Common genetic variants in psychosis across the lifespan from the beginnings of psychosis to cognition in later age

Kepinska, Ada

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King's College London

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Common genetic variants in psychosis across the lifespan
From the beginnings of psychosis to cognition in later age

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Doctor of Philosophy

November 2021
Declaration

This work is less than 100,000 words. Except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This thesis is my own work, with the following exceptions:

• Analyses in Chapters 3 and 4 were based on data from the Schizophrenia: Treatment Resistance and Therapeutic Advances-Genetics (STRATA-G) Consortium. The data were collated by Dr Sophie Smart. Dr Antonio Pardiñas and Dr Smart completed quality control of genetic data. Dr Pardiñas had calculated polygenic scores for schizophrenia which I then used for analyses.

• Analyses in Chapters 5 were based on data from the English Longitudinal Study of Ageing (ELSA). Dr Olesya Ajnakina completed quality control of genetic data and calculated polygenic scores for the whole study sample. I completed statistical analyses under Dr Ajnakina’s supervision and drafted the manuscript in Chapter 5. It underwent revisions in response to feedback from co-authors and as part of peer review prior to acceptance in Translational Psychiatry.

• Systematic review in Appendix A is primarily the work of Dr Sophie Smart. I rated literature and provided feedback on the manuscript draft. The paper also underwent peer review in Psychological Medicine.

• I wrote the first early draft of the literature review in Appendix B. I then rewrote and edited it in close collaboration with senior author, Dr Thomas Pollak. The review was also commented on by co-authors and underwent peer review prior to acceptance in Frontiers in Psychiatry.

This thesis incorporates publications of which I am first author (Chapter 5 and Appendix B). In-depth literature review for Chapter 5 is provided in Chapter 1 and an extended discussion of the paper and its implications is provided in Chapter 6.

Adrianna Patrycja Kępińska, November 2021
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This thesis is dedicated to fellow PhD students who have continued their research in the COVID-19 pandemic.
Abstract

Theory and practice of psychiatric genetics transformed within a decade. Genetics has gone through a century of unsuccessfully trying to find individual specific genes, basic units of heritability, which would explain risk for complex disorders. For the past ten years, genetics has been exploring joint contributions of thousands of genes instead. Research on psychosis has been a strong focus throughout this shift. Psychosis is an umbrella term for multiple types of experience (hallucinations, voices or visions which are not there, delusions, fixed, false beliefs, changes in emotion and drive, and decline in memory and other cognitive skills). Psychosis is a spectrum: it is not distressing and negative to all who experience it and it does not always result in clinical intervention, but also, patients with psychosis live with substantial disability and have a shortened life expectancy, often due to disorders simultaneously present with psychosis (e.g., individuals with schizophrenia lose the equivalent of on average 73% of healthy life per year due to comorbid mental and substance use disorders). Thus, clinical interventions try to alleviate psychosis and improve patient outcomes. As psychosis has a genetic component (its heritability is 70% or more, depending on a psychotic disorder), the aim of psychiatric genetics is to develop measures which would incorporate genetic factors to help explain and predict psychotic symptoms to anticipate, improve, and time, optimal treatment.

As it is now understood that no single candidate gene explains risk for psychosis, a measure including information on multiple genetic variants has been developed. This measure, a polygenic score, is supposed to reflect individual genetic predisposition towards a trait. Polygenic scores sum up information on thousands of common genetic variants into a single score. Each genetic variant is weighted by its effect size from a genome-wide association study (GWAS). For associations with disorder, GWAS compares common genetic variants between participants with a disorder and controls to determine which variants are more statistically likely to be related to the disorder. Polygenic scores have been primarily used to differentiate cases with psychotic disorders from controls. However, psychosis is a complex and heterogenous disorder. It can have different trajectories (from single episodes and complete remission to multiple relapses throughout life), different combinations of symptoms, and symptoms which change over time. Thus, the aim of this
thesis was to explore if polygenic scores for major psychiatric disorders predict specific psychosis manifestations which may impact psychosis trajectories over time: age of onset of psychosis (age of first symptom manifestations which eventually lead to psychiatric intervention; AOO); duration of untreated psychosis (from symptom onset to either hospitalisation or first antipsychotic treatment; DUP); and longitudinal cognitive decline of memory and executive function in later life (after the age of 50).

In Chapter 3, I show that polygenic scores for major psychiatric disorders (schizophrenia, bipolar disorder, major depressive disorder, autism spectrum disorder, and attention deficit hyperactivity disorder) do not predict AOO in first episode psychosis patients from different European cities. In Chapter 4, I demonstrate that these polygenic scores do not predict DUP in first episode psychosis patients. However, in both studies, research sites, from which the data come, are significantly related to AOO and DUP. Finally, in Chapter 5, I show that the polygenic score for schizophrenia predicts baseline cognitive performance, but not decline of memory and executive function, over a decade-long follow-up.

These findings suggest a nuanced picture of common genetic variants in psychosis: they may be related to increased risk for specific psychosis symptoms (e.g., general cognitive deficit), but not all clinical features which are labelled as “psychosis”, such as early psychotic symptoms or cognitive decline over time. Alternatively, findings in this thesis may be a result of differences between research sites in how psychosis manifestations are measured, inaccurate patient recall of their medical history, bias in diagnosis, or suboptimal methods of generating polygenic scores (these issues, and suggestions for future research, are discussed in Chapter 6).
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Abbreviations

ADHD-PGS  Polygenic score(s) for attention deficit hyperactivity disorder
ADHD  Attention deficit hyperactivity disorder
AOO  Age of onset
APOE  Apolipoprotein E
ASD-PGS  Polygenic score(s) for autism spectrum disorder
ASD  Autism spectrum disorder
BoFEP  Bologna FEP
bp  Base pair
BPD  Bipolar disorder
C4  Component 4
CAARMS  Comprehensive Assessment of At Risk Mental States
CAG  Confidentiality Advisory Group
CAPI  Computer-assisted personal interview
CES-D  Center for Epidemiologic Studies Depression Scale
CNV  Copy number variant
DNA  Deoxyribonucleic acid
DSM-5  The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DUP  Duration of untreated psychosis
DZ  Dizygotic
ABBREVIATIONS

E Environment

EHR Electronic health records

EIS Early intervention services

ELSA English Longitudinal Study of Ageing

EUGEI European Network of National Schizophrenia Networks Studying Gene-Environment Interactions

FDR False discovery rate

FEP First episode psychosis

F Homozygosity rate

GAP Genetics and Psychosis

G Genotype

CNIL Commission nationale de l’informatique et des libertés (National Commission on Informatics and Liberty)

GWAS Genome-wide association study

GWEIS Genome-wide environment interaction study

GxE Gene-environment interaction

H^2 Heritability (broad-sense heritability)

h^2 Heritability (narrow-sense/strict-sense heritability)

HLA Human leukocyte antigen

HRA Health Research Authority

HRS Health and Retirement Study

HSC Health and Social Care

HSE The Health Survey for England

HWE Hardy-Weinberg equilibrium

IBD Identity by descent

IBS Identity-by-state
ICD  The International Classification of Diseases, 11th Edition

ID  Identification

INFO  Information metric score

IoPPN  Institute of Psychiatry, Psychology & Neuroscience, King’s College London

IQ  Intelligence quotient

KCL  King’s College London

LASSO  Least absolute shrinkage and selection operator

LD  Linkage disequilibrium

MAF  Minor allele frequency

MDD-PGS  Polygenic score for major depressive disorder

MHC  Major histocompatibility complex

MRC  Medical Research Council

MZ  Monozygotic

NHS  The National Health Service

NIFEPS  Northern Ireland First Episode Psychosis

OPCRIT  Operational Criteria Checklist for Psychotic Illness

OR  Odds ratio

$P_T$  $P$ threshold

PAFIP  Programa de Atención a las Fases Iniciales de Psicosis (First Episode Psychosis Clinical Program)

PCA  Principal component analysis

PC  Principal component

PGC-BIP2  Psychiatric Genomics Consortium Bipolar Disorder Working Group data freeze 2

PGC-BIP3  Psychiatric Genomics Consortium Bipolar Disorder Working Group data freeze 3

PGC-BIP2  Psychiatric Genomics Consortium Schizophrenia Working Group data freeze 2
PGC-SCZ3 Psychiatric Genomics Consortium Schizophrenia Working Group data freeze

PGC Psychiatric Genomics Consortium

PGS Polygenic score(s)

PI Principal investigator

P Phenotype

PRS Polygenic risk score(s)

QC Quality control

REC The Research Ethics Committee

rGE Gene-by-environment covariance

RGPI Resource for Psychoses Genomics, Ireland

RNA Ribonucleic acid

SCAN Schedules for Clinical Assessment in Neuropsychiatry

SCID Structured Clinical Interview for DSM-IV

SCZ-PGS / SZ-PGS Polygenic score for schizophrenia

SCZ Schizophrenia

SD Standard deviation

SE Standard error

SNP Single nucleotide polymorphism

STRATA WS3 STRATA Workstream 3

STRATA-G STRATA-Genetics

STRATA The Schizophrenia: Treatment Resistance and Therapeutic Advances

TIPP Treatment and Early Intervention in Psychosis Program

TOP Thematic Organized Psychosis Research

UCL University College London

UHR Ultra high risk

ÆSOP Aetiology and Ethnicity in Schizophrenia and Other Psychoses
Chapter 1

Introduction

1.1 Psychosis

Psychosis is a broad term for multiple classes of symptoms. These classes are positive and negative symptoms, cognitive impairment, and disorganised or abnormal motor behaviour, as detailed in major categorical diagnostic systems, the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5; American Psychiatric Association, 2013) and the International Classification of Diseases, 11th Edition (ICD-11; World Health Organization, 2018). Positive symptoms include delusions (idiosyncratic beliefs inconsistent with reality), hallucinations (perceptions without necessary stimuli), and thought disorder (neologisms, illogicality and distractibility). Negative symptoms include blunted or flat affect, alogia (impaired thinking which leads to difficulties in verbal expression), avolition (no motivation or drive), asociality (no interest in social activities), and anhedonia (no experience of pleasure). Disorganised or abnormal motor behaviour includes fidgeting, shifting, fiddling, visible slowing of movements and speech, and catatonic symptoms, including posturing, waxy flexibility (decreased response to stimuli, immobility), negativism (passive resistance to instruction or stimuli), mutism (inability to speak), or stupor (rigid poses). Cognitive impairments are of working memory (short-term information storage, important in decision making), attention, concentration, judgement, learning, and orientation. ICD-11 (World Health Organization, 2018) also lists disturbances in self-experience as a symptom category. It is an experience that one’s thoughts, feelings, emotional states, and behaviours are not controlled by an individual themselves, but by an external force.

Psychosis prevalence in England is about 1% (Bebbington and McManus, 2020). Of psychotic disorders, schizophrenia potentially received the most attention as it is the most common psychotic disorder (Perälä et al., 2007). Nonetheless, all psychotic disorders are
a serious public health issue. They are linked to premature mortality of 8-14 years in bipolar disorder and schizophrenia, respectively. Mortality is also higher in men than in women with psychotic disorders (Weye et al., 2020). Finally, psychotic experience and other mental disorders have a bidirectional relationship (McGrath et al., 2016). Psychotic experience (hallucinations, delusions and convictions that one’s thoughts are controlled by external forces) increases risk for later development of 8 other mental disorders (with the highest risk for bipolar disorder). 18 non-psychotic mental disorders increase risk of future psychotic experience (with the highest risk increase linked to anorexia nervosa).

However, different experience may get labelled as psychosis in Western and non-Western cultures. These differences potentially lead to discrepancies in how clinicians apply diagnostic criteria and so, to discrepancies in international psychosis prevalence (Kalra et al., 2012; Luhrmann and Marrow, 2016). Additionally, psychotic-like experience is much more prevalent in the general population than schizophrenia (its mean lifetime prevalence is approximately 6%; McGrath et al., 2015). Not all individuals who experience psychosis consider their experience negative (e.g., threatening their safety) or distressing (Peters et al., 1999, 2017). Also, not all individuals, who experience psychosis, are later diagnosed with a psychotic disorder (Kaymaz et al., 2012). Thus, while this thesis focuses on genetics of psychotic disorder specifically (and psychotic disorder is what is labelled as “psychosis” throughout), psychosis is, in fact, a continuum (van Os et al., 2009).

Psychotic illness fluctuates and can have different trajectories. Psychosis can include prodromal illness: brief or attenuated symptoms and decline in functioning. After prodrome, an episode with strong psychotic symptoms may occur, called first episode psychosis (FEP). FEP may end in remission or recovery. Indeed, 38% of individuals with FEP experience stable recovery (Lally et al., 2017). Other progressions after FEP may include episodic remission and relapses (Emsley et al., 2013). Psychosis is not chronic for all patients who experience psychotic episodes after FEP. Recovery rates have been assessed to be almost half of patients in central south England (Morgan et al., 2014), or as a median of 13.5% in a systematic review and meta-analysis of studies from Europe, Asia, North and South America, Africa, and Australia (Jääskeläinen et al., 2013). Recovery rates may vary because of different criteria for recovery used in studies. For instance, criteria such as patient social inclusion following psychosis are more elusive to assess than symptom remission (Revier et al., 2015). Jääskeläinen et al. (2013) used stringent recovery criteria (clinical remission and improved social functioning for at least two years). This stringency may explain the relatively low median recovery rate in their study.

Remission and recovery in psychosis improves with response to and adherence to treatment. Clinical practice guidelines in the UK (National Institute for Health and Care Excellence, 2014), Australia and New Zealand (Galletly et al., 2016), and the US
(Kreyenbuhl et al., 2010) list cognitive/family therapy and medication as treatment. The main form of treatment is antipsychotics. Antipsychotics are drugs administered orally or via long-lasting injectables. They reduce severity of positive symptoms but are less successful in improving negative and cognitive symptoms (Harvey et al., 2016; Krause et al., 2018; Takeuchi et al., 2017). Antipsychotics also have side effects which often lead patients to voluntarily stop medication. Side effects include: increased risk for metabolic syndromes, acute movement disorders such as dystonias, akathisia, Parkinsonism and tardive dyskinesia (spasms, fidgeting, tremors, slow movements, uncontrollable movements of lips and tongue; Lally and MacCabe, 2015), negative changes in mood and motivation levels (Read and Williams, 2019), weight gain, glucose level increases, and changes in cholesterol levels (Pillinger et al., 2020). Finally, about 20-30% of individuals fail to respond to antipsychotics, i.e., are treatment resistant (Elkis and Buckley, 2016). See Appendix A for a systematic review on predictors of treatment resistant schizophrenia (Smart et al., 2021), of which I am second author.

In my thesis, I explored genetics of psychosis manifestations which potentially contribute to different psychosis trajectories: age of onset, duration of untreated psychosis, and longitudinal cognitive decline. In Chapter 3, age of onset refers to onset of at least one psychotic symptom, which leads to psychiatric intervention, in FEP patients from multiple European samples. Psychosis onset criteria most commonly included presenting with hallucinations, delusions, bizarre behaviour or disorganised speech (but also onset of any extra symptoms out of psychomotor disorders, catatonia, lack of insight, lack of pleasure, difficulties with concentration, depressive/sad mood, and elevated mood or mania). In Chapter 4, duration of untreated psychosis refers to a period between the onset of psychosis (most commonly defined as onset of hallucinations, delusions, bizarre behaviour or disorganised speech) and either hospitalisation for psychosis or start of antipsychotic treatment of FEP patients. In Chapter 5, the psychosis symptoms of interest are longitudinal decline in cognitive performance on tasks measuring verbal memory and semantic fluency. Tasks are completed over follow-up periods by healthy ageing participants (aged 50 years and more).
1.2 Psychosis manifestations over the course of illness

1.2.1 Age of onset

Age of onset of psychosis (AOO; study 1, Chapter 3) is most commonly early adulthood (ages 20-29). Men experience earlier onset (Castle et al., 1998; Häfner et al., 1993; Miettunen et al., 2019; Thorup et al., 2007). The onset before the age of 13, called childhood-onset or very early-onset schizophrenia, is much rarer. Its prevalence is 0.19 per 10,000 for children aged 2 through 12, with a more frequent onset in boys (Burd and Kerbeshian, 1987). Psychoses commencing after the age of 40 account for approximately 20% of cases (late-onset schizophrenia for psychosis after the age of 40, or very-late-onset-schizophrenia-like psychosis for psychosis after the age of 60; Howard et al., 2000; Jeste and Maglione, 2013). Early- and late-onset schizophrenia share several symptoms, including depressive, negative, and deficit symptoms (worse neurocognitive functioning and blunted affect; Vahia et al., 2010). Late-onset schizophrenia has a different gender distribution (more prevalence in women), lower average severity of positive symptoms, and lower average antipsychotic dose requirements (Maglione et al., 2014). Thus, psychoses with a different AOO may be overlapping but different psychosis subtypes (Maglione et al., 2014). They also differ in potential patient outcomes. Longitudinal studies with a mean follow-up of 42 years showed that individuals with childhood-onset schizophrenia frequently presented with poor outcomes. They experienced prolonged paranoia and disorganisation episodes, catatonia lasting for up to 40 years, or multiple shorter episodes of psychosis (Eggers et al., 2000). Also none of the chronic schizophrenia patients remitted completely (Eggers and Bunk, 1997). These findings suggest that outcomes of childhood-onset schizophrenia are poorer than outcomes of adult-onset schizophrenia, although, in contrast, a recent nationwide Danish register study from Vernal et al. (2020) found that patients with early-onset schizophrenia experienced longer hospitalisations in the first two years after diagnosis but had no poorer long-term outcome than patients with adult-onset schizophrenia over a mean 9.5-year-long follow-up.

1.2.2 Duration of untreated psychosis

Duration of untreated psychosis (DUP; study 2, Chapter 4) is a period between the onset of psychosis and exposure to treatment. This definition is broad, and its ending point of exposure is controversial. Exposure has different definitions across studies, including: hospitalisation, start of (antipsychotic) medication or responding to medication over
different time frames (from 3 to 12 weeks), entrance to a specialised therapeutic program, or combination of engagement in a specialised programme and adherence to medication (Golay et al., 2016). In this thesis, DUP is a period between the first occurrence of psychotic symptoms and either first hospitalisation or the start of antipsychotic treatment for psychosis.

A longer DUP has been relatively moderately, but consistently, correlated with poorer psychosis outcome: poor general symptomatic outcome, more severe positive and negative symptoms, less likelihood of remission, and poor social functioning and global outcome (Penttilä et al., 2014). Thus, reducing DUP is an intervention target (Murru and Carpiniello, 2018) to prevent onset or progression of illness (Fusar-Poli et al., 2017). Intervention may be especially beneficial for psychoses with poor prognosis, such as early-onset schizophrenia described above. In early-onset schizophrenia, a longer DUP predicted less longitudinal improvement in symptom severity (Stentebjerg-Olesen et al., 2016).

### 1.2.3 Cognitive decline

In this thesis, cognitive decline in later life is a change in verbal memory and semantic fluency (executive function) over the period of 10 years. Cognitive impairments are psychosis symptoms in both ICD-11 (World Health Organization, 2018) and DSM-5 (American Psychiatric Association, 2013). Yet, controversy surrounds characterisation of cognitive impairment in psychosis. The first issue is how large the cognitive impairment is. Previous research suggested that up to 27% of psychosis patients do not show neuropsychological impairment (Palmer et al., 1997). Meta-analyses suggested a nuanced picture. Bora et al. (2009) found worse cognitive performance in participants with schizophrenia and affective psychosis (schizoaffective disorder and bipolar disorder) in verbal memory, intelligence quotient (IQ), and executive function. Researchers found no group differences in attention, fluency, visual memory or spatial working memory tests. However, as differences between groups were small and effect sizes were heterogenous, Bora and colleagues argued that evidence was insufficient for categorical differences between schizophrenia and other psychoses. A more recent, larger meta-analysis (Lynham et al., 2020) found that participants with schizoaffective disorder had worse cognitive performance than participants with bipolar disorder, but their performance was better than that of participants with schizophrenia. Altogether, meta-analyses suggest that levels of cognitive impairment may differ across psychotic disorders and types of cognitive ability.

The second issue with cognitive impairment in psychosis is if it is stable, present before or at onset of psychosis, or if it worsens over time. Bora and Murray’s (2014) meta-analysis of cognitive change in individuals with FEP, individuals at ultra high risk (UHR) for
psychosis (with brief psychotic symptoms or decrease of functioning and with an increased genetic risk for psychosis), and controls showed no cognitive decline. Cognition in both FEP and UHR patients actually improved at follow-up. Consistently, a longitudinal study by Bonner-Jackson et al. (2010) found no decline in processing speed and stored knowledge in individuals with schizophrenia over 20-year follow-up. Zanelli et al. (2019), over a 10-year follow-up, found deficits at baseline, no decline in executive functions, and no decline in processing speed in schizophrenia patients, consistent with Bonner-Jackson et al. (2010). However, psychosis patients in Zanelli et al. (2019), who were tested on a larger range of cognitive skills than in Bonner-Jackson et al. (2010), also showed declines in verbal knowledge, IQ, and memory. An issue with the studies is that cognitive decline might occur as study participants grow older. Bonner-Jackson et al. (2010) only included relatively young individuals (mean age of 43 years at the 20th-year cognition reassessment; Fett et al., 2019). This issue may impact findings of Zanelli et al. (2019) as well, given that mean age of first-episode patients at follow-up in their study was 28 years.

To address this issue, Fett et al. (2019) studied patients aged 15 to 60 at first admission for psychosis. Patients completed cognitive assessments 2 and 20 years after FEP. Patients showed decline in verbal and visual memory, attention, processing speed, and abstraction-executive function, especially older patients. Fett and colleagues argued that it was evidence for accelerated cognitive ageing in psychosis. Taken together, these results suggest that presence and the extent of cognitive decline may differ between individuals with psychosis, depending on a psychotic disorder and cognitive domain. These findings require extra exploration, especially in the context of cognitive decline in later life.

### 1.3 Genetics in psychiatry

#### 1.3.1 Brief history of psychiatric genetics

Both genes and the environment influence a majority of human traits (Polderman et al., 2015; Turkheimer, 2000). Genes are a basic unit of heritability, i.e., variation in a measurable characteristic of an individual, called a phenotype (Gratten et al., 2014). Attempts at conceptualising heritability go back to antiquity. Heritability of mental illness especially was often at the core of developments in genetics (Schulze et al., 2004).

The first heritability concepts came from Ancient Greek philosophers. Euripides posited that mortality was familial. Plato argued that selective mating could foster ideal members of the state (Schulze et al., 2004). Psychiatric heritability specifically got first conceptualised by a Renaissance physician Paracelsus (Schulze et al., 2004). His
classification of mental health disorders included disorders passed from parents to offspring. In the 19th century, Western psychiatrists grappled with how “insanity” with onset in later life could be hereditary, given that hereditary traits were believed to manifest at birth. This issue led to a reconceptualisation of heredity of insanity as hereditary predisposition (e.g., towards poorer responses to stress which would trigger disorder onset in later life; Kendler, 2020). Psychiatrist Bénédict Morel introduced a concept of degeneration: a heritable pathology of physicality and morality. Degeneration and heredity provided a new conceptual psychiatric model. They would explain how mental disorders can be present even without specific organic lesions (Dowbiggin, 1991).

Then, experiments in crossing pea plants led monk Gregor Mendel to propose laws of inheritance of “cell elements.” These abstract hereditary determinants of specific plant features are a very early conceptualisation of genes (Portin and Wilkins, 2017). Researchers following Mendel, Mendelists, considered inheritance a series of discrete events. These events led to discontinuous variation in qualitative characteristics, i.e., organisms having distinct features which are not measurable as a range (Schulze et al., 2004). In opposition to that view stood scientist Francis Galton. Galton argued that mental traits, such as intelligence or disease, are a combination of both inheritance, or nature, and environment, or nurture (Galton and Galton, 1997). Galton also conceptualised heritability of traits as a continuous normal distribution. This framework, biometry, and the Mendelian school got reconciled by statistician Ronald A. Fisher (Schulze et al., 2004). Fisher (1919) argued that continuous variability of traits could result from additive effects of many discrete (individual) Mendelian factors.

Early 20th century brought early systematic family studies of psychiatric disorders. Ernst Rüdin completed large family studies of schizophrenia characterised in detail (Kendler and Zerbin-Rudin, 1996a,b). Studies exploring heritability of schizophrenia in twin pairs followed in 1928 (Schulze et al., 2004). Following World War II, research on psychiatric genetics lost popularity. Genetics concerned academic psychiatrists for its potential links to eugenics (that Galton, Fisher, and Rüdin all endorsed; Fisher, 1930; Gillham, 2001; Gottesman and Bertelsen, 1996; Mazumdar, 1996). In the 1980s, technologies for locating specific genes—gene mapping—improved. Thus, genetics moved towards a new research paradigm, genetic linkage. Genetic linkage/gene candidate studies search for chromosomal segments (nucleic acid structures containing genes) related to disease. Candidate gene studies focus on whether specific single genes contribute to disease (Claussnitzer et al., 2020).

However, candidate gene studies failed to replicate (Duncan et al., 2019). Also, as new technology enabled rapid generation of large genetic datasets (Farrell et al., 2015), research moved to analysing multiple genetic regions in each study. These studies include:
genome-wide association studies (GWAS; studies analysing thousands of common genetic variants without predefined hypotheses on which variants are related to phenotype); studies on rare genetic variants such as copy number variants (differences in the numbers of repeats of DNA segments in the genome); whole-genome sequencing (research exploring the whole genome); and whole-exome sequencing (research exploring only genes which encode proteins; see Claussnitzer et al., 2020, and Timpson et al., 2018, for reviews). Psychiatric disorders are now understood to be largely polygenic, with contributions from many genes and not a single gene of large effect (Horwitz et al., 2019). Most gene variants, particularly the common variants analysed by GWAS, have a small contribution per gene variant to the risk of disorder (Duncan et al., 2019). To detect this statistical signal, large samples are required. For this reason, research consortia now collect and collate large-scale genetic and phenotypic data (e.g., the Psychiatric Genomics Consortium; Watson et al., 2020).

Developments in the field lead to the present, deemed “the post-GWAS era” (Gallagher and Chen-Plotkin, 2018). Studies focus on extending results from GWAS. For instance, they clarify functions of genes implicated by GWAS, or model relationships between genes and phenotypes. The hope is that this progress in understanding subtle genetic differences could result in personalised psychiatry. Personalised psychiatry is evidence-based treatment tailored to each patient’s unique features, in genetics defined as using results of genetic testing to guide individualised treatment selection (DeRubeis, 2019).

1.3.2 Heritability and genetics of complex traits

In genetics, heritability is a population parameter. Box 1 presents definitions of different types of heritability and their measurement.

Box 1. Multiple definitions of heritability and its measurement.

Nature-nurture models assume that the genotype (G, human genes) and environment (E) contribute to the phenotype (P; P = G + E). Under those models, heritability is a ratio of variances. Heritability (H²) represents the proportion of phenotypic variance ($\sigma_p^2$) resulting from genetic variance ($\sigma_G^2$). This model excludes variation due to fixed factors such as gender, age, or cohort (Visscher et al., 2008):

$$H^2 = \frac{\sigma_G^2}{\sigma_p^2}$$

This general ratio is called broad-sense heritability because genetic variance is attributed to all possible genetic factors. Genetic factors are dominant or recessive.
Dominant alleles are gene variants whose effects mask or override the effects of recessive alleles. Genetic factors also include interactions between genes (Timpson et al., 2018).

Genetic variance can be further partitioned into additive genetic effects, dominance genetic effects, and epistatic genetic effects. Additive genetic effects are summarised average effects of genes from parents. These parental genetic effects result in the mean genotypic value of their offspring. Additive genetic effects are all effects that contribute to genetic variation in the population when genes differ between individuals. Dominance genetic effects come from interactions of genes on the same locus, i.e., genes located on the same chromosome. Epistatic genetic effects come from genes located on different loci (Visscher et al., 2008).

Heritability is a population parameter because populations differ in their genetic background: segregation of alleles which influence traits, allele frequencies, effect sizes of variants, and the mode of gene actions (Visscher et al., 2008). Heritability can also change within a population over time. An example is intelligence, the heritability of which increases throughout development (the finding and potential reasons for it are reviewed in Plomin et al., 2016).

Unlike broad-sense heritability, narrow-sense (strict-sense) heritability, $h^2$, only concerns additive genetic effects. It is the usual parameter in research. Genetic research focuses on narrow-sense heritability because of patterns of gene transmission between parents and offspring (Visscher et al., 2008). This transmission is of one copy of each gene from parents to offspring. Because of this transmission pattern, most relatives (except for identical twins and full siblings) share a single gene copy or no gene copies that are identical by descent. Gene copies identical by descent are inherited from a common ancestor, without any structural changes occurring over time due to e.g. recombination (Thompson, 2013). Dominance and other non-additive genetic effects based on sharing two gene copies do not contribute to phenotypic resemblance between most relatives. Consequently, narrow-sense heritability is the usual parameter in genetic research (Visscher et al., 2008).

To estimate $h^2$, twin heritability is used: comparing monozygotic (MZ, genetically identical) twins to dizygotic (DZ) or fraternal twins who share half of their genetic material. This estimate is completed under a classic assumption that twins share the same environment, called the equal environments assumption (Conley et al., 2013). Under the equal environments assumption, the difference between MZ and DZ twins in genetic contribution to phenotype of interest should be an unbiased estimate of $h^2$ (if this estimate is indeed unbiased remains debated; Felson, 2014).
CHAPTER 1. INTRODUCTION

Twin data estimates of heritability are much larger than another type of estimation, SNP-based heritability. SNP-based heritability estimates are based on single nucleotide polymorphisms (SNPs). SNPs are common genetic variants analysed in GWAS. The gap between twin studies heritability and SNP-based heritability is called missing heritability (Manolio et al., 2009). The main reasons hypothesised for missing heritability are that: 1) twin studies overestimate heritability, 2) genotyping array technologies need to improve to detect rare genetic variants which in fact contribute to heritability, or 3) GWAS are underpowered for now. Yet, as increasingly larger samples are analysed, GWAS will gain statistical power and will uncover more genetic variants, explain more genetic variance, and “close” the missing heritability gap. See Yang et al. (2017) and Young (2019) for issues with and methodologies for improving estimates of SNP-based heritability, and Tropf et al. (2017) on how issues with GWAS other than GWAS sample size influence SNP-based heritability: the fact that GWAS use pooled data and phenotypes are measured differently across cohorts potentially leads to poor consistency of SNP findings and, thus, poor SNP-based heritability estimates.

Narrow-sense heritability assumes no relationships between genes and the environment. Still, there exist two broad categories of relationships: gene-by-environment covariance (whether the environment to which the individual is exposed depends on their genotype; rGE) and gene-environment interaction (whether the individual’s response to the environment depends on their genotype; G×E; Krapohl et al., 2017). Still, these contributions to disorder are important – and understudied. Genotyping technology was initially not developed enough to detect fine differences between individual gene sequences and small sample sizes led to low statistical power in G×E studies (Iyegbe et al., 2014). Also, G×E studies inconsistently compared different variants from the same gene which has hindered comparison of findings and replication (Grabe and Van der Auwera, 2020). Research designs which address relationships between genes and the environment include structural equation modelling and genome-wide environment interaction study (GWEIS). Structural equation modelling explores covariation between multiple gene variants and environmental exposures (Krapohl et al., 2017). GWEIS tests interactions between multiple genetic variants and an environmental exposure on the outcome of interest (Zwicker et al., 2018; also see Almli et al., 2014 for methodological considerations in GWEIS).

1.3.3 Structure of the human genome

Since determining the full sequence of the human genome in 2003 (Collins et al., 2003), research progressed to address relationships between phenotypes and different elements
of the genome. In this thesis, I use GWAS results as a basis for analyses. This section presents key elements of structure of the human genome. GWAS exploits this structure to analyse multiple common genetic variants and associate them with phenotypes. Section 1.4. details the process of conducting GWAS.

The key elements of the human genome structure are:

Deoxyribonucleic acid (DNA): human DNA codes genetic information. DNA is made of nucleotides. Nucleotides contain a pentose sugar, deoxyribose, a phosphate group, and a nitrogen base. The genome contains approximately 3 billion nucleotide base pairs (bp), comprising the 4 nucleotide bases, adenine (A), cytosine (C), guanine (G), and thymine (T). 1.5% of the DNA sequence encodes proteins, basic building blocks of cells and organs (Lander et al., 2001). The remaining DNA sequence encodes non-protein-coding ribonucleic acid (RNA) transcripts or non-coding DNA. Non-protein-coding RNA transcripts regulate temporal and spatial expression of the protein-coding genes. Parts of the non-coding DNA may have no functional role (Kellis et al., 2014).

Chromosomes: units of the human genome, chromosomes are thread-like structures of varying length (International Human Genome Sequencing Consortium, 2004). Chromosomes consist of DNA coiled around stabilising proteins, histones. There are 22 chromosomes: 21, called autosomes, equal between sexes, and a sex chromosome which is either X or Y. Women usually carry two X chromosomes while men carry a copy of X and Y chromosome each. Locations of elements on the chromosome are called loci.

Polymorphisms: changes in the sequence of DNA nucleotides. A replacement of a single nucleotide (1 bp) is a single nucleotide polymorphism (SNP). Insertions or deletions of one or more nucleotides in a chromosomal segment (<50 bp) are collectively known as indels (from insertions and deletions). Polymorphisms result from inheritance or from gamete formation (egg or sperm cell formation). SNPs and indels are the vast majority of genetic variation. Each individual commonly has ~3-4 million SNPs and ~0.4-0.5 million indels. The vast majority have no functional impact on molecular function or phenotypes. Alternatively, SNP/indel combinations may affect gene structure or gene regulation, which in turn change amino acids. These changes may be either benign or causing disease (Lappalainen et al., 2019).

Alleles: polymorphism variants. Each individual has two alleles of each polymorphism because humans inherit one chromosome from each parent. At population level, multiple alleles of each gene exist. Allele frequency is used for association testing between genes and phenotype. For example, logistic regressions compare allele frequencies between cases with a specific phenotype and controls (Mills et al., 2020). Measures of allele frequency includes minor allele frequency (MAF). MAF is the frequency of the least often occurring
allele at a specific location. SNPs with a low MAF (less than 1%; Choi et al., 2020) are usually removed from analyses because the majority of studies are underpowered to detect associations with those rare SNPs (Mills et al., 2020) and because rare SNPs are prone to poor quality genotyping.

Linkage disequilibrium (LD): a phenomenon where a gene pair is not independently inherited from another gene pair at different loci. In other words, inherited alleles are in correlation. LD enables gene mapping. SNPs in strong LD with one another tend to be closely linked which means that alleles which e.g. increase the risk of complex disease are also inherited together (Slatkin, 2008). LD is commonly measured as a squared correlation ($r^2$). This measure is linear in the sample size which is required to detect associations between an observed and an unobserved causal genetic variant potentially in LD (Visscher et al., 2017).

Haplotype: a chromosomal region, also called a haplotype block, in high linkage disequilibrium (it contains highly correlated polymorphisms). Multiple mechanisms lead to the existence of haplotypes, including: recombination during meiosis (recombination of genetic material during cell division), natural selection, mutation, genetic drift (random changes in gene frequency in the population), bottlenecks (abrupt changes in population size, e.g. due to environmental disaster), exchange of individuals among populations, inbreeding between individuals of the same population, and genomic inversions (chromosomal mutation where the region between the breaks in the chromosome rotates 180 degrees before rejoining the end fragments; Slatkin, 2008).

1.4 Genetic architecture and genome-wide association studies

1.4.1 From monogenic architecture and candidate genes to genome-wide association studies and polygenic architecture

As described above, many heritable components contribute to phenotypic variance. This underlying genetic structure is called genetic architecture. Mendel’s findings indicated that a single heritable factor determines phenotypic variation. Thus, this inheritance pattern is called Mendelian, or monogenic, architecture. Studies in the 1980s and 1990s tried to uncover Mendelian patterns in psychiatric disorders. Candidate gene studies focused on selected single loci, thought a priori to be relevant to disorder (Duncan et al., 2019). But, as early as 1967, Gottesman and Shields (1967) argued that complex disorders such as
schizophrenia had complex inheritance patterns instead, where many genes contributed. Additionally, studies in the 1980s (O’Rourke et al., 1982; Tsuang et al., 1982) showed that transmission of a single major gene did not explain family inheritance of schizophrenia.

Consistently with these findings, psychiatric candidate genes failed to replicate (Border et al., 2019; Farrell et al., 2015), including many SNPs from historical schizophrenia candidate genes (Johnson et al., 2017). Potential reasons for this failure were: small sample sizes and low statistical power (Duncan and Keller, 2011); no accounting for population structure (Colhoun et al., 2003), while genetic differences exist between participants of different ancestries (see Section 1.4.3. for extra details); too liberal thresholds for statistical significance (Claussnitzer et al., 2020); no precise replication efforts (studies failed to address the same SNPs, phenotype, and direction of association); publication bias towards positive results; or low quality replications conducted in small samples (Sullivan, 2007).

A move beyond candidate gene studies happened because of large increases in sample sizes (Duncan et al., 2019). Increases by tens of thousands of participants resulted from two efforts. The first effort was to decrease the cost of genotyping arrays, chips with DNA spots which detect gene sequences. Genotyping cost went down from 317K SNPs for over $1,000 circa 2007, to 700K SNPs for $35 in 2020 (Watson et al., 2020). The second effort was to create infrastructure for large research datasets. Consortia pool available genetic and phenotypic data, like the Psychiatric Genomic Consortium (Watson et al., 2020). Biobanks are repositories of biological samples and medical data from large populations, e.g., the UK Biobank (Bycroft et al., 2018).

1.4.2 Conduct of genome-wide association studies

To analyse large genetic datasets, genome-wide association studies (GWAS) are frequently used. GWAS aim to detect associations between allele or genotype frequency and a phenotype. This phenotype can be a disease status. Then, GWAS compares frequencies of SNP variants in samples of cases vs controls. The phenotype can also be a non-disease characteristic, in which case GWAS addresses an unselected sample (Tam et al., 2019). In either GWAS design, the study uses data from thousands of participants to maximise statistical power.

These data are information on participant phenotype and participant genetic data, obtained through whole-genome sequencing or SNP array genotyping. Whole-genome sequencing means obtaining a detailed genome sequence. Still, this genome sequence is not complete, as it often misses repetitive DNA sequences, such as telomeres (nucleotide sequences at the end of chromosomes) or centromeres (domains separating chromosomes.
into arms; after DNA replication, chromosomes are made of two chromatides which are kept together by the centromere; Barra and Fachinetti, 2018). Initiatives are advancing to provide public large-scale sequence data, e.g., the Exome Aggregation Consortium (Lek et al., 2016) and the Genome Aggregation Database (Karczewski et al., 2020).

SNP array genotyping with imputation is currently more cost effective (Yang et al., 2015). In this process, SNPs, and not the whole genome, are measured in the genotype of each individual. SNPs are measured with a genotyping chip, the microarray. The microarray is a glass slide coated with short DNA sequences, called probes. A participant’s DNA gets fragmented and put in a buffer, and the buffer then covers the microarray. If the participant’s DNA fragments match the probes on the microarray, they bind to the probes. Binding occurs because the probes and participant DNA fragments hybridise. As the samples are fluorescently labelled, binding elicits a fluorescent signal. This signal can be read by the computer. Probes also have copies on the microchip so that it can be clarified how many alleles of a polymorphism each participant has. Probes are systematically organised on the microchip. That way, the computer can interpret fluorescent signals by assigning them to SNPs (Alkan et al., 2011).

After genotyping, missing SNPs are imputed. SNPs which have not been directly assayed in the sample are inferred statistically, based on a reference panel of haplotypes. Reference panels are sets of genetic variants from different populations. Reference panels exist because of structural similarities between genomes of unrelated individuals. Parts of gene sequences are identical by descent, i.e., they do not change when inherited through generations. Consequently, short stretches of gene sequences should be identical between the reference haplotypes and haplotypes of genotyped GWAS participants. This sharing of sequences enables prediction of missing SNPs using the reference panel. Imputation increases the total number of SNPs which can be then analysed in GWAS (Marchini and Howie, 2010). Recent research also suggests that low-coverage sequencing (sequencing many individuals at low depth) with imputation effectively detects variation and improves power in GWAS, while being as cost-effective as SNP array genotyping (Li et al., 2021b; Martin et al., 2021).

After genotyping (or genotyping and imputation), raw genetic data go through a process of quality control (QC). QC includes steps such as removal of incomplete data or data from participants whose sex determined via genetic information and stated gender fail to match (Anderson et al., 2010; Marees et al., 2018). QC of samples used in this thesis is detailed in Chapter 2.

Having a phenotype recorded and SNP data quality controlled, GWAS performs thousands of tests for an association between each genetic locus and the phenotype. For
quantitative or continuous traits (e.g., age of disorder onset), linear regression is most commonly used. For dichotomous traits (e.g., disease status), logistic regression is used. Regressions also include potential confounders (e.g., age; sex; birth cohort to control for non-linear effects; or covariates for population stratification, i.e. systematic ancestry differences between participants; Price et al., 2010).

GWAS uses a test statistic which gives a numerical summary of data used to measure support for the null hypothesis (that no difference is detected between cases and controls in GWAS). The test statistic measures if the beta parameter of a particular common genetic variant is significantly different from zero. The GWAS p-value estimates the probability of obtaining a test statistic as extreme as the test statistic which would be present under the null (Mills et al., 2020). The agreed p-value threshold of significance in GWAS is $P < 5 \times 10^{-8}$, a standard significance level of $P < 0.05$ with a Bonferroni correction applied to it to address multiple testing. The Bonferroni correction divides the p-value by the number of statistical tests performed. For GWAS, this number of tests is a million because millions of common genetic variants are tested simultaneously. Still, there have been suggestions for adjusting the $P < 5 \times 10^{-8}$ threshold depending on a sample tested. Even stricter (lower) thresholds were suggested for populations with lower LD, such as participants of African ancestry (Mills et al., 2020), or a less conservative correction than Bonferroni for low-frequency genetic variants. Rarer variants are less frequent so they may require fewer statistical tests in data analysis altogether. Consequently, potentially the correction for the number of tests would be smaller and the p-value threshold could be relaxed (Fadista et al., 2016).

GWAS findings are summarised in a file called summary statistics. Summary statistics aggregate results for each genetic marker (regression coefficients, standard error, p-value etc.), without presenting calls per each SNP in every individual tested in the original GWAS. The statistics come from the full GWAS analysis. Summary statistics can also come from multiple analyses, if they come from a GWAS meta-analysis, a statistical synthesis of multiple GWAS studies on the same topic (Mills et al., 2020). Summary statistics have been developed to avoid privacy concerns over using data from potentially identifiable individuals. Summary statistics also improve logistics of using data, as results based on data from thousands of participants are available in a single file instead (Pasaniuc and Price, 2017).
1.4.3 Accounting for population and family structures in genetic studies

An additional consideration in GWAS and genetic studies are phenomena which change allele frequencies: family structure, cryptic relatedness, population structure, and genetic drift. Family structure is a genetic structure resulting from familial relatedness in samples. Cryptic relatedness is a genetic structure resulting from distant relatedness in samples with unknown family relationships. Population structure is a genetic structure resulting from differences in genetic ancestry (Astle and Balding, 2009). Genetic drift means fluctuations in allele frequencies over time because of random sampling (Masel, 2011). As a result of these phenomena, polymorphisms occur more frequently than expected, unrelated to a trait being studied in GWAS. Consequently, family and population structures can lead to confounding results in genetic studies because they generate spurious associations, i.e., false positives (Cardon and Palmer, 2003).

Multiple methods exist for adjusting for relatedness. Adjustment for population stratification is either with a genetic relatedness matrix or through principal components. Genetic relatedness matrix is a genetic covariance matrix which includes pairwise comparisons of genetic relatedness across the study sample (Yang et al., 2014). Principal component analysis (PCA) is a technique of statistical analysis and visualisation of dimensions in genetic data to pinpoint differences in population structure (Patterson et al., 2006). PCs are calculated on genetic data of unrelated participants from the sample. Following calculation, the genetic relatedness matrix or PCs are included in genetic regression models as covariates (Yang et al., 2014). The method used in this thesis is of PCs. In research on the STRATA sample, PCs used were adjusted for family relatedness, calculated using R packages PC-AiR (Conomos et al., 2015) and PC-Relate (Conomos et al., 2016). In research on the ELSA sample, PCs were calculated with PLINK (Chang et al., 2015).

Of note, ancestry differences are also important in the context of gene discovery and research application. Differences in ancestral origins yield differences in allele frequencies and linkage disequilibriums. These differences result from migration of ancestors, mutation, recombination, and natural selection. Consistent with evolutionary history, diverse samples, especially of African ancestry, have higher levels of genetic variation than individuals of European or Asian ancestry (The 1000 Genomes Project Consortium et al., 2015). Thus, studies on participants of African ancestry could yield more gene discovery per individual than analyses on individuals of European/Asian ancestry only (Morales et al., 2018). Also, genetic models from single populations are not easily translatable across different populations, leading to false positives or ambiguous results in underresearched populations of non-European descent (Peterson et al., 2019). Yet, this lack of diversity is
still an unresolved issue. While studies on e.g. genetics of schizophrenia in East Asian populations were recently published (Lam et al., 2019; The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020), the majority of GWAS data still comes from populations of European descent from the UK, the United States, and Iceland (Mills and Rahal, 2019).

GWAS results are one of the core elements of the polygenic score calculation, discussed in the next section. As the quality of analyses based on GWAS is partially predicated on the quality of GWAS results, benefits and limitations of GWAS are briefly presented in Box 2 and reviewed extensively by Tam et al. (2019).
Box 2. Benefits and limitations of genome-wide association studies.

Benefits of GWAS:

Multiple novel associations were identified between traits and common genetic variants. Unlike candidate gene study results, GWAS results have replicated consistently across increasingly large GWAS samples (Duncan et al., 2019).

The identification of risk loci related to disorder through GWAS has not yet reached a plateau: there are still variants or gene-gene and gene-environment interactions that are yet to be implicated in disorder or other phenotypes (Wray et al., 2018).

GWAS hits (implicated loci) can indicate novel, previously unsuspected biological mechanisms involved in a phenotype. For example, GWAS uncovered multiple, highly statistically significant associations between schizophrenia and the major histocompatibility complex (MHC). MHC is a genomic region involved in immunity (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020; Sekar et al., 2016) and this finding renewed interest in immune mechanisms in psychosis.

GWAS findings can be used to identify patients at high genetic risk of certain disorders, e.g., cancers, or to subtype disorders (Tam et al., 2019).

GWAS findings can inform drug development and repurposing (using existing drugs for novel therapies). GWAS findings can be used as targets for new research hypotheses. GWAS results could also be used to link patients’ genetic background to drug selection and dosage (Giacomini et al., 2017).

GWAS in diverse populations demonstrate ancestry variation in genetics of complex traits (Peterson et al., 2019).

GWAS, while mostly used for research on common genetic variants, can also be used for low-frequency and rare variants. Reference panels for imputation now cover participants from more diverse populations. Panels also now have a larger range of variants and are more specific (The 1000 Genomes Project Consortium et al., 2015).

GWAS can be used to identify monogenic and oligogenic disease genes, i.e. diseases linked to a single gene or a small number of genes (Hirschhorn, 2009).

GWAS summary statistics have multiple applications beyond summarising loci implicated in phenotypes, e.g., forensic analyses, determining SNP heritability, or polygenic scores, among others (Tam et al., 2019).
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Multiple tools enable straightforward GWAS analysis and data management, e.g., the software PLINK, which can be used to conduct computationally efficient genetic data quality control and GWAS analyses (Chang et al., 2015).

GWAS summary statistics can be easily shared through public databases, e.g., the GWAS Catalog (Buniello et al., 2019). Easy access to results facilitates rapid research.

Limitations of GWAS:

Multiple testing in GWAS led to adopting the stringent significance threshold of $P < 5 \times 10^{-8}$ (discussed above). This high threshold means that GWAS requires large samples, or else the study does not have sufficient power to find results which reach this agreed significance threshold (Dudbridge and Gusnanto, 2008). As whole-genome data become more widely used in GWAS, the number of independent tests will increase to address all available genetic variants. Consequently, the issue with multiple testing will become more severe (Pulit et al., 2017). Also, establishing consortia helped with obtaining large samples but they are still an issue in specific circumstances: research in small isolated populations (Hatzikotoulas et al., 2014), research on phenotypes which are difficult to measure or rare, or deep phenotyping (detailed, often multomic characterisations of a phenotype which are time-consuming and expensive; Tam et al., 2019). An option to address multiple testing is to limit the number of tests conducted. For instance, instead of individual SNPs, larger groupings of biologically related genes could be tested, such as gene pathways (The Network and Pathway Analysis Subgroup of the Psychiatric Genomics Consortium, 2015). Alternatively, to correct for multiple testing, less conservative Bayesian methods could replace the Bonferroni correction (Stephens and Balding, 2009).

GWAS findings explain a limited amount of SNP heritability (Young, 2019).

GWAS potentially does not pinpoint causal variants and genes. GWAS may identify regions which are in high correlation with causal variants because of linkage disequilibrium. But these regions may not be the causal variant itself (Altshuler et al., 2008). Another issue is if GWAS will eventually implicate most of the genome, which would make prioritising genes for research difficult (Goldstein, 2009).

GWAS has been unsuccessful in uncovering epistasis, or gene-gene interaction. This failure is potentially due to large sample sizes required (Visscher et al., 2017) and insufficient statistical analysis methods to address the complexity of epistasis. Epistasis brings the issue of multidimensionality. The search for genetic markers moves away from one dimension, just looking at a number of markers in the study,
as GWAS do now, to multiple dimensions to account for interactions between genes. Also, epistasis requires more complex modelling than the additive genetic models, with multiple interaction terms (Wei et al., 2014).

GWAS signals may reflect cryptic relatedness rather than association with phenotype of interest. Perfect matching of cases and controls in large GWAS samples is considered virtually impossible (McClellan and King, 2010). However, additional methods were developed to control for population stratification (Liu et al., 2013); compare with Section 1.4.3.

GWAS results have limited predictive clinical value. Genetic prediction could potentially improve as additional contributing common variants are uncovered in future GWAS. Alternatively, between rare genetic variants of large effect size and common variants of modest effects, i.e., the variants implicated by GWAS, the intermediate type of variants exists. These intermediate, medium-to-strong effects are important contributions to disease (Janssens et al., 2007) but specific variants with such effect sizes have not been uncovered yet (Tam et al., 2019).

1.5 Polygenic scores

1.5.1 Overview of polygenic score calculation

Potential use of GWAS results goes far beyond just identification of loci associated with a trait (see Maier et al., 2018, for a review of multiple methods). As individual contributions of common SNPs to phenotypes are very moderate (Visscher et al., 2017), information about multiple SNPs is combined into a measure, called a polygenic score (PGS, also called polygenic risk score, polygenic composite score, genetic risk score, genome-wide score, or, for a different PGS calculation variation, polygenic hazard score). PGS is a single aggregate score of an individual’s genetic predisposition to a phenotype (or of genetic risk, if the phenotype is a disorder).

Most commonly, PGS are calculated by summarising an individual’s SNPs which were found associated with disease status or a quantitative trait in GWAS, with SNPs weighted by effect size from GWAS, multiplied by the allele count of each of those genetic variants. SNP effect sizes are obtained from the GWAS summary statistics for the phenotype of interest. Effect sizes mean an increase in the quantitative trait value or increase of risk of disease associated with each additional copy of a specific allele. Effect sizes for quantitative traits are reported as beta coefficients because GWAS is based on linear regression. Effect
sizes for risk of disease (case/control status) are log(ORs) because GWAS is based on logistic regression (Choi et al., 2020).

The common model underlying PGS focuses on two aspects: 1) an additive effect of each genetic marker to genetic risk (Chasioti et al., 2019) and 2) how this genetic risk influences a single phenotype. The model underlying PGS does not consider additional elements such as gene expression (processes by which gene nucleotides direct protein synthesis; Maier et al., 2018) or gene-gene and gene-environment interactions (Aschard, 2016).

The description above includes “the common model” because there are certain variations in calculation of PGS. Most PGS are built under an additive inheritance model (SNPs are assumed to have an additive effect on genetic risk). Other inheritance models (dominant, recessive, and heterozygous models) can also be implemented in calculation, e.g. in the PGS software PRSice (Choi and O’Reilly, 2019). Also, PGS can be either a weighted sum of SNPs or a simple sum of SNPs. The simple sum of SNPs assumes that each genetic variant contributes equally to disease. SNPs are not weighted differently using GWAS effect sizes. However, this solution is unrealistic, given that certain variants contribute more strongly to disease risk (Chasioti et al., 2019), e.g., apolipoprotein E (APOE) ε4 allele to cognitive decline, addressed in a study in Chapter 5. Consequently, PGS in this thesis use weighted SNPs. Variations of scores based on different numbers of weighted SNPs are genetic risk scores and polygenic risk scores. Genetic risk scores are based on common genetic variants with genome-wide significance. PGS are an extension of genetic risk scores, summarising a large number of markers, including markers which did not reach genome-wide significance (variants which have a very small individual effect size; Igo et al., 2019). That is because including additional variants improves predictive power of PGS (Agerbo et al., 2015; Purcell et al., 2009).

Yet another variation in PGS calculation is that SNPs are weighted, but not using the GWAS-derived effect sizes. Instead, hazard ratios are used, from a survival model where SNPs are entered as predictors (Desikan et al., 2017). This variation is called a polygenic hazard score. It is commonly used in dementia research (it models genetic risk for time to event, i.e., till the onset of Alzheimer’s disease). Thus, it is not applied in this thesis.

Calculation of PGS as a GWAS-based weighted sum of SNPs is based on two datasets: a GWAS discovery (also called training, or base) dataset and a target (validation) dataset. The discovery dataset consists of summary statistics of the most powerful GWAS available on the phenotype of interest. Usually, it is the largest, most recent GWAS study. Summary statistics include betas/log(ORs) and p-values for each SNP in a genotype-phenotype association. Summary statistics can also include extra information, such as quality of SNP
imputation (see Chapter 2 for how information from summary statistics was used in PGS calculations). The target dataset includes genotypes and phenotypes of individuals in the study for which PGS is generated. The target dataset is often a relatively small sample, much smaller than the discovery sample (Choi et al., 2020).

The discovery and target samples need to be independent. For instance, participants in the sample should not be related. The target sample should also not be included in the original GWAS which is then used to calculate PGS in the target sample. Otherwise, that inclusion will greatly inflate the association between the PGS and the trait tested in the target sample (Wray et al., 2013).

The general formula for PGS is:

\[ PGS_i = \sum_{j=1}^{J} W_j G_{ij} \]

where \( PGS_i \) is a polygenic score for an individual \( i \) (\( i = 1 \) to \( N \)). \( W \) is the GWAS effect size for SNP \( j \) and \( G \) is the number of reference alleles (0, 1, or 2), for individual \( i \) at SNP \( j \).

Important considerations which influence the final PGS are which SNPs are selected for the final PGS and what weights are assigned to SNPs.

### 1.5.2 Single nucleotide polymorphism selection for a polygenic score

Selecting all SNPs may mean that SNPs which are weakly related to the phenotype of interest are included in the PGS. To avoid this issue, SNPs can be preferentially retained, or filtered, using different strategies, p-value thresholding and linkage disequilibrium adjustment, or, less frequently, stepwise regression:

**P-value thresholding.** Thresholding is selecting only SNPs at particular GWAS p-values (Choi et al., 2020). However, that may omit informative markers with small effect size. PGS which include a large number of SNPs have a greater predictive power than PGS based only on SNPs which reached significance in GWAS (Agerbo et al., 2015; Purcell et al., 2009). Consequently, PGS are frequently calculated at a number of different p-value thresholds to find an optimal PGS, explaining the largest amount of variance.

**Linkage disequilibrium (LD) adjustment.** Another issue is whether SNPs in LD—highly correlated—are informative or redundant if they all have similar associations with a phenotype in GWAS. PGS where LD is not taken into account can be less precise (Wu
et al., 2013). Thus, frequently only SNPs which are uncorrelated (not in LD) are included in the final PGS. This SNP selection occurs through processes of LD pruning and LD clumping. In LD pruning, markers within a specific range of the genome, called window, are correlated pairwise. If the correlation is larger than a specified threshold, the marker with the smallest MAF is removed. This pruning goes on for all SNPs. In LD clumping, all SNPs which meet a decided p-value threshold are identified. They are called index SNPs. For each index SNP, clumps are generated. Clumps are made of SNPs which have the following features defined in advance: LD, distance from the index SNP, and the p-value less than the default of 0.01. Index SNP is representative of a corresponding LD region (Chasioti et al., 2019).

Potential issue for LD adjustment is ancestry differences between base and target data. GWAS participants of different ancestries have different LD patterns (Section 1.4.3.). For this reason, target samples are frequently limited to participants from a single ancestry group which matches ancestry in the discovery (GWAS) sample. Recently, novel methods have been developed to improve genetic comparisons between samples of different ancestries, e.g., Popcorn (Brown et al., 2016), which, while accounting for LD, estimates correlations of effect sizes at potentially causal SNPs in trans-ancestral populations, or PRS-CSx (Ruan et al., 2021), which integrates GWAS summary statistics from multiple populations and models population-specific LD patterns to generate polygenic (risk) scores for ancestrally diverse populations.

Another issue for LD adjustment is complex LD in extended genomic regions. Consequently, a single representative SNP from such regions is retained for final analyses (Bogdan et al., 2018) or, frequently, regions with complex LD (e.g., the MHC regions) are removed in QC.

*Stepwise regression*. SNPs are selected based on if they significantly improve the predictive ability of a model. However, the issue with this approach is that it is purely statistical and not biologically informed. It does not take previously established LD structure or disease-variant associations into account (Chasioti et al., 2019). It is not used in this thesis.

After selection of SNPs for the PGS, the other important consideration is how SNPs are weighted before being added up into a final score.
1.5.3 Single nucleotide polymorphism weight selection for a polygenic score

SNP effects are estimated with uncertainty and not all SNPs may influence the trait under study. Consequently, using effect size estimates as originally reported in GWAS may lead PGS to be poor and have high standard error (Choi et al., 2020). To avoid this issue, SNP weights are adjusted with shrinkage. Shrinkage is a statistical technique which reduces (penalises) effect sizes. SNP weighting and penalisation can use frequentist shrinkage regression techniques (e.g. least absolute shrinkage and selection operator; LASSO; Mak et al., 2017), or Bayesian approaches which perform shrinkage via specification of a distribution of priors (Ge et al., 2019; Vilhjálmsdóttir et al., 2015). These Bayesian methods also address LD (see LD adjustment above). They model LD within PGS because they shrink effect sizes according to LD. Penalisation can also be considered a method for SNP weighting and SNP filtering at the same time (Chasioti et al., 2019). Penalisation can set SNP weights of particular SNPs to 0 (e.g. LASSO regression reduces small effects to zero; Choi et al., 2020).

Figure 1.1 below summarises the general process of PGS calculation. PGS can be then applied in association testing, e.g., through regression of the phenotype of interest in the target sample on the PGS, accounting for additional covariates. In this thesis, association testing is between PGS and psychosis subphenotypes, given increasingly explored links between multiple genes and psychosis, as briefly reviewed in the next section.

1.6 Genetics of psychosis

Psychosis has a genetic component. Psychosis heritability estimates in European twin studies are 73% or more, depending on which psychotic disorder is analysed (Hilker et al., 2018). To elucidate genes underlying this genetic component, increasingly large GWAS have been developed over the years. For schizophrenia for example, GWAS progressed from 479 cases in 2008 (O’Donovan et al., 2008) to 69,369 cases, 236,642 controls, and 270 significant loci found in the most recent PGC study (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020). GWAS implicated multiple novel candidate genes. In schizophrenia, implicated genes have multiple roles, among them, encode voltage-gated calcium and chloride channels (CACNA1C, CLCN3), metabotropic receptors (glutamate and gamma aminobutyric acid), and the ligand-gated N-methyl-D-aspartate receptor subunit (GRIN2A). An association also was found with the major histocompatibility complex (MHC), a genic region coding for essential immunity
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Fig. 1.1 Polygenic score calculation.

The score is based on effect sizes for each single nucleotide polymorphism (SNP), obtained from genome wide-association study (GWAS) for a trait of interest (effect sizes in a row at the bottom). Effect sizes are expressed as a value of either betas or of odds ratios, depending on whether the trait of interest is continuous or a disease case/control status, respectively. Effect sizes describe the contribution of alleles (gene variants) per each SNP. Alleles determined by GWAS to be related to the trait are called effect alleles. Effect sizes are applied to a genotype of an individual (middle section of the figure; alleles are represented as blocks and effect alleles are in black). As an individual can have from 0 to 2 copies of each allele, the effect size at each SNP is also multiplied either by 0, 1 or 2, depending on the number of effect alleles present (top row). All information is then added up together to form a single polygenic score (top right-hand corner). Image of nucleotides via BioRender (www.biorender.com) and based on teaching materials by Abdellaoui (2020).
proteins (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020). Complement component 4 (C4) genes within MHC, involved in autoimmunity and innate immune response, account in part for that MHC association signal (Sekar et al., 2016). In bipolar disorder, implicated genes encode ion channels, including \textit{CACNB2} and \textit{KCNB1}, and receptors targeted by antipsychotics, serotonin receptor \textit{HTR6} and melanin-concentrating hormone \textit{MCHR1}. Associations with variants within and distal to MHC were also statistically significant but, unlike in schizophrenia, human leukocyte antigen genes or C4 genes did not account for the association (Mullins et al., 2021). In psychotic experience, implicated genes included a locus in gene \textit{ANK3}, involved in ion channel binding, and a locus in cannabinoid receptor 2 gene \textit{CNR2} (Legge et al., 2019). However, recent PGC work also showed that, while these genes may be new research targets, they are not universal to all populations. For instance, the previously mentioned MHC was associated with schizophrenia in populations of European ancestry but not in populations of East Asian ancestry. The difference potentially stems from a lower MAF in this region compared to cases of European ancestry (Lam et al., 2019).

PGC work also implicated gene sets and pathways (genes typically involved together in a biologically meaningful way) in psychotic disorders. Findings from The Network and Pathway Analysis Subgroup of the PGC implicated histone and cell-cell organisation pathways as involved both in bipolar disorder and schizophrenia (The Network and Pathway Analysis Subgroup of the Psychiatric Genomics Consortium, 2015), and, similarly, more recent GWAS studies listed above also found gene sets and pathways involved in neuronal processes. In schizophrenia, these were gene sets related to neuronal excitability, development, and structure, ion channel complex, voltage-gated calcium channel activity, and synaptic organisation (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020) and in bipolar disorder, calcium signalling, neuronal and synaptic pathways (Mullins et al., 2021).

Genetic analyses also showed a genetic overlap between major psychiatric disorders, i.e., that specific genetic variants are causally associated across disorders. Analysis of GWAS summary statistics with bivariate gaussian mixture models (which quantify polygenic overlap; Frei et al., 2019) estimated that approximately 8600 variants would need to be uncovered in bipolar disorder and 9700 variants in schizophrenia to explain 90% of SNP heritability of these disorders. Of those loci implicated in bipolar disorder, 97% potentially also influence major depression and 96% influence schizophrenia, although with different directions of SNP effects between disorders (Mullins et al., 2021). Analyses using polygenic scores at first tested a link between polygenic scores for major psychiatric disorders and case/control status. A highly statistically significant overlap of polygenic risk was found between bipolar disorder, major depressive disorder, and schizophrenia, and, to a lesser extent (but still statistically significant), between polygenic scores for autism spectrum
disorder with schizophrenia and bipolar disorder diagnosis (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013).

Later research by Ruderfer and colleagues demonstrated that genetic overlap between schizophrenia and bipolar disorder is specific to phenotypic overlaps between disorders. Bipolar disorder polygenic scores were significantly correlated with mania in schizophrenia (Ruderfer et al., 2014) and psychosis in bipolar disorder (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). Schizophrenia polygenic scores were significantly related to psychosis in bipolar disorder and negative symptoms in schizophrenia (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). This research suggests that polygenic scores successfully differentiate between psychosis symptoms rather than only disease status. Consequently, polygenic scores could be applied to explore genetics of psychosis subphenotypes.

1.7 Summary and scope of thesis

In summary, progress in the field of psychiatric genetics led to a paradigm shift from seeking associations between psychosis and single genes (candidate genes) to seeking associations between psychosis and multiple genes, summarised e.g., as polygenic scores. However, polygenic score studies in psychosis suffer from a number of conceptual issues. First, research focuses on genetic contributions to very broad, high-level phenotypes, such as schizophrenia. This focus may not be informative. Psychiatric disorder phenotypes are highly complex and present with great variation (Nunes et al., 2020). Second, as discussed above, psychosis symptoms such as cognitive decline are likely not static and binary (having or not having a symptom). Instead, cognitive decline in psychosis potentially subtly changes over time (Fett et al., 2019; Zanelli et al., 2019). Additional longitudinal studies are required to explore polygenicity of such dynamic psychosis symptoms.

Thus, the overarching aim of this thesis was to explore contribution of common genetic variants to more narrowly operationalised, specific psychosis phenotypes which may impact trajectories of psychosis over time: age of onset of psychosis, duration of untreated psychosis, and cognitive decline of memory and executive function in later life. Few studies to date addressed genetics in age of onset in psychosis (Ahn et al., 2016; Aminoff et al., 2015; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Kalman et al., 2019; Stepniak et al., 2014) and duration of untreated psychosis (Yin et al., 2019b). Also, these studies focused on schizophrenia and bipolar disorder specifically, rather than a broader psychosis spectrum, which is relevant, as psychotic disorders overlap in terms of syndroms and underlying
genetic factors. In research on cognitive decline and psychosis genetics, there are relatively few large-scale, longitudinal studies which have detailed phenotypes available, and which focus on non-clinical ageing populations (Liebers et al., 2016; Ritchie et al., 2020). Such a study design enables exploring the influence of psychosis genetics on cognitive decline, potentially unconfounded by e.g., symptoms of mental disorders comorbid with psychosis (McGrath et al., 2020; Plana-Ripoll et al., 2019) or side effects of medication (Read and Williams, 2019).

The aim of Chapter 3 was to explore whether polygenic risk scores implicated in major psychiatric disorders and previous research on age of onset in schizophrenia and bipolar disorder (polygenic scores for schizophrenia, bipolar disorder, depression, autism spectrum disorders, and attention deficit hyperactivity disorder) are related to age of onset in psychosis in a sample of first episode psychosis patients of predominantly European ancestry.

The aim of Chapter 4 was to explore whether polygenic risk scores implicated in major psychiatric disorders (polygenic scores for schizophrenia, bipolar disorder, major depressive disorders, autism spectrum disorders, and attention deficit hyperactivity disorder) are related to duration of untreated psychosis in a sample of first episode psychosis patients of predominantly European ancestry.

The aim of Chapter 5 was to explore whether polygenic scores for schizophrenia are related to a deficit in cognitive abilities (memory and executive function), a decline in those abilities over time, or both, over 10 years of follow-up in a healthy, ageing, English population sample of European ancestry.

This thesis focused primarily on relationships between psychosis phenotypes and polygenic scores, i.e., scores summarising common genetic variation. Extra genetic factors (APOE alleles, PCs to describe genetic ancestry) and demographic and environmental factors (e.g., baseline age, gender, research site, educational attainment, physical wealth) were included as covariates in analyses. However, other factors may be important, but they were not part of analyses in this thesis, e.g., rare copy number variants, gene expression, gene x environment interactions, or additional environmental exposures. One such exposure is influenza infection. Together with collaborators, I completed a narrative review on mechanisms in which this infection may contribute to later development of schizophrenia (Appendix B, Kępińska et al., 2020).
Chapter 2

Methods

2.1 Overview

This thesis aimed to investigate what types of common genetic variants underlie risk for psychosis phenotypes which potentially impact trajectories of psychosis. I conducted studies in two samples. Research on genetics in age of onset of psychosis and in duration of untreated psychosis was conducted using the Schizophrenia: Treatment Resistance and Therapeutic Advances - Genetics (STRATA-G) Consortium sample. The sample consists of legacy data of patients with different psychotic disorders from across Europe and Brazil (Section 2.2). Research on genetics of cognitive decline was conducted using the English Longitudinal Study of Ageing (ELSA) sample, a longitudinal population sample of healthy, ageing, white English participants (Section 2.3).

2.2 Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA)

2.2.1 General STRATA overview

The Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA) Consortium is an international, UK-based research consortium whose overarching aim was to develop tools for early prediction of treatment resistance (no response to non-clozapine antipsychotics) in psychosis. STRATA also aimed to improve patient stratification in medical intervention for schizophrenia to minimise delays in effective treatment. Finally, STRATA aimed to provide insights into mechanisms of treatment resistance. Informa-
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tion collected included clinical and demographic data, brain scans (including functional
magnetic resonance imaging and positron emission tomography imaging), and omics data, including genetic data. Collection of specific data types was divided into different work-streams. Genomics and pharmacogenomics are the focus of the STRATA Workstream 3 (WS3), entitled “Predicting Response to Antipsychotic Medication using Clinical, Social and Genetic Information” and also known as STRATA-G (see below). STRATA-G pooled longitudinal first-episode psychosis cohorts focusing on phenotype data collected at first episode, treatment resistant status at follow-up, and genetic data.

STRATA has been funded by a Medical Research Grant from the Medical Research Council (MRC). The Consortium is led by Institute of Psychiatry, Psychology & Neuroscience (IoPPN), King’s College London. The leading genomics site is the MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University. The Principal Investigator (PI) of STRATA was initially Professor Shitij Kapur, with Professor James MacCabe taking over the role in September 2016. Data collection and data pooling for the project finished in December 2019.

2.2.2 STRATA-Genetics (STRATA-G)

The aim of STRATA WS3 was to collect DNA from pre-existing cohorts of first episode psychosis patients who have been followed over time to ensure the unbiased assessment of their resistance to antipsychotics and confirm pharmacogenomic predictors of non-response. WS3 PIs were Professor James Walters and Professor Mick O’Donovan at the MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, and Professor James MacCabe and Professor Robin Murray at the IoPPN, King’s College London.

An international collaboration grew out of STRATA WS3, entitled STRATA-Genetics (STRATA-G), and the genetic data in this thesis come from STRATA-G. The STRATA-G cohort comprises of well phenotyped, prospective longitudinal legacy data on treatment resistance of individuals from Europe and Brazil. Data come from the following studies (leading sites given): Aetiology and Ethnicity in Schizophrenia and Other Psychoses (ÆSOP; King’s College London, UK); Genetics and Psychosis (GAP; King’s College London, UK); Northern Ireland First Episode Psychosis (NIFEPS; Queen’s University Belfast, UK); Resources for Genomics, Ireland (RGPI; Queen’s University Belfast, UK); European Network of National Schizophrenia Networks Studying Gene-Environment Interactions (EUGEI; University of Bologna, Italy); Bologna FEP (BoFEP; University of Bologna, Italy); First-Episode Schizophrenia Follow-Up Project (Istanbul University, Turkey; the data were partially included in the EUGEI sample); French Institute of Health and Medical Research (France; the data were part of the EUGEI sample); Treatment and Early Intervention
2.2.3 Ethical approval, consent and administration

Ethical approval for STRATA WS3 was granted by The Research Ethics Committee (REC) at South Central - Oxford C (reference: 15/SC/0021) and the National Health Service (NHS) Health Research Authority (HRA) Confidentiality Advisory Group (CAG; reference: 14/CAG/1044). These approvals also covered STRATA-G. Conduct of STRATA and STRATA-G complied with the principles of the Declaration of Helsinki (1996) and all applicable regulatory requirements, including the HRA UK Policy Framework for Health and Social Care Research Governance Framework, NHS Trusts and NHS Research Offices policies and procedures, and policies stipulated by the REC and CAG. Each collaborator also signed the STRATA-G Scientific Agreement to record that the provided data comply fully with all local, government, and international laws, regulations, and guidelines.

All participants gave informed consent. In studies where minors took part (ÆSOP; RGPI; First-Episode Schizophrenia Follow-Up Project; PAFIP; The West London Longitudinal First-Episode Psychosis Study), parents/legal guardians signed a parental permission consent document.

Materials and data were transferred to King’s College London and Cardiff University under the following agreements: a retrospective letter of agreement for material transfer between University of São Paulo and KCL for the São Paulo data; a declaration of conformity with Reference Methodology MR03 sent to Commission nationale de l’informatique et des libertés (National Commission on Informatics and Liberty) in accordance with French legislation for the French sample; and material and data transfer agreements between KCL, Cardiff University and leading sites for all the remaining institutions belonging to the STRATA Consortium.

Phenotype and genetic data or blood samples for genotyping were transferred from all Consortium leading sites with the exception of ÆSOP, NIFEPS and RGPI. For these three studies, participants’ blood samples for genotyping, a clinical interview, and medical history (later confirmed by patients’ clinical notes) were collected by Dr Sophie Smart.
and Gemma Evans (ÆSOP) and Dr Lina Homman (NIFEPS and RGPI), under STRATA ethical approvals. For detailed process of follow-up, refer to Smart (2019), Section 7.1.3. ÆSOP Follow-up.

2.2.4 STRATA cohorts

The overarching goal of STRATA is to explore factors related to treatment resistance. However, treatment resistance and phenotypes which required follow-ups in STRATA are not the main focus of this thesis. For detailed information on definitions and determination of treatment resistance and length of follow-up for each STRATA cohort, consult Smart (2019), Section 7.3 Cohorts.

Studies included in STRATA focused on first-episode psychosis in individuals diagnosed with psychosis or a psychotic disorder based on a range of diagnostic criteria and instruments: ICD-10, DSM-IV, Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview schedule, Comprehensive Assessment of At Risk Mental States (CAARMS) scale, Structured Clinical Interview for DSM-IV (SCID), or Operational Criteria Checklist for Psychotic Illness (OPCRIT). As all STRATA-G data are legacy data, cohorts differ in their original study design, diagnostic criteria, and exclusion criteria. This information, together with age range, catchment areas, and totals of participants with psychosis (cases only) by each cohort is summarised in Table 2.1. For numbers of participants stratified by treatment resistance status, see Smart (2019), Section 7.4. Defining treatment resistance, Table 1.
<table>
<thead>
<tr>
<th>Study name</th>
<th>Leading site</th>
<th>Study design</th>
<th>Duration</th>
<th>Catchment area</th>
<th>Full sample size in STRATA</th>
<th>Age range</th>
<th>Diagnostic criteria</th>
<th>Exclusion criteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aetiology and Ethnicity in Schizophrenia and Other Psychoses (ÆSOP)</td>
<td>Institute of Psychiatry, Psychology Neuroscience, King's College London (IoPPN, KCL), London, UK</td>
<td>Naturalistic, prospective incidence and case-control study of first-episode psychosis</td>
<td>September 1997 to August 2000</td>
<td>South-east London, Nottingham, and Bristol, United Kingdom</td>
<td>731</td>
<td>16–65 years</td>
<td>ICD-10 diagnosis of F10-F29 or F30-F33 (World Health Organization, 1992)</td>
<td>Psychotic symptoms precipitated by an organic medical cause, DSM-IV, 293.xx; (American Psychiatric Association, 2000), profound learning disability (IQ 50), previous contact with psychiatric services for psychotic symptoms, evidence of transient psychotic symptoms resulting from acute intoxication as defined by ICD-10 (World Health Organization, 1992)</td>
<td>Morgan et al. (2006a) Kirkbride et al. (2006)</td>
</tr>
<tr>
<td>Genetics and Psychosis (GAP)</td>
<td>IoPPN, KCL, London, UK</td>
<td>Population-based incidence and case-control study of first-episode psychosis</td>
<td>December 2005 to October 2008; retrospective data search in electronic health records five years after first contact for psychosis</td>
<td>South London, United Kingdom (individuals who presented to the psychiatric services of the South London and Maudsley National Health Service Foundation Mental Health Trust)</td>
<td>398</td>
<td>18–65 years</td>
<td>ICD-10 diagnosis of F20–F29 and F30–F33 (World Health Organization, 1992), determined using the OPCRIT checklist (McGu芬 et al., 1991) and validated by administration of SCAN (Wing et al., 1990)</td>
<td>Evidence of psychotic symptoms precipitated by an organic cause; transient psychotic symptoms resulting from acute intoxication as defined by ICD-10 (World Health Organization, 1992); head injury causing clinically significant loss of consciousness, and learning disability (IQ 70)</td>
<td>Di Forti et al. (2015); Ajnakina et al. (2017); Murray et al. (2020)</td>
</tr>
<tr>
<td>Northern Ireland First Episode Psychosis (NIFEPS)</td>
<td>Queen's University Belfast, Belfast, Northern Ireland</td>
<td>Naturalistic, prospective, incidence study of first-episode psychosis</td>
<td>January 2003 and December 2004; follow-ups 1 year after baseline and 13 years after baseline as part of STRATA</td>
<td>Belfast, Northern Ireland (individuals who presented to Belfast HSC Trust, the urban Belfast area, and Northern HSC Trust, north-east rural area)</td>
<td>382</td>
<td>18–64 years</td>
<td>Case notes were analysed using the OPCRIT (McGu芬 et al., 1991) to determine ICD-10 diagnosis (World Health Organization, 1992) at 1 year</td>
<td>Evidence of a clear organic cause for psychotic symptoms, a steroid induced psychosis, short-lived psychotic symptoms solely on the basis of uncomplicated alcohol or drug intoxication or withdrawal, past history of psychosis documented in psychiatric records</td>
<td>Turkington et al. (2009, 2016)</td>
</tr>
<tr>
<td>Resource for Psychoses Genomics Ireland (RGPI)</td>
<td>Queen's University Belfast, Belfast, Northern Ireland</td>
<td>Population-based, incidence study of first-episode psychosis</td>
<td>Ongoing, since 2007</td>
<td>Belfast, Northern Ireland (individuals who presented to psychiatric services in the region of the Queen's University Belfast research team)</td>
<td>16+ years</td>
<td>Diagnosis of schizophrenia based on SCID (First et al., 1997)</td>
<td>Substance-induced psychosis or medical disorder responsible for psychosis, learning disability, not having Irish-born grandparents</td>
<td>Casey and Corvin (2008)</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 2. METHODS

An ongoing, naturalistic prospective study of first-episode psychosis

Ongoing, initially conducted between January 2002 and December 2009

Urban area of West Bologna, Italy (referrals to the West Bologna Community Mental Health Centre)

18–64 years

Case notes used to complete the Item Group Checklist, part of SCAN Wing et al. (1990). The Checklist was used to determine if diagnosis is part of the F10-29 and F10-F33 in ICD-10 (World Health Organization, 1992).

ICD-10 diagnosis of F20-F33 (World Health Organization, 1992), confirmed by the OPCRIT (McGuinness et al., 1991) or review of case notes and other relevant information; additionally, SCAN Wing et al. (1990) was used for diagnosis.

ICD-10 diagnosis of F20-F33 (World Health Organization, 1992) confirmed by the OPCRIT (McGuinness et al., 1991); the Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994).

Previous contact with mental health services for psychosis, evidence of psychotic symptoms precipitated by an organic cause, and transient psychotic symptoms resulting from acute intoxication, as defined by the ICD-10 (codes F1X.5 World Health Organization, 1992).

Previous contact with mental health services for psychosis, evidence of psychotic symptoms precipitated by an organic cause, and transient psychotic symptoms resulting from acute intoxication, as defined by the ICD-10 (codes F1X.5 World Health Organization, 1992).

Previous antipsychotic treatment longer than 15 days, prior inpatient care, another DSM-IV Axis I diagnosis, except anxiety disorders, a serious medical illness, having any organic disorder, known to cause psychosis, or an alcohol or drug use disorder.

Meeting threshold criteria for psychosis, as defined by the “Psychosis threshold” subscale of CAARMS (Yung et al., 2005), diagnosis reported by a treating psychiatrist in all medical documents and at the end of any hospitalisation, longitudinal assessment by clinical case managers over the 3 years of treatment. The consensus diagnosis procedure is carried out by a senior psychiatrist and the senior psychologist who is in charge of scale based assessment over the treatment period.

Taking antipsychotic medication for more than a total of 6 months, psychosis related to intoxication or organic brain disease, or IQ 70.

Absence of an organic cause of psychotic symptoms or profound learning disability; and no previous contact with psychiatric services for psychotic symptoms.
CHAPTER 2. METHODS

Oslo, Norway (outpatient and inpatient psychiatric units at 4 university hospitals in Oslo, which provide treatment for patients referred from primary care) May 2003 to July 2007, all patients invited to take part in follow-up one year after baseline.

Prospective study of first-episode psychosis Naturalistic, prospective incidence study of first-episode psychosis Oslo, Norway (outpatient and inpatient psychiatric units at 4 university hospitals in Oslo, which provide treatment for patients referred from primary care) May 2003 to July 2007, all patients invited to take part in follow-up one year after baseline.

The Early Stages of Schizophrenia study

National Institute of Mental Health, Prague, Czech Republic Hospital-based incidence study of first episode schizophrenia Prague and the surrounding area, Czech Republic (Bohunice, a large general psychiatry hospital which serves Prague and northern part of Central Bohemia region) Not reported 18-35 years

Programa de Atención a las Fases Iniciales de Psicosis (First Episode Psychosis Clinical Program; PAFIP)

University College of Santander, Cantabria, Spain (the outpatient clinic and the inpatient unit at the University Hospital Marques de Valdecilla and referrals to the Hospital from throughout Cantabria) Ongoing, since 2001 15+ years

The Brazilian Wellcome Trust São Paulo Study

University of São Paulo, São Paulo, Brazil Naturalistic, prospective incidence study of first episode psychosis São Paulo, Brazil (central, western and northern regions of the city) July 2002 and December 2004 18-64 years

The West London Longitudinal First-Episode Psychosis Study

University College London, London, UK Naturalistic, prospective incidence study of first episode psychosis West London, United Kingdom 1998 to 2008 16-50 years

Abbreviations: HSC, Health and Social Care; CAARMS, Comprehensive Assessment of At Risk Mental States; ICD-10, International Classification of Diseases-10; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; SCAN, Schedules for Clinical Assessment in Neuropsychiatry; OPCRIT, Operational Criteria Checklist for Psychotic Illness; SCID, Structured Clinical Interview for DSM-IV.
2.2.5 Phenotypes in STRATA

2.2.5.1 Data processing

Prior to any analyses, data were processed by Dr Sophie Smart, including: removal of individuals with 100% missing data; assigning each individual a unique STRATA-G identifier (STRATA-G ID); relabelling variables so that they have consistent names and missingness flags across cohorts; resolving impossible values with collaborators, where applicable; and merging data by STRATA-G identifier. Additional details on the process are described in Smart (2019), Section 7.6. Phenotype data.

2.2.5.2 Participant variables

As the overarching goal of STRATA was to explore as many variables as possible in prediction of treatment resistance, the original post-merge dataset includes multiple participant- and disease-status variables which were measured in different original cohorts (not all variables were measured in every cohort). These variables include: accommodation status; age at baseline assessment; alcohol use; body mass index; cannabis use; employment status; ethnicity; gender; highest education qualification; living circumstances (alone or with family/non-family); relationship status; tobacco use; years in education; age of psychosis onset; duration of untreated psychosis; family history of psychosis; family history of mental health disorders; scales measuring schizophrenia symptoms or functioning, including Brief Psychiatric Rating Scale scores; Global Assessment of Functioning scores; Positive and Negative Syndrome Scale scores; Scale for the Assessment of Negative Symptoms; and Scale for the Assessment of Positive Symptoms. For details on cohorts in which each variable was recorded, how each variable was defined, calculated or normalised by Dr Smart (where variables were coded differently between cohorts), consult Smart (2019), Section 7.6.2. Predictors. As studies in this thesis focus on age of onset and duration of untreated psychosis (controlled for a number of variables; see Chapters 3 and 4), only the variables which were the basis of subsequent analyses are detailed below.

2.2.5.2.1 Age of onset

Age of onset (AOO) is a continuous variable for an age at which the participant’s first psychotic symptoms occurred. Where AOO was not recorded in the original cohort, the age of first presentation to clinical services for psychosis was used and the following correction was applied to it to obtain the final AOO variable: ÆSOP collected data for age of first presentation and age of first symptoms, with the mean difference between these ages of
0.547 years. Thus, the final AOO variable was age at first presentation to clinical services for psychosis minus 0.547 years. Age of first presentation to clinical services for psychosis was recorded at baseline for NIFEPS and RGPI (Belfast), GAP (London), and EUGEI (Paris). Age of first presentation to clinical services for psychosis was recorded at the five-year follow-up for BoFEP (Bologna) and so, it was included as age of first presentation to clinical services for this sample.

Table 2.2 includes details of how AOO was ascertained in samples which were included in the final study of genetics of AOO (Chapter 3). Samples included, from London: ÆSOP, GAP, The West London Longitudinal First-Episode Psychosis Study; Belfast: NIFEPS, RGPI; Bologna: BoFEP, EUGEI; Paris: EUGEI; Istanbul: First-Episode Schizophrenia Follow-Up Project; Lausanne: TIPP; Oslo: TOP; and Santander: PAFIP. These samples were included based on non-missingness of any data required in the analyses: genetic data which passed quality control (see Section 2.2.6.2 below), and which could be correctly merged with a participant’s demographic and clinical data, i.e., information on age, gender, research site, and age of psychosis onset.
Table 2.3 Determination of age of psychosis onset, by Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA) cohort

<table>
<thead>
<tr>
<th>STRATA sample</th>
<th>Definition of age of onset (AOO) of psychosis</th>
<th>Determination of AOO</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÆSOP, IoPPN, KCL, London</td>
<td>Age at any first overt psychosis symptom from the following: hallucinations or pseudohallucinations, delusions, thought and speech disorder (incoherence, irrelevance, blocking, neologisms, incomprehensibility of speech), qualitative psychomotor disorder (negativism, mutism or stupor; catatonic excitement; constrained attitudes and postures), bizarre or grossly inappropriate behaviour, reported by patient/relative, or presence of at least two “suggestive” marks of psychosis (self-neglect, excitement, aggression, social withdrawal) used by psychiatrists to reconstruct a likely age of onset</td>
<td>AOO collated from interviews with the patient, a close relative, and clinical notes using the Personal and Psychiatric History Schedule (Jablensky et al., 1992). Also collected the age at which a person first came into contact with secondary mental health services with the presence of psychotic symptoms</td>
<td>Donoghue et al. (2014)</td>
</tr>
<tr>
<td>GAP, IoPPN, KCL, London</td>
<td>Patient-reported age at first psychosis symptoms (such as bizarre behaviour, catatonia, thought disorder, restricted or blunted affect, delusions, hallucinations, thought insertion or withdrawal, running commentary or abusive/accusatory voices, lack of insight, loss of pleasure, tiredness, poor concentration, elevated or excessively self-reproachful/sad mood)</td>
<td>AOO determined based on face-to-face interviews and analysis of mental health records, conducted by qualified psychiatrists and psychologists, using the Operational Criteria Checklists (OPCRIT; McGuffin et al., 1991)</td>
<td>Ajnakina et al. (2017)</td>
</tr>
</tbody>
</table>
Continuous schizophrenia symptoms (having them every day or almost every day up until the initial clinical contact), including dysphoric mood (depression, anxiety, irritability, hostility, restlessness), sleep disturbance, delusions, suspiciousness of others and their motivations, reduced concentration, attention, hallucinations, deterioration in social function, odd behaviours, social withdrawal (isolation), avolition, disorganised thought process and behaviour.

Age at any first overt psychosis symptom of hallucinations or pseudohallucinations, delusions, thought and speech disorder (incoherence, irrelevance, blocking, neologisms, incomprehensibility of speech), qualitative psychomotor disorder (negativism, mutism or stupor; catatonic excitement; constrained attitudes and postures), bizarre or grossly inappropriate behaviour, reported by patient/relative, or presence of at least two “suggestive” marks of psychosis (self-neglect, excitement, aggression, social withdrawal) used by psychiatrists to reconstruct a likely age of onset.

AOO established using the Symptom Onset in Schizophrenia Inventory, determined by the clinician, based on patient report of the their first symptoms, an assessment from an informant, and the clinician’s assessment if symptoms are severe enough to be considered psychosis and not prodrome (Perkins et al., 2000)

Gutiérrez-Galve et al. (2010)

NIFEPS: AOO established in face-to-face clinical assessment, including using the Personal and Psychiatric History Schedule (Jablensky et al., 1992), filled in by the participant and completed separately by next of kin, where available

Hoy et al. (2012); Turkington et al. (2009); Casey and Corvin (2008)

RGPI: AOO established using the Structured Clinical Interview for DSM-IV (First et al., 1997)
BoFEP, University of Bologna, Bologna

Age at first psychosis symptoms, as reported by the patient: presence of hallucinations, delusions, thought disorder, negative symptoms, mania or clinical suspicion of psychosis, as determined by a psychiatrist

EUGEI, University of Bologna, Bologna

Age of onset of psychosis symptoms, as reported by the patient: having either a clear positive symptom (hallucination, delusion) rather than a non-specific disturbance of mood, thinking, behaviour or functioning, for which there is no return to premorbid functioning after the onset of symptoms) or delusions, hallucinations, first rank psychosis symptoms (auditory hallucinations, thought withdrawal, insertion and interruption, thought broadcasting, somatic delusions, feelings controlled by an external force), catatonic symptoms for a week

AOO obtained by asking patients and/or key informants about when patients experienced the first psychotic symptoms. Patients included in the study based on meeting criteria for psychosis in the Personal and Psychiatric History Schedule (Jablensky et al., 1992)

Information collected to determine duration of untreated psychosis (DUP) used to determine AOO: DUP was measured using The Nottingham Onset Schedule (Singh et al., 2005) which determines the chronology of psychosis onset. DUP was based on time from onset of symptoms to first contact with secondary mental health services for suspected psychosis

Taricone et al. (2012)

Jongsma et al. (2018)
CHAPTER 2. METHODS

EUGÉI, French Institute of Health and Medical Research, Paris

Age of onset of psychosis symptoms, as reported by the patient: having either a clear positive symptom (hallucination, delusion) rather than a non-specific disturbance of mood, thinking, behaviour or functioning, for which there is no return to premorbid functioning after the onset of symptoms) or delusions, hallucinations, first rank psychosis symptoms (auditory hallucinations, thought withdrawal, insertion and interruption, thought broadcasting, somatic delusions, feelings controlled by an external force), catatonic symptoms for a week

Information collected to determine duration of untreated psychosis (DUP) used to determine AOO: DUP was measured using The Nottingham Onset Schedule (Singh et al., 2005), as time from onset of symptoms to first contact with secondary mental health services for suspected psychosis

First-Episode Schizophrenia Follow-Up Project, Istanbul University, Istanbul

Age of first identifiable positive symptoms (hallucinations, delusions)

The date of onset of the first identifiable positive symptoms was timed by the senior psychiatrist on the basis of a best-estimate approach using data gathered from medical records, a patient directly, and family interview

TIPP, Lausanne University Hospital, Lausanne

Age at which symptoms (disorganised speech, perceptual abnormalities, hallucinations) have been present for longer than a week, as reported by the patient

Meeting threshold criteria for psychosis, as defined by the “Psychosis threshold” subscale of Comprehensive Assessment of At Risk Mental States (Yung et al., 2005) which includes criteria on how long psychotic symptoms have been present

Jongsma et al. (2018)

Üçok et al. (2004)

Alameda et al. (2017)
Age at first psychosis symptoms, as reported by the patient: delusions, hallucinations, negative symptoms, disorganised speech

Clinical assessment by specifically trained psychologists and physicians, using SCID (Spitzer et al., 1992)

The age at which the first continuous (present most of the time) psychotic symptom emerged (patients included in the sample had diagnoses of DMS-IV schizophrenia spectrum disorders: schizophrenia, schizophreniform disorder, schizoaffective disorder, brief psychotic disorder and psychosis not otherwise specified)

AOO was based on a formal interview for a full assessment of the patient, conducted by an experienced psychiatrist (Pelayo-Terán et al., 2008)
2.2.5.2.2 Duration of untreated psychosis

Duration of untreated psychosis (DUP) is a continuous variable for days between the first occurrence of psychotic symptoms and, depending on the site, either: i) first contact with statutory mental health services; ii) the start of antipsychotic treatment for psychosis; iii) the first hospitalization.

Where DUP was measured in weeks (TOP, French Institute of Health and Medical Research, and The West London Longitudinal First-Episode Psychosis Study), it was converted to days by multiplying by seven. DUP measured in months (The West London Longitudinal First-Episode Psychosis Study) was converted to days by multiplying by 30.417 (both conversions completed by Dr Smart). All definitions of DUP come as determined and provided by researchers at original study sites. The only change to the data was a conversion of all DUP information to days when data were merged into a STRATA Consortium joint dataset.

Table 2.3 details how DUP was ascertained in samples which were included in the final study of genetics of DUP (Chapter 4), i.e., London: ÆSOP, The West London Longitudinal First-Episode Psychosis Study; Belfast: NIFEPS, RGPI; Paris: EUGEI; Istanbul: First-Episode Schizophrenia Follow-Up Project; Lausanne: TIPP; Oslo: TOP; Santander: PAFIP; and Prague: The Early Stages of Schizophrenia study. These samples were included based on non-missingness of any data required in the analyses (genetic data which passed quality control, and which could be correctly merged with a participant’s demographic and clinical data, i.e., information on age, gender, research site, and DUP).
Table 2.4 Determination of duration of untreated psychosis, by Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA) cohort

<table>
<thead>
<tr>
<th>STRATA sample</th>
<th>Determination of duration of untreated psychosis (DUP)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÆSOP, IoPPN, KCL, London</td>
<td>DUP was estimated using the Personal and Psychiatric History Schedule (Jablensky et al., 1992), using information from cases, their relatives, and case records. DUP was a period in weeks from the onset of psychosis (any symptom from hallucinations, delusions, thought and speech disorder, catatonia, stupor, psychomotor change, bizarre behaviour) to first contact with statutory mental health services</td>
<td>Morgan et al. (2006a,b)</td>
</tr>
<tr>
<td>The West London Longitudinal First-Episode Psychosis Study, UCL, London</td>
<td>DUP established using the Symptom Onset in Schizophrenia Inventory (Perkins et al., 2000): a period between the onset of continuous (daily) symptoms (dysphoic mood, disturbed sleep, delusions, suspicious mood, reduced concentration, attention, hallucinations, deteriorated social functioning and isolation, disorganised thought processes and behaviours) and patient starting antipsychotic treatment</td>
<td>Gutiérrez-Galve et al. (2010)</td>
</tr>
<tr>
<td>NIFEPS and RGPI, Queen’s University Belfast, Belfast</td>
<td>NIFEPS: DUP was estimated from patient reports and from information collected using the Personal and Psychiatric History Schedule (Jablensky et al., 1992) from participants and relatives: DUP was a period from the onset of psychosis (any symptom from hallucinations, delusions, thought and speech disorder, catatonia, stupor, psychomotor change, bizarre behaviour) to first contact with statutory mental health services RGPI: period from symptom onset (patient information collected using Structured Clinical Interview for DSM-IV) to first contact with mental health services (First et al., 1997)</td>
<td>Hoy et al. (2012); Turkington et al. (2009); Casey and Corvin (2008)</td>
</tr>
</tbody>
</table>
EUGEI, French Institute of Health and Medical Research, Paris

DUP was measured using The Nottingham Onset Schedule (Singh et al., 2005). DUP is defined as from onset of symptoms (having either a clear positive symptom or delusions, hallucinations, first rank symptoms or catatonic symptoms for a week) to first contact with secondary mental health services for suspected psychosis (Singh et al., 2005; Jongsma et al., 2018).

First-Episode Schizophrenia Follow-Up Project, Istanbul University, Istanbul

The date of onset of the first identifiable positive symptoms was timed by the senior psychiatrist on the basis of a best-estimate approach using data gathered from medical records, a patient directly, and family interview, and it was used to later determine DUP. DUP was defined as a period from the time of onset of first positive symptoms (hallucinations, delusions) to the first hospitalisation (Jongsma et al., 2018; Üçok et al., 2004).

TIPP, Lausanne University Hospital, Lausanne

Expert consensus of senior psychiatrist and senior psychologist who are in charge of assessments over the treatment period: the time between the onset of disorder (obtained using the “Psychosis threshold” subscale of CAARMS; Yung et al., 2005) (disorganised speech, perceptual abnormalities, hallucinations) and onset of treatment. Treatment onset was originally defined in three different ways for comparison purposes. For details, see Golay et al. (2016); STRATA only included information on TIPP patients for whom the end of DUP was initiation of antipsychotic medication (Do, 2021).

TOP, University of Oslo, Oslo

Clinical assessment by specifically trained psychologists and physicians, using SCID-I Spitzer et al. (1992). DUP as the period between first episode of psychosis (delusions, hallucinations, negative symptoms, disorganised speech) and start of antipsychotic medication (Haukvik et al., 2016; Gjerde et al., 2018).
DUP was defined as the period from the emergence of the first continuous psychotic symptoms (symptoms present most of the time, determined by a formal patient interview with an experienced psychiatrist, leading to a diagnosis of one of the DSM-IV schizophrenia spectrum disorders: schizophrenia, schizophreniform disorder, schizoaffective disorder, brief psychotic disorder and psychosis not otherwise specified) to the initiation of adequate antipsychotic drug treatment.

Patients interviewed by a psychiatrist at their first hospitalization for acute and transient psychotic disorders, with the diagnosis made according to Mini-International Neuropsychiatric Interview (Sheehan et al., 1998)

Kolenic et al. (2018)

Pelayo-Terán et al. (2008)
2.2.5.2.3 Age

Study analyses used two continuous age variables, age and age squared. Age at baseline was available in each cohort and it was used as the age variable. This variable was then squared and used as the variable age squared. Age squared was included to control for non-linear effects of age as these effects potentially impact the risk for psychosis (Radua et al., 2018; Schimmelmann et al., 2015).

2.2.5.2.4 Gender

Gender was a binary variable (male/female), recorded in each cohort.

2.2.6 Genotyping in STRATA-G

2.2.6.1 Genotyping process

Genotyping was completed at Cardiff University, with files with genotypes transferred by collaborators via the KCL and Cardiff University secure file transfer systems. DNA was sent to Cardiff University via courier. STRATA-G samples were genotyped using: Infinium CoreExome-24 BeadChip (Illumina Inc., San Diego, CA, USA; ÆSOP), Genome-Wide Human SNP Array 6.0 (Affymetrix Inc, Santa Clara, CA, USA; NIFEPS; RGPI), Infinium HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, CA, USA; BoFEP; EUGEI; GAP; First-Episode Schizophrenia Follow-Up Project; French Institute of Health and Medical Research), Infinium OmniExpress-24 v1.2 BeadChip (Illumina, San Diego, CA, USA; TIPP; The Early Stages of Schizophrenia study; The Brazilian Wellcome Trust São Paulo Study; The West London Longitudinal First-Episode Psychosis Study), Infinium OmniExpress-12 v1_H BeadChip (Illumina, San Diego, CA, USA; TOP), and Infinium OmniExpressExome-8 BeadChip (Illumina, San Diego, CA, USA; PAFIP).

2.2.6.2 Quality control and imputation

2.2.6.2.1 Pre-imputation quality control

Quality control (QC) was completed by Dr Antonio Pardiñas and Dr Sophie Smart. QC followed a number of steps outlined by Anderson et al. (2010). Where it differed, an additional explanation is provided. QC was performed twice, before and after data imputation.
Each cohort went through the first round of QC separately prior to imputation. Homozygosity rate (the proportion of carrying two different alleles of a single nucleotide polymorphism; SNP) was determined to ensure no samples with ambiguous sex were included in the final dataset. Male sex is defined as rate of $F \geq 0.8$ and female sex is defined as $F \leq 0.2$. Individuals with $F$ between $>0.2$ and $<0.8$ were assumed to have genotyping error and were removed from further analysis. Individuals were also removed if their sex failed to match their self-reported gender. To avoid inaccuracy due to inclusion of DNA of low quality or low concentration, SNPs with $<95\%$ call rate were excluded from further analyses. To avoid duplicates and relatedness in the sample (i.e., pairs of individuals who are closer than second-degree relatives), the measure of identity-by-state (IBS) is calculated and applied to the data to remove duplicates and closely related individuals. IBS is an average proportion of alleles shared in common at genotyped SNPs, from autosomes only, by a pair of individuals. IBS was posited to work best on independent SNPs. To ensure that only independent SNPs are analysed, regions in extended LD are typically removed from the dataset, and they were removed in the STRATA QC from the following chromosomes: chromosome 1: 48–52 Mb; 2: 86–100.5 Mb; 2: 134.5–138 Mb; 2: 183–190 Mb; 3: 47.5–50 Mb; 3: 83.5–87 Mb; 3: 89–97.5 Mb; 5: 44.5–50.5 Mb; 5: 98–100.5 Mb; 5: 129–132 Mb; 5: 135.5–138.5 Mb; 6: 25.5–33.5 Mb; 6: 57–64 Mb; 6: 140–142.5 Mb; 7: 55–66 Mb; 8: 8–12 Mb; 8: 43–50 Mb; 8: 112–115 Mb; 10: 37–43 Mb; 11: 46–57 Mb; 11: 87.5–90.5 Mb, 12: 33–40 Mb, 12: 109.5–112 Mb, and 20: 32–34.5 Mb (Price et al., 2008). The remaining regions are pruned so that pairs of SNPs within each window are not correlated ($r^2 > 0.2$).

IBS data can also be used to determine shared ancestry, or identity by descent (IBD). To obtain IBD values for the STRATA sample, the R packages PC-AiR and then PC-Relate were used. PC-AiR (Conomos et al., 2015) performs principal component analysis (PCA) on GWAS SNP data, to summarise differences in population structure as principal components. PC-AiR also accounts for relatedness in the sample so that ancestry inference is not confounded by family structure.

Principal components calculated by PC-AiR became the basis of relatedness estimation using the PC-Relate package. PC-Relate (Conomos et al., 2016) uses principal components to adjust for population structure and accurately estimate genetic relatedness without an underlying model (without prespecified assumptions about underlying population structure). PC-Relate provided IBD values. IBD = 1 denotes duplicates or monozygotic twins, IBD = 0.5 first-degree relatives, IBD = 0.25 second-degree relatives and IBD = 0.125 third-degree relatives. Anderson et al. (2010) note that due to genotyping error, LD and population structure, these theoretical IBD values may differ, so typically IBD $> 0.1875$ is used to flag closely related individuals and IBD $> 0.98$ is used to identify duplicates. One individual from each tested pair is removed at random. In the STRATA
sample, individuals with IBS > 0.1 were flagged as related and individuals with IBS > 0.9 were assumed to be duplicates and removed from further analysis. These more conservative values were selected because the PC-Relate IBS estimator takes ancestry into account when calculating relatedness, so effectively addresses both the relatedness and ancestry confounding.

Finally, before imputation, palindromic SNPs were removed. Palindromic SNPs are SNPs whose alleles are nucleotides which pair with each other on the DNA molecule and are the same on the forward and reverse strands of the DNA molecule (a forward strand is the DNA strand which is read from the 5’ to the 3’ end, an end portion of the DNA molecule with a deoxyribose bound to a single phosphate group. A reverse strand is read from the 3’ to the 5’ end, an end portion of the DNA molecule with a phosphate group bound to a single deoxyribose). There may be issues in data harmonisation between datasets if the effect allele is based on the forward DNA strand in one dataset and on the reverse strand in another dataset. Different studies may report effects of the same SNP using different strands (e.g. the same SNP may be reported with A/G alleles in one dataset and T/C alleles in another dataset). If the alleles are the same on both strands, as palindromic SNPs are, it is difficult to identify which specific alleles between the two datasets are the effect alleles (palindromic SNPs introduce ambiguity as to which allele is the effect allele). Because of that, palindromic SNPs are removed before Hardy-Weinberg equilibrium (HWE) tests to prevent errors in these tests. HWE tests are \( \chi^2 \) goodness-of-fit tests which compare observed to expected genotypes to explore if observed genotypes depart from HWE. HWE is a model which describes the relationship between allele and genotype frequencies, assumed to be constant over generations if there was no selection, mutation, or migration. A liberal HWE test cut-off \( P < 1 \times 10^{-10} \), as used by the Psychiatric Genomics Consortium; PGC) was applied to the STRATA data and did not exclude any SNPs available at that point of the QC.

2.2.6.2.2 Imputation and post-imputation quality control

Following the basic QC, imputation (statistical inferring of missing genetic data) was conducted in the Michigan Imputation Server (Das et al., 2016) using the Haplotype Reference Consortium panel (McCarthy et al., 2016). The post-imputation QC is required as imputed data may include a large number of missing, poor quality, or incorrectly assigned genotypes which need to be removed. The parameters for this QC included: minor allele frequency (MAF) of 10%; probability threshold > 0.9 (probability that the genotype is imputed correctly); missingness < 5%; HWE p-value > 10^{-6}; INFO > 0.8 (information metric score; score assessing the quality of imputation; score of 1 signifies a high degree of certainty in imputation; Coleman et al., 2016).
2.2.7 Inclusion and exclusion of STRATA-G data in polygenic score analyses

In polygenic score calculation (detailed in the next section), GWAS dataset, which is used as basis of the score calculation, and the research sample for which scores are calculated, should not overlap. Otherwise, associations between the polygenic score and the trait tested in the research sample will be inflated (Wray et al., 2013). As this thesis uses GWAS summary statistics from PGC studies, potential overlap between samples included in STRATA-G and PGC GWAS summary statistics had to be addressed.

As reported by PIs of sites belonging to the STRATA Consortium, STRATA-G samples were also shared with PGC Working Groups for schizophrenia and bipolar disorder. In PGC schizophrenia GWAS summary statistics (detailed in the next section), Dr Pardiñas excluded samples (n = 640) which had been identified as having duplicates or had a relatedness coefficient \( \pi^{\text{hat}} \) \( (\text{"pi-hat"}) \) > 0.2 with any STRATA-G sample. “Pi-hat” is a well-established IBD estimator as implemented in PLINK (Chang et al., 2015). “Pi-hat” estimates the probability of two individuals from the same homogenous, random-mating population sharing 0, 1, or 2 alleles IBD. This de-duplication analysis for related and overlapping samples in GWAS summary statistics and STRATA was completed before schizophrenia polygenic score calculation.

Samples from TOP (University of Oslo, Norway) were reported to be part of PGC bipolar disorder GWAS summary statistics. Before including the TOP participants in STRATA-G, they were wholly anonymised so it would not be possible to link an individual in the STRATA-G database back to their data in the TOP database. Therefore, it was not possible to remove individuals from STRATA-G based on the records held in Oslo which state which individuals were included in the PGC bipolar disorder sample. As such, I excluded TOP data from all thesis analyses which required a bipolar disorder polygenic score (also detailed in the next section).

2.2.8 Polygenic scores

As discussed in Chapter 1, polygenic scores (PGS) are sums of weights assigned to individuals’ SNPs (target sample), based on effect size detailed in GWAS summary statistics (discovery sample). PGS for STRATA-G genetic data (target sample) were calculated for major depressive disorder, schizophrenia, bipolar disorder, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder. These PGS were previously inconsistently implicated in relation to age of onset in schizophrenia and bipolar disorder,
but not in a broad psychosis age of onset. The PGS have also not been tested in relation to duration of untreated psychosis.

PGS were calculated under an additive model, summarising weighted SNPs. Summary statistics from the following studies were used as discovery sets for each respective PGS: for major depressive disorder PGS, statistics from a GWAS meta-analysis by Howard et al. (2019); for bipolar disorder PGS, statistics from GWAS by Stahl et al. (2019); for autism spectrum disorder PGS, statistics from a GWAS meta-analysis by Grove et al. (2019); for ADHD PGS, statistics from a GWAS meta-analysis by Demontis et al. (2019); and for schizophrenia PGS, statistics from GWAS by Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) adjusted not to overlap with STRATA. For the schizophrenia PGS, samples were removed if they had been identified as relatives/duplicates of STRATA participants (see previous section) or as patients with treatment-resistant schizophrenia (i.e., prescribed clozapine). The latter was an effort by the STRATA-G Consortium to resolve the conflicting results which exist in the literature on the association between the schizophrenia PGS and treatment resistance. The total sample size of this adjusted GWAS was 20,325 cases with schizophrenia and 30,122 controls.

PGS for each phenotype was calculated multiple times, each time including SNPs at a different GWAS p-value threshold, following recommendations of the PGC: $10^{-6}$, $10^{-5}$, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.5, and 1. To remain consistent with the QC before PGS calculation, which included filtering out poorly imputed SNPs based on the INFO score, INFO score information was also included as part of PGS calculation, where possible. Where GWAS summary statistics provided INFO scores (bipolar disorder, ADHD, and autism spectrum disorder), the PGS were calculated based on GWAS summary statistics filtered with INFO score threshold of 0.9. No covariates were included as part of the PGS calculation. PGS were standardised, i.e., rescaled to have normal distribution (mean = 0 and standard deviation $\approx 1$), and so that they are easier to compare and interpret (Babb de Villiers et al., 2020). All PGSs were calculated using PRSice-2 (version 2.2.13; Choi and O’Reilly, 2019). PRSice was a software of choice because it is a fast and memory-efficient program for PGS calculation (Choi and O’Reilly, 2019).

2.3 The English Longitudinal Study of Ageing (ELSA)

2.3.1 General ELSA overview

The English Longitudinal Study of Ageing (ELSA) is a large longitudinal sample selected to be representative of the English population aged 50 years and older, living in private
households. The overarching aim of the study is to track multiple characteristics of participants as they move from middle to older age, to enable research relevant to policy: on health trajectories, disability, healthy ageing, life expectancy, socioeconomic status in old age, household structures, social networks and support, and relationships between those domains. So far, nine waves of data collection have been completed, i.e., information has been collected every two years since 2002/3 (wave 1) up to 2018/19 (wave 9). All data are available online, open access, to enable wide dissemination by the research community (https://www.elsa-project.ac.uk/accessing-elsa-data).

Collected information includes: 1) answers to a computer-assisted personal interview (CAPI) on socioeconomic status, demographics, health, social care, and a battery of cognitive tasks; 2) a self-completion questionnaire on socioeconomic status, social networks, etc.; 3) detailed measures of function (balance, grip, anthropometric measurements, information on prescribed medication); and 4) blood for biomarkers and genotyping. Information collected in ELSA has been designed in the way that ELSA data are harmonised (highly comparable) with equivalent international longitudinal studies on ageing, such as the Health and Retirement Study in the US.

ELSA is funded by the National Institute on Aging, the primary US Federal agency supporting Alzheimer’s research, and by a consortium of UK government departments: Department for Health and Social Care; Department for Transport; Department for Work and Pensions, which is coordinated by the National Institute for Health Research. Funding has also been provided by the Economic and Social Research Council. ELSA is a collaboration between UCL Research Department of Epidemiology and Public Health; Institute for Fiscal Studies; The School of Social Sciences, The University of Manchester; National Centre for Social Research, or NatCen Social Research; and Norwich Medical School, University of East Anglia. The PI is Professor Andrew Steptoe, head of the UCL Research Department of Behavioural Science and Health.

At the time of working on analyses for this thesis, information was available (and so analysed in the thesis) from eight waves (up to and including 2016/17). Preliminary wave 9 (2018/19) data were released in 2020, with The ELSA Wave 9 report summarising all the data due in October 2020.

2.3.2 Ethical approval and consent

Ethical approval for all ELSA waves was granted by the National Research and Ethics Committee (NHS HRA Research Ethics Service). All participants provided written consent.
2.3.3 The ELSA sample design and administration

The ELSA sample is formed of people living in private accommodation in England, aged 50 and more. The sample is drawn from previous responders to an annual cross-sectional survey of the general population health in England, the Health Survey for England (HSE). To account for ageing and deaths, the core sample gets periodical refreshment cohorts added, i.e., people at younger ages are enrolled so that the sample remains representative of a full age spectrum of late middle age up to old age. Participants are recontacted for follow-up every two years, which forms each wave. Response rates and attrition in ELSA are complicated to analyse, given the question of whether non-responses to the original HSE survey should be counted, and differences in responses between core and refreshment cohorts. Details on response rates, attrition and deaths from waves 1 to 8 are provided in Table 1 of the ELSA cohort profile paper (Zaninotto and Steptoe, 2019).

Data collection includes completion of the CAPI in a face-to-face interview, self-completion questionnaire, and nurse visit. The CAPI and self-completion questionnaire are administered at every wave (every two years). A nurse visit occurs on alternate waves. Blood for biomarkers and genotyping (detailed below, Section 2.3.5.) is drawn during the nurse visit. The ELSA data collection also includes ad hoc modules (additional assessments) administered to selected waves (a life history interview on key life events, geographical mobility family and health history administered to wave 3, 2006/7; a module on preferences for risk and certainty and risk control at wave 5, 2010/11; and nutrition assessment at wave 9, 2018/19). Figure 2.1 on the next page gives an overview of data collection in ELSA: the total sample and refreshment sample sizes throughout waves, refreshments, and at nurse visits.

2.3.4 Phenotypes in ELSA

As the overarching goal of ELSA is to gather as much policy-relevant information on health and life changes in the ageing population as possible, the ELSA data include hundreds of variables collected at multiple waves. Extended details on each measure can be found online in wave reports detailing the process of data collection in each wave (https://www.elsa-project.ac.uk/wave-reports). Additionally, Table “ELSA DATASET WAVES 0 TO 9” breaks down which data domain was collected at which wave (https://www.elsa-project.ac.uk/the-data-we-collect; wave 0 referring to the HSE questionnaire from which wave 1 was recruited). Data collected includes demography; economic status; expectations on mortality, health, employment and wealth; health, disability and health behaviours; biomarkers; psychosocial measures; and cognitive function. Finally, section
Fig. 2.1 Waves of data collection in the English Longitudinal Study of Ageing (ELSA).

Each wave (left) represents a period of data collection (follow-up) of two years (years given in brackets). Participants are drawn from an annual cross-sectional survey of the general population health in England, the Health Survey for England (HSE). Participants complete online questionnaires and are visited by nurses (nurse visit) for health checks and blood sample draws. Life history is an extended participant interview on key life events. Refreshment samples are periodically added to the ELSA sample so that age range in the sample remains consistent as it changes over time due to attrition or participant death. Figure from Zaninotto and Steptoe (2019).
Content of the ELSA cohort profile (Zaninotto and Steptoe, 2019) gives details on which data domains were collected through the CAPI, self-administered questionnaires, or nurse visits.

Below, I only detail the variables which were the basis of subsequent analyses in this thesis (Chapter 5).

2.3.4.1 Participant variables

The focus of the study was on whether common genetic variants are related to cognitive decline of verbal memory and semantic fluency. An extensive set of covariates was also included in analyses, covariates which have been previously shown to impact cognitive function in the ELSA participants (Choi et al., 2020).

2.3.4.1.1 Verbal memory

Verbal memory was measured with a word recall test. Participants have 10 common words read out loud for them and asked to remember the words. Word recall is tested immediately (immediate recall) and then following a short delay filled with other cognitive tests (delayed recall). Respondents are given up to two minutes to recall words in immediate and delayed recall test. The raw total test scores correspond to the number of words recalled, with a maximum score of 20 (Yin et al., 2019a). In the American HRS, an ELSA equivalent, the verbal memory task did not produce floor or ceiling effects (excessive low or high scores) which indicates its good validity (Bonsang et al., 2012). Similarly, in ELSA there was no evidence of floor or ceiling effect (scores were normally distributed). The variable was collected as part of cognitive testing in face-to-face interviews over subsequent waves.

2.3.4.1.2 Semantic fluency (executive function)

Semantic fluency (executive function) was measured with a word finding test: a test of how quickly participants can think of words from a particular category. Participants are asked to name as many different animals as possible in one minute. The total number of given animal names is used as a continuous measure of semantic fluency. The score is measured on a continuum, from 0 to 30, with the higher score an indicator of better performance (Yin et al., 2019a). The scores were normally distributed in ELSA. The variable was collected as part of cognitive testing in face-to-face interviews over subsequent waves.
2.3.4.1.3 Age

Study analyses used two age variables, age and age squared, to account for non-linear effects of ageing. The age variable was collected as part of face-to-face CAPI over subsequent waves.

2.3.4.1.4 Gender

Gender was a binary variable (male/female). The variable was collected as part of face-to-face CAPI over subsequent waves.

2.3.4.1.5 Smoking

Currently smoking was a binary variable (yes/no). The variable was collected as part of face-to-face CAPI over subsequent waves.

2.3.4.1.6 Wealth

Wealth was measured at baseline as a sum of capital from property, possessions, housing, investments, savings, artwork, jewellery and net of debt. Wealth was divided into tertiles: high, intermediate and low levels of wealth. This information was collected as part of face-to-face CAPI over subsequent waves.

2.3.4.1.7 Educational attainment

Educational attainment is a measure of completed years of schooling. It was collected as part of face-to-face CAPI over subsequent waves.

2.3.4.1.8 Depressive symptoms

Depressive symptoms were measured with an abbreviated, 8-item version of the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977), a scale of self-reported depression symptoms experienced over a week. The full CES-D scale includes 20 items, but the psychometric properties of the abbreviated version have been found to be comparable to the original scale (Turvey et al., 1999). A score 4 was used to define participants with
severe depressive symptoms. CES-D scoring was completed as part of face-to-face CAPI over subsequent waves.

2.3.4.1.9 A limiting long-standing illness

Presence of a limiting long-standing illness was a self-reported binary variable (yes/no). The variable was collected as part of face-to-face CAPI over subsequent waves.

2.3.5 Genotyping in ELSA

2.3.5.1 The genotyping process

The ELSA participants were excluded from blood collection if they have not completed a nurse visit, did not give consent for blood samples to be taken, had a clotting or bleeding disorder, history of fit or convulsion, took anticoagulant drugs, were pregnant, or not eligible to fast before giving the blood sample (with the exception of those over 80 years old or diabetic and on treatment).

The genome-wide genotyping was performed at UCL Genomics in 2013-2014. Genotyping was completed on 7,597 ELSA participants of European ancestry using the Illumina HumanOmni2.5 BeadChips (HumanOmni2.5-4v1, HumanOmni2.5-8v1.3). Genotyping was performed in two batches. Batches were compared for allele frequencies after filtering for 5% of missingness. The subsequent correlation between the batches for the number of chromosomes was over 99%. After post-genotyping quality assurance (excluding self-reported non-European data and duplicates), the GWAS data were available for total 7,412 ELSA participants.

2.3.5.2 Quality control

QC was completed by Dr Olesya Ajnakina. QC followed steps outlined in Marees et al. (2018) and was completed using genetic analysis software PLINK 1.9 (Chang et al., 2015).

2.3.5.2.1 Quality control of individual-level data

Individuals were excluded if they had a mismatch between their recorded genetic sex (determined as part of the genetic data QC) and self-reported identification as male or female (stated by participants in the ELSA face-to-face CAPI). To estimate IBD probabilities
CHAPTER 2. METHODS

from participants’ autosomal SNPs, a method of moments was used. The method assesses probabilities of sharing 0, 1 or 2 alleles identical by descent between two individuals. The method of moments is implemented in PLINK 1.9. Individuals with an IBD value of >0.2 were identified and one individual was excluded from each pair at random.

2.3.5.2.2 Quality control of SNP-level data

Checks for heterozygosity were completed on SNPs which were not highly correlated. To obtain the list of such SNPs, regions in extended LD (highly correlated) were first removed from the following chromosomes: chromosome 6: 12.57874–135.837195 Mb (the lactase gene; LCT); 2: 2.55–3.35 Mb (human leukocyte antigen; HLA) and two inversion regions located on 8p23.1 (chromosome 8, 81.305–1.2 Mb) and 17q21.31 (chromosome 17, 40.9–45 Mb; Novembre et al., 2008). The remaining SNPs were then pruned using the ‘10 5 0.1’ parameters: a sliding window which takes blocks of 10 SNPs at the time, removes SNPs with \( r^2 > 0.10 \) from the block, and then shifts the window by 5 SNPs. Additionally, SNPs were removed if they: came from sex chromosomes; came from individuals with extremely low or high heterozygosity score (>3 standard deviations from the mean); had a call rate of <98%; had a MAF of <0.01; had genotype distributions which deviated significantly from HWE (\( P \neq 10^{-4} \)); and had missingness <0.02. Finally, to ensure a large overlap between the GWAS summary statistics (the discovery files) and the ELSA genetic data (the target file), the IDs specific to platform (kgps) to rs numbers (rsids; standard SNP identifiers). Those of the ELSA SNPs whose kgps failed to convert to rsids were removed.

2.3.5.2.3 Population structure

PCA, as implemented in PLINK, was completed on the ELSA genetic data to 1) identify individuals whose genetic ancestry may not match their self-reported ancestry and 2) provide sample eigenvectors which are used to adjust for population stratification in the association analyses (Price et al., 2006; Wang et al., 2009). However, PCA may be biased if it is based on regions in high LD (Novembre et al., 2008). Consequently, regions in high LD (LCT, HLA, 8p23.1 and 17q21.31) were removed and the autosomal SNP data were pruned from the initial pool of SNPs with a missing call rate <5% and MAF >5%. LD pruning was completed on unrelated participants and selected 147,070 SNPs with all pairs having \( r^2 < 0.1 \) in a sliding 10 Mb window. PCs were calculated using PLINK 1.9. The top 10 PCs were retained to account for ancestry differences in genetics of ELSA participants. After initial PCA on all study participants, the visual inspection of PC
distribution highlighted admixture in 65 individuals. The data for these individuals were excluded and the PCs were recalculated on the updated sample.

### 2.3.6 Polygenic scores

PGS in ELSA (target sample) were calculated by Dr Ajnakina. The schizophrenia PGS was previously tested in relation to cognitive decline in other elderly samples, but previous studies either had samples not as large as ELSA (Ritchie et al., 2020) or produced mixed results (Ritchie et al., 2020; Liebers et al., 2016; McIntosh et al., 2013).

PGS were calculated under an additive model, summarising weighted SNPs. Summary statistics from GWAS by Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) were used as discovery sample. All base SNP files from GWAS meta-analysis were converted to National Center for Biotechnology Information (NCBI) build 37 annotation for compatibility with ELSA SNP data.

ELSA is harmonised with (shares questionnaires, tasks, and methodology with) an American equivalent study of ageing, the Health and Retirement Study (HRS). The HRS team tested multiple methods of PGS calculation to find the most predictive PGS. Ware et al. (2017) reported that PGS based on imputed data had lower or comparable predictive power compared to PGS based only on directly genotyped data of the HRS participants. Following this validation in HRS, a comparable population sample, ELSA followed the same methodology. SNP data in ELSA were not imputed and all PGS in ELSA were built using only directly genotyped data.

The HRS team also tested PGS including SNPs at different GWAS p-value thresholds and demonstrated that PGS which include all available SNPs either explain the greatest amount of variance in the trait or are not significantly different from PGS based on other p-value thresholds. The HRS recommendation is to calculate PGS based on all available SNPs (Ware et al., 2017). Accordingly, PGS in the study in Chapter 5 are based on all SNP data (GWAS p-value threshold of 1). As in Ware et al. (2017), where the beta/OR value from the GWAS summary statistics was negative or OR < 1, in ELSA, the betas/ORs were converted to positive values and the reference allele flipped to represent phenotype-increasing PGS. PGS did not include any covariates as part of the score calculation. PGS were standardised to the mean of 0 (standard deviation = 1).

All analyses were completed on individuals of European ancestry. PGS were calculated using PRSice (version 1; Euesden et al., 2015). Power calculations (Section *Calculate power and predictive accuracy of a polygenic score*) show that PGS had sufficient power for subsequent analyses.
Chapter 3

Common genetic variants for major psychiatric disorders do not predict age of onset in psychosis

3.1 Introduction

Psychosis encompasses a spectrum of severe mental disorders, characterised broadly by delusions, hallucinations, thought disorder (neologisms, distactibility, and illogicality), and cognitive impairment. Psychosis outcomes are heterogeneous and consistently linked to a reduction in life expectancy by 10-20 years in psychosis patients compared to the general population (Owen et al., 2016). Thus, prevention and early intervention are crucial. Knowledge of age of onset (AOO) of psychosis might improve the process of organising and timing psychiatric interventions (Jones, 2013).

Psychosis rarely occurs before the age of 13 (childhood-onset schizophrenia; Ahn et al., 2016). The peak in AOO occurs between ages 20-29, with an earlier onset in men (Castle et al., 1998; Häfner et al., 1993; Miettunen et al., 2019; Thorup et al., 2007). A second, smaller peak occurs after the age of 40 and accounts for approximately 20% of cases (late-onset schizophrenia for psychosis manifesting after the age of 40, or very-late-onset-schizophrenia-like psychosis for psychosis manifesting after the age of 60; Howard et al., 2000; Jeste and Maglione, 2013). Early- and late-onset schizophrenia share several symptoms (similar severity of depressive, negative, and deficit symptoms; Vahia et al., 2010), but late-onset schizophrenia occurs more frequently in women, has a lower average severity of positive symptoms, and requires lower average antipsychotic doses as treatment
(Maglione et al., 2014). Thus, early- and late-onset psychoses are increasingly argued to be overlapping but distinct psychosis subtypes (Maglione et al., 2014).

AAO potentially acts as a mediator between gender, familial history of psychosis and clinical outcomes (Goldberg et al., 2011). AAO has also been hypothesised to be a primary characteristic useful in exploring aetiology of schizophrenia, assuming that genes linked to AAO may also determine timing and nature of brain abnormalities which result in schizophrenia (DeLisi, 1992). Indeed, psychosis AOO has a genetic component. Heritability of psychosis AOO is 33% (Hare et al., 2010). Family studies found that individuals with higher familial risk for schizophrenia present with earlier AOO (Esterberg et al., 2010; Hilker et al., 2017; Li et al., 2007). Familial effects impact different types of cognition in families with adult-onset schizophrenia in comparison to families with childhood-onset schizophrenia (Bigdeli et al., 2020). Childhood-onset schizophrenia is also characterised by higher rates of rare copy number variants (CNVs) than adult-onset schizophrenia, including at the 22q11.2 locus, a well-established contributor to genetic risk for schizophrenia (Monks et al., 2014). These CNVs also increase risk of neurodevelopmental disorders such as autism, intellectual disability, and attention-deficit/hyperactivity disorder (ADHD; Aln et al., 2014; Sagar et al., 2013; Vorstman et al., 2006).

Genome-wide association studies (GWAS), which attempt to identify common genetic variants (single nucleotide polymorphisms; SNPs) related to phenotypes, have identified several SNPs associated with AOO in schizophrenia (Bergen et al., 2014; Wang et al., 2011; Woolston et al., 2017) and AOO in bipolar disorder (Belmonte Mahon et al., 2011; Jamain et al., 2014; Nassan et al., 2017). None of these associations have been replicated, but this may be due to small, insufficiently powered samples that fail to detect the small proportion of phenotypic variance explained by each SNP (Visscher et al., 2017). Polygenic scores (PGS), the aggregate measures of SNP effects, for schizophrenia and bipolar disorder, have also failed to predict the AOO of schizophrenia, bipolar disorder, and mania (Aminoff et al., 2015; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Kalman et al., 2019; Stepiak et al., 2014). A single study reported that schizophrenia PGS predicted childhood-onset schizophrenia status and suggested that autism spectrum disorder PGS might be a significant predictor but only at a liberal GWAS significance threshold of P<0.4 (Ahn et al., 2016). Additionally, Grigoroiu-Serbanescu et al. (2020) recently reported that PGS for ADHD differentiated cases with early-onset bipolar disorder (≤21 years) from controls.

Altogether, psychoses with different ages of onset are likely to vary in terms of both their specific phenotypic presentations and genetic influences underlying those phenotypes. However, PGS related to phenotypes beyond schizophrenia and bipolar disorder have not been generally applied to AOO of psychosis. Hence, the aim of this study was to expand on
previous work and test whether psychosis AOO can be predicted by PGS for other major psychiatric disorders. These PGS may act as proxies for biological processes potentially underlying AOO in psychosis. Existing studies have identified polygenic overlaps between psychosis and psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013, 2019) and neurodevelopmental disorders (Murray et al., 2017), including AOO in bipolar disorder and PGS for ADHD (Grigoroiu-Serbanescu et al., 2020). Thus, I selected PGS for the following major psychiatric disorders: schizophrenia (SCZ), bipolar disorder (BPD), major depressive disorder (MDD), autism spectrum disorder (ASD), and ADHD. As the majority of existing literature found no statistically significant associations between AOO in schizophrenia and bipolar disorder and PGS (Amino et al., 2015; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Kalman et al., 2019; Stepniak et al., 2014), I hypothesised that PGS for major psychiatric disorders would not predict psychosis AOO.

3.2 Methods

3.2.1 Sample

I used data from The Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA) Consortium, specifically STRATA Genetics (STRATA-G). STRATA-G is part of the Consortium focused on using genetic and non-genetic information to predict resistance to non-clozapine antipsychotics. Members of STRATA-G collated longitudinal data from prospective, observational studies of first episode psychosis from multiple European clinical research sites. Data in the current study come from the following studies: Aetiology and Ethnicity in Schizophrenia and Other Psychoses (ÆSOP, London); Northern Ireland First Episode Psychosis (NIFEPS) and Resources for Genomics, Ireland (RGPI, Belfast); Bologna FEP (BoFEP, Bologna), Genetics and Psychosis (GAP, London); First-Episode Schizophrenia Follow-Up Project (Istanbul); Treatment and Early Intervention in Psychosis Program (TIPP, Lausanne); Thematic Organized Psychosis Research (TOP, Oslo); European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EUGEI, Paris); First Episode Psychosis Clinical Program (Programa de Atención a las Fases Iniciales de Psicosis, PAFIP, Santander); and The West London Longitudinal First-Episode Psychosis Study (University College London, UK). The exception are analyses using bipolar disorder PGS which exclude data from the TOP sample. These data were excluded because as they had been originally included in the bipolar disorder GWAS used as basis of PGS calculation in this study (see Section Polygenic score analyses below or Section 2.2.7. Inclusion and exclusion of STRATA data in polygenic
score analyses, Chapter 2). Chapter 2, Section 2.2. provides details on each individual sample, including catchment areas, diagnostic instruments, inclusion and exclusion criteria used in each original study (before the start of each study at its respective research site).

After data from all sites had been combined into the STRATA-G dataset, individual data were excluded if participants were missing any of the demographic and clinical data (age, gender, research site, and AOO) or genetic data (which was either because there was no genetic data from a participant or because their data failed to pass quality control; see Chapter 2, Section 2.2.6.2 Quality control and imputation for details on quality control). Ethical approval was granted by Research Ethics Committee at South Central - Oxford C and the National Health Service Health Research Authority Confidentiality Advisory Group. Participants gave informed consent or, where minors took part, parents/legal guardians signed a parental permission consent document.

3.2.2 Study variables

3.2.2.1 Age of onset of psychosis

AOO is an age at which the participant’s first psychotic symptoms occurred. Depending on STRATA cohort, AOO has been ascertained using the Personal and Psychiatric History Schedule (Jablensky et al., 1992), the Symptom Onset in Schizophrenia Inventory (Perkins et al., 2000), the Structured Clinical Interview for DSM (First et al., 1997; Spitzer et al., 1992), the Nottingham Onset Schedule (Singh et al., 2005), or the “Psychosis threshold” subscale of Comprehensive Assessment of At Risk Mental States (Yung et al., 2005). For the majority of sites, diagnostic criteria were comparable and included presenting with at least one symptom out of hallucinations, delusions, bizarre behaviour or disorganised speech. Several, but not all, diagnostic instruments listed above also include additional symptoms to define psychosis onset (psychomotor disorders, catatonia, lack of insight, lack of pleasure, difficulties with concentration, depressive/sad mood, and elevated mood or mania). AOO was reported by patients but, for a number of cohorts, age recalled by the patient was also corroborated with either extra interview with family/key informants and/or analysis of the patient’s health records (First-Episode Schizophrenia Follow-Up Project; BoFEP; ÆSOP and GAP; NIFEPS and RGPI; see Table 2.2 in Chapter 2 for a detailed breakdown).
3.2.2.2 Covariates

I included covariates of age, age squared, gender, 5 principal components (PCs), and STRATA research site. Age was patient age at baseline (as STRATA includes longitudinal data; for information on follow-up for each STRATA cohort, see Smart (2019), Section 7.3 Cohorts). Age squared was based on age at baseline. Age squared was included to control for non-linear effects of age which potentially impact risk for psychosis (Radua et al., 2018; Schimmelmann et al., 2015). Gender was included in light of gender differences in schizophrenia AOO (van der Werf et al., 2014). PCs were included to account for genetic differences resulting from ancestry (Price et al., 2006); see next section for details on PC generation. Finally, STRATA research site was a site of the Consortium where the data originally came from. I included this variable to account for potential differences in AOO measurement between sites.

3.2.3 Genetic data

3.2.3.1 Quality control and data imputation

Genome-wide genotyping was performed either at the original research site or at Cardiff University School of Medicine, using Illumina BeadChips (Infinium CoreExome-24, Infinium HumanCoreExome-24, Infinium OmniExpress-24 v1.2, Infinium OmniExpress-12 v1_H, Infinium OmniExpressExome-8; Illumina Inc., San Diego, CA) and the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc, Santa Clara, CA, USA; see Chapter 2, Section 2.2.6.1. Genotyping process for a detailed breakdown). The data went through quality control (QC), then they were imputed, and finally, they went through the second, post-imputation QC. QC largely followed steps outlined by Anderson et al. (2010). Before imputation, samples were removed based on homozygosity rate (F) between > 0.2 and < 0.8, or if their genetic sex failed to match participants’ reported gender. SNPs were excluded if their call rate was < 95%, if they were in regions in extended linkage disequilibrium (LD) listed by Price et al. (2008), if they were correlated ($r^2 > 0.2$) within a sliding window, if they were palindromic, and if they failed to fit the Hardy-Weinberg equilibrium (HWE) test cut-off $P < 1 \times 10^{-10}$.

PCs were calculated using R package PC-AiR (Conomos et al., 2015). PCs were then used as basis of relatedness estimation, with package PC-Relate (Conomos et al., 2016). PC-Relate provided identity by descent (IBD) estimation values which were used to flag closely related individuals and duplicates in the sample. Individuals were tested in pairs
and removed at random if IBD > 0.1 (indicating relatedness) or IBD > 0.9 (indicating duplicates). After the basic QC, imputation was conducted in the Michigan Imputation Server (Das et al., 2016) using the Haplotype Reference Consortium panel (McCarthy et al., 2016). Then, post-imputation QC was completed on all the remaining and imputed data. Parameters for this QC were: minor allele frequency of 10%; probability threshold > 0.9 (probability that the genotype is imputed correctly); missingness < 5%; HWE p-value > 10^{-6}; INFO > 0.8 (score assessing the quality of imputation; Coleman et al., 2016). Genotyping and QC are described in detail in Chapter 2, Section 2.2.6. *Genotyping in STRATA.*

### 3.2.3.2 Polygenic scoring

Two samples of STRATA-G data were shared with the Psychiatric Genomics Consortium (PGC) Workgroups for schizophrenia and bipolar disorder and included in GWAS for these disorders. However, if GWAS summary statistics come from samples which overlap with samples for which PGS is being calculated, associations between PGS and the trait of interest are inflated (Wray et al., 2013). Consequently, before PGS for schizophrenia were calculated, Dr Antonio Pardiñas removed samples (n = 640) from schizophrenia GWAS summary statistics, which he had identified as having duplicates or as having a relatedness coefficient \( \pi^\sim \) (“pi-hat”) > 0.2 with any STRATA-G sample. “Pi-hat” was implemented in PLINK (Chang et al., 2015). Samples from TOP (Norway) were part of the PGC bipolar disorder GWAS summary statistics. Thus, data from TOP were excluded from all thesis analyses implementing PGS for bipolar disorder.

PGS were calculated under an additive model, whereby SNPs weighted by GWAS effect sizes are added up to form the PGS (compare with Section 1.5. *Polygenic scores*, Chapter 1, for a detailed overview of PGS calculation). Summary statistics from the following studies were used as discovery sets for each respective PGS: for MDD PGS, statistics from a GWAS meta-analysis by Howard et al. (2019); for BPD PGS, statistics from GWAS by Stahl et al. (2019); for ASD PGS, statistics from a GWAS meta-analysis by Grove et al. (2019); for ADHD PGS, statistics from a GWAS meta-analysis by Demontis et al. (2019); and for SCZ PGS, statistics from GWAS by Schizophrenia Working Group of the Psychiatric Genomics Consortium Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) adjusted so that they do not overlap with STRATA. The total sample size of this adjusted GWAS was 20,325 cases with schizophrenia and 30,122 controls.
PGS for each phenotype was calculated at multiple GWAS p-value thresholds for SNP inclusion, following recommendations of the PGC: $10^{-6}$, $10^{-5}$, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.5, and 1. To remain consistent with the QC before PGS calculation, INFO score information was also included as part of PGS calculation, where provided in the GWAS summary statistics (BPD, ADHD, and ASD). These PGS were calculated based on GWAS summary statistics filtered to retain data with INFO score of 0.9 or higher.

Demontis et al. (2019) provided separate ADHD summary statistics for two of their original study samples: one sample with data from participants of different ancestries (Chinese and European ancestries) and one sample with data from participants of European ancestry only. Accordingly, two separate sets of PGS for ADHD were calculated (one based on data from all ancestries and one based European ancestry only). No covariates were included as part of any PGS calculation. PGS were standardised to a mean of 0 (SD ≈ 1) so that results are easier to compare and interpret (interpret how phenotypes are related to increases in PGS SD; Babb de Villiers et al., 2020). All PGS were calculated with PRSice-2 (version 2.2.13; Choi and O’Reilly, 2019).

### 3.2.3.3 Statistical analysis

I used multiple linear regressions to explore if a PGS for a specific disorder (at one of the GWAS p-value thresholds at a time), adjusted for covariates, would predict AOO. I repeated analyses for each PGS at each of the 9 GWAS p-value thresholds. I also repeated analyses with the value of AOO log-transformed. This was to account for the fact that distribution of AOO is skewed (towards psychosis onset in early 20s; Miettunen et al., 2019). Finally, I repeated all analyses on data from two sets of participants. The first set included all participants whose data were available after genetic data QC, of any ancestry (see Section Quality control and data imputation above). The second set was post-QC data which only included participants of European ancestry. For this European ancestry-only group, analyses with PGS for ADHD included PGS calculated with ADHD GWAS data from European ancestry participants only. That is because PGS based on European ancestry GWAS data are the most precise in prediction in European ancestry patient data (Martin et al., 2019b). Value of $R^2$ came from subtracting $R^2$ attributable to a model with covariates alone from $R^2$ for the model including PGS and covariates. I reported the p-value of each regression analysis. To correct for multiple testing, I also implemented p-values corrected for false discovery rate (FDR; Benjamini and Hochberg, 1995), a method commonly used in genetics where tests are positively correlated (Benjamini and Yekutieli, 2001). I calculated FDR-corrected p-values using the `p.adjust` function from the R package `stats`, with the option `method = "fdr"`. All analyses were conducted in R, release 3.6.3 (R Core Team, 2020).
3.3 Results

3.3.1 Sample characteristics

Characteristics of the total sample are presented in Table 3.1. Total sample consisted of 1103 individuals, with a mean age of 27.9 years at first assessment for psychosis (standard deviation [SD] = 9, range 8-61 years) and a mean AOO of psychosis of 26.7 years (SD = 9, range 7.5-61 years). The majority of the sample were men (653 individuals, 59.2%). Data came from 10 first episode psychosis studies in 8 European cities (Belfast, Bologna, Istanbul, Lausanne, London, Oslo, Paris and Santander). Diagnosis at first assessment (when participants were first asked about their age of onset, which is age of onset analysed in this thesis) was available for 836 out of 1103 participants. Diagnoses were based on the International Classification of Diseases, 10th Edition (ICD-10), and the Diagnostic and Statistical Manual of Mental Disorders, 5th and 4th Editions (DSM-5 or DSM-IV). The majority of participants with a diagnosis available (369 participants) had a diagnosis of schizophrenia, followed by schizophreniform disorder, acute and transient psychotic disorders, and psychosis not specified as schizophrenia. The remaining diagnoses (bipolar disorder, mania, schizoaffective disorder, delusional disorder, depression with or without psychosis, or alcohol- or drug-induced psychosis) accounted each for 5% or less of the remaining available diagnoses.

Sample size for analyses of participants of all ancestries, excluding data from TOP (Oslo, due to its overlap with BPD GWAS) was $n = 983$. Sample size of participants of European ancestry only was $n = 684$ for all analyses except analyses implementing BPD PGS, where the sample size was $n = 609$ after exclusion of data from TOP.
Table 3.1 Characteristics of the Schizophrenia: Treatment Resistance and Therapeutic Advances-Genetics (STRATA-G) sample at first presentation (collection of age of onset data)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total sample N = 1103</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%) / Mean (SD)</td>
</tr>
<tr>
<td>Age of onset of psychosis (in years)</td>
<td>26.7 (9)</td>
</tr>
<tr>
<td>Log-transformed age of onset of psychosis</td>
<td>3.2 (0.3)</td>
</tr>
<tr>
<td>Age at baseline assessment (in years)</td>
<td>27.9 (9)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>450 (40.8%)</td>
</tr>
<tr>
<td>Male</td>
<td>653 (59.2%)</td>
</tr>
<tr>
<td>STRATA-G cohort</td>
<td></td>
</tr>
<tr>
<td>ÆSOP, London</td>
<td>56 (5.1%)</td>
</tr>
<tr>
<td>NIFEPS and RGPI, Belfast</td>
<td>60 (5.4%)</td>
</tr>
<tr>
<td>BoFEP and EUGEI, Bologna</td>
<td>35 (3.2%)</td>
</tr>
<tr>
<td>GAP, London</td>
<td>164 (14.9%)</td>
</tr>
<tr>
<td>First-Episode Schizophrenia Follow-Up Project, Istanbul</td>
<td>42 (3.8%)</td>
</tr>
<tr>
<td>TIPP, Lausanne</td>
<td>175 (15.8%)</td>
</tr>
<tr>
<td>TOP, Oslo</td>
<td>120 (10.9%)</td>
</tr>
<tr>
<td>EUGEI, Paris</td>
<td>31 (2.8%)</td>
</tr>
<tr>
<td>PAFIP, Santander</td>
<td>351 (31.8%)</td>
</tr>
<tr>
<td>The West London Longitudinal First-Episode Psychosis, London</td>
<td>69 (6.3%)</td>
</tr>
<tr>
<td>Diagnosis at first presentation</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>369 (33.5%)</td>
</tr>
<tr>
<td>Schizophreniform disorder</td>
<td>132 (12%)</td>
</tr>
<tr>
<td>Acute and transient psychotic disorders</td>
<td>97 (8.7%)</td>
</tr>
<tr>
<td>Psychosis not specified as schizophrenia</td>
<td>80 (7.3%)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>20 (1.8%)</td>
</tr>
<tr>
<td>Other psychoses</td>
<td>138 (12.5%)</td>
</tr>
<tr>
<td>Not available</td>
<td>267 (24.2%)</td>
</tr>
</tbody>
</table>

### 3.3.2 Associations of polygenic scores for major psychiatric disorders with age of onset in psychosis

Summary of results for PGS at each p-value threshold (\(P_T\)) is provided in Tables 3.2-3.13. Briefly, in analyses including all participants (of European and non-European ancestry),
CHAPTER 3. POLYGENIC SCORES & AGE OF ONSET IN PSYCHOSIS

PGS for SCZ, BPD, ADHD, and ASD were not significantly associated with AOO at any p-value threshold of GWAS training sets (p > 0.05). PGS for MDD was significantly related to a lower AOO at p-value thresholds (PT) P_T = 0.0001 (β = -0.02, 95%CI = -0.04 - 0.002, P = 0.03), P_T = 0.1 (β = -0.03, 95%CI = -0.05 - 0.003, P = 0.03), P_T = 0.5 (β = -0.02, 95%CI = -0.04 - 0.002, P = 0.03), and P_T = 1 (β = -0.03, -0.05 - 0.004, P = 0.02).

In analyses of all participants, where AOO had been log-transformed to account for its skewness, PGS for BPD, ASD, and ADHD were not significantly related to AOO. PGS for MDD was significantly related to a lower AOO at P_T = 0.0001 (β = -0.03, 95%CI = -0.05 - 0.01, P = 0.008), P_T = 0.1 (β = -0.03, 95%CI = -0.06 - 0.01, P = 0.009), P_T = 0.5 (β = -0.03, 95%CI = -0.05 - 0.01, P = 0.01), and P_T = 1 (β = -0.03, 95%CI = -0.06 - 0.01, P = 0.007). PGS for SCZ were significantly related to a later AOO at P_T = 0.01 (β = 0.04, 95%CI = 0.01 - 0.06, P = 0.008), P_T = 0.05 (β = 0.04, 95%CI = 0.01 - 0.07, P = 0.02), P_T = 0.5 (β = 0.04, 95%CI = 0.001 - 0.08, P = 0.04), P_T = 1 (β = 0.04, 95%CI = 0.002 - 0.08, P = 0.04).

In analyses which only retained data of participants of European ancestry, only PGS for ASD and ADHD were significantly related to AOO. PRS for ASD were significantly related to an earlier AOO (at P_T = 0.5 and 1, both β = -0.03, 95%CI = -0.05 - 0.002, P = 0.03). PGS for ADHD were also related to an earlier AOO (at P_T = 0.1, β = -0.03, 95%CI = -0.05 - 0.001, P = 0.04; P_T = 0.5, β = -0.03, 95%CI = -0.05 - 0.08, P = 0.03; and P_T = 1, β = -0.03, 95%CI = -0.06 - 0.004, P = 0.02).

Finally, in log-transformed results on AOO in participants of European ancestry only, PGS for MDD was significantly related to an earlier AOO at two thresholds (P_T = 0.1, β = -0.03, 95%CI = -0.06 - 0.003, P = 0.03 and P_T = 1, β = -0.03, 95%CI = -0.06 - 0.001, P = 0.04). PGS for SCZ was significantly related to a later AOO at a single threshold (P_T = 0.01, β = 0.03, 95%CI = 0.001 - 0.06, P = 0.04). PGS for ADHD was significantly related to an earlier AOO at two thresholds (P_T = 0.5, β = -0.03, 95%CI = -0.06 - 0.002, P = 0.04 and P_T = 1, β = -0.03, 95%CI = -0.06 - 0.003, P = 0.03). All other associations between log-transformed AOO and PGS were not significant.

Given that multiple testing was implemented in the study, p-values were FDR-corrected (Benjamini and Hochberg, 1995). None of the associations between PGS and AOO from any sample remained significant after correction for multiple testing.

AOO was significantly related to at least one STRATA research site in every regression model (p-value range = 6.25 x 10^-9 - 0.048).
### Table 3.2 Multiple linear regressions for associations of polygenic scores for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with age of onset of psychosis (in years)

#### BPD-PGS (n = 983)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>B</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>0.12</td>
<td>0.08</td>
<td>0.01</td>
<td>-0.003-0.03</td>
<td>0.0004</td>
<td>0.12</td>
<td>0.47</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.04</td>
<td>0.08</td>
<td>0.004</td>
<td>-0.01-0.02</td>
<td>0.001</td>
<td>0.63</td>
<td>0.82</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.05</td>
<td>0.08</td>
<td>0.01</td>
<td>-0.01-0.02</td>
<td>0.001</td>
<td>0.58</td>
<td>0.80</td>
</tr>
<tr>
<td>0.01</td>
<td>0.10</td>
<td>0.10</td>
<td>0.01</td>
<td>-0.01-0.03</td>
<td>0.001</td>
<td>0.34</td>
<td>0.76</td>
</tr>
<tr>
<td>0.05</td>
<td>0.09</td>
<td>0.14</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0.001</td>
<td>0.54</td>
<td>0.80</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.06</td>
<td>0.20</td>
<td>-0.01</td>
<td>-0.05-0.04</td>
<td>0.001</td>
<td>0.76</td>
<td>0.91</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.17</td>
<td>0.23</td>
<td>-0.02</td>
<td>-0.07-0.03</td>
<td>0.001</td>
<td>0.47</td>
<td>0.79</td>
</tr>
<tr>
<td>1</td>
<td>0.19</td>
<td>0.27</td>
<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.001</td>
<td>0.47</td>
<td>0.79</td>
</tr>
<tr>
<td>1</td>
<td>0.17</td>
<td>0.27</td>
<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.93</td>
<td>0.54</td>
<td>0.80</td>
</tr>
</tbody>
</table>

#### MDD-PGS (n = 1103)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>B</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.05</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.10</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.28</td>
<td>0.74</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.19</td>
<td>0.09</td>
<td>-0.02</td>
<td>-0.04-0.002</td>
<td>0.0004</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.02</td>
<td>0.09</td>
<td>-0.002</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.87</td>
<td>0.94</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.06</td>
<td>0.10</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.56</td>
<td>0.80</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.14</td>
<td>0.10</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.15</td>
<td>0.52</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.24</td>
<td>0.11</td>
<td>-0.03</td>
<td>-0.05-0.003</td>
<td>0.0004</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.22</td>
<td>0.10</td>
<td>-0.02</td>
<td>-0.04-0.002</td>
<td>0.0004</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>-0.23</td>
<td>0.10</td>
<td>-0.03</td>
<td>-0.05-0.004</td>
<td>0.0003</td>
<td>0.02</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATAG research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.3 Multiple linear regressions for associations of polygenic scores for schizophrenia (SCZ-PGS) with age of onset of psychosis (in years)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>( \beta )</th>
<th>95%CI</th>
<th>( R^2 )</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.10</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.0001</td>
<td>0.37</td>
<td>0.76</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.18</td>
<td>0.12</td>
<td>-0.02</td>
<td>-0.05-0.01</td>
<td>0.0002</td>
<td>0.14</td>
<td>0.50</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.14</td>
<td>0.11</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.0001</td>
<td>0.20</td>
<td>0.62</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.01</td>
<td>0.10</td>
<td>-0.001</td>
<td>-0.02-0.02</td>
<td>0</td>
<td>0.91</td>
<td>0.96</td>
</tr>
<tr>
<td>0.05</td>
<td>0.20</td>
<td>0.11</td>
<td>0.02</td>
<td>-0.001-0.05</td>
<td>0.0003</td>
<td>0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>0.1</td>
<td>0.23</td>
<td>0.13</td>
<td>0.03</td>
<td>-0.003-0.05</td>
<td>0.0003</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>0.5</td>
<td>0.25</td>
<td>0.17</td>
<td>0.03</td>
<td>-0.01-0.06</td>
<td>0.0002</td>
<td>0.14</td>
<td>0.50</td>
</tr>
<tr>
<td>1</td>
<td>0.27</td>
<td>0.17</td>
<td>0.03</td>
<td>-0.01-0.07</td>
<td>0.0002</td>
<td>0.11</td>
<td>0.46</td>
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</tbody>
</table>

Note: analyses included covariates of gender, age, age\(^2\), 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; \( \beta \), standardised beta; CI, confidence intervals; \( R^2 \), percentage of variance explained, computed by comparison of \( R^2 \) for a full model (PGS and covariates) to \( R^2 \) of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.4 Multiple linear regressions for associations of polygenic risk scores for attention deficit hyperactivity disorder (ADHD-PGS) and autism spectrum disorder (ASD-PGS) with age of onset of psychosis (in years)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.05</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.59</td>
<td>0.80</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.01</td>
<td>0.09</td>
<td>-0.001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.92</td>
<td>0.96</td>
</tr>
<tr>
<td>0.001</td>
<td>0.08</td>
<td>0.09</td>
<td>0.01</td>
<td>-0.01-0.03</td>
<td>0.001</td>
<td>0.37</td>
<td>0.76</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.01</td>
<td>0.09</td>
<td>-0.001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.04</td>
<td>0.10</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.68</td>
<td>0.86</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.07</td>
<td>0.10</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.50</td>
<td>0.79</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.06</td>
<td>0.10</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.54</td>
<td>0.80</td>
</tr>
<tr>
<td>1</td>
<td>-0.08</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.37</td>
<td>0.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>0.002</td>
<td>0.09</td>
<td>0.0001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.05</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.57</td>
<td>0.80</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.06</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.46</td>
<td>0.79</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.10</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.26</td>
<td>0.71</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.08</td>
<td>0.09</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.38</td>
<td>0.76</td>
</tr>
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<td>0.1</td>
<td>-0.17</td>
<td>0.10</td>
<td>-0.02</td>
<td>-0.04-0.003</td>
<td>0.001</td>
<td>0.10</td>
<td>0.46</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.16</td>
<td>0.11</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.15</td>
<td>0.52</td>
</tr>
<tr>
<td>1</td>
<td>-0.23</td>
<td>0.12</td>
<td>-0.03</td>
<td>-0.05-0.001</td>
<td>0.001</td>
<td>0.06</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.5 Multiple linear regressions for associations of polygenic scores for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with age of onset of psychosis (in years), log-transformed.

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>0.01</td>
<td>0.003</td>
<td>0.02</td>
<td>-0.003-0.04</td>
<td>0.0004</td>
<td>0.11</td>
<td>0.46</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.002</td>
<td>0.003</td>
<td>0.01</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.65</td>
<td>0.84</td>
</tr>
<tr>
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<td>0.002</td>
<td>0.003</td>
<td>0.01</td>
<td>-0.01-0.03</td>
<td>0.001</td>
<td>0.58</td>
<td>0.80</td>
</tr>
<tr>
<td>0.001</td>
<td>0.01</td>
<td>0.004</td>
<td>0.02</td>
<td>-0.01-0.04</td>
<td>0.001</td>
<td>0.24</td>
<td>0.69</td>
</tr>
<tr>
<td>0.01</td>
<td>0.003</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0.001</td>
<td>0.55</td>
<td>0.80</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.002</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.05-0.04</td>
<td>0.001</td>
<td>0.79</td>
<td>0.91</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.08-0.03</td>
<td>0.001</td>
<td>0.45</td>
<td>0.79</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.05-0.09</td>
<td>0.001</td>
<td>0.55</td>
<td>0.80</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.04-0.09</td>
<td>0.001</td>
<td>0.48</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.004</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.01</td>
<td>0.003</td>
<td>-0.02</td>
<td>-0.04-0.003</td>
<td>0.001</td>
<td>0.09</td>
<td>0.44</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.01</td>
<td>0.004</td>
<td>-0.03</td>
<td>-0.05-0.01</td>
<td>0.001</td>
<td>0.008</td>
<td>0.33</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.003</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.45</td>
<td>0.79</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.01</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.39</td>
<td>0.76</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.01</td>
<td>0.004</td>
<td>-0.03</td>
<td>-0.05-0.002</td>
<td>0.001</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.01</td>
<td>0.004</td>
<td>-0.03</td>
<td>-0.06-0.01</td>
<td>0.0001</td>
<td>0.009</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>-0.01</td>
<td>0.004</td>
<td>-0.03</td>
<td>-0.06-0.01</td>
<td>0</td>
<td>0.007</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
## Table 3.6 Multiple linear regressions for associations of polygenic scores for schizophrenia (SCZ-PGS) with age of onset of psychosis (in years), log-transformed

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>β</th>
<th>95% CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.003</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0</td>
<td>0.53</td>
<td>0.80</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.05-0.01</td>
<td>0.0001</td>
<td>0.21</td>
<td>0.63</td>
</tr>
<tr>
<td>0.0001</td>
<td>-0.004</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.0001</td>
<td>0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>0.001</td>
<td>0.004</td>
<td>0.004</td>
<td>0.01</td>
<td>-0.01-0.04</td>
<td>0.0001</td>
<td>0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.004</td>
<td>0.04</td>
<td>0.01-0.06</td>
<td>0.001</td>
<td>0.008</td>
<td>0.33</td>
</tr>
<tr>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01-0.07</td>
<td>0.001</td>
<td>0.02</td>
<td>0.33</td>
</tr>
<tr>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>-0.002-0.07</td>
<td>0.0003</td>
<td>0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.001-0.08</td>
<td>0.0004</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.002-0.08</td>
<td>0.0004</td>
<td>0.04</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age^2, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.7 Multiple linear regressions for associations of polygenic scores for attention deficit hyperactivity disorder (ADHD-PGS) and autism spectrum disorder (ASD-PGS) with age of onset of psychosis (in years), log-transformed

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.001</td>
<td>0.004</td>
<td>-0.004</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.72</td>
<td>0.88</td>
</tr>
<tr>
<td>0.0001</td>
<td>-0.001</td>
<td>0.003</td>
<td>-0.003</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>0.001</td>
<td>0.001</td>
<td>0.004</td>
<td>0.001</td>
<td>-0.02-0.02</td>
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<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td>0.01</td>
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<td>0.004</td>
<td>-0.004</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.73</td>
<td>0.88</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.001</td>
<td>0.004</td>
<td>-0.003</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.81</td>
<td>0.93</td>
</tr>
<tr>
<td>0.1</td>
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<td>0.004</td>
<td>-0.002</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.77</td>
<td>0.91</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.002</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.59</td>
<td>0.80</td>
</tr>
<tr>
<td>1</td>
<td>-0.002</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.01</td>
<td>0.001</td>
<td>0.51</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beta</th>
<th>SE</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>0.003</td>
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<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.86</td>
<td>0.93</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.004</td>
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<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.67</td>
<td>0.85</td>
</tr>
<tr>
<td>0.001</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.59</td>
<td>0.80</td>
</tr>
<tr>
<td>0.01</td>
<td>0.004</td>
<td>-0.003</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.55</td>
<td>0.80</td>
</tr>
<tr>
<td>0.05</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.76</td>
<td>0.91</td>
</tr>
<tr>
<td>0.1</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.53</td>
<td>0.80</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.36</td>
<td>0.76</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.34</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
### Table 3.8 Multiple linear regressions for associations of polygenic scores for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with age of onset of psychosis (in years). Samples of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>( \beta )</th>
<th>95%CI</th>
<th>( R^2 )</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>0.08</td>
<td>0.10</td>
<td>0.01</td>
<td>-0.01-0.03</td>
<td>0.001</td>
<td>0.44</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.05</td>
<td>0.11</td>
<td>0.01</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.63</td>
<td>0.82</td>
</tr>
<tr>
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<td>0.01</td>
<td>0.11</td>
<td>0.001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.90</td>
<td>0.95</td>
</tr>
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<td>-0.00001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>0.05</td>
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<td>0.19</td>
<td>-0.001</td>
<td>-0.02-0.02</td>
<td>0.001</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.18</td>
<td>0.27</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.51</td>
<td>0.80</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.29</td>
<td>0.31</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>1</td>
<td>0.08</td>
<td>0.39</td>
<td>0.003</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.83</td>
<td>0.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beta</th>
<th>SE</th>
<th>( \beta )</th>
<th>95%CI</th>
<th>( R^2 )</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>-0.03</td>
<td>0.13</td>
<td>-0.003</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.84</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.09</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.45</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.23</td>
<td>0.12</td>
<td>-0.03</td>
<td>-0.05-0.001</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>0.004</td>
<td>-0.04</td>
<td>0.12</td>
<td>-0.0001</td>
<td>-0.03-0.03</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>0.09</td>
<td>-0.18</td>
<td>0.13</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.17</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.26</td>
<td>0.14</td>
<td>-0.02</td>
<td>-0.05-0.002</td>
<td>0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>0.21</td>
<td>-0.22</td>
<td>0.13</td>
<td>-0.02</td>
<td>-0.05-0.003</td>
<td>0.001</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age\(^2\), 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; \( \beta \), standardised beta; CI, confidence intervals; \( R^2 \), percentage of variance explained, computed by comparison of \( R^2 \) for a full model (PGS and covariates) to \( R^2 \) of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.9 Multiple linear regressions for associations of polygenic scores for schizophrenia (SCZ-PGS) with age of onset of psychosis (in years). Samples of European ancestry only

<table>
<thead>
<tr>
<th>SCZ-PGS ($n = 684$)</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGS P threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000001</td>
<td>-0.08</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0</td>
<td>0.49</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.17</td>
<td>0.12</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.0003</td>
<td>0.16</td>
<td>0.53</td>
</tr>
<tr>
<td>0.0001</td>
<td>-0.12</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.0001</td>
<td>0.32</td>
<td>0.76</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.17</td>
<td>0.12</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
<td>0.0003</td>
<td>0.16</td>
<td>0.53</td>
</tr>
<tr>
<td>0.01</td>
<td>0.17</td>
<td>0.12</td>
<td>0.02</td>
<td>-0.01-0.04</td>
<td>0.0003</td>
<td>0.17</td>
<td>0.55</td>
</tr>
<tr>
<td>0.05</td>
<td>0.07</td>
<td>0.12</td>
<td>0.01</td>
<td>-0.02-0.03</td>
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<td>0.58</td>
<td>0.80</td>
</tr>
<tr>
<td>0.1</td>
<td>0.05</td>
<td>0.12</td>
<td>0.01</td>
<td>-0.02-0.03</td>
<td>0</td>
<td>0.67</td>
<td>0.85</td>
</tr>
<tr>
<td>0.5</td>
<td>0.09</td>
<td>0.13</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0</td>
<td>0.49</td>
<td>0.79</td>
</tr>
<tr>
<td>1</td>
<td>0.10</td>
<td>0.13</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0.0001</td>
<td>0.44</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
### Table 3.10 Multiple linear regressions for associations of polygenic scores for autism spectrum disorder (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with age of onset of psychosis (in years). Samples of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>B</th>
<th>95% CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>0.03</td>
<td>0.11</td>
<td>0.004</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.78</td>
<td>0.91</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.05</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.69</td>
<td>0.86</td>
</tr>
<tr>
<td>0.0001</td>
<td>-0.11</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.32</td>
<td>0.76</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.18</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.05-0.01</td>
<td>0.001</td>
<td>0.13</td>
<td>0.49</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.25</td>
<td>0.14</td>
<td>-0.02</td>
<td>-0.05-0.001</td>
<td>0.001</td>
<td>0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.25</td>
<td>0.14</td>
<td>-0.02</td>
<td>-0.05-0.002</td>
<td>0.001</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.35</td>
<td>0.16</td>
<td>-0.03</td>
<td>-0.05-0.002</td>
<td>0.001</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>-0.35</td>
<td>0.16</td>
<td>-0.03</td>
<td>-0.05-0.002</td>
<td>0.001</td>
<td>0.03</td>
<td>0.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>B</th>
<th>95% CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>-0.13</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.04-0.01</td>
<td>0.001</td>
<td>0.27</td>
<td>0.73</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.06</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.08</td>
<td>0.12</td>
<td>0.01</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.50</td>
<td>0.79</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.09</td>
<td>0.11</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.41</td>
<td>0.77</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.05</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.71</td>
<td>0.88</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.23</td>
<td>0.13</td>
<td>-0.02</td>
<td>-0.05-0.001</td>
<td>0.001</td>
<td>0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>0.5</td>
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<td>0.13</td>
<td>-0.03</td>
<td>-0.05-0.001</td>
<td>0.001</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>1</td>
<td>-0.27</td>
<td>0.12</td>
<td>-0.03</td>
<td>-0.06-0.004</td>
<td>0.0004</td>
<td>0.03</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; B, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.11 Multiple linear regressions for associations of polygenic scores for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with age of onset of psychosis (in years), log-transformed. Samples of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>0.003</td>
<td>0.004</td>
<td>0.01</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.49</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.004</td>
<td>0.004</td>
<td>0.001</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>0.001</td>
<td>0.001</td>
<td>0.004</td>
<td>0.003</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.82</td>
<td>0.93</td>
</tr>
<tr>
<td>0.01</td>
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<td>0.01</td>
<td>-0.002</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.85</td>
<td>0.93</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.38</td>
<td>0.76</td>
</tr>
<tr>
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<td>0.01</td>
<td>-0.02</td>
<td>-0.04-0.01</td>
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<td>0.22</td>
<td>0.64</td>
</tr>
<tr>
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<td>0.02</td>
<td>-0.004</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.79</td>
<td>0.91</td>
</tr>
<tr>
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<td>0.02</td>
<td>-0.003</td>
<td>-0.03-0.03</td>
<td>0.001</td>
<td>0.85</td>
<td>0.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.002</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.03-0.02</td>
<td>0.001</td>
<td>0.73</td>
<td>0.88</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.004</td>
<td>0.004</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.001</td>
<td>0.39</td>
<td>0.76</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.03</td>
<td>-0.06-0.001</td>
<td>0.001</td>
<td>0.05</td>
<td>0.35</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.001</td>
<td>0.01</td>
<td>-0.003</td>
<td>-0.03-0.03</td>
<td>0.001</td>
<td>0.84</td>
<td>0.93</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.05-0.01</td>
<td>0.001</td>
<td>0.13</td>
<td>0.49</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.03</td>
<td>-0.06-0.003</td>
<td>0.001</td>
<td>0.03</td>
<td>0.33</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.03</td>
<td>-0.06-0.001</td>
<td>0.001</td>
<td>0.06</td>
<td>0.35</td>
</tr>
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<td>0.01</td>
<td>-0.03</td>
<td>-0.06-0.001</td>
<td>0.001</td>
<td>0.04</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
### Table 3.12: Multiple linear regressions for associations of polygenic scores for schizophrenia (SCZ-PGS) with age of onset of psychosis (in years), log-transformed. Samples of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>( \beta )</th>
<th>95%CI</th>
<th>( R^2 )</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.004</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.0001</td>
<td>0.44</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.05-0.01</td>
<td>0.0004</td>
<td>0.12</td>
<td>0.47</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.004</td>
<td>0.01</td>
<td>-0.01</td>
<td>-0.04-0.02</td>
<td>0.0001</td>
<td>0.38</td>
<td>0.76</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.001-0.06</td>
<td>0.001</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>0.05</td>
<td>0.004</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0.0001</td>
<td>0.42</td>
<td>0.78</td>
</tr>
<tr>
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<td>0.003</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.02-0.04</td>
<td>0</td>
<td>0.56</td>
<td>0.80</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.02-0.05</td>
<td>0.0002</td>
<td>0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.02-0.06</td>
<td>0.0002</td>
<td>0.33</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age\(^2\), 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; \( \beta \), standardised beta; CI, confidence intervals; \( R^2 \), percentage of variance explained, computed by comparison of \( R^2 \) for a full model (PGS and covariates) to \( R^2 \) of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 3.13 Multiple linear regressions for associations of polygenic scores for autism spectrum disorder (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with age of onset of psychosis (in years), log-transformed. Samples of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>0.002</td>
<td>0.004</td>
<td>0.01</td>
<td>-0.02-0.03</td>
<td>0.001</td>
<td>0.72</td>
<td>0.88</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.001</td>
<td>0.004</td>
<td>-0.003</td>
<td>-0.03-0.03</td>
<td>0.001</td>
<td>0.83</td>
<td>0.93</td>
</tr>
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Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
3.4 Discussion

3.4.1 Polygenic scores for major psychiatric disorders in prediction of age of onset in psychosis

I used an international sample of first-episode psychosis patients from different European countries to explore if age of onset (AOO) of psychosis is predicted by polygenic scores, proxies for genetic propensity towards major psychiatric disorders (schizophrenia, bipolar disorder, major depressive disorder, attention deficit hyperactivity disorder, and autism spectrum disorder). Most previous studies on associations between schizophrenia/bipolar disorder AOO and polygenic scores reported no statistically significant relationships (Aminoff et al., 2015; Kalman et al., 2019; Stepniak et al., 2014), with the exception of two studies reporting relationships between childhood-onset schizophrenia and PGS for ASD (Ahn et al., 2016) and early-onset bipolar disorder and PGS for ADHD (Grigoroiu-Serbanescu et al., 2020). Thus, I hypothesised no statistically significant relationships between PGS and AOO of a broad psychosis spectrum. I found no significant results for the majority of associations, with the exception of earlier AOO related to PGS for depression and later AOO related to PGS for schizophrenia in samples of participants of any ancestry and in samples of European ancestry, but only where AOO had been log-transformed. Results on PGS for ASD and ADHD were not consistent in the European ancestry-only sample between analyses which used original AOO and AOO log-transformed.

Findings that earlier AOO is related to PGS for depression (PGS-MDD) is potentially consistent with longitudinal studies of comorbidity of depressive symptoms and early-onset schizophrenia (before the age of 14). PGS-MDD is proxy for genes which are likely related to depressive symptoms, and depressive symptoms are present over follow-up in between one-third and over 60% of patients with early-onset schizophrenia (Liangrong et al., 2021; Remschmidt et al., 2007). Also, in patients diagnosed with adolescent-onset schizophrenia (before the age of 16), depressed mood was a strong predictor of self-perceived low quality of life at 25 (explaining 49% of the total variance; Jarbin and Hansson, 2004). Given that they perceive depressive symptoms as impactful, perhaps patients have a clear and relatively accurate recollection of AOO of comorbid disorders. In line with this interpretation, Wittchen et al. (1989) found that, when self-reports from patients with schizophrenia were compared with clinician ratings of age at first occurrence of depressive symptoms, correspondence between the two sources was high (correlation coefficient of .74). Also, it might be that, out of comorbid symptoms, onset of depressive symptoms is more readily noticed and remembered correctly than onset of psychotic symptoms. For instance, it is
difficult to distinguish between hallucinations and delusions and children’s imaginative play (Taylor, 1998).

A finding that later AOO is related to PGS for schizophrenia (SCZ-PGS) is not consistent with previous research which reported no associations between AOO of schizophrenia or bipolar disorder and SCZ-PGS (Kalman et al., 2019; Stepniak et al., 2014). Potentially, a relationship between later AOO and SCZ-PGS could be explained in terms of onset of psychotic disorders being generally very rare in childhood or early adolescence and most frequently occurring later in life, between the ages of 20 and 29 (Miettunen et al., 2019) or, in 20% of cases, after the age of 40 (Howard et al., 2000; Jeste and Maglione, 2013). However, a caveat on both MDD-PGS and SCZ-PGS findings is that none of the associations between PGS and AOO remained significant after applying the FDR correction for multiple testing.

Different explanations have been proposed for this pattern of largely non-significant results on common genetic variants and AOO. Explanations fall broadly into two categories: 1) PGS may not capture genetic information relevant to AOO specifically, and 2) environmental factors may influence risk of AOO more than common genetic factors (Stepniak et al., 2014). Of genetic explanations for no link between PGS and AOO, one interpretation was that PGS may not fully reflect how heterogenous the schizophrenia spectrum is (and this study focused on an even more heterogenous psychosis spectrum). This is because PGS are based on GWAS data which only address the case/control status in psychiatric disorder, rather than genetics underlying heterogeneity (Stepniak et al., 2014). Another interpretation for why PGS currently do not predict AOO was that potentially SNPs aggregated in the PGS do not overlap with genetic variants which increase risk for particular psychosis AOO. For instance, rates of rare genetic variants, CNVs, are higher in patients with childhood-onset schizophrenia than in patients with adult-onset schizophrenia patients or in controls (Ahn et al., 2014; Sagar et al., 2013; Vorstman et al., 2006). Also, PGS do not model pleiotropy (that a gene can influence predisposition for multiple phenotypes). Yet, recent research found a genetic correlation between earlier AOO and more severe negative symptoms in schizophrenia, suggestive of pleiotropy (Musket et al., 2020).

Another type of explanations argues that environmental factors contribute to AOO risk more than common genetic factors. Stepniak et al. (2014) reported no association between SCZ-PGS and SCZ AOO. Instead, researchers found that accumulation of environmental risk factors (perinatal brain insults, cannabis use, neurological trauma, psychological trauma, urbanicity, and migration), especially cannabis use, were highly significantly related to an earlier age of prodrome. In my study, I found significant associations between AOO of psychosis and STRATA-G research sites. Many of the STRATA sites are urban
areas with migrant communities (e.g., London, Bologna or Paris). Nonetheless, research site is the only environmental factor which I have included in my models. Based on my current analyses, it is impossible to clearly establish what specific environmental risk factors underlie the association between sites and AOO in my study. It is especially complex as AOO has been linked to multiple environmental factors; beyond factors addressed by Stepniak et al. (2014), associations have also been reported, e.g., between earlier AOO in psychosis and use of multiple drugs other than cannabis, including alcohol (Large et al., 2011) and stimulants (Rubio-Abadal et al., 2015). However, I have not tested/accounted for them specifically in my analyses.

Taken together, my findings suggest that, currently, multiple psychiatric PGS may not strongly improve prediction of AOO in psychosis. Thus, PGS cannot be used as extra information to time clinical staging better (Murray et al., 2021a), i.e., pinpointing the earliest treatable stage of psychosis to provide treatment most appropriate for that particular disorder stage (Agius et al., 2010). Still, my findings also point to the importance of environmental factors in AOO. Of those risk factors, not all can be straightforwardly targeted with preventive efforts. For instance, urbanicity is a well-established proxy for a psychosis cause, but it is not clear what specific underlying cause urbanicity actually represents. Consequently, it is not clear if urban planning would help ameliorate psychosis (Murray et al., 2021b). Yet, other psychotogenic factors, such as cannabis use, could be targeted with a clear public message about increased health risks of exposure (Murray and Hall, 2020).

### 3.4.2 Methodological considerations and future research

Data in this study come from FEP patients of diverse ethnicities from research sites around Europe, brought together by the STRATA Consortium. This sample composition means that the STRATA sample potentially represents clinical populations better than a sample from a single catchment area/country would. Additionally, the fact that the samples come from FEP patients potentially shortens the time between symptom onset and recall, which could limit inaccurate recall. Also, in several samples patients have had their records of AOO cross-validated with family interviews and health records. The final strength of the STRATA sample is that it includes genetic data from participants of European, African, and Asian ancestries, while commonly researched samples covering only European ancestry do not reflect large global genetic diversity (Peterson et al., 2019).

However, a limitation of my study is that the majority of the sample was still of European descent. Also, all PGS except one (for ADHD) were based on GWAS data from European ancestry samples. Currently, PGS based on European ancestry data
are not highly accurate in prediction in populations of non-European ancestry (Martin et al., 2019b). This suggests that PGS may not detect associations between common genetic variants and AOO, or at least not in samples of non-European ancestry. The second limitation of the PGS analysis is the potential issue of power of original GWAS, used as basis of PGS calculation. GWAS based on large samples have more power to detect effect sizes of SNPs with more precision, which then improves predictive power of PGS (Chatterjee et al., 2013). Thus, current PGS are potentially underpowered and their predictive performance will improve as GWAS sample sizes continue to grow. The third limitation of the PGS analysis, as discussed above, is that PGS does not capture impact of rare genetic variants or genetic interactions, which likely impact AOO. The fourth limitation of the PGS analysis is the issue of multiple testing. To determine what parameters would generate the most predictive PGS (PGS explaining the largest proportion of variance in AOO), multiple PGS including SNPs from different GWAS p-value thresholds needed to be tested. Multiple testing leads to possible inflation of false positive results and power of individual tests to detect statistically significant results decreases drastically, especially after implementing additional statistical control for type I errors (Verhoeven et al., 2005). Using polygenic scoring methods which may require less parameter tuning, e.g., PRS-CS-auto (Ge et al., 2019), would potentially limit the eventual number of tests required (see Section 6.3.3.2., subsection No optimal methodology for PGS calculation for a more detailed discussion of different methods of polygenic scoring).

My findings suggest potential impact of environmental factors on AOO, but, as I did not test multiple, specific environmental factors, it is not clear which factors drive the association between AOO and the STRATA research site. Any interpretations must remain purely speculative. Still, the fact that this finding was the only statistically significant one after multiple testing also suggests that my sample may not be sufficiently powered to detect potential associations. In contrast, Kalman et al. (2021) found associations between PGS for ASD, SCZ, MDD, and educational attainment (years of education) and earlier AOO in bipolar disorder in a sample of 12 977 participants. These findings suggest that sample size of my study may not be sufficiently large to validate any strong conclusions on predictors of AOO.

The final considerations are related to how to measure AOO most reliably. A limitation of the current study is that AOO was measured with different diagnostic tools and based on different definitions of onset (e.g., first occurrence of any psychosis symptom or first occurrence of any positive symptom specifically). Additionally, patients have diagnoses of different psychotic disorders which makes it very likely that patients have to an extent overlapping, but also unique symptom combinations. Consequently, AOO in the STRATA-G sample is likely a very heterogenous phenotype which would explain the null findings.
Accuracy of patient recall may also be an issue, especially if onset of specific symptoms needs to be recalled (e.g., positive symptoms only). Psychiatric disorders may also commence with non-specific symptoms which may be forgotten or incorrectly attributed to a start of illness (Jones, 2013). Additionally, severe symptoms (e.g., cognitive symptoms impacting memory) may make exact recall difficult. However, test-retest reliability for AOO in psychotic disorders, when patient and clinician ratings are compared, has high concordance (over .70) for depressive symptoms and an even higher concordance for psychotic symptoms (Wittchen et al., 1989). Still, the concordance is much lower for individuals who are severely ill at follow-up (Wittchen et al., 1989). These findings suggest that, while measurement of AOO in psychosis may be less impacted by inaccurate recall than generally assumed, future research on AOO should explicitly account for severity of symptoms when assessing AOO. I did not do so in the current study because, out of 1103 participants in total, measures of symptom severity were only available for 267 participants (Positive and Negative Syndrome Scale scores; Kay et al., 1987) and 71 participants (Clinical Global Impression scales ratings; Guy, 1976), measures related to cognitive performance were only available for 169 participants (Wechsler Adult Intelligence Scale; Wechsler, 2008) and 87 participants (National Adult Reading Test; Blair and Spreen, 1989), and a proxy of cognition, years in education, was available for 384 participants (all the measures were available for less than 35% of the total sample). I also do not have detailed information on patient comorbid disorders or symptoms beyond psychosis for the whole STRATA-G sample. This information could potentially elucidate whether links between MDD-PGS and earlier AOO are related to comorbid psychotic (non-affective) and depressive symptoms.

Future research should ensure that data come from well-powered, long-term prospective studies where the time lag between disorder onset and information collection is as brief as possible, where there is detailed information on symptom severity, comorbid disorders, and potential environmental factors impacting psychosis onset, and where data can be cross-validated by multiple clinicians, patient informants, etc.

3.5 Conclusion

My findings suggest that predisposition towards major psychiatric disorders (schizophrenia, major depressive disorder, bipolar disorder, autism spectrum disorder, and attention deficit hyperactivity disorder) due to common genetic variants is not related to age of psychosis onset in an ancestrally diverse sample. Future research should further address potential joint contributions of common and rare genetic factors and environmental factors to age of onset.
Chapter 4

Polygenic scores for psychiatric disorders do not predict duration of untreated psychosis

4.1 Introduction

Duration of untreated psychosis (DUP) describes a period between the onset of psychosis and exposure to treatment. Exposure to treatment has been defined differently across studies, including: hospitalisation, start of medication or response to medication over different time frames (from 3 to 12 weeks), entrance to a specialised therapeutic program, or combination of engagement in a specialised programme and adherence to medication (Golay et al., 2016). A longer DUP has been relatively moderately, but consistently, correlated with poorer psychosis outcome (Penttilä et al., 2014). Thus, reducing DUP is a psychiatric intervention target (Murru and Carpiniello, 2018).

DUP is a multidimensional construct (Murru and Carpiniello, 2018). It has been linked to social factors, such as effectiveness of detection and access to health services (Bak et al., 2003), but it was also postulated to be biological, more specifically, neurobiologically toxic. The neurotoxicity hypothesis (Sheitman and Lieberman, 1998; Wyatt, 1991) postulates that untreated psychosis may cause brain damage (e.g., changes in brain structure or neuronal connectivity). However, evidence for the neurotoxicity hypothesis is inconsistent, potentially due to methodological limitations (small sample sizes and DUP/follow-up periods not long enough to detect a potential toxic effect of active psychosis; Anderson et al., 2014; Bora et al., 2018; McGlashan, 2006; Rund, 2014).
The broader biology underlying DUP remains to be clarified. While schizophrenia and bipolar disorder are known to have a strong heritability component of 60%-80% (Hilker et al., 2018; Johansson et al., 2019), relatively little is known about genetics of DUP specifically. Results on the association between the DUP length and family history of psychosis are contradictory (Chen et al., 2005; Esterberg and Compton, 2012; Norman et al., 2007). Genetic studies addressed individuals at risk of developing psychosis and implicated that transition to psychosis or bipolar disorder is linked to variation in genes including COMT (McIntosh et al., 2007), NRG1 (Bousman et al., 2013; Hall et al., 2006; Keri et al., 2009), DAOA (Bousman et al., 2013; Mössner et al., 2010), IL1B (Bousman et al., 2018), and DGKH (Whalley et al., 2012a). However, these primarily candidate gene findings have not been widely replicated. This is likely because common genetic variants conferring psychosis risk are numerous, of very small individual effect size, and diffusely distributed across the genome. Detection of risk-conferring genetic variants requires large samples, with genomes explored systematically, with genome-wide association studies (GWAS; Duncan et al., 2019).

GWAS provide weightings for how much common genetic variants contribute to disorder, and this information can be combined into a sum called a polygenic score (PGS; Lewis and Vassos, 2020). PGS have been applied to first-episode psychosis (FEP), which operationally corresponds to the first diagnosis of psychosis (Millan et al., 2016). PGS for bipolar disorder have been correlated to brain structures and function in individuals at risk (Whalley et al., 2012b, 2013). PGS for schizophrenia were applied to FEP and prediction of: case-control status (Vassos et al., 2017); progression to schizophrenia rather than other psychoses (Vassos et al., 2017); first-episode symptom dimensions (Sengupta et al., 2017), including in antipsychotic-naive patients pre-treatment and at nine weeks after risperidone treatment (Santoro et al., 2018); and response to antipsychotics (Santoro et al., 2018; Zhang et al., 2019).

However, limited research applied GWAS information to DUP specifically. Recently, Yin et al. (2019b) divided schizophrenia into three distinct subgroups, based on clusters of differences in GWAS-predicted gene expression. The three subgroups differed, among other features, on DUP. Subgroup 1 had a significantly shorter period of untreated psychosis, a higher proportion of positive family history of mental illness, better treatment response and more favourable course of disease than other subgroups. The key gene pathways enriched for subgroup 1 were related to antigen processing and presentation, generation of second messenger molecules, autoimmune thyroid disease, and pyrimidine metabolism. Ajnakina et al. (2021) were the first to apply PGS to predict DUP in first episode psychosis patients from the multicentre European study Gene-Environment Interactions (EU-GEI). Researchers found no significant association between DUP and increases in PGS for schizophrenia, bipolar disorder, major depressive disorder and intelligence quotient.
CHAPTER 4. POLYGENIC RISK & DURATION OF UNTREATED PSYCHOSIS

Given that limited research explored genetics of DUP, the aim of this study was to test whether DUP can be predicted by PGS for other psychiatric conditions. These PGS may act as proxies of different genetic contributions to psychosis. In light of previous research, which identified genetic overlaps between psychosis and major psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013, 2019), I explored if PGS for schizophrenia (SCZ), bipolar disorder (BPD), major depressive disorder (MDD), autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) would predict DUP. DUP was defined as time between onset of psychosis symptoms and either contact with health services for psychosis or start of antipsychotic medication. As very few studies exist on contributions of multiple genetic variants to DUP and studies on genetics of the starting point of DUP, age of psychosis onset, provided inconsistent results (see Chapter 3), I hypothesised that PGS for major psychiatric disorders would not predict DUP.

4.2 Methods

4.2.1 Sample

Data in this study come from an international consortium The Schizophrenia: Treatment Resistance and Therapeutic Advances (STRATA) Consortium, specifically STRATA Genetics (STRATA-G). This theme of the Consortium focuses on uncovering genetic predictors to identify treatment resistance early in the course of psychosis. Consequently, STRATA-G data come from prospective, observational studies of first episode of psychosis. STRATA-G data in this study come from ÆSOP (London), NIFEPS and RGPI (Belfast), First-Episode Schizophrenia Follow-Up Project (Istanbul), TIPP (Lausanne), TOP (Oslo), EUGEI (Paris), PAFIP (Santander), The West London Longitudinal First-Episode Psychosis Study (London), and The Early Stages of Schizophrenia Study (Prague). The exception are analyses using bipolar disorder PGS. These analyses do not include data from TOP, University of Oslo, as these data are part of the bipolar disorder GWAS, used as basis of PGS calculation (compare with Section Polygenic score analyses below or Section 2.2.7. Inclusion and exclusion of STRATA-G data in polygenic score analyses, Chapter 2). For details on each individual sample (catchment areas, diagnostic instruments, inclusion and exclusion criteria used in each original study at its research site), see Chapter 2, Section 2.2.4. After data from separate cohorts have been combined into the STRATA-G dataset, individual data were included if they had complete data on age, gender, research site, DUP, and non-missing genetic data after the process of quality control (see Chapter 2, Section 2.2.6.2 Quality control and imputation). Ethical approval was granted by Research
4.2.2 Study variables

4.2.2.1 Duration of untreated psychosis

Duration of untreated psychosis (DUP) was defined as a period from psychosis symptom onset to either hospitalisation (ÆSOP, EUGEI, NIFEPS and RGPI, First-Episode Schizophrenia Follow-Up Project, The Early Stages of Schizophrenia Study) or start of antipsychotic medication (The West London Longitudinal First-Episode Psychosis Study, TIPP, TOP, PAFIP).

4.2.2.2 Covariates

Covariates were age, age squared, gender, 5 principal components (PCs), and STRATA research site. Age was patient age at baseline (this study only used information from baseline, but see Smart (2019), Section 7.3 Cohorts, for information on follow-up for each STRATA cohort). Age squared was calculated based on age at baseline, to control for potential non-linear effects of age on psychosis risk Radua et al. (2018); Schimmelmann et al. (2015). Gender was included to account for gender differences in schizophrenia AOO (van der Werf et al., 2014). PCs were included to account for genetic differences related to ancestry (see Section 4.2.3. for details on how PCs had been generated Price et al., 2006). Finally, STRATA research site was a site of the Consortium where the data originally came from, included to account for potential differences in measurement of DUP between research sites.

4.2.3 Genetic data

4.2.3.1 Quality control and data imputation

Genome-wide genotyping was performed either at the original research site or at Cardiff University School of Medicine. Genotyping arrays were Illumina BeadChips (Infinium CoreExome-24, Infinium HumanCoreExome-24, Infinium OmniExpress-24 v1.2, Infinium OmniExpress-12 v1_H, Infinium OmniExpressExome-8; Illumina Inc., San Diego, CA)
and the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc, Santa Clara, CA, USA; details on genotyping per individual cohort are provided in Chapter 2, Section 2.2.6.1. *Genotyping process*). Data went through quality control (QC). Then they were imputed and went through a second, post-imputation QC. QC largely followed steps from Anderson et al. (2010). Before imputation, samples were removed if their homozygosity rate (F) was between > 0.2 and < 0.8, or if genetic sex of the sample failed to match a participant’s reported gender. Single-nucleotide polymorphisms (SNPs) were excluded if: their call rate was <95%, if they were in regions in extended linkage disequilibrium (LD), as listed by Price et al. (2008); they were correlated \( r^2 > 0.2 \) within a sliding window; they were palindromic; and if they failed to fit the Hardy-Weinberg equilibrium (HWE) test cut-off \( P < 1 \times 10^{-10} \).

PCs were calculated with R package PC-AiR (Conomos et al., 2015). PCs were then used to estimate relatedness, using R package PC-Relate (Conomos et al., 2016). PC-Relate provided identity by descent (IBD) estimation values which were used to flag closely related individuals and duplicates in the STRATA-G sample. Individuals were tested in pairs and removed at random if IBD > 0.1 (indicating relatedness) or IBD > 0.9 (indicating duplicates).

After the basic QC, genetic data were imputed in the Michigan Imputation Server (Das et al., 2016) using the Haplotype Reference Consortium panel (McCarthy et al., 2016). Then, post-imputation QC was completed on all the data. Parameters for this QC were: minor allele frequency of 10%; probability threshold > 0.9 (probability that the genotype is imputed correctly); missingness < 5%; HWE p-value > \( 10^{-6} \); INFO > 0.8 (score assessing the quality of imputation; Coleman et al., 2016). Genotyping and QC are described in detail in Section 2.2.6. *Genotyping in STRATA*, Chapter 2.

### 4.2.3.2 Polygenic scoring

Genetic STRATA data were shared with the Psychiatric Genomics Consortium (PGC) Workgroups for schizophrenia and bipolar disorder. Any overlaps of samples between GWAS summary statistics and subsequent PGS based on these summary statistics inflate associations between PGS and traits of interest Wray et al. (2013). Thus, before PGS for schizophrenia were calculated, Dr Antonio Pardiñas removed STRATA-G samples from schizophrenia GWAS summary statistics \((n = 640)\), if they were duplicates or had a relatedness coefficient \( \hat{\pi} \) (“pi-hat”) > 0.2 with any STRATA-G sample, implemented in PLINK; Chang et al. (2015). Samples from TOP overlapped with the bipolar disorder GWAS summary statistics. Consequently, TOP samples were excluded from any analyses which implement PGS for bipolar disorder.
PGS were calculated under an additive model: SNPs, weighted by effect sizes from GWAS summary statistics for each respective disorder, were added up to form the PGS (see Section 1.5. *Polygenic scores, Chapter 1, for a detailed overview*). Summary statistics from the following studies were used as discovery sets for each respective PGS: for major depressive disorder PGS, statistics from a GWAS meta-analysis by Howard et al. (2019); for bipolar disorder PGS, statistics from GWAS by Stahl et al. (2019); for autism spectrum disorder PGS, statistics from a GWAS meta-analysis by Grove et al. (2019); for ADHD PGS, statistics from a GWAS meta-analysis by Demontis et al. (2019); and for schizophrenia PGS, statistics from GWAS by Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) adjusted not to overlap with STRATA. The total sample size of this adjusted GWAS was 20,325 cases with schizophrenia and 30,122 controls.

PGS for each phenotype was calculated at the following GWAS p-value thresholds for SNP inclusion (as per recommendations of the PGC): $10^{-6}$, $10^{-5}$, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.5, and 1. To remain consistent with the QC before PGS calculation, INFO score information was also included as part of PGS calculation, where it had been provided in the GWAS summary statistics. PGS for bipolar disorder, ADHD, and autism spectrum disorder were calculated based on GWAS summary statistics filtered to retain data with INFO score of 0.9 or higher. As Demontis et al. (2019) provided separate ADHD summary statistics for two of their original study samples (one sample with data from participants of different ancestries and one sample retaining data from participants of European ancestry only), two separate sets of PGS for ADHD were calculated (one based on data from all ancestries and one based European ancestry only, respectively). No covariates were included as part of any PGS calculation. PGS were standardised to a mean of 0 (SD ≈ 1) to enable easier comparison and interpretation of results (Babb de Villiers et al., 2020). All PGS were calculated with PRSice-2 (version 2.2.13; Choi and O’Reilly, 2019).

### 4.2.4 Statistical analysis

I used multiple linear regressions to explore if a PGS for a specific disorder (at one GWAS p-value threshold at a time, adjusted for covariates), would predict DUP. I repeated analyses for each PGS at each of the 9 GWAS p-value thresholds. I also repeated analyses with the value of DUP log-transformed, to account for skewness of DUP. Finally, I repeated all analyses on data from two sets of participants available post-QC (see Section Quality control and imputation above). The first set came from participants of any ancestry and the second set was a sub-sample which only included participants of European ancestry. For this European ancestry-only group, analyses with PGS for ADHD included PGS calculated with ADHD GWAS data from European ancestry participants only. That is
because PGS based on European ancestry GWAS data are the most precise in prediction in European ancestry patient data (Martin et al., 2019b). Value of $R^2$ came from subtracting $R^2$ attributable to a model with covariates alone from $R^2$ for the full model of PGS and covariates. For each analysis, I reported the $p$-value of a regression analysis and a $p$-value corrected for false discovery rate (FDR; Benjamini and Hochberg, 1995). FDR is used to avoid overly stringent correction for multiple testing, given that genetic tests are frequently related (positively correlated; Benjamini and Yekutieli, 2001). FDR-corrected $p$-values were calculated with the `p.adjust` function from the R package `stats`, with the option `method = "fdr"`. All analyses were conducted in R, release 3.6.3 (R Core Team, 2020).

4.3 Results

4.3.1 Sample characteristics

Total sample consisted of 865 individuals whose characteristics are presented in Table 4.1. The sample consisted primarily of men (57.7% of the sample), whose mean DUP was of 313 days (standard deviation [SD] = 929.5, range 0.1-10976 days). Mean age of participants at baseline assessment was 28.2 years (SD = 9.3, range 8-61 years). As in the case of age of onset (Chapter 3), diagnoses at first assessment were available for a part of the total DUP sample (643 out of was available for 865 participants). Diagnoses were based on ICD-10, DSM-5 or DSM-IV. The majority (263 participants) had a diagnosis of schizophrenia, 129 participants had a diagnosis of schizophreniform disorder, 97 participants had a diagnosis of acute and transient psychotic disorders, and 60 participants had a diagnosis of psychosis not specified as schizophrenia. The remaining diagnoses (bipolar disorder, mania, schizoaffective disorder, delusional disorder, depression with or without psychosis, personality disorder, or alcohol- or drug-induced psychosis) accounted each for 2% or less of the remaining available diagnoses. Data came from 8 European cities (London, Belfast, Istanbul, Lausanne, Oslo, Paris, Santander and Prague). Sample size for analyses of participants of all ancestries, but excluding data from TOP (Oslo, due to its overlap with BPD GWAS) was $n = 745$. Sample size of participants of European ancestry only was $n = 573$ for all analyses except analyses implementing BPD PGS, where the sample size was $n = 498$ after exclusion of data from TOP.
CHAPTER 4. POLYGENIC RISK & DURATION OF UNTREATED PSYCHOSIS

Table 4.1 Characteristics of the Schizophrenia: Treatment Resistance and Therapeutic Advances-Genetics (STRATA-G) sample at first presentation (collection of duration of untreated psychosis data)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample N = 865</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of untreated psychosis (DUP, in days)</td>
<td>313 (929.5)</td>
</tr>
<tr>
<td>Log-transformed DUP (in days)</td>
<td>3.2 (2.5)</td>
</tr>
<tr>
<td>Age at baseline assessment (in years)</td>
<td>28.2 (9.3)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>366 (42.3%)</td>
</tr>
<tr>
<td>Male</td>
<td>499 (57.7%)</td>
</tr>
<tr>
<td>STRATA-G cohort</td>
<td></td>
</tr>
<tr>
<td>ÆSOP, London</td>
<td>47 (5.4%)</td>
</tr>
<tr>
<td>NFEPS and RGPI, Belfast</td>
<td>57 (6.5%)</td>
</tr>
<tr>
<td>First-Episode Schizophrenia Follow-Up Project, Istanbul</td>
<td>42 (4.9%)</td>
</tr>
<tr>
<td>TIPP, Lausanne</td>
<td>116 (13.4%)</td>
</tr>
<tr>
<td>TOP, Oslo</td>
<td>120 (13.9%)</td>
</tr>
<tr>
<td>EUGEI, Paris</td>
<td>30 (3.4%)</td>
</tr>
<tr>
<td>PAFIP, Santander</td>
<td>351 (40.6%)</td>
</tr>
<tr>
<td>The West London Longitudinal First-Episode Psychosis, London</td>
<td>63 (7.3%)</td>
</tr>
<tr>
<td>The Early Stages of Schizophrenia Study, Prague</td>
<td>39 (4.6%)</td>
</tr>
<tr>
<td>Diagnosis at first presentation</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>263 (30.4%)</td>
</tr>
<tr>
<td>Schizophreniform disorder</td>
<td>129 (14.9%)</td>
</tr>
<tr>
<td>Acute and transient psychotic disorders</td>
<td>97 (11.2%)</td>
</tr>
<tr>
<td>Psychosis not specified as schizophrenia</td>
<td>60 (6.9%)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>13 (1.5%)</td>
</tr>
<tr>
<td>Other psychoses</td>
<td>81 (9.4%)</td>
</tr>
<tr>
<td>Not available</td>
<td>222 (25.7%)</td>
</tr>
</tbody>
</table>

4.3.2 Associations of polygenic scores for major psychiatric disorders with duration of untreated psychosis

Detailed results are summarised in Tables 4.2-4.13 below. Briefly, in samples including participants of all ancestries, PGS for BPD was associated with a shorter DUP at two p-value thresholds ($P_T$), $P_T = 0.5$, $\beta = -0.24$, 95%CI = -0.41- -0.06, $P = 0.01$; and $P_T =$
1, $\beta = -0.23$, 95%CI = -0.41- -0.04, P = 0.02. PGS for MDD was associated with a longer DUP ($P_T = 0.5$, $\beta = 0.08$, 95%CI = 0.01-0.14, P = 0.03 and $P_T = 1$, $\beta = 0.08$, 95%CI = 0.01-0.15, P = 0.02). PGS for SCZ was associated with a shorter DUP at a single $P_T$ ($P_T = 0.05$, $\beta = -0.09$, 95%CI = -0.18- -0.01, P = 0.03). Associations between DUP and PGS for ASD and ADHD were not significant.

There were no significant associations between DUP and PGS for ASD, ADHD, MDD, and SCZ in the full sample when DUP had been log-transformed to account for data skewness. However, shorter log-transformed DUP was associated with PGS for BPD ($P_T = 0.0000001$, $\beta = -0.07$, 95%CI = -0.13- -0.02, P = 0.007; $P_T = 0.1$, $\beta = -0.12$, 95%CI = -0.24- -0.01, P = 0.04; $P_T = 0.5$, $\beta = -0.20$, 95%CI = -0.33- -0.06, P = 0.004; and $P_T = 1$, $\beta = -0.17$, 95%CI = -0.30- -0.03, P = 0.02).

In the sample of participants of European ancestry only, none of the PGS were associated with DUP. Finally, in that sample, but in models with DUP log-transformed, shorter DUP was associated with PGS for BPD at four $P_T$ ($P_T = 0.0000001$, $\beta = -0.08$, 95%CI = -0.15- -0.02, P = 0.02, $P_T = 0.1$, $\beta = -0.07$, 95%CI = -0.14- -0.003, P = 0.04, $P_T = 0.5$, $\beta = -0.09$, 95%CI = -0.16- -0.01, P = 0.02, and $P_T = 1$, $\beta = -0.09$, 95%CI = -0.15- -0.01, P = 0.02). All the other PGS were not significantly associated with log-transformed DUP.

Given that multiple testing was implemented in the study, p-values were FDR-corrected (Benjamini and Hochberg, 1995). None of the associations between PGS and DUP from any sample remained significant after correction for multiple testing.

DUP was significantly associated with at least one STRATA research site in every regression model (p-value range = $2 \times 10^{-16}$ - 0.0486).
### Table 4.2 Multiple linear regressions for associations of polygenic scores for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with duration of untreated psychosis (in days)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-52.57</td>
<td>26.42</td>
<td>-0.07</td>
<td>-0.14--0.001</td>
<td>0.001</td>
<td>0.047</td>
<td>0.53</td>
</tr>
<tr>
<td>0.00001</td>
<td>-10.92</td>
<td>27.80</td>
<td>-0.01</td>
<td>-0.08--0.06</td>
<td>0.003</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>0.001</td>
<td>-9.00</td>
<td>27.68</td>
<td>-0.01</td>
<td>-0.08--0.06</td>
<td>0.001</td>
<td>0.75</td>
<td>0.91</td>
</tr>
<tr>
<td>0.01</td>
<td>-32.31</td>
<td>34.34</td>
<td>-0.04</td>
<td>-0.12--0.04</td>
<td>0.0002</td>
<td>0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>0.05</td>
<td>-60.97</td>
<td>47.72</td>
<td>-0.07</td>
<td>-0.16--0.04</td>
<td>0.001</td>
<td>0.20</td>
<td>0.60</td>
</tr>
<tr>
<td>0.1</td>
<td>-65.23</td>
<td>67.87</td>
<td>-0.07</td>
<td>-0.20--0.07</td>
<td>0.0002</td>
<td>0.34</td>
<td>0.70</td>
</tr>
<tr>
<td>0.5</td>
<td>-98.81</td>
<td>80.52</td>
<td>-0.10</td>
<td>-0.25--0.06</td>
<td>0.001</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>1</td>
<td>-248.93</td>
<td>96.44</td>
<td>-0.24</td>
<td>-0.41--0.06</td>
<td>0.007</td>
<td>0.01</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**BPD-PGS (n = 745)**

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>20.16</td>
<td>31.61</td>
<td>0.02</td>
<td>-0.04--0.09</td>
<td>0.001</td>
<td>0.52</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>27.16</td>
<td>29.39</td>
<td>0.03</td>
<td>-0.03--0.09</td>
<td>0.001</td>
<td>0.36</td>
<td>0.70</td>
</tr>
<tr>
<td>0.001</td>
<td>28.23</td>
<td>29.68</td>
<td>0.03</td>
<td>-0.03--0.10</td>
<td>0.001</td>
<td>0.34</td>
<td>0.70</td>
</tr>
<tr>
<td>0.01</td>
<td>27.76</td>
<td>30.83</td>
<td>0.03</td>
<td>-0.04--0.10</td>
<td>0.001</td>
<td>0.37</td>
<td>0.71</td>
</tr>
<tr>
<td>0.05</td>
<td>46.95</td>
<td>33.06</td>
<td>0.05</td>
<td>-0.02--0.12</td>
<td>0.0004</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>0.1</td>
<td>63.42</td>
<td>34.72</td>
<td>0.06</td>
<td>-0.01--0.13</td>
<td>0.002</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>0.5</td>
<td>73.16</td>
<td>37.45</td>
<td>0.07</td>
<td>-0.0001--0.15</td>
<td>0.002</td>
<td>0.05</td>
<td>0.53</td>
</tr>
<tr>
<td>1</td>
<td>74.32</td>
<td>33.80</td>
<td>0.08</td>
<td>0.01--0.14</td>
<td>0.003</td>
<td>0.03</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**MDD-PGS (n = 865)**

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, ; FDR P, false discovery rate-corrected p-value to account for multiple testing
### Table 4.3 Multiple linear regressions for associations of polygenic scores for schizophrenia (SCZ-PGS) with duration of untreated psychosis (in days)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta (unstandardised)</th>
<th>SE</th>
<th>$\beta$ (standardised)</th>
<th>95% CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-37.62</td>
<td>33.48</td>
<td>-0.04</td>
<td>-0.11-0.03</td>
<td>0.0003</td>
<td>0.26</td>
<td>0.62</td>
</tr>
<tr>
<td>0.00001</td>
<td>-8.98</td>
<td>36.21</td>
<td>-0.01</td>
<td>-0.09-0.07</td>
<td>0.001</td>
<td>0.80</td>
<td>0.94</td>
</tr>
<tr>
<td>0.001</td>
<td>18.84</td>
<td>34.76</td>
<td>0.02</td>
<td>-0.05-0.09</td>
<td>0.001</td>
<td>0.59</td>
<td>0.83</td>
</tr>
<tr>
<td>0.01</td>
<td>-45.31</td>
<td>33.49</td>
<td>-0.05</td>
<td>-0.12-0.02</td>
<td>0.001</td>
<td>0.18</td>
<td>0.60</td>
</tr>
<tr>
<td>0.05</td>
<td>-55.74</td>
<td>34.20</td>
<td>-0.06</td>
<td>-0.13-0.01</td>
<td>0.002</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>0.1</td>
<td>-86.07</td>
<td>38.83</td>
<td>-0.09</td>
<td>-0.18-0.01</td>
<td>0.004</td>
<td>0.03</td>
<td>0.49</td>
</tr>
<tr>
<td>0.5</td>
<td>-79.00</td>
<td>46.34</td>
<td>-0.09</td>
<td>-0.18-0.01</td>
<td>0.002</td>
<td>0.09</td>
<td>0.55</td>
</tr>
<tr>
<td>1</td>
<td>-75.72</td>
<td>47.19</td>
<td>-0.08</td>
<td>-0.18-0.02</td>
<td>0.002</td>
<td>0.11</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.4 Multiple linear regressions for associations of polygenic scores for autism spectrum disorder (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with duration of untreated psychosis (in days)

### ASD-PGS ($n = 865$)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>28.16</td>
<td>28.91</td>
<td>0.03</td>
<td>-0.03-0.09</td>
<td>0.001</td>
<td>0.33</td>
<td>0.70</td>
</tr>
<tr>
<td>0.00001</td>
<td>49.08</td>
<td>29.89</td>
<td>0.05</td>
<td>-0.01-0.12</td>
<td>0.002</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>0.0001</td>
<td>33.67</td>
<td>28.87</td>
<td>0.04</td>
<td>-0.03-0.10</td>
<td>0.001</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>0.001</td>
<td>22.77</td>
<td>29.26</td>
<td>0.03</td>
<td>-0.04-0.09</td>
<td>0.0003</td>
<td>0.44</td>
<td>0.75</td>
</tr>
<tr>
<td>0.01</td>
<td>-4.76</td>
<td>29.29</td>
<td>-0.01</td>
<td>-0.07-0.06</td>
<td>0.0003</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
<td>0.05</td>
<td>44.94</td>
<td>33.96</td>
<td>0.05</td>
<td>-0.02-0.12</td>
<td>0.001</td>
<td>0.19</td>
<td>0.60</td>
</tr>
<tr>
<td>0.1</td>
<td>48.60</td>
<td>35.75</td>
<td>0.05</td>
<td>-0.02-0.12</td>
<td>0.002</td>
<td>0.17</td>
<td>0.60</td>
</tr>
<tr>
<td>0.5</td>
<td>72.11</td>
<td>40.22</td>
<td>0.07</td>
<td>-0.01-0.15</td>
<td>0.003</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>1</td>
<td>73.02</td>
<td>40.49</td>
<td>0.07</td>
<td>-0.01-0.15</td>
<td>0.003</td>
<td>0.07</td>
<td>0.53</td>
</tr>
</tbody>
</table>

### ADHD-PGS ($n = 865$)

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>-14.65</td>
<td>30.34</td>
<td>-0.02</td>
<td>-0.08-0.04</td>
<td>0.0001</td>
<td>0.63</td>
<td>0.84</td>
</tr>
<tr>
<td>0.00001</td>
<td>-14.86</td>
<td>30.07</td>
<td>-0.02</td>
<td>-0.08-0.05</td>
<td>0.0001</td>
<td>0.62</td>
<td>0.84</td>
</tr>
<tr>
<td>0.001</td>
<td>13.88</td>
<td>30.82</td>
<td>0.02</td>
<td>-0.05-0.08</td>
<td>0.0001</td>
<td>0.65</td>
<td>0.85</td>
</tr>
<tr>
<td>0.01</td>
<td>-6.83</td>
<td>29.41</td>
<td>-0.01</td>
<td>-0.07-0.06</td>
<td>0.0003</td>
<td>0.82</td>
<td>0.94</td>
</tr>
<tr>
<td>0.05</td>
<td>28.41</td>
<td>34.22</td>
<td>0.03</td>
<td>-0.04-0.10</td>
<td>0.0004</td>
<td>0.41</td>
<td>0.75</td>
</tr>
<tr>
<td>0.1</td>
<td>17.49</td>
<td>33.77</td>
<td>0.02</td>
<td>-0.05-0.09</td>
<td>0.0001</td>
<td>0.61</td>
<td>0.84</td>
</tr>
<tr>
<td>0.5</td>
<td>15.55</td>
<td>33.29</td>
<td>0.02</td>
<td>-0.05-0.08</td>
<td>0.0001</td>
<td>0.64</td>
<td>0.84</td>
</tr>
<tr>
<td>1</td>
<td>13.82</td>
<td>29.95</td>
<td>0.02</td>
<td>-0.05-0.08</td>
<td>0.0001</td>
<td>0.64</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age^2, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.5 Multiple linear regressions for associations of PGS for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with duration of untreated psychosis (in days), log-transformed

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>(\beta)</th>
<th>95%CI</th>
<th>(R^2)</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>-0.17</td>
<td>0.06</td>
<td>-0.07</td>
<td>-0.13- -0.02</td>
<td>0.0002</td>
<td>0.007</td>
<td>0.40</td>
</tr>
<tr>
<td>0.000001</td>
<td>-0.11</td>
<td>0.07</td>
<td>-0.05</td>
<td>-0.10- -0.01</td>
<td>0.003</td>
<td>0.09</td>
<td>0.55</td>
</tr>
<tr>
<td>0.0001</td>
<td>-0.04</td>
<td>0.07</td>
<td>-0.01</td>
<td>-0.07- -0.04</td>
<td>0.005</td>
<td>0.60</td>
<td>0.84</td>
</tr>
<tr>
<td>0.001</td>
<td>-0.13</td>
<td>0.08</td>
<td>-0.05</td>
<td>-0.11- -0.01</td>
<td>0.003</td>
<td>0.12</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.19</td>
<td>0.12</td>
<td>-0.06</td>
<td>-0.14- -0.01</td>
<td>0.003</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.27</td>
<td>0.16</td>
<td>-0.09</td>
<td>-0.19- -0.02</td>
<td>0.003</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.40</td>
<td>0.19</td>
<td>-0.12</td>
<td>-0.24- -0.01</td>
<td>0.002</td>
<td>0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.67</td>
<td>0.23</td>
<td>-0.20</td>
<td>-0.33- -0.06</td>
<td>0.001</td>
<td>0.004</td>
<td>0.40</td>
</tr>
<tr>
<td>1</td>
<td>-0.56</td>
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<td>-0.17</td>
<td>-0.30- -0.03</td>
<td>0.001</td>
<td>0.02</td>
<td>0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>(\beta)</th>
<th>95%CI</th>
<th>(R^2)</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>0.06</td>
<td>0.07</td>
<td>0.02</td>
<td>-0.03- -0.07</td>
<td>0.004</td>
<td>0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>0.000001</td>
<td>0.08</td>
<td>0.06</td>
<td>0.03</td>
<td>-0.02- -0.08</td>
<td>0.004</td>
<td>0.21</td>
<td>0.60</td>
</tr>
<tr>
<td>0.001</td>
<td>0.09</td>
<td>0.06</td>
<td>0.04</td>
<td>-0.01- -0.08</td>
<td>0.004</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
<td>0.04</td>
<td>0.06</td>
<td>0.02</td>
<td>-0.03- -0.07</td>
<td>0.005</td>
<td>0.50</td>
<td>0.79</td>
</tr>
<tr>
<td>0.05</td>
<td>0.12</td>
<td>0.07</td>
<td>0.05</td>
<td>-0.004- -0.10</td>
<td>0.003</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>0.1</td>
<td>0.09</td>
<td>0.07</td>
<td>0.03</td>
<td>-0.02- -0.09</td>
<td>0.004</td>
<td>0.20</td>
<td>0.60</td>
</tr>
<tr>
<td>0.5</td>
<td>0.11</td>
<td>0.08</td>
<td>0.04</td>
<td>-0.02- -0.09</td>
<td>0.004</td>
<td>0.18</td>
<td>0.60</td>
</tr>
<tr>
<td>1</td>
<td>0.09</td>
<td>0.07</td>
<td>0.03</td>
<td>-0.02- -0.09</td>
<td>0.004</td>
<td>0.20</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age\(^2\), 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; \(\beta\), standardised beta; CI, confidence intervals; \(R^2\), percentage of variance explained, computed by comparison of \(R^2\) for a full model (PGS and covariates) to \(R^2\) of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.6 Multiple linear regressions for associations of PGS for schizophrenia (SCZ-PGS) with duration of untreated psychosis (in days), log-transformed

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.07</td>
<td>0.07</td>
<td>-0.03</td>
<td>-0.08-0.03</td>
<td>0.0001</td>
<td>0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>0.00001</td>
<td>-0.01</td>
<td>0.08</td>
<td>-0.01</td>
<td>-0.06-0.05</td>
<td>0.001</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.11</td>
<td>0.07</td>
<td>0.04</td>
<td>-0.01-0.10</td>
<td>0.001</td>
<td>0.13</td>
<td>0.60</td>
</tr>
<tr>
<td>0.001</td>
<td>0.07</td>
<td>0.07</td>
<td>0.03</td>
<td>-0.03-0.08</td>
<td>0.001</td>
<td>0.32</td>
<td>0.69</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.04</td>
<td>0.07</td>
<td>-0.02</td>
<td>-0.07-0.04</td>
<td>0.0002</td>
<td>0.58</td>
<td>0.83</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.09</td>
<td>0.08</td>
<td>-0.03</td>
<td>-0.10-0.03</td>
<td>0.001</td>
<td>0.28</td>
<td>0.64</td>
</tr>
<tr>
<td>0.1</td>
<td>-0.003</td>
<td>0.09</td>
<td>-0.001</td>
<td>-0.07-0.07</td>
<td>0</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.002</td>
<td>0.10</td>
<td>-0.001</td>
<td>-0.08-0.07</td>
<td>0</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.10</td>
<td>0.002</td>
<td>-0.07-0.08</td>
<td>0</td>
<td>0.96</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.7 Multiple linear regressions for associations of PGS for autism (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with duration of untreated psychosis (in days), log-transformed

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>ASD-PGS (n = 865)</th>
<th>ADHD-PGS (n = 865)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>SE</td>
</tr>
<tr>
<td>0.000001</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>0.001</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>0.01</td>
<td>-0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>0.05</td>
<td>-0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>0.1</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>1</td>
<td>0.07</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.8 Multiple linear regressions for associations of PGS for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with duration of untreated psychosis (in days). Samples of participants of European ancestry only.

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95% CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>-0.18</td>
<td>17.73</td>
<td>-0.0001</td>
<td>-0.08-0.08</td>
<td>0.001</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>0.00001</td>
<td>7.02</td>
<td>18.36</td>
<td>0.02</td>
<td>-0.07-0.10</td>
<td>0.001</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>0.001</td>
<td>0.91</td>
<td>18.14</td>
<td>0.002</td>
<td>-0.08-0.08</td>
<td>0.001</td>
<td>0.96</td>
<td>0.99</td>
</tr>
<tr>
<td>0.01</td>
<td>-7.92</td>
<td>21.99</td>
<td>-0.02</td>
<td>-0.10-0.07</td>
<td>0.001</td>
<td>0.72</td>
<td>0.89</td>
</tr>
<tr>
<td>0.05</td>
<td>-44.41</td>
<td>31.30</td>
<td>-0.06</td>
<td>-0.14-0.02</td>
<td>0.004</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>0.1</td>
<td>-26.00</td>
<td>45.00</td>
<td>-0.03</td>
<td>-0.11-0.06</td>
<td>0.001</td>
<td>0.56</td>
<td>0.81</td>
</tr>
<tr>
<td>0.5</td>
<td>-83.54</td>
<td>67.42</td>
<td>-0.06</td>
<td>-0.14-0.03</td>
<td>0.003</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>1</td>
<td>-87.25</td>
<td>73.00</td>
<td>-0.05</td>
<td>-0.14-0.03</td>
<td>0.003</td>
<td>0.23</td>
<td>0.61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>$\beta$</th>
<th>95% CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>25.66</td>
<td>31.60</td>
<td>0.03</td>
<td>-0.04-0.11</td>
<td>0.001</td>
<td>0.42</td>
<td>0.75</td>
</tr>
<tr>
<td>0.00001</td>
<td>40.69</td>
<td>29.61</td>
<td>0.05</td>
<td>-0.02-0.13</td>
<td>0.002</td>
<td>0.17</td>
<td>0.60</td>
</tr>
<tr>
<td>0.001</td>
<td>39.47</td>
<td>29.90</td>
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<td>-0.03-0.13</td>
<td>0.002</td>
<td>0.19</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
<td>34.30</td>
<td>31.09</td>
<td>0.04</td>
<td>-0.03-0.12</td>
<td>0.001</td>
<td>0.27</td>
<td>0.62</td>
</tr>
<tr>
<td>0.05</td>
<td>48.74</td>
<td>33.23</td>
<td>0.06</td>
<td>-0.02-0.13</td>
<td>0.003</td>
<td>0.14</td>
<td>0.60</td>
</tr>
<tr>
<td>0.1</td>
<td>51.23</td>
<td>34.43</td>
<td>0.06</td>
<td>-0.02-0.13</td>
<td>0.003</td>
<td>0.14</td>
<td>0.60</td>
</tr>
<tr>
<td>0.5</td>
<td>70.22</td>
<td>37.12</td>
<td>0.07</td>
<td>-0.003-0.15</td>
<td>0.005</td>
<td>0.06</td>
<td>0.53</td>
</tr>
<tr>
<td>1</td>
<td>55.63</td>
<td>33.72</td>
<td>0.06</td>
<td>-0.01-0.14</td>
<td>0.004</td>
<td>0.10</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.9 Multiple linear regressions for associations of PGS for schizophrenia (SCZ-PGS) with duration of untreated psychosis (in days). Samples of participants of European ancestry only

<table>
<thead>
<tr>
<th>SCZ-PGS (n = 573)</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGS P threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0000001</td>
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<td>29.87</td>
<td>0.03</td>
<td>-0.05-0.10</td>
<td>0.0002</td>
<td>0.51</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>36.97</td>
<td>30.33</td>
<td>0.05</td>
<td>-0.03-0.12</td>
<td>0.002</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>0.0001</td>
<td>33.62</td>
<td>30.10</td>
<td>0.04</td>
<td>-0.03-0.12</td>
<td>0.001</td>
<td>0.27</td>
<td>0.62</td>
</tr>
<tr>
<td>0.001</td>
<td>3.86</td>
<td>30.50</td>
<td>0.01</td>
<td>-0.07-0.08</td>
<td>0.0004</td>
<td>0.90</td>
<td>0.98</td>
</tr>
<tr>
<td>0.01</td>
<td>-9.22</td>
<td>30.52</td>
<td>-0.01</td>
<td>-0.09-0.07</td>
<td>0.0003</td>
<td>0.76</td>
<td>0.91</td>
</tr>
<tr>
<td>0.05</td>
<td>-5.09</td>
<td>30.89</td>
<td>-0.01</td>
<td>-0.08-0.07</td>
<td>0.0004</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
<td>0.1</td>
<td>-7.80</td>
<td>30.94</td>
<td>-0.01</td>
<td>-0.09-0.07</td>
<td>0.0003</td>
<td>0.80</td>
<td>0.94</td>
</tr>
<tr>
<td>0.5</td>
<td>-15.82</td>
<td>31.43</td>
<td>-0.02</td>
<td>-0.10-0.06</td>
<td>0</td>
<td>0.62</td>
<td>0.84</td>
</tr>
<tr>
<td>1</td>
<td>-12.96</td>
<td>31.51</td>
<td>-0.02</td>
<td>-0.10-0.06</td>
<td>0.0002</td>
<td>0.68</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.10 Multiple linear regressions for associations of PGS for autism spectrum disorder (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with duration of untreated psychosis (in days). Samples of participants of European ancestry only

**ASD-PGS (n = 573)**

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000001</td>
<td>18.52</td>
<td>28.85</td>
<td>0.03</td>
<td>-0.05-0.10</td>
<td>0.0002</td>
<td>0.52</td>
<td>0.79</td>
</tr>
<tr>
<td>0.00001</td>
<td>43.67</td>
<td>29.25</td>
<td>0.06</td>
<td>-0.02-0.13</td>
<td>0.003</td>
<td>0.14</td>
<td>0.60</td>
</tr>
<tr>
<td>0.0001</td>
<td>52.54</td>
<td>28.91</td>
<td>0.07</td>
<td>-0.01-0.14</td>
<td>0.004</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>0.001</td>
<td>27.05</td>
<td>29.58</td>
<td>0.04</td>
<td>-0.04-0.11</td>
<td>0.001</td>
<td>0.36</td>
<td>0.70</td>
</tr>
<tr>
<td>0.01</td>
<td>-1.82</td>
<td>29.70</td>
<td>-0.002</td>
<td>-0.08-0.07</td>
<td>0.0004</td>
<td>0.95</td>
<td>0.99</td>
</tr>
<tr>
<td>0.05</td>
<td>36.01</td>
<td>33.75</td>
<td>0.04</td>
<td>-0.04-0.12</td>
<td>0.001</td>
<td>0.29</td>
<td>0.64</td>
</tr>
<tr>
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<td>37.77</td>
<td>35.51</td>
<td>0.04</td>
<td>-0.04-0.12</td>
<td>0.002</td>
<td>0.29</td>
<td>0.64</td>
</tr>
<tr>
<td>0.5</td>
<td>77.63</td>
<td>40.49</td>
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<td>-0.002-0.15</td>
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</tr>
<tr>
<td>1</td>
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<td>0.07</td>
<td>-0.01-0.14</td>
<td>0.004</td>
<td>0.09</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**ADHD-PGS (n = 573)**

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE</th>
<th>$\beta$</th>
<th>95%CI</th>
<th>$R^2$</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000001</td>
<td>-22.65</td>
<td>29.34</td>
<td>-0.03</td>
<td>-0.11-0.05</td>
<td>0.0004</td>
<td>0.44</td>
<td>0.75</td>
</tr>
<tr>
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<td>29.53</td>
<td>-0.03</td>
<td>-0.10-0.05</td>
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<td>0.47</td>
<td>0.78</td>
</tr>
<tr>
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<td>29.62</td>
<td>-0.05</td>
<td>-0.12-0.03</td>
<td>0.002</td>
<td>0.21</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
<td>-12.75</td>
<td>29.22</td>
<td>-0.02</td>
<td>-0.09-0.06</td>
<td>0.0001</td>
<td>0.66</td>
<td>0.86</td>
</tr>
<tr>
<td>0.05</td>
<td>0.77</td>
<td>29.91</td>
<td>0.001</td>
<td>-0.07-0.08</td>
<td>0.0004</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
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<td>19.00</td>
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<td>0.02</td>
<td>-0.05-0.10</td>
<td>0.0001</td>
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</tr>
<tr>
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<td>31.52</td>
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<td>-0.04-0.11</td>
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<td>0.75</td>
</tr>
<tr>
<td>1</td>
<td>33.22</td>
<td>29.17</td>
<td>0.04</td>
<td>-0.03-0.12</td>
<td>0.002</td>
<td>0.26</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age$^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.11 Multiple linear regressions for associations of PGS for bipolar disorder (BPD-PGS) and major depressive disorder (MDD-PGS) with duration of untreated psychosis (in days), log-transformed. Samples of participants of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>(\beta)</th>
<th>95%CI</th>
<th>(R^2)</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
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<td>-0.08</td>
<td>-0.15-0.02</td>
<td>0.002</td>
<td>0.02</td>
<td>0.40</td>
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</tr>
<tr>
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<td>0.08</td>
<td>-0.02</td>
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<td>0.64</td>
<td>0.84</td>
</tr>
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<td>0.10</td>
<td>-0.05</td>
<td>-0.11-0.02</td>
<td>0.003</td>
<td>0.19</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
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<td>0.14</td>
<td>-0.05</td>
<td>-0.12-0.02</td>
<td>0.003</td>
<td>0.17</td>
<td>0.60</td>
</tr>
<tr>
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<td>0.20</td>
<td>-0.04</td>
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<td>0.004</td>
<td>0.27</td>
<td>0.62</td>
</tr>
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<td>0.53</td>
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<td>-0.09</td>
<td>-0.16-0.01</td>
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<td>0.02</td>
<td>0.40</td>
</tr>
<tr>
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<td>-0.09</td>
<td>-0.15-0.01</td>
<td>0.001</td>
<td>0.02</td>
<td>0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>(\beta)</th>
<th>95%CI</th>
<th>(R^2)</th>
<th>P</th>
<th>FDR P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.003</td>
<td>-0.06-0.06</td>
<td>0.004</td>
<td>0.93</td>
<td>0.99</td>
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<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.004</td>
<td>0.55</td>
<td>0.81</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.06</td>
<td>0.08</td>
<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.004</td>
<td>0.47</td>
<td>0.78</td>
</tr>
<tr>
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<td>-0.01</td>
<td>0.02</td>
<td>-0.07-0.05</td>
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<tr>
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<td>-0.03-0.09</td>
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<td>0.01</td>
<td>-0.05-0.07</td>
<td>0.004</td>
<td>0.81</td>
<td>0.94</td>
</tr>
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<td>0.01</td>
<td>-0.05-0.07</td>
<td>0.004</td>
<td>0.75</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age\(^2\), 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; \(\beta\), standardised beta; CI, confidence intervals; \(R^2\), percentage of variance explained, computed by comparison of \(R^2\) for a full model (PGS and covariates) to \(R^2\) of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.12 Multiple linear regressions for associations of PGS for schizophrenia (SCZ-PGS) with duration of untreated psychosis (in days), log-transformed. Samples of participants of European ancestry only.

<table>
<thead>
<tr>
<th>SCZ-PGS (n = 573)</th>
</tr>
</thead>
<tbody>
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<td>PGS P threshold</td>
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</tr>
<tr>
<td>0.00001</td>
</tr>
<tr>
<td>0.001</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.05</td>
</tr>
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<td>0.1</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
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</table>

Note: analyses included covariates of gender, age, $\text{age}^2$, 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; $\beta$, standardised beta; CI, confidence intervals; $R^2$, percentage of variance explained, computed by comparison of $R^2$ for a full model (PGS and covariates) to $R^2$ of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
Table 4.13 Multiple linear regressions for associations of PGS for autism spectrum disorder (ASD-PGS) and attention deficit hyperactivity disorder (ADHD-PGS) with duration of untreated psychosis (in days), log-transformed. Samples of participants of European ancestry only

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
<th>P</th>
<th>FDR P</th>
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</thead>
<tbody>
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<td>0.001</td>
<td>0.02</td>
<td>-0.06-0.06</td>
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<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
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<td>0.08</td>
<td>0.01</td>
<td>-0.05-0.06</td>
<td>0.004</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
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<td>0.07</td>
<td>-0.0001</td>
<td>-0.06-0.06</td>
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<td>-0.08-0.04</td>
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</tr>
<tr>
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<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.004</td>
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</tr>
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<td>0.004</td>
<td>0.47</td>
<td>0.78</td>
</tr>
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<td>0.02</td>
<td>-0.04-0.08</td>
<td>0.004</td>
<td>0.48</td>
<td>0.78</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>PGS P threshold</th>
<th>Beta</th>
<th>SE B</th>
<th>β</th>
<th>95%CI</th>
<th>R²</th>
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<th>FDR P</th>
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<td>-0.01</td>
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<td>0.95</td>
</tr>
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<td>0.64</td>
<td>0.84</td>
</tr>
<tr>
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<td>0.08</td>
<td>0.05</td>
<td>-0.01-0.10</td>
<td>0.002</td>
<td>0.12</td>
<td>0.60</td>
</tr>
<tr>
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<td>0.15</td>
<td>0.08</td>
<td>0.06</td>
<td>-0.004-0.11</td>
<td>0.001</td>
<td>0.07</td>
<td>0.53</td>
</tr>
<tr>
<td>0.5</td>
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<td>0.08</td>
<td>0.03</td>
<td>-0.03-0.09</td>
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<td>0.62</td>
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<td>0.04</td>
<td>-0.02-0.09</td>
<td>0.003</td>
<td>0.23</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Note: analyses included covariates of gender, age, age², 5 principal components, and STRATA-G research site. PGS; polygenic score; PGS P threshold, p-value threshold for SNP inclusion in PGS; SE, standard error of the unstandardised beta; β, standardised beta; CI, confidence intervals; R², percentage of variance explained, computed by comparison of R² for a full model (PGS and covariates) to R² of a model with covariates only; P, p-value; FDR P, false discovery rate-corrected p-value to account for multiple testing.
CHAPTER 4. POLYGENIC RISK & DURATION OF UNTREATED PSYCHOSIS

4.4 Discussion

4.4.1 Polygenic scores for major psychiatric disorders in prediction of duration of untreated psychosis

In this study, I investigated relationships between DUP in an international sample of first-episode psychosis patients and PGS for schizophrenia, bipolar disorder, depression, attention deficit hyperactivity disorder, and autism spectrum disorder. DUP was defined as a period between onset of psychosis and either first hospitalisation for suspected psychosis or start of antipsychotic medication. PGS are summed up common genetic variants, weighted by effect sizes from GWAS, which contribute to genetic predisposition for major psychiatric disorders.

Research applying GWAS findings to DUP is very limited (Yin et al., 2019b; Ajnakina et al., 2021). In light of these findings, I hypothesised there would be no significant relationships between PGS and DUP. Indeed, I found no statistically significant relationships for the majority of PGS. The exceptions were PGS for bipolar disorder (BPD-PGS) and schizophrenia (SCZ-PGS), related to a shorter DUP, and PGS for major depressive disorder (MDD-PGS), related to a longer DUP. However, these findings were not significant in analyses on participants of European ancestry only and in analyses where DUP had been log-transformed to account for data skewness, only BPD-PGS was significantly associated with a shorter DUP.

Associations between PGS and DUP may also be related to how common genetic factors predispose towards severity of different symptoms. For instance, SCZ-PGS was previously found to be positively correlated with negative symptoms in schizophrenia and psychosis in bipolar disorder and BPD-PGS was positively correlated with mania in schizophrenia and psychosis in bipolar disorder (Ruderfer et al., 2014; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018). SCZ-PGS was also related to lower current cognitive performance in SCZ patients, even after controlling for estimated premorbid IQ, in some studies (Legge et al., 2021), but not all (Richards et al., 2019). Onset of severe symptoms may result in a shorter DUP (Demjaha et al., 2009; Drake et al., 2020) because patients may notice severe symptoms—or others may observe these symptoms—leading them to seek treatment rapidly. However, severe symptoms could be interpreted as a contributor to an extended, rather than shortened, DUP instead. Prospective patients may not have enough energy, cognitive capacity or motivation (Schlosser et al., 2014) to seek treatment. Patients with severe symptoms may also be socially isolated (Drake et al., 2000), which may prevent others from offering support resulting in rapid treatment. While this interpretation potentially explains a link
between longer DUP and MDD-PGS, it does not clearly account for relationships between SCZ-PGS or BPD-PGS and a shorter DUP. Another caveat is also that no associations between DUP and PGS reached significance after FDR correction for multiple testing.

Taken together, my findings suggest that common genetic variants for major psychiatric disorders may not confer an increased risk for a particular length of DUP. In contrast, Yin et al. (2019b) predicted schizophrenia subtypes with GWAS data, including a schizophrenia subtype with a shorter DUP. However, these differing findings may be because of differences in methodologies used. Yin and colleagues modelled subtypes of schizophrenia by clustering GWAS-imputed gene expression data and clinical data. In my study, I instead used PGS for multiple psychiatric disorders, including schizophrenia, in patients with different psychoses. Consistently with my findings, in a study with a comparable design (a PGS study of first episode psychosis patients with diagnoses of schizophrenia spectrum disorders, bipolar disorder, psychotic depression and other psychoses), Ajnakina et al. (2021) found no relationships between PGS and DUP.

No significant relationship between PGS for either psychiatric or neurodevelopmental disorders and DUP is informative, as it suggests a potential stronger impact of environmental risk factors than of genetic factors on DUP. Consistently with this interpretation, associations between DUP and STRATA research sites were significant. DUP has been linked previously to multiple social and cultural factors related to access to healthcare. Reduced DUP has been attributed to improvements in diagnostic and therapeutic algorithms (Dell’Osso et al., 2016). Extended DUP has been linked, inter alia, to limited health service accessibility (Murru and Carpiniello, 2018), stigma preventing access to treatment (Hardy et al., 2020), and poor links between first points of contact for patients (such as primary care, emergency rooms, and criminal justice agencies) and psychiatric services (Bhui et al., 2014). Nonetheless, a caveat is that in my study, aside from research site, I did not test relationships between DUP and specific environmental factors. Thus, it is not clear what factors in particular drive length of DUP in STRATA-G.

Still, my findings have potential implications for clinical care. On one hand, my findings suggest that, as PGS do not predict DUP, at least at present, they cannot improve targeted clinical population screening (Murray et al., 2021a) for those at risk for extended DUP. On the other hand, my findings indicate that potentially modifiable factors, likely related to access to healthcare, impact DUP. Also, recent research demonstrated that long DUP is associated with reduced treatment response, response which appears to first worsen quickly and then gradually slower as DUP gets longer (Drake et al., 2020). Taken together, these findings point to the importance of funding psychiatric services which could provide care early on and rapidly. Indeed, early intervention services (EIS), which focus on tailored treatments for individuals at early stages of psychosis, improve several patient outcomes.
These outcomes span decreased hospitalisation risk, improved symptoms, and better global functioning (Correll et al., 2018). Still, EIS remain potentially underused. It is likely because EIS are not optimised for the specific, individual nature of populations which EIS are to serve (size, ethnic and cultural composition, prevalence of untreated psychosis, and common pathways to care in the specific population). Also, patients face difficulties with accessing EIS rapidly and easily, as navigating health services is not user friendly. Finally, patients are unaware that starting treatment rapidly is important for recovery (Malla and McGorry, 2019). Thus, DUp reductions require improving rapid access to psychiatric care through both population-level and public health interventions.

### 4.4.2 Methodological considerations

I analysed data from a FEP sample from an international consortium, STRATA-G. The sample has multiple strengths. The first strength is that FEP data are potentially more reliable records of early stages of disorder/DUP than such data collected at a later time, based on patient recall only. The time lag between disorder onset, first hospitalisation or medication start and having that information recorded would be shortened. This, in turn, potentially limits patient recall bias of their DUp (Dell’Osso et al., 2016). Alternatively, to improve accuracy of recorded DUp, data on first treatment could come from clinicians prescribing treatment (Dell’Osso et al., 2016), or data on prescription or hospitalisation could be cross-checked with patient health records (Simon and VonKorff, 1995). Indeed, records from several STRATA sites (e.g., from Istanbul University Hospital) have been cross-validated by patients, their families and clinicians. The second strength of STRATA-G data is that the data come from multiple European countries which means that the STRATA sample potentially reflects DUp in European patients better than a sample from a single catchment area/country would. The third strength of the STRATA sample is that it includes data from populations of different ancestries. Diversity of data in genetic studies is still lacking, as studies often do not address multiple non-European ancestries (Popejoy and Fullerton, 2016). Consequently, I wanted to include data from as many different populations as available in STRATA-G.

However, a limitation of my study is that the majority of the sample was nonetheless of European ancestry. Also, the majority of GWAS findings, the basis of PGS in this study, comes from samples of European ancestry. As PGS based on European ancestry data are more accurate in individuals of European ancestry than in individuals of other ancestries (Martin et al., 2019b), my analyses likely do not have sufficient accuracy to detect potential genetic associations with DUp in samples of non-European ancestries. Potentially consistently with this interpretation (given that DUp is related to poor patient
outcomes), recent research found that PGS based on the latest schizophrenia GWAS (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020) did not improve performance of predictive models of poor patient outcomes in multiethnic samples of African, admixed American, and European ancestries (Landi et al., 2021). Another limitation is current limited predictive power of PGS. It is likely to improve as over time as GWAS samples, then used as basis of PGS calculation, increase in size (Chatterjee et al., 2013) and have more power to detect associations between SNPs relevant to disorder or cognition.

Another potential limitation is the PGS calculation method in my study, which then requires multiple testing. I tested multiple PGS, with SNPs at different GWAS p-value thresholds. Using multiple tests decreases power of individual tests, in particular after controlling for multiple testing to avoid false positives (Verhoeven et al., 2005). Completing polygenic scoring with a method which would require fewer tests of polygenic scores would potentially improve power of the study to detect associations between PGS and DUP (see No optimal methodology for PGS calculation in Section 6.3.3.2. of Chapter 6 for extra discussion). A general limitation of PGS, regardless of method of their calculation, is that they only capture contributions of common genetic variants to predisposition towards a phenotype. However, as discussed in the previous chapter, phenotypes such as a starting point of DUP, AOO, have been shown to be impacted by rare genetic variation and may also be impacted by gene-gene or gene-environment interactions.

Additional considerations relate to the composition of the STRATA-G DUP sample. In my analyses, to use all available data, I kept data from all participants for whom full information on the study phenotype, genetic predictors and covariates was available (fully quality controlled genetic data for polygenic scoring and principal component calculation, gender, age, research site, and DUP). That resulted in the sample where patients had diagnoses of different psychotic disorders, and this great heterogeneity may be why my results were largely non-significant. In addition, while DUP in 720 out of 865 participants (83% of the sample) was of up to a year, there were also 23 participants with DUP of over 3000 days (over 8 years), with one individual’s untreated psychosis reported as 10976 days (approximately 30 years). It might be that, for participants with such a long DUP, in this extended period, there was an onset of psychosis and then remission, or a brief period of psychotic symptoms, with no need for treatment. Such a period of experiencing psychosis would not really represent DUP (as DUP is a period before treatment) and so, cases with such an extended reported DUP should have been excluded from all analyses as outliers.

As discussed in the previous section, there is also a question of ways in which genetically predisposed severity of symptoms or change in cognitive performance impact length of DUP. A limitation of the current study is that I did not test measures of cognitive
performance, symptom prevalence, or their severity. These measures were not available for the full sample of 865 participants. The Clinical Global Impression Scale severity scale (Guy, 1976) was available for 15 participants, Positive and Negative Syndrome Scale scores (Kay et al., 1987) were available for 211 participants, Wechsler Adult Intelligence Scale (Wechsler, 2008) and National Adult Reading Test (Blair and Spreen, 1989) measures of IQ were available for 140 and 82 participants, respectively, and years in education, proxy of cognitive performance, at the time of first assessment, when DUP was also calculated, were available for 322 participants (measures were available for 37% or less of the total sample).

The data in this study also include DUP defined in different ways, as a period from psychosis onset to either start of antipsychotic treatment or antipsychotic medication. On one hand, it could be argued that hospitalisation and treatment likely occur in close proximity and thus, these DUP are comparable. On the other hand, DUP based on different definitions were found to have different predictive values of patient outcomes, with DUP based on a strict definition (end of DUP defined as adherence to medication and engagement in an early intervention programme) being most predictive (Golay et al., 2016). Thus, it might be that a more stringent definition of DUP would yield measurements of DUP more informative for research purposes.

My study indicates the importance of environmental factors in relation to DUP, but a limitation of my analyses is that it is not clear which specific factors impact DUP in STRATA-G. The environmental covariate of research site may be related to international differences in access to care or cultural stigma. Still, my analyses did not explicitly test for either of those factors, or, indeed, for any additional, individual-specific factors impacting DUP, such as patient comorbidities (Murru et al., 2015). Also, the fact that research site was the only statistically significant result suggests that the sample may be underpowered to detect associations with additional factors relevant to prediction of DUP.

Future research should continue elucidating links between genetics predisposing to disorder, its severity, including pleiotropic contributions towards multiple disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019) and whether they impact DUP specifically. Future research could also explore how a polygenic contribution to traits which may potentially influence individual treatment seeking, such as nervousness or risk-taking (Socrates et al., 2021), is related to DUP. This research should be completed using well-powered, prospective samples which include detailed patient information on symptom severity, duration, and frequency, as well as on enviromental factors which may contribute to the length of untreated psychosis.
4.5 Conclusion

This study shows that genetic propensity towards major psychiatric disorders (schizophrenia, major depressive disorder, bipolar disorder, autism spectrum disorder, and attention deficit hyperactivity disorder) does not predict duration of untreated psychosis in participants of diverse ancestries. These results highlight the impact of non-genetic, potentially modifiable environmental factors instead. Population-level and public health interventions to improve rapid access to psychiatric care could potentially shorten duration of untreated psychosis.
Chapter 5

Schizophrenia polygenic risk predicts general cognitive deficit but not cognitive decline in healthy older adults

5.1 Full copy of the manuscript

Research presented in this chapter is a copy of a complete manuscript (followed by its Supplementary Material), published as:


Literature placing cognitive deficits in the context of psychosis is detailed in Chapter 1, Section 1.2.3. *Cognitive decline*. The study uses sociodemographic, health, cognitive performance, and genetic data from the English Longitudinal Study of Ageing (ELSA). Data, their quality control, polygenic score calculation and later statistical analyses are described in the manuscript and, in extensive detail, in Chapter 2, Section 2.3. Broader implications of the study and its detailed reappraisal are provided in Chapter 6. Dr Ajnakina completed quality control of genetic data and calculated polygenic scores and principal components for the full ELSA dataset. Dr Cadar provided the dataset with selected sociodemographic and cognitive performance variables, required for this study.
(full ELSA data are available online, open access, to enable wide dissemination by the research community: https://www.elsa-project.ac.uk/accessing-elsa-data).

I completed data analyses under Dr Ajnakina’s supervision, wrote the draft of the manuscript, and edited it. All co-authors provided feedback on manuscript drafts. The manuscript also underwent peer review prior to acceptance in *Translational Psychiatry*.

Final formatting follows the house style of the journal.

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Schizophrenia polygenic risk predicts general cognitive deficit but not cognitive decline in healthy older adults

Adrianna P. Kępíńska, James H. MacCabe, Dorina Cadar, Andrew Steptoe, Robin M. Murray and Olesya Ajnakina

Abstract
There has been a long argument over whether schizophrenia is a neurodegenerative disorder associated with progressive cognitive impairment. Given high heritability of schizophrenia, ascertaining if genetic susceptibility to schizophrenia is also associated with cognitive decline in healthy people would support the view that schizophrenia leads to an accelerated cognitive decline. Using the population representative sample of 6817 adults aged >50 years from the English Longitudinal Study of Ageing, we investigated associations between the biennial rate of decline in cognitive ability and the schizophrenia polygenic score (SZ-PGS) during the 10-year follow-up period. SZ-PGS was calculated based on summary statistics from the Schizophrenia Working Group of the Psychiatric Genomics Consortium. Cognition was measured sequentially across four time points using verbal memory and semantic fluency tests. The average baseline verbal memory was 10.4 (SD = 3.4) and semantic fluency was 20.7 (SD = 6.3). One standard deviation (1-SD) increase in SZ-PGS was associated with lower baseline semantic fluency ($\beta = -0.25$, 95%CI = $-0.40$ to $-0.10$, $p = 0.002$); this association was significant in men ($\beta = -0.36$, 95%CI = $-0.59$ to $-0.12$, $p = 0.003$) and in those who were aged 60–69 years old ($\beta = -0.32$, 95%CI = $-0.58$ to $-0.05$, $p = 0.019$). Similarly, 1-SD increase in SZ-PGS was associated with lower verbal memory score at baseline in men only ($\beta = -0.12$, 95%CI = $-0.23$ to $-0.01$, $p = 0.040$). However, SZ-PGS was not associated with a greater rate of decline in these cognitive domains during the 10-year follow-up. Our findings highlight that while genetic susceptibility to schizophrenia conveys developmental cognitive deficit, it is not associated with an ongoing cognitive decline, at least in later life. These results do not support the neo-Kraepelinian notion of schizophrenia as a genetically determined progressively deteriorating brain disease.

Introduction
The question of whether schizophrenia is a neurodegenerative disorder associated with progressive cognitive impairment has been debated for over a century. Many psychiatrists, with support from neuropsychological\(^1\)\(^2\) and neuroimaging\(^3\)\(^4\) reports, believe that the course of schizophrenia is characterised by cognitive decline\(^5\)\(^6\). However, prospective longitudinal studies are scarce. A small decline in cognitive function over a 10-year period following the onset of schizophrenia was reported\(^7\), but the authors could not be certain whether this was due to some intrinsic schizophrenia process(es) or resulted from adverse effects associated with its treatment. There are some suggestions that schizophrenia-related cognitive decline may be particularly pronounced in later life\(^8\)\(^9\). Indeed, an 18-year follow-up study showed that cognition in individuals with a psychotic disorder declined on all but 2 tests compared with controls, with the largest effect among participants who were 50 years or older\(^10\).
Schizophrenia is highly heritable,11 with a polygenic architecture.12 Recent evidence suggests that the polygenic underpinning of schizophrenia overlaps with general cognitive ability.13,14 Family studies also highlight the presence of cognitive impairments in schizophrenia patients prior to the onset of schizophrenia symptoms,15 and in their unaffected relatives but in more attenuated forms.16 Therefore, one of the possible ways to test the view that schizophrenia leads to a greater rate of decline in cognition may be through ascertaining whether genetic susceptibility to schizophrenia is also associated with cognitive decline in healthy people using the polygenic score (PGS) approach. PGS for schizophrenia encompasses multiple common genetic variants of the small effect associated with the illness that are scattered across the whole genome,17 and as such, it indexes susceptibility for this disease.18 Although several longitudinal studies have attempted to establish the effect of the PGS on cognition, they produced mixed results, possibly due to small samples, restricted accountability of important confounders, or limited follow-up time-points with intervals ranging over several decades when measuring cognitive changes in the general population. These inconsistent findings reinforce uncertainty concerning the origins of cognitive impairment in those with schizophrenia.

Therefore, using a large, phenotypically well-defined sample of population-representative older adults, we investigated whether common genetic variants associated with schizophrenia additively confer a stable deficit in cognitive ability, measured sequentially across four time points, or a greater risk of accelerated cognitive decline, or both, over the 10-year follow-up. Assuming a variation in cognitive impairment in schizophrenia is a function of the degree of genetic liability to the disorder, we hypothesised that polygenic score for schizophrenia would be significantly associated with lower cognition at baseline and a greater rate of decline in cognition during follow-up in healthy adults. Additionally, given age and gender differences in cognitive functions and schizophrenia risk,22–24, we also investigated whether the potential relationships of a polygenic score for schizophrenia with cognition at baseline and during follow-up in healthy adults differed by age and gender.

Methods
Sample
We utilised data from the English Longitudinal Study of Ageing (ELSA), which is an ongoing large, multidisciplinary study of a nationally representative sample of the English population aged ≥50 years. The ELSA study started in 2002 (wave 1), with participants recruited from an annual cross-sectional survey that is designed to monitor the health of the general population. The ELSA sample was then followed-up every 2 years. For the present study, baseline data were obtained from either wave 2 (2004–2005) for the core members who started at wave 1, or wave 4 for the participants joining the study at wave 4 through the refreshment sample; the included participants took part in the blood draws during home visits by a nurse. Follow-up data were ascertained from waves 4 (2009–2010) to wave 8 (2016–2017), which is the latest wave of data collection. We excluded participants with diagnosed organic causes of cognitive decline, such as history of dementia and stroke at baseline as well as those with a previous diagnosis of schizophrenia. Those ELSA participants who were included in the study or excluded from the final cohort did not differ in terms of age at baseline; however, the former group included participants with a higher educational attainment, higher accumulated wealth, lower proportion of people with a long-standing limiting health condition, depressive symptoms and smokers compared those respondents who were excluded for relevant variables in this study (Supplementary Table 1). Ethical approval for each of the ELSA waves was granted by the National Research Ethics Service (London Multicentre Research Ethics Committee). All participants gave informed consent.

Study variables
Cognition
Cognition was measured employing tests for verbal memory and semantic fluency, which were chosen because both these cognitive domains are important predictors of clinically significant cognitive decline in healthy older adults.25 To measure verbal memory, immediate and delayed verbal memory were assessed using a word-learning task, which entailed recalling as many out of 10 common words that were read out to them as possible immediately and after a short delay during which they completed other cognitive tests.26 Following the protocol of previous studies,27 the results for immediate and delayed recall were then combined to give an overall verbal memory variable measured on a continuum from 0 to 10 with a higher score indicating a better memory performance. Semantic fluency was measured with a verbal fluency test, where participants were asked to think of as many animal names as they could in 1 min. The total number of animal names written by participants was used as a continuous measure of semantic fluency; the semantic fluency score was measured on a continuum from 0 to 20 with a higher score indicating better performance. Although this task primarily focused on semantic fluency, it combined various aspects of broader executive function including cognitive flexibility, processing speed, inhibitory control, and verbal fluency. As semantic fluency test was not administered at wave 6, in line with previous work in this area,26 there was
a longer follow-up gap in the assessment of this cognitive domain from wave 5 (2010–2011) to wave 7 (2014–2015) (Fig. 1). The distribution of these cognitive domains across all waves of data collection is provided in Supplementary Table 2.

**Covariates**

We included an extensive set of covariates encompassing different aspects of persons’ health and life, which have been shown to affect cognitive function of older English people. Demographic covariates include gender (male) and age; to capture non-linear aging effects which cognition is susceptible to, we also included age$^2$ as a covariate. Social determinants, such as wealth (poorest, middle and wealthiest as reference) and educational attainment, were also included as covariates. To reflect the accumulation of resources at older ages, wealth was measured at baseline by summing wealth from property, possessions, housing, investments, savings, artwork, jewellery and net of debt. The wealth variable was further divided into tertile to represent the groups of individuals with the high, intermediate and low levels of wealth. Educational attainment was measured with how many years of schooling each participant completed. As comorbid health conditions can affect cognitive ageing, we included depressive symptoms and the presence of a limiting long-standing illness as covariates. The presence of a limiting long-standing illness was measured with the participants’ reporting presence of any limiting health illness (coded as “yes”), or absence of any limiting illnesses or health conditions (coded as “no”). Depressive symptoms were measured with an 8-item version of the Centre for Epidemiologic Studies Depression Scale, which has been found to have comparable psychometric properties to the full 20-item scale. A score $\geq$4 was used to define participants with severe depressive symptoms. Behavioural risk factors included smoking status (current smoker and non-smoker was a reference). Because the $\varepsilon$4 allele of the apolipoprotein E gene (APOE-$\varepsilon$4) has previously been associated with cognitive decline in normal aging, we adjusted our analyses for APOE-$\varepsilon$4. Consistent with previous research, APOE-$\varepsilon$4 status was defined according to the absence (APOE $\varepsilon$2/2, $\varepsilon$2/3 and $\varepsilon$3/3) or presence (APOE $\varepsilon$2/4, $\varepsilon$3/4 and $\varepsilon$4/4) of APOE-$\varepsilon$4 alleles. Lastly, genetic ancestry (as was measured with principal components (see below)), was included as covariates to account for any ancestry differences in genetic structures that could bias our results.

**Genetic data**

**Quality control**

The genome-wide genotyping was performed at University College London Genomics in 2013-2014 using the Illumina HumanOmni2.5 BeadChips (HumanOmni2.5-4v1, HumanOmni2.5-8v1.3). Single-nucleotide polymorphism (SNPs) were excluded if they were non-autosomal, the minor allele frequency was <0.01%, if more than 2% of genotype data were missing and if the Hardy-Weinberg Equilibrium $p$-value<$10^{-4}$. Samples were removed based on call rate (<0.99), suspected non-European ancestry, sex difference in allelic frequency of $\geq$0.2, heterozygosity and relatedness. We employed the principal components analysis to identify those individuals who deviated from European ancestry (i.e., ethnic outliers). This set of analyses demonstrated the presence of ancestral admixture in the 65 individuals, who were
subsequently removed; individuals who self-reported they were of non-white ethnicity were also removed. Using the updated sample, we calculated principal component (PCs) (Supplementary Fig. 1), which then were used to adjust for possible population stratification in the association analyses.37,38.

**Polygenic score (PGS) analyses**

PGS for schizophrenia (SZ-PGS) was calculated using summary statistics from genome-wide association study (GWAS) conducted by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC)39. Schizophrenia-associated SNPs, weighted by their effect size derived from the PGC, were summed in a continuous score using PRSice40 following specifications outlined previously.41. As previous research highlighted that PGSs built from directly genotyped data either had more predictive power42 or did not differ significantly from PGSs calculated using imputed data,43 we calculated PGSs based on genotyped data at different p-value cut-offs. Because PGSs including all available SNPs either explain the most amount of variation in a trait or are not significantly different than PGSs based on different p-value thresholds,44 we utilised PGS that was based on a threshold of p-value of 1. To aid the interpretability of the results, SZ-PGS was standardised to a mean of 0 (SD = 1).

**Statistical analysis**

**Association analyses**

To assess the relationships of SZ-PGS with the general cognition and the rate of change in cognitive ability during the 10-year follow-up, we employed linear mixed effect models (LMMs) with maximum likelihood estimation.42. LMMs have been shown to be useful for the analysis of longitudinal data with an evitable loss to follow-up data.43. Having considered linear, quadratic and cubic LMMs, Akaike Information Criterion and Bayesian Information Criterion44,45 showed that the linear model was the most appropriate for our analyses. To investigate whether age influenced the potential relationships between SZ-PGS and cognitive decline over follow-up, we stratified our analyses by age groups, which were formed based on tertiles results (i.e., 50–59 years, 60–69 years and ≥70 years). Given the previously observed gender differences in cognitive functions,22 we additionally performed sex-stratified analyses. We used a significance level of 0.05 (two-tailed) for all analyses. All analyses were conducted in STATA release 14 (STATA Corp LP, USA).

**Calculate power and predictive accuracy of a polygenic score**

To investigate whether SZ-PGS included in the present study had sufficient power to detect relationships with cognition at baseline and a greater rate of decline in cognition during follow-up in healthy adults, we estimated the power of the calculated SZ-PGS using the Avengene package implemented in R18. This showed that SZ-PGS had sufficient power for the subsequent analyses (power=1, p = 6.26 × 10^-17).

**Results**

**Sample characteristics**

The baseline demographic and health characteristics of the total sample are presented in Table 1. The sample comprised 6817 individuals with a baseline mean age of 64.3 years old (standard deviation (SD) = 9.3, range = 50–101); 25.3% (n = 1724) of participants were APOE-ε4 carriers, 46.2% (n = 3159) were men, 30.9% (n = 2108) were unmarried and 32.5% (n = 2154) had a low level of accumulated wealth. The average baseline memory score was 10.4 (SD = 3.4) and the executive function score was 20.7 (SD = 6.3).

**Table 1 Sample characteristics at baseline.**

<table>
<thead>
<tr>
<th>Baseline sample characteristics</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>64.6 (9.3)</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
</tr>
<tr>
<td>50–59 years</td>
<td>2487 (36.5)</td>
</tr>
<tr>
<td>60–69 years</td>
<td>2332 (34.2)</td>
</tr>
<tr>
<td>≥70 years</td>
<td>1998 (29.3)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3667 (53.8)</td>
</tr>
<tr>
<td>Male</td>
<td>3159 (46.2)</td>
</tr>
<tr>
<td>APOE-ε4 present</td>
<td>1724 (25.3)</td>
</tr>
<tr>
<td>Currently smoker</td>
<td>978 (15.2)</td>
</tr>
<tr>
<td>Married</td>
<td>2108 (30.9)</td>
</tr>
<tr>
<td>Wealth</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2154 (32.5)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2234 (33.6)</td>
</tr>
<tr>
<td>High</td>
<td>2251 (33.9)</td>
</tr>
<tr>
<td>Educational attainment (years)</td>
<td>13.7 (3.8)</td>
</tr>
<tr>
<td>Limited life health condition (any)</td>
<td>2132 (31.3)</td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>735 (13.3)</td>
</tr>
<tr>
<td>Cognition</td>
<td></td>
</tr>
<tr>
<td>Memory score</td>
<td>10.4 (3.4)</td>
</tr>
<tr>
<td>Executive function score</td>
<td>20.7 (6.3)</td>
</tr>
</tbody>
</table>

APOE-ε4 two ε4 alleles of the Apolipoprotein E gene, SD standard deviation.
Table 2  Associations between schizophrenia polygenic score (SZ-PGS) and cognitive function in older adults over the 10-year follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Verbal memory</th>
<th></th>
<th>Semantic fluency</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95%CI)</td>
<td>P-value</td>
<td>β (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>−0.07 (−0.14−0.01)</td>
<td>0.091</td>
<td>−0.25 (−0.40−0.09)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>−0.14 (−0.15−0.14)</td>
<td>&lt;0.001</td>
<td>−0.19 (−0.21−0.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>−1.08 (−1.23−0.92)</td>
<td>&lt;0.001</td>
<td>0.02 (−0.29−0.33)</td>
<td>0.906</td>
</tr>
<tr>
<td>Current smoker</td>
<td>−0.10 (−0.33−0.13)</td>
<td>0.386</td>
<td>−0.58 (−1.04−0.12)</td>
<td>0.013</td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>−0.60 (−0.77−0.42)</td>
<td>&lt;0.001</td>
<td>−0.62 (−0.97−0.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education attainment</td>
<td>0.19 (0.17−0.21)</td>
<td>&lt;0.001</td>
<td>0.43 (0.38−0.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>−0.53 (−0.77−0.30)</td>
<td>&lt;0.001</td>
<td>−0.81 (−1.28−0.33)</td>
<td>0.001</td>
</tr>
<tr>
<td>APOE-e4 present</td>
<td>−0.27 (−0.45−0.10)</td>
<td>0.002</td>
<td>−0.31 (−0.66−0.04)</td>
<td>0.074</td>
</tr>
<tr>
<td>Limiting health conditions (any)</td>
<td>−0.18 (−0.35−0.01)</td>
<td>0.041</td>
<td>−0.17 (−0.66−0.04)</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Rate of change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>0.003 (−0.01−0.02)</td>
<td>0.741</td>
<td>0.01 (−0.02−0.03)</td>
<td>0.740</td>
</tr>
<tr>
<td>Age</td>
<td>−0.06 (−0.03−0.09)</td>
<td>&lt;0.001</td>
<td>0.06 (0.002−0.11)</td>
<td>0.044</td>
</tr>
<tr>
<td>Gender</td>
<td>−0.02 (−0.05−0.01)</td>
<td>0.0285</td>
<td>−0.06 (−0.12−0.002)</td>
<td>0.041</td>
</tr>
<tr>
<td>Current smoker</td>
<td>−0.07 (−0.12−0.02)</td>
<td>0.002</td>
<td>−0.05 (−0.14−0.03)</td>
<td>0.229</td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>−0.01 (−0.04−0.03)</td>
<td>0.707</td>
<td>−0.08 (−0.14−0.01)</td>
<td>0.028</td>
</tr>
<tr>
<td>Education attainment</td>
<td>0.002 (−0.002−0.01)</td>
<td>0.255</td>
<td>−0.01 (−0.001−0.002)</td>
<td>0.121</td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>−0.01 (−0.06−0.04)</td>
<td>0.629</td>
<td>−0.05 (−0.15−0.04)</td>
<td>0.258</td>
</tr>
<tr>
<td>APOE-e4 present</td>
<td>−0.08 (−0.12−0.04)</td>
<td>&lt;0.001</td>
<td>−0.10 (−0.16−0.03)</td>
<td>0.005</td>
</tr>
<tr>
<td>Limiting health conditions (any)</td>
<td>−0.03 (−0.06−0.01)</td>
<td>0.126</td>
<td>−0.11 (−0.18−0.05)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-person</td>
<td>0.07 (0.06−0.08)</td>
<td>0.16 (0.13−0.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In initial status</td>
<td>3.69 (3.66−4.28)</td>
<td>15.99 (14.77−17.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In rate of change</td>
<td>0.03 (−0.02−0.08)</td>
<td>0.08 (−0.09−0.26)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The models were further adjusted for age2 to capture non-linear aging effects of which cognition is susceptible to and 4 principal components to account for any ancestry differences in genetic structures that could bias the results.

CI confidence intervals, SZ-PGS polygenic score for schizophrenia, APOE-e4 two e4 alleles of the Apolipoprotein E gene.

*The within-person variance is the overall residual variance in cognition that is not explained by the model. The initial status variance component is the variance of individuals’ intercepts about the intercept of the average person. The rate of change variance component is the variance of individual slopes about the slope of the average person.

Rate of change in cognition over the 10-year follow-up

The rate of decline in cognition over the 10-year follow-up is depicted in Fig. 1. The average biennial rate of decline in verbal memory during the 10 years was 1.21 points (95%CI = 0.99–1.44, p < 0.001). For semantic fluency, the average biennial rate of decline was 1.36 points (95%CI = 1.07–1.65, p < 0.001) over the follow-up period.

SZ-PGS and verbal memory in older adults

There was a trend towards a statistically significant relationship of one standard deviation (1-SD) increase in SZ-PGS with a lower baseline verbal memory score (β = −0.07, 95%CI = −0.14–0.01, p = 0.091) (Table 2). When the analyses were stratified by gender, the results showed that 1-SD increase in SZ-PGS was associated with lower verbal memory score in men (β = −0.12, 95%CI = −0.23 to −0.01, p = 0.040) but not in women (Supplementary Table 3) highlighting a significant interaction with gender. There was also a trend towards significance in the relationship of SZ-PGS with verbal memory score in older adults who were aged 70 years old and older (β = −0.14, 95%CI = −0.28–0.004, p = 0.056) (Supplementary Table 4). Nonetheless, there was no significant association between SZ-PGS and the rate of decline in verbal memory during the 10-year follow-up period.
Although APOE-related factors and APOE-men independently from demographic factors, health-domains during the 10-year follow-up period. Associated with a greater rate of decline in these cognitive domains during the 10-year follow-up period.

Discussion

In the present study, we investigated the relationships of multiple common genetic variants for schizophrenia, which additively indexed susceptibility for this illness, with cognition and rate of decline in cognition during the 10-year follow-up period in a large population-representative sample of older adults. At the core of the study was the notion that if cognitive impairment in schizophrenia is a consequence of genetic liability to the disorder, with greater impairment indicating greater liability, the association between the genetic liability to schizophrenia and cognitive decline would be observed in non-psychotic adults.

In support of the previous findings highlighting molecular genetic overlap between general cognitive ability and risk for schizophrenia in the general population, we found that SZ-PGS was significantly associated with lower verbal memory and semantic fluency scores at baseline in men independently from demographic factors, health-related factors and APOE-e4 status. This is consistent with earlier twin studies which highlighted that boys had higher heritability for a verbal measure of cognitive ability compared to girls, in turn highlighting that this genetic propensity to a higher verbal measure of cognitive ability in men extends to later life. Additive contribution of common genetic markers for schizophrenia was also significantly higher for semantic fluency score among adults who were aged 60–69-year old, supporting the notion that genetic influence on semantic fluency differs by age groups.

In contrast to our hypothesis, however, common genetic variants associated with schizophrenia additively did not confer a greater rate of decline in cognition during the 10-year follow-up in older people from the general population. Although some evidence for gender differences in cognitive aging exist, as further supported by our results, these were not associated with genetic propensity for schizophrenia. The fact that we observed a significant cognitive decline during the follow-up period linked to the effects of APOE-e4, tobacco smoking, educational attainment and lower wealth were inversely associated with semantic fluency during follow-up, SZ-PGS was not associated with a greater rate of decline in these cognitive domains during the 10-year follow-up period.

SZ-PGS and semantic fluency in older adults

1-SD increase in SZ-PGS was associated with lower semantic fluency at baseline ($\beta = -0.25$, 95%CI = −0.40–−0.09, $p = 0.002$). Further analyses showed this association was significant in men ($\beta = -0.36$, 95%CI = −0.59 to −0.12, $p = 0.003$) (Supplementary Table 3) and in those who were aged 60–69 years old ($\beta = -0.32$, 95% CI = −0.58 to −0.05, $p = 0.019$) (Supplementary Table 5). Although APOE-e4, tobacco smoking, educational attainment and lower wealth were inversely associated with semantic fluency during follow-up, SZ-PGS was not associated with a greater rate of decline in these cognitive domains during the 10-year follow-up period.
schizophrenia. Certainly, there is evidence suggesting that loneliness contributes to a worsening in memory and verbal fluency over a decade in the general population. Cumulatively, our results do not support the neo-Kraepelinian notion of schizophrenia as a genetically determined progressively deteriorating brain disease, at least at the molecular level. Therefore, it may be important for optimum clinical care to reconsider the idea of the existence of intrinsically malignant process(es) underlying schizophrenia. This has contributed to an undue pessimism among mental health professionals and their consequent alienation from sufferers and their representatives, who increasingly advocate for the “recovery model” of schizophrenia. Of course, this is not to negate the serious and disabling problems that many patients with schizophrenia experience. Nonetheless, it is still possible that only a subset of the genetic factors for schizophrenia drive cognitive decline observed in patients with this illness, which, due to the nature of the PGS approach, might not have been captured in the present study. Therefore, further analyses, such as pathway-specific polygenic score analyses, genomic structural equation modelling and gene-set enrichment analyses, may be needed before we can draw more precise conclusions of the role schizophrenia risk loci may play in general cognition.

Methodological considerations

We analysed a large nationally-representative cohort of older adults in England who were followed-up every two years. We further benefitted from the availability of repeated measures of cognition across a 10-year span. Our study included a relatively equal proportion of women and men from socio-economically diverse backgrounds. Confidence in these findings is strengthened by the use of a linear mixed model, which is an optimal way to identify the change in continuous dependent variables over time and quantify its association with a range of independent variables, all the while taking the intra-individual and inter-individual variation into account. To avoid providing results of questionable theoretical relevance, we did not present our results as composite scores for broad cognitive functions; instead, we explored verbal memory and semantic fluency separately. The comprehensive inclusion of covariates in the analyses meant that we could control for identified confounding variables reducing any potential risk for biases in our results.

Nonetheless, several methodological limitations warrant a discussion. Although PGSs have the potential to improve health outcomes through their eventual implementation as clinical biomarkers, the poor generalisability of genetic studies across populations is noteworthy. This is because the construction of PGSs is largely dependent on the availability of the summary statistics from genome-wide association studies (GWASs), which are currently predominately based on European participants. Given genetic risk is different in European and non-European individuals, further work is necessary to develop PGSs models in non-white populations. Similarly, by design, polygenic scores do not capture other structural variants beyond common genetic markers of relatively small effects, such as rare variants, poorly tagged or multiple independent variants, gen-by-gene interactions and gene-environment correlation. Therefore, these factors will not be accounted for when applying PGSs in the analyses. Further, the lack of a pre-registered analytical protocol is a notable limitation of the present study. The presence of practice effects may have limited the observed decline in cognitive abilities. However, in line with the previous evidence, the potential practice effects may be insignificant given the relatively long interval between the assessments of cognitive domains (i.e., 2 years). Finally, we assessed several associations, which may raise some concerns over multiple statistical testing. Considering our sample size was large enough to withstand multiple testing without increasing risk for false positive results and the fact that adjusting for multiple statistical testing has significant disadvantages, rather than adjusting our p-values for multiple-testing, we followed the new guidelines for statistical reporting when presenting the results in the present study.

Conclusion

Our findings highlight that while genetic susceptibility to schizophrenia conveys developmental cognitive deficit, it is not associated with an ongoing cognitive decline, at least in later life. Thus, our results do not provide support for the neo-Kraepelinian notion of schizophrenia as a genetically determined progressively deteriorating brain disease.

Acknowledgements

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Data availability

The ELSA data are available in public, open-access repository (the UK Data Archive) which is freely available and can be accessed at https://ukdataservice.ac.uk.
Conflict of interest
RMM has received honoraria from Janssen, Sunovian, Lundbeck and Otsuka. All other authors declare no conflict of interest.

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Published online: 08 December 2020

References
Supplementary Table 1. Comparisons between the ELSA participants who were included in the analyses and those who were excluded

<table>
<thead>
<tr>
<th></th>
<th>Excluded (N=6125)</th>
<th>Included (N=6817)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Socio-demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.53 (10.94)</td>
<td>64.63 (9.46)</td>
<td>t=-0.53, df=12617, p=0.5970</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>2615 (42.69)</td>
<td>3094 (45.93)</td>
<td>$\chi^2=13.57$, df=1, p&lt;0.001</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.44 (3.80)</td>
<td>13.72 (3.79)</td>
<td>t=-3.97, df=11587, p&lt;0.001</td>
</tr>
<tr>
<td>Accumulated wealth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1713 (29.94)</td>
<td>2332 (35.54)</td>
<td>$\chi^2=91.43$, df=2, p&lt;0.001</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1847 (32.28)</td>
<td>2272 (34.62)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2161 (37.77)</td>
<td>1958 (29.84)</td>
<td></td>
</tr>
<tr>
<td><strong>Comorbid health issues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limiting health conditions</td>
<td>2307 (38.27)</td>
<td>2114 (31.38)</td>
<td>$\chi^2=66.66$, df=1, p&lt;0.001</td>
</tr>
<tr>
<td>Depression (score ≥4)</td>
<td>698 (18.89)</td>
<td>735 (13.35)</td>
<td>$\chi^2=51.64$, df=1, p&lt;0.001</td>
</tr>
<tr>
<td><strong>Behavioural outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently a smoker</td>
<td>1072 (17.80)</td>
<td>974 (14.47)</td>
<td>$\chi^2=26.02$, df=1, p&lt;0.001</td>
</tr>
</tbody>
</table>
Supplementary Table 2. Distribution of the cognitive domains across all waves of data collection for the 6817 ELSA participants included in this study

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Wave 1</th>
<th>Wave 2</th>
<th>Wave 3</th>
<th>Wave 4</th>
<th>Wave 5</th>
<th>Wave 6</th>
<th>Wave 7</th>
<th>Wave 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>10.02 (3.32)</td>
<td>10.27 (3.42)</td>
<td>10.45 (3.59)</td>
<td>10.44 (3.54)</td>
<td>10.47 (3.60)</td>
<td>10.62 (3.66)</td>
<td>10.27 (3.80)</td>
<td>9.96 (4.20)</td>
</tr>
<tr>
<td></td>
<td>N=5523</td>
<td>N=5484</td>
<td>N=5118</td>
<td>N=5512</td>
<td>N=5064</td>
<td>N=4689</td>
<td>N=4125</td>
<td>N=3854</td>
</tr>
<tr>
<td></td>
<td>N=5524</td>
<td>N=5490</td>
<td>N=5117</td>
<td>N=5512</td>
<td>N=5057</td>
<td>&quot;</td>
<td>N=4191</td>
<td>N=3729</td>
</tr>
</tbody>
</table>

SD, standard deviation; ELSA, English Longitudinal Study of Ageing
### Supplementary Table 3. Associations between polygenic score for schizophrenia (SZ-PGS) and longitudinal measure of cognitive function in older adults over the 10-year follow-up, stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Verbal Memory</th>
<th></th>
<th>Semantic Fluency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>-0.03 (-0.13-0.08)</td>
<td>0.630</td>
<td>-0.12 (-0.23-0.01)</td>
</tr>
<tr>
<td>Age</td>
<td>0.23 (0.09-0.38)</td>
<td>0.002</td>
<td>0.05 (-0.11-0.22)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>-0.10 (-0.41-0.21)</td>
<td>0.544</td>
<td>-0.13 (-0.46-0.20)</td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>-0.56 (-0.80-0.33)</td>
<td>&lt;0.001</td>
<td>-0.56 (-0.82-0.31)</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>0.21 (0.17-0.22)</td>
<td>&lt;0.001</td>
<td>0.18 (0.15-0.21)</td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>-0.36 (-0.68-0.05)</td>
<td>0.022</td>
<td>-0.79 (-1.18-0.40)</td>
</tr>
<tr>
<td>APOE-ε4 present</td>
<td>-0.29 (-0.53-0.04)</td>
<td>0.020</td>
<td>-0.26 (-0.51-0.01)</td>
</tr>
<tr>
<td>Limiting health conditions</td>
<td>-0.14 (-0.38-0.09)</td>
<td>0.220</td>
<td>-0.23 (-0.48-0.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rate of change</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>-0.01 (-0.04-0.02)</td>
<td>0.433</td>
<td>0.02 (-0.01-0.04)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.02 (-0.02-0.02)</td>
<td>&lt;0.001</td>
<td>-0.02 (-0.02-0.01)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>-0.12 (-0.21-0.02)</td>
<td>0.015</td>
<td>-0.06 (-0.13-0.01)</td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>-0.01 (-0.05-0.04)</td>
<td>0.834</td>
<td>-0.02 (-0.07-0.04)</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>0.003 (-0.003-0.001)</td>
<td>0.344</td>
<td>0.001 (-0.01-0.01)</td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>-0.04 (-0.10-0.03)</td>
<td>0.258</td>
<td>0.04 (-0.08-0.16)</td>
</tr>
<tr>
<td>APOE-ε4 present</td>
<td>-0.09 (-0.14-0.00)</td>
<td>0.001</td>
<td>-0.06 (-0.12-0.01)</td>
</tr>
<tr>
<td>Limiting health conditions</td>
<td>-0.05 (-0.10-0.001)</td>
<td>0.053</td>
<td>-0.02 (-0.06-0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Variance a</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-person</td>
<td>0.08 (0.06-0.09)</td>
<td>0.06 (0.046-0.08)</td>
<td>0.14 (0.10-0.21)</td>
</tr>
<tr>
<td>In initial status</td>
<td>4.07 (3.65-4.54)</td>
<td>3.73 (3.33-4.12)</td>
<td>14.36 (12.78-16.12)</td>
</tr>
<tr>
<td>In rate of change</td>
<td>0.001(-0.0068-0.07)</td>
<td>0.06(-0.002-0.13)</td>
<td>0.18(-0.05-0.41)</td>
</tr>
</tbody>
</table>

CI, confidence intervals; APOE-ε4, two ε4 alleles of the Apolipoprotein E gene; polygenic score for schizophrenia (SZ-PGS).

The models were further adjusted for age to capture non-linear aging effects of which cognition is susceptible to and 4 principal components to account for any ancestry differences in genetic structures that could bias the results.

a The within-person variance is the overall residual variance in cognition that is not explained by the model. The initial status variance component is the variance of individuals’ intercepts about the intercept of the average person. The rate of change variance component is the variance of individual slopes about the slope of the average person.
Supplementary Table 4. Associations between polygenic score for schizophrenia (SZ-PGS) and longitudinal measure of verbal memory in older adults over the 10-year follow-up, stratified by age groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th></th>
<th></th>
<th>Rate of change</th>
<th></th>
<th></th>
<th></th>
<th>Variance a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50-59 years</td>
<td>60-69 years</td>
<td>≥70 years</td>
<td></td>
<td>50-59 years</td>
<td>60-69 years</td>
<td>≥70 years</td>
<td></td>
<td>Within-person</td>
</tr>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>-0.04 (-0.17-0.08)</td>
<td>0.504</td>
<td>-0.03 (-0.16-0.09)</td>
<td>0.590</td>
<td>-0.14 (-0.28-0.004)</td>
<td>0.056</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-1.17 (-1.43- -0.91)</td>
<td>&lt;0.001</td>
<td>-1.22 (-1.47- -0.97)</td>
<td>&lt;0.001</td>
<td>-0.83 (-1.12- -0.53)</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.17 (-0.17-0.51)</td>
<td>0.334</td>
<td>-0.30 (-0.67-0.06)</td>
<td>0.105</td>
<td>-0.29 (-0.81-0.22)</td>
<td>0.263</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>-0.58 (-0.87- -0.28)</td>
<td>&lt;0.001</td>
<td>-0.67 (-0.95- -0.37)</td>
<td>&lt;0.001</td>
<td>-0.42 (-0.74- -0.11)</td>
<td>0.008</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Educational attainment</td>
<td>0.20 (0.16-0.23)</td>
<td>&lt;0.001</td>
<td>0.19 (0.16-0.22)</td>
<td>&lt;0.001</td>
<td>0.20 (0.15-0.24)</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>-0.48 (-0.67- -0.09)</td>
<td>0.017</td>
<td>-0.52 (-0.94- -0.09)</td>
<td>0.017</td>
<td>-0.48 (-0.89- -0.06)</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE-ε4 present</td>
<td>-0.09 (-0.38-0.19)</td>
<td>0.518</td>
<td>0.01 (-0.28-0.29)</td>
<td>0.971</td>
<td>-0.82 (-1.16- -0.48)</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limiting health conditions</td>
<td>-0.30 (-0.61-0.004)</td>
<td>0.053</td>
<td>-0.33 (-0.61- -0.04)</td>
<td>0.024</td>
<td>0.13 (-0.17-0.44)</td>
<td>0.387</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence intervals; APOE-ε4, two ε4 alleles of the Apolipoprotein E gene; polygenic score for schizophrenia (SZ-PGS)

The models were further adjusted for age squared to capture non-linear aging effects of which cognition is susceptible to and 4 principal components to account for any ancestry differences in genetic structures that could bias the results.

aThe within-person variance is the overall residual variance in memory that is not explained by the model. The initial status variance component is the variance of individuals’ intercepts about the intercept of the average person. Likewise, the rate of change variance component is the variance of individual slopes about the slope of the average person.
Supplementary Table 5. Associations between polygenic score for schizophrenia (SZ-PGS) and longitudinal measure of semantic fluency in older adults over the 10-year follow-up, stratified by age groups

<table>
<thead>
<tr>
<th></th>
<th>50-59 years</th>
<th></th>
<th>60-69 years</th>
<th></th>
<th>≥70 years</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td>β (95% CI)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>-0.25 (-0.53-0.03)</td>
<td>0.080</td>
<td>-0.32 (-0.58- -0.05)</td>
<td><strong>0.019</strong></td>
<td>-0.17 (-0.41-0.08)</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.39 (-0.94- -0.17)</td>
<td>0.177</td>
<td>0.03 (-0.50-0.57)</td>
<td>0.900</td>
<td>0.31 (-0.20-0.82)</td>
<td>0.234</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>-0.32 (-1.07- -0.43)</td>
<td>0.407</td>
<td>-0.68 (-1.47-0.10)</td>
<td>0.087</td>
<td>-0.95 (-1.84- -0.06)</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Low level of wealth</td>
<td>-0.11 (-0.75-0.54)</td>
<td>0.745</td>
<td>-0.61 (-1.24-0.01)</td>
<td>0.054</td>
<td>-0.97 (-1.51- -0.42)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Educational attainment</td>
<td>0.50 (0.42-0.57)</td>
<td>0.000</td>
<td>0.43 (0.35-0.50)</td>
<td>&lt;0.001</td>
<td>0.37 (0.30-0.45)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>-0.97 (-1.82- -0.11)</td>
<td>0.027</td>
<td>-0.28 (-1.19-0.62)</td>
<td>0.540</td>
<td>-0.95 (-1.66- -0.24)</td>
<td>0.009</td>
<td></td>
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<tr>
<td>APOE-ε4 present</td>
<td>-0.28 (-0.91-0.35)</td>
<td>0.387</td>
<td>-0.25 (-0.86-0.36)</td>
<td>0.415</td>
<td>-0.42 (-1.01-0.17)</td>
<td>0.168</td>
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<tr>
<td>Limiting health conditions</td>
<td>-0.62 (-1.29-0.05)</td>
<td>0.070</td>
<td>-0.01 (-0.62-0.60)</td>
<td>0.966</td>
<td>-0.01 (-0.53-0.51)</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>Rate of change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SZ-PGS</td>
<td>-0.01 (-0.05-0.04)</td>
<td>0.869</td>
<td>0.01 (-0.04-0.06)</td>
<td>0.696</td>
<td>0.02 (-0.05-0.09)</td>
<td>0.535</td>
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</tr>
<tr>
<td>Gender</td>
<td>-0.01 (-0.10-0.08)</td>
<td>0.879</td>
<td>-0.09 (-0.18-0.01)</td>
<td>0.070</td>
<td>-0.04 (-0.17-0.10)</td>
<td>0.588</td>
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</tr>
<tr>
<td>Current smoker</td>
<td>-0.07 (-0.19-0.05)</td>
<td>0.244</td>
<td>-0.05 (-0.20-0.09)</td>
<td>0.482</td>
<td>0.08 (-0.19-0.35)</td>
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<td>Low level of wealth</td>
<td>-0.06 (-0.16-0.05)</td>
<td>0.300</td>
<td>-0.14 (-0.25- -0.02)</td>
<td>0.017</td>
<td>-0.02 (-0.17-0.13)</td>
<td>0.805</td>
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</tr>
<tr>
<td>Educational attainment</td>
<td>-0.02 (-0.05-0.01)</td>
<td>0.177</td>
<td>0.002 (-0.01-0.01)</td>
<td>0.812</td>
<td>-0.02 (-0.03-0.002)</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>Depression diagnosis</td>
<td>-0.04 (-0.18-0.10)</td>
<td>0.563</td>
<td>-0.07 (-0.23-0.09)</td>
<td>0.405</td>
<td>-0.09 (-0.28-0.11)</td>
<td>0.377</td>
<td></td>
</tr>
<tr>
<td>APOE-ε4 present</td>
<td>-0.06 (-0.14-0.01)</td>
<td>0.134</td>
<td>-0.15 (-0.28-0.03)</td>
<td>0.014</td>
<td>-0.16 (-0.32-0.004)</td>
<td>0.056</td>
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</tr>
<tr>
<td>Limiting health conditions</td>
<td>-0.04 (-0.14-0.07)</td>
<td>0.495</td>
<td>-0.20 (-0.30- -0.09)</td>
<td>&lt;0.001</td>
<td>-0.08 (-0.22-0.07)</td>
<td>0.299</td>
<td></td>
</tr>
</tbody>
</table>

Variance a
- Within-person: 0.09 (0.05-0.17) 0.18 (0.13-0.25) 0.28 (0.19-0.40)
- In initial status: 17.29 (15.13-19.76) 17.54 (15.46-19.90) 12.64 (10.73-14.90)
- In rate of change: 0.10 (-0.19-0.39) -0.06 (-0.35-0.23) 0.13 (-0.24-0.51)

CI, confidence intervals; APOE-ε4, two ε4 alleles of the Apolipoprotein E gene; polygenic score for schizophrenia (SZ-PGS)

The models were further adjusted for age² to capture non-linear aging effects of which cognition is susceptible to and 4 principal components to account for any ancestry differences in genetic structures that could bias the results.

The within-person variance is the overall residual variance in memory that is not explained by the model. The initial status variance component is the variance of individuals’ intercepts about the intercept of the average person. Likewise, the rate of change variance component is the variance of individual slopes about the slope of the average person.
Supplementary Figure 1. Depicts distribution of 10 principal components once 65 individuals with ancestral admixture were removed from the sample.
Chapter 6

Discussion

6.1 General discussion, research implications, and future directions

Psychiatric genetics aims to clarify the underlying biology of mental disorders. Genetic measures are being developed with a view to improving prediction, diagnostic accuracy, drug development, and patient stratification strategies. These strategies should take into account the interactions between genes and environment. Genetic modelling should also address longitudinal trajectories of disease development and course (Sullivan et al., 2018). All these goals are important for psychosis, given that it is a syndrome with a genetic component (heritability of 73% or more; Hilker et al., 2018) and characterised by heterogeneous trajectories (Ensley et al., 2013; Jääskeläinen et al., 2013; Lally et al., 2017; Morgan et al., 2014). This thesis aimed to explore contributions of multiple common genetic variants to specific psychosis manifestations (phenotypes) across the course of illness: age of psychosis onset (Chapter 3); duration of untreated psychosis (Chapter 4); and possible longitudinal decline of verbal memory and executive function (Chapter 5).

These phenotypes are important because they may impact trajectories of psychosis and it is hoped that they can help inform prediction of patient disease course and outcomes. Patients with different ages of onset (AOO) of psychotic disorders have different disorder outcomes (Maglione et al., 2014). Compared to patients with early-onset schizophrenia (before the age of 13), patients with late-onset schizophrenia (over the age of 40) have a lower average severity of positive symptoms and lower average antipsychotic dose requirements (Maglione et al., 2014). Patients with early-onset psychotic disorders have poorer outcomes, e.g., longer hospitalisations in the first two years after diagnosis (Vernal et al., 2020), more prolonged psychotic episodes (Eggers et al., 2000), or no complete
remission over 40 years (Eggers and Bunk, 1997). These findings suggest that psychoses with different AOO may represent distinct psychosis subtypes (Maglione et al., 2014). Duration of untreated psychosis (DUP), a period between psychosis onset and start of treatment, has been linked to patient prognosis. Shorter DUP is associated with a better prognosis, such as a better treatment response, symptom control and overall functional outcome (Murru and Carpiniello, 2018). Finally, cognitive decline is a change in cognitive function, but it is still not clear if there is decline of particular cognitive domains in different psychotic disorders (Bora and Murray, 2014; Lynham et al., 2020). It is also not entirely clear whether cognitive decline in psychosis worsens over time, especially as patients with psychosis age (Fett et al., 2019).

Studies in this thesis found 1) no statistically significant relationships between polygenic scores (PGS) for major psychiatric disorders (measures of common genetic variants predisposing for psychiatric illness) and age of psychosis onset and duration of untreated psychosis, and 2) no statistically significant relationships between PGS for schizophrenia and cognitive decline. Yet, PGS for schizophrenia was related to baseline cognitive performance (at a single time point, at the start of longitudinal measurement of cognitive change). Discussions of strengths and limitations of each study close every research chapter (3-5). The following discussion chapter provides an overview of key thesis findings in relation to the literature, implications of the findings, a discussion of research strengths, limitations and methodological considerations, and closes with suggestions for future research.

6.2 Summary of conducted research and its relation to literature

6.2.1 Age of onset of psychosis and polygenic scores

In Chapter 3, I tested whether AOO of psychosis is associated with polygenic scores for major psychiatric disorders: schizophrenia (SCZ), bipolar disorder (BPD), attention deficit hyperactivity disorder (ADHD), major depressive disorder (MDD) and autism spectrum disorder (ASD). Previously, these associations have only been tested in specific patient populations, testing AOO in bipolar disorder or schizophrenia only (Aminoff et al., 2015; Kalman et al., 2019; Stepniak et al., 2014), or in a specific subtype of psychotic disorder (e.g., childhood-onset schizophrenia only; Ahn et al., 2016). Few studies reported statistically significant relationships between AOO and PGS: Ahn et al. (2016) reported a statistically significant relationship between AOO in childhood-onset schizophrenia and
PGS for ASD and Grigoroiu-Serbanescu et al. (2020) between AOO in early-onset bipolar disorder (≤21 years) and PGS for ADHD.

Therefore, my study aimed to test whether these PGS were associated with AOO in a population of patients with a variety of psychotic disorders. My study used longitudinal data from first episode psychosis (FEP) patients, collated from multiple European sites as part of the STRATA Consortium. This dataset is referred to as STRATA-Genetics (STRATA-G; see Chapter 2, Section 2.2. for details). I tested polygenic scores at multiple p-value thresholds as predictors of AOO in linear regression analyses. Each regression included covariates of principal components (to account for genetic differences in ancestry), age, age squared, gender, and research site. I found an association between earlier AOO and PGS for MDD and later AOO and PGS for SCZ. However, I found no associations when results were corrected for multiple testing, similar to previous literature (Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Stepniak et al., 2014).

The total sample size of this study is comparable to previous studies of AOO and PGS (Ahn et al., 2016; Kalman et al., 2019) and, as my results are generally consistent with previous literature, it may be that there is indeed no relationship between common genetic variants linked to psychiatric conditions and AOO of psychosis. Another possibility is that common genetic variants summarised in PGS are not the only, or potentially optimal, genetic predictors. Copy number variants (CNVs) contribute to schizophrenia and patients with childhood-onset schizophrenia have higher rates of CNVs than both patients with adult-onset schizophrenia and controls (Ahn et al., 2014; Sagar et al., 2013; Vorstman et al., 2006). Alternatively, it might be that my PGS had insufficient prediction power or that my sample is underpowered to detect associations between AOO and PGS. In comparison, associations were recently reported between AOO of bipolar disorder specifically and multiple PGS in a sample consisting of 12,977 participants (Kalman et al., 2021). Additionally, currently there is no single gold standard method for how the PGS itself should be calculated to explain the greatest amount of variance (discussed in more detail in Section 6.3.3.2.).

Finally, while PGS in the current study were not significantly related to AOO, a non-genetic variable of research site was. Previous research on AOO in schizophrenia found that it was not SCZ PGS, but environmental factors, especially cannabis use, which were significantly associated with earlier age at prodromal symptom onset (Stepniak et al., 2014). Also, a higher number of environmental risk factors, especially cannabis use and obstetric complications (very long or very short labour in particular), were associated with earlier AOO in FEP (O’Donoghue et al., 2015). Thus, my results on a relationship between PGS and AOO in psychosis broadly reflect previous findings from FEP and schizophrenia.
However, research site is not a very specific variable and might represent multiple factors. For instance, research site may be related to subtle differences in definitions of AOO (e.g., any first symptoms vs first negative symptoms). Research site may be related to genotyping platforms used at different research sites (see Chapter 2, Section 2.2.6.1. for details, although compare also with Section 2.2.6.2. on quality control which genetic data have undergone before analyses to ensure consistent quality across data batches). Finally, research site may be related to socioenvironmental factors which can influence disease course, e.g., urbanicity (Vassos et al., 2012), neighbourhood characteristics (Ku et al., 2020), or socioeconomic status (Kivimäki et al., 2020). It is unclear which of these factors specifically underlies associations between AOO and research site in my analysis.

### 6.2.2 Duration of untreated psychosis and polygenic scores

Chapter 4 addressed the question of whether duration of untreated psychosis (DUP) is associated with polygenic scores (for SCZ, BPD, ADHD, MDD, and ASD). Previous studies linked polygenic scores for SCZ and BPD to multiple phenotypes in FEP or first diagnosis of psychosis, e.g., case-control status, transition to schizophrenia rather than other psychotic disorders (Vassos et al., 2017), brain structure and function in FEP (Whalley et al., 2012b, 2013), first-episode symptom dimensions (Sengupta et al., 2017), and response to antipsychotics (Santoro et al., 2018; Zhang et al., 2019). However, research on polygenicity of DUP specifically is limited. A single study by Yin et al. (2019b) used GWAS-derived information on gene expression to group schizophrenia patients into three subgroups with different sets of symptoms and characteristics. Subgroup 1, the group with the shortest DUP, had also a higher proportion of positive family history of mental illness, least severe negative symptoms, more favourable disease course, and a better treatment response.

The current study used linear regressions, following study design from Chapter 3 (Section 6.2.1). I tested whether polygenic scores for major psychiatric disorders, calculated at different p-value thresholds, predicted DUP in STRATA-G first episode psychosis data. DUP was defined using different instruments and definitions across research sites (see Table 3 in Chapter 2). DUP was defined as a period from psychosis onset until either start of antipsychotic treatment for psychosis or first hospitalisation for psychosis. PGS for BPD and SCZ were related to a shorter DUP, and PGS for MDD was related to a longer DUP. However, no significant relationships between DUP and polygenic scores (at any p-value threshold) were found after correction for multiple testing. These findings are consistent with the only other study of PGS in prediction of DUP; Ajnakina et al. (2021) also found no association between DUP and PGS for SCZ, MDD and BPD (and
also PGS for intelligence, not tested in my study). My findings, while novel, should be interpreted with some caution, given the already mentioned methodological considerations of predictive value of PGS and potential lack of power of the STRATA-G sample due to its size.

An additional consideration for my findings is how DUP was measured. Golay et al. (2016) showed that different DUP definitions can impact clinical prediction. Researchers used three DUP definitions: DUP as a period between psychosis onset and either initiation of antipsychotic medication; engagement in an early intervention in psychosis programme for young people between 18-35; or combination of engagement in the intervention programme and adherence to medication over the evaluation period (of up to 3 years). Researchers found that DUP calculated based on the most stringent definition (medication adherence and programme engagement), compared to DUP based on the other two definitions, predicted 2 to 5 times more variance in severity of positive and negative symptoms and functional outcome at follow-up of 12, 24 and 36 months. Thus, DUP might be, in fact, a number of distinct phenotypes which potentially need to be explored separately, rather than analysed together as DUP with two different definitions had been in my study. Also, measurement of DUP needs to be more standardised with regards to starting and end points of DUP (a discussion of phenotype measurement in psychiatric genetics follows in Section 6.3.2.)

Findings that DUP related to treatment adherence predicts the most variance in patient outcomes suggests that this specific (sub)type of DUP could be a clinically relevant phenotype to predict. These findings also suggest that, at least for this one subtype of DUP, predictors of adherence to medication or of treatment response could be relevant. I have not examined such predictors in my study. Adherence is difficult to define (partial adherence may or may not have clinical consequences, depending on a patient). Adherence is also difficult to measure (patient self-report is unreliable and more reliable measures of drug levels in plasma/serum are expensive). In other words, no universally reliable and valid predictors of non-adherence exist (Kane et al., 2013). Still, perhaps PGS for treatment response or treatment resistance in schizophrenia (Rees and Owen, 2020) could be an approximate genetic predictor of DUP related to adherence. That is because patients commonly discontinue antipsychotic treatment due to adverse drug reactions or lack of drug effectiveness (Legge et al., 2016). Thus, as treatment resistance may lead to medication non-adherence and so, an extended DUP, maybe PGS for treatment-resistant schizophrenia would be a stronger predictor of DUP related to adherence than the general schizophrenia PGS.

While I found no statistically significant relationships between polygenic scores and DUP, I found a significant relationship between research site and DUP. As already
discussed in previous Section 6.2.1., research site may signify many differences between samples (differences in definitions of DUP, genotyping platforms, urbanicity, neighbourhood characteristics, socioeconomic status), which I have not examined as individual predictors. Differences in DUP have also been reported between participants of different ethnicities. Patients of Black African ancestry had a shorter DUP and patients of Black Caribbean ancestry had a longer DUP, relative to groups of European ancestry (Schoer et al., 2019). However, the current sample had too few individuals of non-European ancestry for a sufficiently powered analysis of differences between groups of different ethnicities. Prolonged DUP has also been linked to increased stigma: shame and fear about the psychosis diagnosis among patients and their families, or lack of reliable information about psychosis from healthcare professionals (Hardy et al., 2020). Finally, there might be differences between samples in prevalence of psychiatric comorbidities. Multiple comorbid symptoms may lead to earlier presentation to mental health services. Extra symptoms may also impact e.g., motivation levels in psychosis patients (addressed in the general discussion of the STRATA sample, Section 6.3.1.1. below). As is the case with the study of AOO, it is unclear which, if any, of these factors underlie associations between DUP and research site in my research, so any interpretations have to remain speculative.

6.2.3 Cognitive decline and polygenic scores

The study in Chapter 5 used linear mixed models to explore if there is a relationship between polygenic scores for SCZ and cognitive skills (memory and executive function) at baseline and over up to ten years in ageing healthy adults. The data come from ELSA (see Chapter 2, Section 2.3), a longitudinal study of ageing. Previous research on cognitive decline in psychotic disorders did not yield consistent results on whether cognitive impairment is progressive (Bora and Murray, 2014; Lynham et al., 2020). Also, few studies addressed factors potentially impacting cognition in psychosis in the context of ageing (Fett et al., 2019). Thus, ELSA is a good sample to explore if genetics of schizophrenia predict cognitive decline. Participants are in the age range (50-101) where cognitive decline would be expected. But the sample is of healthy participants whose health and life changes are closely monitored over follow-ups. Consequently, cognitive change is unlikely to be a by-product of participants’ comorbid illness or medication. In this sample, we found that PGS for SCZ was significantly related to cognitive skills at baseline, but not over follow-up, i.e., SCZ PGS failed to predict cognitive decline. This pattern of results suggests that genetic susceptibility to schizophrenia may confer a developmental deficit, but it may be not related to subsequent cognitive decline. These results are broadly consistent with Liebers et al. (2016)’s findings that SCZ PGS did not predict cognitive decline in the Health and Retirement Study (HRS), a longitudinal study of the ageing
population in the United States (a sister study to ELSA, i.e., an equivalent, using the same measures).

The strength of ELSA comes from a large sample size of ageing participants, followed up for 10 years, tested at two-year intervals to minimise the effects of retesting on test performance. Additionally, because the sample is well-powered, the analyses include multiple covariates linked to cognitive differences in the ageing populations, including age, age squared (to account for non-linear effect of age), gender, principal components (to account for ancestry differences), being a smoker, level of wealth, education attainment, any limiting health conditions, depression diagnosis, and apolipoprotein (APOE) ε4 status, a genetic marker for Alzheimer’s disease.

Our study used linear mixed models to successfully capture cognitive decline and relate it to multiple variables. Recent research called for expanding analyses of long-term data to latent growth curve models, i.e., modelling symptom trajectories (Morgan et al., 2021). Potentially, modelling trajectories of cognitive change would provide an even more detailed picture of relationships between cognition and polygenic scores. For instance, latent growth curve models could be used to model cognitive change from early life, to explore what trajectories of cognitive deficit PGS confer neurodevelopmentally.

6.2.4 Implications

No biological overlap between psychosis manifestations in light of conceptualisations of psychosis: the neurodevelopmental continuum and the neo-Kraepelian notion of schizophrenia. Studies in this thesis found no statistically significant relationships between polygenic scores for major psychiatric disorders and psychosis manifestations: AOO, DUP, and cognitive decline. PGS for schizophrenia was related to baseline cognitive performance (at first measurement in a longitudinal study) in healthy ageing adults. This pattern of findings on cognition potentially contradicts a neo-Kraepelian notion of schizophrenia as a genetically influenced brain deterioration disease. However, findings that schizophrenia and bipolar disorder PGS do not predict even a younger age of psychosis onset may be surprising when considered under the assumption that, instead of progressive deterioration, genetic predisposition for schizophrenia conveys developmental deficits. Non-significant results for PGS for schizophrenia, autism spectrum disorders or attention deficit hyperactivity disorder on AOO are somewhat surprising given genetic evidence for the neurodevelopmental continuum, the concept that severe mental illnesses form a gradient of decreasing neurodevelopmental impairment: intellectual disability, autism spectrum disorders, ADHD, schizophrenia and bipolar disorder (Owen and O’Donovan, 2017). Still, the reason for this
pattern of results might be participant selection, especially of early-onset schizophrenia, given that it is a very rare phenotype (Burd and Kerbeshian, 1987).

Apolipoprotein ε4 and cognitive decline in psychosis. Research from Chapter 5, on genetic factors predicting cognitive decline, included both SCZ PGS and APOE ε4. APOE ε4 allele is a well-established risk factor for Alzheimer's disease (Serrano-Pozo et al., 2021), but not in psychosis. Results on a relationship between APOE alleles and schizophrenia have been non-significant in candidate gene studies, meta-analyses, and a large-scale schizophrenia GWAS (Farrell et al., 2015; González-Castro et al., 2015). More recently, Jonas et al. (2019) argued that APOE ε4 is a psychosis risk factor, but it is age-related. Researchers analysed data from patients with psychosis followed for up to 20 years and found that APOE ε4 was related to worsening severity of hallucinations and delusions after age 40, but not in earlier life. However, as researchers admitted, their results might be due to attrition (participant drop-out): APOE ε4 carriers, because of their trajectory of worsening psychotic symptoms, may be more involved in psychiatric services than other cases and so, more frequently enrolled in research. Another limitation of the study is its sample size of 116 individuals. Findings would need to be replicated in larger samples, ideally representing a greater range of cases with psychosis.

Other recent research, including the study in Chapter 5, addressed healthy individuals without psychosis or dementia. As in our study, others found a significant relationship between cognitive decline in later life and APOE ε4 (Liebers et al., 2016; Ritchie et al., 2020). These studies suggest that APOE ε4 status potentially predicts specific types of cognitive decline beyond dementia, transdiagnostically (e.g., both our study and Liebers et al. (2016) found a statistically significant relationship between verbal memory decline and APOE ε4).

However, a recent study by Henson et al. (2020) calls for a more cautious interpretation. The study is a preregistered report (a study with analysis plan prespecified before data analysis and submitted to a registry so that the analysis plan cannot be changed in reaction to findings before the paper goes to peer review; see Section 6.3.4. Open science below). The study was completed on 610 healthy individuals without dementia. The sample covered adult lifespan (participants had the age range of 18–88 years). Henson and colleagues found no evidence for an age-specific interaction between different types of APOE ε alleles and 6 global measures of brain and cognitive health. An exception was a weak interaction between age and carrying the ε4 allele on decline in fluid intelligence in older age. However, when data were analysed using frequentist statistics, the result did not survive correction for multiple comparisons; when data were analysed using Bayesian statistics, Bayes Factors were around 2, indicating no support for the predicted direction of interactions. As the sample was sufficiently powered to detect effects, researchers overall
questioned effects of \textit{APOE} on cognition across the lifespan, at least in healthy adults. These results suggest that additional research is required on trajectories of change in specific cognitive abilities across the lifespan and in relation to long-term disease course trajectories as well (Martin et al., 2019a).

\textit{Limited identification of psychosis subtypes using common genetic variant information.} Recent research linked genetic information, DUP and AOO. Yin et al. (2019b) found that genetic information predicted subgroups of patients with different DUP and sets of symptoms and Musket et al. (2020) found a significant genetic correlation between schizophrenia AOO and negative symptoms (genetic correlations suggest pleiotropic effects of genes on AOO and schizophrenia symptoms; van Rheenen et al., 2019). In contrast, I found no associations between PGS and psychosis manifestations, associations which would enable the sub-grouping of patients based on their higher genetic propensity towards a specific phenotype.

Altogether, findings of this research suggest a currently limited clinical applicability of polygenic scores as predictors of specific psychosis manifestations, as explored in this thesis, and point to a potential role of specific monogenic predictors, such as \textit{APOE}, and to importance of environmental predictors in models. Consequently, it is worth discussing potential reasons for which polygenic scores were of somewhat limited value in this thesis.

\section*{6.3 Strengths, limitations, and methodological considerations}

\subsection*{6.3.1 Longitudinal cohort studies}

Studies in this thesis used two large-scale samples. Strengths and limitations of the longitudinal cohort design of each sample are discussed in turn.

\subsubsection*{6.3.1.1 STRATA-G}

STRATA-G is the sample of longitudinal psychosis data, commencing at FEP. Two studies based on this sample (Chapter 3 and 4) did not use longitudinal data, but information reported at first episode - AOO and DUP.

STRATA-G is a rich resource which provides multiple data related to health, demographics, and genetics underlying psychosis, with a focus on treatment-resistant psychosis.
The sample combines data from multiple sites in Europe and Brazil which potentially increases generalisability of findings from STRATA-G. The sample represents a wider range of individuals with psychosis than a single sample from one country would.

My studies based on STRATA-G data focused on phenotypes which were not measured longitudinally and are generally related to early stages of illness. Thus, attrition is potentially minimal in the STRATA-G data analysed in this thesis. Also, as the data are on FEP, they are related to a narrow time frame within tight catchment areas (compare with Table 1 in Chapter 2). These features of the sample may limit the impact of selection bias.

However, a potential source of selection bias still is that it is not clear if patients who consent to taking part in research differ from those who do not. In randomised controlled trials, participants with positive symptoms were more suspicious towards researchers and participants with negative symptoms had a lower motivation to participate (Lester and Wilson, 1999). Also, individuals at clinical high risk for psychosis may have greater motivational deficits in early stages of illness due to depressed mood, compared to participants diagnosed with schizophrenia (Schlosser et al., 2014). These findings suggest a caveat that patients who do not consent to taking part in research on early stages of psychosis may indeed differ from those who consent, e.g., in terms of initial symptoms or potential comorbidities. Thus, available samples may not represent the full range of patient experience of early psychosis.

My studies only include data from cases and do not follow a case-control design. This design has been criticised for examining psychiatric phenotypes in genetic research because it can often lead to the comparison of severe cases with very healthy “supernormal” controls. This may can lead to overestimated predictions (Janssens et al., 2011). However, recent research suggests that the use of “supernormal” controls might not impact every genetic study equally strongly: the strength of bias may depend on prevalence of the disorder under investigation (bias may increase with increasing population prevalence; Kendler et al., 2020).

Janssens et al. (2011) also criticised genetic case-control study designs as not reflecting the real-life populations of patients and controls who may potentially have early symptoms or be exposed to multiple risk factors. Although my analyses of STRATA-G data did not implement a case-control design, this criticism potentially applies. My analyses do not address a large number of risk factors. Janssens et al. (2011) also argued that patients with severe disease have been overrepresented in psychiatric genetics research. While not all data analysed in Chapters 3 and 4 come from participants with treatment resistant psychosis, these participants potentially fit the description of the “severe” end of the
patient continuum. The final issue with data commonly used in genetics is the danger of analysing data collected at multiple sites, as is the case of STRATA. There might be multiple sources of variability or bias which may not be documented and made known to all investigators who eventually analyse combined datasets (Lambert and Black, 2012).

6.3.1.2 ELSA

ELSA is a longitudinal population sample of older adults. These features of ELSA were utilised in this thesis as my study addressed cognitive decline (change in cognitive ability over a decade). The design of ELSA has multiple strengths. ELSA has been selected to reflect the population of healthy individuals aged 50+ in England. The sample is periodically refreshed, i.e., additional younger cohorts are enrolled to account for participant ageing and deaths over time. That way, the sample consistently represents the full spectrum of later life, from late middle age to old age. An additional strength of ELSA is that it is an extremely rich data resource. It includes hundreds of variables collected at multiple waves (multiple follow-ups every two years) which can be used to answer a wealth of questions, relevant to policy, on health and life changes in the ageing population. ELSA also offers a degree of flexibility in data collection, in that additional modules with extra questions can and have been introduced in subsequent waves, e.g., on disability and sexual experience (see also https://www.elsa-project.ac.uk/the-data-we-collect for a detailed list; wave 0 refers to responses to the Health Survey for England, HSE, a cross-sectional survey of health in the general population. Wave 1 was recruited from HSE Zaninotto and Steptoe, 2019). Additionally, not only does the sample have multiple variables, but it is also well-powered for analyses given the large sample size (the sample used in research in Chapter 5 was of 6817 participants). Finally, as discussed above, a potential issue with samples in genetic research is that samples fail to represent a broad range of patients and reflect the general population. The ELSA sample is specifically designed to reflect the ageing population, by including people from different age groups, different wealth groups etc., which potentially limits selection bias.

However, a potential issue with ELSA is attrition and how to assess its extent. As detailed in the ELSA cohort paper (Zaninotto and Steptoe, 2019), response rates are complicated to analyse. ELSA includes multiple question modules (see Section 2.3.3., Chapter 2) and participants may respond to some, but not all, modules, or respond in some, but not all, waves. Also, there are differences in responses between the core (the original sample) and refreshment cohorts. Finally, the question remains of how non-responses to the original HSE should be counted, given that participants in the first wave of ELSA were recruited from the HSE, but it was not the start of ELSA proper. Nonetheless, ELSA documentation provides a detailed breakdown of deaths and response rates in
reports for each wave for transparency (downloadable for free from https://www.elsa-project.ac.uk/wave-reports). Also, attrition in ELSA is potentially remedied to an extent by including regular refreshment samples in the general ELSA sample.

Having discussed strengths and limitations specific to each sample, I will now turn to issues underlying every study in this thesis: the issue of how to define psychiatric phenotypes and how it may impact genetic research (Section 6.3.2.); strengths, limitations, and methodological considerations in research using polygenic scores (Section 6.3.3.); and the extent to which research in this thesis followed open science practices (Section 6.3.4.).

### 6.3.2 Defining complex phenotypes in psychiatric genetics

An ongoing discussion in genetics research is how defining phenotypes in GWAS and post-GWAS analyses influences potential findings. The initial strategy of the Psychiatric Genomics Consortium was to obtain case vs control data from multiple cohorts, with “fast phenotype characterization” (Sullivan et al., 2018), i.e., with general phenotypes (research diagnoses) such as schizophrenia or depression, not characterised in greater detail. This strategy rapidly increased sample sizes in the Consortium and sufficiently powered samples to yield statistically significant GWAS results.

However, recent work questioned this approach. (While presented primarily on the example of depression, the arguments apply to psychosis as well, as both depression and psychosis are complex phenotypes.) GWAS analyse data from participants with the same general diagnosis, but whose individual phenotypes are very different. Patient diagnoses might be determined using different measurements, potentially have very different aetiologies (contributions of environmental, genetic and other factors to disorder), and have different clinical features (i.e., different combinations of symptoms, severity, developmental timing, comorbidities, physiology, onset, or number of episodes; Cai et al., 2020). For some disorders, diagnosis is difficult. For example, childhood-onset schizophrenia is very rare and controversial as there is no clear agreement what symptoms or behaviours constitute clinical “psychosis” in children (Bartlett, 2014). Finally, the issue with phenotyping based on a general diagnosis is that particular diagnostic labels may be given (or not given) due to bias (e.g., race- or gender-specific stereotyping in disease definition, bias which reduces access to healthcare, or bias which leads to labelling only individuals with severe symptoms as “cases”). This misdiagnosis may mean that “control” populations in GWAS (diagnosed as healthy) include “true” cases. This bias may result in false conclusions (Dueñas et al., 2020).
In light of these considerations, research in this thesis could be criticised for using summary statistics from recent GWAS of case/control status of major psychiatric disorders. PGS based on current GWAS summary statistics for a single disorder could potentially be considered too “general” and for overly broad phenotypes. It might be that using multiple PGS separately representing genes predisposing for more granular phenotypes (e.g., specific symptoms) would be more informative (Cai et al., 2020). Granular PGS would potentially describe biology of symptoms with greater specificity. (Subsection Validity of polygenic scores in Section 6.3.3.2. below also discusses an extra argument as to why more granular PGS should be used, although the argument addresses PGS created to represent specific biological pathways.)

This thesis addresses one aspect of the issue of the overly broad phenotyping, by not analysing how PGS predispose to case/control status in target samples (STRATA-G and ELSA). Instead, studies here addressed genetic contributions to specific psychosis manifestations. However, it could be argued that quality of phenotype measurement differed between phenotypes.

6.3.2.1 Measurement of age of onset in STRATA-G

AOO measurement is complicated by a number of issues, which also apply to AOO collected in STRATA-G. The first issue is a variety of clinical presentations of psychotic disorders (Dagani et al., 2018). In STRATA-G, different diagnostic instruments were used to determine AOO, depending on the site of an original study (see Table 2.2 in Chapter 2). The majority of criteria overlapped between sites, and onset of psychosis was defined as presenting with at least one symptom out of hallucinations, delusions, bizarre behaviour or disorganised speech. However, several diagnostic instruments used in original STRATA-G studies (but not all) also list additional symptoms as used to define psychosis onset. Those symptoms form a heterogenous list of psychomotor disorders, catatonia, lack of insight, lack of pleasure, difficulties with concentration, depressive/sad mood, and elevated mood or mania. Subsyndromal mood changes (not sufficiently severe to be labelled as a symptom) can be difficult to distinguish from non-clinical mood fluctuations (Dagani et al., 2018). Also, not all diagnostic instruments used in STRATA-G samples specified how severe the symptom has to be or how long it has to be present to be considered a symptom of psychosis and not, e.g., a premorbid state (state prior to onset of disease) or part of an insidious onset (gradual, slow onset).

The second issue in measurement of AOO is no agreed, consistent definitions of illness onset (Dagani et al., 2018). It may be difficult to compare my findings with previous research due to different definitions of onset present in epidemiological studies: first
symptoms (as in my thesis), but also first psychiatric diagnosis and first hospitalisation (Solmi et al., 2021).

The third issue in measurement of AOO is recall error (Dagani et al., 2018). Depending on STRATA-G research site, AOO was determined in two ways: either through patient interview (self-reported) or patient interview corroborated with informants and medical records. Obtaining AOO from patients, retrospectively, may result in recall which is not accurate. Still, corroborating AOO from patient interviews with family interviews and medical records may potentially improve accuracy of collected AOO.

However, recent GWAS of AOO suggest that different ways in which AOO is ascertained result in different findings on associations between AOO and genetic variants. In their study of large-scale UK Biobank and FinnGen data, Feng et al. (2020) addressed AOO of multiple (psychiatric and non-psychiatric) disorders. AOO for each medical condition was captured three times, in three different ways: self-reported age of diagnosis; age of first in-patient ICD-10 diagnosis or hospitalization episode from hospital in-patient records; or age of the earliest event combining self-report, in-patient, primary care, and death records. The three groups of differently captured AOO had different distributions. Also, different numbers of significant GWAS hits were implicated in relation to each AOO group, potentially due to recall bias in AOO collection and/or heterogeneity of AOO definitions. Altogether, this research suggests that studies of AOO measure different phenotypes, which, while they may occur in close temporal succession, are not the same events. This heterogeneity of AOO likely explains null findings in my study in Chapter 3 as well.

6.3.2.2 Measurement of duration of untreated psychosis in STRATA-G

DUP was defined differently between STRATA-G samples, as a period between either psychosis onset and hospitalisation, or psychosis onset and start of antipsychotic medication (see Table 2.3 in Chapter 2 for additional details). Issues with measurement of DUP can be broadly divided into three categories: the issue of defining and reliably measuring the starting point of DUP; the issue of defining and reliably measuring treatment (the end point of DUP); and imprecise operationalisations of what constitutes psychosis occurring between onset and treatment (Compton et al., 2007).

As discussed in the section above, there are multiple issues with a clear demarcation of AOO, which also impacts DUP, as AOO is its starting point. Different diagnostic criteria were used in STRATA-G to determine onset of psychosis which adds to the heterogeneity of the phenotype. The end point of DUP was also defined in different ways (starting antipsychotic medication or hospital admission). These differences in definitions may mean
that at least two different phenotypes (or more) were analysed as a single DUP group, while they potentially should have been analysed separately as different phenotypes (see Section 6.1.2. for a discussion of how different measurements of DUP, based on different definitions, result in different predictions of patient outcomes). However, at least the end points of DUP in different STRATA-G samples may be very similar—starting antipsychotic medication likely occurs very near to hospital admission. Still, retrospective recall of DUP, similarly to recall of AOO, may be impacted by recall bias and, further, by acute psychotic symptoms, disorganisation or cognitive impairments present at the time of assessment (Compton et al., 2007). Also, using initiation of antipsychotic medication as the end point of DUP measurement has been criticised, as a single medication dose is not adequate treatment (Kaar et al., 2020).

Finally, definitions of DUP lack specificity on how psychosis, occurring between onset and treatment, is operationalised. DUP has largely not addressed the frequency or severity of psychotic symptoms before treatment, with the definition of DUP including an implicit assumption that DUP is a continuous illness interval, with persistent symptoms throughout (Compton et al., 2007). As Compton et al. (2007) point out, psychoses of the same duration may represent different overall “doses” of psychosis. For instance, psychoses with more acute onset and worsening, or psychoses with a gradual, or not so acute onset, and worsening, or psychoses which manifest with a combination of different symptoms, each symptom of varying severity and intensity. Measurements of DUP in STRATA-G did not consistently account for severity, frequency of episodes, or mode of onset, as this information was not measured in all of the STRATA-G samples.

6.3.2.3 Measurement of cognitive decline in verbal memory and executive function in ELSA

Cognitive decline in verbal memory and executive function in ELSA was measured with well-established cognitive tests which have been validated in previous studies (Fancourt and Steptoe, 2019; Yin et al., 2019a). These cognitive measures have also been harmonised with (implemented across) other large longitudinal studies of ageing, such as the already mentioned HRS in the US. This means that findings could be straightforwardly replicated in different datasets as measurements are consistent across datasets. Indeed, our findings on no link between verbal ability decline and SCZ PGS are consistent with what Liebers et al. (2016) reported in HRS.
6.3.3 Polygenic scores

Polygenic score is “beguiling in its simplicity” (Lewis and Vassos, 2020): PGS has an intuitive appeal in that it is easy to follow how PGS models polygenicity (Bogdan et al., 2018). That makes a polygenic score a tool which can be easily generated (Chapter 1, Section 1.5) and thus potentially applied widely, as PGS have been increasingly used to address many psychosis manifestations (Section 1.6). On the other hand, PGS could potentially be considered simplistic. Consequently, this section provides a brief appraisal of polygenic scores, to discuss reasons for which results of this thesis should be interpreted with a degree of caution.

6.3.3.1 Strengths of polygenic scores

Immutability of polygenic scores as a measure. The well-established potential strength of polygenic score is its immutability, or that DNA does not change throughout life. This quality of DNA means that participants could be genotyped once and additional or improved PGS calculated for them, as necessary, or as technology progresses for more refined PGS to become available. This feature of PGS also means that a fixed molecular characteristic can be explored while tracing more dynamic characteristics, e.g., progressive change in a phenotype of interest, taking participants’ genetics into account (Harden, 2021). Consistently, this thesis applied polygenic scores as predictors of cognitive change over a decade.

Polygenic score as a basic screening tool for clinical populations. PGS are currently not highly predictive for psychiatric phenotypes in the general population (Lewis and Vassos, 2020, i.e., PGS explain a limited amount of variance attributable to genes). However, Lewis and Vassos (2020) argued that recent research suggests that PGS could be a basic screening tool for specific clinical populations and diagnostic situations. For instance, in FEP, PGS differentiated schizophrenia from other psychosis diagnoses in the top quintile of PGS (Vassos et al., 2017). Perkins et al. (2020) demonstrated that schizophrenia PGS differentiated individuals with high risk for psychosis who then convert to psychosis from non-converters. PGS also modestly improved individual psychosis risk prediction when added to a psychosis risk calculator with non-genetic predictors (stressful life events, trauma, cognitive performance measures, and family history of psychosis). Consistently with this argument, that PGS can be used for discrimination between psychosis subphenotypes in specific populations, this thesis addressed genetics of specific psychosis manifestations.

Similarly to Lewis and Vassos (2020), Wray et al. (2021) argued that PGS could be used in early stages of illness with nonspecific, general symptoms which do not fit a specific
diagnosis to help with clinical decision-making, or to support triage and clinical staging. In a separate review, the same authors (Murray et al., 2021a) also suggested that, for mental health disorders where early intervention improves prognosis, PGS could contribute to risk scores and be combined with basic observational data to trigger in-depth patient assessment. Research in this thesis focused on specific, clinically relevant populations: of psychosis patients at FEP, who would benefit from early intervention, and individuals likely to develop illness or experience change in cognition due to age.

*PGS may (currently) not work as strong individual predictors in psychiatry, but they might be useful in conjunction with copy number variants or environmental information.* CNVs have greater penetrance than common genetic variants, i.e. carrying variants which have greater penetrance is more frequently related with expressing a phenotype, e.g., a disorder (Vassos et al., 2010). In other words, the contribution of CNVs to disorder is greater than of PGS (measures of common genetic variants). Nonetheless, as Lewis and Vassos (2020) argued, PGS still affect risk of disease. Joint contributions of common genetic variation and CNV variation are increasingly explored. Recent studies show that CNV carriers with schizophrenia have higher PGS than healthy controls (Bergen et al., 2019; Tansey et al., 2016) and, among patients with schizophrenia, patients who are CNV carriers have lower PRS than patients who are non-carriers (Bergen et al., 2019). Alternatively, PGS could be used in conjunction with measures of clinical staging or non-genetic risk factors, e.g., measures of stressful life events, trauma, neurocognitive performance, brain imaging or immune biomarkers (Wray et al., 2021). Indeed, this is how PGS have been implemented in the study in Chapter 5, with multiple non-genetic variables relevant to cognitive decline. However, none of the studies in this thesis addressed CNVs.

### 6.3.3.2 Limitations of polygenic scores

*Using polygenic scores to distinguish between phenotypes.* As outlined above, PGS have been used to distinguish between types of phenotypes (e.g., subphenotypes of bipolar disorder and schizophrenia; Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2018; Ruderfer et al., 2014). However, Bogdan et al. (2018) argued for a degree of caution in interpreting such findings. Given shared neural mechanisms across disorders, common genetic variation conferring liability to various disorders is also shared between disorders. Thus, hypotheses on how specific genes are to each disorder have to be clear and nuanced. Potentially, my findings could be criticised on these grounds, that they do not clarify specific biological underpinnings of psychosis symptoms sufficiently.
Statistical power of genome-wide association studies and polygenic scores based on findings of these studies. If different GWAS, used to construct each PGS, are not equally robust (all sufficiently large), that may hinder comparisons between PGS. That is because PGS are based on original GWAS with different statistical power and thus each such PGS may have a different predictive power (Bogdan et al., 2018). However, as GWAS sample sizes increase, so will predictive power of PGS based on summary statistics from such large GWAS (with increases in discovery sample sizes, the estimation error of each SNP effect size shrinks, and that improves predictive power of the PGS; Chatterjee et al., 2013). In light of these considerations, PGS in this thesis have two limitations. The first limitation is that PGS came from GWAS with different sample sizes. Another limitation is that SCZ-PGS and BPD-PGS are not based on the most recent, largest PGC data freezes available. SCZ-PGS are based on the second freeze of the PGC schizophrenia data (PGC-SCZ2 data; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) and BPD-PGS are based on the second freeze of the PGC bipolar disorder data (PGC-BIP2 data; Stahl et al., 2019). These freezes are smaller and less ancestrally diverse than the most recent data freezes, PGC-SCZ3 (The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020) and PGC-BIP3 (Mullins et al., 2021). However, the third PGC data freezes were released after analyses in this thesis had been completed.

Validity of polygenic scores. Validity indicates if the test measures what it intends to measure (Terwee et al., 2007). Four common types of validity are: face validity, if the measure seems to intuitively make sense; construct validity, what a construct is that needs to be measured; content validity, what should be measured to reflect the construct; and criterion validity, to what extent measures correlate with other measurements which they are expected to be related to. Janssens (2019) analysed how validity applies to polygenic scores. I briefly summarise these considerations below and, where applicable, follow with a comment on how they relate to studies from this thesis.

PGS have face validity as they have intuitive logic (Janssens, 2019). However, PGS have also been criticised as too simple of a proxy of genetic liability. A single score potentially results in a loss of information about an individual’s unique genetic profile, which is likely especially important for predicting complex, heterogenous disease or treatment response (Choi et al., 2021).

PGS construct validity depends on whether adding risk alleles up into a single score reflects theoretical models of genetic liability. Janssens (2019) argued that there is potential concern over whether the additive model underlying PGS is correct. It may not correctly capture polygenic liability (Nelson et al., 2013). Also, non-additive models fit genetic data well when effect sizes of loci are small (Moonesinghe et al., 2011; Wray and Goddard, 2010).
These findings would suggest that construct validity of PGS is questionable. However, more recent research on genetic variance in 254,679 unrelated individuals from the UK Biobank suggests that non-additive genetic variance is not a strong contributor to complex traits (Hivert et al., 2021). Still, genes are also involved e.g., in gene-gene interactions or interactions with environmental factors. PGS, including PGS in this thesis, do not address those genetic interactions/models, which potentially limits PGS construct validity to some extent.

The second source of potential issues with construct validity is the inclusion of thousands of single nucleotide polymorphisms (SNPs), which have weights of multiple zeros after the decimal. Janssens (2019) argued that the majority of these SNPs, the ones which are non-significant in GWAS, may improve prediction of PGS very marginally. If contributions of thousands of SNPs are negligible, then strategies for constructing PGS with those SNPs should be clearly justified. In STRATA-G (Chapters 3 and 4), I used PGS which match Janssens’ recommendation and included SNPs at multiple significant p-value thresholds. In ELSA, we used a single PGS constructed with all available SNPs (GWAS p-value threshold of 1). We followed this strategy after it had been first shown that PGS based on all available SNPs explain the largest amount of phenotype variance in HRS, ELSA sister study of a large ageing population in the US (Ware et al., 2017).

PGS criterion validity depends on whether PGS predict disease status (predictive validity) and correlate with measures which they are expected to correlate with (concurrent validity). PGS currently have modest predictive validity but they may have concurrent validity, depending on the modelling implemented (Janssens, 2019). Janssens argued that PGS may not have independent effects on disorder when combined with clinical risk factors or early symptoms as predictors. That is because SNPs may be related to disorder through those clinical risk factors (risk factors serve as intermediate variables). If PGS is put in a prediction model with clinical risk factors, factors which the PGS also predisposes to, SNP effects are conceptually counted twice, through the clinical risk factor and in the PGS itself.

Consequently, Janssens argued that PGS should either be put in models only with factors which do not correlate with PGS (e.g., age and sex), or PGS be developed in a new way, so that they measure a “residual” part of genetic contribution, i.e., a part which is not captured already by including clinical risk factors in the model. Janssens suggested creating multiple pathway-specific PGS which could be then adjusted for clinical variables. This way, modelling could explore if clinical risk factors mediate associations between particular pathways and disease risk. Given this reinterpretation of how modelling with PGS might need to be done, a criticism of my studies could be that none implemented pathway-specific PGS. These PGS could potentially be more precise in pinpointing which
gene groups predispose to psychosis phenotypes. Nonetheless, implementing multiple
PGS would also be problematic in the STRATA-G sample because of the sample size and
decrease in statistical power.

PGS may have content validity, depending on how they are constructed. Janssens
(2019) pointed to the issue of GWAS weights. GWAS weights may be overestimated,
especially if meta-analysed. Sizes of GWAS weights will likely differ between studies
depending on how different original GWAS are planned and if the GWAS weights reflect
the population the subsequent PGS (based on meta-analysed GWAS) is supposed to
describe, e.g., in terms of ancestry or disease status. Samples used in this thesis used
pooled PGC samples as discovery samples for PGS calculation. Nonetheless, meta-analyses
of GWAS findings could be defended as not presenting overestimated effect sizes, as there
are strict quality control standards to prevent errors at every level of meta-analysis (the
study file level, the meta-level across studies and the meta-analysis output level; Winkler
et al., 2014).

No optimal methodology for PGS calculation. As outlined in Chapter 1 (Section 1.5),
there are multiple methods of creating polygenic scores. They can be broadly divided into
two classes, clumping and tresholding (C+T) and methods which perform shrinkage of all
SNPs/model linkage disequilibrium (LD) patterns. In C+T, the only SNPs included in
the calculation of a PGS are SNPs which have an association in GWAS below a certain
prespecified p-value threshold (e.g., \( P < 5 \times 10^{-8} \)). PGS are calculated at multiple p-value
thresholds because the optimal p-value threshold (at which PGS explains the largest
amount of variance in prediction of a trait) is not known a priori (Choi et al., 2020). An
example of a tool implementing C+T in polygenic scoring is PRSice (Choi and O’Reilly,
2019; Euesden et al., 2015), as used in this thesis.

A potential strength of C+T is that it is a relatively straightforward method which
does not require complex Bayesian modelling with additional data (e.g., information on
LD structure). Only the basic GWAS discovery summary statistics and target (validation
sample) data are required. Also, as Pain et al. (2021b) demonstrated, software for
more complex polygenic scoring may encounter technical issues with processing data,
which may make reproducing and comparing results of genetic analyses challenging. In
contrast, software for C+T has been used widely, e.g., PLINK (Chang et al., 2015), a
well-established genetic analysis tool (Lin, 2020), or PLINK-based PRSice (Choi and
O’Reilly, 2019; Euesden et al., 2015). In comparison to recent polygenic scoring methods,
C+T is also the fastest (the least computationally demanding) method of PGS generation
(Pain et al., 2021b). However, a limitation of C+T is that it requires calculating multiple
PGS at different GWAS p-value thresholds. These PGS need to be tested in analyses with
a predicted trait of interest, and then, these multiple comparisons need to be corrected
to prevent false positives (as in Chapters 3 and 4). C+T has also been criticised for discarding LD information which limits prediction accuracy of the final PGS (Vilhjálmsdóttir et al., 2015).

Another class of polygenic scoring methods are methods which perform shrinkage of all SNPs (reduction of their effect sizes) and frequently control for LD by modelling LD patterns. Examples include Bayesian methods such as LDpred (and its updated version, LDpred2) or PRS-CS. LDpred (Vilhjálmsdóttir et al., 2015; Prívé et al., 2020) uses a prior on an effect size of each SNP and LD information from an external reference panel to infer the posterior mean effect size for each SNP, with all SNPs then summarised as a PGS. PRS-CS (Ge et al., 2019) performs shrinkage by placing continuous shrinkage priors on SNP effect sizes (the amount of shrinkage applied to a particular SNP is specific and adaptable to the strength of association for that SNP in GWAS, as based on GWAS summary statistics). Beyond continuous shrinkage, PRS-CS also uses the external LD reference panel to model local LD patterns. PGS-CS updates effect sizes for all SNPs in each LD block together, not for each SNP separately, for greater accuracy of local LD pattern modelling. This information on priors and LD is used together to infer posterior effect sizes of SNPs, eventually summarised as PGS.

An advantage of shrinkage methods over C+T is that, where C+T requires multiple calculations of PGS and then comparisons of different models to find the most predictive PGS parameters (the default is of up to 10 p-value thresholds being tested), shrinkage methods use a comparably small number of parameters. For instance, PRS-CS (Ge et al., 2019) uses 4 default global shrinkage parameters which are tested in a validation dataset to produce the most predictive PGS (Pain et al., 2021b). PRS-CS also includes a version called PRS-CS-auto where a single parameter is automatically learnt from GWAS summary statistics (Ge et al., 2019). This option limits parameter testing required. (However, Ge and colleagues also caution that PRS-CS-auto does not work well in analyses where the genetic architecture is sparse, i.e., involves a small number of causal variants, and where GWAS sample sizes are small, of less than 10,000 cases, although the performance of PRS-CS-auto improves as sample sizes increase.) A disadvantage of shrinkage methods is that they can be computationally challenging and may provide inaccurate adjustment for local LD patterns (Ge et al., 2019).

There is also no established gold standard for polygenic scoring yet. Recent studies assessed multiple methods to benchmark which one generates the most predictive PGS, but findings were not consistent. Kulm et al. (2021) tested 15 PGS calculation methods in the UK Biobank data for 25 traits (including depression and schizophrenia). Researchers reported that PRS-CS, followed by lassosum and LDpred2, provided the greatest average area under the curve improvement (i.e., improvement in the difference in area under the
receiver operating curve for two near-identical logistic regression models, which share the covariates of sex, age and principal components, but one of the models also has a PGS included). Ni et al. (2021) tested 10 methods of PGS calculation in the PGC data on schizophrenia and depression. They found that PGS explaining the greatest percentage of variance were generated with MegaPRS, LDpred2, and SBayesR. All methods performed better than pruning and thresholding (a basic method similar to clumping). In contrast, SBayesR was not found to be one of the top methods in the Kulm et al. (2021) study. Finally, Pain et al. (2021b) tested 8 PGS calculation methods on the UK Biobank and the Twins Early Development Study data for 15 phenotypes (including depression and ADHD). Pain and colleagues found that, on average, the most predictive PGS were calculated with LDpred2, lassosum and PRS-CS and that all methods performed better than pruning and thresholding (although SBayesR had convergence problems if its default settings were implemented, i.e., the SBayesR algorithm would conflict with the data and so analyses would not be completed or would give an erroneous SNP-based heritability estimate of 1).

However, it is somewhat difficult to compare findings across these studies. Although all researchers tested at least some of the same PGS generation methods, researchers did not all test the same full range. Also, Pain et al. (2021b) did not test schizophrenia as an outcome. Of psychiatric phenotypes, they only tested ADHD and depression. Additionally, samples with specific phenotypes had different sizes across studies, which may impact power. For example, the schizophrenia sample in the Kulm et al. (2021) study was 704 cases, while the PGC sample in Ni et al. (2021) included 31,000 cases with schizophrenia and 41,000 controls and the Pain et al. (2021b) study analysed 25,000 cases with depression and 25,000 controls. Finally, while researchers tested some of the same methods for initially generating PGS, each group used different methods of benchmarking PGS performance, i.e., implemented PGS in different types of statistical models. Still, taken together, these findings suggest that newer, more complex Bayesian methods may result in more predictive PGS than basic clumping/pruning and thresholding.

Populations of non-European descent are underserved. As discussed in Chapter 1, results of GWAS studies and, by extension, content of the PGS, will be different in different populations because of population differences in LD, allelic variation at SNPs associated with a phenotype, and admixture (Curtis, 2018; Lam et al., 2019). However, GWAS and PGS studies have shown Eurocentric bias. The majority of data come from participants of European ancestry, with 72% of discoveries reported in data from UK, Iceland and the US (Mills and Rahal, 2019). PGS derived from European ancestry data show poor accuracy in non-European ancestry data. This bias, unless addressed consistently, may exacerbate health disparities (Martin et al., 2019b). A limitation of my thesis is that the majority of work was completed on participants of European ancestry (most STRATA-G data and all ELSA data come from participants of European descent). Also, I did not implement recent
methods for generating PGS, such as PRS-CSx (Ruan et al., 2021), and these methods are geared towards improving predictive power of PGS in trans-ancestral samples.

6.3.4 Open science

Open science has been defined as a collection of actions and as a movement advocating for a more transparent and reproducible research conduct. To this end, researchers and the general public should be given free, easy access to research materials and findings to evaluate and use the research (Nosek et al., 2015; Spellman et al., 2018). The argument for implementing open science is that it would increase credibility of available research. Open science would limit waste of funds on pursuing findings which do not replicate, and on research which is potentially false due to uncaught error, or unclear, incorrectly selected, or fraudulent data analysis practices. Open science would also accelerate discovery by fostering collaboration among researchers (Munafò et al., 2017). Below, I list key open science practices, provide a brief definition of each, and comment on the extent to which research in this thesis has met these standards.

Protecting against cognitive biases: tendency to see patterns in data, where there are none, is often inspired by preexisting hypotheses (Munafò et al., 2017). A potential solution is blinding of data during data cleaning or analyses. However, none of the studies in the thesis were blinded.

Preregistration: registration of the basic study design, primary outcomes, and statistical analysis plan before the data are available and outcomes are known (Munafò et al., 2017). Analyses for this thesis have not been preregistered.

Improving methodological training and independent methodological support: ensuring that research uses correct, up-to-date methods (Munafò et al., 2017). Research in this thesis was supported by a diverse team of supervisors and collaborators (from psychiatry, genetics, bioinformatics and epidemiology) who provided feedback on optimal analysis techniques before the research commenced and in the process.

Collaboration and team science: collaborative work leads to large-scale, sufficiently powered research and increases diversity of research perspectives (Munafò et al., 2017). As described above, the research was highly collaborative. Additionally, it implemented large samples, representative of the English ageing population (ELSA) or reflecting different European first-episode psychosis patient populations (STRATA-G).

Reproducibility: making data, software, research materials and research procedures easily accessible so that results can be re-derived by others (Munafò et al., 2017). Re-
CHAPTER 6. DISCUSSION

prodicibility can mean a spectrum of actions to assess if results and conclusions hold upon reanalysis. Reproducibility is generally defined as using the same openly available code, data and procedures from a publication, as the publication authors used originally. Ideally, the code and data should be linked to the original publication, so that the whole analysis can be easily executed. Another end of the spectrum of reproducibility, and the gold standard, is replicability. Replicability means repeating the analyses in new, independently collected data (Peng, 2011). In genetics, there are multiple aspects to consider for reproducibility. One is reproducibility of data, depending on if genetic data are of consistent quality (Lin, 2020). Indeed, research in this thesis used established, standardised data quality control protocols, e.g., Marees et al. (2018). The other aspect is harmonising phenotypic data, or ensuring that phenotypes are defined and measured in a standardised, consistent way across the dataset(s) (Thorisson et al., 2009). This issue has been discussed in Sections 6.1.3. and 6.3.2. above. Briefly, data from ELSA uses harmonisation of measures with other longitudinal studies of ageing, which makes replication of ELSA findings possible and straightforward. Research from STRATA used different phenotypic measures and different diagnostic criteria across research sites, which potentially makes replicability and reproducibility more challenging.

The ELSA data are available open access (freely downloadable), but STRATA-G data are not. However, I am now completing a detailed documentation report on the data available in STRATA-G which will be timestamped and placed on Open Science Framework (https://osf.io/), an open science platform, to ensure greater transparency of what data are available in STRATA.

In terms of reproducibility of results, the other important aspect is how reproducible code is. Both STRATA-G and ELSA used a freely available software PRSice (Choi and O’Reilly, 2019; Euesden et al., 2015), based on a well-established genetic analysis tool used for GWAS data analysis, PLINK (Lin, 2020), but research in this thesis did not provide open code which can be implemented easily.

Improving the quality of reporting: ensuring that research descriptions are detailed and transparent so that studies can be reproduced/replicated more easily (Munafò et al., 2017). The ELSA study followed the newest available guidelines for statistical reporting. These guidelines place less emphasis on research interpretation in light of p-values only, as p-values frequently get misinterpreted (Harrington et al., 2019).

Protecting against conflicts of interests: clear disclosure of any conflicts of interests and their avoidance, where possible (Munafò et al., 2017). All conflicts of interest have been clearly reported. I have no conflicts of interests in relation to any research completed in this thesis.
Diversifying peer review: ensuring that feedback on academic work comes from the general public and members of the academic community, not a limited number of journal reviewers only (Munafò et al., 2017). Forms of diversifying peer review include, e.g., preprints. Preprints are manuscripts which have not gone through peer review, but they are deposited in a free online repository with a comment section for providing feedback. None of the work in this thesis was preprinted. Still, either I or co-authors covered all published papers in posts on social media platform Twitter, describing main research findings, and we were provided with feedback from the research community there.

Open access: whether manuscripts are free and easy to access (Munafò et al., 2017). Work from Chapters 3 and 4 has not yet been preprinted or submitted for publication. All other work, including papers in Appendices, has been published open access.

Overall, research in this thesis followed some open science practices (diverse collaboration on well-powered samples, published open access) but it should have followed more practices, in particular related to preregistration and reproducibility.

6.3.5 Future directions

This thesis focused on whether common genetic variants predispose to specific psychosis manifestations from across the course of illness. Psychosis phenotypes are dynamic, and research suggests that genetic markers may have different, age-specific effects on symptoms in healthy and clinical populations (see, e.g., Section 6.2.4 on research implications, Apolipoprotein ε4 and cognitive decline in psychosis). Thus, future research should address genetic contributions to trajectories of symptoms across the lifespan in healthy populations and in clinical populations with long-term disease (Martin et al., 2019a) of varying severity (Janssens et al., 2011).

Large-scale datasets with such information are likely to be linked to electronic health records (EHR) to provide information from across the course of disorder. Careful, specific probing of phenotypes will be required to minimise bias potentially encoded in these data. Dueñas et al. (2020) emphasise three key considerations: 1) ensuring that data being analysed keep relatively stable levels of patterns of comorbidities and known risk factors across all groups within the dataset, unless a strong biological reason exists to expect otherwise; 2) considering order and convergence of diagnoses across the lifespan: accounting properly for order, length and times between episodes and treatments which may be different between individuals so that separate, unrelated episodes are not conflated into a phenotype (diagnosis) which is not specific to an individual; 3) using known biology to probe the accuracy of assigned diagnoses, e.g. using PGS or genetic correlations between...
traits to disentangle whether phenotypes originally assigned to individuals based on clinical interview have underlying biology as expected for those phenotypes.

However, PGS are not designed to describe underlying genetic contributions to symptoms exhaustively. Future studies should continue modelling PGS together with rarer but more highly penetrant CNVs (Lewis and Vassos, 2020) to explore their joint and divergent contributions to disorder risk. Also, genetic information which is combined into PGS is itself subject to limitations which should be consistently addressed. GWAS of psychotic disorders in populations of non-European ancestry are increasingly being completed (Lam et al., 2019; Li et al., 2021a; The Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2020) but they should ideally continue to grow to be as large as samples of European ancestry individuals. Future work on GWAS should also continue to improve fine-mapping, i.e. establishing which specific regions within broad loci implicated in GWAS are causal (Benner et al., 2016; Wallace et al., 2015), and refining insights into pathways of action of these loci (Pers et al., 2015; Watanabe et al., 2017).

Additional work on PGS methodology could provide clear guidelines on what SNPs (only from GWAS-significant p-value thresholds or other thresholds) are optimal so that the most predictive PGS are generated (Janssens, 2019). Performance of PGS generation methods (as discussed in Section 6.3.3.2), should be benchmarked more consistently so that it is clear which methods are most likely to generate most predictive scores. Methods should also continue to improve for genetic prediction in ancestrally diverse and admixed populations (Márquez-Luna et al., 2017; Ruan et al., 2021).

Finally, recent research increasingly questions construct validity of PGS (concepts underlying PGS) and additional expansions of PGS have been suggested. Future research could include polygenic measures of greater specificity than currently generated PGS, e.g. PGS predisposing for specific symptoms (Cai et al., 2020) or describing specific biological pathways (Janssens, 2019). Alternatively, functional genomic annotations of PGS have been developed, based on transcriptomic gene expression data. These updated scores, gene expression risk scores (Pain et al., 2021a), are yet to be tested in psychosis. Also, while PGS are based on an additive model, a reformulation of this model has recently been proposed. Omnigenic models posit that interconnected gene regulatory networks influence aetiology of complex traits through a set of core genes (a small group of disease-specific genes) and then perturbations in regulatory elements related to core genes and members of similar pathways, outside core pathways, called peripheral genes (thus, omni, “all”, in the name; Boyle et al., 2017). While later research (Rammos et al., 2019) presented potential core genes for schizophrenia (TCF4, FMR1, upregulation of MIR137 and downregulation of CHD8), the omnigenic model has also been criticised as too simple to actually account for complexity of polygenic disorders (Wray et al., 2018). Thus, future research could
further explore what models could underlie PGS so that PGS become stronger variables in clinical prediction or modelling explaining biology of psychiatric disorder.

6.4 Conclusion

This thesis used European data from first episode psychosis patients and longitudinal data from a British sample of ageing adults to demonstrate that measures of common genetic predisposition, polygenic scores for psychiatric disorders, do not predict specific psychosis phenotypes: age of onset, duration of untreated psychosis or cognitive decline. However, polygenic scores for schizophrenia significantly predicted baseline cognitive performance in older adults. This finding potentially contradicts a neo-Kraepelian argument that schizophrenia is a genetically determined progressively deteriorating brain disease. Null findings on AOO and DUP suggest that polygenic scores for schizophrenia and other major mental health disorders do not describe genetic risks which contribute to these specific psychosis phenotypes. Alternatively, these findings may result from differences between research sites in how AOO and DUP are measured, from bias, or from suboptimal methods of generating polygenic scores. Also, it is not clear which of the particular biological pathways contribute to specific disorder manifestations. Future research should address biological pathways of carefully measured granular psychosis phenotypes in large, longitudinal, ancestrally diverse samples.
References


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REFERENCES


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Appendix A

Predictors of treatment resistant schizophrenia: a systematic review of prospective observational studies

Research presented in this appendix is a copy of a complete manuscript published as:


The manuscript is a systematic literature review. I rated studies for the review independently from Dr Smart and, as all co-authors, provided feedback on the manuscript. The manuscript also underwent peer review prior to acceptance in Psychological Medicine.

Final formatting follows the house style of the journal.

The manuscript is published open access.
Invited Review


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Key words:
First episode; longitudinal; prediction; psychosis; schizophrenia; treatment resistant

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Abstract

Treatment-resistant schizophrenia, affecting approximately 20–30% of patients with schizophrenia, has a high burden both for patients and healthcare services. There is a need to identify treatment resistance earlier in the course of the illness, in order that effective treatment, such as clozapine, can be offered promptly. We conducted a systematic literature review of prospective longitudinal studies with the aim of identifying predictors of treatment-resistant schizophrenia from the first episode. From the 545 results screened, we identified 12 published studies where data at the first episode was used to predict treatment resistance. Younger age of onset was the most consistent predictor of treatment resistance. We discuss the gaps in the literature and how future prediction models can identify predictors of treatment response more robustly.

Predictors of treatment resistant schizophrenia: A systematic review of prospective observational studies

For approximately a third of patients with schizophrenia, standard antipsychotic medications do not adequately alleviate their psychotic symptoms (Conley and Kelly, 2001). This subgroup is termed treatment-resistant schizophrenia (TRS). The most common clinical and research criteria used for TRS is the failure to respond to two trials of non-clozapine antipsychotics, of adequate dose and duration (Suzuki et al., 2011; Howes et al., 2017).

Patients with TRS have higher rates of unemployment, worse quality of life, and poorer social and occupational functioning than people who respond to treatment (Iasevoli et al., 2016). Researchers have estimated that the direct healthcare costs for TRS in the US is 3–11-fold higher than for the schizophrenia population as a whole, with multiple hospitalisations accounting for a large proportion of this cost (Kennedy et al., 2014). In England, 25–50% of the National Health Service’s (NHS) £11.8 billion mental health budget is allocated to schizophrenia services and TRS is thought to contribute a large proportion of these costs (Andrews et al., 2012; Killaspy et al., 2013).

Clozapine is the only antipsychotic recommended for TRS and is more effective than other antipsychotics in alleviating psychotic symptoms in patients with TRS (Kane et al., 1988; Siskind et al., 2016; Taylor, 2017). However, owing to its adverse effects, clozapine is only licenced in the UK (NICE, 2014) and most other developed countries (Warnez and Alessi-Severini, 2014) as a third-line treatment. Nevertheless, evidence suggests that TRS is often not recognised promptly, and that clozapine is offered after a delay of some years or not at all. According to treatment guidelines, the earliest that patients can be diagnosed with TRS, and prescribed clozapine, is 12 weeks after commencing antipsychotic treatment; however, Howes et al. (2012) report an average delay of 3.9 years, suggesting that there is considerable scope to shorten this period of inadequate treatment. Furthermore, patients with a shorter delay before clozapine initiation show a better symptomatic response to clozapine (Yoshimura et al., 2017).

Thus, there is a need to identify patients—who are likely to develop TRS—earlier in the course of their illness and expedite their access to specialist treatment; this may require moving beyond the current definition of TRS towards criteria based upon predictors and biomarkers, which quantify a patient’s risk of developing TRS. If predictors of TRS can be identified, they may be useful in three ways: firstly, to identify TRS patients earlier in treatment so that they can be offered effective treatments earlier; secondly, to identify patients for clinical trials of interventions for TRS; and thirdly, to improve our understanding of the aetiology of TRS.

We present a comprehensive systematic review of all prospective observational studies in schizophrenia populations, which report baseline predictors of TRS. We focused solely on prospective observational studies to draw clearer conclusions regarding the causal relationship...
between predictors and TRS in naturalistic settings over a long follow-up, and because only longitudinal studies can identify risk factors at first episode that might predict TRS.

**Method**

**Inclusion/exclusion criteria**

Studies were included if they met the following inclusion criteria: (1) participants were diagnosed with schizophrenia, schizophreniform disorder, schizoaffective disorder, and/or a psychotic disorder; we did not exclude studies that also included affective disorders or substance-induced psychosis, given the diagnostic uncertainty around the first episode of psychosis; (2) participants were followed from the first episode or first treatment with antipsychotics; (3) the majority of participants were aged between 16 and 64 at baseline (we excluded studies that focused exclusively on children or older adults); (4) data were collected prospectively from the first episode; (5) the outcome was a categorical definition of TRS, established using longitudinal prospective medication history; and (6) a non-TRS comparison group was recruited and followed up in the same manner as the TRS group. Studies were excluded if (1) they were clinical trials, or if non-antipsychotic treatments, such as CBT or ECT, were administered as part of the study procedure; (2) the study focussed exclusively on early or late-onset schizophrenia; or (3) inferential statistics measuring the association between baseline variables and TRS were not reported, and our subsequent requests to the authors for unpublished data were unsuccessful.

**Defining TRS**

Only recently has attention been given to the standardisation of TRS criteria (Farooq et al., 2013; Suzuki et al., 2012; Lee et al., 2015; Howes et al., 2017); therefore, we did not restrict studies to one definition of TRS. We did, however, only include studies with a categorical definition of TRS to capture the key underlying concept at least two treatment failures – and differentiate TRS from relative measures of response/nonresponse. If patients took clozapine at follow-up, we inferred that they met criteria for TRS. Clozapine prescription is likely to underestimate the true proportion of patients with TRS (Howes et al., 2012), but it is a pragmatic criterion, since clozapine is only used for TRS, except in very rare indications (e.g. psychosis in the context of Parkinson’s disease or for people who suffer severe side-effects to other antipsychotics).

**Literature search**

Studies were identified by searching PubMed, PsychINFO (up to October 2017), Medline (up to October 2017), Embase (up to October 2017), and OpenGrey on the 1 November 2017. In addition, we examined the first 20 pages of Google Scholar using terms ‘predictor AND treatment-resistant AND schizophrenia’ on 3 January 2018. No restrictions were placed on the publication date, but searches were restricted to the titles and abstracts of papers (and subject headings in Medline, Embase, and PsychINFO), studies published in English, and studies using human participants. Search terms for PubMed were as follows: ‘(treatment resistant) OR (treatment resistance) OR (treatment refractory)) AND (schizophrenia) AND ((longitudinal) OR (prospective))’. Search strategies for other databases can be found in Appendix 1. We screened the title and abstracts of all identified studies and then performed full-text screening of all potentially eligible studies. Potentially eligible studies were cross-referenced; additional relevant studies were identified by hand-searches of the references, and by screening papers which had previously cited these studies. Each additional paper was also hand-searched until no new studies were identified. When full-text articles were not available, the corresponding author was contacted. Author SES conducted the initial screening, with APK independently screening the studies identified through database searches and all studies identified through cross-referencing.

**Quality assessment**

We followed the PRISMA guidelines for reporting systematic reviews (Liberati et al., 2009). Study quality was assessed using the Newcastle-Ottawa Scale (NOS) for cohort studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Eight items measure the selection, comparability, and outcome of each study. These items were modified for this review, for example, follow-up needed to have been longer than one year to score on the item concerning adequate duration of follow-up (see Appendix 2). Authors SES and APK independently rated each study on the NOS (Appendix 3), any differences in rating were discussed between authors and final ratings were a consensus.

When available, we report adjusted hazard (HR) or odds ratios (OR) with 95% confidence intervals (95% CI) in parentheses, for predictors measured at baseline.

**Results**

A total of 12 studies were identified for inclusion in this review. Study screening is depicted in Fig. 1 and a summary of the number of participants recruited into each study is presented in Table 1. Database searches identified 545 records, 293 of which were duplicates and removed. A total of 252 records were screened and 248 were excluded. The main reasons for exclusion were: the study did not follow participants from the first episode or first treatment with antipsychotics (31%), participants recruited after TRS had been identified (29%), and an outcome other than TRS was reported (23%). The remaining four records were examined in more detail, as were the eight records identified by cross-referencing. Only duplicates were identified through Google Scholar. Of the 12 studies, 11 were published in peer-reviewed academic journals. One study was unpublished (Chan et al., 2014), however, after corresponding with the authors, a full report was identified on the funding body’s website containing enough information to be included in this review (https://rfs1.fhb.gov.hk/app/fundedsearch/projectdetail.xhtml?id=1363).

Of the 12 included studies, eight presented original data and four presented data on additional exposures within the same cohort as a previous study, or a subset thereof. Of these eight, three were population cohort studies. Both Sorensen et al. (2014) and Wimberley et al. (2016b) used Danish population registers: data was extracted from multiple national databases and linked using a unique personal identification number. Additional analyses of Wimberley et al. (2016b)’s data tested whether urbanicity (Wimberley et al., 2016a), the polygenic risk score for schizophrenia (PRS-SZ, Wimberley et al., 2017), functioning (Horsdal et al., 2017b), and C-reactive protein levels (Horsdal et al., 2017a) could predict TRS. The third population
The cohort came from South Korea (Kim et al., 2017). The remaining five studies analysed longitudinal first episode psychosis patient cohorts (Meltzer et al., 1997; Chan et al., 2014; Lally et al., 2016; Üçok et al., 2016; Demjaha et al., 2017).

In population registries, a proxy definition of first-episode psychosis is required. In the Danish studies, the first International Classification of Diseases (ICD; World Health Organization, 1993) diagnosis of schizophrenia was used to define the baseline cohort. The South Korean study used ICD diagnosis of schizophrenia and the first use of antipsychotics to define the baseline cohort. When using diagnoses, the first episode is likely to be later in the disease course, when compared to cohort studies. Additional study characteristics, including information about recruitment, diagnoses, and criteria for TRS and non-TRS can be found in Appendix 4. The variables measured, and tested as predictors of TRS, varied considerably across studies, therefore this information is summarised in Table 2. Appendix 5 contains the unadjusted and adjusted OR/HR, when these were reported.

Predictors of TRS
Chan et al. (2014) analysed a subsample of a first episode cohort who presented to mental health services over a five-year period and used clozapine prescription as a definition of TRS. As this was a case-control study including all patients with TRS and a ratio of two non-TRS patients for every TRS patient, the
prevalence of TRS could not be calculated. The two groups were matched on baseline diagnosis. Chan et al. (2014) included age of onset, duration of untreated psychosis (DUP; days), duration of first episode, years of education, Premorbid Adjustment Scale (PAS) adult (19+ years) subscale score (Cannon-Spoor et al., 1982), substance misuse history, and the number of relapses in the first three years, in a Cox proportional hazard regression. The model significantly predicted TRS (Chi-square = 66.11, df = 7, p = <0.0001). While number of relapses in the first three years significantly predicted TRS, the only baseline predictors significantly associated with TRS were younger age of onset (HR = 0.94) and poorer premorbid functioning (HR = 3.22, 95% CI = 0.83–9.49) and poorer premorbid functioning (indicated by higher scores) according to the PAS (HR = 3.22, 95% CI = 1.43–7.23).

Demjaha et al. (2017) analysed data from the AESOP study, which recruited first episode patients over a three-year period and followed them up ten years later. The researchers entered gender, diagnosis, age of onset, negative symptoms, mode of onset, DUP (weeks), and ethnicity into a multivariate penalised logistic regression. The model selected five variables that predicted TRS: a diagnosis of schizophrenia at baseline (instead of psychotic depression; OR = 0.41, or psychotic mania; OR = 0.52), younger age of onset (years, OR = 0.97), higher severity of negative symptoms (OR = 1.09), an insidious mode of onset (instead of acute; OR = 1.28), and longer DUP (OR = 1.0013). Goodness-of-fit was measured using McFadden’s pseudo R² and correct classification rates were measured using the Brier score. A McFadden’s pseudo R² between 0.20 and 0.40 is considered a good model fit. The Brier score is used to evaluate predictive models; if the incidence of TRS is 23%, as estimated from Demjaha et al. (2017), a Brier score of 0 would be a perfect model while a score of 0.177 would be a non-informative model (Steyerberg et al., 2010). Demjaha et al. (2017) reported a McFadden’s pseudo R² of 0.10 and a Brier score of 0.146, suggesting that their model is not a good fit of the data nor is it a good classifier of TRS.

Kim et al. (2017), in their South Korean population cohort, estimated the cumulative incidence of clozapine use using the Kaplan–Meier method and log-rank test. They reported that younger age of onset predicted TRS. Unlike Chan et al. (2014) and Demjaha et al. (2017), Kim et al. (2017) examined age of onset categorically: defining younger age of onset as those aged between 15–20 years of age, and comparing them to a middle-onset group (21–44 years of age) and a late-onset group (45–64 years of age). Kim et al. (2017) also found, using the Walter–Elwood method (Walter and Elwood, 1975), a higher incidence of clozapine use, in those born during winter (December to February) when compared to those born in summer (June to August). This pattern remained true when stratifying season of birth by age of onset. Kim et al. (2017) reported no measures of overall model fit.

Lally et al. (2016) recruited first episode patients over a five-year period and used electronic medical records to follow them up five years later. They entered age of onset, Positive and Negative Symptom Scale (PANSS; Kay et al., 1987) scores, Global Assessment of Functioning (GAF; Hall, 1995) disability score, and GAF symptom scores into a penalised logistic regression, controlling for living arrangements, employment status, and alcohol/substance misuse during the follow-up period. Lally et al. (2016) included the PANSS total score, the positive, negative and general psychopathology subscale scores, as well as two individual items: lack of insight and conceptual disorganisation. None of the PANSS or GAF variables predicted TRS. Age at first contact with mental health services was split into four categories: 18–20, 21–25, 26–30, >31 years. Only age of onset between 18 and 20 years, compared to all other age groups, significantly predicted TRS (OR = 2.49, 95% CI = 1.25–4.94). The authors did not report the overall model fit. Age of onset was subsequently stratified by gender and ethnicity. Age of onset, between 18 and 20, only predicted TRS in males (OR = 2.13, 95% CI = 1.35–7.23) or those of black ethnicity (OR = 3.71, 95% CI = 1.44–9.56).

Meltzer et al. (1997) recruited patients at first admission to hospital for schizophrenia or schizoaffective disorder and followed them up for approximately four years. The authors examined the age of onset and gender in relation to TRS using a two-way analysis of variance (ANOVA). Gender was not associated with TRS but younger age of onset was. As males had a
Table 2. The variables which have been tested as predictors of TRS in the twelve studies included in this review

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NB: grey squares, variables not significantly associated with TRS; dark grey squares, variables significantly associated with TRS; all analyses using the Wimberley et al., Danish dataset were grouped under Wimberley et al. (2016b)

younger age of onset than females, the researchers examined the associations between age of onset and gender in more depth using simple effects ANOVA. In the non-TRS group, males had a younger age of onset ($F = 6.6, df = 1, p < 0.01$), however, in the TRS group, there was no difference in age of onset between males and females. Meltzer et al. (1997) calculated the conditional probability of a patient having TRS given their age of onset. For those aged between 15 and 18 years old, the probability of developing TRS was between 32% and 38% for both males and females.

Sorensen et al. (2014), in their Danish population cohort, entered a season of birth into a Cox proportion hazard regression adjusted for birth year and gender. The model did not significantly predict TRS. However, the authors found that being born in autumn (September to November), compared to spring (March to May), predicted TRS ($HR = 1.24, 95% CI = 1.06–1.46$). Unlike in Kim et al. (2017)’s study, being born in winter (December to February) failed to predict TRS.

Üçok et al. (2016) analysed a subsample of patients recruited into an ongoing first episode schizophrenia study. Üçok et al. (2016) entered the age of onset, DUP (days), first relapse despite adherence to antipsychotic treatment, relapse in the first six months, and antipsychotic polypharmacy during follow-up, into logistic regression. The authors did not report the overall model fit. Only first relapse despite adherence to antipsychotic treatment and antipsychotic polypharmacy predicted TRS. No baseline variables predicted TRS.

Wimberley et al. (2016b), in their Danish population cohort, entered twenty-three variables into a Cox proportion hazard regression. These variables included: gender, age at first schizophrenia diagnosis as a proxy for age of onset, family history of schizophrenia in first-degree relatives, winter birth (December to March), paternal age, parental loss before the age of 18, living alone, conviction for a violent offence before first schizophrenia diagnosis, level of education, employment status, urbanicity at first schizophrenia diagnosis, admission to psychiatric hospital before first schizophrenia diagnosis, schizophrenia subtype (paranoid vs. all others), comorbid psychiatric diagnosis before first schizophrenia diagnosis, antipsychotic prescription in the year before first schizophrenia diagnosis, antidepressant prescription in the year before first schizophrenia diagnosis, and benzodiazepine prescription in the year before first schizophrenia diagnosis. Goodness-of-fit was measured using McFadden’s pseudo $R^2$ and correct classification rates using Harrell’s C statistic; a C statistic of 0.5 would be a non-informative model while a score of 1 would be a perfect model. Wimberley et al. (2016b) report a McFadden’s pseudo $R^2$ of 0.27 and a Harrell’s C statistic of 0.70, suggesting that this model is a good fit of the data and reasonable classifier of TRS. At baseline, younger age of onset (years, $HR = 0.96, 95% CI = 0.95–0.97$), living in less urban areas (rural vs. capital area, $HR = 1.44, 95% CI = 1.25–1.65$), higher education (higher vs. primary education, $HR = 0.88, 95% CI = 0.79–0.98$), psychiatric hospital admission at diagnosis ($HR = 2.07, 95% CI = 1.87–2.29$), having spent more than 30 bed-days in a psychiatric hospital in the year before diagnosis ($HR = 1.54, 95% CI = 1.35–1.75$), paranoid subtype diagnosis ($HR = 1.24, 95% CI = 1.13–1.37$), comorbid personality disorder ($HR = 1.24, 95% CI = 1.11–1.39$), comorbid suicide attempt ($HR = 1.21, 95% CI = 1.07–1.39$), antipsychotic use ($HR = 1.51, 95% CI = 1.35–1.69$), antidepressant use ($HR = 1.15, 95% CI = 1.03–1.29$), and benzodiazepines use ($HR = 1.22, 95% CI = 1.10–1.37$), all predicted TRS. Data on additional exposures within the same cohort, or a subset thereof, were published separately. Lower levels of urbanicity (Wimberley et al., 2016a) and severely impaired functioning (a GAF functioning score ≤30; Horsdal et al., 2017b) predicted TRS, but the polygenic risk score for schizophrenia (PRS-SZ; Wimberley et al., 2017) and C-reactive protein levels (Horsdal et al., 2017a) did not predict TRS.

**Subcategories of TRS**

Some patients have little or no response to antipsychotic treatment from the onset of their illness, while others initially respond to medication and then later develop TRS. Two of the studies in our review reported comparisons between subgroups TRS patients; early-onset TRS was operationalised as meeting criteria after a period of symptomatic remission. Chan et al. (2014) found no differences, in demographics, clinical characteristics, or premorbid functioning, between early-onset...
TRS ($N = 17, 11.64\%$) and delayed-onset TRS ($N = 129, 88.36\%$). Lally et al. (2016) found no differences in demographics between the two groups, but the early-onset TRS group ($N = 56, 70\%$) had a younger mean age of onset than the delayed-onset TRS group ($N = 24, 30\%$).

**Discussion**

This review identified twelve research papers that examined predictors of TRS. Seven of the studies included in this review tested the age of onset as a predictor, and six reported that younger age of onset predicted TRS. Given that multiple definitions of the age of onset – age of onset of psychotic symptoms, age of first diagnosis of schizophrenia, age of first contact with mental health services – were reported and data was treated both continuously and categorically, this is a robust finding. Other potential risk factors, that have been identified by more than one study, include diagnosis, level of functioning, male gender, and season of birth.

A recent meta-analysis linked younger age of onset to multiple poor outcomes in schizophrenia: more hospitalisations, more negative symptoms, more relapses, poorer social/occupational functioning, and poorer global outcome (Immonen et al., 2017). Many of these poor outcomes have also been associated with TRS. Immonen et al. (2017) found that males had a younger age of onset and, therefore, samples with a higher proportion of males tended to show stronger associations between age of onset and outcomes. In the studies included in this review, the association between age of onset and TRS is unlikely to be wholly confounded by gender, as the proportion of males ranged from 49% (Kim et al., 2017) to 67% (Lally et al., 2016) and the studies which controlled for gender still showed an effect of age of onset (Meltzer et al., 1997; Lally et al., 2016; Wimberley et al., 2016a; Demjaha et al., 2017). In schizophrenia, age of onset has been thought to reflect genetic liability for the disease; younger age of onset has been associated with an increased familial risk of schizophrenia (Hilker et al., 2017; Byrne et al., 2018). Could, therefore, TRS be the result of increased genetic risk? While Wimberley et al. (2017) found no association between PRS-SZ and TRS, other work published by Frank et al. (2014) reports that an increased PRS-SZ is associated with TRS. In addition, rare copy number variations have been associated with both TRS (Martin and Mowry, 2015) and childhood-onset schizophrenia (Addington and Rapoport, 2009). Therefore, patients with TRS, who also have a younger age of onset, may have a more salient genetic influence than later-onset cases, although further work is required to substantiate this claim.

This review complements previous reviews by Gillespie et al. (2017) and Carbon and Correll (2014). Gillespie et al. (2017) examined studies comparing patients with treatment-resistant to patients with treatment-responsive schizophrenia. They included all study methodologies, but excluded studies where treatment-responsiveness was defined solely as not meeting treatment-resistant criteria. Carbon and Correll (2014) examined studies identifying predictors of response and remission. The researchers focused on first-episode psychosis studies where participants were followed up for five years. Some of the predictors of TRS, identified in this review, were found to be associated with less chance of response/remission by Carbon and Correll (2014), e.g. younger age of illness onset, poor premorbid adjustment, being male, lower level of education, living in a rural environment, diagnosis of schizophrenia, longer duration of untreated psychosis, poorer functioning, and worse psychopathology. However, Carbon and Correll (2014) also associated less chance of response/remission with being single, family history of psychosis, greater cognitive dysfunction, more family conflicts, and substance misuse; characteristics not identified as predictors of TRS. There was relatively little overlap between this review and Gillespie et al. (2017)’s review. In terms of studies included, only Meltzer et al. (1997)’s study was included in both reviews. In terms of characteristics associated with TRS, Gillespie et al. (2017) identified five neuroimaging studies, nine gene-association studies, and two studies of neurocognitive function, and these studies were not included in our review. The examination of biological markers, associated with TRS, within longitudinal study designs is rare; this is understandable for genome-wide association studies, which require large sample sizes more easily acquired using a cross-sectional methodology. However, there is a clear gap in the literature investigating biological markers that change over time (for example, proinflammatory cytokines or differently methylated positions within the epigenome) and TRS as an outcome. In terms of neuroimaging research, a review by Nakajima et al. (2015) found only five studies which compared patients with TRS to non-TRS patients, none of which had identified neural correlates of TRS. McGuire and Dazzan (2017) highlight only one study where neuroimaging data predicted a six-year, non-remitting course of illness. Longitudinal imaging studies of TRS are still relatively rare and constitute another gap in the literature.

Of the studies included in this review, few identified characteristics of abnormal neurodevelopment as predictors of TRS, despite neurodevelopmental changes being linked with schizophrenia. The neurodevelopmental theory of schizophrenia proposes that disrupted normal development, in utero or early infancy, leads to deficits in psychophysiological and neurological functioning in childhood or early adolescence, and eventually to prodromal or diagnostic symptoms of schizophrenia (Jablensky et al., 2017; Murray et al., 2017). Previous research has linked characteristics of abnormal development with TRS; higher rates of minor physical anomalies (Lin et al., 2015), more neurological soft signs (de Bartolomeis et al., 2018), poor verbal intelligence and fluency (Kravariti et al., 2018), and poor verbal memory (Joober et al., 2002; de Bartolomeis et al., 2013). None of the studies in this review included variables measuring physiology during development or cognition at the first episode. Only Chan et al. (2014) examined premorbid functioning, retrospectively using the PAS. They found no difference, between the TRS and non-TRS groups, in terms of functioning during childhood, early adolescence, or late adolescence. There was a difference in functioning after the age of 19 and subsequently, worse functioning predicted TRS in their final model. If educational attainment can be considered a proxy for development only lower level of education qualification was found to significantly predict TRS (Wimberley et al., 2016b). The number of years in education was not predictive of TRS (Chan et al., 2014). Abnormal neurodevelopment and neuropsychology have not been sufficiently investigated as potential predictors of TRS.

Our review has illuminated some gaps in the existing literature, where potential predictors have not been fully investigated, however, we believe our review has captured all published work and identified predictors that, with further study, may prove to be clinically useful in determining treatment for patients with schizophrenia.

**Strengths and limitations**

The main strength of this review is that we have focused solely on studies that included temporal forecasting (observations at baseline that are used to predict outcomes at follow-up), and as
such eliminated recall bias and established a key component necessary for predictive models. All the studies included in this review are likely to be sufficiently powered to detect predictors of TRS. All the studies reported large sample sizes, and most followed participants for more than one year. Although no studies reported a priori power analysis, and only Meltzer et al. (1997) reported an ad hoc power analysis, we believe lack of power is unlikely to explain these results.

When attrition reduces the sample size at follow-up of longitudinal studies, consequently, statistical power is also reduced. For the studies we have reviewed, that reported on participants lost to follow-up, it is unlikely that the low attrition rates introduced bias. In particular, many studies used Cox proportional hazard regression; an analytic method that not only takes into account that individuals lost to follow-up may develop TRS, but also that individuals may develop TRS after the study endpoint. However, TRS, in particular, may be biased by attrition. There is a case both that TRS patients may be more likely to drop out of research studies due to their higher severity of symptoms and worse social and occupational functioning, and that responders are more likely to drop out as they lose touch with clinical services, but we are not aware of any published studies examining attrition in relation to treatment response.

One limitation to consider, when discussing the findings from these studies, is that some patients may have been misclassified. None of the studies included in this review explicitly accounted for adherence to medication, therefore characteristics may be predicting nonadherence rather than treatment resistance. None of the studies measured antipsychotic plasma levels, therefore characteristics may be predicting sub-therapeutic drug plasma levels, as a consequence of nonadherence, noncompliance, or pharmacokinetics, rather than treatment resistance. McCutcheon et al. (2015) found that 44% of patients referred to an outpatient service for clozapine treatment had sub-therapeutic conventional-antipsychotic plasma levels. On the other hand, it is unlikely that TRS patients have been wrongly classified as responders because the long follow-up periods allow plenty of time for a diagnosis of TRS to be established. Most studies had follow-ups longer than four years; the average delay before being treated for TRS estimated by Howes et al. (2012). The definitions of TRS, used in these studies, are pragmatic criteria: any predictors identified by these naturalistic studies are generalisable to real-world, clinical settings where adherence, compliance, or drug plasma levels influence treatment.

The use of multiple definitions of TRS is a problem across all TRS literature; Suzuki et al. (2011) reviewed 33 studies of prospective studies of pharmacological interventions for TRS and found that all 33 definitions of TRS were different. Howes et al. (2017) reviewed 42 clinical trials and found only two studies which used identical criteria. In addition, some studies use clozapine prescription as a proxy for TRS. When clozapine is under-prescribed, supposed predictors of TRS may, in fact, represent predictors of clozapine initiation (e.g. clinicians’ attitudes towards clozapine prescription). All of the studies, identified in this review, used existing data, not designed to examine TRS, and researchers had to established proxy definitions based on the data available to them. When evidence concerning predictors of TRS is not consistent, it can be hard to draw a clear conclusion about the validity of the predictor, yet when the evidence is consistent across studies, with different definitions, the predictor in question is highly likely to generalise to other cohorts and have clinical validity.

Finally, we must consider the statistical methodology used to establish predictors. A common misconception is that predictive accuracy can be inferred from explanatory accuracy. However, the two are different and should be assessed separately (Shmueli, 2010). Only three studies included in this review reported the overall model fit, and only two reported statistics that measure the predictive validity of the model. Additionally, in predictive modelling, variable selection and overfitting must be considered. Lally et al. (2016) and Demjaha et al. (2017) attempted to reduce overfitting by penalising regression coefficients. However, none of the studies used holdout data (training data), cross-validation, or external validation to evaluate the predictive power of models; the latter being the current ‘gold-standard’ approach. In terms of variable selection, the only methods reported were LASSO regression (Demjaha et al., 2017) and step-wise selection using statistical significance (Chan et al., 2014; Uçok et al., 2016). Stepwise methods are no longer considered appropriate for explanatory models, but stepwise-type algorithms are very useful in predictive modelling (Shmueli, 2010), as long as the selection criteria rely on predictive power (e.g. Akaike information criterion) rather than explanatory power (e.g. statistical significance), as was the case in these studies. These methodological limitations must be taken into consideration when evaluating predictive models. The studies included in the review, on the whole, report analyses designed to identify explanatory variables of TRS. Future studies will need to use more robust prediction methods before moving from statistical prediction to clinical prediction.

Conclusion
The aim of this systemic literature review was to identify predictors of treatment-resistant schizophrenia from prospective longitudinal studies. In choosing to focus exclusively on longitudinal studies, we have filled a gap in the existing literature, and hope that consolidating this information will be of use to researchers attempting to identify clinical predictors of TRS and the biological mechanisms causing TRS. We have identified earlier age of schizophrenia-onset as a robust predictor of TRS, with evidence that male gender, autumn/winter birth, poor premorbid functioning and rural upbringing may also contribute. We have also highlighted gaps in the literature namely, studies examining neuroimaging, immune, and genetic markers of TRS. Examination of biological markers, particularly within the framework of a prospective longitudinal study, has the potential to go beyond simple prediction and add to our understanding of the underlying causes of TRS. In conclusion, while early identification of TRS is clinically important, we currently have very limited knowledge of its predictors.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291719002083.

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The views expressed in this article are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health and Social Care, the BRC, the MRC, or King’s College London.

Conflict of interest. S.E.S. has received travel and accommodation expenses from H Lundbeck A/S. A.P.K. declares no conflicts of interest. R.M.M. has received honoraria for lectures from Janssen, Otsuka, Lundbeck and others.
Sunovian, J.H.M. has received travel and accommodation expenses and research funding from H Lundbeck A/S.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

**References**


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Appendix B

Schizophrenia and influenza at the centenary of the 1918-1919 Spanish influenza pandemic: Mechanisms of psychosis risk

Research presented in this appendix is a copy of a complete manuscript published as:


The manuscript is a narrative literature review. I wrote the first draft of the review. All co-authors provided feedback on manuscript drafts. Dr Pollak and I edited the manuscript. The manuscript also underwent peer review prior to acceptance in *Frontiers in Psychiatry*.

Final formatting follows the house style of the journal.

The manuscript is published open access.
Schizophrenia and Influenza at the Centenary of the 1918-1919 Spanish Influenza Pandemic: Mechanisms of Psychosis Risk

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Associations between influenza infection and psychosis have been reported since the eighteenth century, with acute “psychoses of influenza” documented during multiple pandemics. In the late 20th century, reports of a season-of-birth effect in schizophrenia were supported by large-scale ecological and sero-epidemiological studies suggesting that maternal influenza infection increases the risk of psychosis in offspring. We examine the evidence for the association between influenza infection and schizophrenia risk, before reviewing possible mechanisms via which this risk may be conferred. Maternal immune activation models implicate placental dysfunction, disruption of cytokine networks, and subsequent microglial activation as potentially important pathogenic processes. More recent neuroimmunological advances focussing on neuronal autoimmunity following infection provide the basis for a model of infection-induced psychosis, potentially implicating autoimmunity to schizophrenia-relevant protein targets including the N-methyl-D-aspartate receptor. Finally, we outline areas for future research and relevant experimental approaches and consider whether the current evidence provides a basis for the rational development of strategies to prevent schizophrenia.

Keywords: infection, epidemiology, autoimmunity, neurodevelopment, maternal immune activation (MIA), influenza, schizophrenia

INTRODUCTION: WHAT IS THE EVIDENCE FOR AN ASSOCIATION BETWEEN INFLUENZA AND SCHIZOPHRENIΑ?

Schizophrenia risk is associated with a variety of environmental and genetic factors (1), including those associated with immunity and inflammation (2). Genome-wide association studies (GWAS) implicate loci at the major histocompatibility complex (MHC) which encodes multiple genes involved in immunity such as the human leukocyte antigen (HLA) genes (3–6) and complement
component 4 (C4) (7), among others, and enhancers related to B-lymphocyte lineages (CD19 and CD20 lines) involved in acquired/adaptive immunity (8). Overall there is strong evidence supporting the involvement of specific immune variants in schizophrenia risk (7), some evidence of convergence across genomics, transcriptomic, and methylomic processes (9), but conflicting evidence for both (i) enrichment of specific immune cell types or pathways (10) and (ii) for genetic overlap between SZ and specific immune diseases (11, 12).

Substantial epidemiological evidence exists suggesting that maternal, perinatal, childhood, and adult infection may all increase the risk of schizophrenia diagnosis (13–19). While many organisms and infection types have been implicated in schizophrenia risk, the influenza virus has special status: not only is maternal influenza infection the most well-replicated infective risk factor for schizophrenia, but the history of schizophrenia research has been shaped at crucial points by observations concerning the apparent, sometimes surprising, role of influenza as an exposure. This review aims to present the current state of knowledge on mechanisms by which influenza infection may confer schizophrenia risk, along with the implications of this understanding for future research, prevention, and treatment.

Before the focus of this review moves to schizophrenia and related psychotic disorders, it should be noted that some of the associations that will be discussed are now thought not to be specific to schizophrenia risk. The late winter/spring season of birth effect has also been reported in bipolar disorder (BD) (20), but the evidence for a link between BD and influenza is somewhat mixed (21) and addressed in limited studies (22). Influenza (including serologically documented infection) has been reported as a risk factor for BD with psychotic features but not nonpsychotic BD [reviewed in (22–24)]. Furthermore, some evidence suggests an association between maternal infection and autism spectrum disorders (ASD) [reviewed in (25)]. A substantial body of work from Scandinavian (largely Danish) health register studies supports the notion that clinically diagnosed maternal, childhood, or adulthood infection is a pluripotent risk factor for the subsequent development of psychiatric disorder, with effects observed across diagnostic boundaries (13–16, 26, 27). Therefore, while the focus of this review is on schizophrenia and psychosis, the potential transdiagnostic relevance of some of the mechanisms reviewed here should not be ignored.

Currently, influenza is regarded predominantly as a respiratory illness, but before the last century a far broader conceptualisation existed. As early as a 1732 epidemic, clinicians made note of the nervous sequelae of infection, with manifestations including neurasthenia, melancholy, hysteria, mental prostration, and insanity (28). According to the historian of medicine Mark Honigsbaum: “in the mid-1890s British medical journals were full of tales of Victorian professionals driven to the brink of madness and beyond by the nervous sequelae of influenza... for some 30 years, from the first epidemics of Russian influenza in the 1890s through to the ‘Spanish’ influenza of 1918–19, the ‘psychoses of influenza’ were a widely recognised psychiatric phenomenon” (29). In 1919, Karl Menninger published a now-classic paper reporting the characteristics of 100 patients with mental disturbances associated with influenza infection admitted in a 3-month period to the Boston Psychopathic Hospital. Of 80 on whom full data were available, 16 were diagnosed with delirium, 25 with “dementia praecox,” 23 with “other psychoses,” and 16 were unclassified (30). Interestingly, two-thirds of the “dementia praecox” patients were reported to have fully recovered at 5-year follow up (31). A further historically important strand of evidence came from von Economo’s (32) research onencephalitis lethargica (EL), a still poorly understood inflammatory CNS condition featuring psychotic and catatonic symptoms, which was broadly contemporaneous with and potentially aetologically related to the 1918-1919 Spanish influenza pandemic. Table 1 provides an overview of historical influenza pandemics that have been linked to the occurrence of psychosis.

While suggestive, these reports do not provide evidence of a causal link between influenza infection and psychotic disorders. Renewed interest in the second half of the 20th century shifted focus towards maternal infection, following consistent findings

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<th>Influenza strain involved</th>
<th>Relationship to psychosis</th>
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<td>1889–1892 influenza pandemic (Russian influenza)</td>
<td>1889–1892</td>
<td>H2N2</td>
<td>psychosis, suicidal thoughts, paranoia following infection</td>
<td>(29, 33, 34)</td>
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<td>1918 Spanish influenza pandemic</td>
<td>1918–1920</td>
<td>H1N1</td>
<td>delirium, dementia praecox, acute psychosis (33); encephalitis lethargica (32) following infection</td>
<td>(31, 32, 35)</td>
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<td>Asian influenza pandemic</td>
<td>1957–1958</td>
<td>H2N2</td>
<td>acute psychotic manifestations: anxiety, confusion, restlessness, paranoia, abnormal electroencephalography 2-10 days after influenza onset, (36); excess of female births with an increased schizophrenia risk five months after the onset of the 1957 epidemic (37); however, no significant excess of schizophrenia cases in births in the 1959 epidemic (37); encephalitis, psychosis, including depressive-type psychosis and repetitive transient psychosis in children following infection</td>
<td>(33, 36–43)</td>
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<td>2009 influenza pandemic (swine flu)</td>
<td>2009–2010</td>
<td>H1N1</td>
<td>encephalitis, psychosis, including depressive-type psychosis and repetitive transient psychosis in children following infection</td>
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of an increased risk for schizophrenia in late winter/spring season births (20, 46), raising the possibility of winter-borne infection as a plausible mechanism. Beginning with Mednick et al.’s 1988 study of a Finnish population exposed to the 1957 influenza A2 pandemic, epidemiological studies in the 1980s–1990s, of an ecological nature, described increased risk for schizophrenia in children who were in utero during an influenza epidemic (37–41). These studies are comprehensively reviewed in (47). Frequently, rates were highest for second trimester exposures, although the first trimester appeared also to be a period of increased risk. Some subsequent studies however—often with more accurate case ascertainment and larger samples—were not able to replicate these initial findings [e.g. (48, 49)]. While estimates of risk varied greatly and heterogeneity in methodology somewhat limits generalisability, a 2010 review calculated that maternal influenza exposure increased schizophrenia risk with an odds ratio of 3.0 and a population attributable proportion of 14% (47).

Partly because of the manifold methodological problems involved in imputing precisely who was exposed to influenza, these ecological studies were followed by so-called “sero-epidemiological” studies, in which infection was verified using archived biological specimens: in one such early study first trimester maternal exposure was associated with a sevenfold increase in offspring schizophrenia risk, with threefold increase in risk associated with early-to-mid gestation exposure (50).

Other studies explored whether other viral and bacterial infections are associated with differential schizophrenia risk. A meta-analysis found that childhood viral infection was associated with a nearly twofold increased risk of adult nonaffective psychosis and that of all childhood infections, viral infections in particular, were associated with a nearly twofold increased risk of adult schizophrenia (18). However, bacterial infections were not associated with risk for psychosis, suggesting that risk may be specific for childhood viral infections.

Some controversy persists as to whether the evidence for maternal influenza as a schizophrenia risk factor is sufficient. A recent review of studies of schizophrenia risk in relation to the 1957 influenza pandemic criticised the serological studies for using strain-specific antibody titres that were too low to be specific for recent infection and so were insufficient as proxy measures of recent infection; furthermore, a pooled meta-analysis of eight ecological studies and one serological study found no overall increased risk of schizophrenia in children of influenza-exposed mothers (51). This review was in turn criticised as inappropriate given the heterogeneity of methods used in the pooled studies (52); furthermore it appeared to omit some relevant serological data [e.g. (39, 40)] and as it focuses on the 1957 pandemic only, it does not include studies on other strains of influenza infection and psychosis.

Complicating interpretation of ecological/epidemiological and serological studies is the fact that obstetric complications are more likely following influenza or influenza-like illness, and that obstetric complications are an independent risk factor for the subsequent development of psychotic disorders and/or symptoms (53–55).

Reconciling Maternal Infection With Influenza With the Neurodevelopmental Hypothesis of Schizophrenia

The late 1980s and 1990s saw the emergence of neurodevelopmental theories offering mechanistic accounts of how schizophrenia develops. The neurodevelopmental hypothesis (56, 57) posits that schizophrenia results from a pathological disruption of normal brain development which commences many years before schizophrenia onset (58). Infection and other insults could disrupt developmental processes such as cell proliferation, cell migration, arborisation, and myelination (59) with resulting brain structural alterations [e.g. ventricular enlargement, grey matter reductions, and white matter disruption; (60)]; activation of pathologically developed brain systems in adolescence or young adulthood then manifests in schizophrenia symptoms (59).

Amongst other criticisms, the theory fails to account for later-onset schizophrenia [45 years or older; (61)] and postadolescence changes (62). Extended neurodevelopmental models posited further “hits,” e.g., genetic and environmental factors first predisposing to schizophrenia prenatally and then later in life (“three-hit” model, (63); multiple hit theory, (64)). Infection is a possible “hit”; for instance, human endogenous retrovirus infections, activated by viruses including influenza, were suggested as late “hits” (64). This theory is consistent with evidence that maternal infection contributes to later increased offspring risk for childhood infections, which in turn contribute to schizophrenia development (65).

There is no clear evidence that genetic liability to schizophrenia increases the likelihood of influenza infection or predisposes to a disrupted immune response to influenza, or that influenza genetic loci are implicated in schizophrenia. In terms of genetic risk for influenza infection, while significant genetic effects accounting for the antibody level in influenza A and B (66, 67) have been reported with $h^2$ (heritability) range of 0.20–0.27 and $c^2$ (shared environment) = 0.19 for influenza A and B (66), results for discrete serostatus (seropositive/seronegative) were significant for influenza B only. However, another GWAS of IgG response to viruses identified HLA class II residues as causal variants and found an overlap between variants affecting the humoral response to influenza A and variants linked to influenza-related autoimmune disorders including narcolepsy (68). Neither of these studies nor any others to date have directly addressed the issue of overlap between genetic risk for schizophrenia and specific risk for influenza infection, although this has been explored for other pathogens (69–71). A UK population-based cohort study of 7,921 mothers found no association between schizophrenia polygenic risk score (PRS) and perinatal infection (using a single “any infection” category) (72). Similarly, a case-control study by Benros et al. explored an association between schizophrenia PRS and a history of hospital contacts for viral infections, including influenza infection: PRS for schizophrenia did not account for the association between hospitalisation for infection and subsequent schizophrenia risk, indicating that schizophrenia risk does not increase proneness to such severe infections (73).
THE INFLUENZA VIRUS AND POTENTIAL PATHOLOGICAL MECHANISMS UNDERLYING THE ASSOCIATION BETWEEN SCHIZOPHRENIA AND INFLUENZA INFECTION

Influenza: Structure and Pathophysiology

The influenza virus is an enveloped RNA virus from the family Orthomyxoviridae, with three genera, influenza A, B, and C (74). Given that influenza type A is responsible for pandemics (75) historically linked to schizophrenia and psychotic symptoms (see Table 1), we will focus on this alone. Influenza A viruses are classified into subtypes based on the antigenic properties of their envelope glycoproteins (see Figure 1), hemagglutinin, and neuraminidase. The viral envelope is a lipid membrane derived from plasma membrane of an infected host cell. Influenza strain targets also differ. Notably, the H5N1 virus and other avian-derived strains are neurotropic while H1N1 is thought not to be (76–78).

For infection to be successful, hemagglutinin binds the influenza virus to its receptors, sialyloligosaccharides, on the host cell surface. The viral envelope and the host cell membrane fuse giving the viral RNA access to the host cell surface. The viral envelope glycoproteins (see Figure 1), hemagglutinin, and neuraminidase. The viral envelope is a lipid membrane derived from plasma membrane of an infected host cell. Influenza strain targets also differ. Notably, the H5N1 virus and other avian-derived strains are neurotropic while H1N1 is thought not to be (76–78).

For infection to be successful, hemagglutinin binds the influenza virus to its receptors, sialyloligosaccharides, on the host cell surface. The viral envelope and the host cell membrane fuse giving the viral RNA access to the host cell (74, 79). Neuraminidase facilitates virus release (74, 75). Following the production of viral particles in the nucleus of the host cell, the host cell lyses and dies (75). Protective immune responses from the cell occur; the viral hemagglutinin, neuraminidase, and matrix 2 (M2) proteins are targeted by antibodies; matrix 1 (M1) proteins are targeted by T cells (80); and nucleoproteins are targeted by T cells (80) and nonneutralizing antibodies (81).

Experimental Paradigms of Maternal Infection

While maternal infection is reported to be a risk factor for schizophrenia, controversy remains concerning which biological processes underlie this risk. There is scant evidence for transplacental passage and persistence of the influenza virus in the offspring brain (82). More likely to be relevant are the effects of infection-induced maternal immune activation (MIA) on the developing brain (83).

MIA cannot be easily modelled in humans and longitudinal, prospective research on effects of infection during pregnancy on human development is scarce (84). Hence, translational animal models of MIA have been developed: these models have been critical in providing causality to the epidemiological data and are starting to provide clues as to the cellular and molecular mechanisms that may underlie the associations (83). Rodents in gestational periods are exposed either directly to a pathogen such as influenza, or more commonly to nonvirulent immune-activating agents such as the viral mimic polyriboinosinic-polyribocytidlic acid (poly(I:C)) or the bacterial endotoxin lipopolysaccharide (LPS), the inflammatory agent turpentine, or specific proinflammatory cytokines (83, 85). These animal models of MIA provide evidence for behavioral, neurochemical, neuroanatomic, and neuropsychologic disruptions in the offspring which map onto endophenotypes observed across human psychiatric disorders with a neurodevelopmental onset (83, 86). Such translational research complements the insights from human epidemiology by establishing causal relationships, identifying cellular and molecular mechanisms and offering the potential to explore therapeutic interventions (85, 86). Frequently, these aforementioned deficits in the MIA model demonstrate a maturational delay, such that they are not evident before young adulthood, and many studies have sought to mitigate these deficits with treatments (e.g., pharmacological, immunological, behavioral) (83). Another important etiologic advance for such animal models is to recapitulate a “two-hit” approach, in which pathology becomes evident in MIA-exposed offspring only after a second hit, such as unpredictable psychological stress (87).

MIA may lead straightforwardly to damage to the foetal brain during the early stages of neurodevelopment (82), but may also provide entry into a deviant trajectory of neural development which predisposes offspring to behavioral deficits depending on the intensity of the infection and when in gestation it occurs [early vs. late—and potentially as late as the lactation stage (88)]. MIA-associated abnormalities have been described, sometimes inconsistently, for multiple brain cell types, all of which are implicated across psychiatric disorders from postmortem data and genetic studies to a greater or lesser extent: Schwann cells (89), astrocytes and microglia (90, 91), hippocampal GABAergic cells (92, 93), dopaminergic neurons (94), and parvalbumin interneurons (95, 96).

Notably, most rodent (and primate) MIA models use a dose of poly(I:C) which models a high intensity, acute and transient (<24 h) infection, the physiological relevance of which could be questioned. Furthermore, factors such as the source, molecular weight, and endotoxin contamination of experimental poly(I:C) may be unrecognised sources of variability in foetal outcomes (97). Although MIA models specifically using pathogens as the immune activating stimulus have become rarer in recent years, primarily due to increasingly stringent safety frameworks around the use of potentially virulent pathogens (98), a series of MIA studies using human H1N1 influenza infection by the group of S. Hossein Fatemi are particularly valuable in elucidating potential mechanisms of psychosis risk. Broadly, maternal human H1N1

![Figure 1](Structure of the influenza virus.)
infection has been demonstrated to cause abnormalities, within the offspring, of the following [summarized in (99) and (59)]:

Gene Expression: the breadth of gene expression changes was greater the later in embryonic development infection occurred; for example embryonic day 16 or 18 (E16 or E18) infection disrupted more genes, across more brain regions, than did E7 infection. Furthermore, infection at later embryonic stages disrupted expression of genes involved in myelination and implicated in schizophrenia risk.

Protein Expression: increase in production of potentially harmful neuronal nitric oxide synthase (nNOS), reduction of reelin expression indicating abnormal neuronal migration and decreased synaptic plasticity, and downregulation of myelin basic protein.

Brain Structure: reduced cerebral cortical volume; increased total brain volume after early embryonic infection, decreased total brain volume after late infection.

Behavior: decreased prepulse inhibition (PPI), increased head twitch response.

Neurotransmitter Levels: reduced serotonin and taurine levels.

Placental Development: increased cytoarchitectural disorganisation, increased presence of immune cells, presence of variously sized thrombi, and dysregulation of placental gene expression.

Additional selected studies, focused on models of infection with influenza virus, are presented in Table 2.

Other animal studies have demonstrated associations between maternal influenza infection and schizophrenia-related neurotransmitter dysfunction including elevated serotonin 5-HT2A receptor expression in the frontal cortex (121), reductions of cerebellar serotonin levels at postpartum days (P) 14 and P35 (111, 115), downregulation of the metabotropic glutamate receptor 2 in the frontal cortex (121), and decrease in dopamine levels at P14 and P56 (115). Changes following poly (I:C) MIA exposure included subtle metabolic perturbations of postnatal prefrontal cortex maturation (124), and dynamic changes in volumes of multiple brain structures (125), including adult changes which can be prevented by periaidolence administration of antipsychotic medication (at nonantipsychotic dose equivalents) (126). Supporting the translational relevance of these studies, there is an emerging parallel literature in humans suggesting that early immune activation affects subsequent brain development and behavior: for example, maternal IL-6 levels during pregnancy predicted greater neonatal amygdala volumes and connectivity, which in turn predicted poorer impulse control at two years of age (127); complementary results for amygdala connectivity and internalizing behaviors have been reported for maternal cortisol levels (128).

An important mediator of the maternal immune response to infection is likely to be disruption of cytokines regulating brain development. Notably maternal infection could dysregulate cytokine networks either by direct transplacental transfer of cytokines to the foetus, by placental cytokine production or by increased foetal production of cytokines, including within the CNS (129). Cytokine dysregulation can result in perturbations of both proinflammatory and antiinflammatory cytokines. The deleterious or protective effects of any individual cytokine are likely determined by its context within a network of proinflammatory and antiinflammatory mediators, dynamically responding to external and endogenous challenges with differential expression in different brain regions over time (130). For example, macrophage-driven expression of antiinflammatory IL-10 in a mouse model can attenuate the long-term effects of prenatal viral infection, but in the absence of inflammatory stimulus, IL-10 itself precipitates offspring behavioral abnormalities (131).

Cytokines are induced in response to inflammation by neurons, astrocytes, and microglia, with the role of activated microglia in schizophrenia pathogenesis being the object of much recent attention (132). MIA exposure leads to alterations of the microglial transcriptome, with an initial shift to a more reactive state proximal to the MIA insult, followed by a delay in the maturation of brain microglia, as compared to controls (133). Alterations in the microglial transcriptome also lead to phagocytic function abnormalities and behavioral abnormalities in the adult MIA offspring (134). Results from the MIA literature are heterogenous with regards to microglial activation in offspring (91). Some studies report increased microglial density [e.g. (135)], morphology [e.g. (136)] or expression of activation markers [e.g. (137)], while other studies using late MIA have failed to demonstrate long-term changes in microglia density, morphology, or activation (138).

Inference regarding the translational relevance of these findings, too, has been limited by a lack of clarity around the utility of the putative human markers of microglial activation such as translocator protein (TSPO), bound by ligands in Positron Emission Tomography (PET) studies, and of microglial markers used in post-mortem studies; notably, in addition to microglia, both astrocytes and vascular endothelial cells show dynamic changes in TSPO expression in response to inflammatory stimuli, and in a mouse model schizophrenia-relevant behavioral abnormalities and increased inflammatory cytokine expression were associated with reduced, rather than increased, prefrontal TSPO levels (139).

Both neurotropic and nonneurotropic influenza strains can cause microglial activation and potentially contribute to inflammation (76, 77). Innate immune training against influenza confers protection against infection with antiviral interferon-stimulated defence genes, including MXA (prevents nuclear import of the virus), IFITM3 and other IFITM proteins (block host-virus cell membrane fusion), and viperin [blocks influenza virus release; (140)]. Innate immune training also promotes disease tolerance of host tissues (140) and previous activation primes microglia to respond strongly to a new stimulus (141). Previous neuropathology potentially attunes microglia to respond more strongly to systemic inflammation (142), including inflammation by chronic mild stress in periaidolence following MIA (86). Consequently, infection could prime microglia towards heightened activation, potentially increasing the risk of developing psychotic symptoms (143); alternatively, the opposite could be true, i.e., that the microglia become tolerant and as such cannot respond flexibly to new stimuli.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Influenza virus type</th>
<th>Animal infected</th>
<th>Animal age at assessment</th>
<th>Behavioral and physical outcomes</th>
<th>Pathological outcomes</th>
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<tbody>
<tr>
<td>Cotter et al.</td>
<td>1996</td>
<td>A/ Singapore/1/57</td>
<td>Mice</td>
<td>Offspring 21 days postpartum</td>
<td>N/A</td>
<td>No excess pyramidal cell disarray when compared with influenza-free, age-matched controls. Cell disarray greater among mice exposed on day 13 of pregnancy</td>
</tr>
<tr>
<td>Fatemi et al.</td>
<td>1998</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Neonate pups at P0</td>
<td>N/A</td>
<td>Increased expression of membrane protein synaptosome-associated protein 25 kDa (SNAP-25), a presynaptic neuronal marker in the neonatal brain: 40%–347% over control in most septal–dorsal hippocampal layers; 10%–114% over control in all mid septo-temporal hippocampus layers, except for the hippocampal plate; but SNAP-25 expression was reduced in all temporal–ventral levels, infected layers by 21%–33% below control except for mild increases of 8.8% and 10% in subplate and hippocampal plate layers</td>
</tr>
<tr>
<td>Fatemi et al.</td>
<td>1999</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Neonate pups at P0</td>
<td>N/A</td>
<td>Changes influencing levels of reelin, a protein responsible for normal lamination of the brain. Significant reductions in reelin-positive cell counts in layer I of neocortex and other cortical and hippocampal layers. Layer I Cajal–Retzius cells produced significantly less reelin. Decreases in neocortical and hippocampal thickness</td>
</tr>
<tr>
<td>Fatemi et al.</td>
<td>2000</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Adolescent offspring (P35) and young adults (P56)</td>
<td>N/A</td>
<td>Changes in the levels of neuronal nitric oxide synthase (nNOS) involved in synaptogenesis and excitotoxicity: increase of 147% in nNOS levels in the brain at P35, with an eventual 29% decrease on P56. Reductions in nNOS in middle and caudal brain areas on P35 and P56. Viral RNA encoding the nonstructural NS1 protein was detected in sections at midbrain levels in most animals. Negative-strand genomic RNA and positive-strand RNA, including mRNA, were found. RNA encoding nucleoprotein and polymerases, which form the replicative complex of the virus, were detected in fewer brains. RNA encoding envelope proteins were found only in occasional brains. No viral cDNA could be identified</td>
</tr>
<tr>
<td>Aronsson et al.</td>
<td>2001</td>
<td>A/WSN/33 (H1N1)</td>
<td>Four-week-old Tapa1 (antigen peptide transporter 1) gene knockout mice</td>
<td>7 days and 10, 12, and 17 months p.i.</td>
<td>N/A</td>
<td>Viral RNA encoding matrix and/or nucleoprotein detected in a proportion of foetal brains and lungs, viral RNA detected in some placentas. RNA persisted for at least 90 days of postnatal life</td>
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<tr>
<td>Aronsson et al.</td>
<td>2002</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 14 of pregnancy</td>
<td>Foetuses at pregnancy day 17; offspring 10, 20, 35, 60, and 90 days of age</td>
<td>N/A</td>
<td>Altered expression of glial fibrillary acidic protein (GFAP), a marker of gliosis, neuron migration, and reactive injury; increases in GFAP-positive density in exposed cortical and hippocampal cells; ependymal cell layer GFAP-IR cell counts showed increases with increasing brain age from P0 to P14 and P35 in infected groups. The GFAP-positive cells in showed increased only in the exposed adult mice</td>
</tr>
<tr>
<td>Fatemi et al.</td>
<td>2002</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Neonates at P0 and 14-week-old offspring</td>
<td>N/A</td>
<td>The rate of pyramidal cell proliferation per unit area decreased from birth to adulthood in both control and exposed groups, nonpyramidal cell growth rate increased only in the exposed adult mice</td>
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<tr>
<td>Fatemi et al.</td>
<td>2002</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Adult offspring</td>
<td>N/A</td>
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<tr>
<td>Shi et al.</td>
<td>2003</td>
<td>A/NWS/33CHINI (H1N1)</td>
<td>Mice on day 9.5 of pregnancy</td>
<td>N/A</td>
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<tr>
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<th>Behavioral and physical outcomes</th>
<th>Pathological outcomes</th>
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<tbody>
<tr>
<td>Asp et al. (108)</td>
<td>2005</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice on day 14 of pregnancy</td>
<td>Offspring sampled at E17 and sex-matched animals on P35, P60, and P90</td>
<td>N/A</td>
<td>Levels of transcripts encoding neuroleukin and fibroblast growth factor 5 were significantly elevated in the brains of the virus-exposed offspring at 90 and 280 days of age, but not at earlier time-points. For neuroleukin, this difference could also be observed at the protein level.</td>
</tr>
<tr>
<td>Fatemi et al. (109)</td>
<td>2005</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Newborn offspring</td>
<td>N/A</td>
<td>Significant upregulation of 21 genes and downregulation of 18 genes in brains of day 0 exposed offspring, including genes involved in signal transduction/cell communication, solute transport, protein metabolism, energy metabolism, nucleic acid metabolism, immune response, and cell growth and maintenance.</td>
</tr>
<tr>
<td>Asp et al. (110)</td>
<td>2007</td>
<td>A/NWS/33 (H1N1)</td>
<td>Newborn offspring on P3</td>
<td>Newborn offspring</td>
<td>N/A</td>
<td>Increased levels of transcripts encoding Gcm1 and syncytin B, but not syncytin A, in NIH-3T3 cells as well as in mouse primary neurons or glia. Overexpression of human GCM1 in NIH-3T3 cells resulted in increased levels of transcripts encoding syncytin B but not syncytin A. Systemic administration of neurotropic influenza A virus resulted in a neuronal infection and increased levels of Gcm1-encoding transcripts in brains of young mice.</td>
</tr>
<tr>
<td>Fatemi et al. (111)</td>
<td>2008</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice on day 18 of pregnancy</td>
<td>Male offspring tested at birth (P0), childhood (P14), adolescence (P35), and young adulthood (P56)</td>
<td>N/A</td>
<td>Altered gene expression of Sema3a, Trf2 and Vldlr and altered protein levels of Foxp2. Embryonic day 18 mother infection led to significant gene alterations in frontal, hippocampal and cerebellar cortices of developing offspring. Significant atrophy in several brain areas and white matter thinning in corpus callosum. Altered levels of serotonin (P14, P35), 5-Hydroxyindoleacetic acid (P14) and taurine (P35). Changes in mRNA and protein levels of nucleolin, aquaporin 4, and connexin 43 (markers involved in ribosomal RNA transcription, potentially viral replication, water transport, and changes in brains of subjects with autism): nucleolin mRNA and aquaporin 4 significantly decreased in neocortex at P0 and P35. Protein levels were significantly upregulated at P35 and P56 in neocortex and P56 in cerebellum. Microcephalin mRNA was significantly decreased in neocortex at P56 and protein levels were significantly decreased at P56 in the cerebellum. Two-fold or greater upregulation of 103 genes and downregulation of 102 genes in cerebellum at P35. Two-fold or greater upregulation of 27 genes and downregulation of 23 genes in the cerebellum at P56. Genes with their regulation disrupted are involved in cell growth and/or maintenance, channel proteins, membrane receptors, signalling, and transcription regulation, among other functions.</td>
</tr>
<tr>
<td>Fatemi et al. (112)</td>
<td>2008</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice on day 9 of pregnancy</td>
<td>Offspring tested at birth (P0), childhood (P14), adolescence (P35), and young adulthood (P56)</td>
<td>N/A</td>
<td>Altered levels of transcripts encoding several key enzymes of the kynurenine pathway observed in the brain on P7 and P13 but not on day P24. On P13, infiltrating T lymphocytes and increased levels of kynurenic acid in the brains of the infected animals.</td>
</tr>
<tr>
<td>Fatemi et al. (113)</td>
<td>2008</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice on day 16 of pregnancy</td>
<td>Offspring at P35 and P66</td>
<td>N/A</td>
<td>(Continued)</td>
</tr>
<tr>
<td>Holtze et al. (114)</td>
<td>2008</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice infected at P3 or P4</td>
<td>Whole brains from both sexes sampled at P7, P13, or P24</td>
<td>N/A</td>
<td>(Continued)</td>
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<tr>
<td>Study</td>
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<tr>
<td>Winter et al. (115)</td>
<td>2008</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice</td>
<td>Mice on day 16 of pregnancy</td>
<td>Male offspring tested at P0, P14, P35, and P56</td>
<td>N/A</td>
</tr>
<tr>
<td>Asp et al. (116)</td>
<td>2009</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice</td>
<td>Wild-type mice and Tap1 gene knockout mice infected at P3 or P4</td>
<td>3–4-months-old male mice</td>
<td>Infected Tap1 gene knockout mice, but not wild type mice, exhibited deficits in working memory, increased rearing activity, and anxiety</td>
</tr>
<tr>
<td>Shi et al. (117)</td>
<td>2009</td>
<td>A/NWS/33CHN1 (H1N1)</td>
<td>Mice</td>
<td>Mice on day 9.5 of pregnancy</td>
<td>Adult offspring and offspring 11 days of age</td>
<td>Male offspring tested at P0, P14, P35, and P56</td>
</tr>
<tr>
<td>Fatemi et al. (118)</td>
<td>2009</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice</td>
<td>Mice on day 16 of pregnancy</td>
<td>Male offspring tested at P0, P14, P35, and P56</td>
<td>Tap1 gene knockout mice, but not wild-type mice, exhibited a reduction in PPI at 5–6 months of age</td>
</tr>
<tr>
<td>Fatemi et al. (119)</td>
<td>2009</td>
<td>A/NWS/33 (H1N1)</td>
<td>Mice</td>
<td>Mice on day 16 of pregnancy</td>
<td>Male offspring tested at P0, P14, P35, and P56</td>
<td>Tap1 gene knockout mice, but not wild-type mice, exhibited a reduction in PPI at 5–6 months of age</td>
</tr>
<tr>
<td>Moreno et al. (121)</td>
<td>2011</td>
<td>A/WSN/33 (H1N1)</td>
<td>Mice</td>
<td>Mice on day 9.5 of pregnancy</td>
<td>Adult offspring (10–12 weeks of age)</td>
<td>Neurons from rat embryos recovered at day 14 of pregnancy; offspring of mice infected in pregnancy tested at 30 and 90 days of age</td>
</tr>
<tr>
<td>Landreau et al. (94)</td>
<td>2012</td>
<td>A/New Caledonia/ 20/99-like (H1N1) (A/NC-L/99), A/Sydney/5/97-like (H3N2) (A/Sy-L/97), A/WSN/33 (H1N1)</td>
<td>Rats and mice</td>
<td>Primary cultures of rat mesencephalon infected after day 14 of pregnancy; mothers on day 9–11 of pregnancy</td>
<td>Neurons from rat embryos recovered at day 14 of pregnancy; offspring of mice infected in pregnancy tested at 30 and 90 days of age</td>
<td>Offspring of mother infected with both influenza virus strains showed behavioral abnormalities in exploration, anxiety and working memory. Behavioral alterations emerged in different neurodevelopmental stages depending on the strain, appearing in adult life in offspring of mothers infected with A/NC-L/99</td>
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(Continued)
In terms of potential downstream consequences of such foetal microglial activation, there is an emerging literature implicating the role of microglia in shaping brain development, including the potential for synaptic pruning via the full or partial engulfment [phago- or trogocytosis (144)] and putative degradation of synaptic inputs, a process mediated at least in the rodent visual thalamus in a complement and activity-dependent manner (145), which may also involve other molecular mediators such as TREM2 for example or the fractalkine receptor [CX3CR1; (146)] [potential mechanisms are reviewed in (147)]. Given the well-replicated finding of reduced dendritic spine number in schizophrenia (148), and evidence that patient-derived microglia-like cells are capable of synapse elimination at least in vitro (149), it is plausible also that maternal infection-induced foetal microglial activation could lead to later psychopathology through upregulation of synaptic pruning mechanisms (or conversely, loss of them: for example, early in development, microglia contact of neurons actually stimulates dendritic spine formation (150) suggesting the underexplored possibility that different pathologies play out in a potentially time- and region-specific manner).

Additionally, influenza infection may lead to placental abnormalities that result in hypoxia and/or nutritional deficiency or foetal brain growth restriction (122). Brain abnormalities may also stem from the maternal immune response whereby maternal autoantibodies are transported via the placenta and interact with foetal brain antigens to disrupt brain development [the teratogenic antibody hypothesis of schizophrenia; (151–153)]. The concept of infection-induced brain autoimmunity is explored in the next section.

**Influenza and Autoimmunity**

Research on autoimmune disorders and schizophrenia dates to the 1950s, when schizophrenia was found to be protective against the development of rheumatoid arthritis (154, 155). Subsequently, the cooccurrence of schizophrenia with celiac disease was noted [e.g. (156)]. Recent meta-analysis suggests a positive association between nonneurological autoimmune disorders and psychosis (157). The risk for developing schizophrenia in people with autoimmune disorders was found to increase in association with increasing number of hospitalisations for infections (158), suggesting a synergistic effect. Drawing on the clinical observation that patients with autoantibody-mediated encephalitis frequently presented with psychosis (159), serological research has reported the presence of these same autoantibodies against cell surface neuronal antigens in some patients with schizophrenia [e.g. (160, 161)]. An extensive literature also focuses on markers of previous infection (usually IgG antibodies to specific pathogens, including influenza) in adults with psychotic disorders, with exposure to several organisms associated with increased schizophrenia risk or risk for a specific psychosis symptom profile (e.g. impaired cognition) (17, 162).

**Viral Infection, Neuronal Surface Autoantibodies and Psychosis: Anti-NMDAR Encephalitis as a Model of Autoimmune Psychosis With Potential Infective Antecedents**

Autoimmune encephalitis (AE) frequently presents with acute psychosis in adults (163, 164). Autoantibodies to a variety of CNS cell surface antigens (neuronal surface autoantibodies; NSAbs) have been implicated in AE, including the NMDAR and more rarely LGI1, CASPR2, AMPAR, GABA<sub>A</sub>R, GABA<sub>B</sub>R, D2R, DPPX, mGluR5, and GlyR (165–169). Of the autoimmune encephalitides associated with the above antigens, NMDAR encephalitis presents most frequently with psychosis. The typical pattern includes prodromal malaise, or influenza-like symptoms, before the emergence of psychiatric symptoms. 4%
of patients show isolated psychotic episodes at presentation or relapse (170) and behavioral and cognitive impairments including psychosis are predominant early symptoms (171). The psychosis reported in anti-NMDAR encephalitis is distinctive, polymorphic (with significant affective elements) and does not correspond clearly to currently existing categories of psychotic disorder in mainstream psychiatric use (172). Anti-NMDAR encephalitis is caused by IgG antibodies directed against an epitope on the N-terminal domain of the NMDA glutamate receptor (173, 174), with intrathecal antibody production by B lymphocyte descendants thought to be essential for pathogenesis.

Anti-NMDAR encephalitis is associated with ovarian teratoma in under a third of cases. An intriguing association between infection and the development of the disorder became apparent when it was observed that a number of patients experiencing “relapses” following herpes simplex virus (HSV) encephalitis had cerebrospinal fluid (CSF) NMDAR antibodies, suggesting that these “relapses” were in fact a postinfectious AE, rather than the result of reinfection or viral reactivation (175). Subsequent work has established that NMDAR antibody production can occur following HSV encephalitis even in the absence of clear “relapse” or encephalopathy (176), and that nonencephalitic HSV infection is also more common in patients with anti-NMDAR encephalitis (177). Other viral pathogens—including Epstein Barr Virus, Human Herpesvirus 6, cytomegalovirus, adenovirus and HIV—have been implicated in this and other autoimmune encephalitides [including those characterized by antibodies to the GABA_A and GABA_B receptors, the AMPA receptor and the dopamine D2 receptor; reviewed in (178)].

A potential association between anti-NMDAR encephalitis and influenza is supported by reports of at least five patients who developed the disorder following influenza vaccination (179–182)—although in none of these cases can causation be proven. A phylogenetic relationship has been suggested between microRNAs related to anti-NMDAR encephalitis and the H1N1 influenza virus, with some authors suggesting a theoretical basis for the possibility that anti-NMDAR encephalitis could be induced by influenza vaccination [(183); see also next section].

Anti-NMDAR encephalitis shares clinical features with EL in children, and indeed NMDAR antibodies have been reported in children with contemporary EL (184). When considered in the light of classic research on EL following the 1918-1919 Spanish influenza pandemic (32), this suggests a potential relationship between influenza infection, NSAbs and psychosis in anti-NMDAR encephalitis. The association between influenza and EL is, however, highly controversial, not least because of temporal and geographical discrepancies between the start of the pandemic and the first recorded EL cases, as well as studies on post-mortem tissue which have frequently failed to find evidence of influenza virus; but given historical issues with case ascertainment and storage of biological samples potentially undermining efforts at viral detection, the association remains a plausible hypothesis for some authors (185).

This interpretation is supported by findings that anti-NMDAR encephalitis may be seasonal, with a peak in incidence during winter (186), potentially converging with seasonality of influenza. Recent research found that Māori and Pacific Island populations have higher incidence and potentially more severe outcomes of anti-NMDAR encephalitis, a finding of significance given that population’s apparent increased susceptibility to severe influenza infection (187, 188).

NMDAR antibodies are of interest in schizophrenia because of their links to the glutamate/NMDAR hypofunction hypothesis of psychotic disorders: NMDAR antibodies found in patients with schizophrenia can disrupt NMDAR dynamics in vivo (189, 190) providing prima facie support for the NMDAR hypofunction hypothesis. Crucially, Hammer et al. (191) reported the presence of influenza virus A or B IgG was significantly associated with NMDAR antibody seropositivity in a large cohort of adult patients with psychotic disorders and disease and healthy controls, a finding that was subsequently replicated in an independent cohort (192).

Acquired Neuronal Autoimmunity and Its Relevance to the Maternal Exposure Model

As described above, infection-induced neuronal autoimmunity may have relevance for some acute psychoses. However neuronal autoimmunity also has relevance in the context of maternal transmission. Maternal-foetal transfer of pathogenic antibodies has long been proposed as a potential mechanism in the development of ASD and, to a lesser extent, for schizophrenia also (153). Although not formally regarded as part of the MIA paradigm, recent animal models have had some successes in recapitulating neurodevelopmental phenotypes in immunisation paradigms whereby maternal antibodies are transferred to the offspring, resulting in neuropathological and behavioral abnormalities (193–195). Two of these studies used CASPR2 antibodies, cell surface IgG antibodies which have been implicated in encephalitis and a variety of peripheral nerve manifestations. Intriguingly, a study by Coutinho et al. found that NMDAR antibodies were more frequent in mothers of children with neurodevelopmental disorders, who themselves (i.e. the mothers) subsequently developed psychosis. This finding was not replicated in another cohort in which the mothers did not go on to develop psychosis, but clearly mandates attempts at replication (196). A recent animal study has shown that maternal-foetal transfer of recombinant NMDAR NR1 antibodies—at levels that did not affect the behavior of the pregnant mother—resulted in impaired neurodevelopmental reflexes, reduced anxiety, motor hyperactivity, and impaired sensorimotor gating, the latter two of which were regarded as psychosis-like phenotypes (197) (but see section “Experimental paradigms of maternal infection” for transdiagnostic relevance of these behaviors).

Influenza and Molecular Mimicry

The association between influenza infection and NMDAR autoantibody status may have structural molecular basis. The influenza A M2 channel and NMDAR share a ligand, the antiviral compound amantadine (198), suggesting putative structural homology which could form the basis for NMDAR autoimmunity occurring after infection. In molecular mimicry,
there is sharing of sequences, such as linear amino acid sequences, by molecules from dissimilar genes or their protein products. In infection, if the virus shares cross-reactive epitopes for B or T cells with the host, the host immune cells can target both the infecting agent and the host itself, potentially inducing autoimmune disease (199). The processes involved include T$_\text{c}$ cells damaging self-tissue by lysis or T$_\text{h}$ cells releasing cytokines. Cytokines in turn activate macrophages or stimulate secretion of antibodies, and antibodies bind to cross-reactive epitopes on the surface of tissues, triggering further cytokine production by macrophages (200). Damaged tissues can also release new self-epitopes which activate autoantigen-reactive T and B cells, recognising those self-epitopes [epitope spreading; (201)].

There are multiple strands of evidence that influenza infection may have an aetiological role in systemic autoimmunity, including in Henoch-Schonlein purpura, type 1 diabetes mellitus and antiphospholipid syndrome [reviewed in (202)]. In one study, influenza vaccination induced autoimmunity (primarily antiphospholipid antibodies) in apparently healthy volunteers (203). H1N1 infection in rabbits has also been shown to induce brain-reactive antibodies, including to a 37kDa target also present in humans (204). Precedent for the role of influenza exposure initiating neurological disorder, potentially via molecular mimicry, exists for Guillain-Barre syndrome (205) and narcolepsy, in which hypocretin-producing neurons could be an autoimmune target due to molecular mimicry between H1N1 virus-derived antigen and a neuronal autoantigen in HLA-DQB1*06:02 positive patients (206, 207); see (208) for an example model of narcolepsy. This association is supported by epidemiological findings of an increased risk of narcolepsy in children following the H1N1 vaccination, Pandemrix (209, 210), and by serological findings that antibodies to influenza nucleoprotein might cross-react with hypocretin receptor 2 in patients with Pandemrix vaccination [(211), although see (212–214)].

Further evidence for molecular mimicry as a bridging link between influenza infection, the adaptive immune response and neurodevelopmental risk for schizophrenia comes from gene sequence overlap between the H5N1 virus and genes abnormally regulated in schizophrenia (215). Furthermore, the H1N1 influenza antiviral protein hemagglutinin was found to share peptide structure with a variety of human axon guidance proteins; the majority of proteins identified as containing homologous sequences are involved in processes which, if disrupted, could lead to deviant neurodevelopmental trajectories. The observed peptide matches were conserved across influenza strains and frequently involved experimentally validated hemagglutinin epitopes (216). Finally, the NMDAR 2A subunit was found to share peptides with several pathogens, including the influenza A virus (217). The findings suggest that anti-pathogen immune responses to the influenza A virus may cross-react with multiple schizophrenia-related proteins. This reaction could potentially trigger processes which may ultimately lead to schizophrenia. Work from our group has confirmed the higher-than-expected overlap between the influenza proteome and schizophrenia-relevant proteins, additionally identifying hemagglutinin as contributing, amongst influenza proteins, the most extensive peptide sharing [Kepinska et al., in submission; see also (218)].

**CONCLUSION AND FUTURE DIRECTIONS**

Converging evidence demonstrates that infection with the influenza virus has a multiplicity of effects on prenatal and postnatal processes which, when disrupted, could result in increased risk of the development of schizophrenia or acute psychoses in adulthood. Figure 2 outlines potential prenatal and postnatal pathogenetic contributions. Nonetheless, it is important to emphasise that infection has been linked with increased risk of several psychiatric disorders (see Introduction). It is therefore not clear to what extent the mechanisms discussed in this review are schizophrenia-specific, or whether, as is highly likely, other factors may shape the clinical expression of disease.

Outstanding questions and possible future experimental approaches are summarized in Box A. Future immunity-focused research on schizophrenia and influenza should further explore the relationship between infection and the innate and adaptive immune response in schizophrenia using animal models and large-scale serological studies in patients at different stages of disease. To date, MIA models typically include very little deep immunophenotyping, and discussion of the adaptive immune response in these models has been almost entirely lacking. Standardised and more sensitive testing technologies are required, including improved noninvasive methods to assess central neuroinflammation in humans and nonhuman animals (222, 223).

Recent developments in stem cell technology suggest the possibility of using induced pluripotent stem-cell (iPSC) microglia-like cells [as per (149)] to assess how influenza infection affects the phenotype of these cells. Potentially, iPSC-derived cerebral organoids [so-called ‘mini brains’ (224)] could offer a window into the effects of influenza infection on relevant aspects of neurodevelopment.

While this paper reviews limited case studies and series indicating that in some instances influenza vaccination has been linked to CNS-directed autoimmunity, there is currently no evidence demonstrating a clear association between influenza vaccination and the development of schizophrenia or other psychotic disorders. The limited reported cases constitute a weight of evidence which is far weaker than the many epidemiological studies supporting the association between maternal influenza infection and schizophrenia. Influenza vaccination—both pandemic and seasonal—has saved and continues to save countless millions of lives worldwide, with an overwhelming evidence base supporting its efficacy. Within this context, influenza vaccination may nonetheless represent an as-yet underutilised opportunity for epidemiological and mechanistic explorations of potential influenza-psychosis associations. For example, healthy volunteers having the vaccination could be assessed using immunophenotyping, brain imaging, and behavioral measures to further characterize the acute response to influenza exposure [analogous to similar...
human studies of the acute response to LPS or typhoid vaccine administration (219–221).

From the perspective of prevention of psychosis, consideration has been given to the potential use of antiviral medication in at-risk pregnant women. Although human studies are lacking, pilot studies in mice suggest that giving oseltamivir to pregnant mice can prevent some influenza-induced changes in the offspring (99). And while oseltamivir is regarded as having a favourable profile in pregnancy, there are no data on the long-term effects on neurodevelopment in human children.

Consideration has also been given to the potential role of influenza vaccines prior to, or during, pregnancy as a preventive measure to limit the prenatal teratogenic influence of viruses (225–227). The seasonal influenza vaccine has established efficacy in preventing maternal infection, as well as partially preventing the infant through passive immunity, and its administration remains best practice for protection of mother and child, with the World Health Organisation recognising pregnant women as a priority vulnerable group. In addition, educating pregnant women to contact their healthcare provider if they have a fever is recommended in order to expedite administration of antiviral medication and supportive care (228). Some authors consider the fact that influenza vaccination is not recommended in the first trimester in some countries as cause for concern, leaving women and the developing foetus vulnerable during a critical neurodevelopmental window (228). Epidemiologically, first trimester (or any other trimester) pandemic influenza vaccination does not appear to be associated with increased childhood morbidity (229). Although neurodevelopmental outcome data are largely lacking, some mouse models suggested that influenza vaccination early in pregnancy can indeed promote behavioral function and neurogenesis in the offspring, and confer protection from the effects of MIA with LPS (230, 231). One note of caution has been raised by a cohort study of nearly 200,000 children in California which reported a small but statistically significantly increased risk of ASD following first
trimester vaccination (232); unsurprisingly the report was controversial, with ensuing disagreement concerning interpretation of the findings and whether the correct statistical measures were used (233, 234).

Given that the vast majority of children of mothers who experience an infection do not develop psychiatric disease, recent consideration has been given to maternal and foetal mechanisms of resilience to perinatal infection and inflammation: these include maternal nutritional status, the microbiome, and a variety of postnatal environmental factors (235). In terms of interventions within the MIA paradigm that have potential widespread relevance, dietary supplementation with omega-3 polyunsaturated fatty acids (PUFAs) may represent an attractive preventative strategy (236, 237).

An increase in our understanding of neuro-immune interactions has enabled a fuller understanding of the mechanistic underpinnings of the neurodevelopmental hypothesis of schizophrenia and have contributed to a more nuanced picture of schizophrenia pathogenesis which can accommodate the influence of influenza infections after the perinatal period. Our understanding of both influenza and schizophrenia has changed immensely since the 1918-1919 pandemic. The development of next-generation genetic, immunological and bioinformatic technologies may bring a resolution of the centuries-old puzzle of the relationship between influenza and psychosis.

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