NEUROIMAGING-BASED GENOMIC PREDICTORS OF ANTIDEPRESSANT RESPONSE

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Contents

List of Abbreviations

5-HT	Serotonin
ADM	Antidepressant Medication
BDNF	Brain-Derived Neurotrophic Factor
BUP-SR	Bupropion-Sustained Release
CBT	Cognitive Behavior Therapy
cDNA	Complementary DNA
CNS	Central Nervous System
COMED	Combining Medications to Enhance Depression Outcomes
CRHR1	Corticotrophin Receptor 1
CSEA	Cell Specific Expression Analysis tool
CTQ	Childhood Trauma Questionnaire
CYP	Cythocrome P450
DBS	Deep Brain Stimulation
DNA	Deoxyribonucleic Acid
DNAm	DNA Methylation
DNRIs	Dopamine-Norepinephrine Reuptake Inhibitors
Drd1+	Dopamine Receptor Type 1 Positive
Drd2+	Dopamine Receptor Type 2 Positive
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
DUL	Duloxetine
ECT	Electroconvulsive Therapy
ELS	Early Life Stress
ENIGMA	Enhancing NeuroImaging Genetics through Meta-Analysis
ESC	Escitalopram
FDG-PET	Fluorodeoxyglucose Positron Emission Tomography
FDR	False Discovery Rate
FKBP5	FK506 Binding Protein 5
GCTA	Genome-wide Complex Trait Analysis Tool
GENDEP	Genome-Based Therapeutic Drugs for Depression
GM	Grey Matter
GO	Gene Ontology

GR	Glucocorticoid Receptor
GREAT	Genomic Regions Enrichment of Annotation Tool
GSK3beta	Glycogen Synthase Kinase-3beta
GWAS	Genome-Wide Association Study
GxE	Gene by Environment
h^2	Heritability
HC	Hippocampus
HDRS	Hamilton Depression Rating Scale
HPA	Hypothalamus-Adrenal-Pituitary
HWE	Hardy–Weinberg Equilibrium
IBD	Identical by Descent
ICV	Intracranial Volume
LAM	Left Amygdala
LD	Linkage Disequilibrium
LPCUN	Left Precuneus
LPMC	Left Premotor Cortex
MAF	Minor Allele Frequency
MAOI	Monoamine Oxidase Inhibitors
MAP	Mood and Anxiety Disorders Program
MARS	Munich Antidepressant Response Signature
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
MDS	Multi Dimensional Scaling
MHC	Major Histocopatibility Complex
MIRT	Mirtazapine
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
NET	Norepinephrine Transporter
Ntsr+	Neurotensin Receptor Positive
PBO	Placebo
PCA	Principal Component Analysis
PGC	Psychiatric Genomics Consortium
PGS	Polygenic Scores
Pihat	IBD proportion

PReDICT	Prediction of Remission in Depression to Individual and Combined Treatments
pT	P-value Thresholds
QC	Quality Control
QIDS	Quick Inventory of Depression Symptomatology
qPCR	Real-time Polymerase Chain Reaction
R	Pearson Correlation Coefficient
R^2	Proportion of Variance Explained
r2	Linkage Disequilibrium Correlation Coefficient
RAI	Right Anterior Insula
RIN	RNA Integrity numbers
RITC	Right Inferior Temporal Cortex
RMC	Right Motor Cortex
RNA	Ribonucleic Acid
ROI	Regions of Interest
SD	Standard Deviation
SERT/*5-HTT*	Serotonin Transporter
SNPs	Single Nucleotide Polymorphisms
SNRIs	Serotonin Norepinephrine Reuptake Inhibitors
SSRIs	Selective Serotonin Reuptake Inhibitors
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
TRD	Treatment-Resistant Depression
TSS	Transcription Start Site
VEN-XR	Venlafaxine-Extended Release

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Abstract

Biomarkers to guide optimal treatment selection are lacking for major depressive disorder (MDD). Despite some promising findings, candidate gene and genome-wide association studies (GWAS) of antidepressant response have met with little success and none has focused on differential outcome to mechanistically different treatments. Development of such biomarkers would allow to better match patients with their most favorable treatment and has direct implications for the development of precision biology-based clinical practice. The overall aim of this thesis is to provide a framework for developing easily accessible predictors of individualized treatment response.

Combining imaging techniques with genetic variation and considering coexisting clinical entities may facilitate identification of novel genetic contributors and enhance our understanding of the neural basis and networks involved in treatment response variation. Using a neuroimaging-based genomics approach, we constructed polygenic predictors based on structural (hippocampus (HC) volume differences) and functional (brain glucose metabolism) neuroimaging endophenotypes of (1) overall treatment outcome and (2) treatment-specific outcome in MDD patients.

The first part of this thesis investigates whether polygenic scores (PGS) derived from single nucleotide polymorphisms (SNPs) influencing HC volume from the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) study (Hibar et al., 2015) could predict clinical improvement of depression in three independent samples. Additionally, we hypothesized that reduction in clinical/environmental heterogeneity could identify subtype specific biomarkers and improve HC-PGS prediction. HC structure and function are implicated in the neurobiology of MDD and treatment response. Better response to treatment has been observed in patients with larger HC volume, patients who have not been exposed to early life adversity, or patients with non-anxious depression.

HC-PGS were unable to predict treatment outcome overall, or when exposure to early life adversity was considered. However, HC-PGS significantly predicted outcome scores in non-anxious patients. Higher HC-PGS reflecting increased HC-volume correlated with better outcomes, agreeing with previous findings relating anxiety and reduced HC-volume with poor outcomes in depression. Gene profiles tagged by the predictive variants are enriched in cortical and hippocampal adult brain regions and cortical and striatal brain cell-type specific expression patterns.

The second part of this thesis focuses on identifying predictors of treatment-specific outcome. Previously resting state activity patterns of six distinct brain regions were reported to predict differential response to either escitalopram (ESC) or cognitive behavior therapy (CBT) (McGrath et al., 2013). We investigate whether PGS based on genetic associations with metabolic activity of these regions or PGS derived from SNPs influencing HC volume differences could predict treatment-specific outcome in an independent cohort; the Prediction of Remission in Depression to Individual and Combined Treatments (PReDICT) sample (B. W. Dunlop et al., 2012). PReDICT enrolled treatment-naïve patients and randomized them to three antidepressant treatments; CBT, ESC and duloxetine (DUL). As such, it is the largest single-site MDD randomized trial comparing CBT to antidepressant medication (ADM) ever performed.

HC-PGS, Insula-PGS and left premotor cortex-PGS predicted differential outcomes to CBT vs. ADM in PReDICT with clinically relevant effect sizes. Genes tagged by SNPs from neuroimaging-based predictive PGS overlapped with previously identified schizophrenia risk variants and were enriched for disease relevant gene ontology (GO) terms and convergent cortical and striatal brain cell-type specific expression patterns.

The approach used in this work contributes to the identification of molecular pathways possibly critical for antidepressant outcomes and offers novel insights into MDD pathophysiological subtypes. We demonstrate that combining neuroimaging and genetic markers as well as accounting for clinical subtypes is essential to identify predictors of antidepressant response and may allow selection of a specific treatment for a specific patients. **Figure 1** provides an overview of this work.

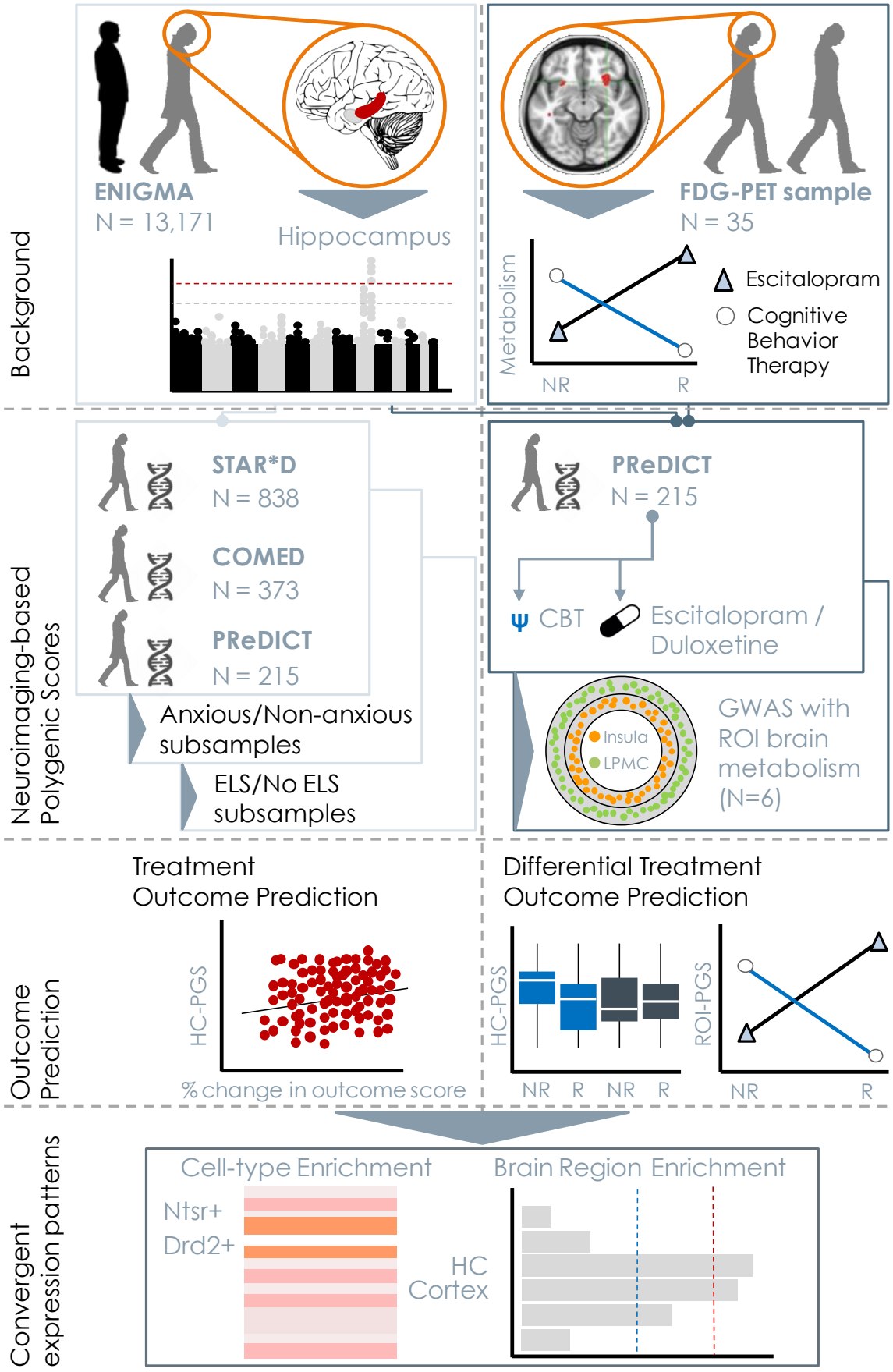


Figure 1. Overview of neuroimaging-based genomic predictors of antidepressant response approach

1. Introduction

The following section provides a general background on the main topics relevant for this research. First, core concepts such as major depressive disorder (MDD) and antidepressant treatment outcome are defined, followed by an overview of the most important risk factors, available treatments and existing biomarkers of antidepressant treatment outcome. The challenges and weaknesses faced in the field are discussed, providing as such a rationale for the aims of this research. To conclude a "combined-approach" strategy to possibly advance future biomarker research in depression is proposed.

1.1. Major Depressive Disorder

MDD is a highly prevalent psychiatric disorder that is currently the third leading cause of disability worldwide and is projected to be the number one cause of disease burden by the year 2030 (Judd et al., 2000; R. C. Kessler et al., 2003; Lepine & Briley, 2011). Lifetime prevalence is estimated to be 17% and it is twice more common in women than in men (R. C. Kessler et al., 2005; Weissman et al., 1993). The impact of MDD on individuals and societies is to a great extent due to the chronic and recurring course of illness, which is often resistant to current available treatments (Paul E. Holtzheimer & Mayberg, 2011; R. C. Kessler, et al., 2003). Persistence of depressive symptoms contributes to increased suicide risk, health-related costs, and productivity loss (Ronald C. Kessler et al., 2006). Predicting which patients will respond to which initial treatment could therefore be of great benefit.

MDD is characterized by a highly heterogeneous range of symptoms. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), MDD patients experience a pathological change of mood for at least two weeks characterized by anhedonia or depressed mood alongside three or more of the symptoms presented in Table 1. One of the instruments most used by clinicians to evaluate the presence of depression symptoms or to assess clinical treatment outcomes is the Hamilton Depression Rating Scale (HDRS); a depression-screening questionnaire originally consisting on 17-items (HAMILTON, 1960). A score of twenty or higher indicates at least moderately severe MDD, clinical remission is often defined as a score of zero to seven at the end of treatment and non-remission as a change in HDRS score of ≤ 30% from start to end of treatment, while response is typically considered as a reduction in HDRS of ≥ 50 % (HAMILTON, 1960).

Table 1. DSM-IV MDD diagnosis criteria. Source: (Association, 2000)

A	Change from previous functioning present during the same two-week period characterized by anhedonia or depressed mood along with five or more of the following symptoms: 1. Depressed mood most of the day, nearly every day, as indicated either by subjective report (e.g., sadness, emptiness) or observation made by others (e.g., appears tearful) 2. Noticeably diminished interest or pleasure in all (or almost all) activities most of the day, nearly every day (as indicated either by subjective account or observation made by others) 3. Change of ≥ 5% of body weight in a month (weight loss/weight gain when not dieting), or decrease or increase in appetite nearly every day 4. Insomnia or hypersomnia nearly every day 5. Psychomotor agitation or retardation nearly every day 6. Fatigue or loss of energy nearly every day 7. Feelings of worthlessness or excessive or inappropriate guilt (may be delusional) nearly every day 8. Decreased concentration or indecisiveness, nearly every day (either by subjective account or observation made by others) 9. Recurrent suicidal ideation without a specific plan, or a suicide attempt or specific plan for committing suicide
B	Symptoms do not meet criteria for a mixed episode
C	Symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning
D	The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism)
E	The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation

In addition to the extensive variety of symptoms comprising MDD, comorbid conditions and concomitant symptoms that are not required for DSM-IV diagnosis, may affect the course of the disorder. For example, anxiety, which co-occurs in 40-50% of patients with MDD (Melartin et al., 2002) is associated with higher illness severity, greater functional impairment (Fava et al., 2004; Fava et al., 2008; Joffe, Bagby, & Levitt, 1993), increased risk of suicide (Fava, et al., 2004; Fava, et al., 2008; Tollefson, Holman, Sayler, & Potvin, 1994) and delayed response to treatment (Clayton et al., 1991; Fava, et al., 2008).

Although anxious depression is currently not categorized as a diagnostic subtype of MDD, evidence suggests that it might be a valid diagnostic class (Fava, et al., 2004; Fava, et al., 2008). Symptom heterogeneity in MDD suggests that distinct depression subtypes may benefit from tailored treatment strategies.

1.2. Pathophysiology of MDD

The pathophysiology of MDD is poorly understood, current findings point to an “individual”-depression hypothesis, where therapies should be tailored to each patients distinct biological features (Hasler, 2010). Among systems observed to be affected in MDD are for example alterations in the hypothalamus-adrenal-pituitary (HPA) axis, structural brain changes (Schmaal et al., 2015), environmental risk factors (e.g., early life adversity), genetic variation and gene by environment (GxE) interactions (Patel, 2013).

In addition, the mechanism of action of many antidepressant drugs points to the involvement of altered neurotransmitter regulation in individuals suffering MDD. Disruptions in dopamine (Dunlop & Nemeroff, 2007; Tham, Woon, Sum, Lee, & Sim, 2011), norepinephrine and serotonin systems have been implicated in the pathogenesis of depression. In the following sections, environmental, genetic, GxE interactions and structural brain changes influencing MDD susceptibility risk are discussed in detail.

1.2.1. Environmental Risk Factors

Adverse traumatic life events, particularly early in life are among the most robust risk factors for depression (R. C. Kessler, 1997). Early life stress (ELS) like childhood trauma, abuse or neglect has been repeatedly linked to greater susceptibility to several psychiatric disorders including MDD (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008). Beyond disease risk, such environmental influences (Nemeroff et al., 2003) are also strong predictors of antidepressant treatment outcomes (Chapman et al., 2004).

For example, in a meta-analysis of ten clinical trials, response to depression treatment was reported to be significantly better in patients who did not experience childhood abuse in contrast to those who did (Nanni, Uher, & Danese, 2012). ELS can induce potential long-lasting epigenomic changes (Divya Mehta et al., 2013) and is considered more profound when compared to stressors during adulthood also in animal studies (Russo, Murrough, Han, Charney, & Nestler, 2012).

Exposures to stress and adversity activate the stress hormone system, in particular during specific developmental stages (Heim & Binder, 2012; Heim, et al., 2008; Kendler, 1998; R. C. Kessler, 1997; Lupien, McEwen, Gunnar, & Heim, 2009). Activation of glucocorticoid receptors (GR) in the brain results from glucocorticoid release following stress exposure. Subsequently, homeostasis of the HPA axis is sought through transcriptional regulation of genes that activate the HPA axis feedback loop. Increased risk to depression and other mood disorders often results when stress exposure becomes chronic, or when this regulation is disturbed. MDD patients for example, show HPA axis hyperactivity and increased levels of cortisol due to glucocorticoid resistance (i.e., impairment of GR-mediated negative feedback) (Pariante, 2006). A disrupted regulation of the stress hormone system has been reported in at least a subset of patients with depression (F. Holsboer, 2000; Pariante & Miller, 2001) and clinical response to treatment has been associated with its normalization (Schüle, 2007). Variation in modulators of the stress-hormone system, like FK506 binding protein 5 (*FKBP5*), have in fact been linked to antidepressant response (E. B. Binder et al., 2004).

While the stress hormone system has central effects on neural processing, it also impacts peripheral tissues, including peripheral blood cells and the immune system. A number of studies reviewed by Raison & Miller highlight the importance of immune function in depression (Raison & Miller, 2011). For instance a recent peripheral blood transcriptome study in a large sample of depressed patients and controls has pointed to immune signaling as an important factor, with increased expression of genes in the interferon α/β signaling pathway observed in MDD patients (Mostafavi et al., 2014).

Greater risk to develop psychiatric disorders due to stressful life events varies among individuals; some individuals are particularly more sensitive than others. These differences in vulnerability may be, to some extent, explained by interaction of these environmental exposures with genetic components or additional environmental factors as well as the presence or absence of clinical concomitant entities (i.e., anxiety). Expressly, the effects of environmental exposures may occur solely on the presence of predisposing genetic factors (Klengel & Binder, 2013). The latter highlights the importance of studying not just genetic or environmental factors, but their interactions influencing disease susceptibility.

1.2.2. Genetic Risk Factors (Polygenic Architecture)

The proportion of variance in liability that can be attributed to genetic risk factors for MDD (i.e., heritability) is estimated to be around 40% (P. F. Sullivan, Neale, & Kendler, 2000); recurrent episodes and early age onset are associated with higher familial aggregation (Kendler, Gatz, Gardner, & Pedersen, 2005). To date, most variants associated to increased risk for complex psychiatric disorders like MDD, contribute however to merely a small fraction of the genetic variation and explain only a small proportion of the heritability (N.R. Wray et al., 2010). Recent findings show that risk-associated single nucleotide polymorphisms (SNPs) are shared between many psychiatric disorders (Lee et al., 2013).

Table 2 shows a summary of MDD genome-wide association studies (GWAS) results. Up to now only very few loci have achieved genome-wide significance (Kohli et al., 2011). Recently whole-genome sequencing analyses identified two novel loci associated with MDD (**Figure 2**) (consortium, 2015). The polygenic architecture of MDD (Patrick F. Sullivan, Daly, & O'Donovan, 2012), where many genes of small effect are likely to contribute to disease risk, suggests that , outcome to antidepressant treatment is likely to be influenced too by multiple genes, as well as by their interactions with each other or with the environment (Almasy & Blangero, 2001).

Table 2. Summary of MDD GWAS studies. Table lists the number of cases and controls for each GWAS and summarizes results. Meta-analyses = M, Replication = R. Ripke et al., 2013; N. R. Wray et al., 2012, include imputed data. Top associated SNPs are listed for each study. Source: Flint & Kendler, 2014

Study	Discovery sample	SNP Discovery	P-value Discovery	Position Discovery	M/R* Sample	SNP M/R*	P-value M/R*	Position M/R*
Lewis et al., 2010	3,230	rs9416742	1.3×10^{-7}	chr10: 60542444	3,336	rs606149	2.5×10^{-6}	chr1: 193921298
Muglia et al., 2010					3,141	rs4238010	5.8×10^{-6}	chr12: 4118067
Sullivan et al., 2009	3,541	rs2715148	7.7×10^{-7}	chr7: 82449785	11,972	rs2715148	8.2×10^{-1}*	chr7: 82449785
Shyn et al., 2011	2,857	rs12462886	1.7×10^{-6}	chr19: 29263440	7,385	rs1106634	6.7×10^{-7}	chr8: 20065799
Shi et al., 2011	2,656	rs17077450	1.8×10^{-7}	chr18: 65285279				
Wray et al., 2012	6,104	rs182358	8.8×10^{-6}	chr1: 97462900	12,664	rs12446956	1.1×10^{-6}	chr16: 73501786
Ripke et al., 2013b	18,759	rs11579964	1.0×10^{-7}	chr1: 224538690	57,478	rs1969253	4.7×10^{-6}*	chr3: 183876262
Kohli et al., 2011	719	rs1545843	5.5×10^{-8}	chr12: 84563818	14,373	rs1545843	1.4×10^{-9}*	chr12: 84563818
Rietschel et al., 2010	1,968	rs2765493	2.2×10^{-7}	chr1: 157797750	2,918	rs7713917	1.4×10^{-6}	chr5: 78828999
CONVERGE, 2015	10,640	rs12415800	1.9×10^{-8}	chr10: 69624180	6,417	rs12415800	7.7×10^{-4}	chr10: 69624180
CONVERGE, 2015	10,640	rs35936514	1.2×10^{-8}	chr10: 126244970	6,417	rs35936514	1.6×10^{-5}	chr10: 126244970

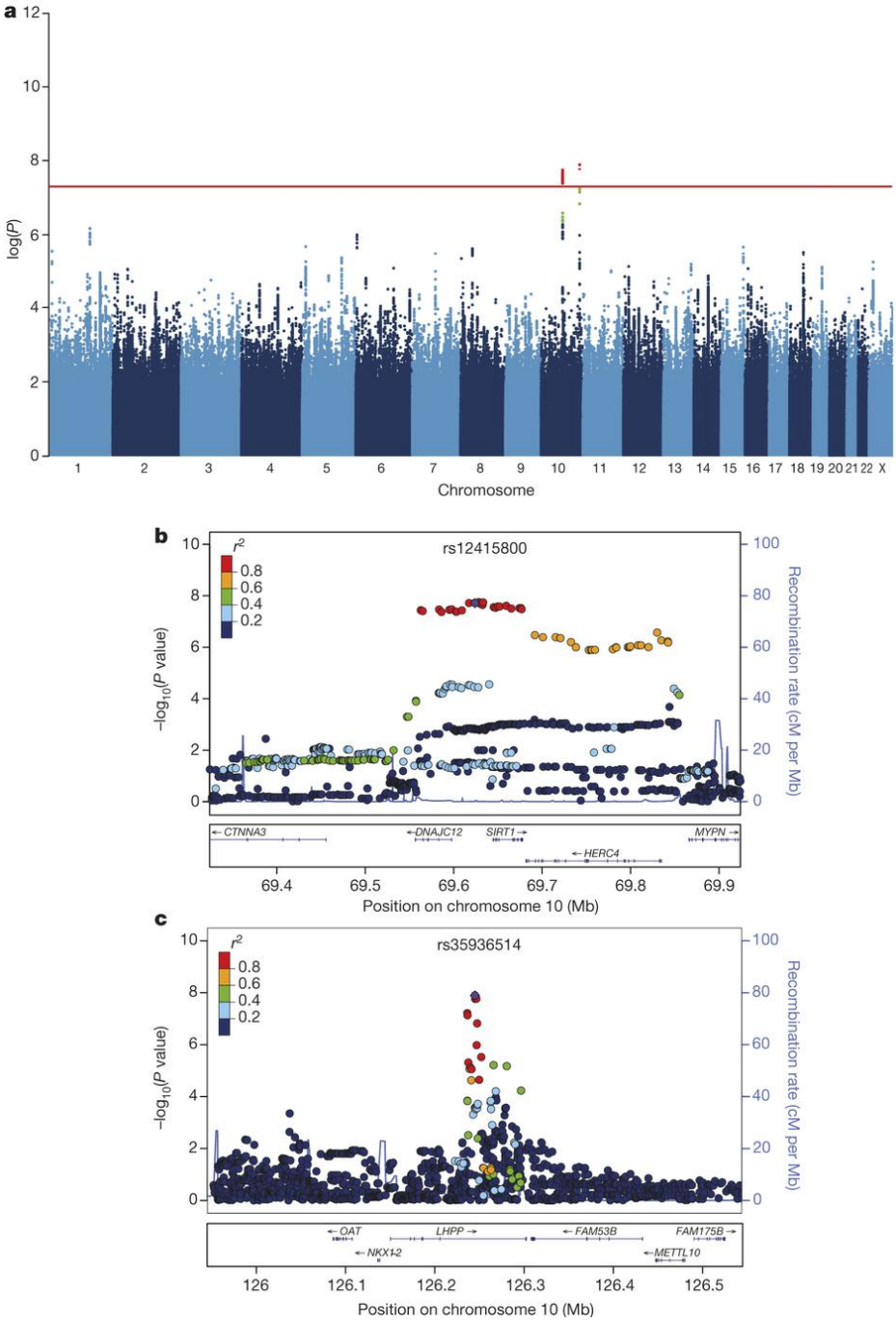


Figure 2. Loci associated with MDD in the CONVERGE sample. a) Manhattan plot of genome-wide association for MDD. b) LocusZoom20 regional association plot of the *SIRT1* region on chromosome 10. c) LocusZoom20 regional association plot at the *LHPP* gene on chromosome 10. For b and c the -$\log_{10}$ (P-value) of imputed SNPs associated with MDD is shown on the left y axis. The recombination rates expressed in centimorgans (cM) per Mb (NCBI Build GRCh37) are shown on the right y axis. Position in Mb is on the x axis. Linkage disequilibrium of each SNP with the top SNP, displayed as a large purple diamond, is indicated by its color. Source: (consortium, 2015)

1.2.3. Gene by Environment Interactions

Depression risk is influenced to different degrees by a combination of environmental and genetic factors in each particular individual. Disease predisposition variability highlights the importance of studying GxE interactions (i.e., the influence of environmental factors while taking into account genetic risk as well) in relation to MDD.

The first evidence of genetic predisposition modulating environmental exposure linked to depressive symptoms was shown for a polymorphism in the promoter of the serotonin transporter (*5-HTT* or *SERT*) gene interacting with stressful life events (Caspi et al., 2003). Polymorphisms in the corticotrophin receptor 1 (*CRHR1*) increased MDD risk in adulthood in individuals exposed to ELS (Bradley et al., 2008). Further evidence demonstrates a *CRHR1* by childhood trauma interaction modulating HPA axis reactivity in depression (Heim, et al., 2008; Tyrka et al., 2009). Other examples of GxE interactions on MDD include the *GR* gene (Bet et al., 2009), *FKBP5* gene (Appel et al., 2011; Zimmermann et al., 2011), brain-derived neurotrophic factor (*BDNF*) (Aguilera et al., 2009; J. M. Kim et al., 2007), and dopamine receptor 2 (Vaske, Makarios, Boisvert, Beaver, & Wright, 2009) are illustrated in **Figure 3**.

Epigenetic modifications, particularly deoxyribonucleic acid (DNA) methylation has been proposed as a possible mechanism underlying GxE interactions in relation to stress-related psychiatric disorders like MDD, specifically for *FKBP5* and childhood trauma (Klengel et al., 2013). Growing evidence of GxE interactions (Bradley, et al., 2008; Caspi, et al., 2003; Heim, et al., 2008; Klengel, et al., 2013; Divya Mehta, et al., 2013; Zimmermann, et al., 2011) supports the existence of possibly more biologically homogeneous MDD subtypes, further emphasizing that subjects with different types of depression will likely respond to specific treatments customized to their individual biology.

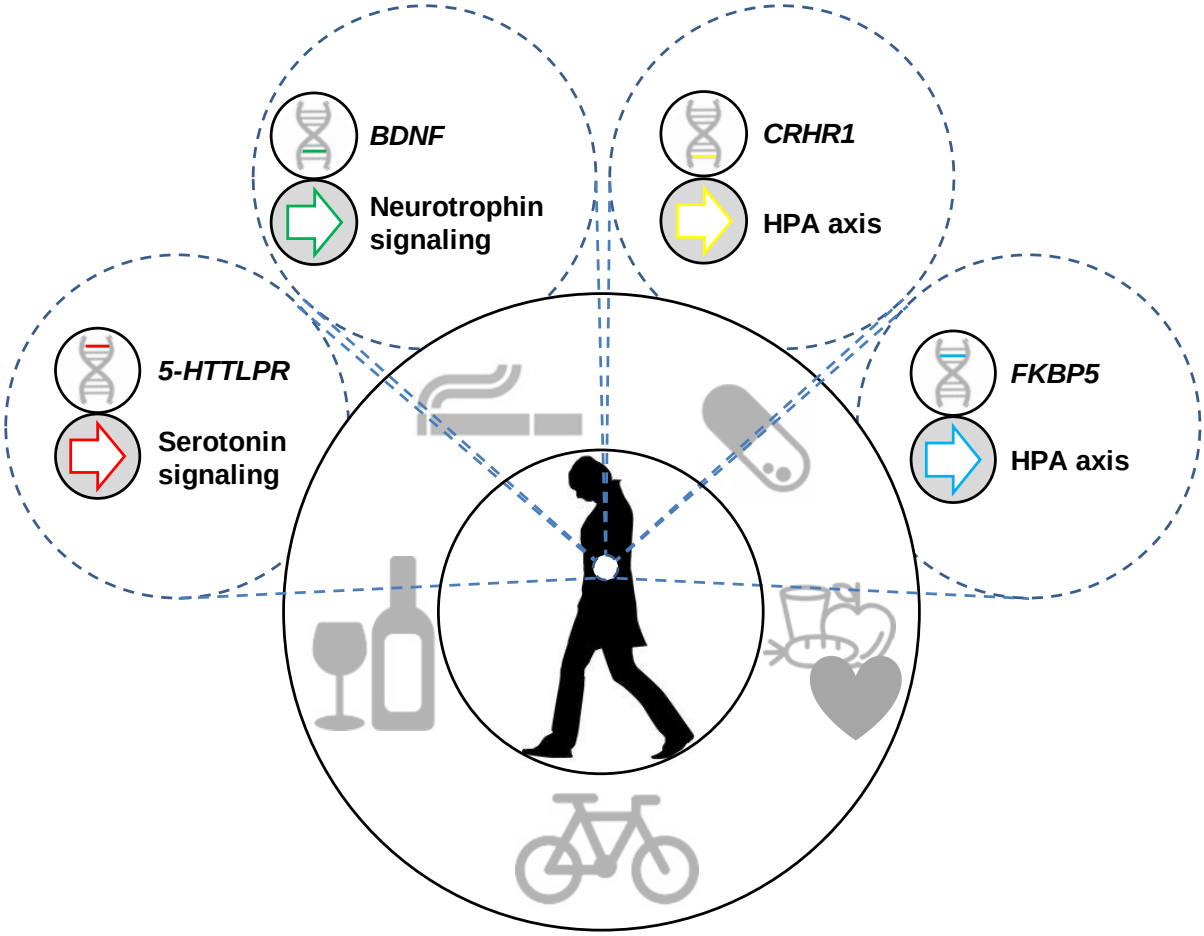


Figure 3. Gene by environment interactions resulting in major depressive disorder. Serotonin transporter linked-promoter region = *5-HTTLPR*, corticotrophin receptor 1 = *CRHR1*, brain-derived neurotrophic factor = *BDNF*, FK506 binding protein 5 = *FKBP5*. Colored bars in genes represent genetic variation. Based on: (Klengel & Binder, 2013)

1.2.4. Structural Brain Changes

Neuroimaging techniques emerged as a tool for non-invasive exploration of neural mechanisms involved not just in normal cognition processes but also in psychiatric disease states. The association between depression and regional brain volume abnormalities has been of interest since more than two decades. Functional and structural modulation of the hippocampus (HC) by antidepressant medication (ADM) and stress (Rao et al., 2010) (a highly relevant environmental MDD risk factor (**see section 1.2.1**)), has motivated the investigation of HC volume changes related to depression. By dissecting the extent and temporal trajectories of HC structural differences, studies have sought to elucidate the link between environmental risk-factors, HC integrity and MDD features such as treatment resistance and chronicity (G. MacQueen & Frodl, 2011).

To date, several magnetic resonance imaging (MRI) meta-analyses have sought to identify structural brain alterations in depressed patients (Campbell, Marriott, Nahmias, & MacQueen, 2004; Cole, Costafreda, McGuffin, & Fu, 2011; Du et al., 2014; Hajek, Kozeny, Kopecek, Alda, & Höschl, 2008; Hamilton, Siemer, & Gotlib, 2008; Kempton et al., 2011; Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009; Videbech & Ravnkilde, 2004; Zhao et al., 2014). The most consistent finding across studies was decreased bilateral HC volume in recurrent MDD as compared to controls. Data on volumetric changes in healthy at-risk populations is sparse, but taken together evidence is in favor of reduced HC volume in healthy individuals at familial risk for depression (Chen, Hamilton, & Gotlib, 2010; Lenze, Xiong, & Sheline, 2008).

The largest effort to identify regional brain volume alterations in MDD was carried out by the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA)-MDD working group. This study comprised 1,728 MDD patients and 7,199 controls from fifteen research centers. The main finding was in fact reduced bilateral HC volume in MDD patients relative to controls; this effect was driven mainly by recurrent MDD (Schmaal, et al., 2015).

Changes in grey matter (GM) and HC volume have not just been linked to MDD but also to treatment outcomes. For instance poor treatment outcome after five weeks has been observed in patients with reduced baseline left HC

volume (Samann et al., 2013). This result was consistent with a previous study that observed increased baseline bilateral HC volume and better treatment outcome after eight weeks of ADM (G. M. MacQueen, Yucel, Taylor, Macdonald, & Joffe, 2008). While findings reflect early treatment outcomes only (6-8 weeks), long-term outcome to antidepressant treatment has also been related to changes in HC volume. For example, increased relapse during a 2-year follow-up was observed in MDD patients with baseline reduced bilateral HC volume while no differences were found between HC volume of non-relapsing patients and healthy controls (Kronmüller et al., 2008). A previous study found significantly smaller baseline HC in patients whole relapsed within a 1-year follow-up as well (Frodl et al., 2004). Taken together, these findings point to reduced HC volume as an important determinant of short and long-term outcomes in depression.

1.2.5. Genetic Contribution to Brain Structures and Psychiatric Risk

Common genetic variation contributes to enduring structural brain changes which occasionally result in behavioral changes and increase disease risk. Several imaging-genomics studies have investigated the association of common gene variants, brain volume and stress-related phenotypes.

A number of candidate gene studies have reported suggestive SNP influences on GM volumes. For example, variants in the glycogen synthase kinase-3beta (*GSK3beta*) gene have been associated with right HC volumes as a function of MDD status (Inkster et al., 2009). Recent findings showed GxE interaction effects on HC volume between three gene-variants (Catechol-o-methyl transferase Val158Met, *BDNF* Val66Met, *5-HTTLPR*) and cumulative environmental adversity (Rabl et al., 2014).

Structural brain volume differences, similar to other complex traits, are likely to exhibit an underlying polygenic architecture. Efforts carried out by the ENIGMA consortium to identify genome-wide significant risk variants influencing brain structures investigated seven subcortical regions including the hippocampus. This large effort included more than thirty thousand subjects from 50 cohorts. Five novel genetic variants were associated with putamen and caudate nucleus volumes and two with HC (rs77956314, rs61921502) (Hibar et al., 2015) (**Figure 4**).

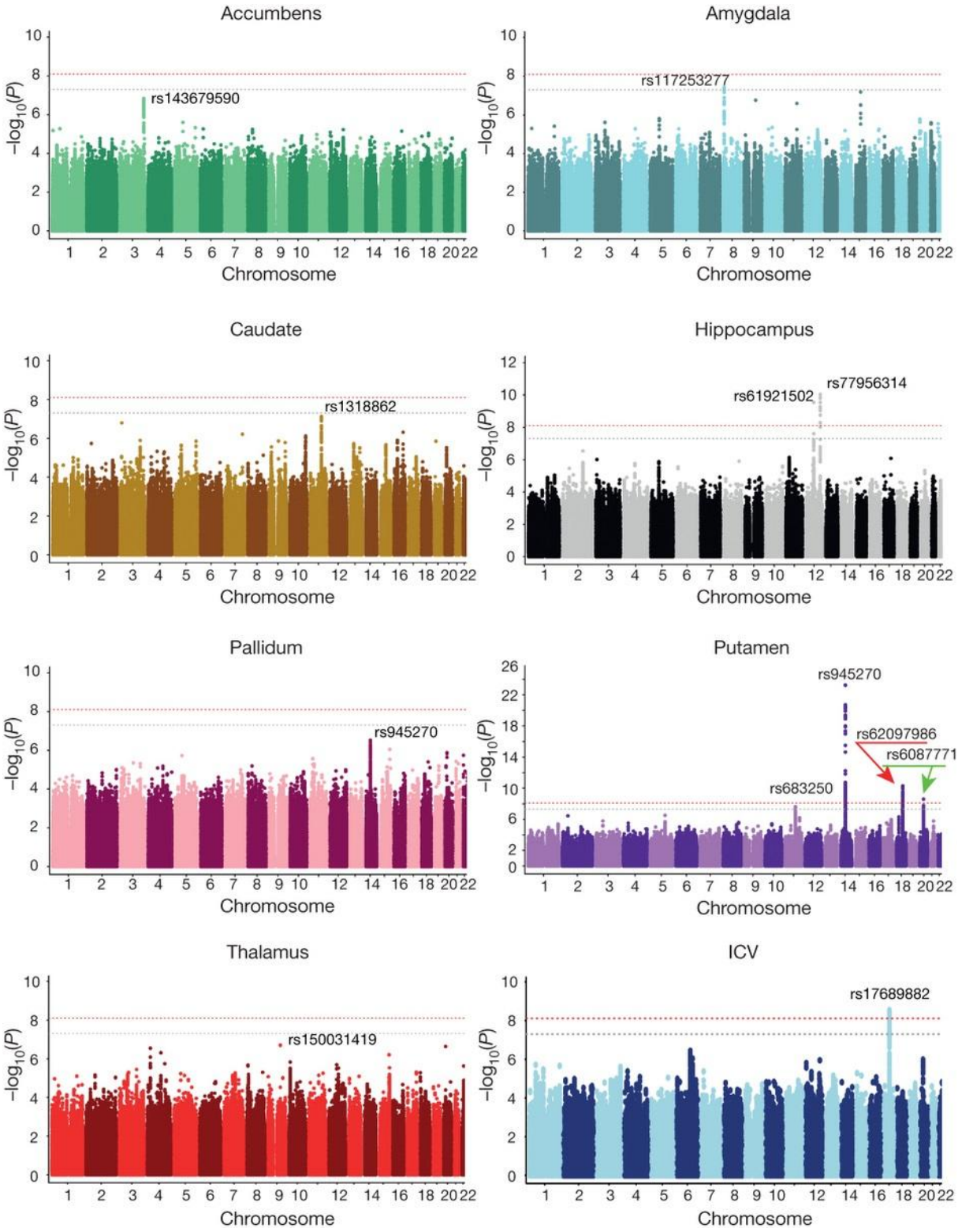


Figure 4. Common genetic variants associated with subcortical volumes and intracranial volume (ICV). Grey dotted line shows genome-wide significance threshold of $p = 5 \times 10^{-8}$ and red dotted line shows multiple tests-corrected threshold of $p = 7.1 \times 10^{-9}$. Source: (Hibar, et al., 2015)

1.3. Antidepressant Treatments and Outcome

Antidepressant treatment outcome is highly variable; approximately only 30% of patients achieve remission after the first given treatment (Gaynes et al., 2009). Due to the high prevalence and recurrence of MDD, prevention and adequate treatment selection are considered a worldwide health priority. MDD treatment outcome has improved with the development of several classes of ADM, which have shown more efficacy than placebo (Fountoulakis & Möller, 2011; Fournier et al., 2010), however high rates of treatment resistance and non-remission are still an important contributor of disease burden.

Effective treatments of depression aim to completely restore psychological functioning as well as social and work-related productivity. Failure to achieve remission is related to greater functional impairment and earlier reversion of depression symptoms (Gaynes, et al., 2009; Papakostas et al., 2004). Achieving remission in the early stages of MDD is consequently of high importance for outcome in the long-term. Optimal treatment selection could help maintain favorable long-standing outcomes.

Common clinical outcome measures used in pharmacogenetic (Investigators, Investigators, & Investigators, 2013; Rudolf Uher et al., 2010) or neuroimaging (Dunlop, Kelley, McGrath, Craighead, & Mayberg, 2015; McGrath, et al., 2013) studies aiming to identify predictors of antidepressant treatment outcomes, are percentage improvement (i.e., continuous measure of change) and remission or response (i.e., categorical measures). These are defined by HDRS scores (HAMILTON, 1960) or outcome scores from other depression rating scales like the Quick Inventory of Depression Symptomatology (QIDS) (A. John Rush et al.).

The recommended first-line treatment options for MDD are cognitive behavior therapy (CBT), ADM or a combination of both. Variable rates of remission to ADM and/or CBT highlight the different molecular mechanisms possibly underlying these treatments. While different neural circuits and brain regions are affected by these treatments in depressed patients (Boccia, Piccardi, & Guariglia, 2015), these may partially overlap (Barsaglini, Sartori, Benetti, Pettersson-Yeo, & Mechelli, 2014). To date, there are no available methods to match initial treatment choices to each patient’s unique biological type appropriate for clinical implementation.

1.3.1. Cognitive Behavior Therapy

CBT aims to increase awareness of abnormal negative thoughts, and introduce better adaptive behaviors. Initially CBT screens for irrational or maladaptive beliefs, replaces them with new concepts and coping skills and ultimately consolidates the learned behaviors through practice and follow-up sessions (Gatchel & Rollings, 2008). How and which neural mechanisms are exactly influenced by CBT in depressed patients is still under investigation. Ideal treatment outcome biomarkers would assist identification of those patients who would profit more from CBT in comparison to one of the other first-line treatments or vice versa.

1.3.2. Antidepressant Medication

Several classes of ADM are known, for example selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), tryciclic antidepressants, monoamine oxidase inhibitors (MAOI), serotonin modulators, dopamine-norepinephrine reuptake inhibitors (DNRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and norepinephrine-serotonin modulators, among others (Nelson, Pikalov, & Berman, 2008). The scope of this research focuses on SSRIs and SNRIs.

SSRIs are the most commonly prescribed antidepressants due to the lower presence of side effects and their high selectivity for the serotonin transporter (*SERT*) (Papakostas, Thase, Fava, Nelson, & Shelton, 2007). SSRIs increase serotonin (5-HT) levels in the central nervous system (CNS) by selectively inhibiting reuptake of the neurotransmitter in neuronal synapses. In contrast, SNRIs block the reuptake of not just serotonin but also norepinephrine resulting in indirect increase of dopamine levels (Stahl, Grady, Moret, & Briley, 2005). Most studies so far have focused on identifying biomarkers of antidepressant response to different ADM, however the lack of reliable effect sizes and results make these unsuitable for clinical application.

1.3.3. Deep Brain Stimulation

Approximately 10 to 20% of patients with depression do not respond to standard interventions, including electroconvulsive therapy (ECT) (Lozano et al., 2008). Deep brain stimulation (DBS) of particular brain target areas (i.e., subcallosal cingulate gyrus) (P. E. Holtzheimer et al., 2012) represents an adequate option for such treatment-resistant depression (TRD) patients. In fact, it has been shown that 75% of patients with treatment-resistant depression experienced a prolonged response after DBS in this brain region (Lozano, et al., 2008).

DBS appears to block the firing of neurons, thus regulating the metabolic equilibrium in the brain. However, the precise mechanism of action is still unknown. Typically electric pulses are delivered fourteen hours a day through a pacemaker which can be controlled and programmed from outside the body after it is inserted in a surgical procedure, (Volkmann, Herzog, Kopper, & Deuschl, 2002). Usually MDD patients that undergo DBS have not responded to various treatments (i.e., minimum 3 to 4 antidepressant treatments) and have had MDD for a long period of time (i.e., one year) (P. E. Holtzheimer, et al., 2012).

1.4. Biomarkers of Antidepressant Treatment Outcome

Depression is common and treatable, similar to other medical illnesses, developing biomarkers to optimize treatment selection in MDD for a given patient is a current goal. Biomarker studies for antidepressant response have focused to a great extent on single candidate gene variants influencing response to ADM, however a large amount of depressed patients does not benefit from ADM, and sometimes remission is only achieved after multiple trials with diverse doses or ADM classes (Trivedi et al., 2006).

The ability to predict treatment outcomes at the level of individual patient could be improved by the development of easily obtainable biomarkers that beyond foreseeing if a patient will improve over the course of overall treatment, also, predict whether an individual will respond to a specific treatment and not to an alternative treatment. Such biomarkers should be able to differentiate among current first-line antidepressant treatments (i.e., antidepressant medications and CBT).

Up to now no study has searched for genetic predictors of differential response to mechanistically different treatments. Development of such treatment-specific biomarkers would allow clinicians to offer treatment possibilities tailored to each patient's individual pathology (Florian Holsboer, 2008). In addition, careful consideration of possible MDD subtypes and simultaneously occurring clinical entities, above improving biomarker identification, might provide insight into subtype-specific pathological mechanisms of depression. The below sections briefly describe current available depression treatment outcome biomarkers.

1.4.1. Pharmacogenetic Biomarkers

Variability in treatment response is to some extent influenced by genetic variation. In fact the proportion of variance in antidepressant response explained by common genetic variants is estimated to be around 42% (Tansey et al., 2013). Pharmacogenetic studies aim to elucidate the genetic background underlying differences in treatment outcome by investigating the influence of gene variants on either pharmacokinetics or pharmacodynamics of drug treatment. Despite efforts made so far, results from studies focused on candidate genes are controversial, identification of genes possibly regulating antidepressant response failed to replicate often. **Table 3** and **Table 4** include a summary of the most relevant pharmacokinetic and pharmacodynamic findings in depression (Chiara Fabbri & Serretti, 2015).

1.4.2. Pharmacokinetic Biomarkers

Studies of pharmacokinetic biomarkers in depression focus on genes regulating metabolism, absorption, distribution and excretion of ADM (Lanni, Racchi, & Govoni, 2013) like cythocrome P450 (*CYP*); a large gene family encoding drug metabolism enzymes. Among those currently used in clinical practice are CYP2D6 and CYP2C19 (**Table 3**); highly polymorphic enzymes involved in ADM metabolism (Narasimhan & Lohoff, 2012). Based on CYP polymorphisms individuals are classified according to the allele-dependent rate at which ADM is metabolized (Drago, De Ronchi, & Serretti, 2009). The metabolizer status (i.e., poor, intermediate, extensive and ultra-rapid metabolizer (Charlier et al., 2003; Tsai et al., 2010)) determines the efficacy of the drug, whether an individual is prone to side effects, and ideally how this relates to clinical response. CYP2D6 and CYP2C19 genotypes can be obtained using genotyping chips such as the AmpliChip CYP450 (Roche Diagnostics) to guide ADM choice and dose. Their direct association with response is debatable, making clinical application limited (Narasimhan & Lohoff, 2012).

Candidate gene studies have correlated ADM outcomes with genetic variation in the gene encoding an ATP-binding P-glycoprotein transporter; *ABCB1* (Manfred Uhr et al., 2008) (**Table 3**) situated in the blood-brain barrier from which many ADMs are known to be substrates. Polymorphisms in the *ABCB1* gene (**Table 3**) regulate thus intra-cerebral concentrations of these drugs which may perhaps consequently affect clinical response (Manfred Uhr, et al., 2008). Nevertheless these findings require replication in larger clinical samples in order to achieve clinical application.

Table 3. Summary of pharmacokinetic studies including known associations with antidepressant outcomes. Source: (Chiara Fabbri & Serretti, 2015)

Gene	Function	Polymorphisms	Main findings	Study
CYP2D6	Enzyme involved in ADM metabolism	*1 (wild type), *4, *5, and *10 (none or reduced activity), gene duplications	Increased treatment efficacy in the intermediate metabolizer group; Increased risk of treatment failure in ultrarapid metabolizers; Increased side effects in non-extensive metabolizers	Kawanishi et al., 2004; Müller et al., 2013; Peters et al., 2008; Rau et al., 2004; Tsai et al., 2010; Zackrisson et al., 2010
CYP2C19	Enzyme involved in ADM metabolism	*1 (wild type), *2, and *3 (no activity), *17 (increased activity)	Increased side effects in poor metabolizers; poor metabolizers classified as citalopram tolerant may show increased remission probability	Müller et al., 2013; Yin et al., 2006; Mrazek et al., 2011
ABCB1	Encodes ATP-dependent P-glycoprotein transporter located in blood-brain barrier	rs2032582, rs1045642, rs2032583, rs2235040	Increased treatment efficacy in rs2032582 TT genotype, rs2032583 C allele, rs2235040 A allele	Niitsu et al., 2013; Kato et al., 2008; Nikisch et al., 2008; Uhr et al., 2008; Sarginson et al., 2010

1.4.3. Pharmacodynamic Biomarkers

Pharmacodynamic studies investigate genes involved directly with ADM effects, and their target systems. Even though the exact pharmacodynamic mechanisms of ADM remain unclear, candidate gene studies have focused on systems previously linked to the pathophysiology of depression (**see section 1.2**) like the serotonin, dopamine, monoaminergic, norepinephrine, glutamatergic and stress hormone systems (Drago, et al., 2009; C. Fabbri, Di Girolamo, & Serretti, 2013; Narasimhan & Lohoff, 2012; Perlis, 2014).

Among genes proposed to regulate antidepressant response are *BDNF* (Aguilera, et al., 2009; J. M. Kim, et al., 2007), *FKBP5* (Appel, et al., 2011; E. B. Binder, et al., 2004; Klengel, et al., 2013; Zannas & Binder, 2014; Zimmermann, et al., 2011), *CRHR1* (Bradley, et al., 2008; Tyrka, et al., 2009) and *5-HTTLPR* (Aguilera, et al., 2009; Caspi, et al., 2003; J. M. Kim, et al., 2007; Porcelli, Fabbri, & Serretti, 2012; Serretti, Kato, De Ronchi, & Kinoshita, 2007) (**see Table 4**). Some of these have been suggested to interact with environmental factors like childhood trauma (Aguilera, et al., 2009; Appel, et al., 2011; Bet, et al., 2009; Klengel, et al., 2013) or life stress (Caspi, et al., 2003; J. M. Kim, et al., 2007) to influence treatment outcome variability. The field has in recent years mostly shifted to an unbiased genome-wide approach (**see section 1.4.4**), without improved success or clinical practice applications possibly in part due to lack of consideration of MDD's polygenic architecture.

Table 4. Summary of pharmacodynamic studies of antidepressant outcomes. Source: (Chiara Fabbri & Serretti, 2015)

Gene	Function	Polymorphisms	Main findings	Study
FKBP5	Regulation of Akt activity; regulation of gluco-corticoid receptor sensitivity	rs1360780, rs3800373, rs4713916, rs352428	Increased treatment efficacy in rs1360780 TT genotype, rs352428 G allele, rs4713916 A allele, and rs3800373 C allele	Binder et al., 2004; Niitsu et al., 2013; Lekman et al., 2008; Kirchheiner et al., 2008; Ellsworth et al., 2013; Zou et al., 2010
BDNF	Neurotro-phic factor	rs6265 (196G/A; Val66Met)	Better response in the rs6265 heterozygous genotype, especially in Asians, or better response in Met allele carriers	Niitsu et al., 2013; Tsai et al., 2003; Yoshida et al., 2007; Zou et al., 2010 and Choi et al., 2006; Alexopoulos et al., 2010; Taylor et al., 2010; Kocabas et al., 2011; El-Hage et al., 2015
HTR2A	Serotonin main excitatory receptor	rs7997012, rs6311, rs6313	SNPs in the downstream or first intron region may modulate SSRI response	Fabbri et al.,2014; Noordam et al.,2015; Niitsu et al., 2013; Tiwari 2013
GNB3	G protein beta polypeptide 3, involved in generation of second messenger cascades	rs5443 (C825T)	Better response in rs5443 T allele carriers	Niitsu et al., 2013; Zill et al., 2000; Serretti et al., 2003; Lee et al., 2004; Keers et al., 2010;
SLC6A4	Serotonin reuptake into the pre-synaptic neuron; target of several anti-depressant classes	5-HTTLPR, rs25531, STin2	5-HTTLPR S allele correlates with poor outcome in Caucasians treated with SSRI. LG variants (5-HTTLPR L allele + rs25531 G allele equivalent to 5-HTTLPR S allele	Porcelli et al., 2012; Gudayol-Ferre et al., 2012; Bousman et al., 2014; Staeker et al., 2014

1.4.4. Genome-wide Genetic Biomarkers

Among candidate biomarkers, genotypes are easily obtainable in clinical settings and have been extensively studied as predictors of outcomes to depression treatments. However, despite some promising initial findings (M. Uhr et al., 2008), efforts to identify genetic predictors of antidepressant response have met with particularly little success (Clark et al., 2011; Garriock et al., 2010; Gvozdic, Brandl, Taylor, & Muller, 2012; Ising et al., 2009; Laje & McMahon, 2011; Rudolf Uher, Investigators, Investigators, & Investigators, 2013; Rudolf Uher, et al., 2010). These studies were underpowered, as several genes with small effects are likely to influence treatment outcome variability and a large number of samples are required to detect such associations.

Even in a large meta-analysis of three large cohorts; the Genome-Based Therapeutic Drugs for Depression (GENDEP) project (Rudolf Uher, et al., 2010), the Munich Antidepressant Response Signature (MARS) project, and the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, including a total of more than 2,200 patients, only one imputed loci was found associated with treatment outcome at genome-wide significance level ($p < 5 \times 10^{-8}$) but could not be confirmed after follow-up genotyping (**Figure 5**) (Investigators, et al., 2013). Additionally, SNPs suggestively associated ($p < 5 \times 10^{-6}$), explained only 2% variance in treatment response (Investigators, et al., 2013).

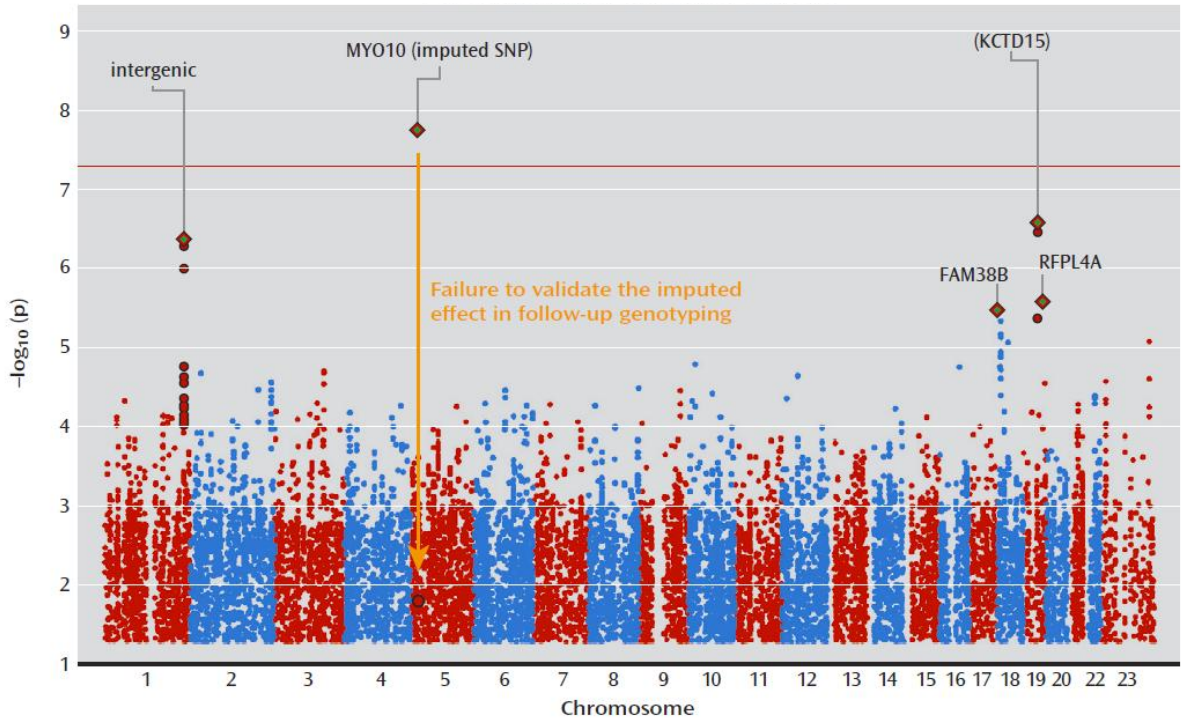


Figure 5. Manhattan plot of genome-wide meta-analytic results of antidepressant treatment outcome (percentage improvement after 12 weeks) in entire analyzed samples from three studies. The y axis plots indicate -$\log_{10}$ P-values of association. Gene symbols indicate the gene on which the associated SNP ($p \leq 5 \times 10^{-6}$) is located, or, if the gene symbol is in parentheses, the nearest gene up to 100 kb away from the associated SNP. Source: (Investigators, et al., 2013)

1.4.5. Epigenetic Biomarkers

Depression risk factors identified so far, comprise genetic and environmental influences as well as their interactions. Increasing evidence suggests the additional role epigenetic modifications contributing to susceptibility for complex traits possibly through gene expression regulation. Epigenetic mechanisms like DNA methylation (DNAm) have been proposed as one of the plausible underlying mechanisms of GxE interactions influencing neuropsychiatric disorders.

Even though DNAm has tissue specific patterns, its validity as a biomarker in brain relevant disorders cannot be disregarded (E. Hannon, Lunnon, Schalkwyk, & Mill, 2015; Shah et al., 2015). Several studies have now actually showed concordance between brain and blood DNAm patterns, increasing as such the reliability of peripheral tissues to study the involvement of epigenetic mechanisms on psychiatric traits (Davies et al., 2012; Farre et al., 2015; E. Hannon, et al., 2015; Walton et al., 2015). DNAm changes influenced by DNA sequence variation in the developing human brain are actually enriched amongst SNPs increasing risk for neurodevelopmental disorders (i.e., schizophrenia) (Eilis Hannon et al., 2015).

Among candidate genes, altered DNAm in *FKBP5* (Klengel, et al., 2013) and *BDNF* (Kundakovic et al., 2015) mediated by ELS increases risk for developing psychiatric disorders later in life. *BDNF* methylation is as a matter of fact proposed as a plausible relevant biomarker for early detection of psychiatric disease development in adulthood (Kundakovic, et al., 2015). Global epigenetic modifications are altered in depressed patients as well (Byrne et al., 2013), suggesting that epigenetic alterations may also be relevant in antidepressant treatment outcome variability. Changes in *BDNF* methylation and antidepressant response in MDD patients have in fact been reported (Lopez et al., 2013). Larger studies with better symptomatically characterized samples comparing different antidepressant treatments in previously untreated patients are to date not available.

1.4.6. Gene Expression Biomarkers

Gene expression signals measured in peripheral blood can aid the development of biomarkers for psychiatric disorders. In fact, several molecular signatures associated to psychiatric traits like postpartum depression (D. Mehta et al., 2014), Parkinson's disease (Scherzer et al., 2007), post-traumatic stress disorder (Divya Mehta, et al., 2013), suicidality (Le-Niculescu et al., 2013), bipolar disorder (Le-Niculescu et al., 2009), among others, have been identified in peripheral-blood gene expression.

Most biomarkers studies of antidepressant outcomes have not been performed in human samples (Mamdani et al., 2011), and no study so far has looked for transcriptome signatures across mechanistically different treatments. Recently, expression profiling related changes in 127 transcripts to depression and proposed retinoid-related orphan receptor alpha as a biomarker for antidepressant response (Hennings et al., 2015).

Given that both CNS and peripheral processes like immune responses, are involved in the etiology of depression, blood gene expression transcripts represent more than just a surrogate measure of brain-related processes. In addition, there is a significant correlation between the transcriptome of several CNS tissues and whole-blood in humans, with relevant candidate genes for disorders like schizophrenia also expressed in both tissues (P. F. Sullivan, Fan, & Perou, 2006). Use of peripheral blood gene expression may be consequently a useful molecular signature for complex psychiatric diseases.

1.4.7. Neuroimaging Biomarkers

Another approach to address challenges faced when trying to identify biomarkers of antidepressant response is to study the correlation between genetic risk and quantitative endophenotypes, measurable constructs that may lie in greater etiological proximity to the underlying genetic risk for MDD, thus serving as more powerful starting point to explore genetic risk than clinical outcomes (i.e., percentage improvement or remission after treatment) itself (Almasy & Blangero, 2001; Gottesman, II & Gould, 2003). In this context, neuroimaging structural measures and metabolic activity represent a promising endophenotype to examine.

Previous studies have shown for example that pre-treatment activity of specific brain regions may predict likelihood and efficacy to respond to particular antidepressant treatments (Brody et al., 2001; Conway et al., 2012; Dougherty et al., 2003; Ketter et al., 1999; Konarski et al., 2009; Mayberg et al., 1997; C. L. McGrath et al., 2014; Pizzagalli et al., 2001; Siegle et al., 2012). Specifically, McGrath and colleagues showed that resting state pre-treatment brain activity (measured by fluorodeoxyglucose positron emission tomography, FDG-PET) of six brain regions of interest (ROI), including the right anterior insula (RAI), right motor cortex (RMC), left premotor cortex (LPMC), right inferior temporal cortex (RITC), left amygdala (LAM), and left precuneus (LPCUN) identified patient subgroups responding to either Escitalopram (ESC) or CBT (Dunlop, et al., 2015; McGrath, et al., 2013).

As for structural neuroimaging biomarkers decreased HC volume predicts poor treatment outcomes in depression, correlating not only with early response (Samann, et al., 2013) but also long-term treatment outcome (Frodl et al., 2008; Kronmüller, et al., 2008). Even after 3 years post-depressive episode, patients with smaller hippocampal volumes show a correlation to negative clinical outcome (**Figure 6**).

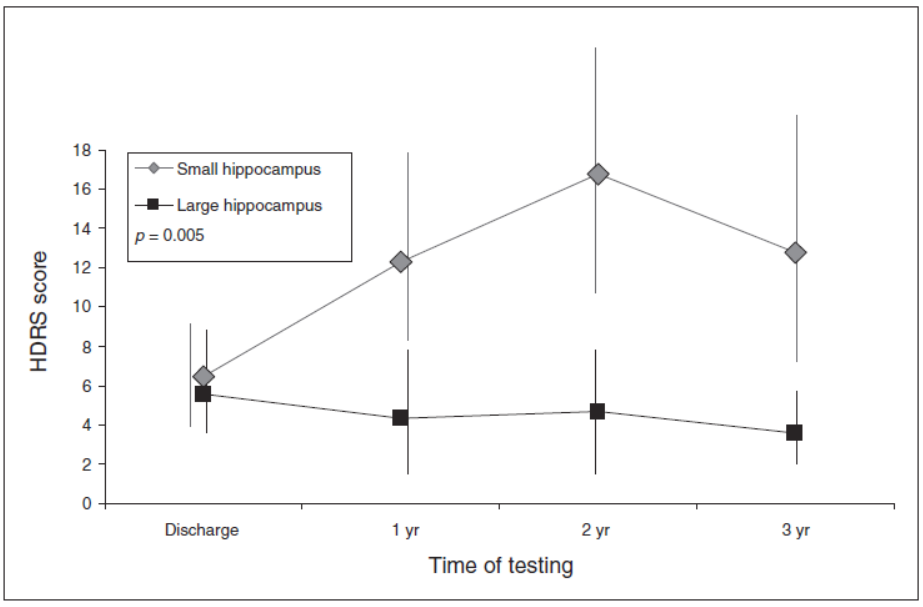


Figure 6. HDRS scores for patients with depression hippocampal volume. Large hippocampal volume (N = 11), compared with those with a small hippocampal volume (N = 8). P-value derived by multivariate analysis of variance, and it refers to the difference between groups in the HDRS scores at 1, 2 and 3 years. Source: (Frodl, et al., 2008)

1.5. A Neuroimaging-based Genomics Approach

Depression treatments exhibit a diverse degree of success; 70% of patients fail to achieve clinical remission with the first treatment. Currently no method or biomarker exists to match the initial antidepressant treatment option to each patient depression type and only one test based on ADM metabolizing enzyme polymorphisms has been translated into clinical practice (**see section 1.4.2**). Even though several gene candidates influencing outcome together with well known environmental factors like ELS have been identified, results are still inconclusive. Candidate gene studies or single SNP associations are unlikely to reflect MDD's polygenic nature and GWAS of antidepressant response have not yet identified robust candidates. Limited neuroimaging studies have acknowledged preferential outcomes to CBT or ADM based on pre-treatment metabolic rates of specific brain areas, providing some validity to the existence of biologically distinct depression subtypes with need for customized therapies (Konarski, et al., 2009; McGrath, et al., 2013).

The aims of this thesis (**see section 2**) are motivated by the urgent need to bring insight into the possible mechanisms influencing variation in treatment response overall and specific to first line treatments of depression. We sought to take into consideration MDD's complexity and heterogeneous clinical features by combining not only multiple genetic factors but several stratums of information (e.g., neuroimaging structural and functional phenotypes previously related to antidepressant treatment outcome) with special consideration of comorbid phenotypes and environmental exposure. Combining multiple layers of information may bring additional insights into the multiple systems involved in MDD.

Recent identification of genetic variation associated to HC volume differences has become available (Hibar, et al., 2015). The correlation of smaller HC volumes with bad outcomes to antidepressant treatment, makes HC volume differences an ideal candidate to enhance biomarker identification. Brain imaging measures are not as easily obtained as genotype variants; genome-wide genotyping comes at reduced time and costs. The approach introduced in this research used genetic variants modifying HC volumes identified by ENIGMA (**see section 1.2.5**) as easily obtainable "surrogates" of HC volume instead of HC volume per se to predict antidepressant response.

This is also the first attempt to look at mechanistically different treatments like CBT and ADM. In this context, pre-treatment activity of specific brain regions predicted efficacy to respond to CBT or ESC (**see section 1.4.7**) (McGrath, et al., 2013). Availability of genome-wide genotypes and brain activity of these ROIs motivated the idea to use these as functional “endophenotype” of outcome to specific treatments. The work in this thesis involved the largest single site randomized trial of treatments for MDD ever performed (**see section 4.2.3**) with only previously untreated patients randomized into CBT or ADM. Identifying biomarkers that classify pathophysiologically comparable individuals who respond to a particular treatment strategy would be meaningful in order to optimize clinical treatment choices and improve the patient’s quality of life.

2. Aims

This section states the aims of this thesis which are motivated by the present challenges faced in the field, where the polygenic nature and phenotypic complexity of MDD slows the advances in development of individually-tailored treatments. The approach presented here combines multiple layers of information including genetics and neuroimaging techniques; starting with structural, followed by functional neuroimaging methods like FDG-PET. The following studies sought to gain insight into specific signatures of clinical response and frame the sections in which the methods and results are divided in this work.

The overall aims of this thesis are to make use of alternative frameworks that assist the identification of: (1) treatment outcome biomarkers as well as clinical/environmental-feature specific biomarkers (**see section 2.1**) and (2) biomarkers of mechanistically different treatments (CBT or ADM) (**see section 2.2**) in human samples.

2.1. Treatment Outcome Polygenic Predictors

Common genetic variation influences HC volume. MDD as well as treatment outcomes have been associated with HC volume changes (**see section 1.2.4**). The purpose of this study is to build HC-based polygenic predictors of MDD treatment outcomes from summary association results from the largest to date genome-wide association study on human subcortical brain structures from the ENIGMA consortium (**see section 1.2.5**) (Hibar, et al., 2015).

Additionally, we hypothesized that taking into account MDD clinical diagnostic subtypes or environmental exposure to stress would increase power and allow uncovering of robust subtype-specific predictors of treatment outcomes. We tested whether HC-derived polygenic constructs could predict response overall and in subgroups of depression with specific clinical features that have been associated with differential treatment response in three independent samples; the STAR*D sample (**see section 4.2.2**), the Prediction of Remission in Depression to Individual and Combined Treatments (PReDICT) sample (**see section 4.2.3**) sample, and the Combining Medications to Enhance Depression Outcomes (COMED) sample (**see section 4.2.4**). Particularly we assessed for differences in anxious and non-anxious diagnostic subtypes within MDD patients as well as for exposure to early life stress. Both anxiety (Fava, et al., 2008) and ELS (Nemeroff, et al., 2003) are related to negative antidepressant outcomes.

2.2. Treatment-Specific Polygenic Predictors

We hypothesized that the combined use of quantitative structural and functional neuroimaging endophenotypes (i.e., HC-volume and metabolic activity) and multiple genetic variants could predict differential treatment outcome to CBT or ADM in MDD. To examine this hypothesis, HC-based polygenic predictors from summary association results from the ENIGMA consortium GWAS on human subcortical brain structures (**see section 1.2.5**) (Hibar, et al., 2015) were constructed for an independent sample (PReDICT) and then tested whether these could predict treatment-specific outcomes.

Next, we identified genetic variants associated with metabolic activity of six brain regions previously shown to predict treatment-specific response (McGrath, et al., 2013). Subsequently, polygenic scores based on the identified variants were calculated for individuals in a second independent MDD sample, and tested whether these polygenic scores could predict treatment-specific outcomes in this cohort. Beyond providing a framework for developing easily measured predictors of individualized treatment response, this approach may contribute to the identification of molecular pathways critical for treatment-specific outcomes, thereby offering novel insights into mechanisms of antidepressant action and MDD pathophysiological subtypes.

3. Neuroimaging-based Genomic Predictors Technical Background

The purpose of this section is to describe critical methods involved in the construction of polygenic scores (PGS). As GWAS summary results constitute the first step in PGS calculation, quality control of genome-wide genotypes and other decisive factors in GWAS such as population stratification are discussed in this section. This section provides a general mini-guide for PGS computation and discusses the most common confounders and available methods.

3.1. Genome-wide Genotyping

Methods for identification of common genetic variation specifically SNPs, have become widely available and affordable. SNPs are single base pair DNA sequence variations present within a population. SNPs are composed in general by two alleles; one is typically less frequent (i.e., minor allele); the minor allele frequency (MAF) is characteristically not less than 1%.

In the human genome, about one polymorphism exists per every 300 bases on average, that is, close to ten million single sites. These ten million SNPs represent 90% of the common sequence variation among individuals (Consortium, 2003; Kruglyak & Nickerson, 2001). Genotyping methods screen for the presence of specific alleles in an individual, within this work we focused on the technical description of whole-genome SNP genotyping arrays.

Genome-wide genotyping enables fast detection of thousands of genetic variants in a given sample. Genotyping arrays are usually composed of a solid surface of oligonucleotide probe-containing beads ("SNP tags") targeting a specific locus in the genome. These SNP tags take into account linkage disequilibrium (LD) correlation coefficients (r2) (**see section 3.1.1**) allowing thus for SNP detection beyond the array content.

The array content is often chosen based on information provided by sequence variation catalogues like the HapMAP project (Consortium, 2003); a catalogue describing which, where and how common genetic variation occurs in the human population. In the genotyping assay, the sampled DNA binds to its complementary sequence in the array as it is hybridized. Then, single base extension incorporates one of four labeled nucleotides, which confers the allele specificity. Two color readouts, one for each allele, are then detected for every SNP by a scanner once laser-excited. Lastly, intensity values for each color convey information about the present alleles at a given locus (Perkel, 2008).

3.1.1. GWAS Quality Control

GWAS pre-processing includes stringent quality control (QC) measures to avoid confounding of results. Alleles of SNPs around 1 MB in the genome are in LD (i.e., correlated and possibly inherited together) (Consortium, 2005). LD is preferably measured as the correlation coefficient r2; ranging from 0 to 1, where one is complete LD and zero represents LD independence. According to the Hardy-Weinberg equilibrium (HWE) principle, in absence of disturbing factors and random mating, genetic variation (i.e., allele frequencies and genotypes) remains unchanged (in equilibrium) across generations in a large population (Hardy, 1908). HWE rarely occurs in nature since it can be influenced by multiple factors like non-random mating, mutations, natural selection, genetic drift, etc. (Hardy, 1908). Allele frequencies can be estimated under HWE for a given population. For a locus with two alleles, the HWE equation states:

$$p^2 + 2pq + q^2 = 1$$

where p^2 is the genotype frequency of homozygotes for one allele, $2pq$ the frequency of heterozygotes and, q2 the frequency of the homozygotes for the other allele (Hardy, 1908).

Significant deviations from expectation serve in GWAS as a measure of genotype quality (J. Xu, Turner, Little, Bleecker, & Meyers, 2002), for example sample contamination, which induces identical by descent (IBD) (see more details below) inflation estimates (Purcell et al., 2007).

Another confounding factor in GWAS is unknown relatedness in the sample. Relatedness can be estimated from uncorrelated SNPs (i.e., not in LD) as the proportion of alleles that are IBD. By definition alleles are IBD in two (or more) individuals if they were inherited from a common ancestor (Powell, Visscher, & Goddard, 2010). LD-independent markers with sample-wise genotype call rate ≥ 0.98, SNP call rate ≥ 0.98, HWE P-value ≥ 1×10^{-5} and MAF ≥ 1 to 5% are generally kept and carried forward in GWA studies. Typically only unrelated individuals (IBD proportion (Pihat) < 0.0625) are kept in association analyses.

3.1.2. Population Stratification

Genetic predictors of disease risk and predictors of antidepressant response are often specific for one population (Elisabeth B. Binder et al., 2010; Garriock, et al., 2010; Porcelli, et al., 2012). The use of multi-ethnic cohorts can allow detection of potentially stable predictors and improve application in clinical practice across ancestries (Yudell, Roberts, DeSalle, & Tishkoff, 2016). Spurious associations however, can result from population structure (i.e., systematic allele frequency ancestry differences between controls and affected individuals) (A. L. Price, Zaitlen, Reich, & Patterson, 2010).

This problem can be accounted for by methods like EIGENSTRAT; a method using principal component analysis (PCA) to compute ancestry-independent genome-wide associations. PCA is typically applied as a reduction method of high-dimensional data; lower numbers of axes (eigenvectors) of continuous variation independent of each other are identified. EIGENSTRAT includes this eigenvectors representing genomic regions that cause specific groupings of individuals as covariates in the association analyses (A. L. Price et al., 2006). Other methods like Genome-wide Complex Trait Analysis tool (GCTA) (Yang, Lee, Goddard, & Visscher, 2011) uses PCA as well, to calculate eigenvectors that can be included as covariates in association analyses performed in whole-genome association tools like PLINK (Purcell, et al., 2007).

3.2. Polygenic Scores

The phenotypic complexity and polygenic etiology of MDD complicates the identification of relationships between single genes and clinical outcomes (Patrick F. Sullivan, et al., 2012) and contributes to the lack of consistency in genetic predictors identified for antidepressant response. Rather than examining the influence of single gene variants in complex traits like MDD, biomarker identification may profit from methods that reflect the polygenic nature of the disease.

Polygenic contribution to complex disorders like depression (i.e., schizophrenia) was proposed nearly half a century ago (I. I. Gottesman & Shields, 1967). The largest GWAS performed so far including 36,989 schizophrenia patients and more than 113,000 controls found 108 loci contributing to disease risk, further suggesting that common complex diseases have a polygenic architecture (Ripke et al., 2014). GWAS findings thus far stimulated the development of methods to capture this signature. Polygenic scores (PGS) add the allelic effects of multiple genetic variants (Naomi R. Wray et al., 2014) and as such, may provide better insight into the genetic architecture of complex traits.

PGS require a minimum of two independent samples – a discovery and a target sample - with no close relatives included. First a GWAS is conducted in the discovery sample, from which the effects sizes (i.e., odds ratios in case-control GWAS or Betas in quantitative trait GWAS) and reference alleles from SNPs below certain P-value thresholds (pT) of association are used to create PGS in the target sample. **Figure 7** shows the general steps involved in PGS construction.

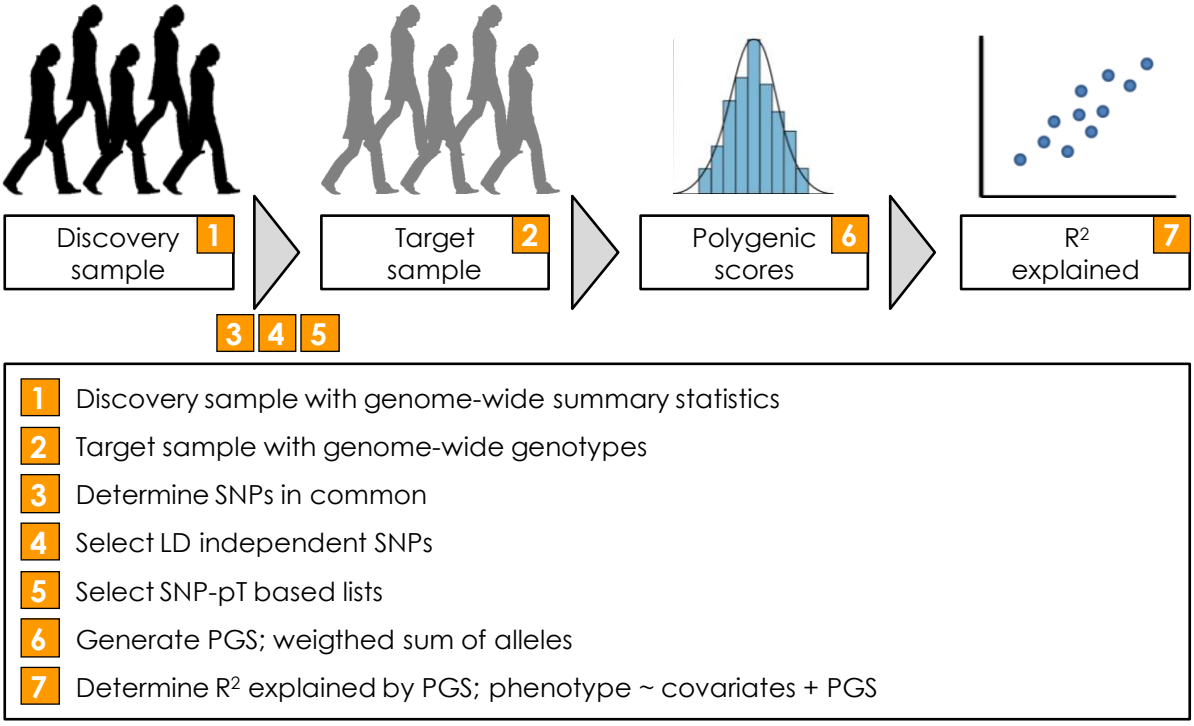


Figure 7. Miniguide to method: polygenic scores. Adapted from (Naomi R. Wray, et al., 2014)

PGS are calculated for each individual by summing the count of reference alleles at each locus (in the target sample) weighted by the value of the effect size (from the discovery sample) for each respective SNP, divided by the total number of SNPs at a given P-value of association. Lastly, PGS are regressed on the target sample phenotypes accounting for possible confounding factors by including them as covariates in the models (Naomi R. Wray, et al., 2014).

When building PGS, the correlation structure of the genome is important to consider; keeping LD independent SNPs in r2 < 0.2 within 500 kb is usually recommended (Naomi R. Wray, et al., 2014). In addition, to avoid confounding, the major histocompatibility complex (MHC) region of chromosome six is usually excluded due to high levels of LD. Given the absence of set criteria to establish P-value thresholds in polygenic scoring a recent (Euesden, Lewis, & O'Reilly, 2015); PRSice, creates high-resolution PGS by testing a large range of P-value thresholds and evaluates which pT maximizes the variance accounted for (R^2) by the scores.

4. Methods

General methods that apply to both (1) treatment outcome and (2) treatment-specific outcome studies are first described in this section (i.e. general parameters and procedure used in high-resolution PGS calculation, functional annotation of significant PGS, enrichment of specific brain cell-types and regions and enrichment with psychiatric susceptibility GWAS SNPs). Next, samples used to derive and further construct PGS are described in detail, including treatment outcome variables and clinical subgroup definitions, as well as genotyping QC. In addition, specific parameters used in PGS calculation and outcome prediction, schematic overviews and statistical power are explained in the respective methods section for each study.

4.1. General Methods

4.1.1. Neuroimaging-based High-resolution PGS Construction

SNPs and weight effect sizes based on GWAS summary statistics results from discovery samples (i.e., ENIGMA for HC-PGS or FDG-PET sample for ROI-PGS) are used to generate neuroimaging-based PGS (i.e., HC-PGS for treatment and treatment-specific outcome overall or ROI-PGS for treatment-specific) to test whether the multiallelic effect of respective SNP associations with HC-volume or ROI-metabolism could predict (1) treatment response overall in STAR*D, PReDICT and COMED and (2) treatment-specific response in PReDICT.

Given the absence of set criteria to establish thresholds in polygenic scoring and to evaluate which threshold maximizes the variance R^2 accounted for by the scores, we used PRSice (Euesden, et al., 2015), which uses a range of P-value thresholds creating high-resolution PGS. Lists of SNPs based on association pT, ranging from a minimum P-value threshold to a maximum P-value threshold were produced based on HC and ROI summary statistics. Number of thresholds tested and specific parameters used are stated in the respective methods section for each study.

HC or ROI association results were clumped individually using a stringent linkage disequilibrium threshold (r2 < 0.2 across 500 kb) to ensure only independent signals formed part of the scores (Naomi R. Wray, et al., 2014). Due to high levels of linkage disequilibrium in the MHC region of chromosome six, this locus was excluded from HC and ROI GWAS summary statistics to avoid confounding.

HC-PGS or ROI-PGS were calculated for individuals in each of the target samples as defined by PLINK's scoring method (Purcell, et al., 2007) through PRSice. Each score is computed by summing the number of reference alleles at each locus in each individual in a given target sample (i.e., STAR*D, COMED or PReDICT) multiplied by the value of the Beta (derived from HC-volume associations or ROI-metabolism associations) for that respective SNP, divided by the total number of SNPs at each of the different pT defined by the upper and lower P-value threshold limits (**see section 3.2** or **Figure 7** for details on PGS general method).

Linear regressions were then used to assess whether neuroimaging-based PGS could predict percentage change in outcome scores overall or treatment-specific according to each study (**see section 4.2.5** and **4.2.6** for treatment outcome overall and **section 4.3.4** for treatment-specific outcome) on the target samples. Significance was set as suggested in PRSice at $P < 0.004$ (Euesden, et al., 2015). Specific parameters and significance thresholds accounting for number of brain regions tested in the ROI-PGS predictions are included in the respective methods section for that study (**see section 4.3.4**). For all neuroimaging-based PGS we report the proportion of variance R^2 for the best-pT PGS after fitting covariates (specific covariates described in respective sections of each study). To further validate predictions, percent change in outcome scores was permuted 1000 times, and permutation-based P-values were derived for significant PGS respectively.

4.1.2. Functional Annotation and Ontology Enrichment of PGS

Gene annotation (hg19 assembly) of significant HC-PGS and ROI-PGS, as well as enrichment of loci within different gene ontology (GO) terms was performed with the Genomic Regions Enrichment of Annotation Tool (GREAT) (McLean et al., 2010). SNPs from significant HC-PGS in target samples were annotated together and carried out to cell type enrichment. Because ROI-PGS SNPs are derived from different brain regions, significant ROI-PGS SNPs were annotated and used in enrichments separately. In addition SNPs in common between significant ROI-PGS SNPs were also annotated.

4.1.3. Enrichment of Specific Brain Cell Types and Brain Regions

Cell type-specific and brain region-specific enrichment for genes annotated to HC-PGS SNP overlap, ROI-PGS overlap and individual ROI-PGS was performed using the Cell Specific Expression Analysis (CSEA) Tool (http://genetics.wustl.edu/jdlab/csea-tool-2/) (Doyle et al., 2009). We assessed significant neuroimaging-based PGS annotated genes for enrichment of 35 broad and specific cell type/region gene sets across multiple brain regions (CSEA specificity threshold set to 0.05) derived from a translational profiling approach isolating transcriptomes in mouse and humans. We report enrichments at Benjamini-Hochberg corrected $P < 0.05$ calculated in CSEA.

4.1.4. Enrichment of Psychiatric Susceptibility GWAS SNPs

To test whether neuroimaging-based PGS SNPs were enriched among SNPs associated with psychiatric disorder first the overlap of PGS SNPs with variants conferring susceptibility for psychiatric disorders of the Psychiatric Genomics Consortium (PGC) was calculated. Then we performed permutation analysis to determine if this overlap was significant by sampling 1000 sets of SNPs (drawn without replacement). The SNP sets were LD independent ($r2 < 0.2$ across 500 kb), matched on SNP count and MAF distribution by dividing the SNPs into non-overlapping MAF-bins, each of the width 0.05 as previously described (Nicolae et al., 2010). Permutations resulted in 1000 overlapped null proportions. Empirical P-values were defined as the number of null proportions greater than the observed overlap proportion. The PGC MDD, PGC schizophrenia-2, PGC cross-disorder and non-psychiatric trait data (height and diabetes) was downloaded from the PGC website (http://www.med.unc.edu/pgc)

4.2. Treatment Outcome Polygenic Predictors

4.2.1. ENIGMA Sample (Discovery Sample)

Sample Description

The ENIGMA consortium is a network of researchers from more than seventy institutions world-wide seeking to comprehend brain function, structure, and disease through integration of clinical, genetic and neuroimaging measures (Thompson et al., 2014). Findings of the ENIGMA-MDD working group linked reduced HC-volume to MDD (**see section 1.2.4**). Recently, ENIGMA conducted the largest meta-analysis GWAS of intracranial volume (ICV) and seven subcortical regions derived from MRI. This study included 30,717 individuals from fifty cohorts; among other findings they identified two genome-wide association signals influencing HC-volume (**see section 1.2.5**).

The ENIGMA sample was been described in detail in Hibar et al., 2015. In brief, the sample was comprised by a discovery sample of 13,171 and a replication sample of 17,546 subjects of European descent, with age range of 9 to 97 years old. Healthy subjects as well as individuals with several mood disorders including anxiety, Alzheimer's disease, attention-deficit/hyperactivity disorder, bipolar disorder, epilepsy, MDD and schizophrenia were included. Written informed consent was obtained from all participants.

Imaging Protocols and Meta-GWAS Results

Standardized imaging processing, genotyping QC and imputation protocols were designed and performed by ENIGMA. Imaging protocols are available online (http://enigma.ini.usc.edu/protocols/imaging-protocols/) and described in detail in Schmaal et al., 2015. Heribitability (h^2) estimation of mean volumes of the eight brain structures and calculation methods are described in Hibar et al., 2015. All region volumes showed high heritability (e.g., hippocampus (h^2 = 0.79; 0.74 – 0.83)).

Meta-GWAS were initially run in the discovery sample (N = 13,171) for ICV and seven subcortical brain regions (nucleus accumbens, caudate, putamen, pallidum, amygdala, hippocampus and thalamus) aiming to identify SNPs contributing to volume differences. Multi dimensional scaling (MDS) was implemented to calculate components that were included as covariates in models to avoid confounding due to

population stratification. All analyses controlled for age, age^2, gender, MDS components, ICV (for subcortical regions) and diagnosis (if applicable) (Hibar, et al., 2015). A significance P-value threshold of 7.1×10^{-9} was set after calculating the number of trait independent tests (N = 7).

All significant SNPs ($p < 7.1 \times 10^{-9}$) identified in the discovery sample were replicated in the second sample, including those contributing for HC-volume (**Table 5**). Further analyses showed that SNP contributions were not correlated to mean age of samples, suggesting that effects are stable across lifetime. Furthermore results remained consistent after excluding diagnosed mood disorder patients. For SNPs associated at $p < 1 \times 10^{-5}$, effect size correlation of full sample and when patients were excluded was high ($r > 0.99$), further supporting no contribution of disease status to these effects. Detailed results are described in Hibar et al., 2015.

The scope of this research focuses only on HC-volume GWAS findings, since HC-volume differences have been linked to MDD and antidepressant response (**see section 1.2.4**). Full summary association results from HC-volume meta-analysis (discovery cohort), were used to build HC-based PGS for this study (**see section 4.2.5**).

Table 5. Genome-wide significant SNPs contributing to Hippocampal volume . Effect sizes are given in units of mm3 per effect allele. The variance explained gives the percentage variance explained by a given SNP after correcting for covariates. The percentage difference in volume per effect allele (Diff. / Allele) is based on the absolute value of the final combined effect divided by a weighted average of the brain volume of interest across all sites in the discovery sample multiplied by 100. Source: (Schmaal, et al., 2015)

Hippocampal volume associated variants			
	Marker	rs77956314	rs61921502
	A1	T	T
	A2	C	G
	Allele Frequency	0.91	0.84
Discovery cohort	Effect (se)	−54.21 (8.37)	43.40 (6.89)
	P-value	9.33×10^{-11}	2.92×10^{-10}
	Sample size	13.163	13.163
Replication cohort	Effect (se)	−57.43 (12.69)	26.81 (13.32)
	P-value	6.04×10^{-6}	0.044
	Sample size	4.027	3.046
Discovery + replication cohorts	Effect (se)	−55.18 (6.99)	39.90 (6.12)
	P-value	2.82×10^{-15}	6.87×10^{-11}
	Total sample size	17.19	16.209
	Variance explained (%)	0.36	0.26
	Diff. / Allele (%)	1.40	1.01

4.2.2. STAR*D Sample (Target Sample 1)

Sample Description

The STAR*D is a clinical trial assessing antidepressant treatment (A. J. Rush et al., 2004) initially including 4,041 MDD outpatients recruited across more than forty centers in the United States. Eligible participants were 18 to 75 years old with a primary diagnosis of non-psychotic unipolar MDD, assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders (Association, 2000) and confirmed through an evaluation by a study psychiatrist.

Participants required a score of ≥ 14 at baseline on the HDRS (17-items) to be eligible for participation. Written informed consent was obtained from all participants. The sample considered in this work consisted only of subjects of European descent, with complete outcome measures as well as genotype information. This sample has been previously utilized and described in a treatment outcome meta-GWAS (Investigators, et al., 2013). STAR*D phenotype and genotype data are available through the National Institute of Mental Health (NIMH) Human Genetic Initiative (https://www.nimhgenetics.org/).

Treatment Protocol

Patients were treated with 20-60 mg/day doses of ESC. Depression severity was rated every two weeks for the initial six weeks using the clinician rated and self-report versions of the 16-item QIDS (A. John Rush, et al.).

Clinical Outcome

The primary outcome measure was the 17-item HDRS, however percent change in QIDS was available for a higher number of individuals (Investigators, et al., 2013) and consequently used for this study (N = 838). Antidepressant response to treatment was defined as a reduction in the QIDS self report score of ≥ 50% after six weeks of treatment. Anxious depression was defined as MDD with high levels of anxiety symptoms, as reflected in a HDRS anxiety/somatization factor score including six items (psychiatric anxiety, somatic anxiety, gastrointestinal somatic symptoms, general somatic symptoms, hypochondriasis, and insight) ≥ 7 (Fava, et al., 2008). Out of 838 patients, 436 had non-anxious depression and 402 had anxious depression. Early life stress measures were unavailable for this sample.

Genotyping and QC

Genome-wide genotypes were obtained from 1,948 participants; of whom 1,491 were of European descent (Investigators, et al., 2013) and were considered for this study. Genome-wide (Affymetrix Human Mapping 500K and Genome-wide Human SNP array 5.0) genotypes were measured in peripheral blood DNA drawn at baseline. SNPs with sample-wise call rate ≥ 0.99, SNP call rate ≥ 0.98, HWE P-value ≥ 1×10^{-5} and MAF ≥ 0.05 were selected, allowing for a total of 264,466 markers. Subjects of European ancestry that passed QC and had complete outcome measures at week six (N = 838) were included in PGS analyses. Based on IBD (**see section 3.1**) estimates in PLINK (Purcell, et al., 2007), all subjects in the sample were unrelated (Pihat < 0. 0625).

4.2.3. PReDICT Sample (Target Sample 2)

PReDICT included never treated MDD patients randomized to twelve weeks of ADM or CBT. As the PReDICT study was used only as a secondary target sample for these analyses, and is of higher relevance for the second study included in this thesis, the sample is described in detail in a following section (**see section 4.2.3**).

Clinical and Environmental Subgroups

Anxious depression in PReDICT was defined as MDD with high levels of anxiety symptoms, as reflected in a HDRS anxiety/somatization factor score ≥ 7 including the same items as for the STAR*D sample (Fava, et al., 2008). ELS was defined with the childhood trauma questionnaire (CTQ). The CTQ assesses five types of childhood trauma: physical, sexual and emotional abuse, and physical and emotional neglect. CTQ scores for none, mild, moderate and severe trauma have been established for each type of abuse (Bernstein et al., 2003). First participants were classified into two categories for each type of abuse (physical, sexual, and emotional): those with CTQ scores in the none to mild range and those with CTQ scores in the moderate to severe range. Then a compound variable across all of the 3 types of abuse was created. Participants were lastly classified into two categories: those with no type of abuse in the moderate to severe range and those with at least 1 type of abuse in the moderate to severe range.

From the entire sample, 215 subjects had valid outcome data (HDRS score) over twelve weeks (**see section 4.3.3**). Of these, 128 had non-anxious depression and 87 had anxious depression, 97 had no ELS and 118 had ELS.

4.2.4. COMED Sample (Target Sample 3)

Sample Description

The COMED study was a single-blind, randomized, placebo-controlled trial for first-step MDD treatment. Subjects were 18 to 75 years of age, met DSM-IV criteria for either recurrent (≥ 1 prior major depressive episode, MDE) or chronic (current MDE for ≥ 2 years) MDD based on a clinical interview. Eligible participants had to be in the index episode for ≥ 2 months to reduce the likelihood of placebo response and to have a score ≥ 16 on the HDRS. Eligible subjects could not have had any psychotic illness, bipolar disorder, or be in need of hospitalization (A. J. Rush et al., 2011).

Treatment Protocol

Subjects were randomized to one of three treatment possibilities: the SSRI-ESC + placebo (PBO); bupropion-sustained release (BUP-SR) + ESC; or venlafaxine-extended release (VEN-XR) + mirtazapine (MIRT). The consent and study procedures were approved by the Institutional Review Boards at the National Coordinating Center (The University of Texas Southwestern Medical Center at Dallas), the University of Pittsburgh Data Coordinating Center, each participating Regional Center, and relevant clinical site.

Sociodemographic and illness features were gathered at baseline. The anxiety subscale of the baseline HRSD established the presence of anxious features (Fava, et al., 2008) and the self-report Psychiatric Diagnostic Screening Questionnaire was used to establish the presence of current Axis I disorders (A. J. Rush et al., 2005). Outcome assessments were collected at baseline and at all subsequent treatment visits for 12 weeks.

Clinical Outcomes

The primary outcome measure was the 16-item QIDS self report (A. John Rush, et al.). Antidepressant response to treatment was defined as a reduction in the QIDS score of ≥ 50% after six weeks of treatment. Genotypes were obtained for 476 subjects (3 of which were replicates); 373 subjects had valid outcome data (QIDS-SR score) over six weeks.

Clinical and Environmental Subgroups

Anxious depression was defined as MDD with high levels of anxiety symptoms, as reflected in a HDRS anxiety/somatization factor score ≥ 7 including the same items as for the STAR*D sample. ELS was defined as in the PReDICT sample. Of the 373 subjects with valid outcome data, 92 had non-anxious depression and 281 had anxious depression, 174 had no ELS and 199 had ELS.

Genotyping and QC

Genotyping was performed for 476 individuals using the HumanOmni2.5S Illumina array. All relatives of individual subjects (n = 14, Pihat ≥ 0.125) based on mean IBD in PLINK (Purcell, et al., 2007)) values were excluded from the sample. After also removing three replicate samples, 459 remained for further QC. We selected for SNPs with sample-wise call rate ≥ 0.98, SNP call rate ≥ 0.98, $HWE_{P\text{-value}} \geq 1 \times 10^{-5}$ and MAF ≥ 0.05, allowing for a total of 1,301,806 markers in 444 individuals. Because of the extensive coverage of the genotyping array, and the population admixture of the sample, no imputation was performed.

4.2.5. HC-based Polygenic Predictors of Treatment Outcome

SNPs and weight effect sizes based on ENIGMA GWAS for HC volume (Hibar, et al., 2015) were used to generate HC-based polygenic scores (HC-PGS) to test whether the multiallelic effect of these SNP associations with HC-volume could predict treatment response in STAR*D, PReDICT and COMED.

Lists of SNPs (N = 5557) based on association pT, ranging from $P = 1 \times 10^{-5}$ to P = 0.5 (increments of 0.00009) were produced based on HC summary statistics (*PRSice command line: slower 0.00001 \ sinc 0.00009 \ supper 0.5 \ remove.mhc T \ report.individual.scores T \ clump.r2 0.2 \ clump.kb 500*). Clumping parameters are stated in the general **section 4.1.1**. HC-PGS were calculated for individuals in each of the target samples; first in STAR*D (N = 838), then PReDICT (N = 215) and finally COMED (N = 373). Scores were calculated as described in **section 4.1.1** at each of the different pT (P-value of association derived of HC-GWAS).

Linear regressions were then used to assess whether HC-PGS could predict percentage change in outcome scores (QIDS scores from baseline to week six in STAR*D, as well as in COMED and HDRS change from baseline to week twelve in PReDICT) in target samples. PCAs were calculated for each of the target samples using GCTA (Yang, et al., 2011) and included as covariates, as well as gender, and treatment (applicable for COMED and PReDICT only). Age was not included as covariate given that ENIGMA SNP HC-volume associations effects were corrected for and independent of age (Hibar, et al., 2015). Significance was set at P < 0.004 for HC-PGS prediction. Given the sample size of STAR*D, this was defined as “discovery”-target sample and COMED and PReDICT as “replication”-target samples for HC-PGS predictions, where nominal significance (P < 0.05) was considered as replication P-value.

4.2.6. HC-based Polygenic Predictors of Clinical/Environmental Feature-Specific Treatment Outcome

To test whether reduction in clinical heterogeneity in MDD could improve HC-prediction of antidepressant outcome, the target samples were stratified into anxious and non-anxious subsamples. HC-PGS were calculated for the "discovery"-target (STAR*D) and "replication"-target (PReDICT and COMED) anxious and non-anxious sub-samples (N = 3 anxious subsamples, N = 3 non-anxious subsamples). Linear regressions to predict treatment response were conducted in each subsample as described for the main HC-analyses (**section 4.2.5**). As STAR*D patients underwent ESC treatment only, post-hoc models were also tested only in the ESC and ESC-PBO subsets of PReDICT and COMED samples (N = 78, N = 122, respectively) to investigate the direction of significant PGS.

We also explored whether ELS in MDD patients influenced HC-prediction of treatment outcome following the same procedure as for anxiety. In this case, because information on early life adversity was not available for STAR*D, COMED was used as "discovery"-target sample and PReDICT as "replication"-target sample. A general overview of the methods used in **sections 4.2.5** and **4.2.6** is illustrated in **Figure 8**.

4.2.7. Statistical Power

Statistical power was estimated for a quantitative trait (percent change in QIDS or HDRS score from baseline to end of treatment) using R (Gauderman, 2002; Lee & Wray, 2013) (https://www.r-project.org/). Assuming a significance threshold of 0.004, we have 100% power to detect whether HC-PGS explain clinically relevant effect sizes (6.3% of variation in percent improvement) in STAR*D sample (N = 838), 97.2% in PReDICT (N = 215) and 99.6% in COMED (N = 371) (Gauderman, 2002; Lee & Wray, 2013; Team, 2014). This amount of explained variance has been described as clinically relevant for pharmacogenetic studies of antidepressant response (R. Uher, Tansey, Malki, & Perlis, 2012).

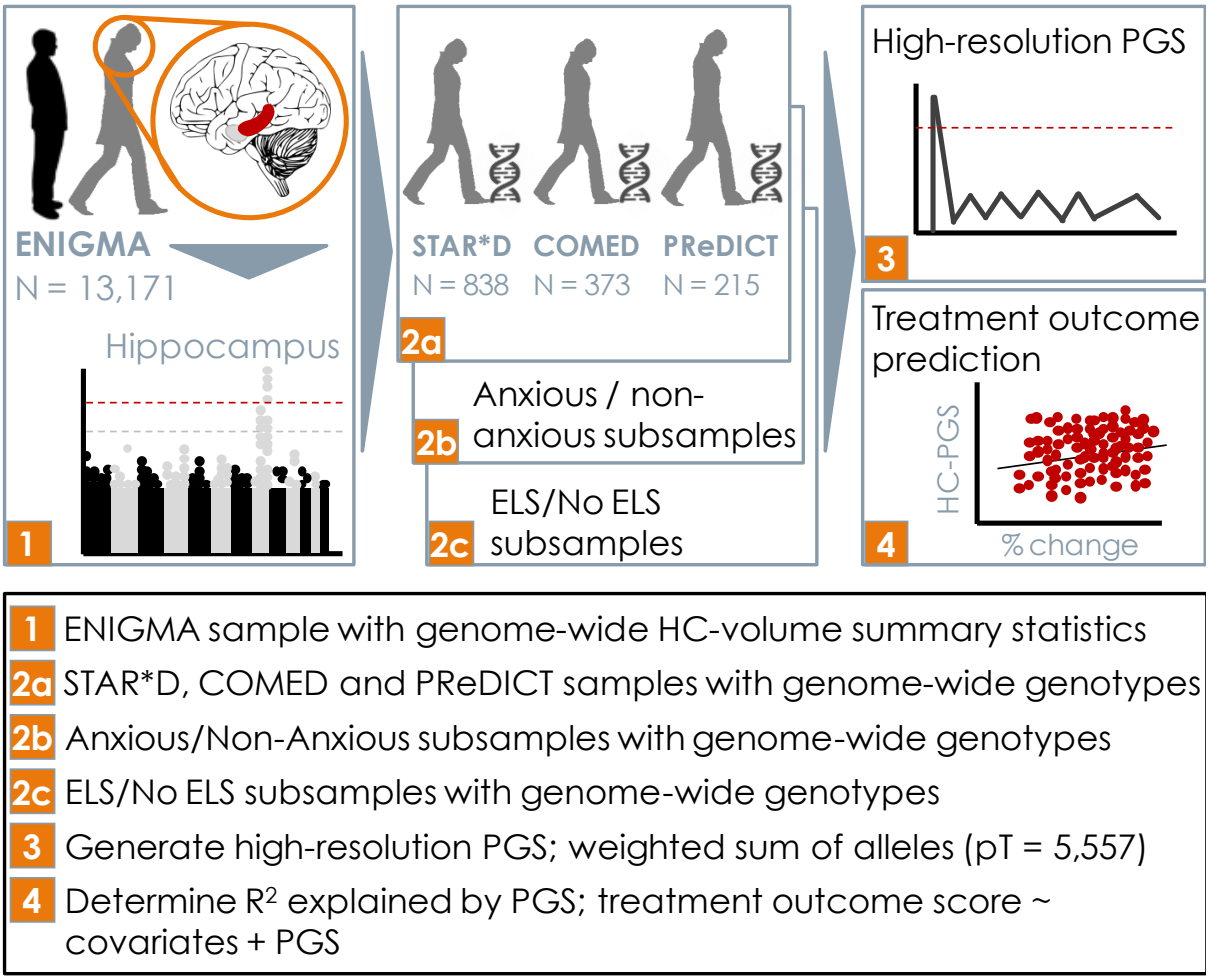


Figure 8. Overview of polygenic predictors of treatment outcome methods

4.3. Treatment-Specific Outcome Polygenic Predictors

4.3.1. ENIGMA Sample (Discovery Sample 1)

The ENIGMA sample is described in a previous section (**see section 4.2.1**) and in full detail in Hibar *et al*., 2015. Full summary association results from HC-volume meta-analysis, were used to build HC-based PGS for the entire PReDICT sample (**see section 4.2.3**).

4.3.2. FDG-PET Sample (Discovery Sample 2)

Sample Description

Subjects were recruited through the Mood and Anxiety Disorders Program (MAP) at Emory University. Study protocol and inclusion/exclusion criteria have been previously described (Boadie W. Dunlop et al., 2012; McGrath, et al., 2013). Briefly, eligible participants were 18-60 year old outpatients with a primary diagnosis of MDD, assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders (Association, 2000) and confirmed through an evaluation by a study psychiatrist. Participants required a score of ≥ 18 at screening and ≥ 15 at baseline on the HDRS (17 items) to be eligible for randomization. The Emory Institutional Review Board approved the study as registered at clinicaltrials.gov (NCT00367341). Written informed consent was obtained from all participants.

Treatment Protocol

Although treatment consisted of two phases (Callie L. McGrath et al., 2014; McGrath, et al., 2013), only phase1 was considered in this study. Briefly, in phase 1, participants underwent FDG-PET scans and were afterwards randomly assigned (1:1) to a 12-week regimen of ESC (10-20 mg/day) or 16 sessions of CBT. Raters, blind to treatment assignment, assessed changes in symptom severity weekly for the initial six weeks, then every two weeks through week twelve.

Clinical Outcomes

Remission was defined as an HDRS score ≤ 7 at both weeks 10 and 12. Non-remission was defined as a change in HDRS score of ≤ 30% from baseline to phase one endpoint. Partial responders defined by a HDRS score > 30% but not achieving remission (≥ 50% but with HDRS score > 7) as well as non-completers were not included in the analyses to remain consistent with the initial analyses of these data (Dunlop, et al., 2015; McGrath, et al., 2013).

Neuroimaging Endophenotypes

Prior to treatment randomization, a resting state FDG-PET scan was performed. Image pre-processing and analysis have been previously described (C. L. McGrath, et al., 2014; Callie L. McGrath, et al., 2014; McGrath, et al., 2013). The six brain regions (right anterior insula, right motor cortex, left premotor cortex, right inferior temporal cortex, left amygdala, and left precuneus) found to significantly differentiate patients by response outcomes to ESC or CBT (McGrath, et al., 2013) were used as quantitative traits to conduct GWAS. Activity in regions without significant associations with differential treatment outcome (four with previously reported metabolic changes in MDD: right and left occipital cortex, right Brodmann area 9, left Brodmann area 9, and two not associated with either MDD or treatment outcome: right cerebellum and left cerebellum) was used as negative control.

Genotyping and QC

Genome-wide genotypes (Illumina OmniExpress array) were measured in peripheral blood DNA drawn at baseline randomization visit. SNPs with sample-wise call rate ≥ 0.99, SNP call rate ≥ 0.98, $HWE_{P\text{-value}} \geq 1 \times 10^{-6}$ and MAF ≥ 0.05 were selected, allowing for a total of 604,640 markers for subsequent analysis. Out of 38 individuals included in the analysis of McGrath et al., 2013, one did not agree to DNA collection and two were excluded for low genotyping. A total of 35 patients were included in the GWAS (11 remitters and 7 non-remitters to CBT, 11 remitters to and 6 non-remitters ESC). Based on IBD (**see section 3.1**) estimates in PLINK (Purcell, et al., 2007), all subjects in the sample were unrelated (Pihat < 0. 0625).

4.3.3. PReDICT Sample (Target Sample)

Sample Description

The PReDICT study enrolled subjects through MAP at Emory University. The study design has been published previously (B. W. Dunlop, et al., 2012). Ethical approval was given by The Emory Institutional Review Board and the Grady Hospital Research Oversight Committee and the study was registered at clinicaltrials.gov (NCT00360399). Briefly, PReDICT enrolled 344 treatment-naïve 18-65 year old adults with primary diagnosis of non-psychotic MDD.

Participants were eligible for randomization if they met DSM-IV criteria for current MDD (HDRS score ≥ 18 at screening and ≥ 15 at baseline) and if they had never previously received treatment for mood disorders. The current study included subjects from the per-protocol completer dataset (N = 234); patients who met all inclusion/exclusion criteria, had no major protocol violations, completed 12 weeks of treatment, and whose week 12 given ADM did not contradict the randomized treatment assignment. Of these participants who agreed to provide DNA, five did not pass genotyping-QC and three were removed for relatedness based on IBD (see more details below). A total of 215 patients were included in the analyses. Basic demographic and clinical variables are reported in **Table 8**.

Treatment Protocol

Participants were randomly assigned to one of three possible treatments: ESC 10-20 mg/day; Duloxetine (DUL) 30-60 mg/day; or CBT delivered as 16 one-hour individual sessions. The initial phase treatment consists of a 12-week period with one of these monotherapies. Non-responders were eligible for an additional 12-week combined treatment of medication and CBT (results to be reported elsewhere). Symptoms severity was assessed weekly by blinded raters for the first 6 weeks, then every two weeks for the second 6 weeks.

Clinical Outcomes

Identically to the discovery sample 2, remission was defined as an HDRS score ≤ 7 at week 10 and week 12 and non-remission as an HDRS score improvement of ≤ 30% from baseline to endpoint.

Genotyping and QC

Genome-wide genotypes (Illumina OmniExpress array) were measured in peripheral blood DNA drawn at baseline randomization. SNPs with sample-wise call rate ≥ 0.99, SNP call rate ≥ 0.98, $HWE_{P\text{-value}} \geq 1 \times 10^{-6}$ and MAF ≥ 0.05 were selected, allowing for a total of 587,665 markers. All relatives of individual subjects (N = 3, Pihat ≥ 0.125) were excluded, as well as those with low genotyping (N = 5). The entire PReDICT sample after QC (N = 215) was included in the HC-PGS treatment specific polygenic prediction (**see section 4.3.4**). Genotyped remitters and non-remitters who completed 12-week treatment were included in the functional neuroimaging-based polygenic prediction analysis (N = 138) (28 remitters and 14 non-remitters to CBT, 36 remitters and 13 non-remitters to ESC, 40 remitters and 7 non-remitters to DUL). Confirmatory secondary analyses include the entire sample (N = 215) (**see section 4.3.4**).

4.3.4. HC-based Polygenic Predictors of Treatment Specific Outcome

SNPs and weight effect sizes based on ENIGMA meta-GWAS for HC volume (Hibar, et al., 2015) were used to generate HC-PGS to test whether SNP associations with hippocampal volume could predict treatment response to CBT or ADM in PReDICT. Lists of SNPs (N = 5,557) based on association pT, ranging from $P = 1 \times 10^{-5}$ to P = 0.5 (increments of 0.00009) were produced based on HC summary statistics (*PRSice command line: slower 0.00001 \ sinc 0.00009 \ supper 0.5 \ remove.mhc T \ report.individual.scores T \ clump.r2 0.2 \ clump.kb 500*). Clumping parameters are stated in the general section (**see section 4.1.1**).

HC-PGS were calculated for individuals in the entire PReDICT (N = 215) sample. Scores were calculated as described in **section 4.1.1** at each of the different pT (P-value of association derived of HC-GWAS). Linear regressions were then used to assess whether HC-PGS could predict percent change in HDRS from baseline to week 12 in PReDICT depending on treatment (as interaction term; treatment: HC-PGS) with either CBT or ADM. PCAs were calculated using GCTA (Yang, et al., 2011) and included as covariates, as well as gender in predictions. Age was not included as covariate given that ENIGMA SNP HC-volume associations effects were corrected for and independent of age (Hibar, et al., 2015). Post-hoc, models were also tested in the Caucasian subset of the entire PReDICT sample (N = 113). Significance was set at P < 0.004 for HC-PGS prediction.

4.3.5. Functional Neuroimaging Polygenic Predictors of Treatment Specific Response

To test whether the combined effect of SNP associations with the activity of each of the ROIs associated with differential response could predict treatment-specific response in a second sample (PReDICT), we first conducted association analyses between RAI , RMC, LPMC, RITC, LAM, LPCUN (N = 6 regions) brain activity and genome-wide SNPs in the first sample of 35 MDD patients (McGrath, et al., 2013). Principal components to account for population stratification were calculated using GCTA (Yang, et al., 2011) and included as covariates together with age and gender in every GWAS. Summary statistics from these GWAS were then used to generate polygenic scores in PReDICT using PRSice (Euesden, et al., 2015). Clumping parameters are stated in the general section (**see section 4.1.1**).

Due to the sample size of the GWAS discovery sample 2 (N = 35) a more stringent range of P-value thresholds was used to create PGS. Lists of SNPs (N = 1113) based on association pT, ranging from $P = 1 \times 10^{-5}$ to P = 0.1 (increments of 0.00009) were produced based on each ROI activity summary statistics separately (*PRSice command line: slower 0.00001 \ sinc 0.00009 \ supper 0.1 \ remove.mhc T \ report.individual.scores T \ clump.r2 0.2 \ clump.kb 500*). Each individual's PGS was calculated as defined as described **section 4.1.1**.

Linear regressions were then used to assess whether these functional neuroimaging-based PGS could predict percent change in HDRS from baseline to week 12 in PReDICT depending on treatment (as interaction term; treatment : PGS) with either CBT or ADM. Because PReDICT participants were ethnically diverse (**Figure 9**), PCAs were calculated and included as covariates, as well as age and gender (for general methods on population stratification **see section 3.1.2**) To be consistent with McGrath et al., 2013 (FDG-PET sample) only remitters and non-remitters were included in these analyses (N = 138). Post-hoc, models were also tested only in the Caucasian subset of this sample (N = 70).Confirmatory secondary analyses, however, evaluated outcomes in all subjects (N = 215). Significance was set at P < 0.004 for each ROI-PGS prediction; however, because of the correlation between the brain activity of the six brain regions (**Figure 10**), we calculated the number of independent tests (Cheverud, 2001) (N = 5) and set P < 0.0008 as threshold accounting for number of scores and regions tested.

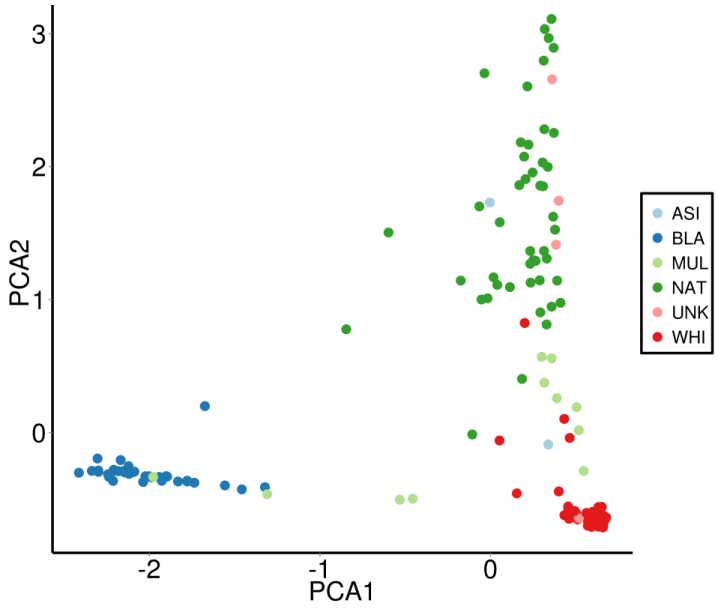


Figure 9. PCA plot of PReDICT sample shows good concordance between self-reported ethnicity (legend) and estimated ethnicity by principal component analysis. Asian (ASI), African-American (BLA), Multiple (MUL), Native-American (NAT), Caucasian (WHI), Unknown (UNK)

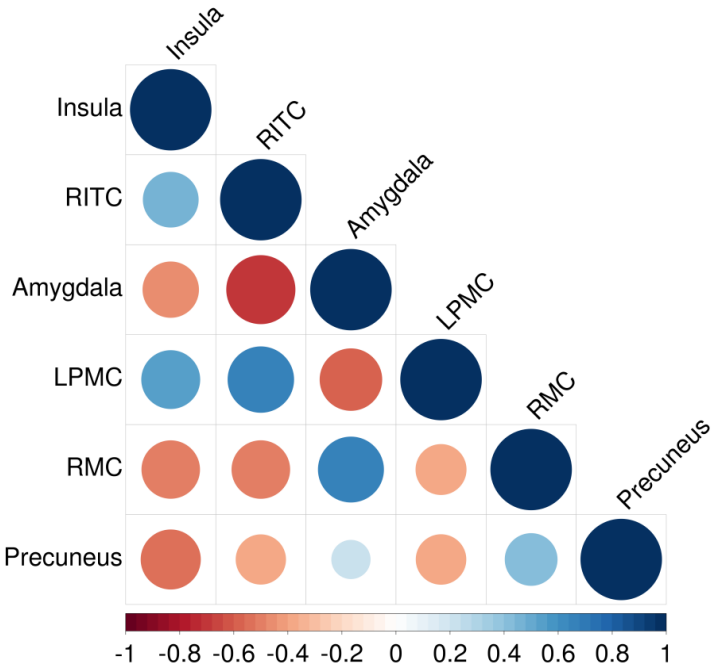


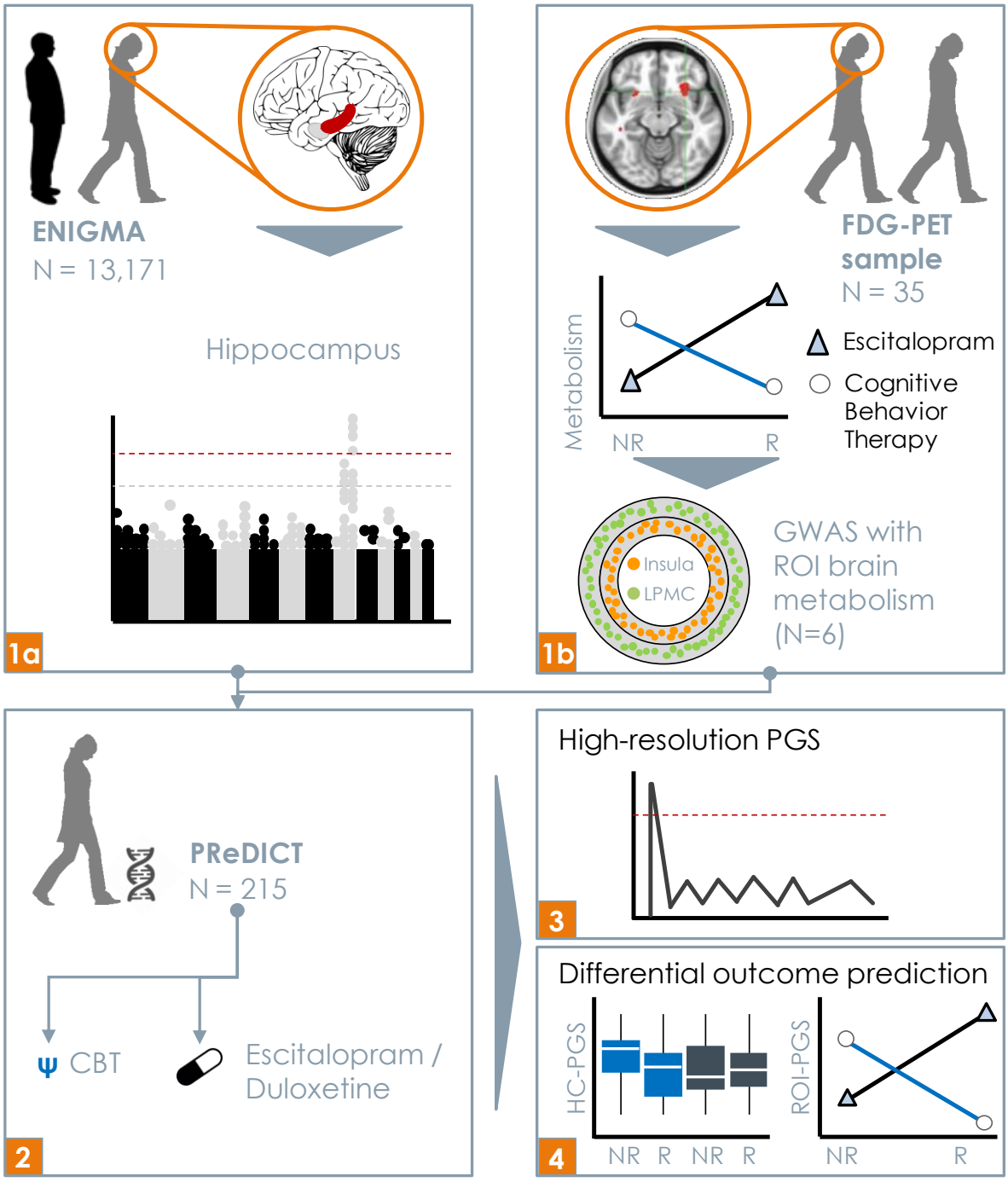
Figure 10. Pearson correlation between brain activitiesof right anterior insula (RAI), right inferior temporal cortex (RITC), left amygdala (LAM), left premotor cortex (LPMC), right motor cortex (RMC) and left precuneus (LPCUN). Size of circle represents strength of correlation and the color gradient represents the strength and direction of correlation, where blue is positive and orange is negative correlation

To verify that PGS based on activity from non-associated brain regions or clinical response alone based PGS could not predict treatment-specific response, we conducted further GWAS between the brain activity from six non-associated regions (right and left occipital cortex, right and left Brodmann area 9, and right and left cerebellum) in the first sample (N = 35), including PCAs, gender and age as covariates.

Quantitative trait association analysis was then performed to test for association between SNPs and percentage improvement in HDRS scores after 12 weeks of treatment. PGS based on each of the results of these seven association analyses were calculated and linear regressions to predict treatment response in the second sample were conducted as described for the main analyses in this section. A general overview of the methods used in this section is illustrated in **Figure 11.**

4.3.6. Statistical Power

Statistical power was estimated for a quantitative trait (percent change in HDRS score from baseline to twelve weeks) using R (Gauderman, 2002; Lee & Wray, 2013) (https://www.r-project.org/). Assuming a significance threshold of 0.004, we have 97.2% power to detect whether HC-PGS explain clinically relevant effect sizes (6.3% of variation in percent improvement) in PReDICT (N = 215). ROI-PGS were sufficiently powered (88.7%) to detect clinically relevant effect sizes of the PGS in the remitters and non-remitters (N = 138) PReDICT sample at $P < 0.0008$ (Gauderman, 2002; Lee & Wray, 2013; Team, 2014). This amount of explained variance has been described as clinically relevant for pharmacogenetic studies of antidepressant response (R. Uher, et al., 2012).



1a ENIGMA sample with HC-volume GWAS summary statistics

1b FDG-PET with ROI metabolism GWAS summary statistics

2 PReDICT samples with genome-wide genotypes

3 Generate high-resolution PGS; weigthed sum of alleles (pT_{ENIGMA} = 5,557, $pT_{FDG\text{-}PET}$=1,113)

4 Determine R^2 explained by PGS (HC-based PGS or ROI-based PGS): treatment outcome score~covariates + treatment:PGS

Figure 11. Overview of polygenic predictors of treatment-specific response methods

5. Results

The following results demonstrate that combining neuroimaging and genetic markers as well as accounting for clinical subtypes is essential to identify predictors of antidepressant response. The first results show that clinical heterogeneity dissection in MDD reveals potential structural neuroimaging-based polygenic predictors of antidepressant response. Both structural and functional neuroimaging-based PGS predict outcomes to CBT and ADM as well. Gene profiles tagged by predictive HC-PGS variants are enriched in cortical and hippocampal adult brain regions. ROI-PGS SNPs overlap with previously identified schizophrenia risk variants from the Psychiatric Genomics Consortium. Finally convergent cortical and striatal brain cell-type specific expression patterns for both HC-PGS and ROI-PGS variants were identified.

5.1. Treatment Outcome Polygenic Predictors

Socio-demographic factors baseline depression rating scale scores, response rates, medication and comorbidity rate of anxiety disorders for the STAR*D, COMED and PReDICT samples are listed in **Table 6**, **Table 7** and **Table 8**. There were no differences in demographic and clinical characteristics in the STAR*D sample and between treatment groups in the PReDICT sample or the COMED sample.

Table 6. Characteristics of MDD patients in STAR*D sample (N = 838)

MDD Patients in STAR*D Sample			**p**
Response Status (N / %)			
Non-responders	376 (44.96)		
Partial-responders	462 (55.14)		
Anxious Status (N / %)			0.24
Non-anxious	436 (52.02)		
Anxious	402 (47.98)		
Gender (N / %)			0.78
Female	495 (59.06)		
Male	343 (40.94)		
	Mean	**SD**	**p**
HDRS Baseline	21.23	5.06	0.087
Age	43.11	13.26	0.33

Table 7. Characteristics of MDD patients in COMED sample(N = 373)

	Bup / Escit (N = 123)		Escit / Placebo (N = 122)		Ven / Mirt (N = 128)		p
Response Status (N / %)							
Non-responders	58 (47.16)		56 (45.90)		53 (41.40)		
Responders	65 (52.84)		66 (54.10)		75 (58.60)		
Early Life Stress (N / %)							0.08
no ELS	56 (45.52)		54 (44.26)		64 (50)		
ELS	67 (54.48)		68 (55.74)		64 (50)		
Anxious Status (N / %)							0.18
Non-anxious	24 (19.51)		38 (31.14)		30 (23.43)		
Anxious	99 (80.49)		84 (68.86)		98 (76.56)		
Ethnicity (N / %)							0.56
Asian	3 (2.4)		0		0		
Black	28 (22.76)		25 (20.4)		29 (22.65)		
Hawaiian, Pacific Islander	1 (0.08)		0		0		
Native-American	1 (0.08)		4 (3.27)		0		
Other	7 (5.6)		4 (3.27)		5 (3.9)		
White	87 (70.73)		91 (74.59)		94 (73.43)		
Gender (N / %)							0.65
Female	87 (70.74)		84 (68.85)		90 (70.31)		
Male	36 (29.26)		38 (31.14)		38 (29.69)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
HDRS Baseline	24.08	4.41	23.04	4.71	24.5	5.14	0.49
Age	42.92	12.77	45.45	12.28	42.64	11.62	0.33

5.1.1. HC-based PGS do not Predict Treatment Response Overall in Independent Samples

To test whether HC-based PGS could successfully predict treatment response in independent samples we constructed high-resolution HC-PGS from SNPs at many P-value thresholds (N_{pT} = 5,557) and determined the most informative pT predicting treatment outcome in the target samples (STAR*D, PReDICT and COMED). None of the pT from HC-based PGS were able to predict response in either of the target samples at P < 0.004 (**Figure 12a, b** and **c**).

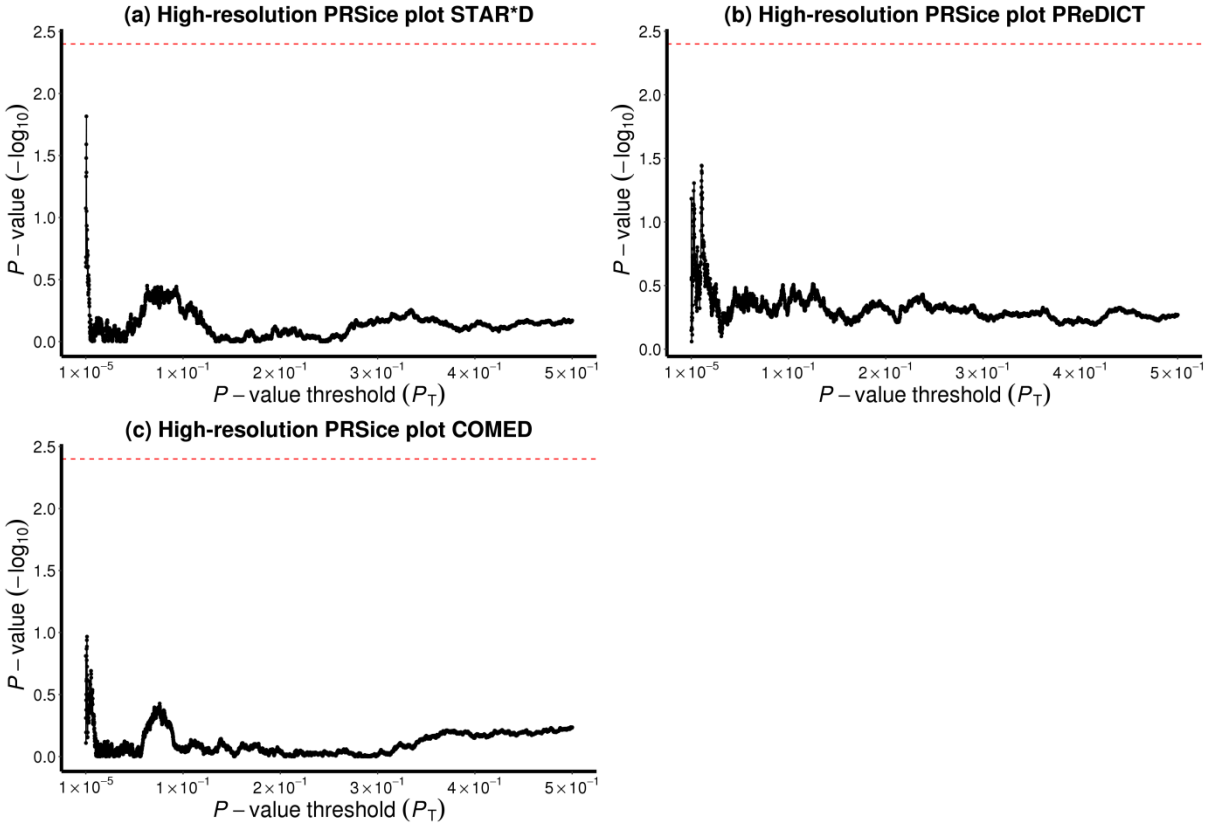


Figure 12. High-resolution PRSice plots. Red line represents PRSice multiple testing threshold P < 0.004. X axis shows range of pT tested, y axis shows -$\log_{10}$ P-value of treatment outcome prediction. (a) HC-PGS STAR*D plot, (b) HC-PGS PReDICT plot and (c) HC-PGS COMED plot

5.1.2. Reduction of Clinical Heterogeneity Improves HC-PGS Prediction of Treatment Response in Independent Samples

Non-anxious MDD might represent a more homogeneous phenotype with more robust genetic predictors for antidepressant response. We hypothesized that reduction in clinical heterogeneity in MDD could improve prediction of treatment outcome. Consequently, target samples were stratified into anxious and non-anxious subsamples. High-resolution HC-based PGS were constructed at several P-value thresholds (N_{pT} = 5,557) and the most informative HC-PGS pT predicting treatment outcome in the target subsamples (STAR*D, PReDICT and COMED) was determined.

None of the pT from HC-based PGS were able to predict response in either of the anxious target sub-samples at $P < 0.004$. On the other hand, HC-PGS predicted percent improvement in outcome scores (QIDS scores from baseline to week six in STAR*D and HDRS week twelve scores in PReDICT) in the non-anxious STAR*D ($P = 0.003$) and PReDICT ($P = 0.006$) target subsamples (N_{STAR*D} = 436, $N_{PReDICT}$ = 128) with moderate percentages in variance explained (R^2_{STAR*D} = 3.6, $R^2_{PReDICT}$ = 2.4, respectively), suggesting that reducing clinical heterogeneity might indeed advance the identification of molecular predictors of response.

Replication was not achieved in the non-anxious COMED sample ($P = 0.112$), possibly due to the low number of subjects with non-anxious depression (N = 92) as compared to those with anxious-MDD (N = 281) (**Figure 13**). In addition after predicting randomly permuted response status 1000 times with the best-fit HC-PGS from STAR*D and PReDICT non-anxious subsamples, only two and six P-values respectively were lower than those initially achieved ($p_{permSTAR*D} < 0.002$, $p_{permPReDICT} < 0.006$).

The best-fit HC-PGS predicted outcome at $P_{STAR*D} = 0.003$, $P_{PReDICT} = 0.006$, respectively and included similar amount of SNPs; 114 SNPs-$_{STAR*D}$ and 145 SNPs-$_{PReDICT}$ with a portion of them in common across samples (N = 61 SNPs) (**Figure 14**).

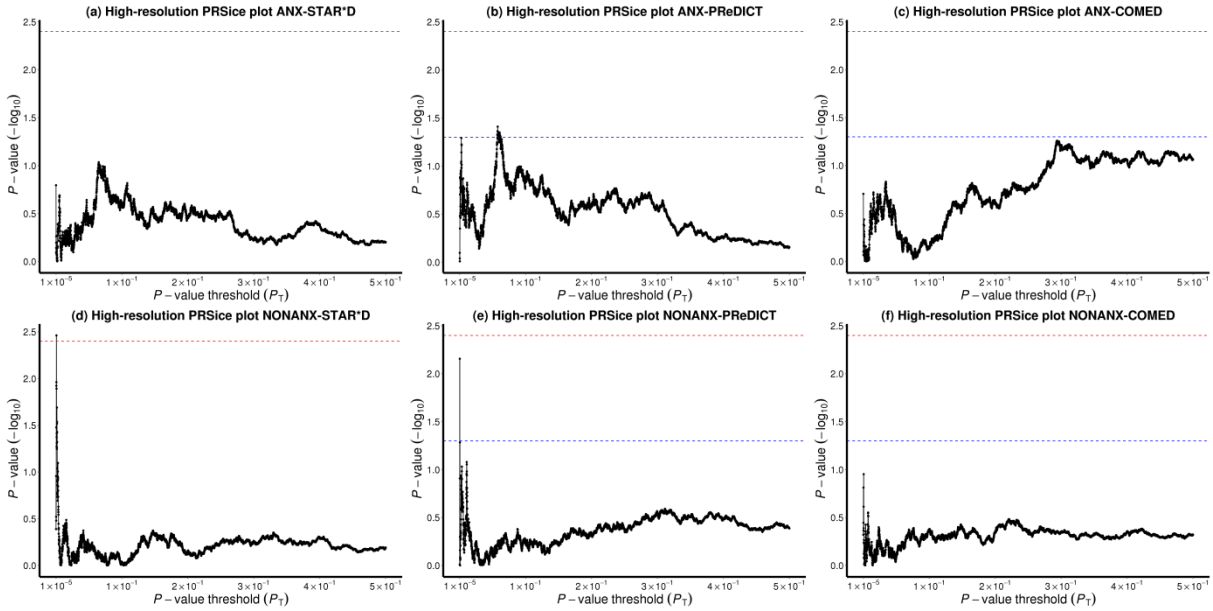


Figure 13. High resolution PRSice plots anxious and non-anxious MDD. Red line represents PRSice multiple testing threshold P < 0.004, blue line represents replication P-value threshold P < 0.05. X axis shows range of pT tested, y axis shows -$\log_{10}$ P-value of treatment outcome prediction. (a) HC-PGS anxious STAR*D plot, (b) HC-PGS anxious PReDICT plot, (c) HC-PGS anxious COMED plot, (d) HC-PGS non-anxious STAR*D plot, (e) HC-PGS non-anxious PReDICT plot and (f) HC-PGS non-anxious COMED plot

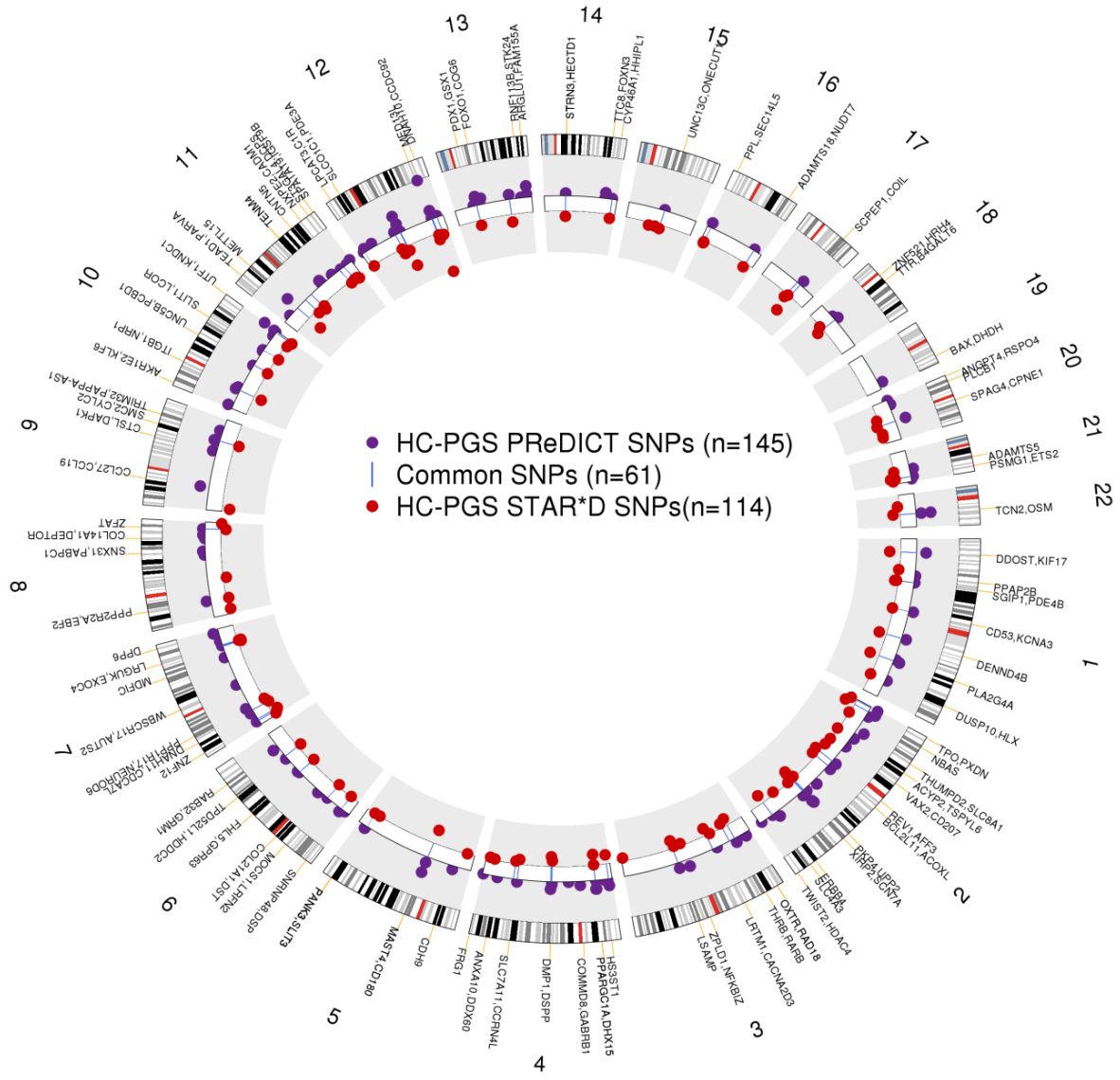


Figure 14. Circular plot of annotation of HC-PGS STAR*D (red) and HC-PGS PReDICT SNPs (purple). Blue lines represent location of common SNPs between PGS

Higher HC-PGS (reflecting a genetic load for increased HC volume) associated better outcomes, while lower HC-PGS (reflecting a genetic load for decreased HC volume) associated nonresponse (**Figure 15**) in STAR*D and PReDICT non-anxious subsamples. Post-hoc analyses when only ESC patients from PReDICT where included significantly predicted differential response and in the same direction (P = 0.0046, R^2 = 1.02, N = 78) as in the non-anxious STAR*D MDD sample (**Supplementary Figure 1**). For ESC-PBO patients from COMED, prediction was not significant, possibly due to the reduced power resulting from low non-anxious MDD patients (N = 38).

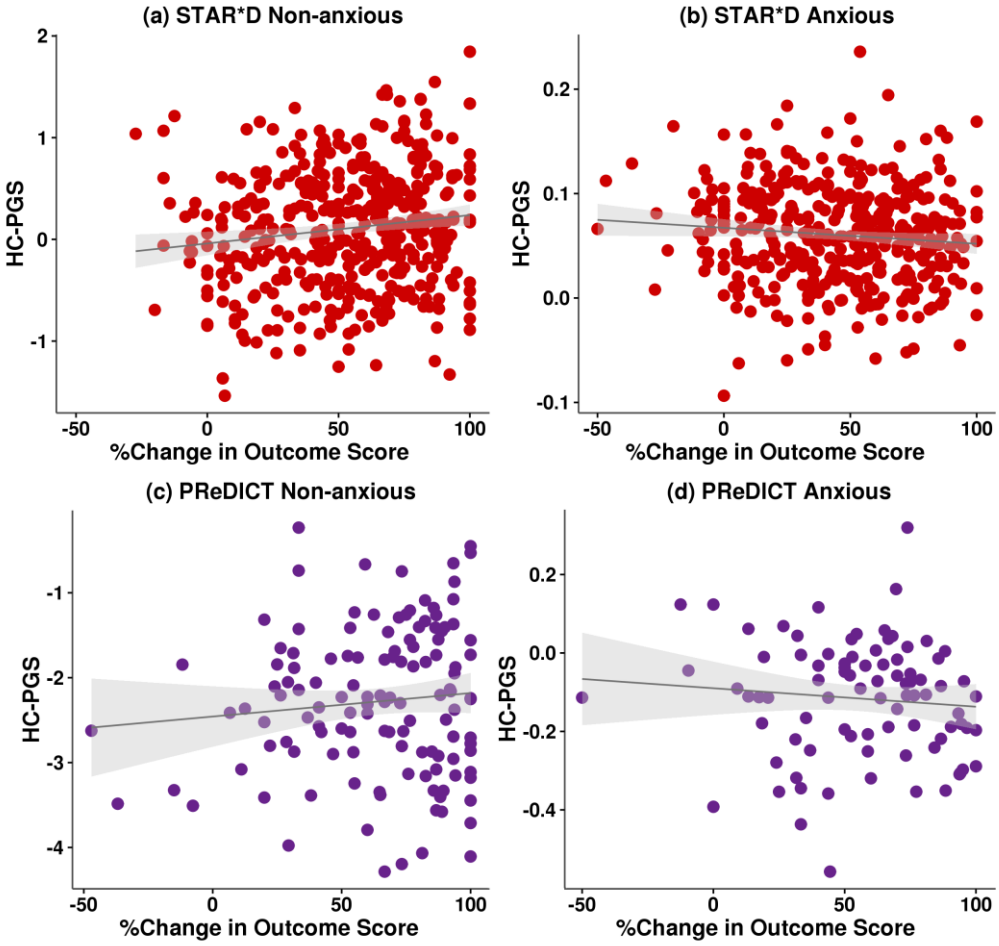


Figure 15. HC-based polygenic scores in STAR*D and PReDICT. (a) Scatter plot of HC-PGS in STAR*D non-anxious sample (N = 436, P = 0.003) (c) Scatter plot of HC-PGS in PReDICT non-anxious sample (N = 128, P = 0.006). (b, d) Scatter plots of HC-PGS in STAR*D and PReDICT anxious samples (N = 402, N = 87, P > 0.05)

5.1.3. Early Life Stress does not Improve HC-PGS Prediction of Treatment Response in Independent Samples

ELS contributes to depression risk later in life, thus we hypothesized that taking into account early life adversity could improve HC-prediction of outcome. Target samples were stratified into MDD subsamples with and without ELS. None of the pT (N_{pT} = 5,557) from HC-based PGS were able to predict response in either the discovery-target sample (in this case COMED) at $P < 0.004$ or the replication target samples (PReDICT) at $P < 0.05$ (**Supplementary Figure 2**).

5.1.4. Gene Annotation of HC-PGS and GO Enrichment

Gene annotation (**Figure 14**) and GO enrichment was performed separately for SNPs in the top HC-PGS of STAR*D and PReDICT together. Out of 198 HC-PGS SNPs ($N_{STAR*D\text{-}SNPs}$ = 53, $N_{PReDICT\text{-}SNPs}$ = 84, $N_{common\text{-}SNPs}$ = 61), 194 mapped to at least one gene locus. No GO terms where enriched.

5.1.5. HC-PGS Genes Map to Brain Cell-type Specific Expression Patterns

To explore which cellular populations may be most representative of the significant HC-PGS SNPs from non-anxious target subsamples together, enrichment for cell-type specific genes across multiple cell-types was performed. HC-PGS gene profiles of annotated SNPS (198 SNPs, **Figure 14**) were enriched for Ntsr+ (neurotensin receptor positive) neurons ($P_{Bonferroni} = 0.009$) as well as dopamine receptor type 2 positive (Drd2+) medium spiny neurons of the striatum ($P_{Bonferroni} = 0.011$) (**Figure 16**).

5.1.6. HC-PGS Genes Map to Brain Region Specific Expression Patterns

HC-PGS gene profiles of annotated SNPS (198 SNPs, **Figure 14**) were enriched for cortex and hippocampal adult brain regions ($P = 0.002$, $P_{Bonferroni} = 0.006$, $P = 4.27 \times 10^{-4}$, $P_{Bonferroni} = 0.003$) (**Figure 17**).

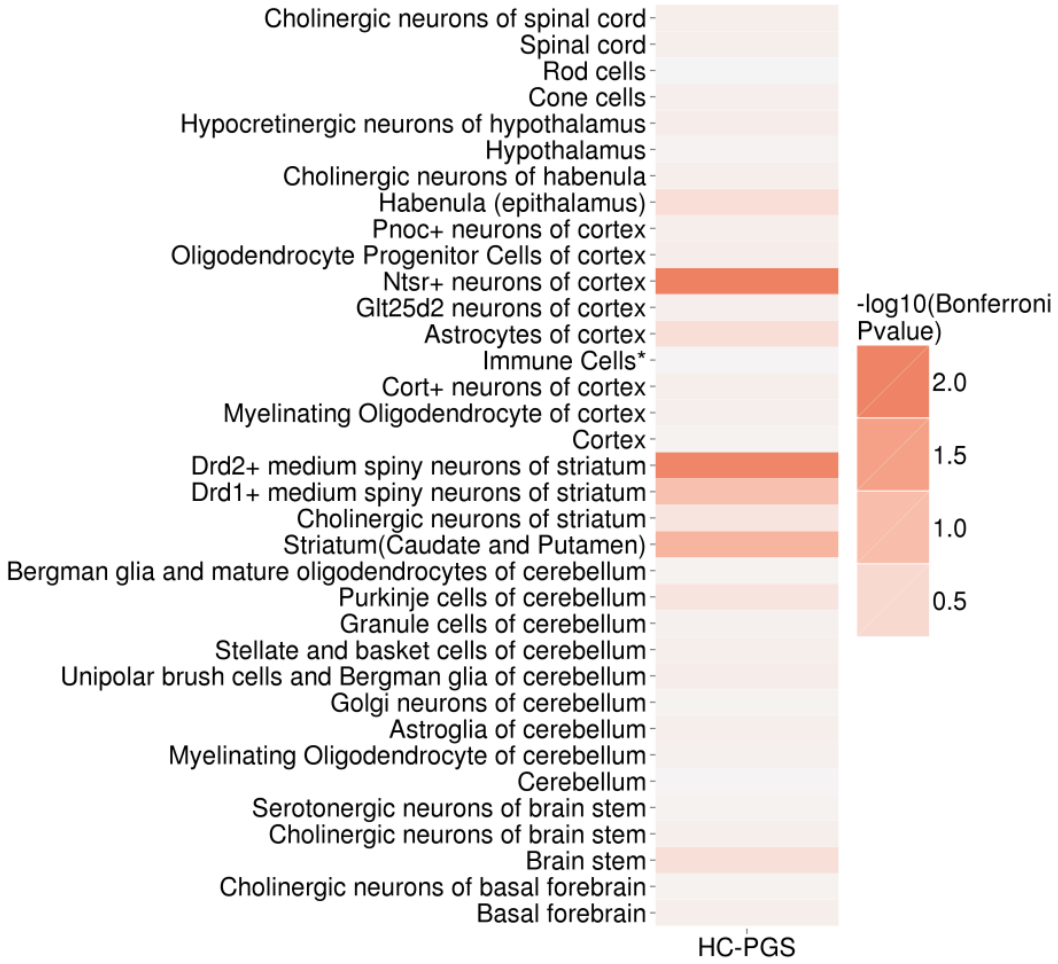


Figure 16. Cell-type specific enrichment of HC-PGS gene profiles. Color gradient represents Bonferroni corrected significant $-\log_{10}$ P-values. Transcripts were enriched for Ntsr+ and Drd2+ neurons ($P_{Bonferroni} = 0.009$, $P_{Bonferroni} = 0.011$)

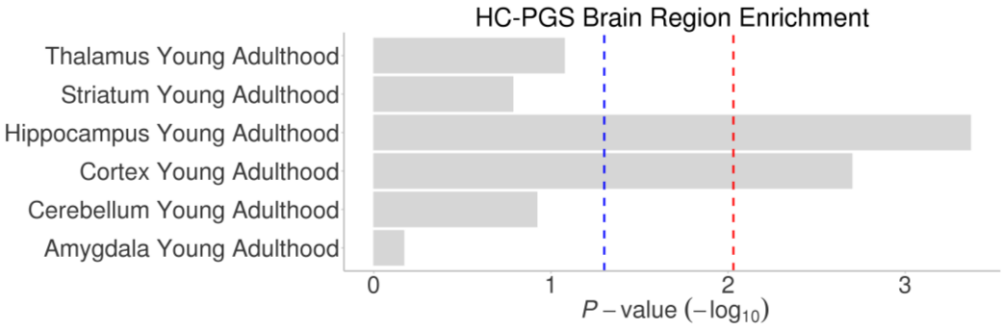


Figure 17. Brain-region specific enrichment of HC-PGS gene profiles. Transcripts were enriched in the cortex and hippocampus regions ($P = 0.002$, $P_{Bonferroni} = 0.006$, $P = 4.27 \times 10^{-4}$, $P_{Bonferroni} = 0.003$). Red line represents Bonferroni corrected P-value. Blue line represents nominal significance $P < 0.05$

5.2. Treatment-Specific Outcome Polygenic Predictors

There were no differences in demographic and clinical characteristics between treatment groups in the PReDICT sample (**Table 8**).

Table 8. Characteristics of MDD patients in PReDICT sample(N = 215)

	CBT (N = 64)		ESC (N = 78)		DUL (N = 73)		p
Response Status (N / %)							
Remitters	28 (43.75)		36 (46.15)		48 (65.75)		
Non-responders	14 (21.87)		13 (16.67)		7 (9.59)		
Partial-responders	22 (34.37)		29 (37.18)		26 (35.62)		
Early Life Stress (N / %)							0.3577
no ELS	33 (51.5)		30 (38.5)		34 (46.57)		
ELS	31 (48.5)		48 (61.5)		39 (53.43)		
Anxious Status (N / %)							0.0605
Non-anxious	39 (60.93)		45 (57.70)		44 (60.27)		
Anxious	25 (39.07)		33 (42.30)		29 (39.73)		
Ethnicity (N / %)							0.115
Asian	0		2 (2.56)		0		
Black	5 (7.81)		17 (21.79)		15 (20.55)		
Multiple	3 (4.69)		6 (7.69)		4 (5.48)		
Native-American	11 (17.19)		18 (23.08)		17 (23.29)		
Unknown	2 (3.12)		2 (2.56)		0		
White	43 (67. 19)		33 (42. 31)		37 (50.68)		
Gender (N / %)							0.998
Female	35 (54.68)		43 (55.12)		40 (54.79)		
Male	29 (45.32)		35 (44.88)		33 (45.21)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
HDRS Baseline	18.75	3.52	19.62	3.69	18.84	3.37	0.256
Age	39.8	11.7	42.2	11.7	38.9	11.66	0.205

5.2.1. HC-based PGS Predict Treatment-specific Response in a Second Sample

To test whether HC-based PGS could successfully predict treatment response in PReDICT we constructed high-resolution HC-PGS from SNPs at 5,557 P-value thresholds and determined the most informative pT predicting treatment-specific outcome. Three pT from HC-PGS were able to predict response specific to CBT or ADM at P < 0.004 (**Figure 18**). The best-fit HC-PGS included 145 SNPs (**Figure 19**) and predicted differential treatment response with P = 0.00053 and R^2 = 4.6. In addition after predicting randomly permuted response status 1000 times with the best-fit HC-PGS, only one P-value was lower than those initially achieved (p_{perm} < 0.0009).

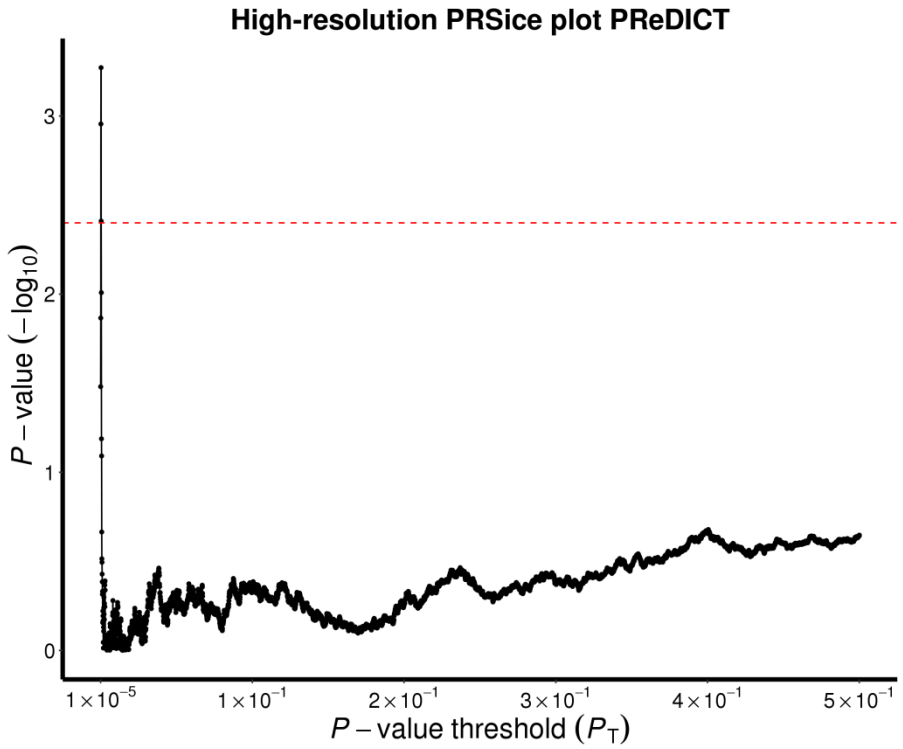


Figure 18. High-resolution PRSice Plot for HC-PGS in PReDICT sample. Red line represents PRSice multiple testing threshold P < 0.004. X axis shows range of pT tested, y axis shows -$\log_{10}$ P-value of treatment outcome prediction

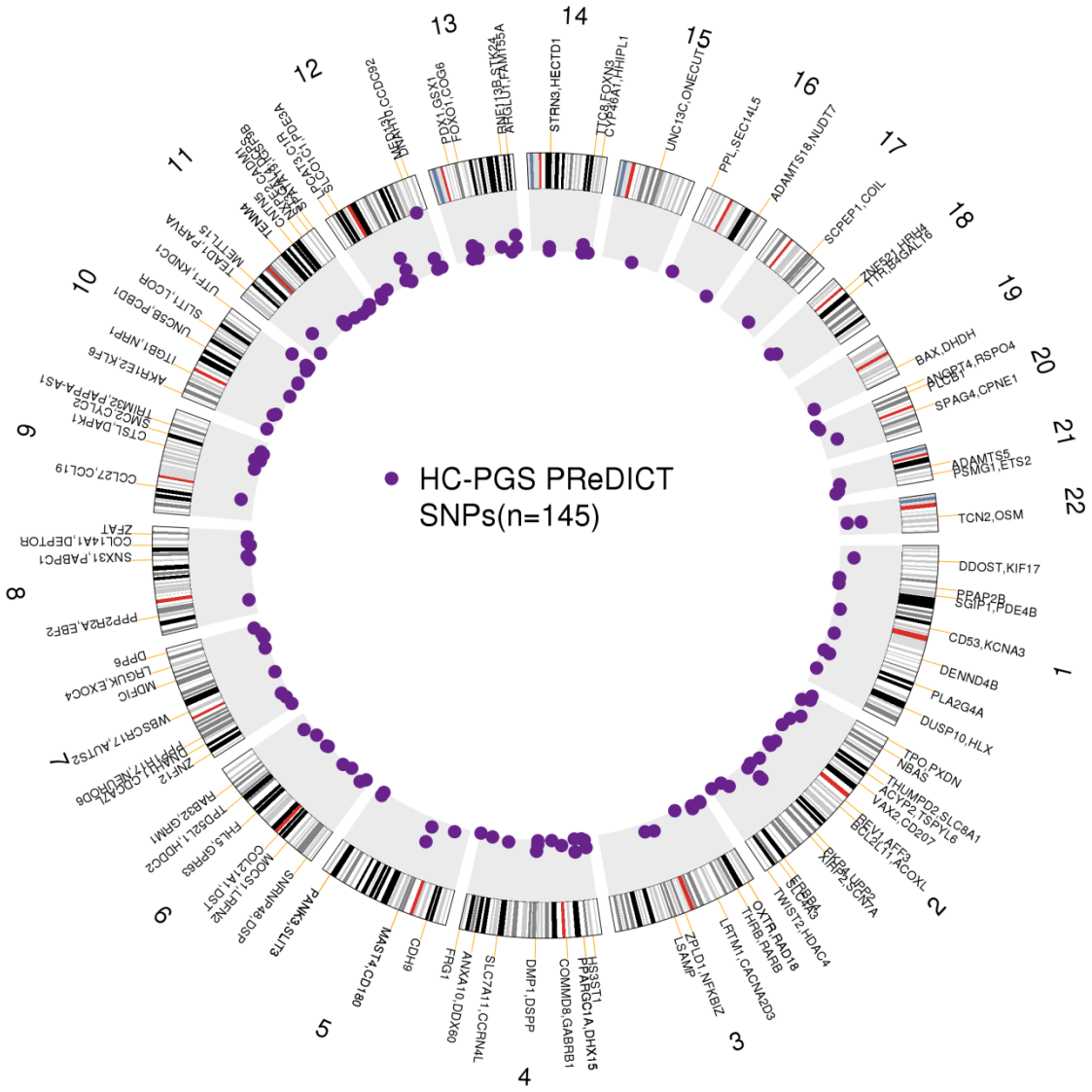


Figure 19. Circular plot of annotation of HC-PGS PReDICT SNPs (purple).

Higher HC-PGS (reflecting a genetic load for increased HC volume) associated with response to ADM and nonresponse to CBT, while lower HC-PGS (reflecting a genetic load for decreased HC volume) associated response to CBT and non-response to ADM (**Figure 20a** and **b**). Post-hoc analyses when only Caucasian patients from PReDICT where included (N = 113) while not significant at P < 0.004 (possibly due to reduced sample size), the direction remained the same (P = 0.03) (**Figure 20c** and **d**).

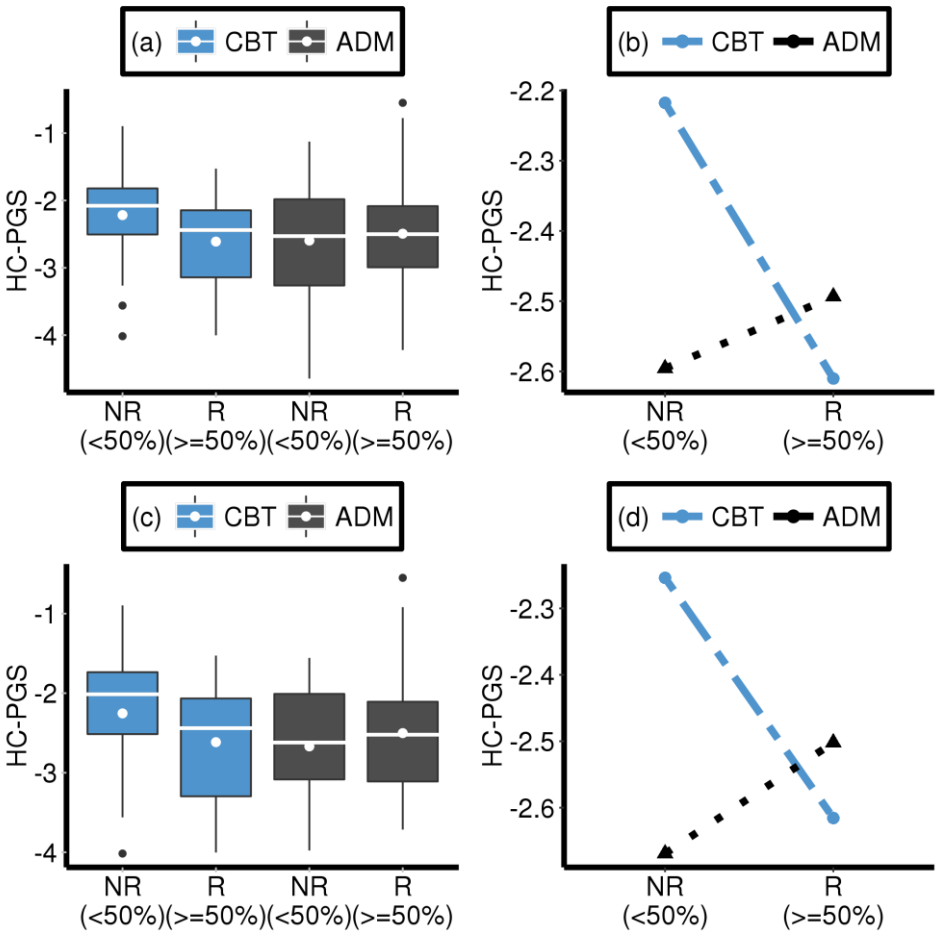


Figure 20. Hippocampal-based polygenic scores in PReDICT by treatment groups. R: responders, NR: non-responders. (a) Scatter plot of HC-PGS and %change in HDRS score at week12 stratified by treatment (N = 215, P = 0.00053). (b) Interaction plot of mean HC-PGS stratified by outcome group and treatment (c) Caucasians scatter plot of HC-PGS and %change in HDRS score at week12 stratified by treatment (N = 113, P = 0.03). (b) Caucasians interaction plot of mean HC-PGS stratified by outcome group and treatment

5.2.2. Gene Annotation of HC-PGS and GO Enrichment

Gene annotation (**Figure 19**) and GO enrichment was performed for SNPs in the best-fit HC-PGS. Out of 145 HC-PGS SNPs, 143 mapped to at least one gene locus. No GO terms where enriched.

5.2.3. HC-PGS Genes Map to Brain Cell-type Specific Expression Patterns

To explore which cellular populations may be most representative of the significant HC-PGS SNPs enrichment for cell-type specific genes across multiple brain regions and cell-types was performed. HC-PGS gene profiles of annotated SNPS (145 SNPs, **Figure 19**) were enriched for Ntsr+ (neurotensin receptor positive) neurons ($P_{Bonferroni}$ = 0.013) as well as dopamine receptor type2 positive (Drd2+) medium spiny neurons of the striatum ($P_{Bonferroni}$ = 0.013) (**Figure 21**).

5.2.4. HC-PGS Genes Map to Brain Region Specific Expression Patterns

HC-PGS gene profiles of annotated SNPS (145 SNPs, **Figure 19**) were enriched for cortex and hippocampal adult brain regions (P = 0.008, $P_{Bonferroni}$ = 0.025, P = 0.006, $P_{Bonferroni}$ = 0.025) (**Figure 22**).

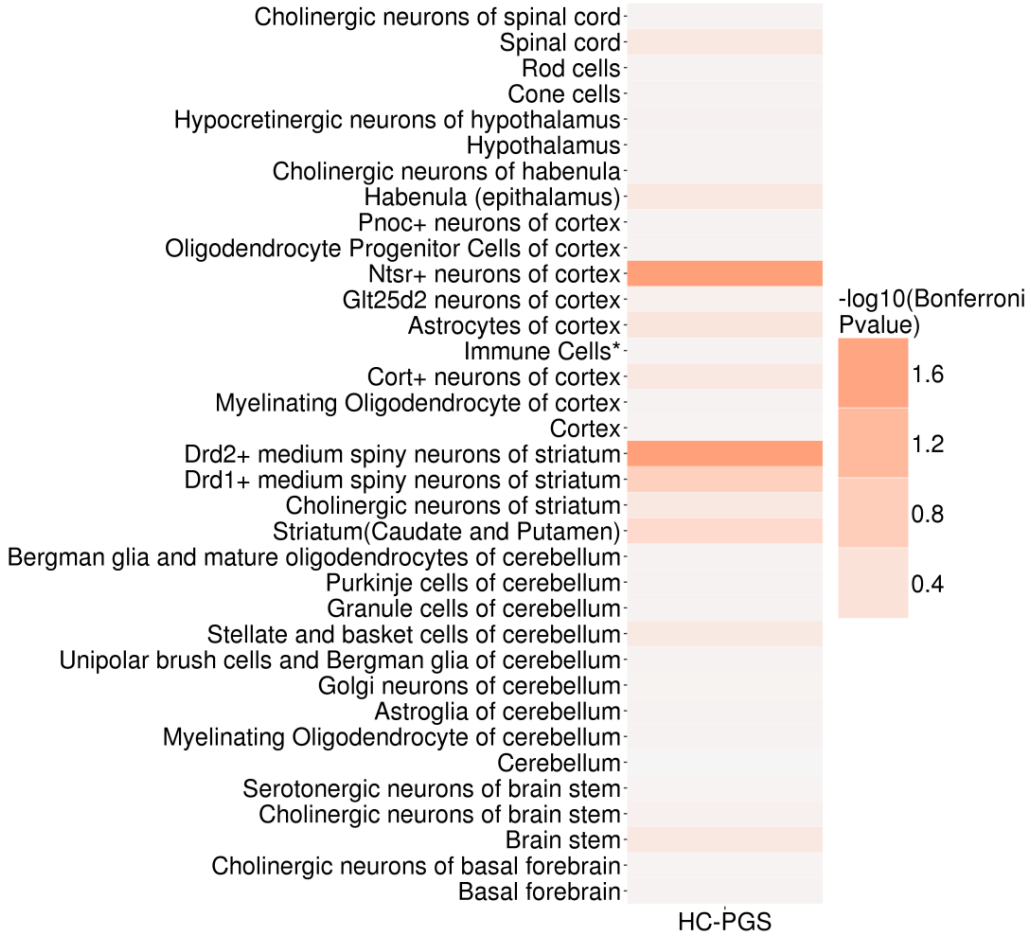


Figure 21. Cell-type specific enrichment of HC-PGS gene profiles. Color gradient represents Bonferroni corrected significant $-\log_{10}$ P-values. Transcripts were enriched for Ntsr+ and Drd2+ neurons ($P_{Bonferroni}$ = 0.013, $P_{Bonferroni}$ = 0.013)

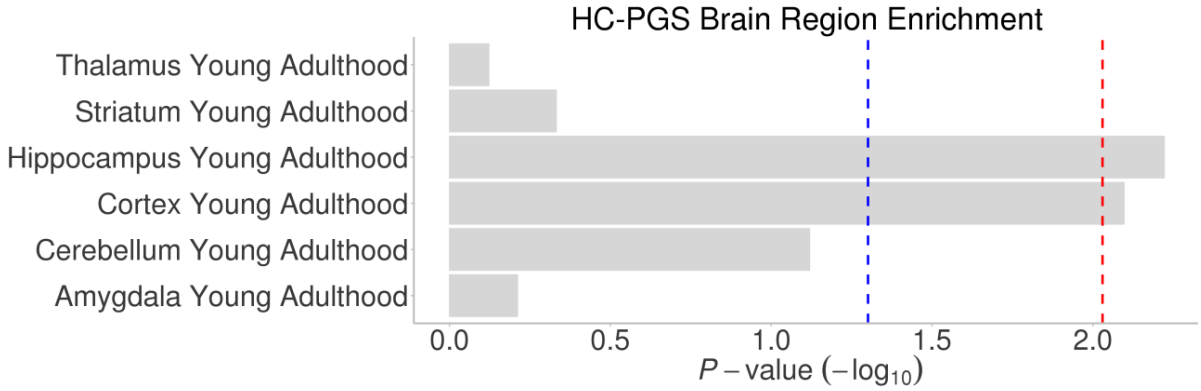


Figure 22. Brain-region specific enrichment of HC-PGS gene profiles. Transcripts were enrichted in the cortex and hippocampus regions (P = 0.008, $P_{Bonferroni}$ = 0.025, P = 0.006, $P_{Bonferroni}$ = 0.025). Red line represents bonferroni corrected P-value. Blue line represents nominal significance $P < 0.05$

5.2.5. PGS Derived from GWAS with Insula and LPMC Activity Predict Treatment-specific Response in a Second Sample

To test whether neuroimaging-based PGS could successfully predict treatment response in an independent sample (PReDICT), we ran GWAS with the activity of the 6 predictive brain regions (see **Figure 10** for correlation matrix of ROIs brain activity). No genome-wide significant results were observed and inflation factor λ was < 1.02 for all analyses (**Supplementary Figure 3**), indicating no or negligible population stratification. Next, we constructed high-resolution PGS from each of these GWAS from SNPs at several P-value thresholds (N_{pT} = 1113 for each region) and determined the most informative pT predicting differential treatment outcome in the second sample.

RMC, RITC, LAM and LPCUN-PGS did not predict response in the second sample at any pT at P < 0.0008 or P < 0.004. Insula and LPMC-PGS, however, predicted treatment-specific percent improvement in HDRS after twelve weeks in the PReDICT sample (N = 138 patients, excluding partial responders) at several thresholds (N = 12 thresholds for PGS-Insula and N = 6 for PGS-LPMC) at P < 0.0008, with relatively large percentages in variance explained (R^2 = 10.4–10.8, R^2 = 10.5–10.9, respectively). At P < 0.004 N = 317 and N = 150 thresholds were significant for PGS-Insula and PGS-LPMC, respectively (**Figure 23**). A threshold P-value of pT < 0.01198 was the best-fit for PGS-Insula; it included 4,292 SNPs and predicted differential treatment response with P = 0.00053 and R^2 = 10.8. For PGS-LPMC pT < 0.0199 was the best-fit including 6,500 SNPs, and predicting the outcome at P = 0.00061 with R^2 = 10.8. In addition after predicting randomly permuted response status 1000 times with the best-fit pT PGS-Insula and PGS-LPMC no P-values were lower than those achieved by both best-fit PGS (p_{perm} < 0.001).

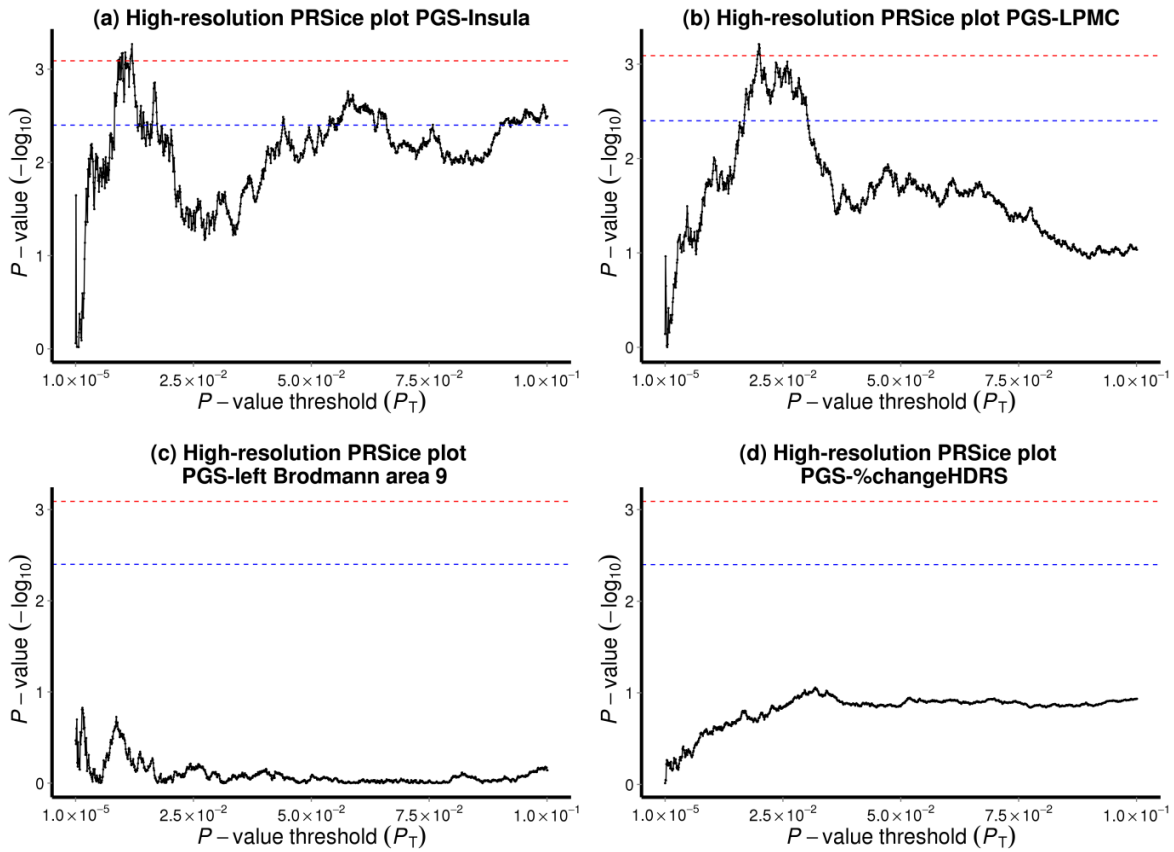


Figure 23. High resolution PRSice plots. Red line represents region-wide significant threshold P < 0.0008, blue line represents PRSice multiple testing threshold for a single region P < 0.004. X axis shows range of pT tested, y axis shows -$\log_{10}$ P-value of treatment outcome prediction. (a) PGS-Insula plot. (b) PGS-LPMC plot. (c) PGS-Left Brodmann area 9 plot (d) PGS-percent change in HDRS after twelve weeks plot

For both PGS-Insula and PGS-LPMC, higher PGS associated with remission to CBT, while lower PGS associated with non-remission to CBT (**Figure 24a, b, e** and **f**) in the remitter/non-remitter sample (N = 138). Moreover, both PGS-Insula and PGS-LPMC differed more between remitters and non-remitter in the CBT than in the ADM group. When only Caucasians where included, PGS-LPMC significantly predicted differential response and in the same direction (P = 0.017, R^2 = 5.5, N = 70). For PGS-Insula, while not significant, the direction remained equal (P = 0.291, R^2 = 1.3, N = 70) (**Supplementary Figure 4**).

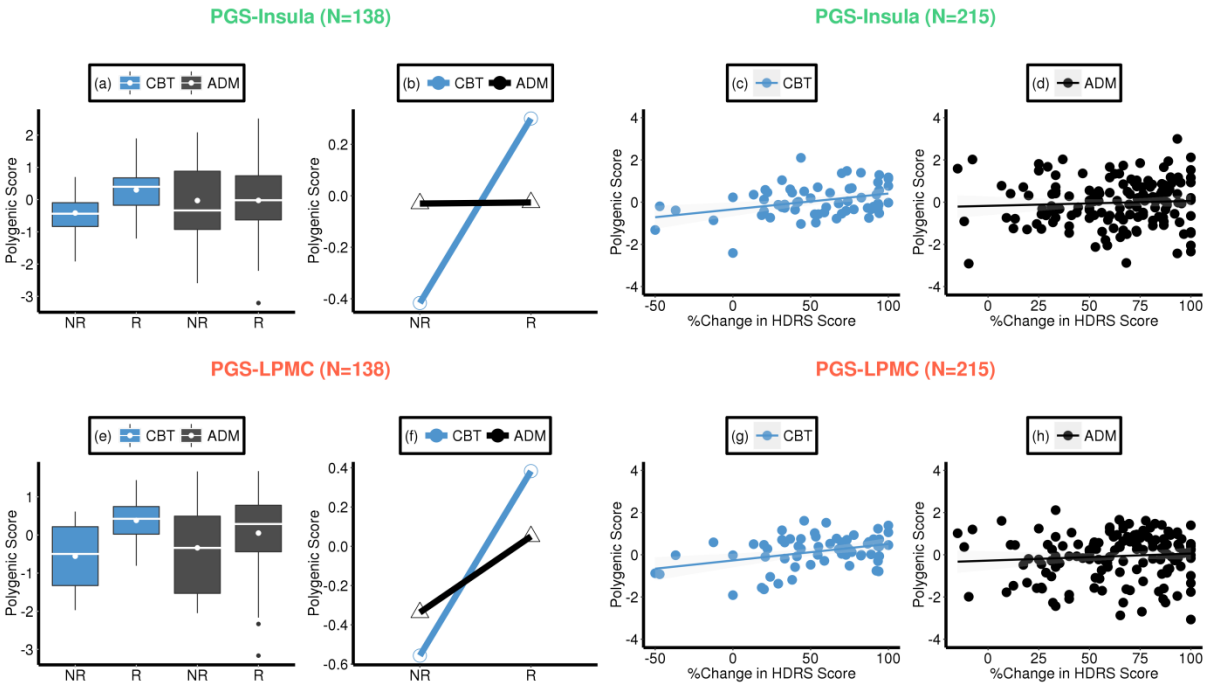


Figure 24. Polygenic scores in PReDICT based on right anterior insula and left premotor cortex activity stratified by groups. R: remitters, NR: non-remitters. (a) Boxplot of insula Z-score transformed PGS (Z-PGS) stratified by treatment and outcome (N = 138, P = 0.00053), white dot represents the mean. (b) Interaction plot of mean Insula Z-PGS stratified by outcome group and treatment (N = 138, P = 0.00053). (c, d) Scatter plots of Insula-based Z-PGS and %change in HDRS score at week 12 stratified by treatment group for most significant pT prediction in the whole PReDICT sample (N = 215, P = 0.00234). (e) Boxplot of LPMC Z-PGS stratified by treatment and outcome (N = 138, P = 0.00061). (f) Interaction plot of mean LPMC Z-PGS stratified by outcome group and treatment (N = 138, P = 0.00061). (g, h) Scatter plots of LPMC-based polygenic Z-scores and %change in HDRS score at week 12 stratified by treatment group for most significant pT prediction in the whole PReDICT sample (N = 215, P = 0.0052)

Secondary analyses showed that treatment-specific PGS-Insula predicted response at eleven pT in the whole PReDICT sample (N = 215) at P < 0.004 (R^2 = 5.3–5.8). pT < 0.00928 (3,366 SNPs, P = 0.00234, R^2 = 5.8) accounted for the highest variance and predicted response in the same direction as in the smaller sample (**Figure 24c** and **d**). PGS-LPMC did not predict response at P < 0.004 in the whole PreDICT sample, however pT < 0.0199 was, as in the previous analysis, the most predictive pT (P = 0.0052, R^2 = 5.5) (**Figure 24g** and **h**).

To examine specificity of these endophenotype-based PGS, we conducted GWAS with the activity of six non-predictive regions as well as with percent change in HDRS in the first sample and constructed high-resolution PGS based on these in PReDICT. None of the pT from any of the random neuroimaging-based or clinical response based PGS (**Figure 23c** and **d**) were able to predict response in PReDICT (neither in the whole sample or when excluding partial responders).

5.2.6. Genes Annotated to PGS-Insula and PGS-LPMC SNPs are Enriched for Relevant GO Terms

Gene annotation (**Figure 25**) and GO enrichment was performed separately for SNPs in the top PGS-Insula, top PGS-LPMC and overlapping SNPs between both PGS. Out of 4,292 PGS-Insula SNPs, 4,149 mapped to at least one gene locus. The most enriched GO for PGS-Insula genes was myelin assembly (GO:0032288, Binomial $FDR_{q\text{-value}} = 5.045 \times 10^{-4}$, Fold Enrichment = 2.669, which corresponds to 10 of the 17 genes in the GO) (**Supplementary Figure 5**).

Out of 6,500 PGS-LPMC SNPs, 6,311 mapped to at least one gene locus. Genes annotated to PGS-LPMC were enriched for regulation of membrane repolarization (GO:0060306, Binomial $FDR_{q\text{-value}} = 2.33 \times 10^{-3}$, Fold Enrichment = 2.0041), regulation of potassium ion transmembrane transporter activity (GO:1901016, Binomial $FDR_{q\text{-value}} = 2.53 \times 10^{-3}$, Fold Enrichment = 2.2456) and dopamine transport (GO:0015872, Binomial $FDR_{q\text{-value}} = 3.01 \times 10^{-2}$, Fold Enrichment = 2.3560), among others (**Supplementary Figure 5**). The overlap between PGS-Insula and PGS-LPMC consists of 536 SNPs (**Figure 25**), out of which 524 mapped to at least one gene. Genes annotated to overlapping SNPs showed enrichment for glycosaminoglycan binding (GO: 0005539, Binomial $FDR_{q\text{-value}} = 4.01 \times 10^{-2}$, Fold Enrichment = 2.3741).

Figure 25. Circular plot of genome-wide annotation of PGS-Insula (green) and PGS-LPMC SNPs (orange). Blue lines represent location of common SNPs between PGS. Gene names represent the suggestive associations for either PGS-Insula or PGS-LMPC ($P < 5 \times 10^{-4}$). Grey gradient represents strength of association in GWAS with Insula brain activity and GWAS with LPMC brain activity, where lighter grey represents the most associated SNPs for each GWAS

5.2.7. PGS-Insula and PGS-LPMC Genes Map to Convergent Brain Cell-type Specific Expression Patterns

To explore which cellular populations may be most representative of the PGS-Insula and PGS-LPMC gene profiles, enrichment for cell-type specific genes across multiple brain regions and cell-types was performed. Despite low overlap in genes and SNPs between PGS-Insula and PGS-LPMC (**Figure 25**), both were enriched for cortical neurons ($P_{Bonferroni}$ = 0.011, $P_{Bonferroni}$ = 0.01), particularly Ntsr+ (neurotensin receptor positive) neurons ($P_{Bonferroni}$ = 2.54 × 10^{-6}, $P_{Bonferroni}$ = 3.46 × 10^{-9}) as well as dopamine receptor type1 (Drd1+ ($P_{Bonferroni}$ = 0.005, $P_{Bonferroni}$ = 0.019)) and type 2 positive (Drd2+) medium spiny neurons of the striatum ($P_{Bonferroni}$ = 3.59 × 10^{-6}, $P_{Bonferroni}$ = 2.61 × 10^{-4}) (**Figure 26**). The overlapped genes between PGS-Insula and PGS-LPMC were enrichment only for Ntsr+ cortical neurons ($P_{Bonferroni}$ = 1.71 × 10^{-5}).

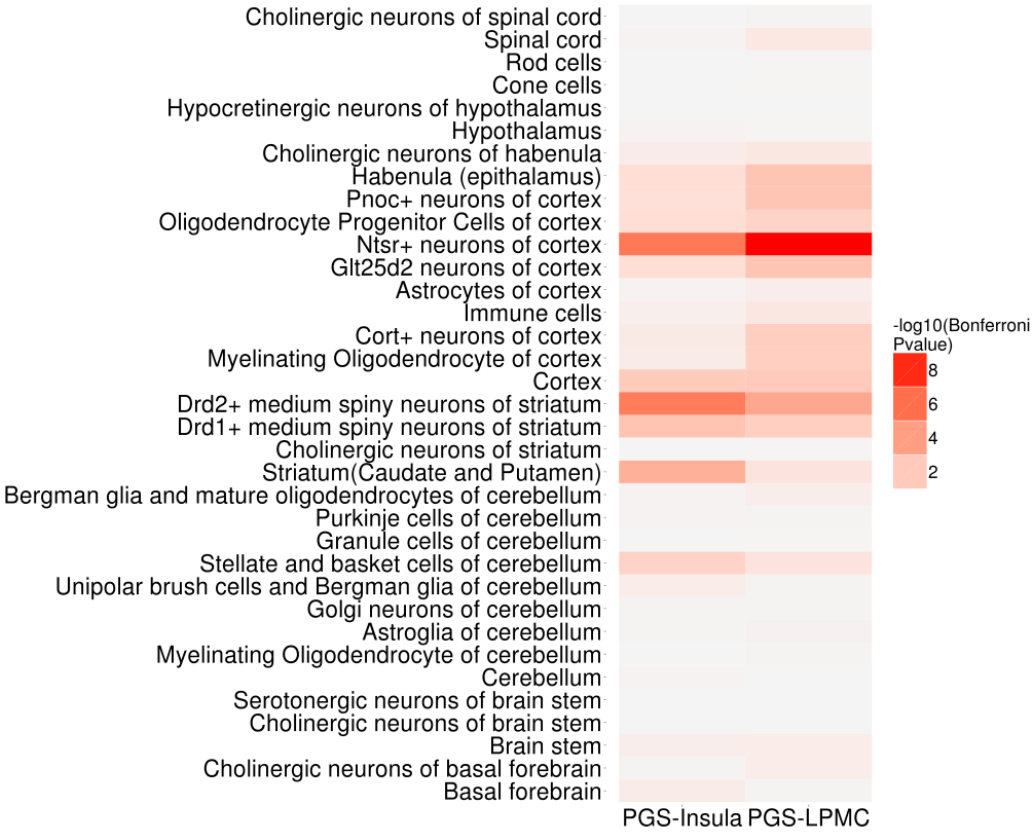


Figure 26. Cell-type specific enrichment of PGS-Insula and PGS-LPMC transcripts. Color gradient represents Bonferroni corrected significant -$\log_{10}$ P-values. Genes mapped by both PGS were enriched for cortical neurons ($P_{Bonferroni}$ = 0.011, $P_{Bonferroni}$ = 0.01), particularly Ntsr+ (Neurotensin receptor positive / Corticothalamic) neurons ($P_{Bonferroni}$ = 2.54 × 10^{-6}, $P_{Bonferroni}$ = 3.4 × 10^{-9}) and also Drd1+ (dopamine receptor type 1 positive) ($P_{Bonferroni}$ = 0.005, $P_{Bonferroni}$ = 0.019) and Drd2+ (dopamine receptor type 2 positive) medium spiny neurons of the striatum ($P_{Bonferroni}$ = 3.59 × 10^{-6}, $P_{Bonferroni}$ = 2.61 × 10^{-4}). The transcripts that overlapped between PGS-Insula and PGS-LPMC transcripts were enrichment only for Ntsr+ cortical neurons ($P_{Bonferroni}$ = 1.71 × 10^{-5}). PGS-Insula transcripts were enriched also for striatum (caudate and putamen) ($P_{Bonferroni}$ = 6.11 × 10^{-4}) and stellate and basket cells of cerebellum ($P_{Bonferroni}$ = 0.027). PGS-LPMC transcripts also showed significant enrichment for Pnoc+ (prepronociceptin positive) ($P_{Bonferroni}$ = 0.006), Cort+ (Cort+ interneurons of cortex) ($P_{Bonferroni}$ = 0.016) and Glt25d2 (Glycosyltransferase 25 family member 2 positive/ Corticospinal, corticopontine) neurons of cortex ($P_{Bonferroni}$ = 0.006), habenula (epithalamus) ($P_{Bonferroni}$ = 0.006), myelinating oligodendrocytes ($P_{Bonferroni}$ = 0.019) and oligodendrocyte progenitor cells of cortex ($P_{Bonferroni}$ = 0.027)

5.2.8. PGS-Insula and PGS-LPMC SNPs Overlap with Genome-wide Significant Schizophrenia Associated Loci

Risk genetic loci have shown to overlap across psychiatric disorders (Lee, et al., 2013), thus we examined the overlap between the best threshold SNPs of PGS-Insula and PGS-LMPC with loci previously shown to confer risk for psychiatric disorders (MDD, Schizophrenia and cross-disorder) (Ripke, et al., 2014; Ripke et al., 2013; Smoller et al., 2013). Both PGS-Insula SNPs and PGS-LMPC SNPs were significantly enriched (P = 0.0009, P = 0.0049) among genome-wide associated schizophrenia loci, and the enrichment was consistent also across lower association P-value thresholds; $P < 5 \times 10^{-7}$ (P = 0.0009, P = 0.0199) and $P < 5 \times 10^{-6}$ (P = 0.0109, 0.0029) (**Supplementary Figure 6a** and **b**). Next we tested for overlap with SNPs in the PGC MDD and cross-disorder GWAS at $P < 5 \times 10^{-6}$, $P < 5 \times 10^{-5}$, and $P < 5 \times 10^{-4}$. Neither PGS-Insula nor PGS-LPMC SNPs were enriched for SNPs in either of these studies at any of the thresholds tested.

Lastly we conducted the analysis with non-psychiatric traits using genome-wide significant loci associated with height (Lango Allen et al., 2010) and diabetes (Morris et al., 2012). There was no significant overlap of either PGS-Insula or PGS-LPMC with loci associated with height (P = 0.0739, 0.0529) (**Supplementary Figure 6c** and **d**) or diabetes (P = 0.172, P = 0.359) associated SNPs.

6. Discussion

This section summarizes the findings of this thesis. It states the strengths, limitations and novel features of the neuroimaging-based polygenic predictors approach. As a final point, the potential importance of cell-types and brain regions expression patterns in the context of MDD and treatment response is discussed.

Prediction of antidepressant response has proven a difficult task, especially with easily obtainable biomarkers, such as genotypes. Favored outcomes to mechanistically different antidepressant treatments, such as CBT, ADM, ECT, or DBS support the existence of biologically distinct subtypes of depression. Identifying the underlying differences would allow to better match patients to their biological optimal treatment and is therefore of tremendous importance. Symptom improvement observed in depressed patients if the adequate treatment is chosen is often identical, suggesting the existence of a common signature of clinical response across treatments too, possibly via effects in converging downstream systems, such as the stress hormone system or the immune system. Furthermore, the presence of clinical entities like anxiety or stress exposure influencing MDD susceptibility and treatment response suggests that depression subtypes might benefit from tailored therapeutic strategies.

Using a combined approach this thesis presents an alternative framework to address the above statements. Polygenic scores derived from genetic variants influencing structural brain volume changes, expressly HC-volume, predicted treatment outcome scores in MDD non-anxious patients in two independent samples. Gene profiles based on these HC-PGS were enriched for hippocampal and cortical brain regions, as well striatal brain cell-type signatures. HC-based PGS and functional neuroimaging endophenotype-based PGS were also successful at predicting treatment-specific outcomes in depressed patients and were enriched as well for disease relevant GO terms and convergent cortical and striatal brain cell-type specific expression patterns and overlap with previously identified risk loci for schizophrenia.

This novel technique for identifying treatment selection biomarkers combines four features that differ from previous approaches. First, for genetic associations, we did not use treatment outcome per se but structural brain differences (HC-volume) related to treatment outcome (Samann, et al., 2013) and MDD (Hibar, et al., 2015; Schmaal, et al., 2015) as well as functional neuroimaging endophenotypes (brain metabolic activity) predictive of outcome (McGrath, et al., 2013). Second, to acknowledge the polygenic nature of such phenotypes we built polygenic predictors from these associations. Another important factor was the application of high-resolution PGS. As illustrated in **Figure 12**, **Figure 18** and **Figure 23** fixed

thresholds, as opposed to the sliding ones used here, would have missed the most predictive thresholds in both studies. Lastly, we aimed to reduce sample heterogeneity by considering clinical and environmental factors in outcome prediction and for the second study we did not attempt to predict treatment outcome overall, but focused on differential outcome to CBT versus ADM phenotypes. Taken together, these features provide a framework with possible implications for the development of precision medicine-based clinical practice.

In the first study included in this thesis, we hypothesized that taking into account depression diagnostic subtypes (anxious and non-anxious) or environmental exposure to stress would increase power and allow identification of genetic predictors for antidepressant treatment response. This is particularly relevant given the high comorbidity between depression and anxiety (Melartin, et al., 2002) and the differential response between anxious and non-anxious subtypes of MDD (Clayton, et al., 1991; Fava, et al., 2004; Fava, et al., 2008; Joffe, et al., 1993; Tollefson, et al., 1994). Early life stress is also a strong predictor of stress-related psychiatric disorders in adulthood (Aguilera, et al., 2009; Chapman, et al., 2004; Heim, et al., 2008) and poor treatment outcomes for depression (Nanni, et al., 2012; Nemeroff, et al., 2003).

Heritability estimation of HC-volume is around 80%, which is a much higher genetic contribution than treatment response alone (h^2 ~ 42%). HC-PGS were unable to predict treatment outcome overall in any of the target samples, or when exposure to early life stress was considered (**Supplementary Figure 2**). As previously proposed, ELS interaction with genetic variation, might be mediated by epigenetic mechanisms (Klengel, et al., 2013), which may explain in part the lack of improved prediction. Epigenetic signatures have proved to improve phenotypic prediction over and above genetic factors for complex traits (Shah, et al., 2015). Future studies exploring gene by environment interactions on depression or treatment outcome, should considered the contribution of epigenetic modifications such as DNAm.

When only non-anxious STAR*D patients were included in the analyses HC-PGS predicted outcome scores (**Figure 12**). Replication of HC-PGS outcome prediction was achieved in the PReDICT non-anxious target subsample, and in the same direction as in STAR*D (**Figure 15**). In both STAR*D and PReDICT non-anxious subsamples, higher HC-PGS reflecting larger HC-volume correlated with better

outcomes, and lower HC-PGS reflecting smaller HC-volume with non-response. This is in agreement with previous findings relating anxious depression (Fava, et al., 2008; A. J. Rush et al., 2008) and decreased HC-volumes (Frodl, et al., 2008; Kronmüller, et al., 2008; Samann, et al., 2013) with poor outcomes. No replication was achieved in the COMED replication-target sample, most likely due to the small sample size of the non-anxious subgroup (N = 92).

Gene profiles of predictive HC-PGS mapped to expression profiles of hippocampus and cortex in adulthood as well as to particular neuronal subtypes (X. X. Xu, Wells, O'Brien, Nehorai, & Dougherty, 2014). HC-PGS genes were enriched for Ntsr+ cortical neurons and Drd2+ medium spiny neurons of the striatum. D2 neurons receive a broad number of signals from cortical areas, evidence indicates the importance of the corticostriatal neurocircuity in antidepressant response (Gershon, Vishne, & Grunhaus, 2007; Lobo et al., 2013; Nestler, 2015; Schmidt et al., 2012; Vialou et al., 2010). For instance animal studies show that induction of ΔFosB -a relevant transcription factor promoting reward and motivation- in the striatum, mainly in Drd1+ and Drd2+ medium spiny neurons is required for fluoxetine antidepressant action (Lobo, et al., 2013; Nestler, 2015; Vialou, et al., 2010). Human imaging studies show abnormal activity both in cortex and striatum in patients with MDD (J. L. Price & Drevets, 2012). Abnormal striatum activity mediates for example the appearance of depressive symptoms in relation to early life stress in humans (Hanson, Hariri, & Williamson). These studies further suggest that HC-PGS tag previously identified pathways and mechanisms relevant not just for major depression but for neural mechanisms involved in treatment response as well.

A number of limitations should be considered, to start, one has to note some differences between the target samples (STAR*D, PReDICT and COMED) that may limit their comparability, which include differences in outcome measures (clinician rated HDRS vs. patient rated QIDS) as well as the treatment regimen (diverse fixed randomized regimen). The QIDS assessment questionnaire is considered closely equivalent to the HDRS and the majority STAR*D reports have relied on this outcome score (A. J. Rush, et al., 2008; Trivedi, et al., 2006). Despite of these differences HC-PGS were able to predict outcome scores in STAR*D and across treatment groups in PReDICT, which consisted of never before treated patients. These findings propose that careful selection of clinical subtypes within MDD is important when

examining genetic predictors of antidepressant response and that non-anxious depression may represent a more homogeneous subtype of depression.

The second study demonstrates the effectiveness of PGS derived not only from structural brain changes, but also from brain metabolism endophenotypes as a potential biomarker of CBT or ADM outcomes. Notably, PGS derived from activity of brain regions not associated with differential outcome in the FDG-PET sample (**see section 4.3.2**) or from clinical response alone were not predictive in the second sample. In contrast to previous pharmacogenetic studies (Chiara Fabbri, Porcelli, & Serretti, 2014; Kato & Serretti, 2010; Rudolf Uher, et al., 2013), ours is the first to investigate predictors for differential response to two mechanistically different treatments, CBT and ADM.

HC-PGS were able to predict differential response to treatment in the entire PReDICT sample. Higher HC-PGS, reflecting a genetic load for increased HC volume associated with favorable outcome to ADM and poorer outcomes to CBT. Larger HC volume has in fact been associated with increased response to ADM (Samann, et al., 2013). Interestingly HC volume differences observed in post-traumatic stress disorder patients undergoing CBT (Levy-Gigi, Szabó, Kelemen, & Kéri) and social anxiety (Kawaguchi et al., 2014) have been associated with clinical response as well. A number of studies have in fact pointed HC volume differences as a crucial determinant for favorable outcomes in depression (Frodl, et al., 2008; Goldapple et al., 2004; Kronmüller, et al., 2008; G. MacQueen & Frodl, 2011; Samann, et al., 2013).

Even though HC-PGS were derived from a sample of individuals with European descent, response prediction was achieved in a multi-ethnic cohort. Notably, the direction of the prediction was the same in the Caucasian sample (**Figure 20**) of PReDICT but, as expected, power was limited. Gene profiles were enriched in cortical and hippocampal adult regions as well as for Ntsr+ cortical neurons and Drd2+ medium spiny neurons of the striatum. The fact that all neuroimaging-based PGS were enriched for these cell types, suggest an important role of these particular cells and structures in the pathophysiology of depression and its treatment.

PGS-Insula and PGS-LPMC successfully predicted treatment-specific clinical response in PReDICT. Importantly, the effect sizes of PGS-insula and PGS-LPMC were in a range that has been described as clinically relevant for antidepressant response. A 3-point decrease in HDRS has been described as clinically significant (NICE, 2004) and in a placebo-controlled trial. This reduction corresponds to 6.3% of the variance in outcome explained by treatment and has been defined as a benchmark for pharmacogenetic studies (R. Uher, et al., 2012). Our polygenic predictors explained a higher amount of variance (10.8%) than this benchmark in the sample of remitters vs. non-remitters.

Although the prediction of non-response vs. remission is most relevant clinically, the PGS from these two regions were also able to significantly predict differential response in the whole sample (up to 5.8% of variance explained) including intermediate responders, supporting the robustness of the predictions across a range of clinical improvement. The polygenic predictors from both the RAI and LPMC brain regions were in the same direction, congruent with the initial prediction in the neuroimaging study (McGrath, et al., 2013), and it is especially noteworthy that, PGS from both regions were best at predicting remission vs. non-response to CBT. Psychotherapy-responsive MDD may represent a more homogenous biological subtype than ADM-responsive depression. In addition there may be added heterogeneity within ADM remitters and non-remitters due to potential responsiveness to alternative ADM classes or presence of drug resistant patients requiring non pharmacological interventions.

Besides clinically relevant effect sizes, a robust biomarker should optimally work in different populations. Often, genetic predictors, including for antidepressant response, are specific for one ethnicity (Elisabeth B. Binder, et al., 2010; Garriock, et al., 2010; Porcelli, et al., 2012). In our study, both the genome wide association study with ROIs and the test of the predictors were performed in multi-ethnic cohorts. To avoid confounding due to population stratification, we used PCAs from genotype distributions as covariates in all analyses and this allowed detecting predictors with some stability across ethnicities. In fact, when using the same predictors from the multi-ethnic cohort in the largest ethnic subset of PReDICT (Caucasians), the direction of the prediction was identical to the whole sample (**Supplementary Figure 4**), but, as expected in a smaller sample, less significant.

Polygenic scores are likely more robust across different ethnicities than single variant predictors as ethnic variability in minor allele frequency of a subset of markers may be buffered by the remaining variants of the scores, which in our case included up to 6,500 SNPs for PGS-Insula and PGS-LPMC. Studies in larger, ethnically more homogenous samples will, however, be needed to fully explore the validity of predictors derived from multi-ethnic cohorts in specific ethnicities.

PGS derived from the activity of two of the six ROIs initially described to predict differential treatment response in the first sample were also predictive of the same phenotype in the second sample. However, no PGS derived from the activity of six non-predictive brain regions or from clinical response alone were predictive in the second sample (**Figure 23c** and **d**). This suggests that PGS derived from endophenotypes associated with the outcome are more powerful than those derived from the outcome itself. Unfortunately, unlike the ENIGMA sample, our neuroimaging sample was too small to identify reliable SNP h^2 for the neuroimaging traits in questions, so we can only speculate that these have a higher genetic contribution than treatment response alone.

Of the six ROIs predictive of differential response in (McGrath, et al., 2013) only PGS derived from RAI and LPMC predicted treatment-specific response in the second sample. The insula was the strongest neuroimaging predictor in the first sample, lending face validity to the PGS from this region as strong predictors. The correlation between LPMC and RAI metabolism was not the strongest ($r = 0.52$) among the 5 other regions contributing to the initial outcome prediction (**Figure 10**), suggesting some independent contribution of these regions. This is also supported by the small overlap of SNPs (N = 536 SNPs or 12 to 8.2%) and associated genes (N = 524 genes or 12.6 to 8.3 %) between the two predictive PGS derived from these regions (**Figure 25**).

SNPs within the most predictive PGS from both the insula and the LMPC were significantly enriched among SNPs with genome-wide and also sub-threshold significance in schizophrenia (Ripke, et al., 2014) but not height or diabetes, suggesting some specificity to psychiatric disorders. No significant overlap with associated SNPs in other psychiatric disorders could be observed, however, except for schizophrenia, a large number of robust genome wide significant associations have not yet been observed for these disorders, likely for current lack of larger

samples (Patrick F. Sullivan, et al., 2012). Nonetheless, the significant overlap with SNPs associated with schizophrenia is of high interest, as first, this disorder has the second highest genetic correlation with depression (0.43 ± 0.06) (Lee, et al., 2013) and second, the right insula is one of three brain regions for which convergent gray matter loss has been reported across six different psychiatric disorders, including schizophrenia and major depression (Goodkind et al., 2015).

In addition to significant overlap with genetic associations with schizophrenia, predictive PGS from both regions showed significant enrichments for disease- and region-relevant GO terms. Genes annotated to PGS-Insula were most enriched for the myelin assembly GO term. Interestingly, myelin maps in humans show distinct features for the insular cortex, this region being the most lightly myelinated cortical region (Glasser & Van Essen, 2011).

Genes annotated to PGS-LPMC activity were enriched for GOs related to membrane repolarization and potassium transport but also dopamine transport. A number of publications have noted the relevance of dopamine and dopamine transport for the function and connectivity of the premotor cortex (Habak et al., 2014; Damian Marc Herz et al., 2014; Damian M. Herz et al., 2014; Kwon & Jang, 2014). In addition, dysregulation in both myelin and dopamine have been implicated in the pathogenesis of depression (Dunlop & Nemeroff, 2007; Gershon, et al., 2007; Tham, et al., 2011).

Finally, we mapped the genes annotated to predictive PGS to gene expression signatures of specific brain regions and neuronal cell types (X. X. Xu, et al., 2014). Both PGS-Insula and PGS-LPMC genes were most enriched for signatures of cortical and striatal neurons, in particular Ntsr+ cortical neurons and Drd1+ and Drd2+ medium spiny neurons of the striatum. This overlapping enrichment resulted from a direct overlap in annotated genes for the two PGS for the cortical Ntsr+ cells, as well as a functional convergence of independent gene sets from the two PGS for the medium spiny neurons. This overlap in functional annotation suggests that the PGS from these regions may contribute to common neurocircuit functions. Dopamine function was enriched, within GOs and cell types and, in fact, a number of recent studies have implicated dopaminergic inputs into medium spiny neurons of the ventral striatum with stress-induced behaviors (Francis et al., 2015; Plattner et al., 2015; Tye et al., 2013). Ntsr+ neurons are corticothalamic projection neurons from

layer 6 which have been shown to activate layer 5a output neurons (J. Kim, Matney, Blankenship, Hestrin, & Brown, 2014). Layer 5 neurons provide the strongest cortico-striatal input and previous studies have shown a critical role for cortico-striatal projection neurons in antidepressant response, predominantly to SSRIs (Habak, et al., 2014). Furthermore, neurotensin has been associated with the pathology of other psychiatric disorders like schizophrenia (E. B. Binder, Kinkead, Owens, & Nemeroff, 2001).

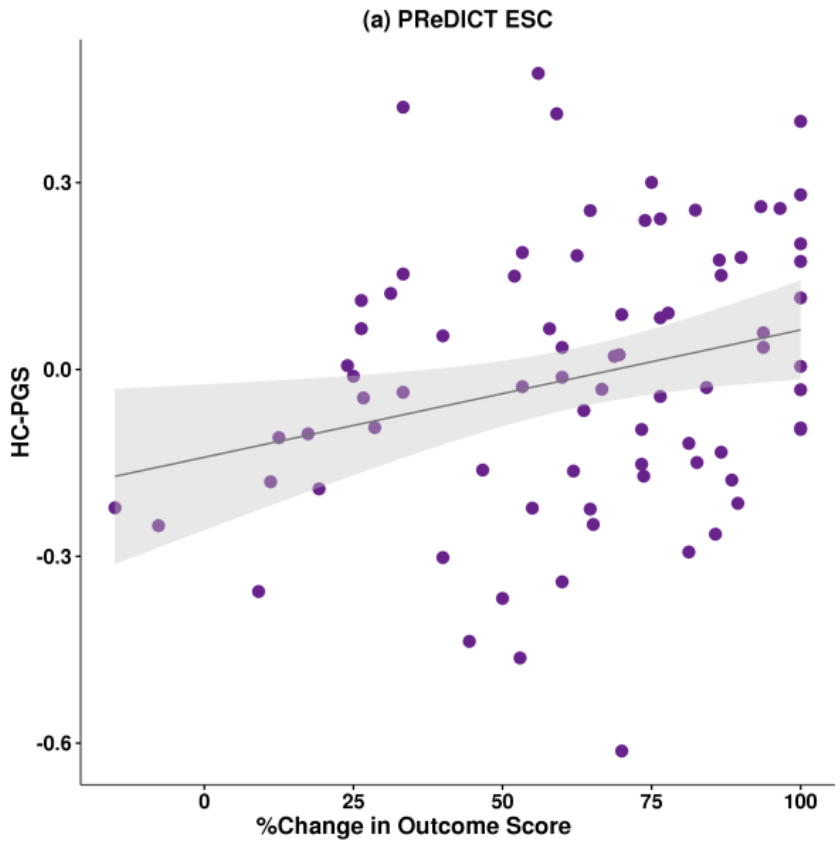
7. Conclusions

The analyses described above suggest that a combined approach using quantitative neuroimaging endophenotypes and polygenic markers could be a promising method to identify molecular predictors that could ultimately be used in clinical settings to inform individualized antidepressant treatment selection.

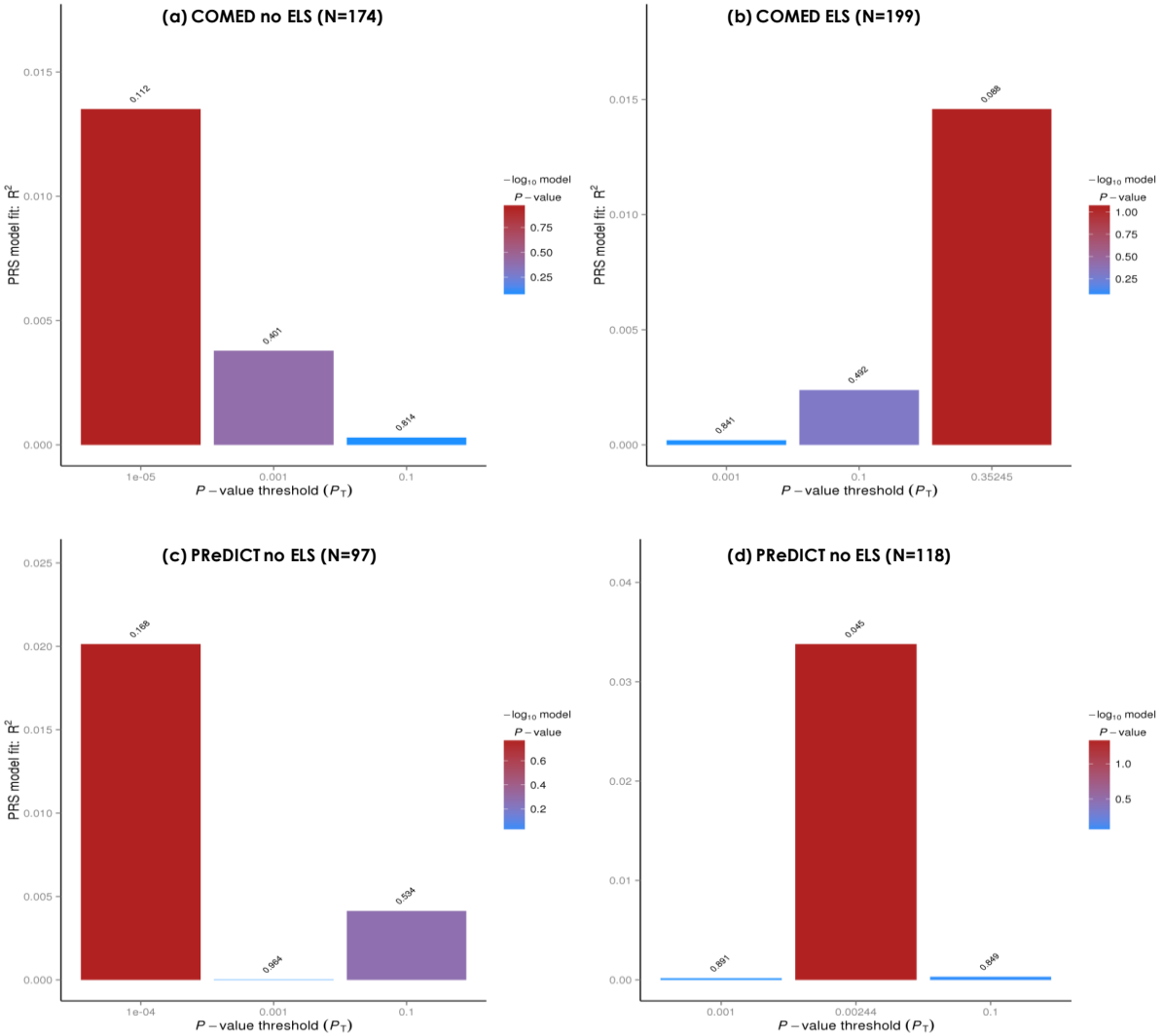
While these findings are assuring, they have to be considered preliminary as they were generated from small samples and additional validation in other cohorts will be necessary. The PReDICT cohort included patients never treated for depression in the past; this might facilitate prediction at initial presentation for treatment. It is also apparent that genetic predictors alone will not be able to explain all variance. Combined predictors, joining genetic, endophenotype and clinical measures will likely be necessary. Previous studies have shown success in combining clinical with genetic factors in enhancing prediction of antidepressant treatment response (Ising, et al., 2009).

We note that these results should be considered preliminary as they resulted from a novel approach that to our knowledge has not been implemented before. Overall, these results represent an initial step towards optimizing prediction of treatment response and highlight the fact that MDD is a heterogeneous clinical entity consisting of several clinical subtypes, each with possibly specific genetic makeup. Stratifying MDD into clinical subtypes in order to decrease phenotypic heterogeneity can enhance identification of genetic predictors of treatment response. Enhancing our ability to match patients to optimal treatment modalities will be an exciting step forward in the march towards more effective treatments of depression. We emphasize that structural and functional neuroimaging in combination genetic markers have significant potential for the development of prognostic biomarkers of clinical response to treatments for depression.

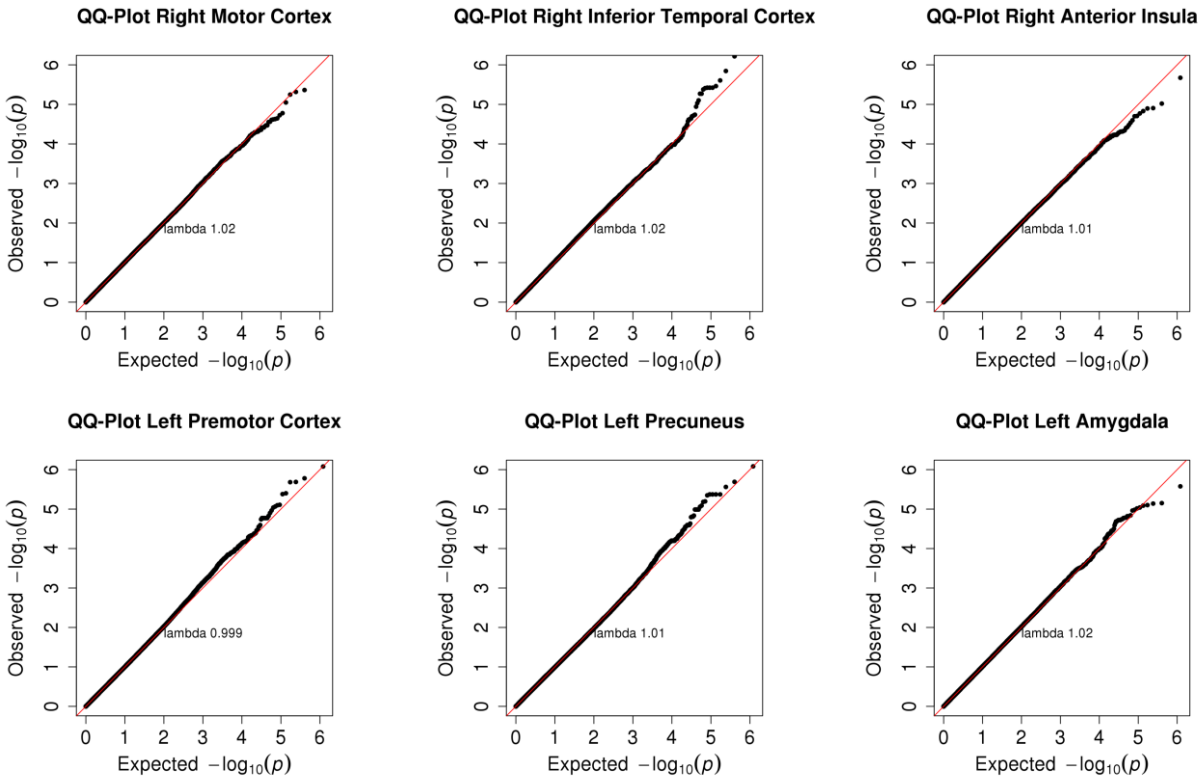
8. Supplementary Figures



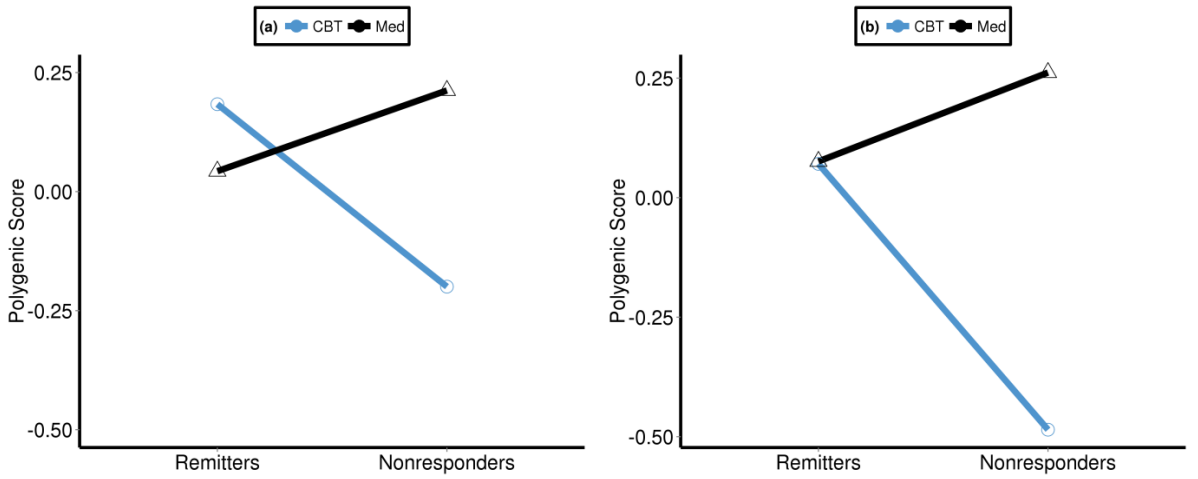
Supplementary Figure 1. HC-PGS Scatter plot PReDICT escitalopram sample. Y axis represents HC-PGS. X axis represents change in HDRS outcome score



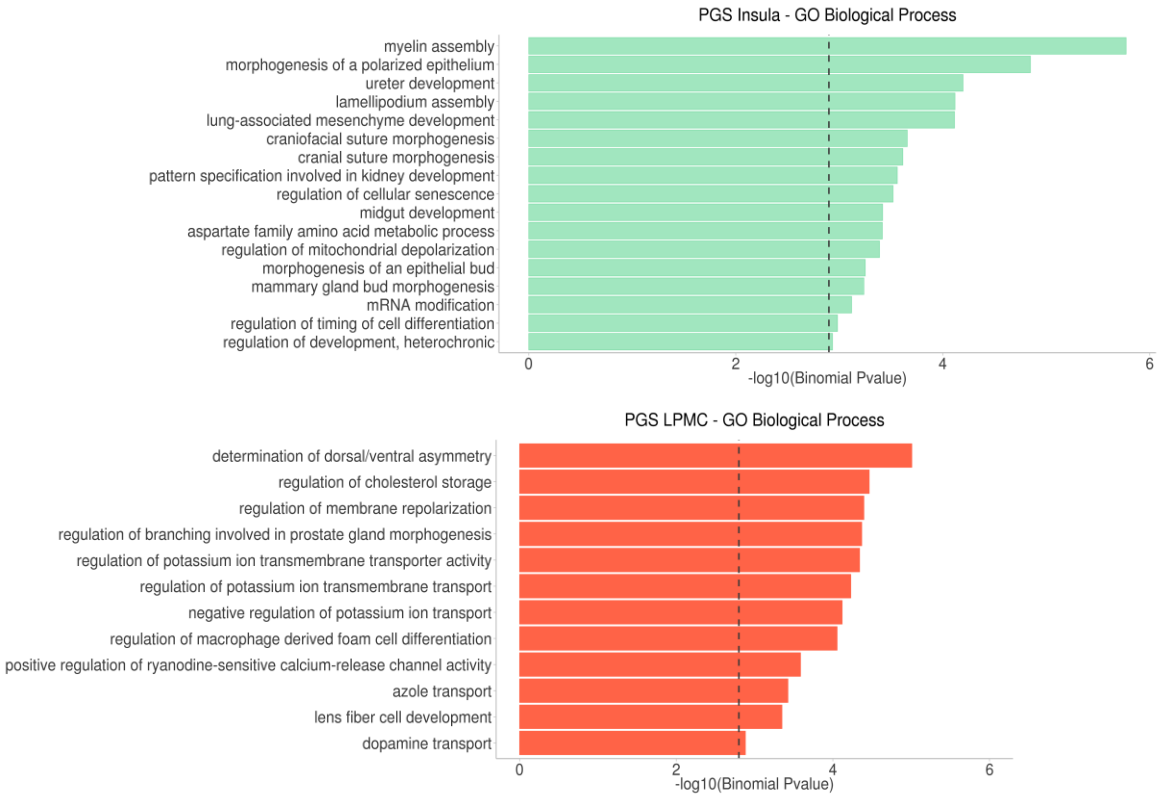
Supplementary Figure 2. HC-PGS barplots of most significant pT stratified by early life stress (ELS) in (a) ELS (b) no ELS COMED subsamples, (c) ELS and (d) no ELS PReDICT subsample. No pT was significant at P < 0.004



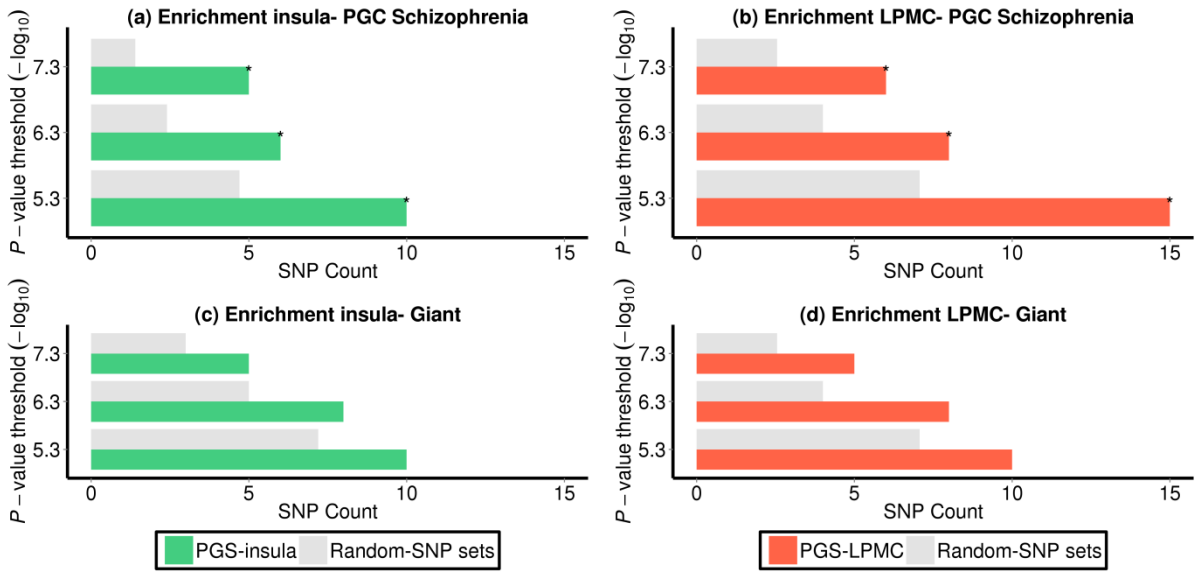
Supplementary Figure 3. QQ plots for genome-wide association analysis in FDG-PET sample. Inflation factor lambda shows no indication of population stratification



Supplementary Figure 4. Interaction plot of mean Insula Z score transformed-PGS (a) and mean LPMC Z score transformed-PGS (b) stratified by outcome group and treatment in remitters and non-remitters for Caucasians only (N = 70)



Supplementary Figure 5. Gene ontology enrichment for Insula-based PGS and LPMC-based PGS. Dashed line represents significant binomial FDR-q value after correction for multiple testing



Supplementary Figure 6. Enrichment of PGS-Insula and PGS-LPMC with PGC Schizophrenia and Giant SNP variants

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Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt worden ist. Die vorgelegte Dissertation wurde weder ganz, noch in wesentlichen Teilen bei einer anderen Prüfungskommission vorgelegt.

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_____ München, den 30. März 2016

Tania Carrillo Roa