting an interaction of OR>4.5 with 95% confidence in our population. These calculations have been made using the Quanto 1.2.4 software using the gene-environment module (John Morrison and W. James Gauderman at the University of Southern California).

8.5.4 Statistical Analysis

Data was recorded in IBM SPSS version 20.0 and analysed in the software package UNPHASED 3.1.7 (Dudbridge, 2008) as a way of combining related and unrelated individuals.

Based on the existing literature and on the findings reported in the previous chapter I used history of OCs as the environmental exposures of interest. AK573765-TWIST2 rs9751357, CACNA1C rs4765905, CEACAM21 rs4803480, CNNM2 rs7914558, CSMD1 rs10503253, Erbb4 rs1851196, ITIH3/4 rs2239547, LOC645434-NMBR rs2066036, LRRFIP1 rs12052937, MIR137 rs1625579, MMP16 rs7004633, NKAPL rs1635, NRG1 rs12807809, NT5C2 rs11191580, PCLO rs6979348, PLXNA2 rs752016, PGBD1 rs2142731, PCGEM1 rs17662626, RELN rs7341475, SDCCAG8 rs6703335, STT3A rs548181, TCF4 rs17512836, UGT1A1 rs741160, rs10489202, rs16887244 alleles were chosen as the genetic variants.

Hardy-Weinberg equilibrium (HWE) test of SNP was performed using Pedigree Statistics - 0.6.12 (c) (Wigginton and Abecasis, 2005).

χ^2 tests and t-tests (or ANOVA) were used to test for associations between the potential confounding variables and the genotype. Further χ^2 tests were carried out to establish whether OCs were more likely to occur in individual carriers of a particular genotype class (a Gene x Environment correlation).

Each SNPs association was investigated using a likelihood-based association test for nuclear families and unrelated subjects under the assumption of Hardy-Weinberg equilibrium, implemented in the software package UNPHASED 3.1.7 (Dudbridge, 2008). Forward stepwise regression within each SNPs was applied to determine independent association signals. Using UNPHASED, for each SNP, one marker at a time was tested for association to case/control status and the presence of OCs. In order to determine whether there was a gene x environment interaction among the SNPs investigated and OCs, effect modification by OCs was explored. Adjustment for multiple testing was performed using Bonferroni and Holmes (1979) correction.

8.6 Results

8.6.1 Description of the Whole Sample

I obtained genotyping data on 123 cases, 93 siblings and 35 controls, with an overall call rate of 79.4%; marker rs10489202 failed.

8.6.1a Demographic Characteristic of the Sample

For those subjects on whom I obtained DNA, cases were significantly younger (mean age 22.7 years; SD 4.7) than the control group (mean age 31.1 years; SD 8.1) (mean

difference -9.66, SE 0.87, 95% CI 6.69- 10.13; $p<0.001$). Moreover patients were more likely to be male ($p<0.001$) (TABLE 8.2).

	Cases n= 128	Controls n= 123	P Value
	Mean (SD)	Mean (SD)	
Age	22.7 (4.7)	31.1 (8.1)	$p<0.001$
	n (%)	n (%)	
Gender	Male 86 (58.5%)	61 (41.5%)	$p<0.001$

TABLE 8.2 Demographic Characteristics of Cases and Controls. P values from t-tests and χ^2 tests.

Analyzing the allelic distribution of each gene by gender no significant difference were found ($p>0.05$).

8.6.1b Genetic Characteristics of the Sample

I analyzed the allelic distribution of each gene together with the Hardy-Weinberg Equilibrium (HWE) (TABLE 8.3).

		Cases n= 123	Siblings n=93	Controls n=35	HWE
MRI137 rs1625579 (77 undetermined)	A/A	65 (70. %)	58 (69%)	22 (68.8%)	$p=0.16$
	A/C	23 (25%)	22 (26.2%)	7 (21.9%)	
	C/C	4 (4.3%)	4(4.8%)	3 (9.4%)	
PLXNA2 rs752016 (39 undetermined)	C/C	3 (3.2%)	4 (4.6%)	2 (6.2%)	$p=0.74$
	C/T	24 (25.8%)	18 (20.7%)	9 (28.1%)	
	T/T	66 (71%)	65 (74.7%)	21(65.6%)	
SDCCAG8 rs6703335 (45 undetermined)	A/A	11 (12.1%)	21 (24.7%)	6 (20%)	$p=0.87$
	A/G	43 (47.3%)	38 (44.7%)	15 (50%)	
	G/G	37 (40.7%)	26 (30.6%)	9 (30%)	
PCGEM1 rs17662626 (24 undetermined)	A/A	78 (73.6%)	69 (78.4%)	29 (87.9%)	$p=0.08$
	A/G	25 (23.6%)	16 (18.2%)	4 (12.1%)	
	G/G	3 (2.8%)	3 (3.4%)	0 (0%)	
ErbB4 rs1851196 (42 undetermined)	A/A	74 (81.3%)	72(84.7%)	28 (84.8%)	$p=1$
	A/G	14 (15.4%)	13 (15.3%)	5 (15.2%)	
	G/G	3 (3.3%)	0 (0%)	0 (0%)	
UGT1A1-HJURP	C/C	18 (17.1%)	9 (10.1%)	2 (5.9%)	$p=0.01^*$

rs741160	C/T	27 (25.7%)	26 (29.2%)	11 (32.4%)	
	T/T	60 (57.1%)	54 (60.7%)	21 (61.8%)	
(23 undetermined)					
AK573765-TWIST2 rs9751357	A/A	45 (57.7%)	42 (58.3%)	10(43.5%)	p=1
	A/G	28 (35.9%)	23 (31.9%)	12 (52.2%)	
	G/G	5 (6.4%)	7 (9.7%)	1 (4.3%)	
(78 undetermined)					
LRRFIP1 r12052937	C/C	51 (58.6%)	50 (59.5%)	19 (65.5%)	p=0.35
	C/G	32 (36.8%)	28 (33.3%)	7 (24.1%)	
	G/G	4 (4.6%)	6 (7.1%)	3 (10.3%)	
(41 undetermined)					
ITIH3/4 rs2239547	A/A	34 (45.3%)	35 (53.8%)	6 (26.1%)	p=0.68
	A/G	33 (44%)	25 (38.5%)	10 (43.5%)	
	G/G	8 (10.7%)	5 (7.7%)	7 (30.4%)	
(88 undetermined)					
RELN rs7341475	A/A	1 (1.1%)	4 (2.4%)	0 (0%)	p=0.74
	A/G	24 (26.1%)	21 (26.8%)	11 (35.5%)	
	G/G	67 (72.8%)	60 (70.9%)	20 (64.5%)	
(42 undetermined)					
NKAPL rs1635	G/G	83 (89.2%)	77 (90.6%)	31 (93.9%)	p=1
	G/T	10 (10.8%)	7 (8.2%)	2 (6.1%)	
	T/T	0 (0%)	1 (1.2%)	0 (0%)	
(40 undetermined)					
PGBD1 rs2142731	C/C	1 (1.1%)	0 (0%)	0 (0%)	p=0.2
	C/T	8 (8.4%)	5 (5.7%)	3 (8.8%)	
	T/T	86 (90.5%)	82 (94.3%)	31 (91.2%)	
(35 undetermined)					
LOC645434-NMBR rs2066036	C/C	24 (25.3%)	19 (22.4%)	7 (23.3%)	p=0.87
	C/G	39 (41.1%)	43 (50.6%)	15 (50%)	
	G/G	32 (33.7%)	23 (27.1%)	8 (26.7%)	
(41 undetermined)					
PCLO rs6979348	C/C	44 (47.7%)	39 (50%)	11 (40.7%)	p=0.6
	C/T	31 (38.1%)	34 (43.6%)	10 (37%)	
	T/T	17 (18.5%)	5 (6.4%)	6 (22.2%)	
(54 undetermined)					
rs16887244	A/A	48 (54.5%)	55 (64.7%)	20 (62.5%)	p=1
	A/G	36 (40.9%)	25 (29.4%)	8 (25%)	
	G/G	4 (4.5%)	5 (5.9%)	4 (12.5%)	
(46 undetermined)					
MMP16 rs7004633	A/A	58 (69.9%)	55 (67.1%)	25 (86.2%)	p=0.77
	A/G	23 (27.7%)	25 (30.5%)	1 (3.4%)	
	G/G	2 (2.4%)	2 (2.4%)	3 (10.3%)	
(57 undetermined)					
CSMD1 rs10503253	A/A	4 (4.4%)	1 (1.3%)	2 (6.7%)	p=0.38
	A/C	25 (27.5%)	17 (22.1%)	8 (26.7%)	
	C/C	62 (68.1%)	59 (76.6%)	20 (66.7%)	
(53 undetermined)					
CNNM2 rs7914558	A/A	7 (10.1%)	8 (11.4%)	2 (8%)	p=0.52
	A/G	36 (52.2%)	22 (31.4%)	6 (24%)	
	G/G	26 (37.7%)	40 (57.1%)	17 (68%)	
(87 undetermined)					
NT5C2 rs11191580	C/C	1 (1.1%)	4 (4.7%)	0 (0%)	p=0.15
	C/T	12 (13.3%)	10 (11.8%)	5 (15.2%)	
	T/T	77 (85.6%)	71 (83.5%)	28 (84.8%)	
(43 undetermined)					
STT3A rs548181	A/A	3 (3.8%)	1 (1.4%)	1 (4.5%)	p=0.09
	A/G	19 (23.8%)	11 (15.3%)	2 (9.1%)	
	G/G	58 (72.5%)	60 (83.3%)	19 (86.4%)	
(77 undetermined)					

NRGN rs12807809 (66 undetermined)	C/C	5 (5.8%)	3 (3.9%)	0 (0%)	p=0.04*
	C/T	18 (20.9%)	13 (17.1%)	2 (8.7%)	
	T/T	63 (73.3%)	60 (78.9%)	21 (91.3%)	
CACNA1C rs4765905 (61 undetermined)	C/C	17 (19.3%)	9 (12.2%)	3 (10.7%)	p=0.13
	C/G	35 (39.8%)	30 (40.5%)	10 (35.7%)	
	G/G	36 (40.9%)	35 (47.3%)	15 (53.6%)	
TCF4 rs17512836 (41 undetermined)	C/C	0 (0%)	2 (2.3%)	0 (0%)	p=1
	C/T	9 (10%)	5 (5.7%)	2 (6.2%)	
	T/T	81 (90%)	81 (92%)	30 (93.8%)	
CEACAM21 rs4803480 (67 undetermined)	A/A	14 (16.3%)	7 (10.1%)	2 (6.9%)	p=0.43
	A/G	46 (53.5%)	28 (40.6%)	11 (37.9%)	
	G/G	26 (30.2%)	34 (49.3%)	16 (55.2%)	

TABLE 8.3 Allelic Frequencies of Genes and Hardy-Weinberg Equilibrium (HWE). Marker rs10489202 failed.

Two genes polymorphisms allele breached a Hardy-Weinberg Equilibrium (HWE) p-value of 0.05, namely UGT1A1-HJURP rs741160 and NRGN rs12807809 (TABLE 8.3). Although, traditionally a $p < 0.05$ was considered to breach the HWE, recent GWAS have suggested a much higher threshold of significance (10^{-4}) before discarding a SNP on the assumption of genotyping error (WTCC, Nature 2007).

Therefore a small difference, even though statistically significant, between expected and observed frequencies of a particular SNP can be explained by population mixture rather than simply by genotyping error. For instance, even the White British, are a heterogeneous population, having been shaped by several waves of immigration from elsewhere in Europe.

After Bonferroni and Holm (1979) correction for multiple testing, the HWE p value in rs741160 and rs12807809 were no longer significant ($p_c \leq 0.002$), thus none of the genes showed significant difference in distribution.

8.6.2 Genes Allele and Psychotic Disorder Correlation

There was evidence of a correlation between selected genotype and psychosis.

In particular, CEACAM21 rs4803480 risk allele “A” increases the risk of the occurrence of psychosis about 3 fold (OR= 2.79, 95% CI 1.42- 5.48; $p= 0.002$). After Bonferroni and Holm (1979) correction for multiple testing ($p_c \leq 0.002$) this remained significant.

8.6.3 Genes Allele and Obstetric Complication Correlation

There was evidence of a correlation between MIR137 rs1625579 ($\chi^2=4.29$; $p=0.04$) and LRRFIP1 rs12052937 ($\chi^2=4.64$; $p=0.03$) polymorphisms and history of OCs.

After Bonferroni and Holm (1979) correction for multiple testing ($p_c \leq 0.002$) neither of them remained significant.

8.6.4 Genes Allele x OCs

The association of the interaction between risk allele of each gene polymorphisms x OCs with presence of a psychotic disorder was then investigated. No significant interaction between OCs and the selected genotypes were found (TABLE 8.4).

Gene	SNPs	GxE	P Value
MIR137	rs1625579	$\chi^2=-0.43$	p=0.51
-	rs10489202	NA	NA
PLXNA2	rs752016	$\chi^2=0.28$	p=0.59
SDCCAG8	rs6703335	$\chi^2=0.95$	p=0.33
PCGEM1	rs17662626	$\chi^2=0.98$	p=0.32
ErbB4	rs1851196	$\chi^2=0.88$	p=0.35
UGT1A1-HJURP	rs741160	$\chi^2=0.01$	p=0.92
AK573765-TWIST2	rs9751357	$\chi^2=1.12$	p=0.29
LRRFIP1	rs12052937	$\chi^2=3.25$	p=0.07
ITIH3/4	rs2239547	$\chi^2=0.56$	p=0.45
RELN	rs7341475	$\chi^2=1.30$	p=0.25
NKAPL	rs1635	$\chi^2=0.09$	p=0.76
PGBD1	rs2142731	$\chi^2=0.96$	p=0.33
LOC645434-NMBR	rs2066036	$\chi^2=1.89$	p=0.17
PCLO	rs6979348	$\chi^2=0.20$	p=0.65
Xxx	rs16887244	$\chi^2=2.79$	p=0.09
MMP16	rs7004633	$\chi^2=0.43$	p=0.51

CSMD1	rs10503253	$\chi^2=-0.35$	p=0.55
CNNM2	rs7914558	$\chi^2=0.27$	p=0.60
NTSC2	rs11191580	$\chi^2=1.03$	p=0.31
STT3A	rs548181	$\chi^2=0.16$	p=0.68
NRGN	rs12807809	$\chi^2=0.01$	p=0.91
CACNA1C	rs4765905	$\chi^2=3.28$	p=0.07
TCF4	rs17512836	$\chi^2=0.29$	p=0.59
CEACAM21	rs4803480	$\chi^2=0.21$	p=0.66

TABLE 8.4 Likelihood Ratio χ^2 of OCs X Gene in Increasing the Risk of Psychosis. Each SNPs association was investigated using a likelihood-based association test for nuclear families and unrelated subjects under the assumption of Hardy-Weinberg equilibrium, implemented in the software package UNPHASED 3.1.7 (Dudbridge, 2008).

These findings indicate that the genotypes I examined do not modify the effect of OCs on the risk of a psychosis.

8.7 Summary of Results

In this study I selected known genotype variants of 25 genes from the GWAS findings published so far in an attempt to show an interaction between each SNPs risk allele and OCs.

Among them CEACAM21 was associated with psychosis while none was associated with likelihood to have had OC. Moreover my results do not suggest any interaction between the presences of OCs and selected genotype on the risk of a psychotic disorder.

8.8 Discussions

Since 2008 GWAS have identified a substantial number of polymorphisms associated with schizophrenia, although these findings are characterized by small effect sizes (O'Donovan et al., 2009). Moreover, it is likely that the effect of genes on the disease causal path might partly depend on environmental exposures through interaction and/or via epigenetic mechanism (Tsuang et al., 2004; Rutter and Mill, 2009).

Results from recent GWAS show several interesting matches between genes and ischemia/hypoxia or vascular factors but so far no studies have yet provided direct evidence for their interaction with hypoxia during pre- and perinatal life in increasing the risk for psychosis (Schmidt-Kastner et al., 2012). In this chapter I investigated the interaction among genotype variants of a total of 25 SNPs that have been chosen looking at the GWAS conducted on schizophrenia and psychosis in general with OCs in increasing the risk of psychosis.

Although I have failed to show any evidence for an interaction the final sample size (n= 262) was underpowered. Therefore these findings do not provide definite evidence against a possible role of the above genotypes in modifying the effect of OCs on risk of psychotic disorders.

8.9 Limitations

As part of the same sample, limitations are already discussed in previous Chapters (CHAPTER 6 and 7).

In particular for this set of analyses the final (n= 262) was underpowered to estimate whether there was any interaction of $OR < 4.5$ in our population, according to the QUANTO estimate. It is possible that a larger sample size may have detected an increased risk for psychosis due to Gene x Environment that the present study did not have sufficient power to assess.

CHAPTER 9

Gene X Environment: a Case- Only Study

9.1 Introduction

As discussed earlier, OCs increase risk of schizophrenia but not everyone that experienced OCs develops psychosis later in life. In this Thesis, in an attempt to replicate the findings of Nicodemus et al. (2008), I selected known genotype variants of 4 genes, namely AKT1, BDNF, DTNMBP1, and GRM3, to look at their interaction with OCs in increasing the risk of psychosis (CHAPTER 7). There were no statistically significant findings but because of limitations of power, I was unable to definitively exclude interaction. Thus, this interaction model has not yet been fully empirically tested.

A case-only design can be a valid approach to evaluate gene-environment interaction in disease etiology (Piegorsch, et al., 1994; Khoury and Flanders, 1996; Yanget al., 1997; Andrieu and Goldstein, 1998; Weinberg and Umbach, 2000). As explained in Khoury and Flanders, 1996, the odds ratio relating the exposure and the allele among case subjects only is a function of the odds ratios for the exposure alone, the genotype alone, and their joint effects in a standard case-control study as follow:

$$COR = OR_{ge} / (OR_e \times OR_g) \times Z.$$

COR is the case-only odds ratio and Z refers to the odds ratio among control subjects relating the exposure and the susceptibility genotype. It can be summarized as $COR = ad/bc$ (FIGURE 9.1).

Exposure	Susceptibility genotype	
	-	+
-	a	b
+	c	d

* COR, case-only odds ratio = ad/bc . Under assumption of independence between exposure and genotype among controls: $COR = OR_{ge}/OR_e \times OR_g = SIM$, where SIM is the synergy index.

FIGURE 9.1 Gene-Environment Interaction Analysis in the Context of a Case-Only Study. COR, case-only odds ratio = ad/bc . Under assumption of independence between exposure and genotype among controls. Taken from Khoury and Flanders, 1996.

If the genotype and the exposures are independent, Z becomes unity and the odds ratio obtained from a case-only study becomes simply the synergy index on a multiplicative scale derived from a regular case-control study (Khoury and Flanders, 1996).

Thus, in order to overcome difficulties associated with the recruitment of an appropriate control group and to have greater statistical power with fewer subjects, in this chapter I perform a case only design statistical analysis in a group of affected subjects to examine GxE interaction.

9.2 Aims

I aim to:

1. Test if each of the selected genetic variants is associated with an increased risk of OCs;
2. Test if each of the selected genetic variants interacts with exposure to OCs to moderate the risk of psychotic disorders.

9.3 Hypotheses

I expect the following SNPs to interact with exposure to OCs in increasing the risk of psychotic disorders:

8. AKT1 rs1130233 “G” allele
9. AKT1 rs2494735 “C” allele
10. AKT1 rs3803300 “G” allele.
11. BDNF rs2049046 “T” allele.
12. BDNF rs56164415 “A” allele.
13. DTNBP1 rs875462 “G” allele.
14. GRM3 rs7808623 “T” allele .

9.4 Methods

The presence of OCs was assessed through maternal interview on 377 psychotic cases.

9.4.1 Recruitment and Assessment

I increased the available sample of patients thanks to collaboration with the Psychosis Incidence Cohort Study (PICOS), Verona (Lasalvia et al., 2012). Under the overall supervision of Professor Mirella Ruggero and Dr Sarah Tosato the project aims to clarify, in a cohort of patients diagnosed with psychosis, the role of specific factors (i.e clinical, environmental, genetic and functional) in predicting the clinical and social outcome. The project also aims to acquire knowledge on environmental, psychological, biological and clinical factors related to the characteristic clinical picture, and on predictors of outcome and response to treatment.

The project used similar diagnostic criteria and recruitment strategy to collect OCs data. OCs was retrospectively collected from mothers and coded using the Lewis-Murray scale as explained in details in **CHAPTER 5**.

Overall the sample comprises 112 individuals who are participants in the MFS, 33 from the GAP and a total of 232 individuals recruited from PICOS. To maximise statistical power, participants from the MFS and GAP were collapsed in one group called “UK”.

9.4.2 Genotyping

DNA material was obtained from 290 subjects in total. AKT1 rs1130233, rs3803300 and rs2494735 alleles, BDNF rs2049046 and rs56164415 alleles, DTNBP1 rs875462 allele and GRM3 rs7808623 allele were chosen as the genetic variants.

The DNA was extracted using a standard phenol-chloroform extraction procedure. TaqMan® Gene Expression Assays Protocol was used to conduct the entire genotyping following the manufacturer instructions (**CHAPTER 7**).

9.4.3 Sample Size and Power Calculation

In order to have 80% power and a significance level of 0.05% (two-tailed) a sample of 203 case is required to be able to detect an OR= 2. This assumes for an exposure of 30% among the general population (Cannon et al., 2002) and with a mean allele frequency of 0.5. Overall I collected genetic data on 290 subjects, thus I have the 80% power of detecting an interaction of OR>1.8 with 95% confidence in our population. These calculations have been made using the Quanto 1.2.4 software using the gene-environment module (Gauderman and Morrison, 2002).

9.4.4 Statistical Analysis

Data was recorded and analysed in IBM SPSS version 20.0. Based on the existing literature and on the findings reported in **CHAPTER 6**, I used history of OCs as the

environmental exposures of interest. AKT1 rs1130233, rs3803300 and rs2494735 alleles, BDNF rs2049046 and rs56164415 alleles, DTNBP1 rs875462 allele and GRM3 rs7808623 alleles were chosen as the genetic variants.

χ^2 tests and t-tests (or ANOVA) were used to test for associations between the potential confounding variables and the genotype. Further χ^2 tests were carried out to establish whether OCs were more likely to occur in individual carriers of a particular genotype class (a Gene x Environment correlation).

Logistic regression was used to analyse the association between candidate genotype and presence of a psychotic disorder along with the exposure to OCs. Separate analyses were run for each exposure of interest, and an interaction between exposure and genotype was included in the model. The interaction term was used to identify the effect of genotype on presence of psychosis, conditional on history of OCs.

9.5 Results

9.5.1 Description of the Whole Sample

9.5.1a Clinical Characteristics of the Sample

Subjects included in the study consisted in 377 subjects who met ICD10 criteria for a diagnosis of non-organic psychosis (F20-F29 and F30-F33).

9.5.1b Demographic Characteristic of the Sample

As I mentioned above the sample comprises 112 individuals who are participants in the MFS, 33 from the GAP and a total of 232 individuals recruited from PICOS. To maximise statistical power participants from the MFS and GAP were collapsed in one group called “UK”. The sample includes only Caucasians.

The demographic characteristics for the two groups are shown in **TABLE 9.1**.

		UK n= 145	PICOS n= 232	P Value
		Mean (SD)	Mean (SD)	
	Age	23.11 (4.9)	30.49 (9.4)	p<0.001
	Maternal Age at Delivery	26.98 (4.5)	27.26 (5.9)	p=0.003
	Maternal Age at Interview	57.89 (6.5)	57.42 (8.7)	p=0.62
		n (%)	n (%)	
Gender	Male	97 (66.9%)	135 (58.2%)	p=0.06
	Female	48 (33.1%)	97 (41.8%)	

TABLE 9.1 Demographic Characteristics of the Sample. P values from t-tests and χ^2 tests.

The mean age of the whole sample is 27.77 years (SD 8.79); among the groups, UK patients were more than 7 years younger than PICOS patients (-7.38 years old, SE 0.87, 95% CI -9.09 - -5.67, $F = 74.83$; $p < 0.001$). There was no significant group difference for gender with an excess of males in both patients' group ($\chi^2 = 2.86$; $p = 0.06$).

9.5.1c Maternal Characteristics of the Sample

The demographic characteristics for the two groups are shown in **TABLE 8.1**. The mean age of the mother at the time of the interview was 57.67 years (SD 7.62). The groups differ in the measure of maternal age at delivery, with UK mothers being slightly younger than PICOS (-0.28 years, SE 0.63; 95% CI -1.53 – 0.96, $F = 9.29$, $p = 0.003$).

9.5.2 Obstetric Complications

The rate of definite OCs for each groups are displayed in **TABLE 9.2** and individual complications in **TABLE 9.3**.

	UK n= 145	PICOS n= 232	P Value
	n (%)	n (%)	
Definite OCs	56 (38.9%)	62 (26.7%)	p=0.01

TABLE 9.2 Rates of Definite OCs in Each Group. P values from χ^2 tests.

38.9% of UK psychotic patients had a positive history for OCs compared to 26.7% of the PICOS subjects ($\chi^2=6.11$, $p=0.01$).

	UK n (%)	PICOS n (%)	P value
Rubella	-	-	-
Syphilis	-	-	-
Rhesus incompatibility	0 (0%)	3 (1.3%)	p=0.66
Antepartum hemorrhage	6 (4.1%)	15 (6.6%)	p=0.23
Severe Pre-eclampsia	16 (11%)	4 (1.8%)	p<0.001*
Premature rupture of membrane	0 (0%)	5 (2.3%)	p=0.49
Labour > 36 hours	9 (6.3%)	14 (6.4%)	p=0.58
Birth weight 2000 gr or less	2 (1.4%)	6 (2.7%)	p=0.34
Complicated twin birth	-	-	-
Cord prolapse	0 (0%)	9 (4.1%)	p=0.01*
Gestational age <37 weeks	9 (6.2%)	17 (7.7%)	p=0.38
Gestational age >42 weeks	5 (3.5%)	0 (0%)	p=0.79
Emergency Caesarian Section	5 (3.1%)	0 (%)	p=0.47
Breach or abnormal presentation	7 (4.9%)	11 (4.9%)	p=0.6
Mid to high forceps	19(13.2%)	2 (0.9%)	p<0.001*
Incubator > 4 weeks	3 (2.1%)	19 (8.4%)	p=0.01*

TABLE 9.3 Individual Obstetric Events on Lewis-Murray Scale. I did not rate as a “Definite” OCs labour lasting less than 3 hours.

9.5.3 Gene-Environment Interaction Analyses In The Context Of a Case-Only Study

The association of the interaction between of OCs and selected genotype with presence of a psychotic disorder was investigated using the COR analysis (Khoury and Flanders, 1996).

9.5.3a AKT1 x OCs Interaction and Risk of Psychosis

rs1130233 x OCs

I obtained AKT1 rs1130233 genotyping data on 241 cases, with an overall call rate of 83.1% (TABLE 9.4).

I found no significant difference in AKT1 rs1130233 allelic distribution by gender ($\chi^2=0.18$; $p=0.91$), or between groups ($\chi^2=2.92$; $p=0.23$).

		UK n= 112	PICOS n= 129	P Value
		Mean (SD)	Mean (SD)	
Age		23.02 (4.9)	30.12 (8.9)	p<0.001*
		n (%)	n (%)	
Gender	Male	72 (64.3%)	73 (56.6%)	p=0.14
AKT1 rs1130233 allelic frequency	A/A	61 (54.5%)	81 (62.8%)	p= 0.23
	A/G	46 (41.4%)	46 (35.7%)	
	G/G	5 (4.5%)	2 (1.6%)	

TABLE 9.4 Characteristics of Patients with AKT1 rs1130233 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the AKT1 rs1130233 genotype and history of OCs in neither of the two groups (UK: $\chi^2=1.21$, $p=0.55$; PICOS: $\chi^2=1.06$, $p=0.59$). Moreover the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs1130233 genotype (TABLE 9.5).

	TOTAL n= 151		UK n= 112		PICOS n= 129	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
A/A	99	43	39	22	60	21
A/G	56	35	24	21	32	14
G/G	5	2	3	2	2	0
OR	1.4		1.5		1.18	
95% CI	0.81-2.40		0.70-3.24		0.53-2.61	
P value	p=0.23		p=0.29		p=0.69	

TABLE 9.5 AKT1 rs1130233 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

rs2494735 x OCs

I obtained AKT1 rs2494735 genotyping data on 224 cases, with an overall call rate of 77.2% (TABLE 9.6).

I found no significant difference in AKT1 rs2494735 allelic distribution by gender ($\chi^2=1.93$; $p=0.38$), or between groups ($\chi^2=0.49$; $p=0.78$).

		UK n= 110	PICOS n= 114	P Value
		Mean (SD)	Mean (SD)	
Age		23.02 (4.9)	30.73 (9.2)	p<0.001*
		n (%)	n (%)	
Gender	Male	70 (63.6%)	61 (53.5%)	p=0.08
AKT1 rs2494735	C/C	16 (14.5%)	14 (12.3%)	p= 0.78
allelic frequency	C/T	55 (50%)	55 (48.2%)	
	T/T	39 (35.5%)	45 (39.5%)	

TABLE 9.6 Characteristics of Patients with AKT1 rs2494735 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the AKT1 rs2494735 genotype and history of OCs in either of the two groups (UK: $\chi^2=3.21$, $p=0.2$; PICOS: $\chi^2=1.19$,

p=0.55). In addition the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs2494735 genotype (TABLE 9.7).

	TOTAL n= 224		UK n= 110		PICOS n= 114	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
C/C	17	12	6	9	11	3
C/T	70	40	33	22	37	18
T/T	60	24	26	13	34	11
OR	1.4		1.59		1.35	
95% CI	0.83-2.68		0.70-3.59		0.58-3.17	
P value	p=0.18		p=0.26		p=0.49	

TABLE 9.7 AKT1 rs2494735 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

rs3803300 x OCs

I obtained AKT1 rs3803300 genotyping data on 241 cases, with an overall call rate of 83.1% (TABLE 9.8). I found no significant difference in AKT1 rs3803300 allelic distribution by gender ($\chi^2=2.05$; p=0.29), or between groups ($\chi^2=2.27$; p=0.32).

		UK n= 97	PICOS n= 144	P Value
		Mean (SD)	Mean (SD)	
Age		22.94 (5.1)	30.04 (9)	p<0.001*
		n (%)	n (%)	
Gender	Male	66 (64.1%)	78 (56.5%)	p=0.15
AKT1 rs3803300 allelic frequency	A/A	88 (83.5%)	113 (81.9%)	p= 0.32
	A/G	17 (16.5%)	22 (15.9%)	
	G/G	0 (0%)	3 (1.2%)	

TABLE 9.8 Characteristics of Patients with AKT1 rs3803300 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the AKT1 rs3803300 genotype and history of OCs in either of the two groups (UK: $\chi^2=0.03$, $p=0.53$; PICOS: $\chi^2=1.14$, $p=0.93$). The effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the AKT1 rs3803300 genotype (TABLE 9.9).

	TOTAL n=240		UK n= 102		PICOS n= 138	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
A/A	133	65	52	33	81	32
A/G	25	14	10	7	15	7
G/G	2	1	0	0	2	1
OR	1.14		1.1		1.19	
95% CI	0.57-2.28		0.38-3.18		0.47-3.03	
P value	p=0.72		p=0.86		p=0.71	

TABLE 9.9 AKT1 rs3803300 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

9.5.3b BDNF x OCs Interaction and Risk of Psychosis

rs2049046 x OCs

I obtained BDNF rs2049046 genotyping data on 236 cases, with an overall call rate of 81.4% (TABLE 9.10).

I found no significant difference in BDNF rs2049046 allelic distribution by gender ($\chi^2=1.13$; $p=0.57$), or between groups ($\chi^2=2.52$; $p=0.28$).

		UK n= 100	PICOS n= 136	P Value
		Mean (SD)	Mean (SD)	
Age		23.08 (4.8)	30.03 (9)	p<0.001*
		n (%)	n (%)	
Gender	Male	65 (65%)	75 (55.1%)	p=0.08
BDNF rs2049046	A/A	24 (24)	31 (22.8%)	p= 0.28
allelic frequency	A/T	45 (45	74 (54.4%)	
	T/T	31 (31%)	31 (22.8%)	

TABLE 9.10 Characteristics of Patients with BDNF rs2049046 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the BDNF rs2049046 genotype and history of OCs in either of the two groups (UK: $\chi^2_{22.35}$, p=0.31; PICOS: $\chi^2_{1.77}$, p=0.41). Furthermore the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the BDNF rs2049046 genotype (TABLE 8.11).

	TOTAL n= 236		UK n= 100		PICOS n= 136	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
A/A	34	21	14	10	20	11
A/T	79	39	22	22	57	17
T/T	44	18	21	10	23	8
OR	0.75		1.04		0.57	
95% CI	0.40-1.41		0.41-2.64		0.24-1.34	
P value	p=0.37		p=0.93		p=0.20	

TABLE 9.11 BDNF rs2049046 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

rs56164415 x OC

I obtained BDNF rs56164415 genotyping data on 248 cases, with an overall call rate of 85.5% (TABLE 9.12).

I found no significant difference in BDNF rs56164415 allelic distribution by gender ($\chi^2_{2.31}$; p=0.31), or between groups ($\chi^2_{1.57}$; p=0.46).

		UK n= 107	PICOS n= 141	P Value
		Mean (SD)	Mean (SD)	
Age		23.1 (5)	30.06 (9)	p<0.001*
		n (%)	n (%)	
Gender	al	72 67.3%)	78 (55.3%)	p=0.04*
BDNF rs56164415	A/A	1(0.9%)	0 (0%)	p= 0.46
allelic frequency	A/G	11 (10.3%)	12 (8.5%)	
	G/G	95 (88.8%)	129 (91.5%)	

TABLE 9.12 Characteristics of Patients with BDNF rs56164415 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the BDNF rs56164415 genotype and history of OCs in either of the two groups (UK: $\chi^2=1.64$, $p=0.44$; PICOS: $\chi^2=1.14$, $p=0.23$). Again the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the BDNF rs56164415 genotype (TABLE 9.13).

	TOTAL n= 247		UK n= 106		PICOS n= 141	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
A/A	1	0	1	0	0	0
A/G	15	8	8	3	7	5
G/G	148	75	54	40	94	35
OR	0.99		0.45		1.92	
95% CI	0.40-2.41		0.11-1.80		0.57-6.44	
P value	p=0.98		p=0.25		p=0.29	

TABLE 9.13 BDNF rs56164415 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

9.5.3.c DTNBP1 rs875462 x OCs Interaction and Risk of Psychosis

I obtained DTNBP1 rs875462 genotyping data on 225 cases, with an overall call rate of 77.6% (TABLE 9.14)

I found no significant difference in DTNBP1 rs875462 allelic distribution by gender ($\chi^2=1.17$; $p=0.56$). Between groups there was a significant expression of the minor allele “G” in the PICOS subjects ($\chi^2=10.33$; $p=0.006$).

		UK n= 97	PICOS n= 144	P Value
		Mean (SD)	Mean (SD)	
Age		22.94 (5.1)	30.04 (9)	$p<0.001^*$
		n (%)	n (%)	
Gender	Male	58 (68.2%)	78 (55.7%)	$p=0.04^*$
DTNBP1 rs875462 allelic frequency	A/A	52 (61.2%)	70 (50%)	$p= 0.006^*$
	A/G	33 (38.8%)	55 (39.3%)	
	G/G	0 (0%)	15 (10.7%)	

TABLE 9.14 Characteristics of Patients with DTNBP1 rs875462 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the DTNBP1 rs875462 genotype and history of OCs in either of the two groups (UK: $\chi^2=0.83$, $p=0.25$; PICOS: $\chi^2=1.82$, $p=0.40$). Moreover the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the DTNBP1 rs875462 genotype (TABLE 9.15).

	TOTAL n=225		UK n= 85		PICOS n= 140	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
A/A	79	43	32	20	47	23
A/G	59	29	17	16	42	13
G/G	12	3	0	0	12	3
OR	0.83		1.51		0.60	
95% CI	0.47-1.45		0.62-3.64		0.29-1.28	
P value	$p=0.51$		$p=0.36$		$p=0.19$	

TABLE 9.15 DTNBP1 rs875462 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

9.5.3d GRM3 rs7808623 x OCs Interaction and Risk of Psychosis

I obtained GRM3 rs7808623 genotyping data on 255 cases, with an overall call rate of 87.93% (TABLE 9.16).

I found no significant difference in GRM3 rs7808623 allelic distribution by gender ($\chi^2=0.54$; $p=0.76$), or between groups ($\chi^2=3.8$; $p=0.15$).

		UK n= 115	PICOS n= 140	P Value
		Mean (SD)	Mean (SD)	
Age		22.94 (5.1)	30.04 (9)	$p<0.001^*$
		n (%)	n (%)	
Gender	Male	76 (66.1%)	79 (56.4%)	$p=0.07$
GRM3 rs7808623 allelic frequency	G/G	96 (83.5%)	111 (79.3%)	$p= 0.15$
	G/T	17 (14.8%)	29 (20.7%)	
	T/T	2 (1.7%)	0 (0%)	

TABLE 9.16 Characteristics of Patients with GRM3 rs7808623 Genotype. P values from t-tests and χ^2 tests.

There was no evidence of a correlation between the GRM3 rs7808623 genotype and history of OCs in either of the two groups (UK: $\chi^2=4.89$, $p=0.09$; PICOS: $\chi^2=0.63$, $p=0.28$). Moreover the effect of OCs on the likelihood to suffer from a psychotic disorder was not modified by the GRM3 rs7808623 genotype (TABLE 9.17).

	TOTAL n= 255		UK n= 115		PICOS n= 140	
	OCs		OCs		OCs	
	No	Yes	No	Yes	No	Yes
G/G	141	66	60	36	81	30
G/T	26	19	7	9	19	10
T/T	0	2	0	2	0	0
OR	1.73		2.62		1.42	
95% CI	0.90-3.29		0.93-7.36		0.59-3.40	
P value	$p= 0.97$		$p=0.68$		$p=0.37$	

TABLE 9.17 GRM3 rs7808623 x OCs Analyses In The Context Of a Case-Only Study. ORs calculated using Logistic Regression tests.

9.6 Summary of Results

In order to overcome difficulties which I run across in **CHAPTER 6**, comprising the recruitment of appropriate control group, and to have greater statistical power with fewer subjects, in this chapter I performed a case only design statistical analysis in an attempt to replicate the findings of Nicodemus et al. (2008).

In my sample the selected genotypes do not modify the effect of OCs on the risk of a psychosis.

9.7 Discussions

GxE research represents an important approach to explain the occurrence of psychosis. In **CHAPTER 7** I aimed to address the question of whether or not genotype variants of AKT1, BDNF, DTNBP1 and GRM3 interact with OCs in increasing the risk of psychosis. Because of limitation of power, I was unable to provide definite evidence against a possible role of those genes in modifying the effect of OCs on risk of psychotic disorders. As explained in the introduction a case-only design can be a valid approach to evaluate gene-environment interaction in disease etiology (Piegorsch, et al., 1994; Khoury and Flanders, 1996; Yanget al., 1997; Andrieu and Goldstein, 1998; Weinberg and Umbach, 2000). Thanks to collaboration with the Psychosis Incidence Cohort Study (PICOS), Verona (Lasalvia et al., 2012) in this chapter I was able to definitively exclude an interaction between the above genetic variants and OCs in increasing the risk of psychosis.

9.8 Limitations

Various authors have highlighted advantages of the case-only design in detecting gene-environment interactions in observational studies (Piegorsch, et al., 1994; Khoury and Flanders, 1996; Yanget al., 1997; Andrieu and Goldstein, 1998; Weinberg and Umbach, 2000; reviewed by Albert et al., 2001).

Of course, the case-only design is subject to methodological problems such as uncontrolled confounding, exposure misclassification, and nonresponse bias (Albert et al., 2001). On the other hand in its favor, the case-only design is immune to bias from poor control selection and to exposure misclassification that is differential by disease status (Albert et al., 2001). In addition case-only design can be highly sensitive to the assumption of independence between the environmental exposure and the genetic marker (Albert et al., 2001).

In conclusion, the case-only approach is nonetheless a useful tool, if used cautiously, for assessing interaction when the independence assumption is justified by empirical evidence or when selection of appropriate controls is difficult or impossible.

As shown in **CHAPTER 7**, in this study the assumptions of independency between exposure to OCs and AKT1, BDNF, GRM3 and DTNBP1selected SNPs are met, thus this case-only design results in efficient estimates of GxE interaction.

CHAPTER 10

Is Hypoxia The Remote Controller of Schizophrenia Genes?

10.1 Introduction

Recent advances in molecular technologies have made mouse models the first choice for studying most human genetic diseases and for schizophrenia have provided evidence in support of the abnormal neurodevelopment hypothesis (reviewed by Chen et al., 2006). Perinatal hypoxia models in rats and mice have revealed several behavioural, pharmacological, neurochemical and neuroanatomical abnormalities in adulthood with relevance to schizophrenia (Brake et al., 1997; Brake et al., 2000; El-Khodori and Boksa, 2000; Wakuda et al., 2008). As described in previous chapters (CHAPTER 7, 8 and 9), gene variants could influence the response to environmental risk factors such as OCs in the aetiology of schizophrenia. Alternatively, hypoxic regulation of genetic expression could be a potential mechanism in the pathogenesis of schizophrenia (Nicodemus et al., 2008; Joo et al., 2009). It has therefore been suggested that schizophrenia may arise from alterations in how some genes are “turned on or off” due to exposure to non-genetic factors (Rutten and Mill, 2009).

In this Chapter, I examined the genetic expression and methylation status of rat brain following hypoxic insult at three developmental stages.

10.2 Genes Selected for the Analysis

Genes were selected on the basis of the GWAS published in 2011 (GWAS, 2011); in particular among them, I selected the genes that are expressed in rat brain (namely CCDC68, CNNM2, CSMD1, MMP16, STT3A, TCF4 and TRIM26).

10.2.1 Coiled-Coil Domain Containing 68 (CCDC68)

CCDC68 gene encodes for a human protein, the Coiled-Coil Domain Containing Protein – 68, whose function is not presently understood.

10.2.2 Cyclin M2 (CNNM2)

This gene encodes a member of the ancient conserved domain containing protein family. CNNM2 is a member of a family of four proteins, CNNM1-4. Members of this protein family contain a cyclin box motif and have structural similarity to the cyclins and may play an important role in magnesium homeostasis in kidney (The National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database). The highest expression of CNNM2 is reported to be ubiquitous in kidney and brain (Stuiver et al., 2011). In kidney CNNM2 transcript is up regulated under conditions of Mg^{2+} deficiency in mouse distal convoluted tubule cells suggesting a specific response to extracellular Mg^{2+} concentrations and thus involvement of CNNM2 in Mg^{+} transport (Stuiver et al., 2011). Tissue damage resulting from trauma to the CNS appears to result from secondary, delayed biochemical changes that follow primary mechanical injury (Vink et al., 1988). Mg^{2+} is essential for a number of critical enzyme reactions, including those of glycolysis, oxidative and substrate level phosphorylation, protein synthesis, phospholipid synthesis, and in maintaining membrane stability; changes in free Mg^{2+} after brain trauma may represent a critical early factor leading to irreversible tissue damage and possible impairment of DNA synthesis (Vink et al., 1988; Blair et al., 1989). Zhang and

colleagues studying primary hippocampal neurons show an increasing of 1.51-fold in Mg^{2+} after 1 hour of oxygen-glucose deprivation suggesting that there may be a Mg^{2+} overload inside the neurons induced by hypoxia (Zhang et al., 2011). In this regards hypoxia induce cellular potential depolarization that cause a subsequent intracellular magnesium surge (Kato et al., 1998). CNNM2 expression regulation may be explained by high concentration of extracellular Mg^{2+} induced by hypoxia.

10.2.3 CUB and Sushi Multiple Domains 1 (CSMD1)

This gene encodes a protein, the CUB and Sushi multiple domains 1. It is a potential tumour suppressor (NCBI RefSeq). CSMD1 is a novel multiple domain complement-regulatory protein highly expressed in regions of neuronal differentiation and outgrowth, remaining high in the adult in areas of great neuronal plasticity, such as the cerebral cortex and especially, the hippocampus (Kraus et al., 2006).

Schizophrenia is established as a brain developmental disorder and it is becoming increasingly clear that immune molecules contribute to brain development and function (Kraus et al., 2006). Epidemiologic studies have often correlated the risk of schizophrenia to hyperactivation of the peripheral immune systems i.e., prenatal infections and autoimmune disorders (Brown and Susser, 2002; Ortega-Hernandez et al., 2009; reviewed by Håvik et al., 2011). Recent studies on candidate genes also suggest immunity pathways as major risk factors for schizophrenia (Guilloux et al., 2010). Acute hypoxia and reperfusion activates the neuroimmune system (Johnson et al., 2007). Restoration of blood flow to ischemic tissue initiates a cascade of inflammatory events, including complement activation (C-activation) which all contribute to post ischemic injury (Jang and Rabb, 2009). Complement regulatory proteins may be important in protecting synapses from aberrant elimination during development and disease. These results suggest that CSMD1 may be an important

regulator of complement activation and inflammation in the developing CNS (Kraus et al., 2006). Genetic association of CSMD molecules to schizophrenia may reflect a heritable impairment in the regulation of the classical complement cascade (Havik et al., 2011).

10.2.4 Matrix Metalloproteinase 16 (MMP16)

MMP16 codes for proteins of the matrix metalloproteinase (MMP) family involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis (Andersson et al., 1996). Most MMP's are secreted as inactive pro-proteins which are activated when cleaved by extracellular proteinases (NCBI RefSeq). A reduction in MMP16 expression has been shown on exposure to hypoxia in PC-3 prostate cancer (Kakkad et al., 2010).

10.2.5 Transcription Factor 4 (TCF4)

TCF4 encodes transcription factor 4, a basic helix-loop-helix transcription factor. TCF4 is widely expressed in human tissues and appears to be involved in multiple biological processes (reviewed by Navarrete et al., 2012). In the brain TCF4 encodes a transcription factor involved in the development of a subset of neural progenitors (Flora et al., 2007). It is likely to play a major role in neurodevelopment, especially as haploinsufficiency has been associated with Pitt-Hopkins syndrome, a severe epileptic encephalopathy with mental retardation and intermittent hyperventilation. Thus showing the importance of a proper gene dosage and tight control of TCF4 expression during brain developmental (Zweir et al., 2007; Navarrete et al., 2012). Brzozka et al. (2011) found that TCF4-overexpressing transgenic mice show schizophrenia-associated behavior (Brzozka et al., 2010). Moreover it seems plausible- based on the

genomics, biological function and interaction of TCF4 in the context of schizophrenia- suggest that TCF4 plays an important role during CNS development and in acute functional effects in neurons (Navarrete et al., 2012).

10.2.6 Tripartite Motif Containing 26 (TRIM26)

TRIM26 code a member of the tripartite motif (TRIM) family protein. The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. The protein localizes to cytoplasmic bodies. Although the function of the protein is unknown, the RING domain suggests that the protein may have DNA-binding activity. The gene localizes to the major histocompatibility complex (MHC) class I region on chromosome 6(NCBI RefSeq). Alternatively spliced transcript variants encoding the same protein have been found for this gene. Recent studies have shown that members of the TRIM superfamily are expressed in response to IFNs and are involved in a range of biological processes associated with innate immunity (Ozato et al., 2008). In addition to their role in innate immunity, TRIM proteins are involved in some genetic disorders, neurological disorders and cancers suggesting an important role in variety of different biological processes (Meroni and Diez-Roux, 2005).

10.2.7 Subunit of the Oligosaccharyl-Transferase Complex, Homolog A (SST3A)

SST3A encodes for a component of the N-oligosaccharyl transferase enzyme. SST3A seems to be involved in complex substrate specificity (by similarity) (NCBI RefSeq). Mutations in a gene encoding an ortholog of STT3a cause salt/osmotic stress hypersensitivity in Arabidopsis (Koiwa et al., 2003).

10.3 DNA Methyltransferases (DNMT)

DNA methylation plays an important role in genomic imprinting through regulation of gene expression, and is essential for development. Several DNA methyltransferases (DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L) have been identified. DNMT1 preferentially methylates hemimethylated DNA and is thought to be a maintenance DNA methyltransferase. DNMT3A and DNMT3B appear to function as de novo methyltransferases since they can methylate unmethylated and hemimethylated DNA with equal efficiencies. Thus, the study of DNMT expression can be a valid approach for an indirect evaluation of rat brain following hypoxic insult.

10.4 Aims

The aims of the present study are to:

- 1.** Examine whether rats that had experienced perinatal asphyxia (A-rats) during birth show any abnormality in gene expression (CCDC68, CNNM2, CSMD1, MMP16, STT3A, TCF4 and TRIM26) in Prefrontal Cortex (PFC), Hippocampus (HiP) and Striatum (STR) at three developmental periods, post natal day (PND) (T1: 0 week), adolescence (T2: 5 weeks old) and adulthood (T3: 12 weeks old).
- 2.** Measure DNMTs expression in PFC, HiP and STR at two developmental periods, PND and adolescence (T1 and T2) in order to have an indirect measure of the methylation status of different brain regions followed by hypoxic insult.

10.5 Hypotheses

1. I expect that rats exposed to hypoxia during birth (A-rats) will show an abnormal gene expression in PFC, HiP and STR at three developmental stages
2. I expect the methylation status of different brain regions to be related to the perinatal asphyxia exposure.

10.6 Methods

I will briefly outline any general materials and methods used in the research reported in this chapter.

10.6.1 Animals and Hypoxia

All experiments were performed in accordance with the Guide for Animal Experimentation at the Hamamatsu University School of Medicine.

Following the methods previously described by Bjelke and Wakuda, intrauterine anoxia was induced immediately after isolation of the uterus in rats delivered by caesarian section (C- section) (Bjelke et al., 1991; Wakuda et al., 2008). Pregnant Sprague–Dawley rats (Japan SLC, Hamamtsu, Japan) within the last day of gestation were anesthetized by diethyl ether and hysterectomized. The isolated intact uterus including the fetuses was placed in a water bath at 37C to induce 15 minutes of asphyxia (100% survival). After delivery, the umbilical cord was legated and the pups were left to recover on a heating pad for 40 minutes. Rats that had delivered normally were used as surrogate mothers, and their pups were used as vaginal delivered pups.

Each surrogate mother received four pups from another surrogate mother, four C-section delivered pups and four asphyxia exposed pups identified by different ear tags.

They were housed three per cage in polycarbonate cages (42.5×26.6×18.5) and maintained in a controlled environment with 12-h light/ 12-h dark cycle (lights on at 7:00 a.m.) at $22 \pm 2^{\circ}\text{C}$ (relative humidity $55 \pm 10\%$), with free access to food and water.

After rats were sacrificed and the brain removed, the Prefrontal Cortex (PFC), Hippocampus (HiP) and Striatum (STR) were immediately dissected. Coronal brain slices of the brain were prepared using Stainless Steel Rat Brain Slicer. The slices were immediately transferred to ice-cold buffer. Samples were stored at -80°C until RNA extraction.

The animals at T1, T2 and T3 were divided in three groups based on the circumstance of delivery: Vaginal delivery (V group), Cesarean section (C group) and Cesarean section with 15 minutes of perinatal asphyxia (A group).

10.6.2 RNA Extraction

10.6.2a Preventing RNA Degradation

The major source of failure in any attempt to produce RNA is contamination by ribonuclease. Ribonucleases (RNases) are very stable enzymes hard to inactivate and great care must be taken to avoid introducing them into RNA preparations both during and after extraction. The following procedures were routinely used to prevent RNA degradation:

- Gloves were always worn and sterile laboratory techniques used at all times

- Sterile, disposable plastic ware was used where possible
- Non-disposable glassware was treated to remove RNase's (baked at 200 °C overnight, and then rinsed with Diethyl Pyrocarbonate (DEPC) -treated water)
- Chemicals used in RNA isolation and analysis were kept separate from other uses
- Any solutions made-up were treated by addition of DEPC to 0.05% and overnight incubation at room-temperature, followed by autoclaving for 30 minutes to remove any traces of the DEPC

10.6.2b Extraction of RNA from Brain Tissue

Brain tissue was obtained from Sprague–Dawley rats, deep-frozen at -80 °C. The following protocol, using TRIzol reagent (Life Technologies, Carlsbad, CA), was used to extract RNA from the tissue:

- Up to 200 mg of tissue homogenized in 2 ml TRIzol reagent
- 1 ml transferred to each of two 2.0 ml Eppendorf tubes
- Incubated at room temperature (20 to 25 °C) for 5 minutes
- 0.2 ml chloroform (CHCl₃) added to each tube
- Vortex vigorously for 15 seconds
- Incubated at room temperature for 2-3 minutes
- Spun at 12,000g for 15 minutes at 4°C
- Combined aqueous phases transferred to a new 2.0 ml Eppendorf tube
- Precipitated with 0.5 ml Isopropanol

- Incubated at room temperature for 10 minutes
- Spun at 12,000g for 15 minutes at 4°C and supernatant immediately decanted
- Pellet disrupted in 1 ml 75% ethanol and centrifuged at 7,500g for 5 minutes, immediately decanted supernatant and carefully removed excess with pipette tip
- Dried and resuspended in 100 µl DEPC-dH₂O

10.6.2c RNA Sample Clean-up

All traces of genomic DNA need to be removed from samples before applications such as Reverse Transcript- Polymerase Chain Reaction (RT-PCR) can be performed. DNase I (Invitrogen Cat. No. 18068015) was thus used to degrade both double-stranded and single-stranded DNA endonucleolytically.

The DNase digestion reaction was set up as follows:

Following extraction from tissue, RNA samples were cleaned-up using RNeasy spin column kits (Qiagen, Crawley, UK) following the manufacturers protocol.

- Bring RNA to 100 µl with dH₂O
- Add 350 µl buffer RLT and mix by light vortex (Buffer RLT must have 10 µl of 2-mercaptoethanol added per 1 ml of buffer beforehand)
- Add 250 µl 100% ethanol, mix by pipetting up/down
- Apply sample (700 µl) to RNeasy spin column, centrifuge 10,000 rpm for 15 sec, RT
- Transfer the column into a new 2 ml collection tube and wash with 350 µl buffer RW1, centrifuge as above

- Transfer the column into a new 2 ml collection tube, add DNase I mix (80ml) to the spin column, and incubate for 15 min at room temperature
- Add 350 µl buffer RW1, and centrifuge as above
- Transfer the column into a new 2 ml collection tube, wash with 500ml buffer RPE, and centrifuge as above
- Transfer the column into a new 2 ml collection tube, wash with 500ml 80% EtOH, and centrifuge 10,000 rpm for 2 min at room temperature
- Transfer the column into a new 2 ml collection tube, open the lid of the spin column, and centrifuge full speed for 5min at room temperature
- To elute, transfer the column to a 1.5 ml tube, pipet 50 µl of RNase-free water directly onto the column membrane
- Incubate RT 1 min and centrifuge full speed for 2min at room temperature

10.6.2d RNA Quality Control

The RNA quality was calculated after measuring absorbance with a spectrophotometer at 260 nm. Following extraction, each RNA sample was quantified using the NanoDrop ND-1000 spectrophotometer. Good quality RNA should have an OD260 / OD280 ratio of between 1.7 and 2.1, and ratios smaller than this can occur because of factors like protein contamination. After quantification, samples were stored in 1.5 ml Eppendorf tube at -20°C prior to further use.

10.6.3 cDNA Synthesis

Following extraction of RNA, cDNA synthesis was set up as follows using III First-Strand Synthesis System for RT-PCR (Invitrogen Cat. No. 18080-051) following the manufacturers' protocol:

- Combine the following in a 0.5-ml tube:
 1. Up to 5 μ g of total RNA
 2. Primer 1 μ l of 50 μ M oligo(dT)₂₀
 3. 10 mM dNTP mix 1 μ l
 4. DEPC-treated water to 10 μ l
- Incubate at 65°C for 5 min, then place on ice for at least 1 min.
- Prepare the following cDNA Synthesis Mix, adding each component in the indicated order:
 1. 10X RT buffer 2 μ l
 2. 25 mM MgCl₂ 4 μ l
 3. 0.1 M DTT 2 μ l
 4. RNaseOUT™ (40 U/ μ l) 1 μ l
 5. SuperScript™ III RT (200 U/ μ l) 1 μ l
- Add 10 μ l of cDNA Synthesis Mix to each RNA/primer mixture, mix gently, and collect by brief centrifugation. Incubate 50 min at 50°C
- Terminate the reactions at 85°C for 5 min. Chill on ice.

- Collect the reactions by brief centrifugation. Add 1 µl of RNase H to each tube and incubate for 20 min at 37°C.

10.6.3a cDNA Quality Control

Following extraction, each cDNA sample was diluted in 50 µl DEPC-dH₂O and quantified using the NanoDrop ND-1000 spectrophotometer. Clean cDNA should have an OD₂₆₀ / OD₂₈₀ ratio of between 1.7 and 2.1, and ratios smaller than this can occur because of factors like protein contamination. Samples were stored in 1.5 ml Eppendorf tube at -20°C prior to further use.

10.6.4 Quantitative Real-Time RT-PCR

10.6.4a Primer Design

Primer specificity was initially assessed *in silico* by searching NCBI and ENSEMBL Browsers for nontarget complementary sequences throughout the genome. Probe and primer sets for the candidate genes tested (CCDC68, CNNM2, CSMD1, MMP16, STT3A, TCF4 and TRIM26) were designed using Oligo7 and Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). The specificity of the primer was evaluated by whole genome alignment using the BLAST.

The primer design strategy is outlined in **FIGURE 10.1** along with the primer sequences which are summarized in **TABLE 10.1**.

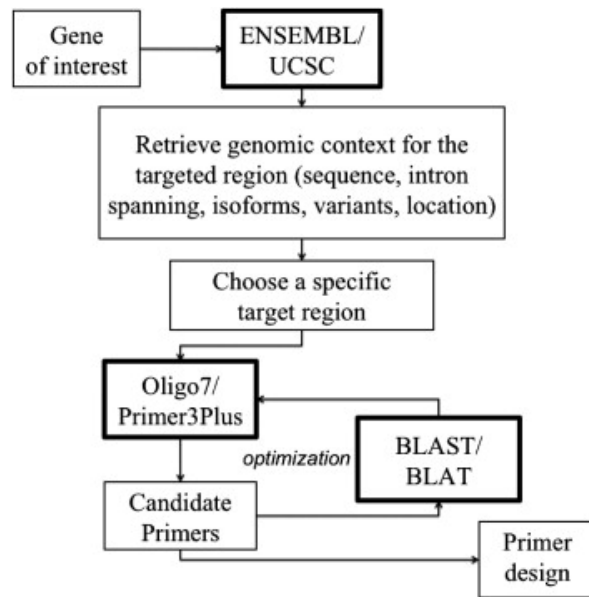


FIGURE 10.1 Primers Design.

Gene	Forward primers	Reverse primers
CCDC68	CACACTGGACGGAATCTCCT	TCGTAGCCGAATCAACATCA
CNNM2	TTGTCAGCAGGACAGAGGTG	GTCGCTCCGACTGAGAGAGT
CSMD1	ATCATTACCAGGGCACCAG	TTTCATGGCCAGCATAGC
MMP16	AGCTTTCGTCCACAAGGAAA	CCTTGAGGATGGATCTTGGA
STT3a	GAACATTGGCTGGTCAGGAT	TCAGTGCGAAGCATATCAGG
TCF4	CGAATCACATGGGTCAGATG	GGAGCAGTGTTTATGTGGGTTT
TRIM26	AAGGCAAGCTGCTGAGAGAC	ACTGGCCGGTGTAGGTAATG
Internal Control		
GAPDH	GACATGCCGCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

TABLE 10.1 Primers Sequences.

10.6.4b RT- PCR Procedure

Real-time PCR was performed the SYBR GREEN I PCR Master Mix (Qiagen, Hilden, Germany). The optimization of the real-time PCR reaction was performed according to the manufacturer's instructions but scaled down to 16µl per reaction. The PCR conditions were standard (SYBR-Green I core reagent protocol) and all reagents were provided in the SYBR-Green I core reagent kit (Qiagen, Hilden, Germany).

Relative expression of specific candidate genes was determined by comparing their expression of to a set of four housekeeping control genes: b-actin (ACTB), CYCRO, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following PCR cycling conditions were used:

- 50°C for 2 minutes
- 95°C for 15 minutes
- 95°C for 15 seconds
- 60°C for 1 minute

Repeat 40 cycles.

Using the comparative gene expression method, a validation experiment to check the efficiencies of the assays were equal was performed. After optimization, nucleotide primers were used at various concentrations for the detection and quantification of GAPDH, signal and coding CCDC68, CNNM2, CSMD1, MMP16, STT3A, TCF4 and TRIM26 (primer sequences table 1). In particular GAPDH expression served as a control for mRNA expression. Quantitative RT-PCR was performed in triplicate for each sample (5 each group) on an ABI Prism 7900HT. Gene expression changes were quantified using the delta-delta C_T method. In particular the expression data

produced were analyzed and converted into threshold cycle values (CT values) using Excel (Microsoft Office Excel 2003).

10.7 Statistical Analysis

All the statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corporation 1989, 2011).

Values were expressed as means $\pm$ Standard Error of the Mean (SEM) and compared using repeated measures ANOVA and post hoc comparisons with Tukey's Multiple Comparisons Test, differences among experimental conditions were considered statistically significant when $p \leq 0.05$.

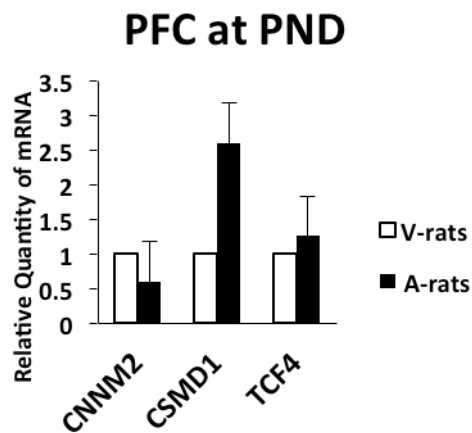
10.8 Results

10.8.1 Genetic Expression of Different Brain Regions Followed by Hypoxic Insult at Birth

10.8.1a T1: Post Neonatal Day

Examining genes expression abnormalities at T1, of 7 genes tested, CNNM2 was significantly down regulated in PFC (mean difference -0.73, SE 0.21, $F(2,18)=6.02$; $p=0.009$), HiP (mean difference -0.58, SE 0.20, $F(2,16)=5.19$; $p=0.027$) and STR (mean difference -0.91, SE 0.28, $F(2,15)=5.61$; $p=0.015$) of A-rats compared with controls (**FIGURE 10.2b**). In contrast, as result of hypoxia, CSMD1 (mean difference 1.14, SE 0.48, $F(2,18)=4.71$; $p=0.025$) and TCF4 (mean difference 0.33, SE 0.08, $F(2,18)=11.60$; $p=0.003$) were overexpressed in PFC of A-rats (**FIGURE 10.2a**).

(A)



(B)

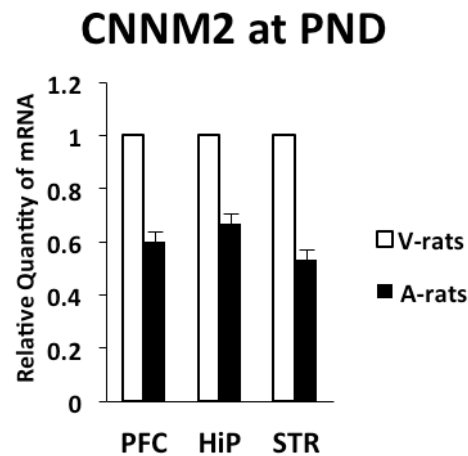


FIGURE 10.2 Gene Expression Differences Following Hypoxia at PND. (A) Genetic expression changes in PFC. (B) CNNM2 deregulation in PFC, HiP and STR of A-rats compared to V-rats. Data represent Mean $\pm$ SEM, $p < 0.05$.

10.8.1b T2: Childhood

Looking at the genetic expression of A-rats at 5 weeks I observed a significant reduction of most of the genes examined in PFC, namely CNNM2 (versus C rats: mean difference -0.61, SE 0.15, $F(2,18)=9.20$; $p=0.003$ / versus V rats: mean difference 0.47, SE 0.14, $F(2,18)=9.20$; 0.011), CSMD1 (mean difference -0.59, SE 0.21, $F(2,18)=4.18$; $p=0.031$), MMP16 (mean difference -0.83, SE 0.26, $F(2,18)=5.23$; $p=0.015$) and STT3a (mean difference -0.68, SE 0.22, $F(2,18)=5.39$; $p=0.019$) (**FIGURE 9.3a**). SST3a was under expressed in STR (versus C rats: mean difference -0.90, SE 0.23, $F(2,18)=8.13$; $p=0.004$ / versus V rats: mean difference 0.57, SE 0.21, $F(2,18)=8.13$; 0.038) (**FIGURE 10.3c**). Interestingly I found TRIM26 elevated in HiP (mean difference 0.97, SE 0.30, $F(2,17)=6.24$; $p=0.016$) and STR (mean difference 0.84, SE 0.23, $F(2,18)=7.52$; $p=0.007$) compared with controls (**FIGURE 10.3d**).

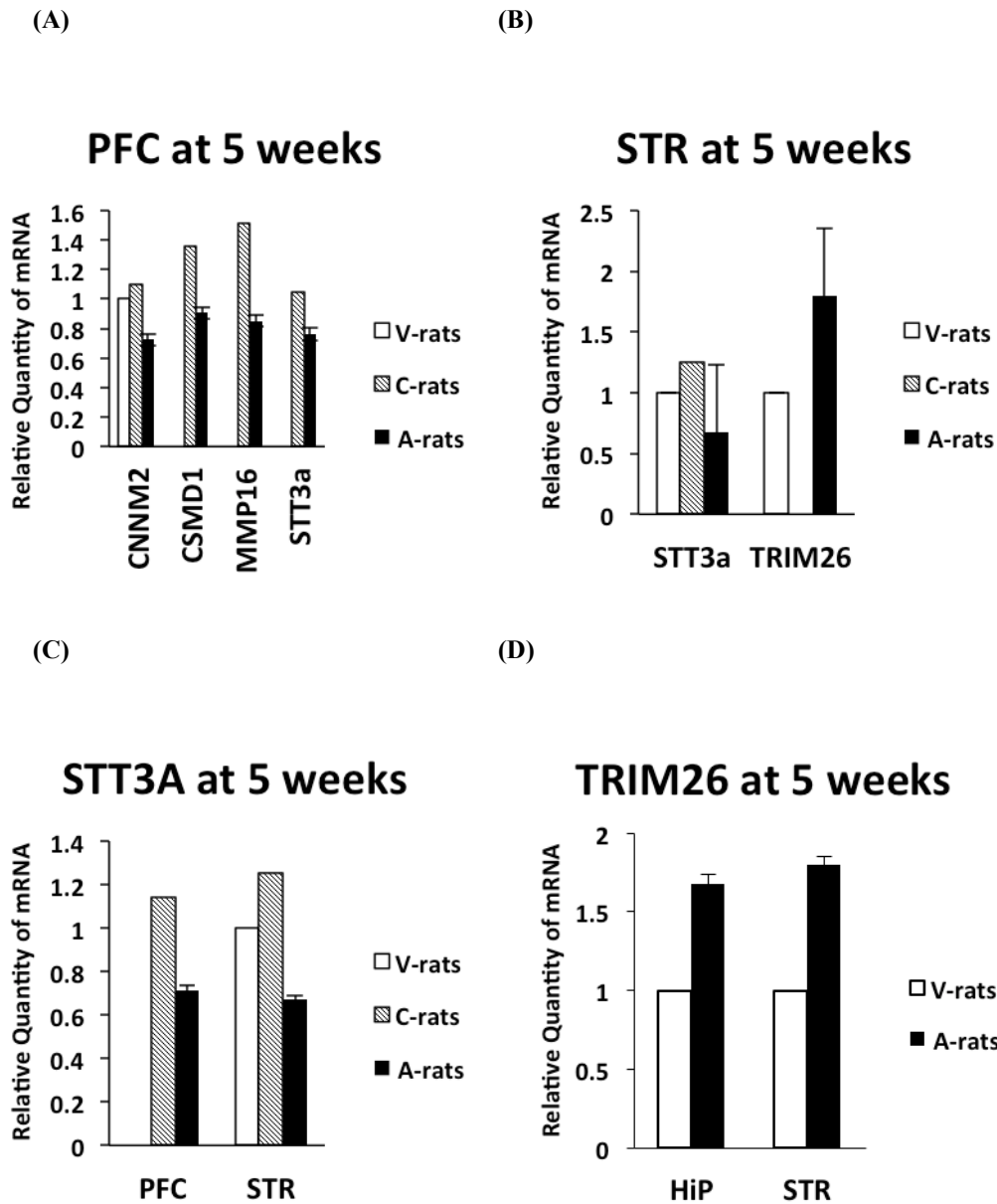


FIGURE 10.3 Gene Expression Differences Following Hypoxia at 5 Weeks. (A) and (B) genetic expression changes in PFC and STR. (C) STT3a deregulation in PFC and STR of A-rats compared controls. (D) TRIM26 over expression in HiP and STR of A-rats. Data represent Mean $\pm$ SEM, $p < 0.05$.

10.8.1c T3: Adulthood

Global hypoxia at birth did not affect the genetic expression in PFC, HiP and STR of 12 weeks rats.

10.8.2 Methylation Status of Different Brain Regions at T1 and T2 Following Hypoxic Insult at Birth

Looking at DNMTs expression abnormality following hypoxia I didn't find any significant abnormality at T1. On the other hand, at T2 I found an over expression in the HiP of 5 weeks old rats of DNMT3b (mean difference 0.73, SE 0.17, F (2,17)=9.43; p=0.003) (FIGURE 10.4a) and an over expression of in the STR of 5 weeks old rats of DNMT3a (mean difference 0.36, SE 0.13, F (2,17)=5.02; p=0.034) (FIGURE 10.4b)

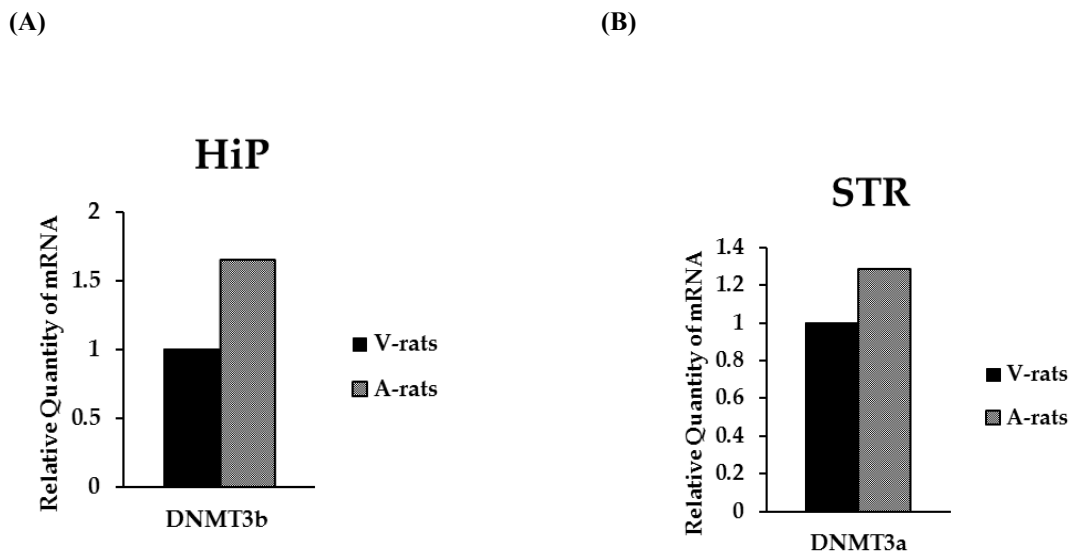


FIGURE 10.4 DNMTs Expression Abnormality Following Hypoxia at 5 Weeks. (a) DNMT3b over expression in HiP. (b) DNMT3a over expression in STR. Data represents Mean $\pm$ SEM, $p < 0.05$.

10.9 Summary of Results

Following hypoxic insult, 7 genes were analyzed for expression as above, namely CCDC68, CNM2, CSMD1, MMP16, STT3A, TCF4 and TRIM26. Overall, many of these genes had heterogeneous pattern of expression, with specific up regulation or

down regulation in A-rats. In general, at post neonatal day CNNM2 was down regulated, whereas CSMD1 and TCF4 were up regulated; at 5 weeks CNNM2, CSMD1, MMP16, STT3a were down regulated, whereas TRIM26 was overexpressed. In addition in my sample I found an over expression of DNMT3b and DNMT3a at 5 weeks.

10.10 Discussions

Increasing evidence from animal studies indicates that both prenatal and early postnatal environmental factors can result in altered epigenetic programming and subsequent changes in the risk of developing disease (Jirtle and Skinner, 2007). In particular many OCs seem to compromise neurodevelopment inducing the oxygen deprivation in the foetus (Mc Grath and Murray, 2003). Nevertheless, no studies have yet examined the direct link between early environmental exposures such as OCs with epigenetic profiles and risk of psychotic disorders.

In my study rats showed a heterogeneous pattern of expression overall the brain in many of the genes tested following hypoxic insult. It seems that the hypoxic regulation of expression of some of these genes could be a potential key player in the aetiopathogenesis of schizophrenia. Moreover at 5 weeks DNMT3b and DNMT3a were over expressed. DNA methylation plays an important role in genomic imprinting through regulation of gene expression, and is essential for development. In mice after 30 min of cerebral artery occlusion mutants heterozygous for a DNA methyltransferase gene deletion (*DnmtS/1*) were resistant to mild ischemic damage, suggesting that increased DNA methylation contributes to poor tissue outcome after mild ischemic brain injury (Endres et al., 2000). DNMT3a and DNMT3b appear to

function as de novo methyltransferases. Both of them could then contribute to the generation of the genome methylation pattern, suggesting a hypoxic regulation to the genetic expression throughs epigenetic mechanism.

It is likely that genes play an important role in mediating the reactions of the CNS to environmental stimuli such as hypoxia. Epigenetic regulation of gene expression is a plausible mechanism behind abnormal gene expression and aberrant neurodevelopmental processes under the effect of perinatal hypoxia–ischemia (Schmidt-Kastner et al., 2012). My study shows that hypoxia in the prenatal and perinatal period regulates the expression of specific genes suggesting a possible contribution to the neurodevelopmental alterations later found in schizophrenic patient. Changes in de novo methylation due to hypoxic insult could also contribute to silencing of the promoters for specific genes and lead to delayed ischemic brain injury.

CHAPTER 11

General Discussion and Future Directions

11.1 Initial Hypotheses and Final Results

In this last chapter findings obtained from the different investigations undertaken in the PhD project are discussed. Based on them, main conclusions are drawn, and directions for future research are presented. **TABLE 5.1 (CHAPTER 5)** summarized the main investigations and related hypotheses (and associated statistical analyses) of the project. **TABLE 11.1** summarizes the results for each of the initial hypotheses.

	Aims	Hypothesis	Results
1	Investigate obstetric history in psychotic patients, their unaffected siblings and healthy controls	I expect psychotic patients to be more likely than controls to have experienced a definite OC	OC increases the risk of psychosis of OR=1.9
2	a) Replicate Nicodemus et al. (2008) b) Examine whether genes deriving from the most recently available GWAS play a role in the onset of psychotic disorders in interaction with OCs	I expect the effect of OCs on odds of psychotic disorder to be conditional on the individual's genotype	My results do not provide definite evidence against a possible role of selected genotypes in modifying the effect of OCs on risk of psychotic disorders
3	Examine whether rats that had experienced perinatal asphyxia during birth show any abnormality in gene expression and methylation status	I expect the hypoxic rats to show abnormal gene expression and methylation status compared to controls	Following hypoxic insult many genes had heterogeneous pattern of expression and the DNA showed an abnormal methylation status

TABLE 11.1 Summary of Results for Each Initial Investigation

This study confirms that patients are more likely than non- psychotic individuals to report an OC, with an effect for OCs in increasing the risk for psychosis of about 1.9, similar to the widely reported risk of 2.0 for schizophrenia (Cannon et al., 2002). As I discussed in the Introduction, OCs are among the best-documented environmental risk factors for schizophrenia, with the first evidence for an association with schizophrenia dated 1934 (Rosanoff et al., 1934). It is now established that the risk of developing schizophrenia is increased in individuals who were exposed to various complications during pregnancy (Cannon et al., 2002). Moreover recent epidemiological studies further document the relationship between obstetric complications and psychosis-like symptoms or deficit in cognitive function (Zammit et al., 2009).

OCs increase risk of schizophrenia but not everyone that experienced OCs develops psychosis later in life. Variability in people's responses to environmental risk factors suggests that genes and environments operate together to produce psychosis (Tsuang et al., 2004; Di Forti et al., 2007b). It is likely that genes play an important role in mediating the reactions of the CNS to environmental stimuli such hypoxia. A difficult challenge is to identify plausible susceptibility genes to investigate how, and if, they moderate the effect of obstetric complications on the risk of psychosis. In this study I selected genotype variants of a total of 32 SNPs to investigate the interaction with OCs in increasing the risk of psychosis. In particular this is the first study examining the interaction between OCs and GWAS selected genes in increasing the risk of psychosis. Despite all the effort I spent to recruit a decent sample, the present study did not have sufficient power to detect any Gene x Environment interaction. Thus my results, although negative, do not provide definite evidence against a possible role of selected genotypes in modifying the effect of OCs on risk of psychotic disorders.

For several labour and delivery complications associated with increased incidence of

schizophrenia perinatal hypoxia is highly likely to be a common factor and the incidence of schizophrenia increases linearly with the number of hypoxia-associated birth complications (Cannon et al., 2002). Many obstetric complications seem to compromise neurodevelopment inducing the oxygen deprivation (hypoxia) in the foetus (Mc Grath and Murray, 2003). Increasing evidence from animal studies indicates that both prenatal and early postnatal environmental factors can result in altered epigenetic programming and subsequent changes in the risk of developing disease (Jirtle and Skinner, 2007; Pishva et al., 2014). This is the first study examining the direct link between early environmental exposures to OCs and abnormal epigenetic profile of psychosis related genes. In particular rats following hypoxic insult showed a heterogeneous pattern of expression in many genes tested. Moreover DNMT3b and DNMT3a were over expressed at 5 weeks. DNMT3a and DNMT3b appear to function as de novo methyltransferases, which may contribute to the generation of the genome methylation pattern.

Thus my findings suggest that OCs are involved in the aetiopathogenesis of schizophrenia probably through hypoxic regulation of gene expression via epigenetic mechanism.

11.2 Discussions

Many studies have uncovered the strong association between specific OCs and an increased risk of developing schizophrenia (Cannon et al., 2002). On the other hand GWAS provided a significant contribution in identifying novel common and rare genetic variants associated with psychotic disorder (GWAS, 2011). It is very likely that OCs and genotype are independent of each other, which is striking enough to

suggest that the key to understand why some subjects who experienced OC develop psychotic disorders while most come to no harm, is to investigate the interplay between the exposure to specific OCs with genetic variants.

Despite there being many SNPs identified so far, the effects of the genotype on phenotype will be far easier to assess if I know what those genes identified code for. In recent years Mittal et al. (2008) directly addressed ways of linking genes to the environment through various models including different varieties of the Multi-factorial Polygenic Threshold Model (MPTP):

- *Phenocopy Model*

A specific population of affected individuals may not be genetically predisposed to schizophrenia, but may have, at some point in time, been overexposed to a particular stimulus which exceeded the threshold for liability. This model is not particularly well-suited to fitting genetic predispositions. However the external stimulus that breaches the phenotypic threshold for this disease can be interpreted to be an OC, suggesting that an OC is more likely to cause schizophrenia, regardless of genotype.

- *Gene-Environment Covariation Model*

Genetics and OCs might both be confounders and not contribute to the etiology of schizophrenia - together they may increase the liability of onset, but individually are not responsible for the onset. The delicate balance between gene and environment interplay is enough to elicit a synergistic effect in predisposing a subject to schizophrenia.

- *Gene-environment Interaction and Additive Influences*

Disease-promoting genes may have a more severe effect coupled with an environmental influence, rather than by themselves. This model also suggests that the

two (i.e. the OC and the gene) can occur independently of each other, but the presence of both exacerbates the effect in bringing on the disease.

Mittal's study concluded that different types of OCs could influence the liability to schizophrenia via different specific mechanisms (Mittal et al., 2008).

GxE research represents an important approach to explain the occurrence of psychosis but not the only way to explain how hypoxia in the prenatal and perinatal period could influence the liability to the illness later in life. Potentially overlapping gene-environment mechanism and/or epigenetic mechanism seem to be a plausible explanation (Mittal et al., 2008). Moreover the absence of clear genetic effects in psychosis supports the concept that the biological risk factors are epigenetic in form rather than solely DNA sequence based (Rutten and Mill, 2009). Epigenetic processes regulate key neurobiological and cognitive processes in the brain through regulation of gene expression. Epigenetic mechanisms are influenced by a spectrum of external environmental factors including early environmental factors, diet, toxins, drugs, and stress. In addition polymorphisms can also exert an effect on gene function via epigenetic processes (Gamazon et al., 2013). These suggest a common pathway behind both genetic and environmental effect possibly through gene-environment interaction (Dempster et al., 2013).

OCs occur at critical periods early in development, a time of rapid cell replication when the epigenome is known to be particularly labile in response to external factors and the standard epigenetic signals driving development and tissue differentiation are being established (Rutten and Mill, 2009; Dempster et al., 2013). In rats following hypoxic insult many of the genes had heterogeneous pattern of expression, suggesting

an important role for genes in mediating the reactions of the CNS to environmental stimuli such hypoxia.

11.3 Conclusion

Nobody would dispute that research into gene environment interaction gave an important contribution to the etiology of psychotic disorders while trying to explain variations in the effect of environmental factors on behavior. Most scientists would accept that variations in the effects of environment on health in general are often mediated by the individual genotype make-up.

My findings do not support a GxE between OCs and selected genetic polymorphisms in increasing the risk of psychosis. On the other hand they suggest that hypoxia in the prenatal and perinatal period seems to regulate the expression of specific genes contributing to the neurodevelopmental alterations later found in schizophrenic patient.

Findings from this PhD project add to the understanding in the extent to which epigenetics provides useful information to explain early environmental exposure impact on psychotic disorders. It can be relevant for future studies whose major challenge would be the inclusion of an epigenetic assessment together with genetic and environmental data. Thus, the key to understand why some people who experience OCs develop psychotic disorders while most come to no harm is to investigate the interplay between the exposure to hypoxia in the prenatal and perinatal period and the epigenome.

In conclusion epigenetics would help to map new pathophysiological pathways

underlining psychosis. Epigenetic mechanisms may mediate the link between environment and the development of psychosis.

11.4 Future Directions

Although much research that has gone into determining the pathophysiology of psychotic disorders, only parts of the puzzle have been found.

The evidence for OCs increasing schizophrenic liability has been established for some time, and further research into this area is perhaps not necessary. In the next decade or so, the focus should probably move to larger case-control studies that incorporate and interpret demographic information rather than use it to adjust statistical values only.

The ongoing research into genetic information should also continue. My findings didn't show any interaction between genes and OCs in increasing the risk of psychosis. Hopefully, more research will continue into the role of gene-environment interaction in the aetiology of schizophrenia and lead to methods of primary prevention. However, these will need to be carried out on a much larger scale than in this PhD. It also gives a flicker of hope to other mental illnesses, and further controlled trials may well act as a double-edged sword in finding a pathophysiology and a specific intervention.

Moreover, a better understanding of the epigenetic mechanisms of certain environmental factors, such as hypoxia, could help to draw a more precisely biological process behind the neurodevelopmental nature of psychosis. Nevertheless, no studies have yet examined in human the direct link between early environmental

exposures with epigenetic profiles and risk of psychotic disorders. Although the epigenetic profile of somatic cells is mitotically heritable, there is evidence that epigenetic mechanisms may be heritable during meiosis in humans and thus potentially transmitted across generations (Rutten and Mill, 2009). This field has reopened the Lamarckian concept of heritability of acquired traits receiving support from rodent studies (Pishva et al., 2014). Thus epigenetic profiles and their subsequent behavioral expression seem to be transmitted to subsequent generations (Pishva et al., 2014). It needs to be established whether epigenetic inheritance is indeed relevant for humans (Pishva et al., 2014). It may be worth exploring whether the exposure to early environmental factors such as OCs induces any abnormal epigenetic profiles in the offspring and whether subsequent phenotypes are specifically transmitted to the offspring. These findings may have strong implications for understanding the impact of environmental influences on epigenome and may help to unveil the missing heritability of psychosis.

Longitudinal cohort studies with data collection on environmental exposures, genetic predisposition and epigenetic profiles are therefore needed.

11.5 Implications of Using Obstetric Records on Clinical Setting

At the moment, pre-natal screening exists for either very common or very serious conditions in the UK. However, family history of psychotic illness is not routinely taken into account, and extra care during the birth, or being equipped with demographic and past family OC history, could play a key role in diverting OCs and the repercussions they have on both mother and child. If OCs were to follow the

phenocopy model, then primary prevention of OC should be sufficient in reducing psychosis liability.

For those neonates who have suffered from adverse pre and perinatal conditions, careful monitoring and early intervention may lead to better management and early diagnosis of psychosis.

ACKNOWLEDGMENT

Thanks to my supervisors and many of you among friends, family and colleagues that made all of this possible.

And thanks to few others for making me strong.

There won't be any name, for my grandparents that can't hear theirs be called anymore and for the little one that never had the chance to have one.

What's in a name? That which we call a rose by any other name would smell as sweet
(William Shakespeare).

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APPENDIX

MATERNAL INTERVIEW SCHEDULES

GAP ID Number:

.....

Subject's Initials:

.....

Rater's Initials:

.....

Date of Completion:

..... / /

	Yes	No	Comments	Page
Obstetric Complications Questionnaire	☐ ₁	☐ ₂		I
Early Developmental	☐ ₁	☐ ₂		19
Assessment of Premorbid Schizoid-Schizotypal Traits	☐ ₁	☐ ₂		14

OBSTETRIC COMPLICATIONS

IDENTIFICATION FORM

Mother's date of birth

..... / /

Father's date of birth

..... / /

Total number of children

.....

Children's date of birth and gender
(tick the box of proband)

Male Female

☐₁ / / ☐₁
☐₂

☐₂ / / ☐₁
☐₂

☐₃ / / ☐₁
☐₂

☐₄ / / ☐₁
☐₂

☐₅ / / ☐₁
☐₂

Birth position of proband

..... (1=1st, 2=2nd etc)

Place of birth of proband

Town, Country

Post code.....

Population.....

BRIEF MATERNAL HEALTH QUESTIONNAIRE

Have you ever suffered from any of the following diseases?

No Yes Unknown

1. Endocrinological and/or metabolic diseases

☐ 0 ☐ 1 ☐ -77

1a. Any thyroid disorders

☐ 1

1b. Any glandular disorders

☐ 2

1c. Type I Diabetes

☐ 3

1d. Other

☐ 4

If yes, please note details

.....

2. Cerebro-cardiovascular diseases

☐ 0 ☐ 1 ☐ -77

2a. Rheumatic heart disorder

☐ 1

2b. Congenital heart disorder

☐ 2

2c. High blood pressure

☐ 3

2d. Low blood pressure

☐ 4

2e. Coagulation defects

☐ 5

2f. Hemostatic disorders

☐ 6

2g. Anemia

☐ 7

2h. Other

☐ 8

If yes, please note details

.....

3. Respiratory diseases

☐ 0 ☐ 1 ☐ -77

3a. Idiopathic Asthma

☐ 1

3b. Allergic Asthma

☐ 2

3d. Other

☐ 3

If yes, please note details

.....

4. Nervous system diseases

☐ 0 ☐ 1 ☐ -77

4a. Epilepsy

☐ 1

If yes, please note details

.....

5. Gynaecological diseases

☐ 0 ☐ 1 ☐ -77

If yes, please note details

.....

6. Any other diseases

☐ 0 ☐ 1 ☐ -77

If yes, please note details

.....

[Part I] PREGNANCY COMPLICATIONS

1. When did you first know that you were pregnant? / /

No Yes Unknown

2. Do you recall anything in particular about the pregnancy? ☐ 0 ☐ 1 ☐ -77
If yes, please note details

.....

.....

3. Proband's birth weight grams/lb

4. Proband's birth age weeks

5. Did you suffer any of the following before becoming pregnant with the proband?

No Yes Unknown

5.1 Rhesus disease/incompatibility ☐ 0 ☐ 1 ☐ -77

5.2 Spontaneous abortion/miscarriage ☐ 0 ☐ 1 ☐ -77

.....trimester

5.2a. During which trimester

5.2b. Total number of the events

5.3 Rubella ☐ 0 ☐ 1 ☐ -77

5.4 Syphilis ☐ 0 ☐ 1 ☐ -77

6. During the pregnancy, did any of the following occur?

	No	Yes	Unknown	Trimester	N of episode
6.1 Falls, trauma	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.2 Hyperemesis/ excessive vomiting	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.3 Threatened miscarriage/ any ante-partum haemorrhage	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.4 Albuminuria/ presence of albumin in the urine	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.5 High blood pressure	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.6 Pre- eclampsia	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐
6.7 Eclampsia	☐ 0	☐ 1	☐ -77	1st ☐ 2nd ☐ 3rd ☐	Tot ☐

MATERNAL INTERVIEW SCHEDULES

6.8 Not putting on weight as expected

☐ 0

☐ 1

☐ -77

1st ☐ 2nd ☐ 3rd ☐

Tot ☐

6.9 Abdomen not growing as expected

☐ 0

☐ 1

☐ -77

1st ☐ 2nd ☐ 3rd ☐

Tot ☐

6.10 Putting on excessive weight

☐ 0

☐ 1

☐ -77

1st ☐ 2nd ☐ 3rd ☐

Tot ☐

6.11 Other

☐ 0

☐ 1

☐ -77

1st ☐ 2nd ☐ 3rd ☐

Tot ☐

If yes, please give description of problems

.....
.....
.....
.....
.....

GESTATIONAL INFECTIONS

7. During the pregnancy, do you remember having suffered any of the following?

Trimester	No	Yes	Unknown
7.1 Herpes/cold sores	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.1. Chickenpox	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.2. Rubella	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.3. Cytomegalovirus infection	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.4. Measles	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.5. Toxoplasmosis	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.6. Malaria	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.7. Tuberculosis	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.8. Typhoid fever	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.9. Listeria	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.10. Salmonella	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.11. Syphilis	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 ☐ -77	
7.12. Respiratory tract infection	☐ 0 ☐ 1	☐ -77	

MATERNAL INTERVIEW SCHEDULES

7.13.	Gastro-enteritis	1st ☐ 2nd ☐ 3rd ☐	☐ 0	☐ 1	☐ -77
7.14.	Urinary tract infections	1st ☐ 2nd ☐ 3rd ☐	☐ 0	☐ 1	☐ -77
7.15.	Jaundice	1st ☐ 2nd ☐ 3rd ☐	☐ 0	☐ 1	☐ -77
7.16.	Fever/temperature/pyrexia	1st ☐ 2nd ☐ 3rd ☐	☐ 0	☐ 1	☐ -77
7.17.	Any other infections/diseases	1st ☐ 2nd ☐ 3rd ☐	☐ 0	☐ 1	☐ -77

If yes, please give description of problems

.....

MATERNAL SUBSTANCE USE

ALCOHOL

8.1 During the pregnancy how often did you have a drink containing alcohol? ☐ -77

Never	☐	Trimester
Monthly or less	☐	1st ☐ 2nd ☐ 3rd ☐
2-4 time per month	☐	1st ☐ 2nd ☐ 3rd ☐
2-3 time per week	☐	1st ☐ 2nd ☐ 3rd ☐
4 or more time per week	☐	1st ☐ 2nd ☐ 3rd ☐
Everyday	☐	1st ☐ 2nd ☐ 3rd ☐

8.2 How many UNITS of alcohol did you have on a typical day when you were drinking? ☐ -99

		Trimester											
Trimester		☐	1 ☐	2 ☐	3 ☐	10 to 14	☐	1 ☐	2 ☐	3 ☐			
1 or 2	☐					15 to 19	☐						
3 or 4	☐					20 to 29	☐						
5 or 6	☐					30 or more	☐						
7 to 9	☐												

Units of alcohol

2 units= A pint of standard beer/ lager
 1 unit= A single measure of spirit/small glass of wine

MATERNAL INTERVIEW SCHEDULES

3 units= A pint/can of strong beer/lager
1.5 units= A bottle of alcopop (e.g. Smirnoff Ice)

CIGARETTES

9.1 How many cigarettes did you smoke per day on average?

☐ -77

Never smoked	☐	Trimester
6-9 cigarettes	☐	1st ☐ 2nd ☐ 3rd ☐
10-20 cigarettes	☐	1st ☐ 2nd ☐ 3rd ☐
>20 cigarettes per day	☐	1st ☐ 2nd ☐ 3rd ☐

9.2 Did anyone in your house smoke while you were pregnant? No ☐ 0 Yes ☐ 1 unknown ☐ -77

Total number of people smoking

.....

6-9 cigarettes	☐
10-20 cigarettes	☐
>20 cigarettes per day	☐

STREET DRUGS

10. How often did you use the following substances while you were pregnant?

☐ -77

	Amphetamin e	LSD	Heroin	Methadone	Cocaine	PCP/ Ketamine	Cannabis	Khat	Inhalants
Never	☐	☐	☐	☐	☐	☐	☐	☐	☐
Monthly or less	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐
More than once a month	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐
Weekly	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐	☐ 1st ☐ 2nd ☐ 3rd ☐
More than once a week	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐	☐ 1st ☐ 2nd ☐

MATERNAL INTERVIEW SCHEDULES

	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐
Every day	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1st ☐	1st ☐	1st ☐	1st ☐	1st ☐	1st ☐	1st ☐	1st ☐	1st ☐
	2nd ☐	2nd ☐	2nd ☐	2nd ☐	2nd ☐	2nd ☐	2nd ☐	2nd ☐	2nd ☐
	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐	3rd ☐

MEDICINES

11. During the pregnancy did you take any medication?

No ☐ 0 Yes ☐ 1 unknown

☐ -77

duration	No	Yes	Unknown	Trimester	Dose and
11.1 Anti-inflammatory, Analgesic and anaesthetic	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Anti-rheumatic	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.3 Migraine medication	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.4 Smooth muscle relaxants	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.5 Anticonvulsives	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.6 Hormones	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.7 Antihistamines	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.8 Immunosuppressive	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Anticoagulants	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Blood pressure medication	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Psychopharmaca	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Antibiotics	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		
11.2 Vaccinations	☐ 0 1st ☐ 2nd ☐ 3rd ☐	☐ 1 1st ☐ 2nd ☐ 3rd ☐	☐ -77		

MATERNAL INTERVIEW SCHEDULES

11.2 Vitamins

☐ 0 ☐ 1 ☐ -77
1st ☐ 2nd ☐ 3rd ☐

11.2 Folic acid/B9

☐ 0 ☐ 1 ☐ -77
1st ☐ 2nd ☐ 3rd ☐

12. Did you breast feed?
Yes ☐ 1

No ☐ 0

If yes, please give length of
time

.....

12a. If yes, did you take any of the previous substances during breast feeding?

☐ -99

duration	No	Yes	Unknown	Dose and
<u>ALCOHOL</u>	☐ 0	☐ 1	☐ -77	
<u>CIGARETTES</u>	☐ 0	☐ 1	☐ -77	
<u>STREET DRUGS</u>	☐ 0	☐ 1	☐ -77	
Amphetamine	☐			
LSD	☐			
Heroin	☐			
Methadone	☐			
Cocaine	☐			
PCP/ ketamine	☐			
Cannabis	☐			
Khat	☐			
Inhalants	☐			
<u>MEDICINES</u>	☐ 0	☐ 1	☐ -77	
Anti-inflammatory, Analgesic anaesthetic	☐	and		
Anti-rheumatic	☐			

MATERNAL INTERVIEW SCHEDULES

Migraine medication	☐	
Smooth muscle relaxants	☐	
Anticonvulsives	☐	
Hormones	☐	
Antihistamines	☐	
Immunosuppressive	☐	
Anticoagulants	☐	
Blood pressure Medication	☐	
Psychopharmaca	☐	
Antibiotics	☐	
Vaccinations	☐	
Vitamins	☐	
Folic acid/B9	☐	

Notes

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MATERNAL INTERVIEW SCHEDULES

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[Part 2] LABOUR AND DELIVERY COMPLICATIONS

	No	Yes	
Unknown			
1. Do you recall any difficulties <u>during the birth of your son/daughter?</u>	☐ 0	☐ 1	
☐ -77			
	<i>If yes, please note details</i>		
			
2. Did you give birth to twins?	☐ 0	☐ 1	☐ -77
2a. If yes, who came first?			
2b. How much later?	min		
3. How long did the labour last?	hours		
3a. > 24 hours	☐ 0	☐ 1	☐ -77
3b. > 36 hours	☐ 0	☐ 1	☐ -77
3c. < 3 hours	☐ 0	☐ 1	☐ -77

4. Was the labour induced?

☐₀ ☐₁ ☐₋₇₇
If yes, please note details

.....

5. Did you have a premature rupture of membrane?

☐₀ ☐₁ ☐₋₇₇

5a. > 24 h before delivery?

☐₀ ☐₁ ☐₋₇₇

5b. > 12 h before delivery?

☐₀ ☐₁ ☐₋₇₇

6. Was it a caesarean birth?

☐₀ ☐₁ ☐₋₇₇

6a. If yes, why was it done?

.....

6b. Was it done in emergency?

☐₀ ☐₁ ☐₋₇₇

7. Did the baby come out head first?

☐₀ ☐₁ ☐₋₇₇

7a. If not, was it a face birth?

☐₀ ☐₁ ☐₋₇₇

7b. Was it a forehead birth?

☐₀ ☐₁ ☐₋₇₇

7c. Was it a breech birth?

☐₀ ☐₁ ☐₋₇₇

7d. Other abnormal presentation?

☐₀ ☐₁ ☐₋₇₇
If yes, please note details

.....

8. Were forceps used/was a suction delivery?

☐₀ ☐₁ ☐₋₇₇

9. Did you have any bleeding during the delivery?

☐₀ ☐₁ ☐₋₇₇

10. Did you have any bleeding soon/after the delivery?

☐₀ ☐₁ ☐₋₇₇

11. Did you have any problem with the placenta?

☐₀ ☐₁ ☐₋₇₇

11a. If yes, was it an ablatio placenta?

☐₀ ☐₁ ☐₋₇₇

11b. Was it a placenta praevia?

☐₀ ☐₁ ☐₋₇₇

12. Was the cord prolapsed?

☐₀ ☐₁ ☐₋₇₇

13. Was the cord around the baby's neck?

☐₀ ☐₁ ☐₋₇₇

14. Did rhesus disease occur?

☐₀ ☐₁ ☐₋₇₇

Notes

.....

[illegible]

12

4. Do you know the Apgar score of your baby?

☐0 ☐1 ☐-77
If yes, note the 1st, 5th, 10th minutes score

.....

5. Did your baby require resuscitation?

☐0 ☐1 ☐-77

6. Was the baby ventilated?

☐0 ☐1 ☐-77

7. Was the baby intubated?

☐0 ☐1 ☐-77

8. Was the baby in incubator?

☐0 ☐1 ☐-77

.....days

9. Did the baby have any malformations at birth?

☐0 ☐1 ☐-77
If yes, please note details

.....

10. Did the baby have any infections at/soon after birth?

☐0 ☐1 ☐-77

10.1 Congenital syphilis

☐0 ☐1 ☐-77

10.2 Toxoplasmosis

☐0 ☐1 ☐-77

10.3 Listeria

☐0 ☐1 ☐-77

10.4 Rubella

☐0 ☐1 ☐-77

10.5 Cytomegalovirus

☐0 ☐1 ☐-77

10.6 Herpes virus

☐0 ☐1 ☐-77

10.7 Smallpox

☐0 ☐1 ☐-77

10.8 Hepatitis A or B

☐0 ☐1 ☐-77

10.9 Coxsackie A or B

☐0 ☐1 ☐-77

10.10 HIV

☐0 ☐1 ☐-77

10.11 Malaria

☐0 ☐1 ☐-77

10.12 Enterovirus

☐0 ☐1 ☐-77

10.13 Congenital typhoid fever

☐0 ☐1 ☐-77

10.14 Pneumonia

☐0 ☐1 ☐-77

10.15 Tuberculosis

☐0 ☐1 ☐-77

10.16 Parvovirus

☐0 ☐1 ☐-77

10.17 RSV (Respiratory Syncytial Virus)

☐0 ☐1 ☐-77

10.18 Other

☐0 ☐1 ☐-77

If yes, please note details

11. Did the baby have to have any treatment/medication at/soon after birth?

☐ 0 ☐ 1 ☐ -77
If yes, please note details

12. Did the baby have to have a blood transfusion at/soon after birth?

☐ 0 ☐ 1 ☐ -77
If yes, please note details

Notes

EARLY DEVELOPMENTAL QUESTIONNAIRE

- | | No | Yes | Unknown |
|---|----------------------------|----------------------------|------------------------------|
| 1 Did you breast feed your baby? | ☐ 0 | ☐ 1 | ☐ -77 |
| 1.1 If yes, from what age to what age (in months) | | | |
| | | From..... To..... | |
| 2 In the first few weeks after birth, or as a young baby, did s/he suffer from any infection affecting the central nervous system or brain like meningitis or encephalitis? | ☐ 0 | ☐ 1 | ☐ -77 |
| 2.1 If yes, what? | | | |
| | | | weeks |
| 2.2 At what age? | | | |
| 2.3 Were they admitted to hospital? | ☐ 0 | ☐ 1 | ☐ -77 |

2.4	If yes, where?	
		☐ ₀ ☐ ₁ ☐ ₋₇₇
2.5	Were there any long lasting effects? (Prompt if necessary: such as epilepsy/convulsions/fits weakness or learning problems)	
2.6	If yes, what?	
2.7	Where were they treated?	
2.8	Was any treatment given, such as anticonvulsants, physiotherapy, other?	
		<i>If yes, please note details</i>
		
		
		
		
3	What about later in childhood, perhaps during the school years, did s/he suffer from any similar infection affecting the central nervous system or brain, again like meningitis or encephalitis?	☐ ₀ ☐ ₁ ☐ ₋₇₇
3.1	If yes, what?	
3.2	At what age?	weeks
3.3	Were they admitted to hospital?	☐ ₀ ☐ ₁ ☐ ₋₇₇
3.4	If yes, where?	
3.5	Were there any long lasting effects? (Prompt if necessary: such as epilepsy/convulsions/fits weakness or learning problems)	☐ ₀ ☐ ₁ ☐ ₋₇₇
		
3.6	If yes, what?	
3.7	Where were they treated?	
3.8	Was any treatment given, such as anticonvulsants, physiotherapy, other?	
		<i>If yes, please note details</i>
		
		
		
		
4	Did your child suffer any serious head injury whilst a baby or later in childhood?	☐ ₀ ☐ ₁ ☐ ₋₇₇
4.1	If yes, at what age?	months /years
4.2	Was your child knocked out, or did they	☐ ₀ ☐ ₁ ☐ ₋₇₇

lose consciousness at all?

4.3 If yes, for how long? minutes/hours

4.4 Was your child admitted to hospital for treatment for this head injury? ☐₀ ☐₁ ☐₋₇₇

4.5 If yes, where?

4.6 Did s/he have a head scan or CT scan? ☐₀ ☐₁ ☐₋₇₇

4.7 Were there any long-lasting effects, like epilepsy/ convulsions/fits, or learning problems, speech problems or problems with weakness or in walking? ☐₀ ☐₁ ☐₋₇₇

4.8 Did your child have to have any long-term treatment, like anticonvulsants for fits, or physiotherapy or help with learning? ☐₀ ☐₁ ☐₋₇₇

4.9 If yes, what?

4.10 Where was this given?

5 Did your child ever receive any special help at school or go to a special school? For example, some children need special help with reading, or arithmetic/mathematics. ☐₀ ☐₁ ☐₋₇₇

5.1 If yes, what?

5.2 How old were they when it started? years

5.3 Who gave it / where was it given?

5.4 How old were they when it stopped? years

6 Did your child ever need to see a psychologist or educational psychologist or did they have their special educational needs assessed (were they "statemented")? ☐₀ ☐₁ ☐₋₇₇

.....years

6.1 If yes, how old were they?

6.2 Who did they see?

6.3 Where was that?

6.4 Why was that?

7 Did your child ever need to see a psychologist, doctor or other specialist because of his/her behaviour? (Were they ever referred to a child guidance clinic or other type of clinic?) ☐₀ ☐₁ ☐₋₇₇

.....

MATERNAL INTERVIEW SCHEDULES

7.1	If yes, why?	years		
7.2	How old were they?	years		
7.3	Where was that?			
7.4	Did they get any treatment or other special help?	☐ 0	☐ 1	☐ -77
7.5	If yes, what?			
8	Did your child ever need to see a psychologist, doctor or other specialist because of problems at school?	☐ 0	☐ 1	☐ -77
8.1	If yes, why?			
8.2	How old were they?	years		
8.3	Where was that?			
8.4	Did they get any treatment or other special help?	☐ 0	☐ 1	☐ -77
8.5	If yes, what?			
9	Did your child ever have to see a child psychiatrist or psychologist for any other reason?	☐ 0	☐ 1	☐ -77
9.1	If yes, why?			
9.2	How old were they?	years		
9.3	Where was that?			
9.4	Did they get any treatment or other special help?	☐ 0	☐ 1	☐ -77
9.5	If yes, what?			
10	Did your child reach all his/her milestones at the same time as his/her brothers and sisters, or other children of a similar age? For example, crawling, walking talking, stopping wetting the bed, that sort of thing.	☐ 0	☐ 1	☐ -77
10.1	If not, were they earlier or later in any of these compared with other children?	☐ 0	Earlier	
		☐ 1	later	
10.2	If they were later, what did you notice?			
10.3	Which milestones were later?			
11	Did your child ever have to see a doctor or other specialist because of problems with the way they developed, like walking or talking later than other children?	☐ 0	☐ 1	☐ -77
11.1	If yes, why?			

MATERNAL INTERVIEW SCHEDULES

11.2 How old were they? years

11.3 Where was that?

11.4 Did they get any treatment or other special help? ☐0 ☐1 ☐-77

11.5 If yes, what?

12 Now I'd like to ask you about each of the milestones, so thinking back to the first few years of their life:

12.1 At what age did s/he sit up? ☐1 3-6 months
☐2 7-12 months
☐3 over 12 month
☐-77 don't know

12.2 At what age did s/he crawl? ☐1 6-12 months
☐2 12-18 months
☐3 over 18 month
☐-77 don't know

12.3 At what age did s/he walk? ☐1 under 1 year
☐2 1-2 years
☐3 3-4 years
☐4 over 4 years
☐-77 don't know

12.4 At what age did s/he speak single words (other than "mama" or "dada") ☐1 9-13 months
☐2 14-18 months
☐3 19-24 months
☐4 2-3 years
☐5 3-4 years
☐6 over 4 years
☐-77 don't know

12.5 At what age did s/he string two or more words together? ☐1 9-13 months
☐2 14-18 months
☐3 19-24 months
☐4 2-3 years
☐5 3-4 years
☐6 over 4 years
☐-77 don't know

13 Did your child have any serious illnesses, either as a baby or later while they were at school – I mean illnesses other than the usual mild childhood illnesses? ☐0 ☐1 ☐-77

13.1 If yes, what?

13.2 How old were they? months/years

13.3 Were they admitted to hospital? ☐0 ☐1 ☐-77

- 13.4 If so, where?
- 13.5 Did they get any treatment or other special help?
- 13.6 If yes, what?
- 14 While they were growing up, was there anything you thought was different about compared with other children? ☐ 0 ☐ 1 ☐ -77
- 14.1 If so, what?
- 14.2 How old were they? months/years
- 14.3 Did you or his /her teachers do anything about this?
- 15 Is there anything about while they were a child that you think may be important or significant in any way, but that we have not covered? ☐ 0 ☐ 1 ☐ -77
- If yes, please note details*
-
-
-
-

ASSESSMENT OF PREMORBID SCHIZOID-SCHIZOTYPAL TRAITS

Information from either parent

The starred questions must be asked and then followed by further questions to define the degree of any abnormality.

N.B. For cases all questions refer to enduring personality traits present before the first episode of psychosis.

* **Cases:** We are interested in the sort of person that was before he/she first became ill at the age of (work out age from baseline file).

I. Social Isolation

- * *How social and outgoing was he/she?*
- * *Did he/she have many close friends?*

- Not withdrawn, active social interaction** ☐₁
- Casual friends only** ☐₂
- Moderately withdrawn, excessive daydreaming and fantasy, or deviant friendship patterns only (with children older or younger only, or relatives only, or with 1 or 2 individuals only)** ☐₃
- Unable to form relationships, withdrawn isolated – no friends** ☐₄

2. Affect

** How warm and affectionate was he/she?*

- Warm spontaneous shows of affection** ☐₁
- Displays affection with family, but often cold and aloof with others** ☐₂
- Hardly ever displays affection even with close relations** ☐₃
- Cold and aloof with all** ☐₄

3. Suspiciousness/Sensitivity

** Was he/she at ease when with other people, or did he/she sometimes appear to be unduly anxious or suspicious?*

** Did he/she mind being criticised?*

- Neither suspicious nor unduly socially anxious** ☐₁
- Undue social anxiety or occasionally suspicious or occasionally sensitive to criticism** ☐₂
- Marked social anxiety or suspicious/distrustful or hypersensitive to imagined criticism** ☐₃
- All characteristics listed in 1 to 3 above plus believed that someone was deliberately trying to harm him/her** ☐₄

4. Thought Content/Beliefs

** Did he/she ever express any strange or unusual ideas, or behave oddly?*

- No abnormality** ☐₁
- Occasionally (less than once a month) expresses odd ideas, strange or unusual perceptions, magical thinking or ideas of reference or occasional odd behaviour** ☐₂
- Often (more than once a month)** ☐₃
- Above features predominate (daily)** ☐₄

5. **Speech**

- * *Was his/her speech ever odd, vague or difficult to follow?*
 * *Did he/she ever use odd words or words in an unusual way?*

- Normal ☐₁
 Occasionally (less than once a month) deviant (see below) ☐₂
 Often (more than once a month) deviant (see below) ☐₃
 Consistent pattern of clearly deviant speech ☐₄

(Deviant speech: ie digressive, over elaborate, vague, circumstantial. Difficult to carry out meaningful, goal directed conversation)

6. **Antisocial behaviour (i.e. with delinquent peer group)**

- * *Did he/she ever commit any of the following: Lying, stealing, fire-setting, violence, drugs, truancy, vandalism, sex offences?*
 If yes: *Did he/she do this alone or with others?*

NB. Rate based only on those activities carried out with others.

- None ☐₁
 Mild ☐₂
 Moderate (leading to complaints by neighbours, teachers, police etc) ☐₃
 Severe and repeated (child arrested on more than one occasion or parents sought help from outside agencies) ☐₄

7. **Asocial, Antisocial behaviour (i.e. carried out alone)**

NB. Rate from answer to question 6 based on activities carried out alone.

- None ☐₁
 Mild ☐₂
 Moderate (leading to complaints by neighbours, teachers, police etc) ☐₃
 Severe and repeated (child arrested on more than one occasion or parents sought help from outside agencies) ☐₄

8. **Any Other Abnormalities**

Try to obtain verbatim account of premorbid personality

MATERNAL INTERVIEW SCHEDULES

*** How would you describe before they first became unwell?**

What was his/her mood like?

Was he/she a calm sort of person who took life as it came?

How did he/she cope with the normal demands of life?

What was his/her temper like?

What sort of standards does he/she have (at home, at work for example)

- | | |
|----------------------------|---------------------------------------|
| Normal | ☐ ₁ |
| Mildly abnormal | ☐ ₂ |
| Moderately abnormal | ☐ ₃ |
| Severely abnormal | ☐ ₄ |

9. **Total score**