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**Major Depressive Disorder: prevalent, heterogeneous, and disabling**

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV and DSM-5) classifies major depressive disorder (MDD) in individuals with depressive symptoms for at least two weeks nearly every day, which consist of at least one of the two core symptoms of depressed mood or decreased interest (anhedonia), added to a total of five symptoms when also considering the seven secondary symptoms of weight or appetite change, change in sleep, psychomotoric change, fatigue or loss of energy, feelings of guilt or worthlessness, decreased ability to concentrate, and thoughts of dead or suicide. MDD is considered a common disorder as it affects around 15% of people sometime in life,\(^1,2\) a number far exceeding the approximate 1% of people diagnosed with psychiatric disorders such as schizophrenia (SCZ), bipolar disorder (BIP) or autism (ASD).\(^1\) The first onset of MDD can occur at all stages in life, but most often MDD presents between the age of 20 and 40 to have a chronic course of more than 24 months in approximately 20% of patients.\(^3,4\) Notably, MDD affects women twice as often as men.\(^5,6\) As a consequence of the wide diagnostic criteria requiring only five of a total of nine symptoms, the clinical presentation of MDD varies considerable from one patient to the other. The DSM IV and DSM 5 acknowledges this heterogeneity by defining subtypes of MDD, such as: *melancholic depression* characterized by i.a. loss of pleasure in all activities (severe anhedonia), lack of response to positive stimuli, excessive weight loss, early-morning waking and a clear day-pattern of symptoms; *atypical depression* characterized by i.a. weight gain and increased sleep; *catatonic depression* characterized by i.a. almost full inability to speak or move; and a *depression with psychotic features* with delusions concerning guilt, punishment, disease or financial debt possibly accompanied by auditory hallucinations from a devaluing nature. Some milder forms of MDD are self-limiting and require no other interventions than psychoeducation about symptoms and lifestyle advice, whereas more severe forms of MDD requires therapies ranging from cognitive behavioral therapy or antidepressant therapy for moderate episodes, tricyclic antidepressants with lithium for severe episodes, up to electroconvulsive therapy for episodes with severe motoric and psychotic features as well as for therapy resistant severe episodes.\(^7\) Despite the range of therapeutic strategies available, not all MDD can be treated, leading to live long symptoms in some. Indeed, the suicide rate amongst MDD patients in the USA has been estimated at approximately 3.4% more often in male (7%) than in female (1%) patients.\(^8\) People suffering from MDD are often unable to participate in working and social life, and the World
Health Organization has predicted that by 2030 MDD will be globally leading in disease burden.9

The etiology of Major Depressive Disorder remains largely unknown
The etiology and pathophysiology of MDD is largely unknown, in particular when compared to other medical conditions such as, for example, diabetes mellitus, which pathophysiology has been pinpointed to failure of the islets of Langerhans in the pancreas to produce insulin (type I) or by peripheral insulin resistance (type II). Nevertheless, despite the largely unknown etiology of MDD, many associates have been identified leading to hypotheses about MDD’s pathophysiology. For example, MDD is known to be associated to lower educational attainment,10 stressful life-events, childhood trauma,11,12 and personality characteristics, but also to medical conditions such as diabetes mellitus13 and cardiovascular disease,14 and neurobiological measurements such as hypothalamic-pituitary-adrenal axis indicators,15 hippocampal volume loss,16 and inflammation.17,18 Childhood trauma, often defined as trauma before the age of 16, is one of the most notable risk factors, with an OR for MDD between 2 and 3, that also increases risk amongst MDD patients to suffer from psychotic features, to attempt suicide and to achieve poorer treatment outcome.12 In a step towards understanding MDD’s etiology, Kendler et al. have suggested a developmental model where three broad pathways interact; internalizing factors (genetic risk factors, neuroticism, low self-esteem, early-onset anxiety, and past history of major depression), externalizing factors (genetic risk factors, conduct disorder, and substance misuse) and adversities.19,20 From a purely biological perspective, MDD has been hypothesized to arise from synaptic deficiency of monoamines (serotonin, dopamine, noradrenalin) given the effectiveness of synaptic monoamine increasing medication such as selective serotonin reuptake inhibitors, but this hypothesis is now considered too simplistic.21,22 It is clear that MDD is associated to many social, psychological and biological factors, but its etiology still remains largely unknown, as do the reasons for its association with these factors. Some associations may be causal, others may be a consequence of MDD, and yet others may be due to a shared etiology, which may include shared genetic risk.

Relevance of genetic research
Genetic research might reveal itself as powerful catalyzer of research in MDD’s etiology in the years to come. Firstly, the nature of the association between MDD risk and genetic variants is unique, because the direction of causality is certain:
genetic risk variants impact MDD and not visa versa. Notably, this property is already lost in the next physiological level, because the association between MDD and the expression of a gene may be attributable to gene-expression impacting MDD but also to MDD impacting gene-expression via e.g. increased stress. Secondly, genome-wide association studies (GWAS) provide the opportunity for hypothesis-free testing of all possible pathophysiological pathways potentially inspiring novel therapies. Thirdly, genetic research can help to understand why individuals differ in their vulnerability for MDD by, for example, assessing the proportion of variation in MDD risk in the population attributable to genetic effects expressed as the so called heritability \( h^2 \). Fourth, theory about the distribution of genetic variants and mating patterns can help to understand why psychiatric disorders still exist in the population despite their unfavorable effects on reproductive fitness, which may be of minor interest for MDD (impacted by very little natural selection) but can be much more relevant for disorders such as schizophrenia (SCZ) and autism (ASD) associated with a clear reduced fecundity.\(^{23}\) Fifth, genetic variants that influence multiple traits can potentially help to understand, at least in part, some of the many comorbidities associated with MDD. Importantly, the points of relevance described here have not yet been fulfilled as psychiatric genetic research has met major challenges, but suggest nevertheless the exciting potential of psychiatric genetic research in general.

**Genetic research in MDD**

Genetic research in MDD had been quickly evolving in the years up to 2011, when this PhD project commenced. Until around 2009, genetic research on Major Depressive Disorder (MDD) had mainly focused on twin and family studies, linkage studies, and candidate gene studies, but has since increasingly concentrated on genome-wide association studies (GWAS) with case-control data. Population based twin and family studies pointed to an heritability for MDD in the general population of around \( h^2 \approx 0.35 \) by considering MDD risk in relation to the expected genetic similarity between family members without requiring information on genotypes.\(^{24-26}\) Notably, increased heritability estimates have been reported for hospitalized depression (0.48-0.75)\(^{27}\) and for lifetime diagnosis based on repeated assessments in women (0.66).\(^{28}\) Linkage studies provided the first attempts to find specific genetic regions associated to MDD in large pedigrees or sib-pair studies by testing for linkage between genetic loci and disease status. However, linkage studies can only detect genetic regions with large effect on MDD risk, and did not lead to consistent findings.\(^{29}\) In candidate gene studies, a single or couple of genetic variants were being tested based on a
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priori hypotheses about gene function by comparing individuals with MDD to healthy unrelated controls, and although these studies did point to some potentially associated genes\textsuperscript{30} these results showed little consistency.\textsuperscript{31} Around 2005, new hope arose as -following sequencing of the first human genome costing 3 billion US dollar and published in 2001.\textsuperscript{32} techniques for genotyping had progressed to provide the opportunity to genotype at relatively low cost over 500,000 genome-wide single nucleotide polymorphisms (SNP), which resulted in published MDD GWAS results from 2009 onwards.\textsuperscript{33,31,34–37} Nevertheless, the largest GWAS up to 2011, comprising 5763 MDD cases and 6901 healthy controls, found no genome-wide significantly associated SNP, which was disappointing to many.\textsuperscript{31}

Datasets used in this thesis
This thesis analyzes both empirical data and simulated data. The empirical data come from the Netherlands Study of Depression and Anxiety (NESDA),\textsuperscript{38} Netherlands Twin Registry (NTR),\textsuperscript{39} and Psychiatric Genomics Consortium (PGC).\textsuperscript{40} NESDA is an ongoing longitudinal cohort study of MDD and anxiety disorders whose nearly 3,000 subjects were recruited from mental health care settings, general practitioners, and the general population in the period from 2004 to 2007. NTR has been collecting data on Dutch twin families since 1991 and comprises data on nearly 90,000 adult individuals. NESDA and NTR collaborate in their genetic research on MDD where NESDA provides most of the cases and NTR most of the controls that were all genotyped together.\textsuperscript{29} The PGC is an ever-growing international collaboration combining genotype data from cohorts from multiple countries (the USA, Australia, Germany, Denmark, Sweden, the UK, and NESDA and NTR from the Netherlands).

Part A: Genetic effects and environment in depression

Why were no genome-wide significant loci for MDD found by 2011?
A sample of 5763 cases and 6901 controls for a GWAS on MDD was considered very large at the time, and the lack of significantly associated loci in MDD, as well as the dearth of findings for other traits, inspired development of novel methods and reconsideration of the expected genetic architecture (number of risk loci, their frequency, their effect sizes, and the way in which they act together). First, it should, however, be noted that testing loci in around 1,000,000 independent genomic regions requires control of false positive findings by setting a stringent
level of significance at $5 \times 10^{-8}$ (0.05/1,000,000). Naturally, large sample sizes are needed to balance this high multiple testing burden. Nevertheless, despite this stringent significance threshold, the MDD GWAS was still expected to detect 20% of risk loci with an odds ratio (OR) of 1.2 or larger, and having found none implicated effective loci for MDD would likely have much smaller effects.\textsuperscript{31} Because the loci tested were still expected to explain (a large part of) the heritability estimated at 35% from family studies, it was concluded that MDD is most likely affected by many loci with small effect pointing to a polygenic genetic architecture. This polygenic architecture was further confirmed by methods developed just before 2011 to test for the overall effect of all loci at once. Polygenic risk scores were constructed in a target sample by counting the number of risk alleles based on GWAS results from an independent discovery sample, and significantly predicted a small proportion of variation in MDD.\textsuperscript{41} These findings were in line with results from another method, genomic-relatedness-matrix restricted maximum likelihood (GREML), which compares concordance in disease status within pairs of individuals to their genetic relatedness based on SNP data, to find that a considerable proportion of variation in MDD was explained by genotyped SNPs, referred to as the SNP-heritability.\textsuperscript{42} In addition, So et al developed a method to assess the SNP-heritability from z-statistics from GWAS results,\textsuperscript{43} which showed similar estimates of a SNP-heritability of around 0.3 for MDD\textsuperscript{42} in data from the Netherlands Study of Depression and Anxiety (NESDA)\textsuperscript{38} and Netherlands Twin Registry (NTR).\textsuperscript{39} Following these considerations about the genetic architecture, it was assumed in 2011 (when this PhD project commenced) that significantly associated SNPs would be found for MDD with increasing sample size. These considerations were, notably, not unique for MDD and also applicable for other traits, such as schizophrenia (SCZ) and height, where additional significant SNPs were also anticipated with increasing sample size.

**Challenges unique for genetic research MDD**

In addition to the challenge for most traits introduced by the high polygenicity and small SNP effect sizes, the GWAS on MDD also met more unique challenges as it should be noted that other traits had already detected significant loci by 2011, such as the seven loci found for SCZ.\textsuperscript{44} Although the SCZ GWAS contained more cases (12,945) than the MDD GWAS (5763), the difference in results was also considered to be attributable to the different disease-characteristics of MDD compared to SCZ. First of all, family studies pointed to a lower heritability for MDD (0.35) compared to SCZ (0.8),\textsuperscript{1} which already suggested that loci would be harder to find for MDD than for SCZ (assuming roughly the same number of
effective loci for both traits implying smaller average effect sizes for MDD). However, other factors were also considered relevant, such as the high prevalence of MDD and its diagnostic heterogeneity.

To understand the impact of the high lifetime prevalence of MDD (15% compared to 1% for SCZ) on the power to detect associated loci, it is helpful to consider the liability-threshold model, which assumes that MDD and SCZ are underpinned by an unobserved disease-liability resulting from both genetic and environmental effects (typically assumed normally distributed), and that individuals are affected when they exceed a liability threshold (defined by the population prevalence). Under this model, individuals with MDD have less extreme MDD-liability values than individuals with SCZ have SCZ-liability values, because MDD has a lower disease-threshold following from its higher prevalence. A GWAS on MDD can, thus, be compared to a GWAS on height comparing individuals with average height of e.g. 170 (controls) to individuals with height of 180 (cases), whereas SCZ can be thought of as comparing individuals with height of 172 to individuals with height of 210. This illustrates why GWAS on MDD have less power than GWAS on SCZ, and it has indeed been suggested that GWAS on MDD would require four times the number of cases than GWAS on SCZ. Second, it can be seen that the average MDD-liability in MDD controls (individuals without MDD) is lower than the SCZ-liability in SCZ controls, which suggests that screening of controls is more important for MDD than for SCZ; a difference further exaggerated by the later onset of MDD and thus larger uncertainty of disease-status in controls. The importance of screening controls in relation to the disease prevalence is addressed in Chapter 6 of this thesis with respect to the SNP heritability.

Another factor often discussed in relation to the lack of significant GWAS findings for MDD is its heterogeneity and nosological uncertainty. First of all, MDD isn’t based on etiology of disease but on clustering of psychological and physical symptoms, which is in line with the other psychiatric disorders, but not with somatic disorders such as, for example, diabetes mellitus. Moreover, compared to other psychiatric disorders, MDD has a relatively wide range of diagnostic criteria leading to large heterogeneity in symptoms: of the nine MDD criteria a minimum of only 5 are required (including at least one of two core symptoms) resulting in 227 possible combinations of symptoms to meet MDD diagnosis. The number of possible combinations increases even further as some of the criteria are loosely defined to include two opposing symptoms, such as either gain or loss in both the weight-criterion and sleep-criterion, and feelings of either psychomotor agitation or retardation. One could hypothesize that different
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combinations of DSM-IV criteria (leading to a MDD diagnosis) might be attributable to different pathological pathways, which could partly explain why GWAS’s on MDD lack power to detect associated loci. It has, furthermore, been hypothesized that etiology of MDD could differ across different environmental conditions (irrespective of the combination of diagnostic criteria); a phenomenon referred to as gene-by-environment interaction.

Gene-by-environment interaction in MDD with candidate genes

Research on gene-by-environment interaction (GxE) tests whether genetic effects are moderated by environmental conditions resulting in a combined impact of environmental and genetic effects different from the sum (or product) of their individual effects. If GxE-effects were to exist in MDD, they would form an additional challenge for GWAS to detect genetic effects when environmental conditions are not appropriately accounted for. Studies on GxE-effects in MDD are, therefore, relevant to inform optimal GWAS design, but also to gain insight in potential different pathophysiological pathways across environmental strata, or environmental potentiating of genetic effects. In principal, GxE-studies can be conducted for all genetic variants across numerous environmental conditions, and many different genes and environmental factors have indeed been studied in the candidate-gene era. The most illustrative example in this aspect is the 2003 paper of Caspi and colleagues, in which childhood trauma was found to increase the impact on MDD of the length polymorphisms in the serotonin-transporter-linked polymorphic region (5-HTTLPR). This interaction-effect was considered a scientific break-through at the time that fitted well with the hypothesized relevance of the serotonin transporter, which is the target of antidepressant medication inhibiting serotonin reuptake in synapses. However, the initial finding was followed by numerous conflicting replication efforts, and even meta-analyses lead to opposing conclusions. It had been argued that the conflicting findings in replication efforts were attributable to differences in study design, but the 2011 study of Fergusson et al followed a very similar design to the original paper to find no evidence for the interaction-effect reported by Caspi et al. Taken all together, it seems unlikely that the original finding is generalizable to other cohorts. In addition, a critical review suggested that the GxE-literature from the candidate-gene era suffered from publication bias, because 96% of novel GxE-studies yielded significant results compared to only 27% of replication studies, and because smaller replication studies reported more significant results than the larger samples (with the threshold for significance set at 0.05 typical for candidate gene studies). In Chapter 2 of this thesis, a well-described large
sample containing individuals from NESDA and NTR adds to the discussion by testing for interaction between childhood trauma and other environmental conditions with 5-HTTLPR, while also considering the single nucleotide polymorphism, rs25531, that has been found to moderate the function of 5-HTTLPR.  

**Gene-by-environment interaction in MDD with genome-wide information**

Although research on main genetic effects had evolved from candidate-genes to a hypothesis-free GWAS approach, research on GxE-effects has not yet followed this progress. As discussed above, the GWAS on main genetic effects lacked power with a total of 5763 cases and 6901 controls collected from many contributing cohorts, and detailed information on environmental conditions was available for only some of the cohorts contributing to the overall GWAS sample. As a consequence, power was lacking to test single-SNP GxE-effects for all SNPs as this would require a genome-wide significance threshold of $5 \times 10^{-8}$. Nevertheless, GWAS samples had also been applied for polygenic risk score (PRS) analysis to capture the effect of all genotyped SNPs at once by utilizing SNP-effect estimates from an independent discovery sample. Notably, PRS analyses require a significance threshold of only approximately 0.05 (because only 1 test in target set), given the availability of independent discovery results. Furthermore, contrary to single SNPs (or candidate genes), the PRS had a repeatedly confirmed effect on MDD making it a more feasible instrument to test for GxE than single loci (assuming it is unlikely that single loci have completely opposing effects across different environmental conditions). The interpretation of GxE results with PRS (PRSxG) is more complex than the interpretation of GxE for candidate genes, but the relevance of PRSxG is still found in its potential to point to environmental conditions with increased genetic effects informing optimal GWAS design, and in its possible contribution to obtain insight in MDD’s heterogeneity.

In Chapter 4 of this thesis, the impact of PRS on MDD is compared between individuals exposed and individuals not exposed to childhood trauma in NESDA. This single-cohort finding is, subsequently, tested in Chapter 5 with data from the international collaboration of the Psychiatric Genomics Consortium (PGC), which allows combining data of several cohorts to optimize sample size.

**MDD and educational attainment**

In addition to aiming to detect causal genetic risk variants, genetic research can also contribute by testing whether shared genetic effects can help to explain comorbidities or phenotypic association. An association of particular interest is
the increased risk for MDD in individuals with lower educational attainment (EA), which has been confirmed in various western countries with a three percent decrease in MDD risk per additional year of education estimated in a meta-analysis of 37 studies. This association might reflect an impact of lower EA on increased MDD risk (via e.g. less effective coping strategies), an impact of MDD on one’s possibilities to obtain his or her full educational potential, or a third factor impacting both EA and MDD risk. Such a third factor could, for example, comprise of certain personality characteristics and of shared genetic effects. Analyses for the causes of the association between MDD risk and lower EA are relevant to further understand the etiology of MDD, but also as this might inform future preventions programs to reduce this deleterious link. In Chapter 3 of this thesis different methods are, therefore, applied to test if the association between MDD risk and lower EA could be attributable to shared effects of genotyped SNPs.

Part B: Methodological aspects of study design, SNP-heritability, power and assortative mating

Different types of GWAS design
The GWAS cohorts that contribute to the Psychiatric Genomics Consortium (PGC) have recruited cases and controls with different strategies, which is likely to impact results from association testing but also estimates of the SNP heritability (the proportion of population variance in disorder risk attributable to genome-wide genotyped SNPs). Some of the MDD cohorts have ascertained cases from clinical settings and others from population; while most cohorts have carefully screened controls some do not. Nevertheless, all MDD cohorts have recruited unrelated controls, which contrasts some of the ADHD and autism cohorts that apply proband-parents trio data. Trio data of affected probands and their parents is essential to detect de novo mutations, perform imprinting studies, and obtain accurately phased haplotypes, but also provides pseudocontrols constructed from the non-transmitted parental alleles for association testing. Pseudocontrols have regularly been applied in candidate gene studies to protect against population stratification, but have also been taken forward for GWAS studies where other methods are also available to protect against stratification with GWAS data such as genomic principal components or mixed model association analysis. In Chapter 6 of this thesis, the GWAS-properties of the trio design and use of unscreened controls are addressed by deriving the expected SNP-heritability and power to detect a risk variant, while also considering that some of
the trio cohorts overrepresented multiplex family (with more than one affected proband), and while taking into account that assortative mating has been found to occur for most psychiatric traits.59

Assortative mating
Interestingly, a number of studies have found evidence for assortative mating for psychiatric traits (a population spouse-correlation in risk for psychiatric disease). Depending on the mechanisms leading to assortment, there are different consequences of assortative mating. Under phenotypic assortment, assortative mating impacts on the genetic architecture of traits and on genetic tests. In Chapter 6 of this thesis, the consequence are derived of assortative mating for SNP heritability estimates and power to detect single risk variants, and in Chapter 7 boundaries are defined for the genetic consequences of assortative mating for psychiatric traits in terms of the population disease prevalence and heritability in the next generation.

Aims of this thesis
This thesis aims to study the complex link between genetic effects and environment in depression in real data, and to explore boundaries for some of the consequences of GWAS study design and assortative mating from a theoretical perspective. Chapter 2 contributes to the debate on the possible moderating effect of 5-HTTLPR on the link between childhood trauma and depression by testing this GxE-effect in NESDA and NTR. Chapter 3 contributes to the research on the many phenotypic associates of MDD by testing whether the deleterious link between lower education attainment and increased MDD risk can be explained by genome-wide genotyped SNPs. Chapter 4 adds a hypothesis to the literature on heterogeneity of MDD’s genetic effects by testing for interaction between polygenic risk scores and childhood trauma in depression. Chapter 5 places the findings from Chapter 4 in a broader context by analyzing childhood trauma and polygenic risk for MDD in the large international Psychiatric Genomics Consortium. Chapter 6 aims to serve decisions for GWAS study design by addressing the consequences of the trio design and unscreened controls on estimates of SNP-heritability and power to detect genetic risk variants. Chapter 7 contributes to the literature on assortative mating by exploring boundaries for the genetic consequences of assortative mating with respect to population prevalence and heritability in the next generation.
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