DNA Methylation Signatures in Panic Disorder

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List of Abbreviations

AD	Anxiety disorders
APA	American Psychiatric Association
CD4T	CD4 T cells
CD8.naive	Naive CD8 T cells
CD8pCD28nCD45Ran	Differentiated (Memory and Effector) CD8 T cells
CGIs	CpG Islands
DEX	Dexamethasone
DHS	DNase I hypersensitive site
DNAmAge	DNA Methylation Age
DNMTs	DNA Methyltransferases
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders Fourth Edition
ECG	Electrocardiography
EEG	Electroencephalography
ESCs	Embryonic Stem Cells
EWAS	Epigenome-wide Association Study
FDR	False Discovery Rate
FunNorm	Functional Normalization
GxE	Gene by Environment
Gran	Granulocites
GWAS	Genome-wide Association Study
M-CIDI	Munich version of the Composite International Diagnostic Interview
MDD	Major Depressive Disorder
Mono	Monocytes
MPIP	Max Planck Institute of Psychiatry
NK	Natural Killer cells
NS	Not Significant
OR	Odds Ratio

PCA	Principal Component Analysis
PDA	Panic Disorder with Agoraphobia
PD	Panic Disorder without agoraphobia
PlasmaBlast	Plasmablast cells
PTSD	Post-Traumatic Stress Disorder
QC	Quality Control
QQ plot	Quantile-Quantile plot
SCID	Structured Clinical Interviews for DSM-IV
SNP	Single Nucleotide Polymorphism
SVA	Surrogate Variable Analysis
TSSs	Transcription Start Sites
UTR	Untranslated Region

List of Gene Symbols

ADCYAP1	Adenylate Cyclase Activating Polypeptide 1
ADCYAP1R1	ADCYAP1 Receptor Type I
ASB1	Ankyrin Repeat And SOCS Box Containing 1
BDNF	Brain Derived Neurotrophic Factor
COMT	Catechol-O-Methyltransferase
CRH	Corticotropin Releasing Hormone
CRHR1	Corticotropin Releasing Hormone Receptor 1
FHIT	Fragile Histidine Triad
FKBP5	FK506 Binding Protein 5
GAD1	Glutamate Decarboxylase 1
HECA	Hdc Homolog, Cell Cycle Regulator
HTR1A	5-Hydroxytryptamine Receptor 1A
HTR2A	5-Hydroxytryptamine Receptor 2A
MAOA	Monoamine Oxidase B
NPSR1	Neuropeptide S Receptor 1
OXTR	Oxytocin Receptor
SGK1	Serum/Glucocorticoid Regulated Kinase 1
SLC6A4	Solute Carrier Familiy 6 Member 4
TMEM132D	Transmembrane Protein 132D
TMEM16B	Transmembrane Protein 16B
PKP1	Plakophilin 1

Abstract

Panic Disorder (PD) affects about 7.9 million Europeans, with women affected twice as likely as men, causing substantial suffering and high economic costs. The etiopathogenesis of PD remains largely unknown, but both genetic and environmental factors contribute to risk. PD constitutes a strong psychological stressor, and it is therefore an important risk factor for accelerated aging. It is known that environmental factors can influence DNA methylation, and epigenetic regulation of genomic functions has been related to increased susceptibility for psychiatric disorders like PD. Therefore, an Epigenome-Wide Association Study (EWAS) was conducted in the MPIP Panic Cohort I to compare medication-free PD patients (n=89) with healthy controls (n=76) stratified by sex. Replication was sought in an independent sample (MPIP Panic Cohort II) consisting of 131 cases and 169 controls, and functional analyses were conducted in a third sample (MPIP Dexamethasone Treatment Study, N=71). DNA methylation was assessed in whole blood using the Infinium HumanMethylation450 BeadChip and epigenetic age was calculated with the Horvath DNA methylation-based predictor of aging. One genome-wide association surviving FDR of 5% (cg07308824, P=1.094 x 10-7, P-adj=0.046) was identified in female PD patients (N=49) compared to controls (N=48). The same locus, located in an enhancer region of the *HECA* gene, was also hypermethylated in female PD patients in the replication sample (P=0.035) and the significance of the association improved in the meta-analysis (P-adj=0.004). Methylation at this CpG site was associated with *HECA* mRNA expression in another independent female sample (N=71) both at baseline (P=0.046) and after induction by dexamethasone (P=0.029). 5 of 15 candidates previously reported as associated with PD or anxiety traits also showed differences in DNA methylation after gene-wise correction and included *SGK1, FHIT, ADCYAP1, HTR1A, HTR2A*. Epigenetic age was accelerated in PD patients with agoraphobia of the MPIP Panic Cohort II compared to PD patients without, and effects were stronger in females. Our study examines epigenome-wide differences in PD

patients and epigenetic age acceleration in peripheral blood for PD. Our results point to possible sex-specific methylation changes in the *HECA* gene for PD and suggest age acceleration in PD patients with agoraphobia but overall highlight that this disorder is not associated with extensive changes in DNA methylation in peripheral blood.

1. Introduction

Anxiety is a normal reaction to stress and can even be beneficial in some situations. Anxiety disorders differ from normal feelings of nervousness or anxiousness, often stress-induced, by being persistent (e.g. typically lasting 6 months or more) and involving excessive fear or anxiety that can interfere with the ability of leading a normal life (American Psychiatric Association, 2013). Anxiety disorders are the most common type of psychiatric disorders and affect nearly 30 percent of adults (Kessler et al., 2007; Craske and Stein, 2016). Despite the high prevalence rates, anxiety disorders are often under-recognized and under-treated. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (American Psychiatric Association, 2013), anxiety disorders include:

1) Panic Disorder (PD)
2) Agoraphobia
3) Separation Anxiety Disorder
4) Social Anxiety Disorder (Social Phobia)
5) Specific Phobia
6) Selective Mutism
7) Generalized Anxiety Disorder (GAD)

Anxiety disorders tend to be highly comorbid with each other, but they can be differentiated by close examinations since they differ from one another in the types of objects or situations that induce fear, anxiety or avoidance behaviour, and the associated cognitive ideation (American Psychiatric Association, 2013).
Many of the anxiety disorders develop in childhood, tend to persist if not treated, and occur more frequently in females than in males (approximately 2:1 ratio) (American Psychiatric Association, 2013). Anxiety disorders represent a heterogeneous group of disorders, probably with no single unifying etiology.

1.1. Panic Disorder

Panic Disorder (PD) is the most disabling anxiety disorder, causing substantial suffering and high economic and social costs. It affects about 7.9 million Europeans with women being twice as likely to be affected as men (Wittchen et al., 2011). PD is characterized by sudden episodes of acute anxiety (panic attacks) occurring without any apparent reason. It can be accompanied by a persistent concern of having additional attacks or worry about the possible consequences of the attacks (e.g. suffering of a heart attack, dying, losing control) and significant behavioural changes to avoid future panic attacks (Table 1) (Goodwin et al., 2005) . First onset for PD is in adolescence and early adulthood and it is highly comorbid with other mental disorders, especially agoraphobia (Noyes et al., 1986). Despite the substantial long-term disability, PD appears to be under-diagnosed and under-treated in mental health settings. According to the DSM-5 "Panic disorder is associated with high levels of social, occupational, and physical disability, considerable economic costs, and the highest number of medical visits among the anxiety disorders, although the effects are strongest with the presence of agoraphobia. Panic attacks and a diagnosis of panic disorder in the past 12 months are related to a higher rate of suicide attempts and suicidal ideation even when comorbidity and a history of childhood abuse and other suicide risk factors are taken into account" (American Psychiatric Association, 2013). Early to middle 20s is the typical mean age of onset and a small number of cases begin in childhood. Onset after age 45 years is unusual but can occur (American Psychiatric Association, 2013).

Table 1. Diagnostic criteria for panic disorder. The table is based on DSM-5 (American Psychiatric Association, 2013).

A.	**Recurrent unexpected panic attacks** A panic attack is an abrupt surge of intense fear or intense discomfort that reaches a peak within minutes, and during which time four (or more) of the following symptoms occur:
1.	Palpitations, pounding heart, or accelerated heart rate
2.	Sweating
3.	Trembling or shaking
4.	Sensations of shortness of breath or smothering
5.	Feelings of choking
6.	Chest pain or discomfort
7.	Nausea or abdominal distress
8.	Feeling dizzy, unsteady, light-headed, or faint
9.	Chills or heat sensations
10.	Paresthesias (numbness or tingling sensations)
11.	Derealization (feelings of unreality) or depersonalization (being detached from oneself)
12.	Fear of losing control or „going crazy“
13.	Fear of dying
B.	**At least one of the attacks has been followed by 1 month (or more) of one or both of the following:**
1.	Persistent concern or worry about additional panic attacks or their consequences (e.g. losing control, having a heart attack, “going crazy”)
2.	A significant maladaptive change in behavior related to the attacks (e.g. behaviors designed to avoid having panic attacks, such as avoidance of exercise or unfamiliar situations)
C.	**The disturbance is not attributable to the physiological effects of a substance** (e.g. a drug of abuse, a medication) **or another medical condition** (e.g. hyperthyroidism, cardiopulmonary disorders)
D.	**The disturbance is not better explained by another mental disorder** (e.g. the panic attacks do not occur only in response to feared social situations, as in social anxiety disorder; in response to circumscribed phobic objects or situations, as in specific phobia; in response to obsessions, as in obsessive-compulsive disorder; in response to reminders of traumatic events, as in posttraumatic stress disorder; or in response to separation from attachment figures, as in separation anxiety disorder)

1.1.1. Risk and Prognostic Factors

The APA classifies the risk factors that can influence the onset of panic disorder in three main categories and describes them as follows (American Psychiatric Association, 2013):

1. *Temperamental:* negative affectivity (neuroticism) (i.e., proneness to experiencing negative emotions) and anxiety sensitivity (i.e., the disposition to believe that symptoms of anxiety are harmful) are risk factors for the onset of panic attacks.
2. *Environmental:* sexual and physical abuse during childhood are more common in PD compared to other anxiety disorders. Smoking represents also a risk factor for panic attacks and panic disorder.
3. *Genetic and physiological:* It is believed that multiple genes confer vulnerability to panic disorder. However, the exact genes, gene products, or functions related to the genetic regions implicated remain unknown. Current neural systems models for panic disorder emphasize the amygdala and related structures, much as in other anxiety disorders. There is an increased risk for panic disorder among offspring of parents with anxiety, depressive, and bipolar disorders. Respiratory disturbance, such as asthma, is associated with panic disorder, in terms of past history, comorbidity, and family history (American Psychiatric Association, 2013).

1.1.2. Sex-Related Diagnostic Issues

Relevant differences in the clinical features between males and females are not described so far. However, there is some evidence for sexual dimorphism, with an association between panic disorder and the catechol-O-methyltransferase (*COMT*) gene in females only (American Psychiatric Association, 2013).

1.2. Agoraphobia

The essential feature of agoraphobia is anxiety about being in places or situations from which escape might be difficult (or embarrassing) or in which help may not be available in the event of having a panic attack or panic-like symptoms (School of Health and Related Research (ScHARR), 2004). This leads to a pervasive avoidance of a variety of situations that may include: using public transportation, being in open spaces, being in enclosed places, standing in line or being in a crowd, being outside of the home alone in other situations (American Psychiatric Association, 2013). In most severe forms, agoraphobia can cause individuals to become completely home-bound, unable to leave their home and dependent on others for services or assistance to provide even for basic needs (American Psychiatric Association, 2013).

Agoraphobia is diagnosed irrespectively of the presence of panic disorder. The percentage of individuals with agoraphobia reporting panic attacks or panic disorder preceding the onset of agoraphobia ranges from 30% in community samples to more than 50% in clinical samples (American Psychiatric Association, 2013). The majority of individuals with panic disorder show signs of anxiety and agoraphobia before the onset of panic disorder. Every year approximately 1.7% of adolescents and adults have a diagnosis of agoraphobia. Females are twice as likely as males to be affected. Agoraphobia may occur in childhood, but incidence peaks in late adolescence and early adulthood. In two-thirds of all cases of agoraphobia, initial onset is before 35 years. Heritability for agoraphobia ranges up to 61% (American Psychiatric Association, 2013). Negative events in childhood (e.g. separation, death of parent) and other stressful events, such as being attacked or mugged, are associated with the onset of agoraphobia. The majority of individuals with agoraphobia also have other mental disorders, most frequently other anxiety disorders (e.g. specific phobias, panic disorder, and social anxiety disorder), depressive disorders (MDD), PTSD, and alcohol use disorder. Whereas other anxiety disorders (e.g. panic disorder) frequently precede onset of agoraphobia, depressive disorders and substance use disorders

typically occur secondary to agoraphobia (American Psychiatric Association, 2013).

1.3. Pathophysiology of Anxiety Disorders

Two are the most acknowledged contributors to psychopathology: genetic vulnerability and environmental stressors.

Several approaches have been used to define the genetic contribution to psychiatric disorders like anxiety disorders, including family studies, twin studies, linkage studies, association studies, GxE studies, molecular, cellular and clinical studies (Figure 1).

1.3.1. Genetic Risk Factors

1.3.1.1. Family and Twin Studies

Family and twin studies provided consistent evidence that panic disorder is familiar and heritable. The overall heritability of PD is substantial with heritability estimates up to 48% (Hettema et al., 2001).

1.3.1.2. Linkage Studies

Having established that genetic factors influence PD, many linkage studies have been performed to map the relevant loci. Linkage analysis have implicated several chromosomal regions (Smoller et al., 2008) and the strongest evidence was found for the 13q locus when the phenotype was defined as a syndrome that included PD as well as several other medical conditions (mitral valve prolapse, serious headaches, and/or thyroid problems) (Weissman et al., 2000; Hamilton et al., 2003).

In the largest analysis to date, Fyer et al. reported genome-wide significant linkage to 15q using a broad panic phenotype that included sporadic and limited symptom panic attacks in addition to PD (Fyer et al., 2006). However, there has been little consistency in linkage scans for PD (Smoller et al., 2008).

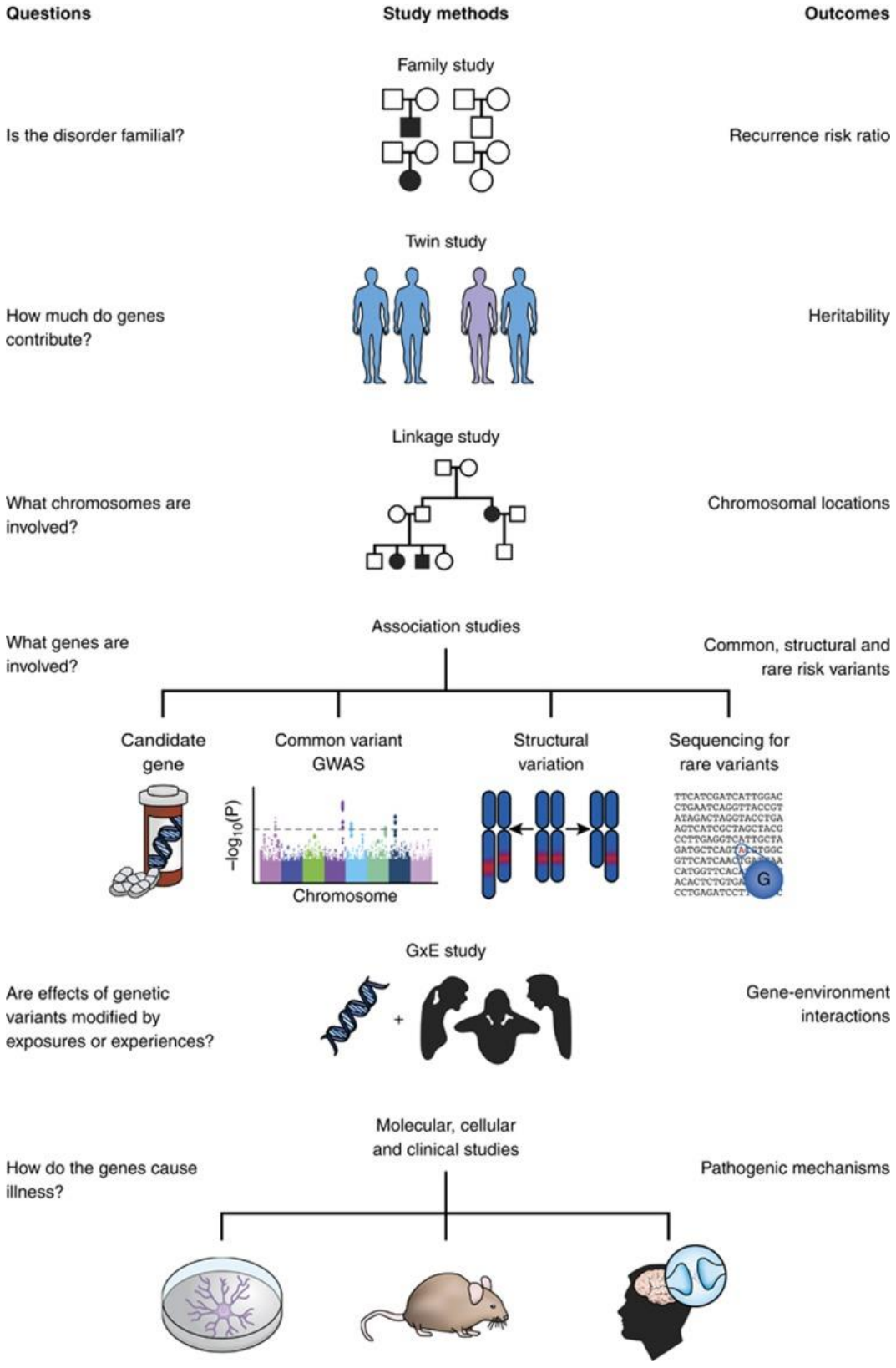


Figure 1. Summary of psychiatric genetics methods. Source: Smoller (2016)

1.3.1.3. Genome-Wide Genetic Associations

Genome-wide association studies (GWAS) enable a so-called “unbiased” search for risk loci by examining variants across the genome instead of limiting the search to hypothesized candidates (as in candidate gene studies) (Smoller, 2016).

The first GWAS of PD was published in 2009 (Otowa et al., 2009), included 200 cases and 200 controls of the Japanese population and reported significant variants in *TMEM16B* and *PKP1*, but these findings could not be replicated by the same investigators in a larger independent sample (Otowa et al., 2010; Smoller, 2016). However, more robust support has emerged for transmembrane protein 132D (*TMEM132D*) in three independent samples consisting of a total of 909 cases and 915 controls. Risk genotypes identified in this study were associated with higher TMEM132D mRNA expression in human post-mortem frontal cortex. These results were further supported by a mouse model in which high anxiety-related behaviour was associated with a TMEM132D SNP and correlated with expression of TMEM132D mRNA in the anterior cingulate cortex (Erhardt et al., 2011). It has been suggested that this gene has a role in threat processing, but its function is not fully understood (Haaker et al., 2014; Smoller, 2016).
This study had an adequate power to detect the reported effect size of OR 1.4 in the combined sample of 909 cases, even if the size of the discovery sample was modest. In complex psychiatric disorders genetic effect sizes of this magnitude are probably the exception, therefore more modest effects in other important genes might have been missed (Wellcome Trust Case Control Consortium, 2007; Baum et al., 2008; Erhardt et al., 2011). A summary of GWAS of anxiety disorders is reported in Table 2.

Table 2. GWAS of anxiety disorders. Source: Shimada-Sugimoto et al. (2015)

Reference	Phenotype	Dataset	Most significant finding	P-value	OR
(Otowa et al., 2009; Otowa et al., 2010)	PD	200 cases, 200 controls (GWAS); 558 cases, 566 controls (Replication)	*TMEM16B* (12p13)	$3.73x10^{-9}$ (GWAS); NS (Replication)	22.1
(Erhardt et al., 2011)	PD	216 cases, 222 controls (GWAS); 693 cases, 693 controls (Replication)	*TMEM132D* (12q24)	7.73×10^{-7} (GWAS); 1.36×10^{-6} (all samples)	2.2
(Otowa et al., 2012)	PD	718 cases, 1717 controls (GWAS); 329 cases, 861 controls (Replication)	*BDRKB2* (14q32)	4.43×10^{-6} (GWAS); 1.32×10^{-5} (all samples)	1.31
(Kawamura et al., 2011)	PD	535 cases, 1520 controls (genome-wide copy number variation analysis)	Common duplication (16p11.2)	3.5×10^{-6}	2.35
(Trzaskowski et al., 2013)	Anxiety-related behaviours	2810 7-year-old children (GWAS); 4804 children (Replication)	*STXBP6, NOVA1* (14q12) (Negative Cognition); *CAP2* (6p22.3) (Anxiety Composite)	4.12×10^{-7} (Negative Cognition); 6.27×10^{-7} (Anxiety Composite)	-
(Schosser et al., 2013; Otowa et al., 2014)	Anxiety in MDD	1522 MDD (1080 with anxiety) cases, 1588 controls	*DSCAM* (21q22.2)	3.27×10^{-7}	1.53
(Otowa et al., 2014)	GAD, PD, agoraphobia, social phobia, specific phobia	2540 European Americans, 849 African Americans	*MFAP3L* (4q32.3)	8.63×10^{-7}	-

1.3.2. Environmental Risk Factors

Psychiatric disorders are multifactorial diseases that emerge through the interplay between environmental factors and genetic predisposition. The aim of gene-by-environment interaction (GxE) studies is exactly to determine the extent to which genetic predisposition in combination with environmental determinants shapes the risk for psychiatric disorders (Halldorsdottir and Binder, 2017).
Different hypothesis have been developed over the years to explain how genes and environment contribute to disease risk. According to the "diathesis-stress" hypothesis, genes and adversity, independently and in combination, increase the liability to disorder (Smoller, 2016). This model however focuses only on negative environmental influences. An alternative model, known as the differential-susceptibility perspective, has been proposed by Belsky and colleagues (Belsky et al., 2007; Belsky and Pluess, 2009). According to the latter, no genotype is inherently good or bad, but individuals vary in their susceptibility to both negative and positive environmental influences.

GxE studies available so far have focused on a small number of predominantly functional candidate markers in a limited number of genes and no genome-wide search for GxE in common psychiatric disorders, including anxiety disorders (AD), has been conducted (Shimada-Sugimoto et al., 2015). The lack of such studies in this field is due to the need of larger samples and of a more precise definition of "environmental" or "candidate stressors" (Klauke et al., 2010), which are very important factors for the design of future genome-wide GxE studies in AD.

Epidemiologic and twin studies (Kendler et al., 2016; South et al., 2016; Torvik et al., 2016) support the importance of adverse events occurring early in life in increasing the risk for psychiatric disorders, including mood and anxiety disorders but also psychoses and personality disorders (Kessler et al., 2010; Binder, 2017). Adverse life events have been shown to associate with specific epigenetic modifications, such as DNA methylation, which may mediate the

lasting cellular consequences of these exposures in psychiatric disorders (Slatkin, 2009), also in the context of GxE (Klengel and Binder, 2015).

1.4. Epigenetics

The word “epigenetic” literally means “in addition to changes in genetic sequence”. The term has evolved to include any process that alters gene activity without changing the DNA sequence, and leads to modifications that can be transmitted to daughter cells (although experiments show that some epigenetic changes can be reversed) (Weinhold, 2006). Epigenetic modifications are heritable changes in gene expression not encoded by the DNA sequence. They include DNA methylation, the histone code, noncoding RNA, and nucleosome positioning, along with DNA sequence. Epigenetic processes are natural and essential to many organism functions, but if they occur improperly, there can be major adverse health and behavioural effects.

DNA methylation, catalysed by the DNA methyltransferases (DNMTs), is considered a key player in epigenetic silencing of transcription and is one of the most studied epigenetic modifications in human cells. Changes in DNA methylation patterns play a critical role in development, differentiation and diseases such as multiple sclerosis, diabetes, schizophrenia, aging, and multiple forms of cancer (Bibikova et al., 2011).
DNA methylation may regulate the chromatin status via the interaction of DNMTs together with other modifications and with components of the machinery mediating those marks (Jin et al., 2011).

1.4.1. DNA Methylation

DNA methylation is a heritable epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring (Figure 2) of DNA by DNA methyltransferases (DNMTs) (Robertson, 2005).
In mammals, DNA methylation occurs at cytosines in any context of the genome (Lister et al., 2009). However, more than 98% of DNA methylation occurs in a

CpG dinucleotide context in somatic cells, while as much as a quarter of all methylation appears in a non-CpG context in embryonic stem cells (ESCs) (Lister et al., 2009). DNA methylation is typically removed during zygote formation and then re-established in the embryo at approximately the time of implantation (Zhu, 2009). Most DNA methylation is essential for normal development, and it plays a very important role in a number of key processes including genomic imprinting, X-chromosome inactivation, and suppression of repetitive element transcription and transposition and, when dysregulated, contributes to diseases like cancer (Robertson, 2005; Gopalakrishnan et al., 2008; Jin et al., 2008; Jin et al., 2009).

The methylation pattern in mammalian genomes is bimodal, with most of the genomes methylated except for short DNA stretches called CpG islands (CGIs), which are generally protected from methylation (Siegfried and Simon, 2010).

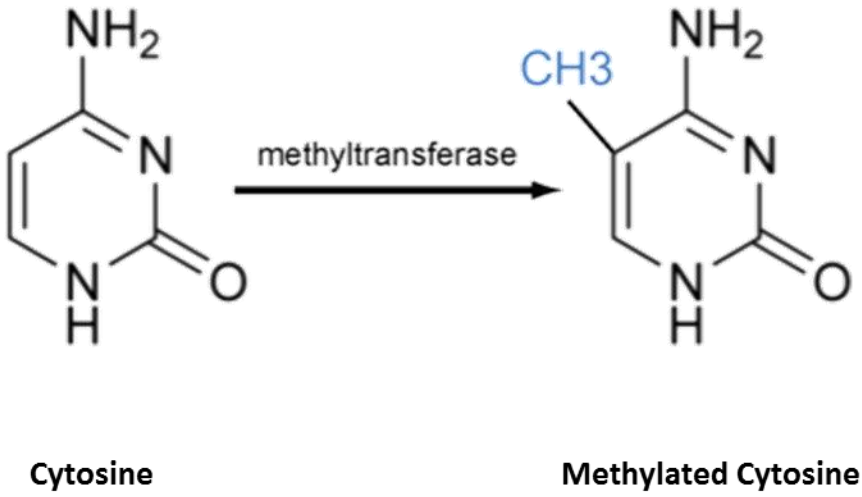


Figure 2. DNA methylation
Modified from: http://www.ks.uiuc.edu/Research/methylation/

1.4.2. CpG Islands

CpG islands were first described 30 years ago (Bird, 1986). In mammals CpG dinucleotides are under-represented in the genome with the exception of short DNA stretches called CGIs. This uneven distribution is also associated with a bimodal pattern of cytosine methylation (almost all CpG dinucleotides are methylated with the general exception of CGIs). The higher CpG density in the unmethylated CGIs is probably due to the lower mutation rate of unmethylated cytocines. Deamination of cytosines in methylated CpG dinucleotides produces thymine, which is stable, whereas deamination of unmethylated cytosine produces uracil, which can be removed by uracil glycosylase. The consequence is that unmethylated regions maintain their CpG density whereas methylated regions lose their CpG density due to mutations. Indeed, CpGs located within CGIs are more highly conserved between human and chimpanzee. Moreover, even among CGIs, methylated islands have diverged at faster rates than the nonmethylated CGIs (Siegfried and Simon, 2010).

DNA methylation across CpG sites in the genome is typically regarded as bimodal, with CpG-rich regions known as CpG islands, often associated with transcription start sites (TSSs), typically showing hypomethylation, and other CpG sites showing hypermethylation (Wagner et al., 2014).

1.4.3. DNA Methylation and Gene Expression

The relationship between methylation and gene expression is complex, with high levels of gene expression often associated with low promoter methylation (Kass et al., 1997) (Figure 3) but elevated gene body methylation (Jones, 1999), and the causality relationships have not yet been determined (Wagner et al., 2014).
Methylation has been shown to be highly variable across cell types with variable sites falling in two broad categories: those with inverse correlation between DNA methylation and chromatin accessibility and constitutive DNA hypomethylation (Thurman et al., 2012). In the study of (Wagner et al., 2014), CpG probes where methylation levels correlated negatively with gene expression were for the most

part located in regions with marks of regulatory activity (H3K4me3 or DHS). In contrast, positively correlated probes were slightly more often seen with the inactive gene-associated marker H3K27me3 when compared with negatively correlated probes (Wagner et al., 2014).

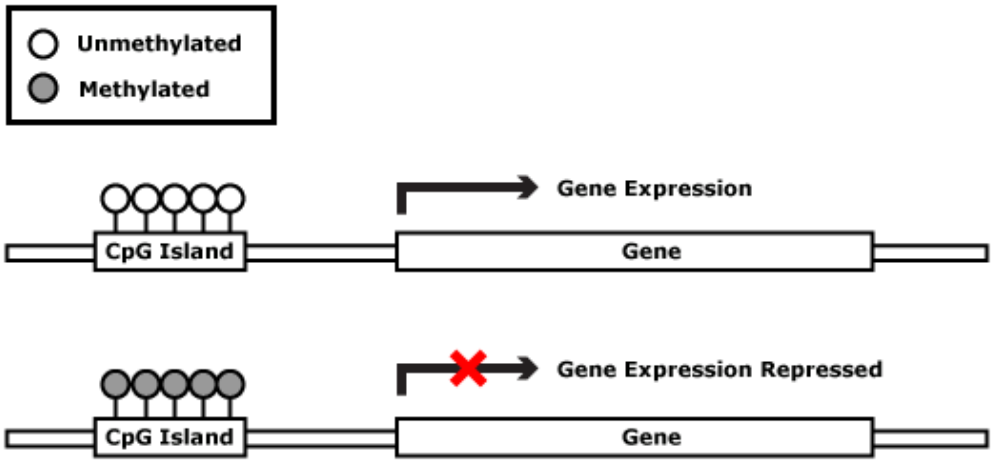


Figure 3. Consequences of DNA methylation on gene expression
Source: http://missinglink.ucsf.edu/lm/genes_and_genomes/methylation.html

1.4.4. Epigenome-Wide Association Studies

Although the genome gives information about genome sequence and structure, the human epigenome provides functional aspects of the genome. Epigenome-wide association studies (EWAS) provide an opportunity to identify genome-wide epigenetic variants that could be associated with human phenotypes (Verma, 2012; Flanagan, 2015). The epigenome is especially intriguing as a target for study, as epigenetic regulatory processes are, by definition, heritable from parent to daughter cells and are found to have transcriptional regulatory properties. As such, the epigenome is an attractive candidate for mediating long-term responses to cellular stimuli, such as environmental effects modifying disease risk. When a pattern of changes of DNA methylation is found to occur

repeatedly at specific loci, discriminating the phenotypically affected cases from control individuals, this is regarded as an indication that epigenetic perturbation has taken place that is associated, possibly causally, with the phenotype. This approach is described as an epigenome-wide association study (EWAS) (Rakyan et al., 2011), and takes its cue from the association of genetic variability with phenotypes in genome-wide association studies (GWAS) (Birney et al., 2016).

1.5. Technical Background

Epigenetic variation can contribute to the development of a disease or be a consequence of it (also known as reverse causality). Distinguishing between the two processes presents a major challenge for EWASs (Paul and Beck, 2014).
Array-based assays have been widely adopted to study DNA methylation owing to their low costs, ease of use and high throughput. The Illumina Infinium 450K BeadChips are among such assays and have been the platform of choice for epigenome-wide association studies. They can quantify CpG and a very small fraction of non-CpG methylation at single-base resolution (Plongthongkum et al., 2014).

1.5.1. Illumina 450K Array Design

The Illumina 450K BeadChip interrogates more than 485,000 methylation sites per sample at single-nucleotide resolution and can analyse twelve samples in parallel. It covers 99% of RefSeq genes, with an average of 17 CpG sites per gene region distributed across the promoter, 5´ UTR, first exon, gene body, and 3´ UTR. It covers 96% of CpG islands, with additional coverage in island shores and the regions flanking them (Figure 4). Each sample is measured on a single array, in two different color channels (red and green) (Figure 5). For each CpG, there are two measurements: a methylated intensity and an unmethylated intensity. Depending on the probe design, the signals are reported in different colors:

- for Type I design, both signals are measured in the same color: one probe for the methylated signal and one probe for the unmethylated signal.

- for Type II design, only one probe is used. The Green intensity measures the methylated signal, and the Red intensity measures the unmethylated signal (Bibikova et al., 2011).

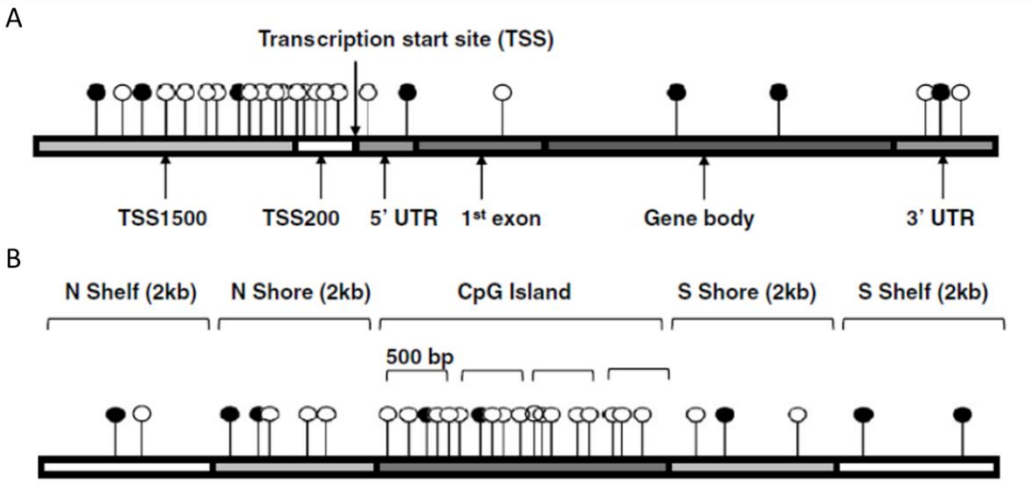


Figure 4. Illumina Infinium 450K BeadChip probe distribution. Source: (Bibikova et al., 2011)
4A. Coverage of NM and NR transcripts from UCSC database.
Each transcript was divided into "functional regions" — TSS200 is the region from Transcription start site (TSS) to − 200 nt upstream of TSS; TSS1500 covers − 200 to − 1500 nt upstream of TSS; 5′ UTR, 1st exon, gene body and 3′ UTR were also covered separately.
4B. Coverage of CpG islands and adjacent regions.
CpG islands longer than 500 bp were divided into separate bins. The 2 kb regions immediately upstream and downstream of the CpG island boundaries, or "CpG island shores", and the 2 kb regions upstream and downstream of the CpG island shores, referred to here as "CpG island shelves," were also targeted separately.

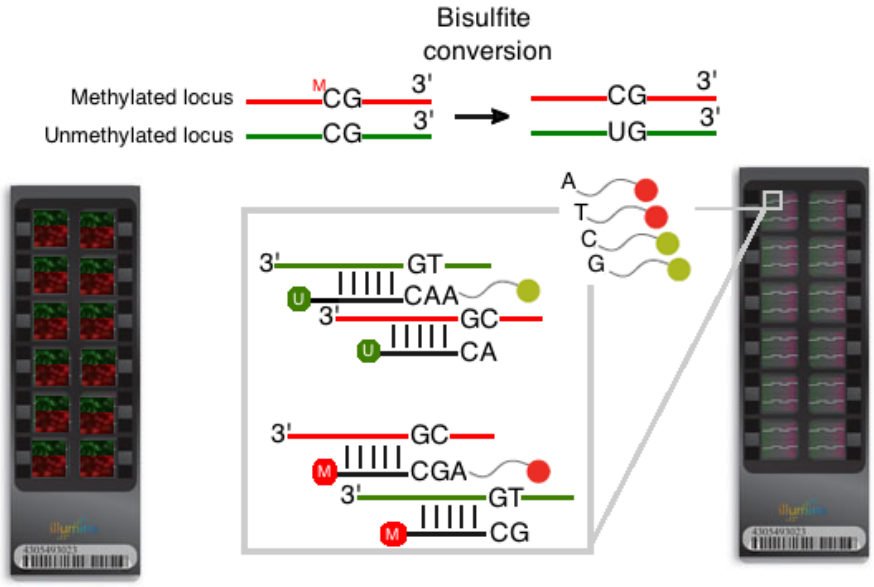


Figure 5. Illumina Infinium 450K BeadChip principle. Modified from: https://www.illumina.com/products/methylation_450_beadchip_kits.html

Two measures are commonly used to report methylation levels: β-values and M-values.

β-value:

$$\beta = \frac{M}{M + U + \alpha}$$

where *M* and *U* denote the methylated and unmethylated signals respectively; α is a constant (by default, α=100) that regularizes the β-value when both methylated and unmethylated probe intensities are low.

The β-value statistic results in a number between 0 and 1, or 0 and 100%. Under ideal conditions, a value of zero indicates that all copies of the CpG site in the sample were completely unmethylated (no methylated molecules were

measured) and a value of one indicates that every copy of the site was methylated (Du et al., 2010).

M-value:

$$Mval = log\left(\frac{M}{U}\right)$$

The M-value is calculated as the log ratio of the intensities of methylated probe versus unmethylated probe.
An M-value close to 0 indicates a similar intensity between the methylated and unmethylated probes, which means the CpG site is about half-methylated. Positive M-values mean that more molecules are methylated than unmethylated, while negative M-values mean the opposite (Du et al., 2010).

The β-value has a more intuitive biological interpretation, the M-value is on the other hand more statistically valid for the differential analysis of methylation levels (Du et al., 2010). In this thesis, M-values were used for differential methylation analysis and β-values to show the results.

1.6. DNA Methylation Age (Epigenetic Clock)

DNA methylation changes during physiological processes like aging, but also during disease status, e.g. aging associated diseases (Bjornsson et al., 2008; Christensen et al., 2009; Rakyan et al., 2010; Hernandez et al., 2011; Heyn et al., 2012; Horvath et al., 2012; Horvath, 2013).

Considering the unprecedented growth rate of the world´s aging population, there is a clear need for a better understanding of the biological aging process and the determinants of healthy aging (Jylhava et al., 2017). Towards this aim, several DNA methylation-based predictors of aging have been developed (Bocklandt et al., 2011; Hannum et al., 2013; Horvath, 2013; Weidner et al., 2014). Among these, a composite predictor comprised of 353 Cytosine-

phosphate-Guanosine sites (CpGs) across the genome ('epigenetic clock') was shown to strongly correlate with chronological age across multiple tissues (r=0.96) in humans (Horvath, 2013) with a small mean deviation from calendar age (3.6 years), suggesting its usefulness as a biomarker in aging-related research (Zannas et al., 2015). The algorithm behind the DNA methylation age calculation uses a penalized regression model to predict the CpG sites and has been developed in 8000 samples, covering the entire adult life span and different ethnic populations.

Accelerated epigenetic aging (Δ-age) calculated using this predictor, defined as the difference between DNA methylation-predicted age (DNAmAge) and chronological age, has been associated with aging-related diseases and other phenotypes, including cancer, obesity, cytomegalovirus infection, Down's syndrome, PTSD, physical and cognitive decline, all-cause mortality, the presence of higher self-control, lower socioeconomic status, and lifetime stress (Horvath, 2013; Horvath et al., 2014; Boks et al., 2015; Kananen et al., 2015; Marioni et al., 2015b; Marioni et al., 2015a; Miller et al., 2015; Zannas et al., 2015). The correlation with all-cause mortality was confirmed in a study that included 13 cohorts for a total of 13,098 individuals from three ethnic groups (Chen et al., 2016). Moreover, epigenetic estimates that incorporated information on blood cell composition led to the smallest p-values for time to death (p=7.5 x 10^{-43}). The latter study could strengthen the evidence that epigenetic age predicts all-cause mortality above and beyond chronological age and traditional risk factors. This suggests that the epigenetic age of blood tissue is one of the mediating processes of chronological age on mortality and that this is independent of age-dependent changes in blood cell composition.

2. Aims

Exploring DNA methylation might give us an integrated view of both environmental and genetic risk factors. While epigenetic changes including DNA methylation are mainly tissue specific, some sites show cross tissue relevance (Farre et al., 2015; Hannon et al., 2015) and furthermore changes in peripheral tissues such as blood could serve as potential biomarker for disease risk.

To date, a few studies have investigated differences in DNA methylation in candidate genes in PD (Bayles et al., 2013; Domschke et al., 2013; Prelog et al., 2016; Ziegler et al., 2016), but genome-wide analysis of the peripheral blood methylome of PD patients as compared to controls are currently lacking. To perform such a study in two independent samples of patients with PD vs control was the aim of the study. To reduce confounding due to effects of drug treatment, both patients and controls were free of psychotropic medication. Given that both the prevalence of PD as well as DNA methylation pattern show large sex differences (Yousefi et al., 2015), a sex-stratified analysis was undertaken and complemented by a meta-analysis.

Previous studies report that hits identified in genome-wide association studies (GWAS) show changes in DNA methylation in peripheral blood, e.g. in schizophrenia (Montano et al., 2016) or bipolar disorder (Houtepen et al., 2016). For this reason, in addition to an unbiased approach, I also investigated DNA methylation changes in candidate genes that have emerged from genome-wide genetic studies either in humans or animals (Erhardt et al., 2011; Knoll et al., 2016; Nieto et al., 2016).

Panic disorder is known to be a strong stressor and psychological stress is an important risk factor for accelerated aging and aging-related diseases. PD has a high comorbidity rate with other psychiatric disorders like agoraphobia and depression, and with other medical conditions e.g. cardiovascular disorders, asthma, epilepsy (American Psychiatric Association, 2013). Agoraphobia constitutes a stronger stressor for patients with PD, therefore PD patients with

comorbid agoraphobia are classified as more severely ill compared to PD patients without (American Psychiatric Association, 2013).

It has been proven that aging and aging-related diseases are associated with changes in DNA methylation (Bjornsson et al., 2008; Christensen et al., 2009; Rakyan et al., 2010; Hernandez et al., 2011; Heyn et al., 2012; Horvath, 2013), and was therefore used so far as a useful biomarker of aging-related research. Previous studies show indeed a correlation of DNA methylation age with morbidity and mortality (Horvath et al., 2015; Marioni et al., 2015b; Chen et al., 2016; Christiansen et al., 2016).

Given these previous findings, I wanted to investigate whether age acceleration is occurring in PD patients and if there is a difference among PD patients with agoraphobia and PD patients without. To answer these questions, I calculated epigenetic aging (Δ-age) using the Horvath DNA methylation-based predictor (Horvath, 2013).

3. Materials and Methods

3.1. Samples and Study Design

3.1.1. MPIP Panic Cohort I and II

PD patients included in the MPIP panic cohort I and II were recruited in the anxiety disorders outpatient unit at the Max Planck Institute of Psychiatry (MPIP) in Munich (Erhardt et al., 2011). PD was the primary diagnosis; mild secondary depression was allowed (Table 3). The diagnosis was ascertained by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders DSM-IV criteria. All patients underwent the Structured Clinical Interviews for DSM-IV (SCID I and II). PD due to a medical or neurological condition or the presence of a comorbid Axis II disorder was an exclusion criterion. All patients included in the current analyses were not taking any psychotropic medications for at least 4 weeks before the blood draw and underwent a thorough medical examination including EEG, ECG and detailed hormone laboratory assessment.

Control subjects were recruited from a Munich-based community sample and screened for the absence of axis I psychiatric disorders using the Munich version of the Composite International Diagnostic Interview (M-CIDI) (Wittchen, 1997). Controls were age- and sex-matched with patients.
All subjects were Caucasian and provided written informed consent. The Ethics Committee of the Ludwig Maximilians University, Munich, Germany, in accordance with the Declaration of Helsinki approved all procedures.

3.1.2. MPIP Dexamethasone Treatment Study

Glucocorticoid-induced methylation and gene expression changes were examined in an independent sample of 71 Caucasian female subjects (29 healthy probands and 42 depressed) recruited at the MPIP. Recruitment strategies and characterization of participants have been previously described (Arloth et al., 2015). Baseline whole blood samples were obtained at 6 p.m. after 2 hours of

fasting and abstention from coffee and physical activity (baseline). Subjects then received 1.5 mg oral dexamethasone (DEX) and a second blood draw was performed at 9 p.m. three hours after DEX ingestion (post-DEX). The study was approved by the local ethics committee and all individuals gave written informed consent.

Table 3. Characteristics of the participants included in the study

Variable	Controls	Cases	Total
MPIP Panic Cohort I			
Participants, N (%)	76 (46%)	89 (54%)	165
Male , N (%)	28 (17%)	40 (24%)	68 (41%)
Female, N (%)	48 (29%)	49 (30%)	97 (59%)
Age, years (SD)	37 (7.5)	36 (10.4)	
Diagnosis	None	PDA 72% PD 28% Comorbidity: MDD 13.5%	
MPIP Panic Cohort II			
Participants, N (%)	169 (56%)	131 (44%)	300
Male , N (%)	48 (16%)	48 (16%)	96 (32%)
Female, N (%)	121 (40%)	83 (28%)	204 (68%)
Age, years (SD)	38 (7.2)	38 (11.6)	
Diagnosis	None	PDA 61% PD 39% Comorbidity: MDD 13%	
MPIP Dexamethasone Treatment Study			
Participants, N (%)	29 (41%)	42 (59%)	71
Male , N (%)	0	0	0
Female, N (%)	29	42	71
Age, years (SD)	44 (11.4)	44 (13.7)	
Diagnosis	None	MDD	

3.2. Methylation Data

3.2.1. MPIP Panic Cohort I and II

Genomic DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen). DNA quality and quantity was assessed using NanoDrop 2000 Spectrophotometer (Thermo Scientific) and Quant-iT Picogreen (Invitrogen). To minimize batch effects, samples were randomized with respect to case-control status, sex and age.

Genomic DNA was bisulfite converted using the Zymo EZ-96 DNA Methylation Kit (Zymo Research) and DNA methylation levels were assessed for >480,000 CpG sites using the Illumina HumanMethylation450 BeadChip array. Hybridization and processing were performed according to the instructions of the manufacturer.

3.2.2. MPIP Dexamethasone Treatment Study

Genomic DNA was extracted from whole blood using the Gentra Puregene Blood Kit (QIAGEN) and processed as for the MPIP Panic cohorts. DNA methylation levels were assessed for >480,000 CpG sites using the Illumina HumanMethylation450 BeadChip arrays.

3.3. Quality Control of Methylation Data

3.3.1. General

The Bioconductor R package *minfi* (version 1.10.2) was used for the quality control of methylation data including intensity read outs, normalization, cell-type composition estimation, β- and M-value calculation. Outliers, i.e. samples whose behaviour deviated from that of others, were excluded from the analysis as well as samples with a discordant methylation-predicted vs reported sex (Supplementary Table S21).

Failed probes were excluded based on a detection p-value larger than 0.01 in >50% of the samples. X chromosome, Y chromosome, and non-specific binding probes were removed (Chen et al., 2013). I also excluded probes if single

nucleotide polymorphisms (SNPs) were documented in the interval for which the Illumina probe is designed to hybridize. Probes located close (10 bp from query site) to a SNP, which had a minor allele frequency of ≥0.05, as reported in the 1000 Genomes Project, were also removed. This yielded a total of around 425,000 CpG sites in the discovery and replication sample for further analysis.

The data were then normalized with functional normalization (FunNorm)(Fortin et al., 2014), an extension of quantile normalization included in the R package *minfi.* Batch effects were identified by inspecting the association of principal components of the methylation levels with possible technical batches using linear regressions and visual inspection of PCA plots using the Bioconductor R package *shinyMethyl* (version 0.99.3). Identified batch effects (i.e. bisulfite conversion plate and plate position) were removed using the Empirical Bayes' (EB) method *ComBat* (Johnson et al., 2007). Batch corrected M-values after *ComBat* were used for all further statistical analyses.

3.3.2. Biological and Non-Biological Confounders

There is a number of other additional factors that influence data analysis which are not related to the scientific question that I want to answer, but rather due to the methodology itself, that introduces unwanted variability to the data. These additional factors, defined as confounders, can be classified in two main categories:

1) Biological (or non-technical) confounders
2) Non-biological (or technical) confounders

3.3.2.1. Biological Confounders

Biological confounders include:

1. Cell subtype proportional heterogeneity (Houseman)

   There is a potential for cell subtype proportional heterogeneity to influence the DNA methylation patterns observed in pools of cells. This was highlighted by Houseman and colleagues in a study showing that

altering the proportions of purified cells in a mixture generates different DNA methylation profiles, reflecting the distinctive DNA methylation patterns of each cell type present (Houseman et al., 2012). It was subsequently shown that cell subtype effects accounted for a major proportion of the epigenetic changes associated with ageing in a re-analysis of five studies of peripheral blood leukocytes (Jaffe and Irizarry, 2014). These findings of the influence of cell subtype heterogeneity prompted the development of new analytical approaches to account for this effect (Houseman et al., 2012; Houseman et al., 2014). Even when cells are "purified" using cell surface markers, (Wijetunga et al., 2014) found evidence for further cell subtypes with distinctive DNA methylation patterns. It is, therefore, likely that even when using purification techniques, a pool of cells is composed of multiple epigenomes, generating what can be defined as "meta-epigenome" (Wijetunga et al., 2014).

2. Age, sex and race

   In our study design and during the selection process of the samples to be included in the study, I already took into account that there is a sex-bias in the incidence of the disease (occurring panic disorder twice more often in females compared to males) and a difference in terms of age. I also considered that usually the distribution of age and sex is different in patients compared to healthy controls, that is why I matched cases and controls according to age and sex. I applied the same principle also when performing the experiments, and designed it in order to have the same ratio of males/females and a balanced age distribution across the methylation chips.

For all these reasons, age, sex and Houseman-calculated cell count were always included as covariates in the regression models. As showed in the MDS plots (Supplementary Figure 1-2), all the individuals included in the study are Caucasians and there was no indication to include the PCs for ethnicity as covariates.

3.3.2.2. Non-Biological Confounders

„Batch effects“ are non-biological experimental variations commonly observed between multiple groups of samples (batches) in high-throughput experiments, e.g. microarray experiments, that can „confound“ the results by adding variation to the data, thus decreasing the statistical power to detect biological phenomena. For example, batch effects may occur if a subset of experiments was run on Monday and another set on Tuesday, if two technicians were responsible for different subsets of the experiments, or if two different lots of reagents, chips or instruments were used.

Batch effects can be reduced through data normalization and through a good experimental design, i.e. the study groups have to be equally represented throughout the experiment (in this case in the different chips used). However still high levels of systematic heterogeneity in the data often remains and it can obscure biological phenomena under study; for this reason, it is necessary to correct the data for batch effects.

There are two main ways to correct for batch effects: (1) directly removing known batch effects using ComBat and (2) identifying and estimating surrogate variables for unknown sources of variation in high-throughput experiments (Leek et al., 2010).

3.3.3. Batch Correction

3.3.3.1. ComBat

ComBat uses an empirical Bayes approach to estimate and to remove batch effects and it also avoids over-correcting, which is critical for the use with small batches. Location and scale parameters, representing mean and variance, are estimated for each batch and each gene independently and combined with empirical Bayes to remove batch effects (Johnson et al., 2007). ComBat has been successfully applied to several datasets (Walker et al., 2008; Chen et al., 2011; Luo et al., 2012; Chmielewski et al., 2014), and using a single reference sample for each batch, its usefulness has been demonstrated for cross-sectional data

(Walker et al., 2008). This method assumes that the batches are known (Muller et al., 2016).

3.3.3.2. Surrogate Variable Analysis (SVA)

In addition to the measured variable(s) of interest, there will tend to be sources of signal due to factors that are unknown, unmeasured, or too complicated to capture through simple models and this is true even for well-designed, randomized studies (Leek and Storey, 2007).

Surrogate variables are covariates constructed directly from high-dimensional data (like gene expression/RNA sequencing/methylation/brain imaging data) that can be used in subsequent analyses to overcome these problems by adjusting for unknown, unmodeled, or latent sources of noise (Jaffe et al., 2015).

Defining a precise biological question is a crucial step for genomics investigation in general, and for this type of analysis in particular. In performing SVA, effects specified in the model will be preserved while systematic heterogeneity is identified and subsequently adjusted for in subsequent statistical analysis. If our biological question is for example whether there is a difference between cases and controls, we can “protect for” case-control status and correct for the remaining variance. This approach has previously been shown to result in more accurate and stable gene rankings, improved false discovery estimation and correct p-value distributions (Leek and Storey, 2007, 2008; Leek et al., 2010).

3.4. Expression Data

3.4.1. MPIP Dexamethasone Treatment Study

Whole blood RNA was collected using PAXgene Blood RNA Tubes (PreAnalytiX), processed as described previously (Arloth et al., 2015). Blood RNA was hybridized to Illumina HumanHT-12 v3 and v4 Expression BeadChips arrays. All gene expression array probes have been subjected to an extensive quality control including filtering by low p-detection value, variance stabilization and normalization (VSN) (Lin et al., 2008) as previously described in (Zannas et al., 2015). Cellular composition was estimated by using CellCode (Chikina et al.,

2015).

3.5. Statistical Analysis

3.5.1. MPIP Panic Cohort I and II

3.5.1.1. Epigenome-Wide Association Study

Linear regression models were fit for each probe to test for a case vs control difference within the R package MatrixEQTL (version 2.1.1) (Shabalin, 2012). Sex, age and imputed white blood cell distribution from the Houseman projection (Houseman et al., 2012) were included as covariates. Population stratification was investigated using multidimensional scaling and could not be observed (Supplementary Figure 1-2). Significance after multiple testing was adjusted using false discovery rate (FDR) of 5%. As a first step all the samples of every cohort (Table 3) were analysed together but, given the higher prevalence of PD in females, I also performed a sex-stratified analysis, first in the MPIP Panic Cohort I and then in the MPIP Panic Cohort II. A fixed-effect meta-analysis across both samples was performed following identification of hits in the individual analyses.

3.5.1.2. Targeted Gene Analysis

High number of studies showed mostly single SNP associations in different genes with PD, however, the replicability of these findings was low. Therefore, I used three lines of approaches to select candidate genes for the targeted methylome analysis:

1) candidate genes from human genetic studies confirmed in the recent meta-analysis study of different international PD cohorts (*TMEM132D*, *COMT*, *NPSR1* and *HTR2A*) (Howe et al., 2016),
2) and/or having additional evidence from translational studies for anxiety and stress-related phenotypes (*CRH*, *CRHR1, ADCYAP1, ADCYAP1R1, FKBP5, SGK1, BDNF, HTR1A*) (Blaya et al., 2010; Ressler et al., 2011;

Konishi et al., 2014; Straube et al., 2014; Han et al., 2015; Cattaneo and Riva, 2016; Weber et al., 2016; Zannas et al., 2016) and lastly,

3) genes containing loci with previous evidence for differential methylation in PD and anxiety disorders (*GAD1, OXTR*) (Domschke et al., 2013; Ziegler et al., 2015).

All the genes examined (N=15) showed previous evidence of association with stress-related phenotypes not only in clinical (human) studies but also in preclinical (animal) studies (Leonard et al., 2008; Benekareddy et al., 2011; Erhardt et al., 2011; Desbonnet et al., 2012; Mustafa et al., 2015; Bahi et al., 2016; Knoll et al., 2016; Nieto et al., 2016).

The CpGs lying within the target genes were selected from the meta-analysis results of the EWAS and FDR correction of 5% was applied for the number of CpGs included in the gene.

3.5.1.3. Disease Association Analysis

To investigate a possible enrichment for specific pathways, I conducted a disease association analysis using Web Gestalt (Zhang et al., 2005; Wang et al., 2013), DAVID (Huang da et al., 2009a, b) and the R-package DOSE (Yu et al., 2015). Tested genes for a disease enrichment were annotated from CpG sites with P-value < 0.001 in the meta-analysis results of the cases vs controls EWAS in the whole sample (N_{genes}=312), in the female subset (N_{genes}=428) and in the male subset (N_{genes}=379). The analysis was background corrected for the Illumina HumanMethylation450 BeadChip array annotated genes.

3.5.1.4. DNA Methylation Age Calculation

DNA methylation age was calculated from peripheral blood of patients and controls included in the MPIP Panic Cohort I (N=165) and II (N=300). DNA methylation-based age prediction was performed using the R code and statistical pipeline developed by Horvath (Horvath, 2013). This predictor was developed using 82 Illumina DNA methylation array datasets (n = 7,844) involving 51 healthy tissues and cell types (Horvath, 2013). The raw data were normalized using BMIQ

normalization method (Teschendorff et al., 2013) implemented in the Horvath DNA methylation-based age predictor R script (Horvath, 2013). I then tested whether epigenetic age acceleration (Δ-Age), calculated by subtracting the actual chronological age from DNA methylation age (Horvath, 2013), was associated with 1) case-control status; 2) the presence/absence of agoraphobia in PD patients. Since DNA methylation age is calculated from raw beta values, technical batches identified for MPIP Panic Cohort I and II (96-well plate) were included as covariates in the linear regression model together with age, sex and cell counts (Houseman and Horvath cell counts, specifically: PlasmaBlast, CD8pCD28nCD45Ran, CD8.naive, CD4T, NK, Mono, Gran).

3.5.2. MPIP Dexamethasone Treatment Study

Methylation levels of cg07308824 were tested for association with gene expression levels of the *HECA* mRNA (ILMN_1770667) using a linear mixed effects model within the lme4 package (Bates et al., 2015).

4. Results

4.1. Identification of Hidden Confounders

MPIP Panic Cohort I (as well as MPIP Panic Cohort II) went through the quality control (QC) steps described in the Materials and Methods section. This QC pipeline has been used successfully previously (Zannas et al., 2015; Emeny et al., 2017), I was therefore expecting to see similar results as previous studies. Surprisingly, the result of our first analysis in the MPIP Panic Cohort I showed a high inflation of test statistic (Figure 6).

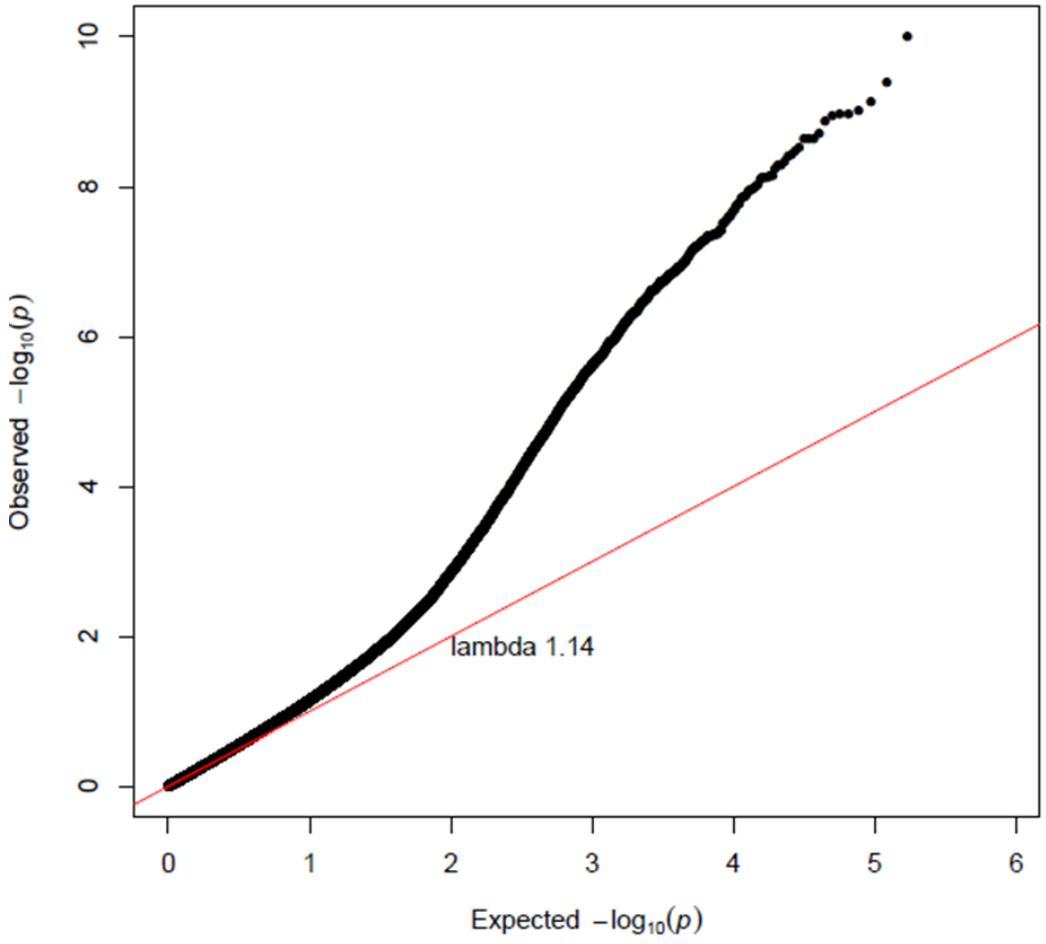


Figure 6. QQ-plot of the first case-control EWAS in the MPIP Panic Cohort I. Expected vs Observed $-\log_{10}(p)$ of the results for the case-control comparison (linear model, N=187). Lambda indicates the genomic inflation factor and it is defined as the ratio of the median of the empirically observed distribution of the

test statistic to the expected median (thus quantifying the extent of the bulk inflation and the excess false positive rate).

Considering the small sample size (N=187), this result was clearly not due to a real case-control difference, but rather to hidden confounders which were still there even after batch correction. To exclude that a technical problem occurred during the experiment, I performed the same case-control analysis in the other samples (N=609, 399 MDD patients, 210 controls) that were processed together, in the same batch, with the MPIP Panic Cohort I. The results in this case were not significant and the QQ-plot showed no inflation (Figure 7). This confirmed that the inflation present in the MPIP Panic Cohort I was not due to a technical problem, but rather to unknown confounders.

To investigate the possible sources of variability in the data, I used the Surrogate Variable Analysis (SVA) (Leek and Storey, 2007) approach, which has the aim of detecting hidden variability in a given dataset while protecting for the phenotype of interest. I therefore calculated the surrogate variables (SVs) protecting for the case-control status; I then checked for the correlation with our known batches (Supplementary Figure 3) and corrected including them in the regression model (Figure 8). None of the tested models with the SVs included performed much better than the initial model batch corrected using Combat.

I then tested whether the model that I were using, the linear model (which is the model of election for methylation analysis due to the distribution of the data), was not the appropriate one, and applied a logistic regression (glm, family=binomial). The latter is in general useful in predicting a binary outcome from a set of continuous predictor variables, but it did not perform better than the linear model in our analysis (Figure 9).

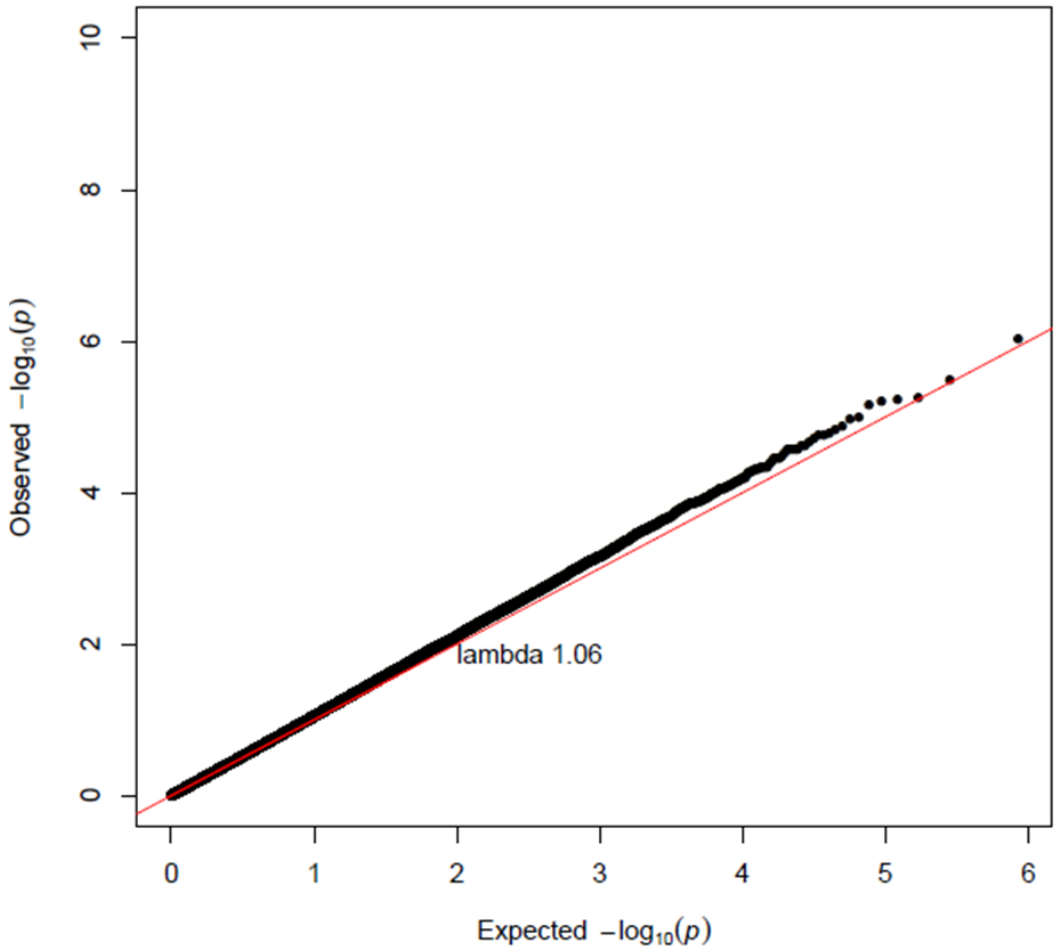


Figure 7. QQ-plot of the case-control EWAS results in the MDD cohort (N=609). Linear model of the case-control analysis.

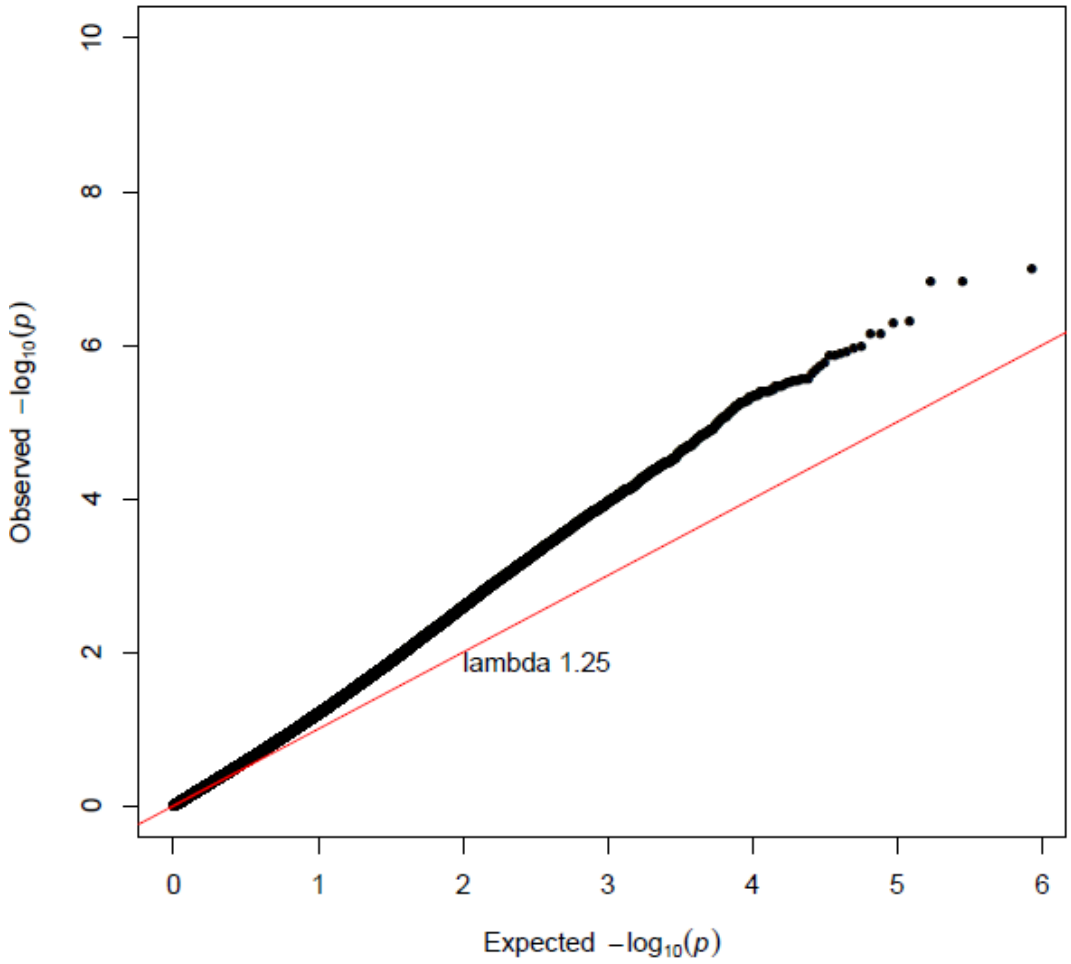


Figure 8. QQ-plot of the first case-control EWAS in the MPIP Panic Cohort I with SVs included in the model.
Linear model with the first five surrogate variables included as covariates.

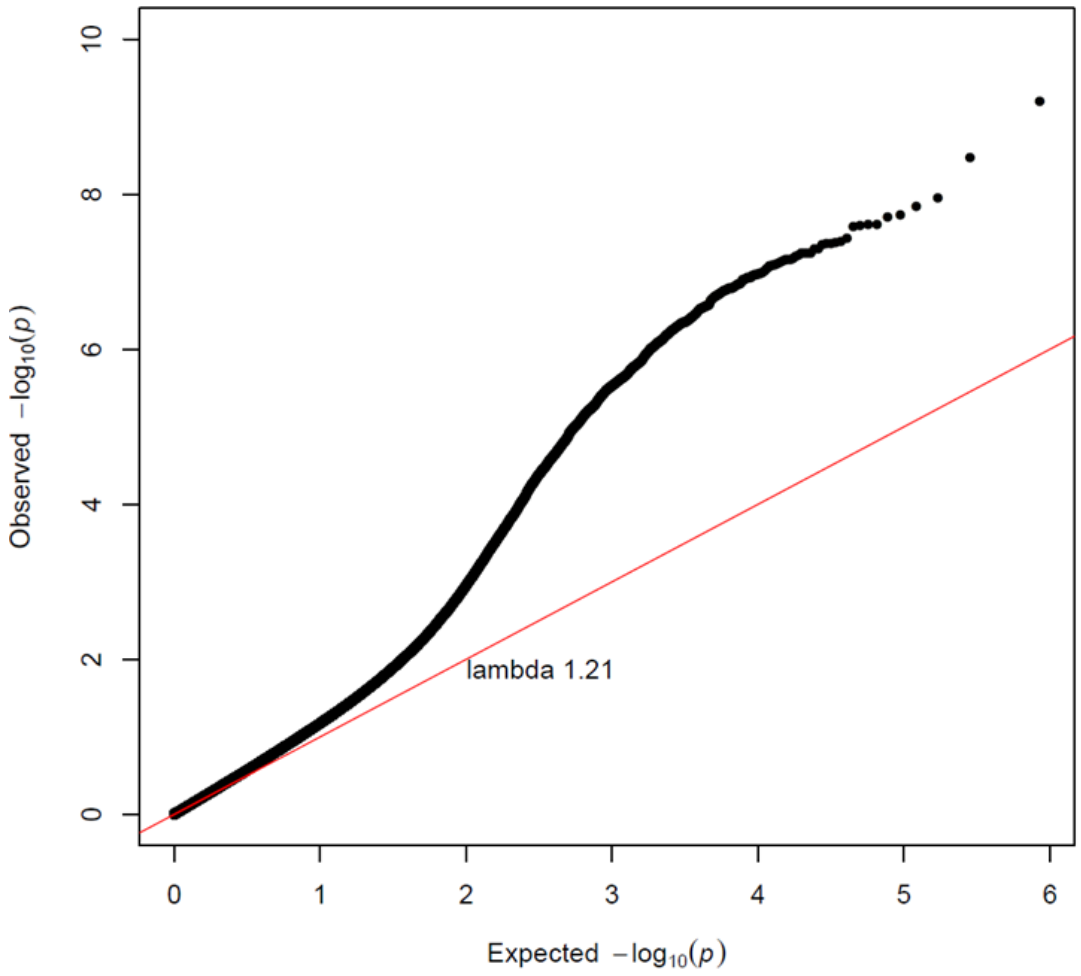


Figure 9. QQ-plot of the first case-control EWAS in the MPIP Panic Cohort I with glm applied and SVs included in the model.
Logistic regression with the first five surrogate variables included as covariates.

For this reason, I went deeper into the phenotypic characterization and went back to the laboratory to get more information about the samples, e.g. DNA extraction method, storage information, collection date. I then performed again the analysis considering the additional information. Strikingly, I realized that the processed samples were stored differently: only a few DNA samples (N=21) included in the MPIP Panic Cohort I, all (except one) controls, were stored at -20°C while the rest of the samples was stored at 4°C. I hypothesized that this could be the factor possibly introducing a strong bias in the case-control analysis. However, it was a confounder I could not detect when correlating the principal

components with the known batches, even after including the storage information (Figure 10). The principal component analysis (PCA) did not reveal any cluster neither considering all the samples that were processed together (MPIP Panic Cohort I-MDD, Figure 11) nor analysing the MPIP Panic Cohort I alone (Figure 12). I therefore considered these samples as biological outliers and excluded them from the analysis. The resulting QQ-plot, after repeating the analysis without the outlier samples, does not show an inflation anymore (Figure 13), proving that the different storage conditions might have been the hidden confounder I was looking for.

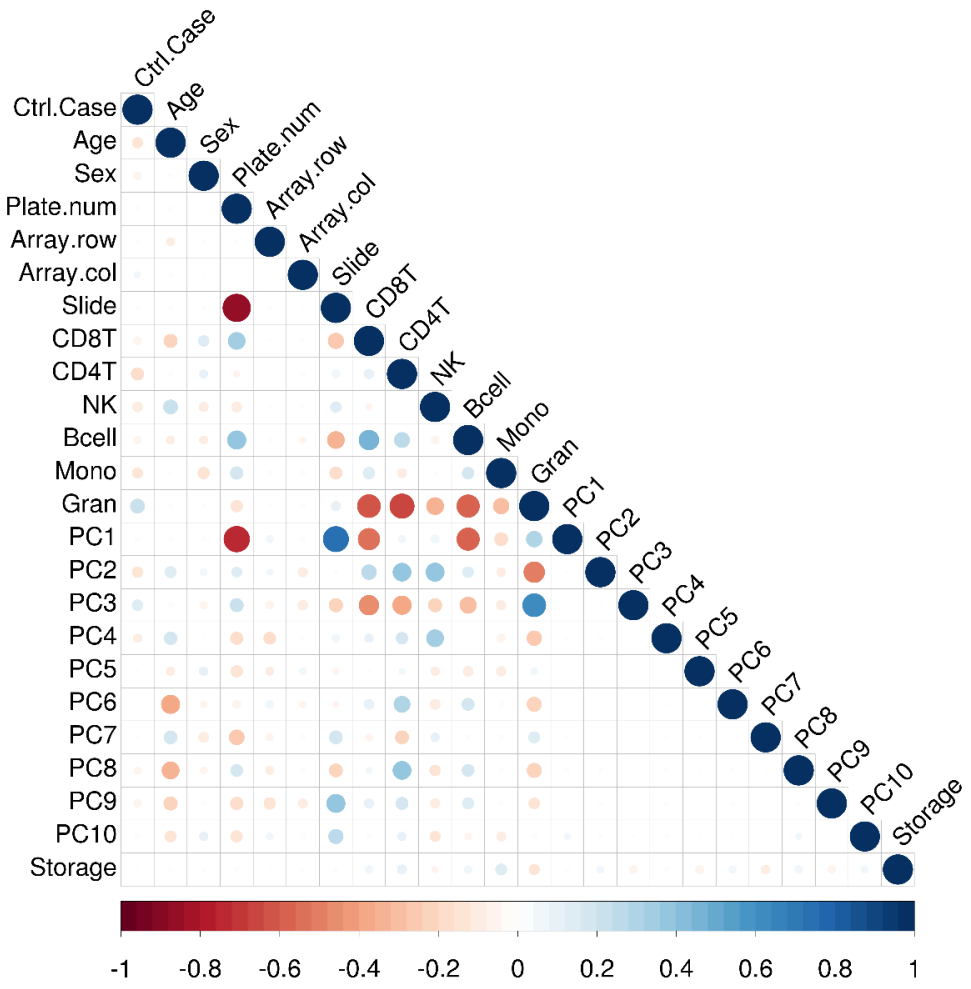


Figure 10. Correlation plot for the MPIP Panic Cohort I-MDD data (N=699) after normalization, before batch correction.

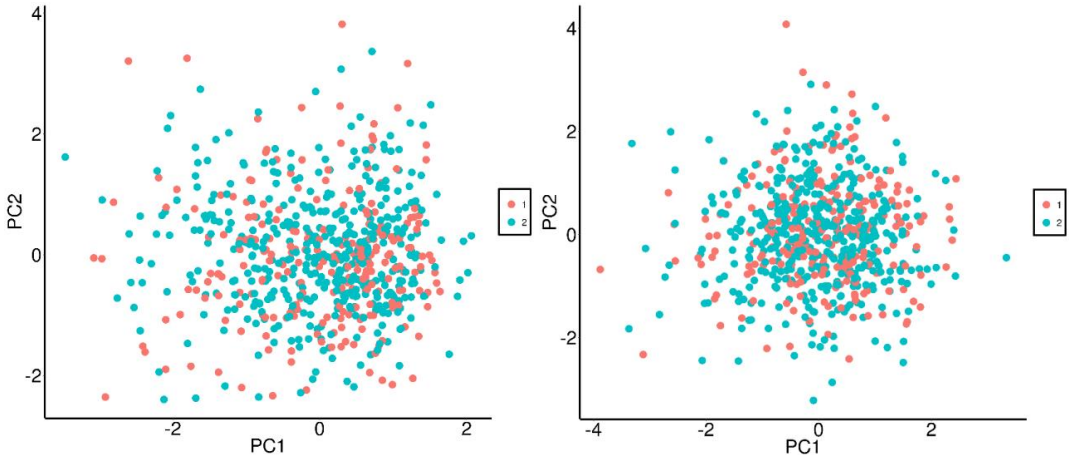


Figure 11. Principal Component Analysis in the MPIP Panic Cohort I-MDD data (N=699) before (left) and after (right) batch correction for the Storage variable (1= extracted DNA stored at -20 °C, 2= extracted DNA stored at 4 °C).

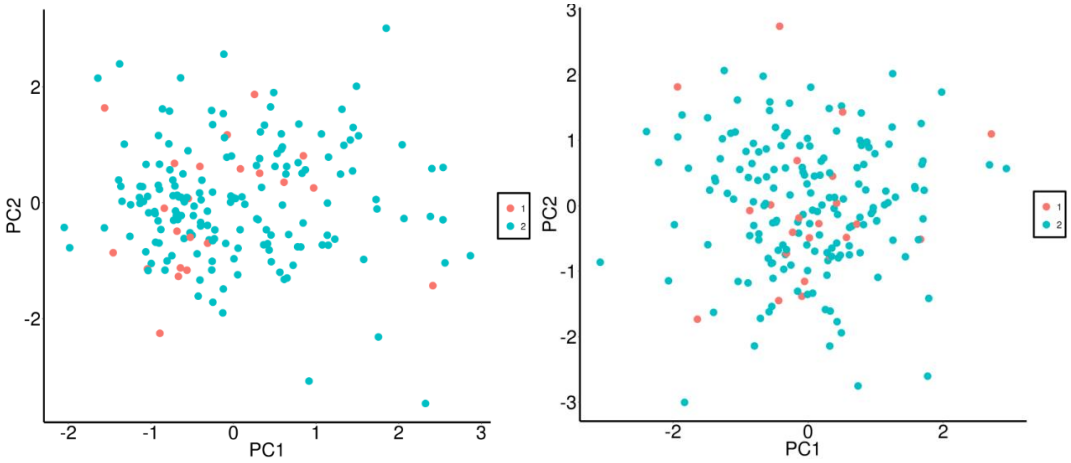


Figure 12. Principal Component Analysis in the MPIP Panic Cohort I (N=187) before (left) and after (right) batch correction for the Storage variable (1= extracted DNA stored at 4 °C, 2= extracted DNA stored at -20 °C).

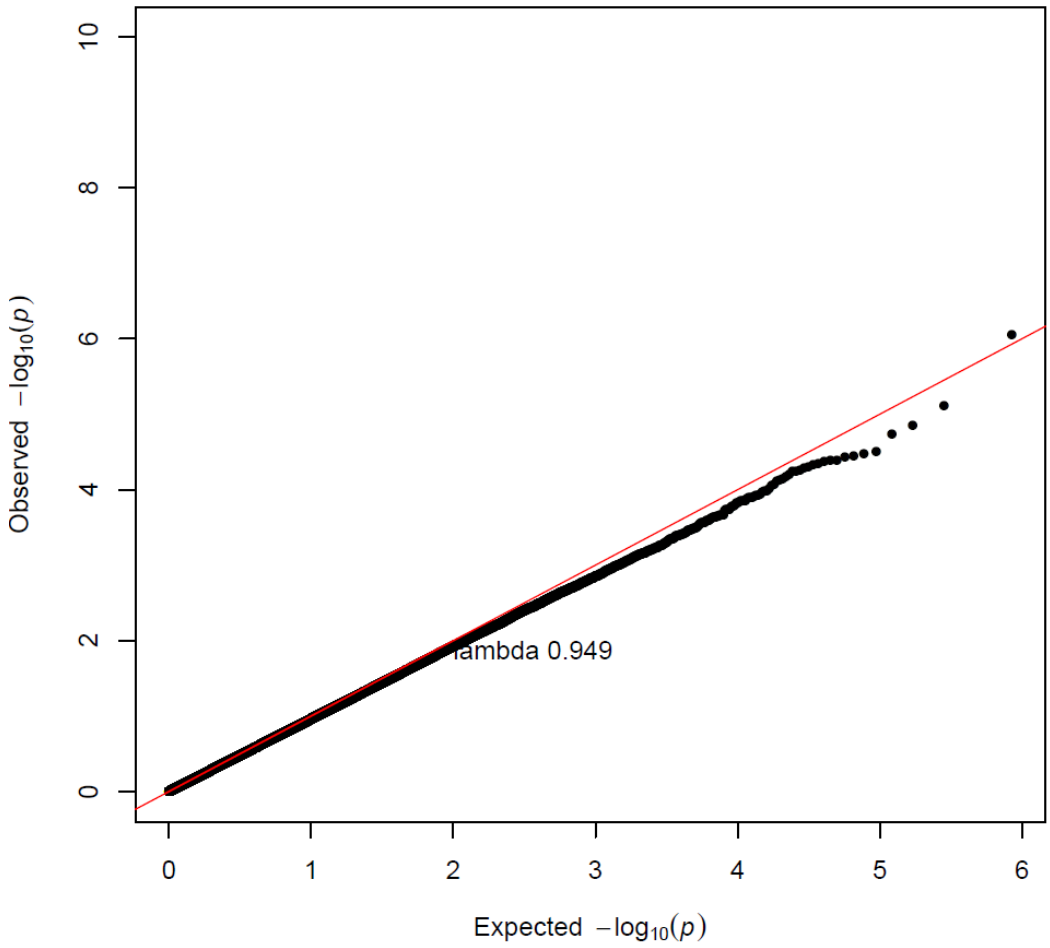


Figure 13. QQ-plot of the case-control EWAS in the MPIP Panic Cohort I without the outlier samples.

4.2. Epigenome-Wide Association Study (EWAS)

Genome-wide associations were performed in the MPIP Panic Cohort I (discovery sample), combined as well as stratified by sex. While no association survived correction for multiple testing in the overall samples and the male subset, one genome-wide association, cg07308824, surviving FDR of 5% (p= 1.094 x 10^{-7}, *p-adj*=0.046) was observed in the female subset of the MPIP Panic Cohort I (discovery sample). QQ plots for each of the analyses are presented in Figure 13 and Supplementary Figures 4-8. cg07308824 is located in the promoter of the *HECA* gene and was hypermethylated in female PD patients (N=49) compared to

controls (N=48). The association and the direction of the association could be replicated in the MPIP Panic Cohort II (replication sample, *p*=0.035) and yielded a combined *p-value* of 1.651 x 10^{-8} in the meta-analysis, that would again survive correction for multiple testing (*p-adj*=0.004) (Figure 14-15).

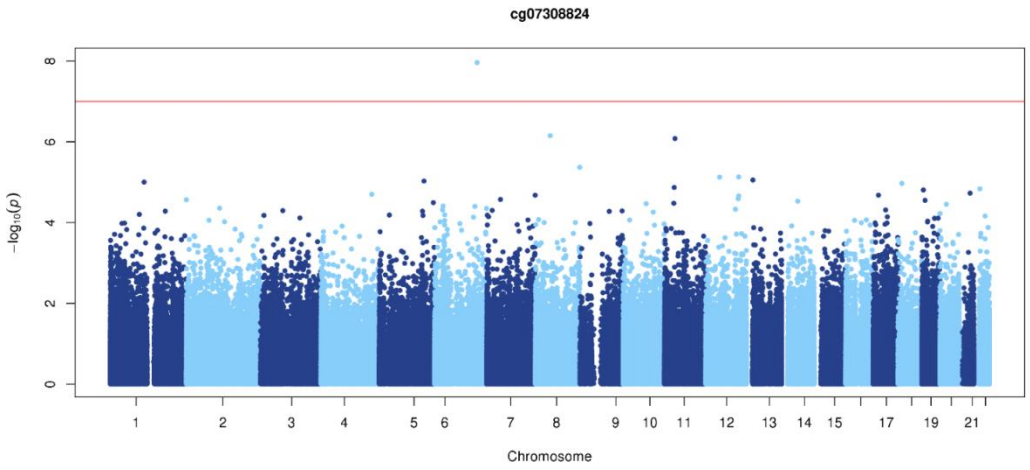


Figure 14. Manhattan plot of the Panic Disorder EWAS in females (meta-analysis results). The x-axis shows chromosomal position and the y-axis shows $-\log_{10}(P)$. The red line represents the multiple test threshold ($p < 1.09 \times 10^{-7}$).

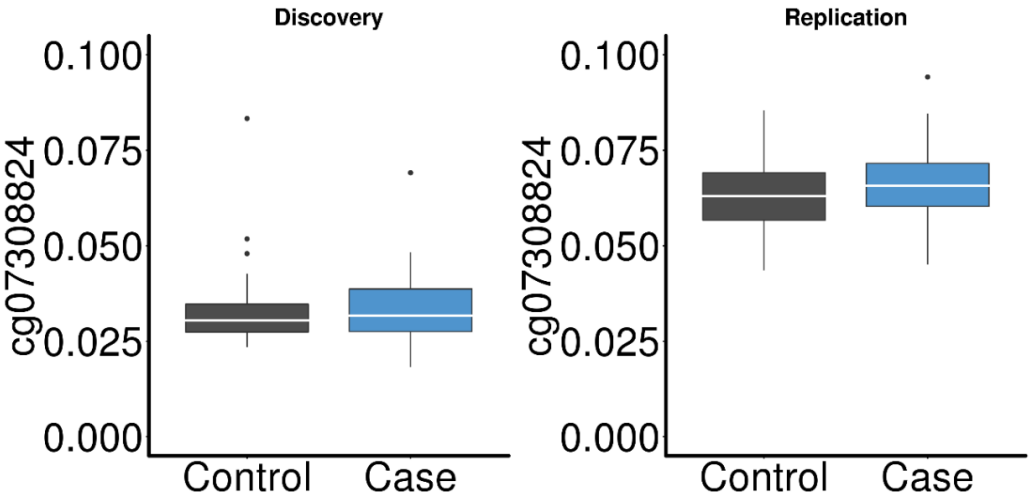


Figure 15. Box plot of DNA methylation levels for the genome-wide significant CpG, in the MPIP Panic Cohort I (discovery, *p-adj*= 0.046) and MPIP Panic Cohort II (replication, *p*=0.035).

4.2.1. Targeted Gene Analysis

The targeted gene analysis (see Supplementary Table 1-15 for the complete list of genes tested) using the meta-analysis results, yielded in females one significant CpG each (surviving 5% FDR correction over the CpGs in the gene) in *ADCYAP1 (PACAP)* (*p-adj*=0.010) and *HTR1A* (*p-adj*=0.041) (Figure 16). The same analysis yielded one significant CpG in *SGK1* (*p-adj*=0.035) (Figure 17) in the whole sample and in males in *FHIT* (*p-adj*=0.010) and two significant CpGs in HTR2A (*p-adj*=0.015 and *p-adj*=0.029) (Figure 18) (Table 4). Single nominal associations have been found in the genes *ADCY1P1R1, BDNF, COMT, CRH, CRHR1, GAD1, OXTR* and *TMEM132D*. No differential methylation was detected for NPSR1 between cases and controls.

Table 4. Targeted gene analysis results for the significant CpGs

Sample	*Gene*	*CpG*	*P-adj Meta-analysis*
Whole	***SGK1***	cg00959636	0.035
Males	***FHIT***	cg07351758	0.010
	HTR2A	cg09361691	0.015
		cg06476131	0.029
Females	***ADCYAP1 (PACAP)***	cg13940693	0.010
	HTR1A	cg16280141	0.041

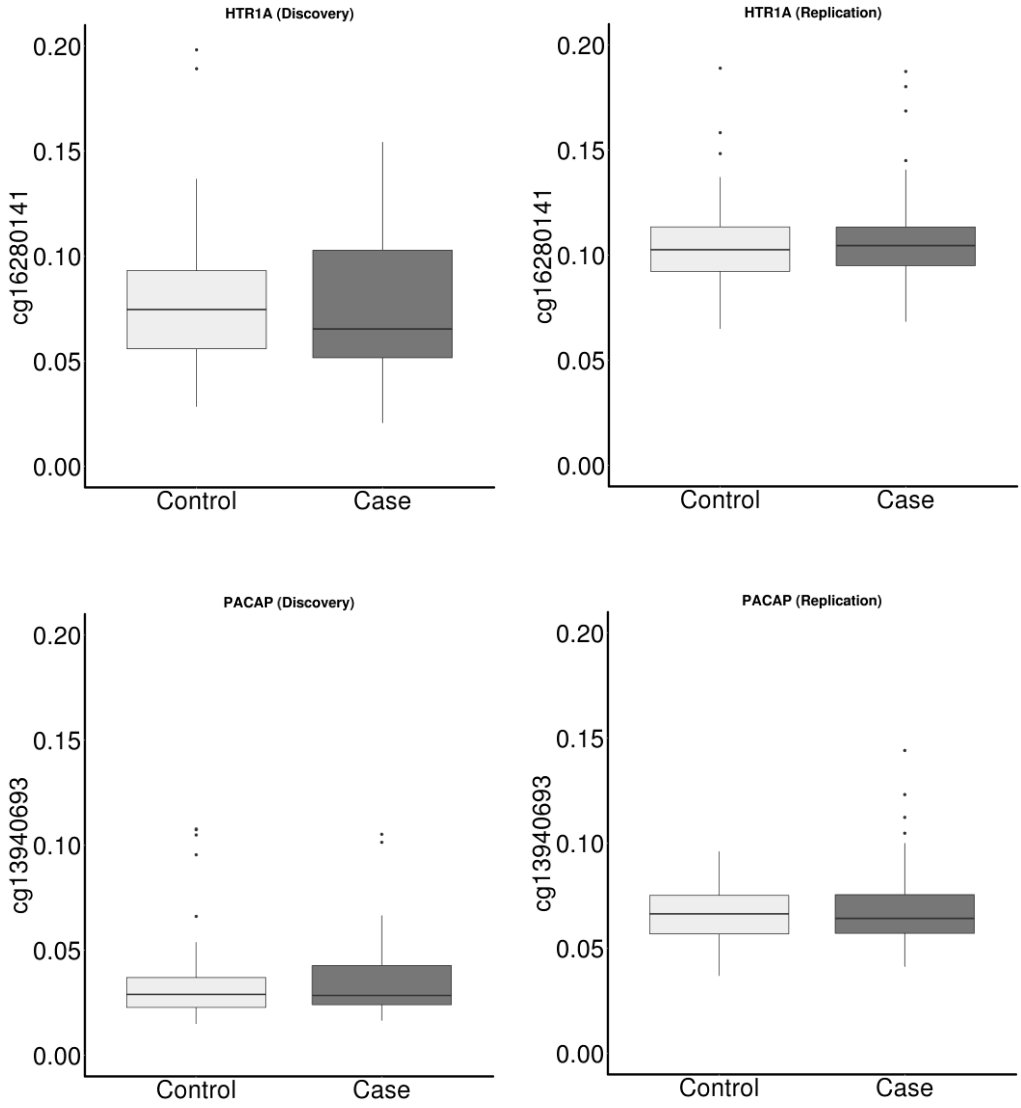


Figure 16. Box plots of DNA methylation levels for the significant CpGs in the gene-targeted analysis in the MPIP Panic Cohort I (discovery) and MPIP Panic Cohort II (replication) in females.

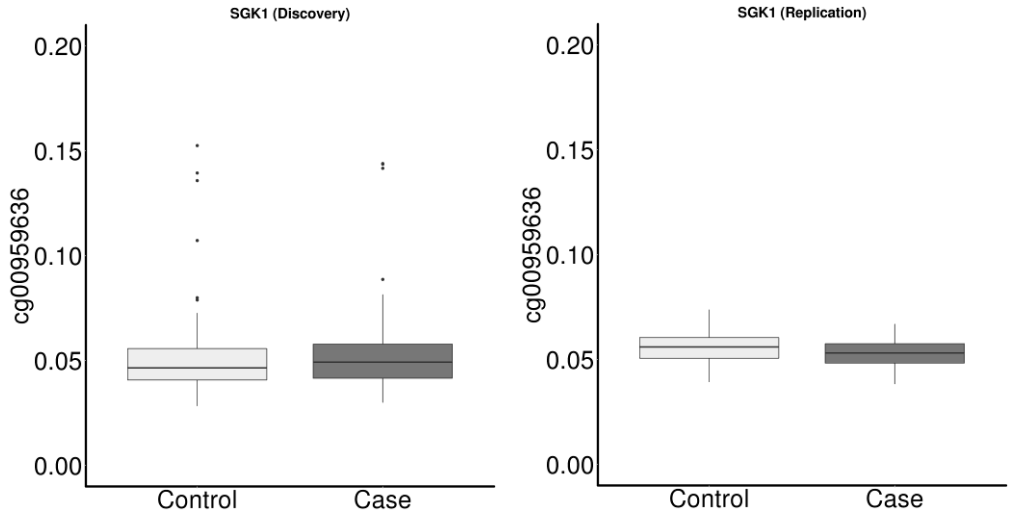
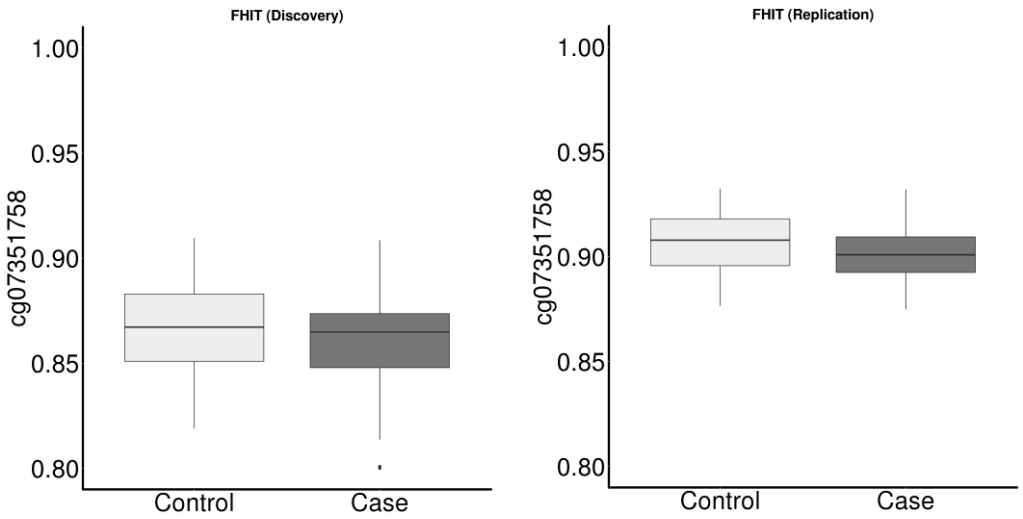


Figure 17. Box plots of DNA methylation levels for the significant CpGs in the gene-targeted analysis in the MPIP Panic Cohort I (discovery) and MPIP Panic Cohort II (replication) in the whole sample.



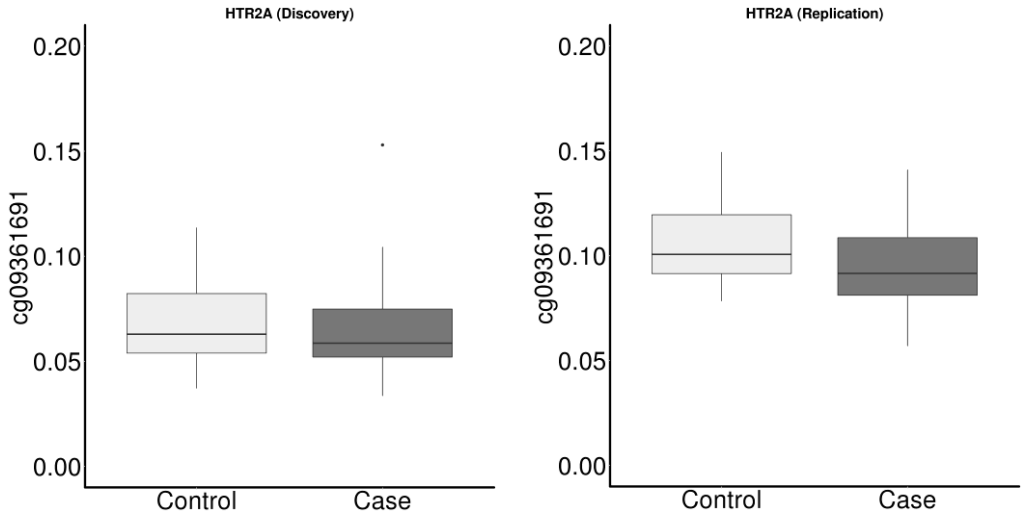


Figure 18. Box plots of DNA methylation levels for the significant CpGs in the gene-targeted analysis in the MPIP Panic Cohort I (discovery) and MPIP Panic Cohort II (replication) in males.

4.2.2. Functional Characterization of Significant Results

To assess the functionality of the significant CpG methylation site, association of methylation levels with gene expression of the *HECA* mRNA was tested. Methylation at this CpG site was associated with mRNA expression of *HECA* (ILMN_1770667) both at baseline (p=0.046) and after induction by dexamethasone (p=0.029) (Figure 19). Gene expression was significantly altered in the sample following dexamethasone induction (p=8.78e-05) but not DNA methylation (p=0.796) (Figure 20), indicating that the significant association between gene expression and DNA methylation is specific and not due to dexamethasone.

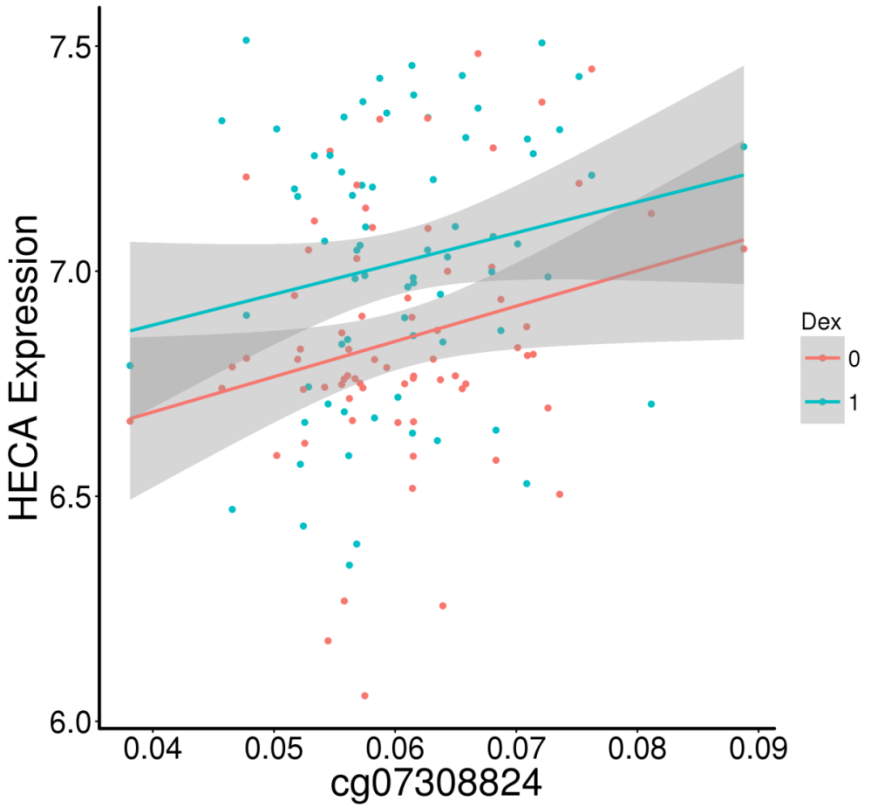


Figure 19. Functional characterization of significant results.
Scatterplot showing the association between DNA methylation (x-axis, beta values) and gene-expression (y-axis, VSN normalized array probe intensity) in an independent female sample at baseline (p=0.046) and after induction by dexamethasone (p=0.029).

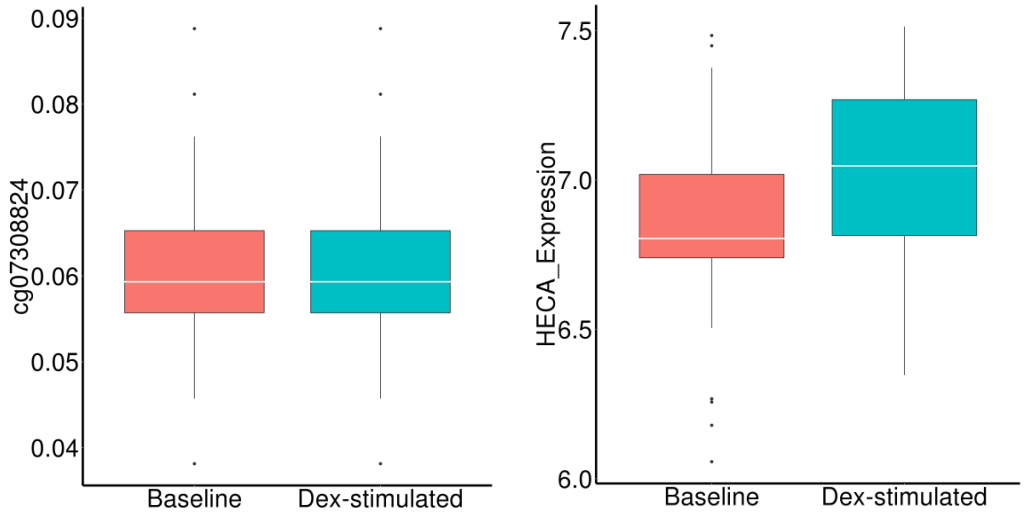


Figure 20. Box plot of DNA methylation and gene expression levels in the MPIP dexamethasone treatment study.
DNA methylation levels on the left (*p*=0.796) and gene expression levels on the right (*p*=8.78e-05) at baseline and after dexamethasone induction in the MPIP dexamethasone treatment study.

4.2.3. Functional Annotation of the *HECA* Locus

I further investigated the functional relevance of cg07308824 in the UCSC Genome Browser (Kent et al., 2002), located in the intragenic and enhancer region of the Homo sapiens headcase homolog (Drosophila) (HECA) gene on Chromosome 6 (Figure 21). An overlap was observed between the location of cg07308824 probe and histone 3 lysine 27 acetylation (H3K27Ac) on 7 cell lines from ENCODE (Rosenbloom et al., 2013), suggesting that the sequence where the probe is located is functional (Creyghton et al., 2010).

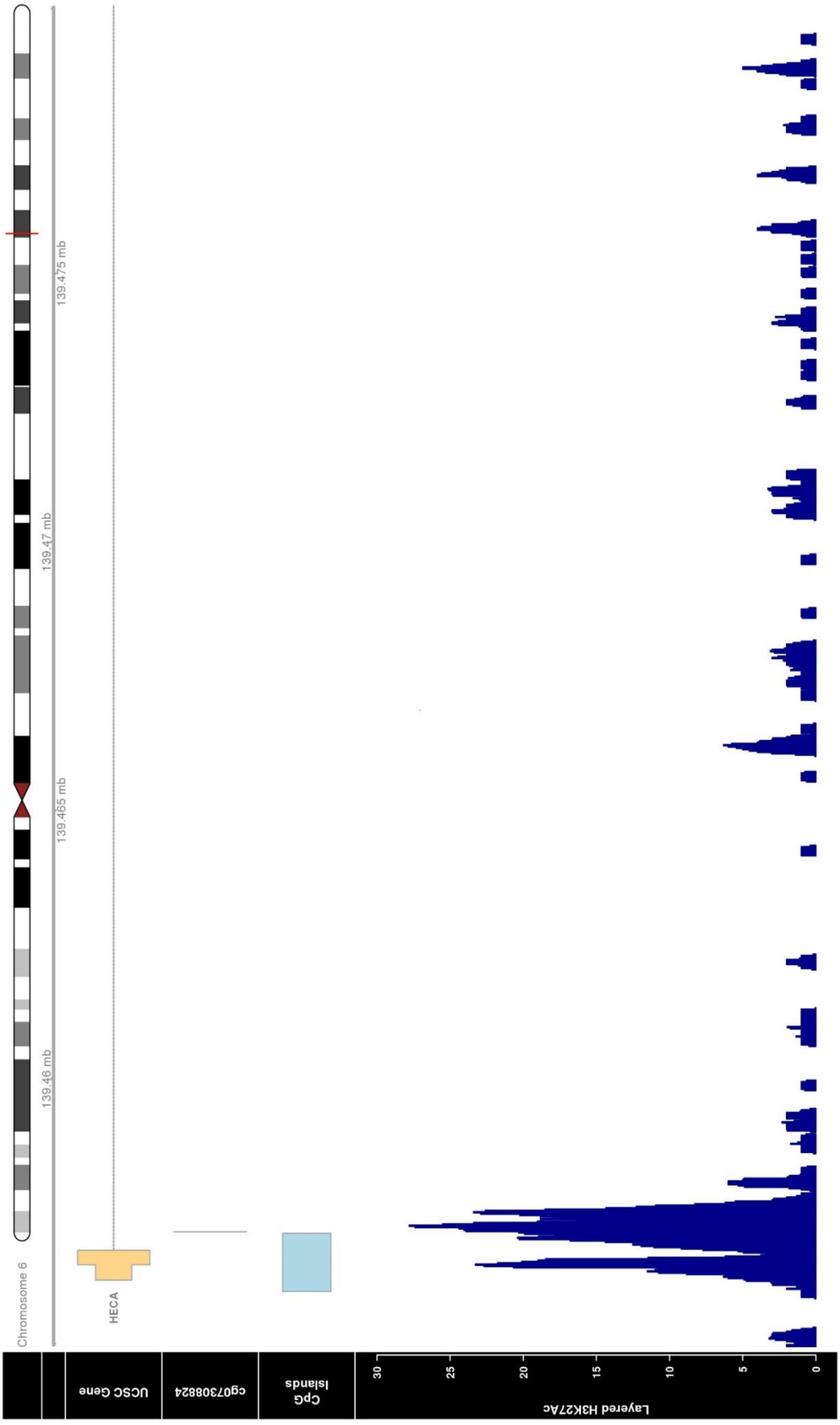

Chromosome 6
139.46 mb
139.465 mb
139.47 mb
139.475 mb
HECA
UCSC Gene
cg07308824
CpG Islands
Layered H3K27Ac
30
25
20
15
10
5
0

Figure 21. Annotation of the genome-wide significant CpG located in the HECA gene. The top panel contains the *HECA* gene model, located on Chr 6. The other two panels show the genome-wide significant CpG and the CpG island where the CpG is located. The bottom panel shows the levels of enrichment of the H3K27Ac mark in the *HECA* gene. Data were obtained from UCSC Genome Browser and plotted using the R package Gviz (Hahne and Ivanek, 2016).

4.2.4. Disease Association Analysis

After the characterization of the genome-wide significant hit, I also wanted to investigate whether genes annotated to the top CpGs (*p-value* < 0.001) were enriched for specific diseases, especially psychiatric disorder. For this purpose I ran a disease association analysis using two web tools (Web Gestalt and DAVID) (Zhang et al., 2005; Huang da et al., 2009a; Wang et al., 2015) and the R-package DOSE (Yu et al., 2015). As for the previous analysis, I first analysed the whole sample of the MPIP Panic Cohort I and MPIP Panic Cohort II and then I stratified by sex.

Looking broadly at the enrichment found for all the diseases in all the tools used, there was no disease that was enriched in all the three tools (Table 5).
Looking more closely at disease enrichment with a focus on psychiatric disorder, an enrichment could be found in the whole sample (bipolar disorder, *p*=1.9e-2; mental disorders, *p*=2.5e-2) and in females (response to antipsychotic treatment, *p*=5.3e-2; ADHD, *p*=9.5e-2) using DAVID (Huang da et al., 2009a, b) (Table 6).

Table 5. Disease association analysis (General overlap)
The table shows disease association results overlapping between the different pathway analysis tools used. Genes included in the analysis were annotated from CpGs with P < 0.001 in the meta-analysis results of the cases vs controls EWAS.

Pathway tool	*Whole sample*	*Males*	*Females*
Disease association overlapping between tools	Germ Cell and Embryonal Neoplasm, Amyotrophic Lateral Sclerosis, Mental Disorders	Type 2 Diabetes, Carcinoma	Muscular Disease
Web Gestalt	-	*p*=0.12	*p*=0.001
DAVID	*p*=2.2e-2, *p*=3.6e-2, *p*=2.5e-2	*p*=2.8e-2, *p*=7.6e-2	-
DOSE (R-package)	*p*=0.150, *p*=0.07, *p*=0.154	-	*p*=0.121

Table 6. Disease association analysis (Psychiatric disorders)
The table shows disease association results for psychiatric disorders. Genes included in the analysis were annotated from CpGs with P < 0.001 in the meta-analysis results of the cases vs controls EWAS.

Pathway tool	*Whole sample*	*Males*	*Females*
Disease association with psychiatric disorders	Bipolar disorder, Mental Disorders		Response to antipsychotic treatment, ADHD
Web Gestalt	-	-	-
DAVID	*p*=1.9e-2, *p*=2.5e-2	-	*p*=5.3e-2, P=9.5e-2
DOSE (R-package)	-	-	-

4.2.5. Blood-Brain Correlation of the *HECA* Locus

No significant correlations were found between cg07308824 methylation levels and four different brain regions (i.e. prefrontal cortex, superior temporal gyrus,

entorhinal cortex and cerebellum) in a linear regression model (Hannon et al., 2015) (Supplementary Figure 6).

4.3. DNA Methylation Age and Agoraphobia

Psychological stress is an important risk factor for accelerated aging and aging-related diseases, including cardiovascular disease, immune dysregulation, and late-life neuropsychiatric disorders. Stress-related psychiatric disorders, including anxiety, PTSD and major depression, are themselves a risk for such diseases (Danese et al., 2008; Vaccarino et al., 2013; Meneghetti et al., 2017). The molecular mechanisms that link psychological stress with accelerated aging and aging-related diseases remain largely unknown. It has already been reported that stressors can induce lasting changes in DNA methylation (Weaver et al., 2004; Klengel et al., 2013), therefore one plausible mechanism that can act as a mediator in this process is epigenetic regulation through DNA methylation.

PD is a strong stressor for the people affected and so far no studies have been carried out to determine whether patients affected by PD also develop age acceleration. I was interested in investigating whether age acceleration was present in PD patients compared to controls, and whether age acceleration could be detected in the most severe patients, specifically the ones affected by panic disorder with agoraphobia.

I first compared the Δ-age in PD patients with healthy controls in the whole MPIP Panic Cohort I (N=165, p=0.980) and II (N=300, p=0.282) and found no significant differences. I then stratified for sex and found no significant results in males (MPIP Panic Cohort I: N=68, p=0.835; MPIP Panic Cohort II: N=95, p=0.467) as well as in females (MPIP Panic Cohort I: N=97, p=0.964; MPIP Panic Cohort II: N=204, p=0.402) (Figure 22-23).

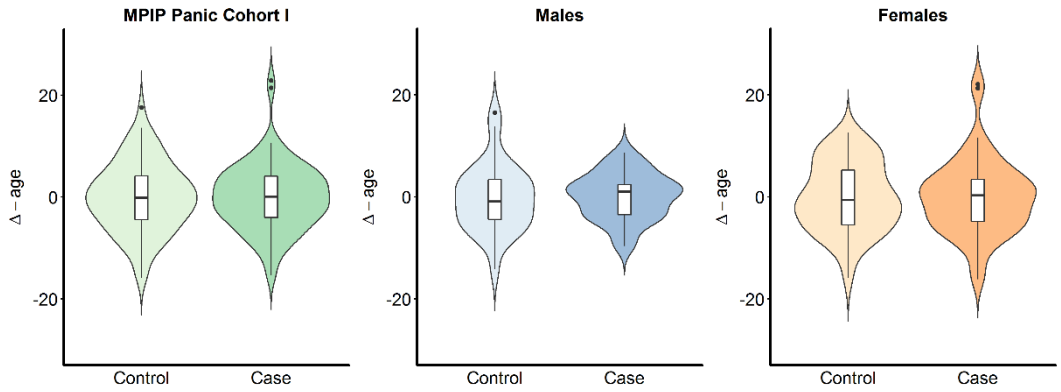


Figure 22. Violin plots of Δ-age by control-case status in the MPIP Panic Cohort I sample (from the left: whole sample, males only, and females only).

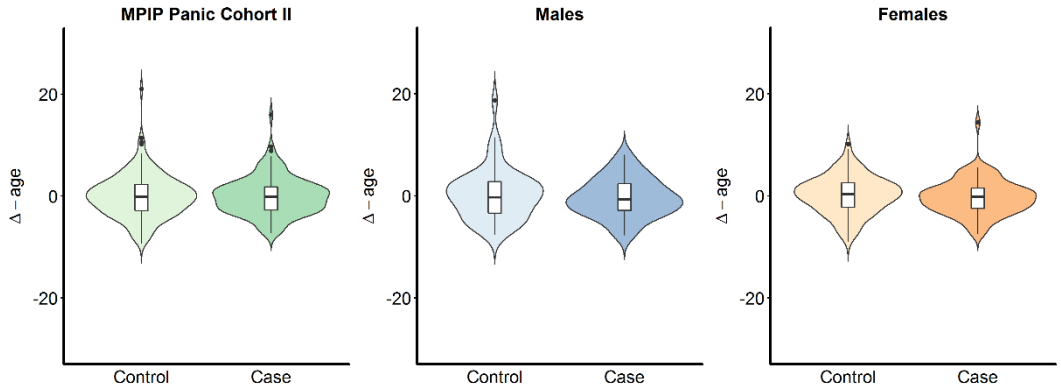


Figure 23. Violin plots of Δ-age by control-case status in the MPIP Panic Cohort II sample (from the left: whole sample, males only, and females only).

Because it is known that the symptoms of panic disorder are stronger in presence of agoraphobia (American Psychiatric Association, 2013), I hypothesized that epigenetic age acceleration (Δ-age) would be positively associated with patients with agoraphobia and not in patients without. I then therefore restricted the analysis to patients only.

I performed a first analysis in each dataset (MPIP Panic Cohort I and II) overall and then stratified by sex. I could confirm our hypothesis in the MPIP Panic Cohort II, where the Δ-age was positively associated with agoraphobia in the

whole dataset (N=131, *p*=0.016) and the association was stronger if females were analysed separately (N=83, *p*=0.005). Results were not significant in males. No association between Δ-age and agoraphobia was detected in the MPIP Panic Cohort I (Figure 24-25).

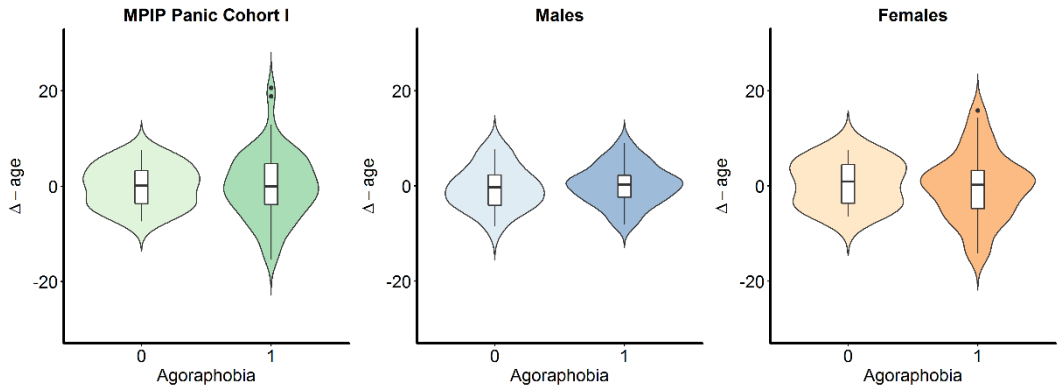


Figure 24. Violin plots of Δ-age by agoraphobia status in the MPIP Panic Cohort I sample (from the left: whole sample, males only, and females only).

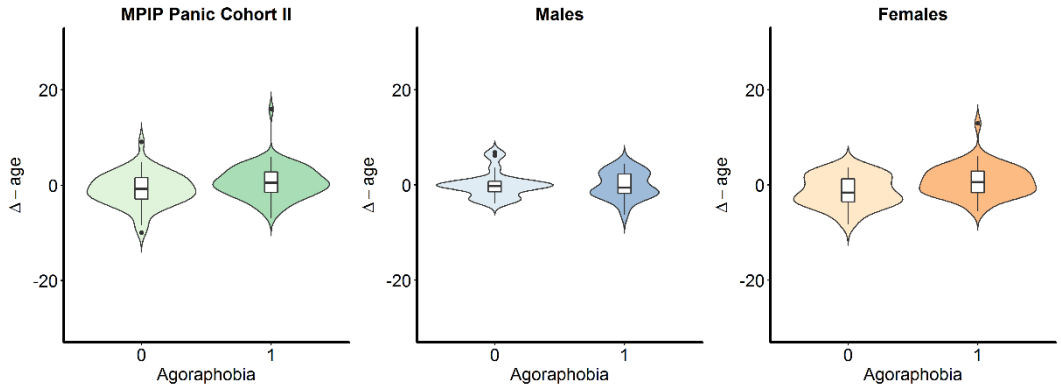


Figure 25. Violin plots of Δ-age by agoraphobia status in the MPIP Panic Cohort II sample (from the left: whole sample, males only, and females only).

5. Discussion

Panic disorder is a multifactorial disorder where both genetic and environmental factors contribute to its onset. Susceptibility genes for PD have been reported by previous genetic studies (Erhardt et al., 2011; Erhardt et al., 2012) (Howe et al., 2016) (Weber et al., 2016) (Gottschalk and Domschke, 2016), but the mechanism involved in the pathogenesis of PD still remains to be clarified. Epigenetics is considered to be a mediator of environmental stressors on the genome, DNA methylation in particular. In this study I investigated whether DNA methylation is involved in panic disorder analysing blood samples of medication-free patients and healthy controls. Our results suggest that sex-specific DNA methylation changes are occurring in panic disorder patients and not in healthy controls and that epigenetic age is accelerated in PD patients with agoraphobia compared to PD patients without. This is, to our knowledge, the first study of this kind on PD in the European population.

5.1. Hidden Confounders

Our results on the MPIP Panic Cohort I show the crucial importance of a good randomization process and a good experimental design. Even if our study was accurately randomized with cases and controls matched for age and sex, and distributed accordingly in different plates and slides, there were other variables, i.e. DNA storage, that introduced a considerable bias in our first analysis. Therefore, this demonstrates that the more information can be taken into consideration about the samples, the less will be the confounders in the analysis of the generated data. Crucial would be to take into account all the possible confounders already in the pre-experimental phase, so that batch effects can be avoided already in the laboratory. It is indeed known that if strong batch effects are present, it is not possible to disentangle the confounder from the experimental question under study (e.g. in a case-control study, if cases are separated in different slides from controls), so it is impossible in that case to get

rid of the batch effect. Therefore, the experiment has to be repeated with well-randomized samples for it to be analysable.

In my analysis, I was probably not able to eliminate the hidden confounders with the surrogate variable analysis (SVA) because I was protecting for the case-control status and only one out of 21 samples stored at -20°C was a control while the others were cases.

I also could not see these outliers in the principal component analysis (PCA) of all the samples processed together (MPIP Panic Cohort I-MDD), because the difference in the storage treatment was randomly distributed between cases and controls (Figure 11). Surprisingly, a cluster was not present even when the PCA was performed on the MPIP Panic Cohort I only (Figure 12).

5.2. EWAS

Genome-wide association of whole blood DNA methylation with PD cases and matched controls identified a locus (cg07308824), which was hypermethylated in female PD patients compared to healthy controls. This locus was also associated with case-control status in females in another independent sample and results were further confirmed with a meta-analysis (N=301). No methylation differences were identified at genome-wide level in the whole sample. This is the first and biggest EWAS for PD in a population with European background.

The methylation locus that I identified in females is located in the intragenic and enhancer region (Kent et al., 2002) of the Homo sapiens headcase homolog (Drosophila) (*HECA*) gene on Chromosome 6. The *HECA* gene is a cell cycle regulator and may play an important role in human cancers e.g. hepatocellular carcinoma (Wang et al., 2015); however, only a few publications about this gene are available to date. The potential functional relevance of cg07308824 was further investigated in the UCSC Genome Browser (Kent et al., 2002). An overlap was observed between the location of cg07308824 probe and histone 3 lysine 27 acetylation (H3K27Ac) on 7 cell lines from ENCODE (Rosenbloom et al., 2013) (Figure 21). H3K27Ac was previously found near to active regulatory elements

suggesting that the sequence where the probe is located is functional (Creyghton et al., 2010). The *HECA* gene is expressed in brain at lower levels compared to blood (Supplementary Figure 9) but I could not see any correlation between the methylation levels of the significant CpG found in blood and brain, which could indicate that the relevance of these results might be limited to blood. No significant correlations were found between cg07308824 methylation levels and four different brain regions (i.e. prefrontal cortex, superior temporal gyrus, entorhinal cortex and cerebellum) in a linear regression model using a publicly available data set (Hannon et al., 2015)(Supplementary Figure 10).

It is noteworthy considering that methylation levels of the identified locus showed a significant correlation with gene expression levels of the *HECA* gene in another independent female sample, which points to the functional relevance of the observed methylation change. The fact that the direction of the association is positive (higher gene expression correlated with higher methylation) may be explained by the intragenic location of the significant locus. It has indeed previously been shown (Wagner et al., 2014) that a positive correlation with gene expression is expected for CpG probes located in the body of the gene and a negative correlation is expected for CpG probes located close to a gene's TSS. The authors also report that however this is only partially verified, with one-third of the latter type showing a positive correlation and nearly half of the former type showing a negative correlation.

Notably, the significant changes in DNA methylation presented here are small (0.08%) but replicable. This is in line with results from the recent small Japanese EWAS in PD (Shimada-Sugimoto et al., 2017), where 40 significant CpGs with overall low methylation differences have been detected. Sex-specific associations were not reported, most likely because of the small sample size. Furthermore, similar effect sizes were observed in other methylome studies of complex disorders e.g. rheumatoid arthritis (Liu et al., 2013), multiple sclerosis (Huynh et al., 2014), Alzheimer's disease (Lunnon et al., 2014) and schizophrenia (Montano et al., 2016). The latter study is to date the largest case-control EWAS in the psychiatric field, with 689 schizophrenia patients and 645 controls included in the analysis. A bigger (N=1522) epigenome-wide association study on anxiety in a

population-based cohort was recently published, suggesting the involvement of the *ASB1* gene in anxiety-related phenotypes (Emeny et al., 2017).

As epidemiological studies clearly show the relevant contribution of the environment to the development of anxiety disorders (South et al., 2016; Torvik et al., 2016) and that these influences result in DNA methylation changes (Teh et al., 2014), a strategy including higher sample size and integrating specific environment-related factors (such as number or structure of life events) might be more successful to detect higher case-control methylation differences in PD.

Female-specific DNA methylation changes in PD have been previously shown both in mice (Papale et al., 2016) and in humans. A female-specific association and/or correlation of negative life events with decreased overall methylation levels has been shown for GAD1 and MAOA (Domschke et al., 2012; Domschke et al., 2013). In contrast, female-specific effects in terms of increased methylation levels of promoter region were observed in the FOXP3 gene for PD (Prelog et al., 2016). Sex-specific findings regarding the methylation pattern have been also detected in depression, which is highly comorbid with PD, and psychosis (Byrne et al., 2013; Melas et al., 2013).

Interestingly, the disease association analysis shows enrichment for psychiatric disorders in the whole sample and in females, but not in males.

However, one of the limitations of the study is the lower number of males compared to females, which is due to the higher prevalence of the disease in the latter. This might also explain why no significant results were found in the male subset but only in females. For this reason a bigger study with a higher number of subjects, with possibly the same ratio between males and females, is necessary in order to confirm the sex-specificity of our findings.

Another limitation is that I could not correct for the smoking status of the subjects, due to lack of information. I verified though that our significant genome-wide hit was not one of the top-associated CpGs in the biggest EWAS for cigarette smoking (Joehanes et al., 2016).

A targeted gene approach was subsequently applied, with the aim of investigating if genes previously associated with PD or anxiety-related

phenotypes are affected at the methylation level. Five of the genes I analysed, i.e. HTR1A, HTR2A, ADCYAP1 (PACAP), FHIT and SGK1 showed different methylation patterns in PD patients compared to controls. For these genes, the evidence for a correlation with anxiety disorders at the genetic level could be confirmed in our data at the epigenetic level. SGK1 (serum/glucocorticoid regulated kinase 1) is one of the key player in the mediation of fast and chronic stress response and, therefore, could be implicated in the transition of the environmental stress influences via methylation (Cattaneo and Riva, 2016). It seems to play a role in the expression of conditioned fear in the animal model (Knoll et al., 2016) and is one target of miRNAs in the glucocorticoid pathway affecting neurogenesis and leading to anxiogenic and depressiogenic behaviour in mice (Jin et al., 2016). Additional evidence from human studies point to the implication of this gene in the pathophysiology of traumatic stress, e.g. PTSD (Licznerski et al., 2015) and our results point to a possible involvement in PD as well. The second gene which is implicated in the stress response regulation is PACAP (Pituitary adenylate cyclase-activating polypeptide). Ressler et al. could demonstrate a female specific significant correlation of the PACAP38 peptide concentration in blood with PTSD symptoms and diagnosis (Dias and Ressler, 2013). In line with these previous findings, I could also demonstrate a female specific significant methylation difference in one locus between cases and controls, suggesting that this gene may play an important role in long-lasting stress dependent pathophysiology in PD or other anxiety disorders. Similarly, a female specific methylation difference could be shown for the gene HTR1A. This serotonin receptor is the most abundant of all serotonin receptors in the brain and HTR1A variants have been shown to be associated with depression and defensive behaviour in PD patients (Straube et al., 2014). So far, there is no evidence for sex specific implication of HTR1A gene in PD or other mental disorders. There are instead already previous studies showing an association of HTR2A and PD (Unschuld et al., 2007; Howe et al., 2016), and supporting evidence comes from our results in males. Similarly, variants in the gene FHIT (fragile histidine triad) were nominally associated in GWAS studies with anxiety (Luciano et al., 2012) and PD (Erhardt et al., 2011) and were not sex specific. In a

recent huge meta-analysis for broad depression phenotype, several variants in FHIT were among the most significant hits (Direk et al., 2016). However, there was no difference in the burden of depressive symptoms or depression diagnosis between the male and female group in our study, therefore, I cannot refer our finding to depression as bias. For HTR1A, HTR2A and FHIT, sex-specific effects presented here need to be replicated and elucidated in further studies.

5.3. DNA Methylation Age

Another aim of the study was to determine the effect of PD on epigenetic aging, as measured with the epigenetic clock (Horvath, 2013). I found that in the MPIP Panic Cohort II there was an increased epigenetic age acceleration in PD patients with agoraphobia compared to patients without and that results are stronger if females are analysed separately. This might be explained by the higher severity of PD in presence of agoraphobia (American Psychiatric Association, 2013). The fact that the association is stronger in females is in line with the other results of this study, which so far pointed at each level of our analysis towards a sex-specific mechanism that may underlie the onset and pathophysiology of panic disorder. The results could not be confirmed in the MPIP Panic Cohort I, where no significant differences could be found. I also investigated whether there was age acceleration in PD patients compared to controls, but results were not significant neither in the whole samples, nor in the sex-stratified analysis, in both MPIP Panic Cohort I and II. This might be explained by the heterogeneity of the samples in terms of age. It was showed by Zannas et al. that the effect of personal life stress on Δ-age is stronger in older as compared to younger people (Zannas et al., 2015). This suggests that the effects in our study, if present, might be diluted by the age-range.

To overcome the problem of the precision of the prediction of DNA methylation age, other predictors optimized for a different age-range have been developed. The Horvath age predictor was developed from primarily adult samples and has been used to accurately predict chronological age in children and adults (Horvath, 2013). It is known however that a prediction model is, in general,

weakest at the extremes of the distribution. Knight et al. therefore designed a predictor optimized to accurately estimate gestational age (GA) that can also be informative of developmental stage (Knight et al., 2016).

5.4. Limitations of the Study

Psychiatric disorders are complex and heterogeneous disorders that bear the disadvantage that the primary organ affected, the brain, is usually not available. On the other hand, the use of post-mortem brain tissue samples, while informative, has also limits and cannot capture the fluid state of the epigenome in vivo (Tylee et al., 2013). For this reason surrogate tissues that are more easily accessible, e.g. blood, have been often used as a potential proxy and source of biomarkers that may reflect the state of illness in the brain (Tylee et al., 2013). The limitations of the latter are certainly that their results only allow for indirect conclusions about the associated biological processes in living brain tissue. Walton et al. used a within-subject design to investigate the blood-brain correspondence of DNA methylation and found that most DNA methylation markers in peripheral blood do not reliably predict brain DNA methylation status (Walton et al., 2016). However, a subset of peripheral data may proxy methylation status of brain tissue. Therefore, the authors suggest that restricting the analysis to these markers can identify meaningful epigenetic differences in brain disorders (Walton et al., 2016).

Horvath et al. investigated the effects of age on genome-wide methylation levels in leukocytes and brain tissue samples (frontal and temporal cortex, pons, and cerebellum). According to their review (Horvath et al., 2012), a number of factors, such as the location of the specific CpG site (island, non-island, proximity to promoter) and the participation of the gene in developmentally regulated gene expression programs influence age-related changes in methylation (Tylee et al., 2013). Their results moreover indicated a high correlation between blood and brain methylation levels (approximate r=0.9); the effects of age on methylation levels were also moderately conserved (approximate r=0.3). Their conclusion was that the blood-brain methylome is more closely correlated than blood-brain gene

expression values as detected by microarray (approximate r=0.6) (Tylee et al., 2013). In the same direction, Kaminsky et al. observed a significant positive relationship between age and methylation levels at specific CpG sites in both blood and brain samples (Kaminsky et al., 2012).

A number of studies (Kronfol and Remick, 2000; Felger and Lotrich, 2013; Emeny et al., 2017) have implicated immune changes as one possible contributor to the pathophysiology of psychiatric disorders, therefore peripheral blood may give direct mechanistic insight into the brain (Klengel and Binder, 2015).

In summary, not all the findings deriving from peripheral blood reflect what is happening in the brain, but a close correspondence may indeed exist for many genes. The results presented in this thesis should be therefore considered with caution.

6. Conclusions and Outlook

The work described in this thesis examines epigenome-wide differences in peripheral blood for PD patients and suggests that possible sex-specific methylation changes are occurring, specifically in the *HECA* gene. Moreover, epigenetic age seems to be accelerated in PD patients with agoraphobia compared to patients without, with effects being stronger in females.

However, these results have to be considered preliminary and their validity might be limited to peripheral blood. Therefore, more studies with bigger sample sizes and possibly in different biological tissues have to be performed to gain more insights into the role of DNA methylation and the *HECA* gene in PD as well as the sex-specificity. To address these questions, a bigger replication sample should also include an equal number of males and females.

As a second layer of analysis, epigenetic age acceleration should be measured in other cohorts of PD patients with and without agoraphobia to confirm our findings of age acceleration occurring in PD patients with agoraphobia.

7. Supplementary Material

Supplementary Tables 1-15. Targeted gene analysis

List of all the CpGs tested for every target gene. Beta and P-values refer to the EWAS meta-analysis results in the whole sample and stratified by sex.

ADCYAP1 (PACAP)

	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00067606	-0.0504	0.4317	0.949447	-0.2272	0.03499	0.28668	0.0449	0.5866	0.900692
cg04105966	-0.0066	0.878	0.949447	-0.0781	0.3986	0.8712	-0.0082	0.8722	0.9198
cg06372303	0.0034	0.9629	0.9629	-0.0466	0.7303	0.8712	0.1528	0.1048	0.4062
cg07211875	0.0098	0.8622	0.949447	0.0276	0.7744	0.8712	0.0425	0.5534	0.900692
cg07376535	0.045	0.496	0.949447	-0.0348	0.7729	0.8712	0.1765	0.03384	0.24714
cg07788286	-0.0116	0.8967	0.949447	-0.3214	0.06718	0.30231	-0.028	0.798	0.9198
cg10384245	0.045	0.3765	0.949447	0.0368	0.6656	0.8712	0.0847	0.2008	0.4062
cg11402363	0.0815	0.2328	0.949447	0.0413	0.7456	0.8712	0.0261	0.7596	0.9198
cg11771234	0.0213	0.7908	0.949447	0.2461	0.0894	0.32184	-0.0479	0.6312	0.900692
cg11859607	-0.0491	0.352	0.949447	-0.0317	0.7281	0.8712	0.0068	0.9198	0.9198
cg13940693	0.0084	0.8805	0.949447	-0.1812	0.03518	0.28668	0.2438	0.000593	**0.010674**
cg14200170	-0.127	0.03223	0.58014	0.0071	0.9451	0.9481	-0.0347	0.6505	0.900692
cg14479567	0.0301	0.7072	0.949447	-0.0782	0.5644	0.8712	0.136	0.1837	0.4062
cg14489474	-0.0192	0.8281	0.949447	-0.0642	0.6738	0.8712	-0.2295	0.04119	0.24714
cg14908653	-0.0248	0.7178	0.949447	-0.2592	0.04778	0.28668	-0.0192	0.8178	0.9198
cg17439660	0.0661	0.3505	0.949447	0.0834	0.4905	0.8712	0.1198	0.2031	0.4062
cg21331088	-0.0123	0.7441	0.949447	0.0675	0.2967	0.8712	-0.0696	0.1557	0.4062
cg22374233	-0.0528	0.3309	0.949447	-0.006	0.9481	0.9481	0.0956	0.1964	0.4062

ADCYAP1R1 (PACAP receptor)

	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg01556466	-0.1996	0.03595	0.670383	-0.1541	0.3582	0.618709	-0.1724	0.1913	0.841067
cg02418899	-0.0239	0.7897	0.9983	0.0307	0.8359	0.9329	0.1	0.3876	0.841067
cg02846790	0.0589	0.3935	0.830722	-0.0105	0.9329	0.9329	-0.0195	0.8211	0.976041
cg03447880	-0.0453	0.322	0.830722	-0.0539	0.5013	0.793725	-0.0366	0.5312	0.841067
cg04879561	-0.0016	0.9859	0.9983	-0.1654	0.2648	0.54188	0.0909	0.4223	0.841067
cg10000602	-0.1618	0.1722	0.670383	-0.4688	0.012	0.228	-0.0014	0.9929	0.9951
cg11218385	-0.0547	0.3655	0.830722	-0.0556	0.6069	0.887008	-0.0797	0.2933	0.841067
cg12140543	0.1192	0.2112	0.670383	0.0215	0.8924	0.9329	0.068	0.584	0.853538
cg13886135	0.0369	0.693	0.9983	-0.3401	0.04424	0.366637	-0.089	0.4496	0.841067
cg14785679	0.0227	0.7944	0.9983	-0.2577	0.05789	0.366637	-0.0187	0.8704	0.976041
cg16621855	0.0237	0.6881	0.9983	-0.0185	0.853	0.9329	0.012	0.8733	0.976041

cg17822807	0.0309	0.6739	0.9983	0.2252	0.09804	0.406283	0.0645	0.4884	0.841067
cg18421840	2.00E-04	0.9983	0.9983	-0.1469	0.2852	0.54188	6.00E-04	0.9951	0.9951
cg19317517	-0.1125	0.1589	0.670383	-0.2136	0.1283	0.406283	0.077	0.4528	0.841067
cg21619594	-0.0942	0.2117	0.670383	-0.1646	0.1855	0.440563	0.1132	0.2545	0.841067
cg21844005	-0.0281	0.7024	0.9983	0.1969	0.1213	0.406283	0.0733	0.4511	0.841067
cg22963629	-0.0108	0.8824	0.9983	-0.1733	0.1785	0.440563	0.0236	0.804	0.976041
cg24384519	-0.0066	0.9329	0.9983	-0.0557	0.6671	0.90535	0.2632	0.0117	0.2223
cg25195987	0.1254	0.1718	0.670383	-0.0373	0.8174	0.9329	0.1264	0.2964	0.841067

BDNF

	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00298481	-0.1265	0.285	0.855536	0.1718	0.3485	0.7675	0.097	0.5367	0.934125
cg01418645	0.078	0.3517	0.855536	-0.0977	0.4971	0.771184	0.1157	0.2892	0.934125
cg05189570	0.0183	0.8676	0.915208	-0.107	0.573	0.771184	0.08	0.5722	0.934125
cg06025631	0.0686	0.4779	0.855536	0.2301	0.1765	0.735417	0.0388	0.7473	0.934125
cg06260077	-0.0075	0.938	0.938	0.0983	0.5861	0.771184	-0.0024	0.9835	0.9835
cg06979684	-0.0738	0.3139	0.855536	-0.2008	0.1077	0.6975	-0.1534	0.09701	0.934125
cg07159484	0.0165	0.7782	0.915208	0.1106	0.2676	0.7675	0.1126	0.1304	0.934125
cg07238832	-0.0485	0.6191	0.915208	-0.2511	0.134	0.6975	0.0158	0.8999	0.940833
cg08388004	0.1013	0.2613	0.855536	-0.1435	0.3347	0.7675	0.1149	0.3399	0.934125
cg09492354	-0.0477	0.6261	0.915208	0.0184	0.9114	0.96625	-0.1694	0.1906	0.934125
cg10558494	-0.0967	0.2255	0.855536	-0.2112	0.1395	0.6975	0.2338	0.01978	0.4945
cg14291693	-0.0895	0.3045	0.855536	-0.0999	0.4771	0.771184	0.0543	0.6402	0.934125
cg15014679	-0.0583	0.4299	0.855536	0.0429	0.7265	0.864881	-0.0516	0.5985	0.934125
cg15313332	0.0617	0.4583	0.855536	0.0624	0.6666	0.83325	0.0605	0.5703	0.934125
cg18117895	-0.0904	0.2966	0.855536	-0.1263	0.4298	0.7675	-0.0523	0.6224	0.934125
cg18354203	-0.0276	0.6872	0.915208	-0.1422	0.2245	0.7675	-0.033	0.7032	0.934125
cg18595174	-0.0445	0.5585	0.915208	-0.0171	0.8961	0.96625	-0.0218	0.8232	0.940833
cg20108357	0.0865	0.2285	0.855536	-0.1808	0.1364	0.6975	0.0169	0.8554	0.940833
cg20954537	-0.0354	0.7109	0.915208	0.0945	0.5578	0.771184	0.1082	0.3856	0.934125
cg23426002	-0.0692	0.4791	0.855536	-0.1361	0.4151	0.7675	-0.0407	0.7343	0.934125
cg23619332	-0.0184	0.8786	0.915208	-0.0191	0.9276	0.96625	0.0976	0.5288	0.934125
cg23947039	0.1282	0.01451	0.36275	0.221	0.03315	0.6975	0.0266	0.6894	0.934125
cg25962210	0.0639	0.4228	0.855536	0.1512	0.3036	0.7675	0.0548	0.5954	0.934125
cg26057780	-0.0133	0.864	0.915208	-0.0024	0.987	0.987	-0.0114	0.9032	0.940833
cg27193031	-0.0243	0.7932	0.915208	-0.1441	0.3757	0.7675	0.0579	0.6272	0.934125

COMT

	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00107488	0.0065	0.9401	0.9802	0.1968	0.1889	0.611578	-0.1524	0.1805	0.736447
cg00465975	-0.0069	0.9244	0.9802	0.07	0.5822	0.860119	-0.0169	0.8565	0.8834

cg03205258	-0.005	0.8893	0.9802	-0.0318	0.5999	0.860119	-0.0068	0.8834	0.8834
cg03724721	-0.0253	0.7949	0.9802	0.0681	0.7012	0.860119	0.0843	0.478	0.736447
cg04856117	-2.00E-04	0.9983	0.9983	-0.1824	0.1728	0.611578	0.0153	0.8826	0.8834
cg06045576	0.1157	0.204	0.935894	0.0784	0.6336	0.860119	-0.2391	0.03039	0.464
cg06346307	0.0518	0.5645	0.935894	-0.4143	0.004372	0.126788	0.0222	0.8528	0.8834
cg06787004	0.108	0.2796	0.935894	0.0729	0.6573	0.860119	0.1988	0.1327	0.736447
cg06860277	0.0781	0.4868	0.935894	0.0689	0.7224	0.860119	0.0402	0.7786	0.8834
cg07579946	0.0504	0.5809	0.935894	-0.0743	0.6332	0.860119	0.1277	0.284	0.736447
cg08289189	-0.0089	0.8944	0.9802	-0.0694	0.5459	0.860119	0.0445	0.6038	0.833813
cg08730070	0.1632	0.04414	0.320015	0.2221	0.1532	0.611578	0.0999	0.3163	0.736447
cg09926649	-0.0831	0.2905	0.935894	-0.169	0.1792	0.611578	-0.0159	0.8779	0.8834
cg10122187	0.0221	0.7038	0.971914	-0.0258	0.7844	0.860119	-0.0853	0.2765	0.736447
cg10253022	0.0793	0.3768	0.935894	-0.1159	0.4682	0.860119	0.0839	0.4529	0.736447
cg11361387	-0.0887	0.1439	0.83462	-0.1254	0.1898	0.611578	0.0718	0.3931	0.736447
cg12728623	0.0632	0.5121	0.935894	0.1581	0.3218	0.777683	-0.0564	0.6594	0.833813
cg13175282	0.2176	0.01142	0.320015	0.0379	0.8008	0.860119	0.1246	0.2558	0.736447
cg16834011	0.1582	0.04337	0.320015	0.0384	0.7707	0.860119	0.2201	0.032	0.464
cg18731680	-0.0442	0.4924	0.935894	0.0352	0.7272	0.860119	-0.0702	0.4219	0.736447
cg18773129	0.0376	0.6234	0.951505	0.0064	0.9598	0.9598	0.0447	0.6613	0.833813
cg19930203	-0.162	0.0234	0.320015	-0.0174	0.8799	0.911325	0.0444	0.6474	0.833813
cg20709110	-0.0033	0.9464	0.9802	0.0964	0.244	0.7076	-0.0699	0.2569	0.736447
cg21905167	0.0423	0.5738	0.935894	-0.0626	0.6084	0.860119	-0.1944	0.05099	0.492903
cg21919834	0.0318	0.6771	0.971914	-0.2054	0.1658	0.611578	0.1107	0.233	0.736447
cg22546130	-0.0231	0.7766	0.9802	-0.3316	0.01465	0.212425	0.186	0.07032	0.50982
cg23601416	0.0841	0.32	0.935894	-0.147	0.3039	0.777683	0.0963	0.3826	0.736447
cg25836061	-0.0451	0.4905	0.935894	-0.1444	0.1616	0.611578	0.0652	0.4825	0.736447
cg27521571	0.0523	0.4265	0.935894	-0.05	0.6345	0.860119	0.0763	0.3681	0.736447

CRH									
	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00603617	0.1035	0.2519	0.431829	0.0085	0.958	0.958	-0.0175	0.8787	0.8787
cg03405789	-0.1706	0.02053	0.10688	0.0112	0.936	0.958	-0.1043	0.2391	0.35865
cg08215831	0.07	0.1503	0.3006	-0.0141	0.8841	0.958	0.1449	0.01404	0.08424
cg15971888	-0.0269	0.7758	0.7758	-0.1252	0.497	0.958	-0.0546	0.6326	0.75912
cg17305181	-0.0466	0.3381	0.4508	-0.0711	0.4154	0.958	0.0911	0.1394	0.248914
cg18640030	0.1051	0.02672	0.10688	0.0258	0.7515	0.958	0.089	0.1452	0.248914
cg19035496	0.0306	0.5702	0.68424	0.2023	0.04534	0.54408	0.0972	0.1404	0.248914
cg20329958	-0.1223	0.1017	0.24408	-0.1603	0.1738	0.958	-0.1859	0.05638	0.18723
cg21240762	0.1022	0.3175	0.4508	0.1002	0.5859	0.958	0.029	0.8195	0.8787
cg21878188	0.048	0.6343	0.691964	-0.1515	0.3789	0.958	0.2398	0.06241	0.18723
cg23027580	0.2001	0.009545	0.10688	0.0882	0.5185	0.958	0.2669	0.005015	0.06018
cg23409074	0.0995	0.0688	0.2064	-0.0484	0.6452	0.958	0.0608	0.3636	0.4848

CRHR1									
	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00022871	0.0498	0.637	0.975	-0.0833	0.63	0.987553	0.0666	0.624	0.763688
cg00025823	0.0099	0.8965	0.975	0.0873	0.5061	0.987553	0.0659	0.5093	0.730414
cg04856689	-0.0952	0.1	0.823175	-0.156	0.128	0.608	-0.0457	0.5304	0.730414
cg07778819	-0.0903	0.1733	0.823175	0.0083	0.9416	0.9922	0.0538	0.5382	0.730414
cg11338426	-0.172	0.1522	0.823175	-0.3406	0.1064	0.608	-0.0979	0.506	0.730414
cg11731737	0.0045	0.9309	0.975	-0.0199	0.8275	0.987553	0.0164	0.8008	0.845289
cg13521908	-0.1154	0.2961	0.892156	0.0257	0.8836	0.987553	-0.2714	0.0674	0.730414
cg13947929	-0.054	0.4226	0.892156	-0.0012	0.9922	0.9922	0.0683	0.4239	0.730414
cg15607306	0.066	0.4151	0.892156	0.0319	0.8166	0.987553	0.0015	0.9889	0.9889
cg16642545	0.0608	0.4768	0.90592	-0.1068	0.4444	0.987553	-0.1061	0.3516	0.730414
cg16830379	-0.0824	0.3382	0.892156	-0.2499	0.08074	0.608	-0.0785	0.4758	0.730414
cg18534039	-0.0078	0.9323	0.975	-0.0653	0.7171	0.987553	0.0503	0.6598	0.763688
cg24063856	-0.0368	0.5671	0.975	-0.0778	0.5036	0.987553	-0.0333	0.6833	0.763688
cg24353392	0.003	0.975	0.975	-0.1281	0.4467	0.987553	0.1821	0.1323	0.730414
cg24394631	0.0156	0.7464	0.975	-0.0667	0.4246	0.987553	0.0592	0.3558	0.730414
cg26656751	-0.0145	0.7419	0.975	-0.1694	0.0119	0.2261	-0.0373	0.5311	0.730414
cg27410679	0.0867	0.3052	0.892156	0.0731	0.5889	0.987553	-0.0864	0.4364	0.730414
cg27503360	-0.1991	0.05718	0.823175	-0.0279	0.8809	0.987553	-0.18	0.1794	0.730414
cg27551605	-0.0097	0.8815	0.975	0.0193	0.8578	0.987553	0.1369	0.1014	0.730414

FHIT									
	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00071984	0.1004	0.174	0.72501	-0.02	0.8903	0.9912	0.094	0.2899	0.9447
cg00506250	-0.0727	0.5265	0.982695	-0.1749	0.3757	0.9912	-0.003	0.9838	0.9951
cg00658590	0.1067	0.2702	0.820393	0.0401	0.8001	0.9912	0.0962	0.436	0.989733
cg00721771	0.0645	0.3802	0.982695	0.0014	0.9912	0.9912	0.1003	0.31	0.9447
cg01556706	0.0182	0.7415	0.982695	-0.146	0.1233	0.80145	-0.0193	0.7822	0.9951
cg02923224	0.0343	0.6361	0.982695	-0.0808	0.5541	0.9912	0.046	0.613	0.9951
cg03060986	0.2573	0.002328	0.090792	-0.1763	0.2237	0.9912	0.1497	0.1831	0.826367
cg03319184	-0.0027	0.9677	0.9892	0.0017	0.9877	0.9912	-0.0384	0.6607	0.9951
cg03610148	0.1937	0.05035	0.63102	-0.0018	0.991	0.9912	0.2637	0.04792	0.6357
cg04216480	0.0827	0.2945	0.820393	-0.026	0.8453	0.9912	0.1799	0.08276	0.6357
cg04383442	0.1798	0.05617	0.63102	-0.148	0.3314	0.9912	0.0331	0.7881	0.9951
cg04835638	0.1208	0.1859	0.72501	0.0535	0.7414	0.9912	0.2417	0.0362	0.6357
cg05645292	0.1099	0.1488	0.72501	0.0926	0.4818	0.9912	-0.0221	0.8179	0.9951
cg05709770	-0.1124	0.1202	0.72501	-0.2657	0.03914	0.305292	0.01	0.9124	0.9951
cg07351758	-0.0112	0.9048	0.982695	-0.6192	0.000274	**0.010694**	0.1809	0.1136	0.6357
cg08223225	-0.0015	0.9892	0.9892	-0.0515	0.8039	0.9912	0.2412	0.07833	0.6357

cg10763247	0.1066	0.2341	0.820393	-0.1009	0.4984	0.9912	0.0111	0.9248	0.9951
cg11815980	-0.0256	0.7883	0.982695	-0.0703	0.6783	0.9912	0.0922	0.4568	0.989733
cg13679804	0.0153	0.7701	0.982695	0.0627	0.4471	0.9912	0.0545	0.456	0.989733
cg13745692	0.0543	0.5126	0.982695	0.0468	0.7276	0.9912	0.0724	0.5194	0.9951
cg14147855	-0.0305	0.7321	0.982695	-0.0088	0.9542	0.9912	7.00E-04	0.9951	0.9951
cg15135842	0.0198	0.8306	0.982695	-0.1549	0.3627	0.9912	-0.0535	0.6371	0.9951
cg15238012	-0.0655	0.4165	0.982695	-0.1304	0.3482	0.9912	0.0924	0.3613	0.989733
cg15970800	0.0303	0.7785	0.982695	0.0448	0.8196	0.9912	0.02	0.8825	0.9951
cg16806041	-0.0484	0.5667	0.982695	-0.0395	0.7892	0.9912	0.0181	0.8687	0.9951
cg17087356	0.1739	0.06472	0.63102	0.1753	0.2861	0.9912	0.0325	0.7865	0.9951
cg17894779	0.0977	0.2918	0.820393	-0.0815	0.6151	0.9912	0.1838	0.1141	0.6357
cg19282443	0.1679	0.1161	0.72501	0.0901	0.6283	0.9912	-0.0136	0.92	0.9951
cg19729536	-0.0066	0.9323	0.982695	-0.0545	0.6815	0.9912	-0.0207	0.8386	0.9951
cg20366397	0.0305	0.7811	0.982695	-0.1105	0.5522	0.9912	0.0604	0.6695	0.9951
cg20517149	-0.0577	0.4933	0.982695	-0.3129	0.02887	0.305292	-0.0653	0.5301	0.9951
cg22380007	0.0081	0.8393	0.982695	-0.2264	0.00176	0.03432	0.0521	0.2877	0.9447
cg22533480	-0.0134	0.8636	0.982695	0.0472	0.7022	0.9912	-0.181	0.07068	0.6357
cg23222057	-0.0064	0.9299	0.982695	-0.2589	0.03471	0.305292	-0.0534	0.5666	0.9951
cg23737061	-0.0633	0.4357	0.982695	-0.09	0.4774	0.9912	-0.0899	0.4052	0.989733
cg25724751	0.0331	0.6593	0.982695	-0.1619	0.1836	0.9912	-0.0011	0.9913	0.9951
cg25921543	0.044	0.6272	0.982695	0.0909	0.5333	0.9912	0.1528	0.1907	0.826367
cg26358659	0.0211	0.8371	0.982695	-0.2083	0.247	0.9912	0.0218	0.8667	0.9951
cg27254860	0.105	0.1714	0.72501	0.0114	0.933	0.9912	0.0976	0.3149	0.9447

FKBP5									
	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00140191	-0.0095	0.9156	0.9156	-0.076	0.6362	0.956114	0.0457	0.682	0.909333
cg00862770	-0.0144	0.875	0.9156	-0.2893	0.05151	0.41208	0.0807	0.496	0.878109
cg02665568	0.0397	0.6025	0.9156	-0.0746	0.5369	0.956114	-0.0551	0.5872	0.878109
cg03546163	0.0154	0.8468	0.9156	-0.0303	0.8366	0.956114	0.0743	0.4578	0.878109
cg06087101	-0.1129	0.09849	0.7904	-0.1248	0.3068	0.867733	0.0451	0.6037	0.878109
cg07061368	-0.0127	0.8722	0.9156	-0.0317	0.8151	0.956114	-0.0798	0.4227	0.878109
cg07633853	-0.037	0.6138	0.9156	-0.2626	0.0283	0.41208	-0.172	0.07264	0.878109
cg08586216	-0.0167	0.8784	0.9156	0.1918	0.3254	0.867733	0.0043	0.975	0.975
cg10300814	0.0487	0.6327	0.9156	-0.0059	0.9747	0.9852	0.0242	0.8557	0.94528
cg10913456	-0.0824	0.2729	0.9156	-0.1859	0.1979	0.867733	-0.0489	0.5884	0.878109
cg14284211	-0.0296	0.5765	0.9156	0.0247	0.7918	0.956114	0.0098	0.8862	0.94528
cg14642437	0.0135	0.8462	0.9156	-0.0527	0.6653	0.956114	0.1002	0.2648	0.878109
cg16052510	0.0568	0.3671	0.9156	-0.0764	0.4941	0.956114	0.0474	0.5502	0.878109
cg17085721	0.0286	0.736	0.9156	0.0026	0.9852	0.9852	0.0953	0.3893	0.878109
cg18726036	0.112	0.09913	0.7904	-0.0578	0.6615	0.956114	-0.0273	0.7471	0.919508
cg19014730	0.1435	0.1482	0.7904	-0.2024	0.2339	0.867733	0.1177	0.3461	0.878109

GAD1									
	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00224929	-0.1151	0.2565	0.582955	-0.0017	0.9926	0.9926	0.089	0.4797	0.9953
cg00729049	-0.0639	0.3315	0.6375	-0.0601	0.5836	0.892059	-0.0209	0.7982	0.9953
cg01089249	0.0021	0.9756	0.9756	-0.3051	0.006905	0.172625	0.0619	0.4815	0.9953
cg01089319	-0.0659	0.1748	0.517222	-0.1306	0.1276	0.556591	-0.0415	0.4882	0.9953
cg01763173	0.1152	0.144	0.517222	-0.1679	0.2026	0.556591	0.0336	0.7518	0.9953
cg02723395	0.0045	0.9298	0.968542	-0.1428	0.111	0.556591	0.0019	0.9766	0.9953
cg04105250	0.0493	0.4382	0.695313	-0.0047	0.9669	0.9926	0.1051	0.1991	0.9953
cg07420274	-0.0169	0.7377	0.922125	-0.0281	0.7614	0.964643	5.00E-04	0.9942	0.9953
cg08863440	-0.1331	0.006858	0.17145	0.0072	0.9323	0.9926	-0.006	0.9258	0.9953
cg09144707	0.0111	0.8725	0.968542	0.0548	0.6711	0.932083	0.0955	0.2618	0.9953
cg11281641	0.0324	0.6359	0.922125	-0.0311	0.8103	0.964643	-0.0278	0.7345	0.9953
cg11348701	-0.1768	0.0494	0.30875	-0.0967	0.5006	0.892059	-0.0689	0.5692	0.9953
cg11582100	0.0139	0.8867	0.968542	-0.0867	0.6066	0.892059	0.0358	0.7731	0.9953
cg14005211	-0.0698	0.04549	0.30875	-0.12	0.0499	0.425417	-0.0434	0.3229	0.9953
cg14486905	-0.0578	0.1862	0.517222	-0.0986	0.1816	0.556591	0.064	0.252	0.9953
cg14914809	-0.0296	0.7286	0.922125	-0.184	0.2449	0.556591	-0.0326	0.752	0.9953
cg15126544	0.0712	0.1489	0.517222	-0.1252	0.1661	0.556591	0.1095	0.06797	0.849625
cg15306595	-0.2275	0.03319	0.30875	-0.117	0.5319	0.892059	0.0297	0.8302	0.9953
cg15753746	-0.1095	0.1203	0.517222	0.1533	0.167	0.556591	6.00E-04	0.9953	0.9953
cg16911423	0.089	0.445	0.695313	-0.0499	0.8094	0.964643	0.0822	0.5667	0.9953
cg19009018	0.0927	0.2564	0.582955	-0.1659	0.2248	0.556591	0.0544	0.6077	0.9953
cg19538089	-0.0079	0.9169	0.968542	-0.0214	0.8637	0.981477	0.0853	0.3836	0.9953
cg19846314	-0.0378	0.3886	0.693929	0.1399	0.05105	0.425417	-0.0109	0.8495	0.9953
cg21535772	-0.0975	0.3259	0.6375	-0.0975	0.5732	0.892059	0.0456	0.7215	0.9953
cg26391350	-0.0384	0.6655	0.922125	-0.0802	0.5764	0.892059	0.23	0.05303	0.849625

HTR1A									
	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg01020744	0.0106	0.8343	0.903825	0.0908	0.3018	0.594425	0.0226	0.7247	0.817267
cg02266732	-0.0476	0.3816	0.674818	0.0273	0.792	0.812	0.0213	0.7544	0.817267
cg04694812	-0.1448	0.01779	0.23127	-0.1747	0.07498	0.324913	0.0712	0.4038	0.61061
cg04799838	0.1181	0.1346	0.660833	0.0964	0.4784	0.616318	0.138	0.1705	0.484714
cg07839533	-0.0996	0.1525	0.660833	-0.1644	0.1596	0.5187	-0.1075	0.2272	0.484714
cg08259925	0.0332	0.571	0.674818	0.068	0.5215	0.616318	0.0904	0.2423	0.484714
cg09698471	0.0487	0.477	0.674818	-0.2473	0.03323	0.324913	-0.0121	0.8856	0.8856
cg10588470	0.0412	0.3416	0.674818	0.1407	0.06787	0.324913	0.0609	0.261	0.484714
cg13666507	-0.052	0.3586	0.674818	-0.0714	0.4872	0.616318	0.1212	0.09676	0.484714
cg15092168	-0.0382	0.521	0.674818	0.0844	0.3658	0.594425	-0.0968	0.2391	0.484714
cg16280141	-0.0454	0.4468	0.674818	-0.1288	0.2331	0.594425	0.2164	0.003174	**0.041262**

cg23448729	0.0521	0.3248	0.674818	0.0914	0.3227	0.594425	-0.0507	0.4421	0.61061
cg27615388	0.0017	0.9608	0.9608	-0.0139	0.812	0.812	0.0314	0.4697	0.61061

HTR2A

	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00308665	0.0488	0.4868	0.9857	-0.0733	0.5726	0.68712	-0.0276	0.7543	0.9396
cg02250787	-0.0416	0.5508	0.9857	-0.2273	0.07555	0.210432	0.0146	0.8669	0.9396
cg06476131	-0.013	0.8378	0.9857	-0.316	0.004871	**0.029226**	0.1765	0.02728	0.1761
cg09361691	-0.1354	0.03219	0.38628	-0.3527	0.001296	**0.015552**	0.0408	0.6169	0.9396
cg11514288	0.0015	0.9795	0.9857	-0.1098	0.2933	0.43995	0.0407	0.5913	0.9396
cg12089079	-0.0781	0.2738	0.8214	-0.2426	0.08768	0.210432	-0.0283	0.7408	0.9396
cg12367389	0.0055	0.9411	0.9857	-0.0061	0.9661	0.9661	-0.0068	0.9396	0.9396
cg14059288	-0.1516	0.1409	0.7188	-0.2898	0.1119	0.2238	0.2735	0.02935	0.1761
cg15894389	-0.0171	0.7837	0.9857	-0.1105	0.2905	0.43995	0.0515	0.5294	0.9396
cg16188532	-0.0779	0.1797	0.7188	-0.2064	0.03577	0.14308	-0.0227	0.7607	0.9396
cg20102280	-0.0093	0.8987	0.9857	-0.0619	0.6432	0.701673	0.1157	0.2079	0.8316
cg26950475	-0.0013	0.9857	0.9857	-0.0998	0.3847	0.512933	-0.0196	0.8505	0.9396

NPSR1

	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00081087	-0.0708	0.3385	0.866125	-0.0697	0.5941	0.965413	-0.0105	0.9111	0.9111
cg03382549	0.1207	0.1725	0.866125	0.0056	0.9706	0.9706	0.2105	0.05748	0.24908
cg05399607	0.0043	0.9622	0.9622	-0.21	0.1605	0.6435	0.1293	0.2702	0.71916
cg06506864	0.0469	0.3728	0.866125	-0.0798	0.3933	0.748614	0.1704	0.007977	0.09581
cg08251685	-0.0691	0.4108	0.866125	-0.0245	0.8733	0.9706	0.0175	0.8675	0.9111
cg15754660	0.0855	0.09754	0.866125	0.1037	0.2475	0.6435	0.071	0.2766	0.71916
cg17744825	-0.0339	0.722	0.9386	0.0176	0.9133	0.9706	0.119	0.3397	0.736017
cg19194095	0.0039	0.9622	0.9622	-0.1729	0.2049	0.6435	0.0873	0.4303	0.799129
cg20495677	0.0635	0.3644	0.866125	0.0068	0.9536	0.9706	0.0213	0.8139	0.9111
cg20842782	-0.0159	0.8542	0.9622	0.1281	0.4031	0.748614	0.0262	0.8106	0.9111
cg23448390	-0.0662	0.533	0.866125	-0.4318	0.02289	0.29757	0.0512	0.6924	0.9111
cg23862011	0.0454	0.6039	0.8723	-0.0116	0.9414	0.9706	0.038	0.7336	0.9111
cg24929847	0.0733	0.4825	0.866125	-0.3039	0.08601	0.559065	0.3266	0.01474	0.09581

OXTR

	Whole			Males			Females		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00078085	-0.0551	0.3384	0.661556	0.0346	0.7427	0.881282	-0.0451	0.5208	0.9555
cg00385883	0.162	0.1131	0.661556	-0.0794	0.6432	0.881282	0.3078	0.02286	0.169975
cg02192228	0.088	0.1461	0.661556	0.0399	0.6974	0.881282	-0.0012	0.9884	0.9884

cg03987506	-0.0473	0.4157	0.661556	0.0186	0.8568	0.9273	-0.0361	0.6191	0.9555
cg04523291	0.0508	0.3698	0.661556	-0.0093	0.9273	0.9273	0.1559	0.02615	0.169975
cg08535600	0	0.9995	0.9995	-0.1099	0.2265	0.881282	0.0181	0.7881	0.9555
cg09353063	0.0666	0.2741	0.661556	0.0388	0.7329	0.881282	0.0472	0.541	0.9555
cg12695586	-0.0267	0.6667	0.733092	-0.1694	0.1618	0.881282	0.0337	0.6621	0.9555
cg15317815	0.0197	0.6767	0.733092	0.034	0.6542	0.881282	0.1222	0.05432	0.235387
cg17285225	-0.0627	0.2339	0.661556	-0.0337	0.7149	0.881282	-0.0261	0.6983	0.9555
cg19619174	0.0986	0.2742	0.661556	-0.2726	0.07895	0.881282	0.1683	0.132	0.429
cg23391006	-0.0601	0.458	0.661556	0.048	0.7457	0.881282	-0.0249	0.8162	0.9555
cg27501759	-0.0199	0.5233	0.68029	0.0178	0.7278	0.881282	0.0061	0.882	0.9555

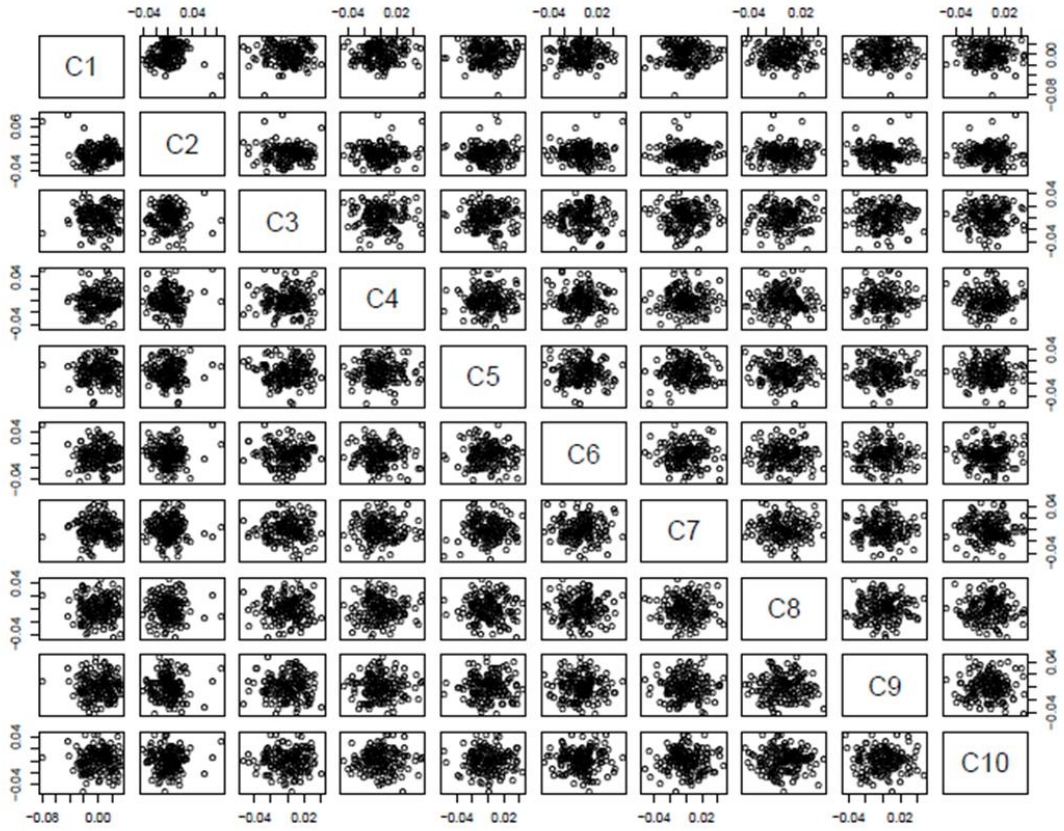
SGK1									
	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg00959636	-0.3081	0.000787	**0.035424**	-0.0941	0.5429	0.939635	-0.0021	0.9856	0.9856
cg01059669	-0.1247	0.1967	0.5901	0.1557	0.337	0.871816	-0.1666	0.1743	0.6678
cg02904344	0.0517	0.5148	0.858	0.1559	0.2766	0.871816	0.0453	0.6538	0.8406
cg03146155	0.0765	0.2543	0.6585	-0.0719	0.5347	0.939635	-0.0878	0.2901	0.713045
cg03400131	-0.0144	0.8348	0.9684	0.02	0.8799	0.960239	0.0422	0.6169	0.824824
cg03762694	-0.1551	0.05531	0.3957	0.1272	0.3681	0.871816	-0.1048	0.3082	0.713045
cg03944089	0.0093	0.8968	0.9684	-0.0517	0.6711	0.953743	0.0929	0.3133	0.713045
cg04060943	0.0431	0.6092	0.877091	-0.1797	0.2277	0.871816	0.0985	0.3486	0.713045
cg04905719	-0.0536	0.3023	0.680175	-0.0263	0.7418	0.953743	-0.085	0.2081	0.6678
cg05183646	-0.0835	0.1623	0.521679	-0.2501	0.02184	0.480488	-0.0899	0.2205	0.6678
cg05966641	0.0029	0.9684	0.9684	-0.2313	0.07036	0.63324	-0.0256	0.7723	0.923566
cg06358608	-0.0329	0.6432	0.877091	0.0762	0.5397	0.939635	0.0724	0.427	0.7686
cg06642177	0.0945	0.007257	0.163283	0.0159	0.7968	0.960239	0.0319	0.494	0.793929
cg06849960	-0.1124	0.2532	0.6585	-0.2079	0.2853	0.871816	-0.1347	0.2537	0.713045
cg07340870	0.2117	0.05854	0.3957	0.2113	0.2364	0.871816	0.2343	0.1282	0.6678
cg08239804	0.0299	0.7262	0.961147	-0.297	0.03905	0.480488	-0.0556	0.6232	0.824824
cg08550353	0.0321	0.6409	0.877091	0.1066	0.3565	0.871816	0.0638	0.468	0.788167
cg08640361	0.1429	0.1342	0.50325	0.0185	0.9075	0.960239	-0.1126	0.3455	0.713045
cg08647910	-0.0548	0.4741	0.826442	-5.00E-04	0.9974	0.9974	-0.0064	0.9465	0.9856
cg08698685	0.0084	0.8757	0.9684	-0.0367	0.6733	0.953743	0.0174	0.8055	0.929423
cg09315391	-0.004	0.9668	0.9684	-0.112	0.447	0.939635	0.0928	0.4729	0.788167
cg09404376	0.1271	0.0718	0.3957	0.101	0.416	0.936	0.1743	0.05934	0.5841
cg09872934	-0.0159	0.8251	0.9684	-0.1379	0.2846	0.871816	-0.0184	0.8376	0.9423
cg10105971	-0.0449	0.6336	0.877091	-0.0271	0.8566	0.960239	-0.0832	0.5142	0.797897
cg11856561	0.0101	0.9266	0.9684	-0.2646	0.1481	0.833063	0.1756	0.2106	0.6678
cg12009778	0.0321	0.7607	0.9684	-0.3621	0.04271	0.480488	0.2384	0.07788	0.5841
cg13307058	0.1	0.09686	0.43587	0.0762	0.5068	0.939635	0.0021	0.9774	0.9856
cg14905466	0.0363	0.5627	0.877091	0.0397	0.7217	0.953743	0.0038	0.9607	0.9856
cg17284168	-0.0591	0.5934	0.877091	0.0893	0.6447	0.953743	-0.0827	0.5454	0.8181

cg17689707	-0.1106	0.158	0.521679	0.0208	0.8779	0.960239	-0.0498	0.6197	0.824824
cg18566177	0.0087	0.9256	0.9684	-0.012	0.9389	0.960239	0.0361	0.7638	0.923566
cg20393620	-0.0121	0.8861	0.9684	0.0443	0.7363	0.953743	0.0178	0.8794	0.965195
cg20655113	0.0267	0.7832	0.9684	0.0691	0.7113	0.953743	0.1472	0.2075	0.6678
cg20822858	-0.0811	0.4775	0.826442	0.0205	0.9191	0.960239	0.169	0.2226	0.6678
cg21064939	-0.1945	0.04844	0.3957	-0.2798	0.1248	0.833063	-0.3021	0.0128	0.192
cg21078322	0.0748	0.3227	0.6915	-0.0365	0.7874	0.960239	-0.085	0.3702	0.724304
cg21366688	0.0704	0.3438	0.703227	-0.0606	0.627	0.953743	0.1606	0.09767	0.627879
cg21676440	-0.1223	0.2896	0.680175	-0.2162	0.2948	0.871816	-0.2585	0.07001	0.5841
cg21834463	0.037	0.4706	0.826442	-0.0832	0.3456	0.871816	-0.1629	0.01225	0.192
cg23347562	0.0789	0.457	0.826442	-0.0389	0.8407	0.960239	0.1646	0.2177	0.6678
cg24688636	-0.1439	0.06378	0.3957	-0.2034	0.1302	0.833063	-0.2642	0.006513	0.192
cg25025235	-0.0972	0.2634	0.6585	-0.095	0.5276	0.939635	-0.0643	0.5671	0.82321
cg25661219	-0.156	0.07914	0.3957	-0.3501	0.03569	0.480488	-0.0299	0.7799	0.923566
cg26557834	0.1684	0.03655	0.3957	0.1328	0.3486	0.871816	0.1004	0.3188	0.713045
cg27289153	0.1291	0.1258	0.50325	-0.0731	0.6134	0.953743	0.0838	0.423	0.7686

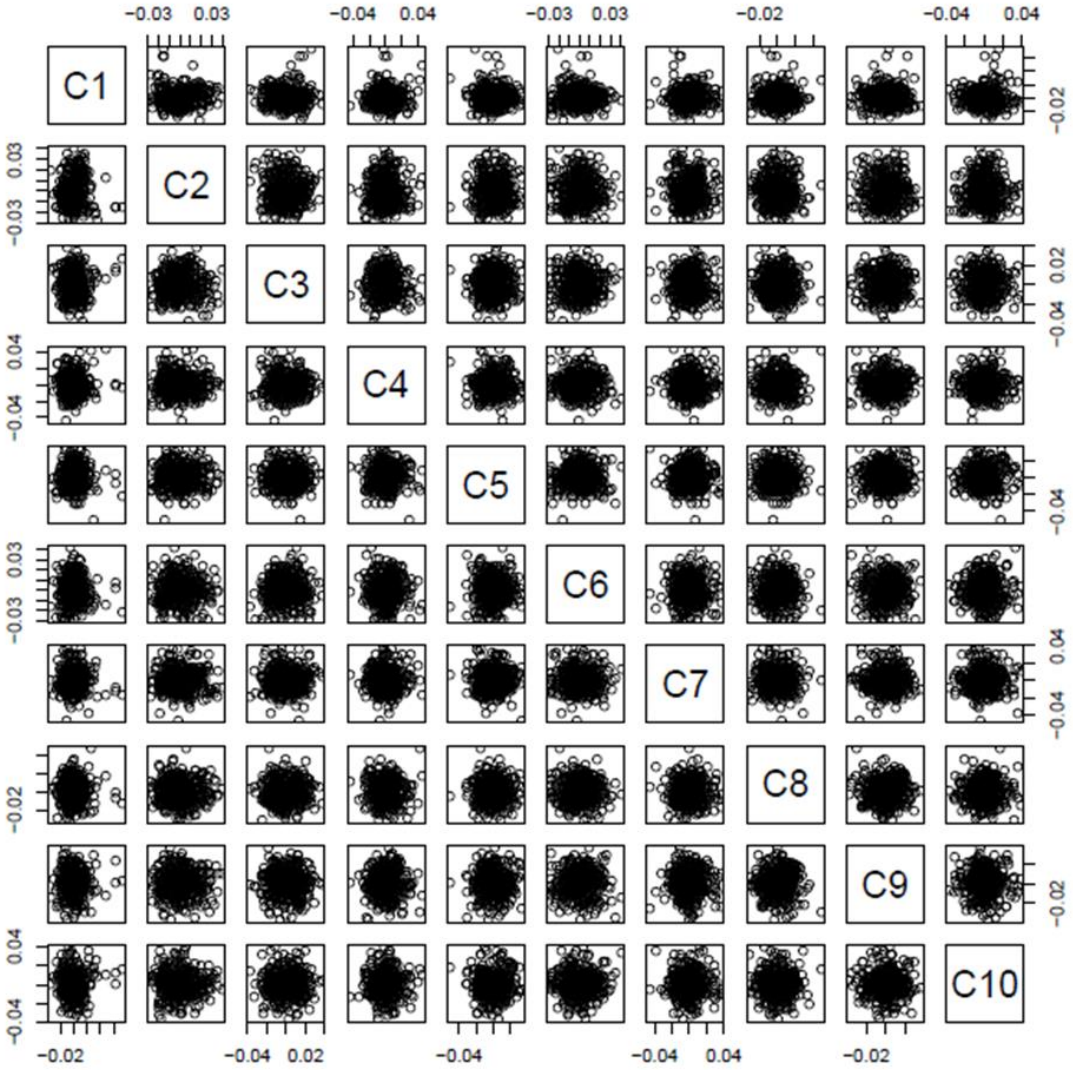
TMEM132D									
	Whole			**Males**			**Females**		
Probe	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**	**Beta**	**P-value**	**FDR (Gene)**
cg01163404	0.1089	0.1225	0.601033	0.2281	0.05266	0.410459	0.0125	0.8917	0.9854
cg01202700	0.2627	0.01158	0.211335	0.3553	0.05984	0.410459	0.2938	0.02614	0.432744
cg01831527	-0.0222	0.7099	0.94071	0.0595	0.5857	0.92345	-0.0815	0.2604	0.746164
cg02365079	0.1364	0.04654	0.283118	0.2054	0.08221	0.500111	0.0107	0.9031	0.9854
cg02767665	0.0241	0.7505	0.94071	0.0209	0.8775	0.957934	0.0881	0.3673	0.812512
cg03283235	-0.0326	0.6895	0.94071	0.0119	0.9325	0.9775	-0.0019	0.9854	0.9854
cg03420866	-0.0508	0.537	0.94071	0.1719	0.2448	0.770758	-0.0181	0.8623	0.9854
cg03469054	0.0441	0.4531	0.94071	-0.0715	0.5112	0.92345	0.1036	0.1493	0.746164
cg03685843	-0.0021	0.9685	0.981951	-0.1253	0.2045	0.746425	-0.0747	0.2763	0.746164
cg04386563	-0.0081	0.8993	0.976613	-0.117	0.2773	0.778573	0.0609	0.4494	0.849815
cg04414975	-0.081	0.3413	0.94071	-0.307	0.02555	0.410459	-0.0691	0.5355	0.849815
cg04729491	0.0398	0.7088	0.94071	-0.0291	0.8782	0.957934	-0.0106	0.9377	0.9854
cg04925956	0.0389	0.5332	0.94071	-0.0241	0.8394	0.957934	-0.0518	0.4992	0.849815
cg05160910	0.0375	0.6074	0.94071	0.2321	0.06185	0.410459	0.0973	0.3156	0.794441
cg05384697	-0.1853	0.04234	0.280984	-0.1445	0.4073	0.838892	-0.1897	0.08699	0.58473
cg05479657	0.0645	0.4126	0.94071	-0.0498	0.6908	0.92345	-0.0398	0.6969	0.97721
cg05742082	-0.0227	0.7898	0.960923	-0.0993	0.564	0.92345	0.0962	0.3383	0.796642
cg06200996	-0.0308	0.5584	0.94071	-0.0505	0.5793	0.92345	-0.0432	0.5236	0.849815
cg06679878	-0.0165	0.8482	0.967478	-0.3303	0.02196	0.410459	-0.2043	0.06154	0.546364
cg07056260	-0.0643	0.3118	0.94071	-0.1391	0.1953	0.746425	-0.0224	0.7859	0.97721
cg07067993	-0.0376	0.4332	0.94071	-0.0713	0.3777	0.838892	0.0873	0.1595	0.746164
cg07230440	0.0776	0.1235	0.601033	0.0329	0.7351	0.941444	-0.0527	0.3882	0.819269
cg07350016	0.0513	0.5975	0.94071	-0.0794	0.6195	0.92345	0.039	0.7624	0.97721

cg08261450	0.0405	0.5438	0.94071	-0.1287	0.2721	0.778573	-0.0408	0.6287	0.936635
cg08546107	-0.0013	0.9872	0.9872	-0.1925	0.2152	0.748076	0.1208	0.2362	0.746164
cg09044656	0.0068	0.9204	0.976613	0.0904	0.4137	0.838892	0.0965	0.2862	0.746164
cg10639585	0.028	0.6388	0.94071	0.046	0.6713	0.92345	0.0061	0.9347	0.9854
cg11023224	0.0052	0.9411	0.981433	-0.1195	0.3893	0.838892	0.1089	0.1996	0.746164
cg11160362	0.108	0.6069	0.94071	0.0122	0.9699	0.9775	-0.2488	0.3928	0.819269
cg11230248	0.0825	0.2952	0.94071	0.1943	0.1686	0.733865	0.0137	0.89	0.9854
cg11496226	-0.1054	0.02166	0.225883	-0.0574	0.4759	0.890787	-0.1544	0.006169	0.225169
cg12072740	-0.0412	0.5541	0.94071	-0.2433	0.04073	0.410459	-0.0985	0.266	0.746164
cg12820134	0.0178	0.8193	0.967478	-0.1296	0.3286	0.814923	-0.0641	0.529	0.849815
cg13090220	0.1599	0.04151	0.280984	0.1352	0.3276	0.814923	0.1136	0.2506	0.746164
cg13123585	-0.0419	0.5674	0.94071	-0.1381	0.2477	0.770758	0.003	0.9756	0.9854
cg13916352	-0.0723	0.4593	0.94071	-0.1895	0.2534	0.770758	-0.0561	0.6555	0.93898
cg14504768	-0.0147	0.8417	0.967478	0.071	0.5863	0.92345	0.0547	0.5513	0.856274
cg14918019	0.0365	0.6602	0.94071	-0.0041	0.9775	0.9775	0.0093	0.9281	0.9854
cg15617706	0.2868	0.005854	0.142447	0.0345	0.8459	0.957934	0.2738	0.0431	0.524383
cg15936861	0.0317	0.718	0.94071	-0.2418	0.1079	0.562621	0.1396	0.2007	0.746164
cg16048915	0.056	0.4032	0.94071	-0.0314	0.788	0.957934	0.0605	0.4764	0.849815
cg16533379	0.0551	0.3138	0.94071	0.0192	0.8424	0.957934	0.0035	0.9588	0.9854
cg17157798	-0.0147	0.8296	0.967478	-0.1897	0.1064	0.562621	0.0837	0.3527	0.804597
cg17186073	-0.0772	0.09916	0.556822	-0.112	0.1709	0.733865	-0.047	0.4626	0.849815
cg17444697	0.0559	0.3957	0.94071	0.0451	0.7068	0.92345	0.0866	0.2815	0.746164
cg17513770	0.0176	0.7401	0.94071	-0.0047	0.9605	0.9775	0.104	0.1184	0.720267
cg17718276	-0.0548	0.3384	0.94071	0.2271	0.04171	0.410459	-0.0024	0.972	0.9854
cg17735631	0.0077	0.9231	0.976613	0.1239	0.3474	0.818071	0.0746	0.4832	0.849815
cg17883960	0.031	0.6779	0.94071	-0.0057	0.9644	0.9775	0.0627	0.5133	0.849815
cg18180056	0.0718	0.4296	0.94071	-0.1	0.5439	0.92345	0.0359	0.7525	0.97721
cg18437033	-0.0617	0.5145	0.94071	-0.0232	0.8792	0.957934	0.0698	0.5676	0.863225
cg18723572	0.166	0.01526	0.214377	0.0948	0.4355	0.853908	0.2139	0.01315	0.319983
cg18758559	0.0026	0.9648	0.981951	0.0885	0.4003	0.838892	-0.0786	0.2807	0.746164
cg19070138	0.1849	0.001056	0.077088	0.1522	0.1168	0.568427	-0.0032	0.9649	0.9854
cg19700087	0.0244	0.6669	0.94071	0.0375	0.7084	0.92345	0.1107	0.1331	0.746164
cg19790509	0.0827	0.2906	0.94071	-0.0765	0.5785	0.92345	0.0718	0.4571	0.849815
cg20168964	0.0867	0.1801	0.730406	0.027	0.8083	0.957934	-0.0264	0.7513	0.97721
cg20327057	0.2712	0.003043	0.11107	0.0846	0.6043	0.92345	0.2435	0.02964	0.432744
cg20470734	0.048	0.4505	0.94071	-0.194	0.05933	0.410459	0.0168	0.8443	0.9854
cg21903395	-0.0221	0.6757	0.94071	0.0363	0.7003	0.92345	0.0197	0.7755	0.97721
cg23266743	-0.0606	0.4741	0.94071	-0.0486	0.7506	0.944721	-0.0156	0.8861	0.9854
cg23733052	0.19	0.03625	0.280984	-0.1412	0.3326	0.814923	0.0758	0.5326	0.849815
cg23805623	0.0288	0.5975	0.94071	-0.181	0.06041	0.410459	0.0655	0.3302	0.796642
cg23917477	-0.0209	0.7603	0.94071	0.0514	0.6656	0.92345	0.2477	0.004213	0.225169
cg24008358	0.0659	0.1666	0.7154	0.1575	0.06094	0.410459	-0.0162	0.7898	0.97721
cg25015139	-0.0301	0.6985	0.94071	-0.1862	0.1872	0.746425	0.1165	0.231	0.746164

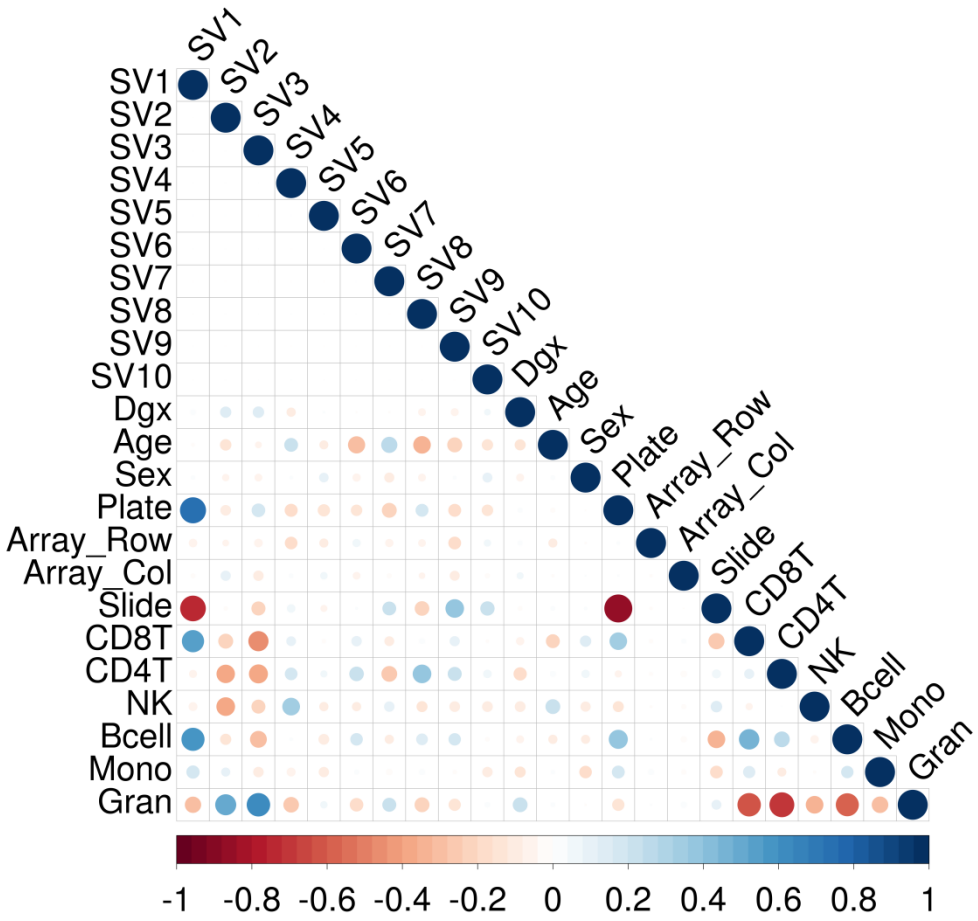
cg25102216	0.1816	0.02538	0.231593	-0.029	0.8573	0.957934	0.1068	0.2708	0.746164
cg25625370	0.0714	0.1421	0.648331	0.0379	0.6591	0.92345	0.1122	0.06271	0.546364
cg26322591	-0.0173	0.7326	0.94071	-0.2447	0.009236	0.410459	0.1045	0.08811	0.58473
cg26364947	0.0555	0.2	0.768421	-0.0056	0.9423	0.9775	0.0241	0.656	0.93898
cg26411747	0.2294	0.01762	0.214377	0.1354	0.4445	0.853908	0.1603	0.1791	0.746164
cg26614129	0.0106	0.8801	0.976613	0.0496	0.686	0.92345	-0.1679	0.06736	0.546364
cg27463181	0.0093	0.892	0.976613	0.1093	0.3349	0.814923	-0.031	0.7374	0.97721



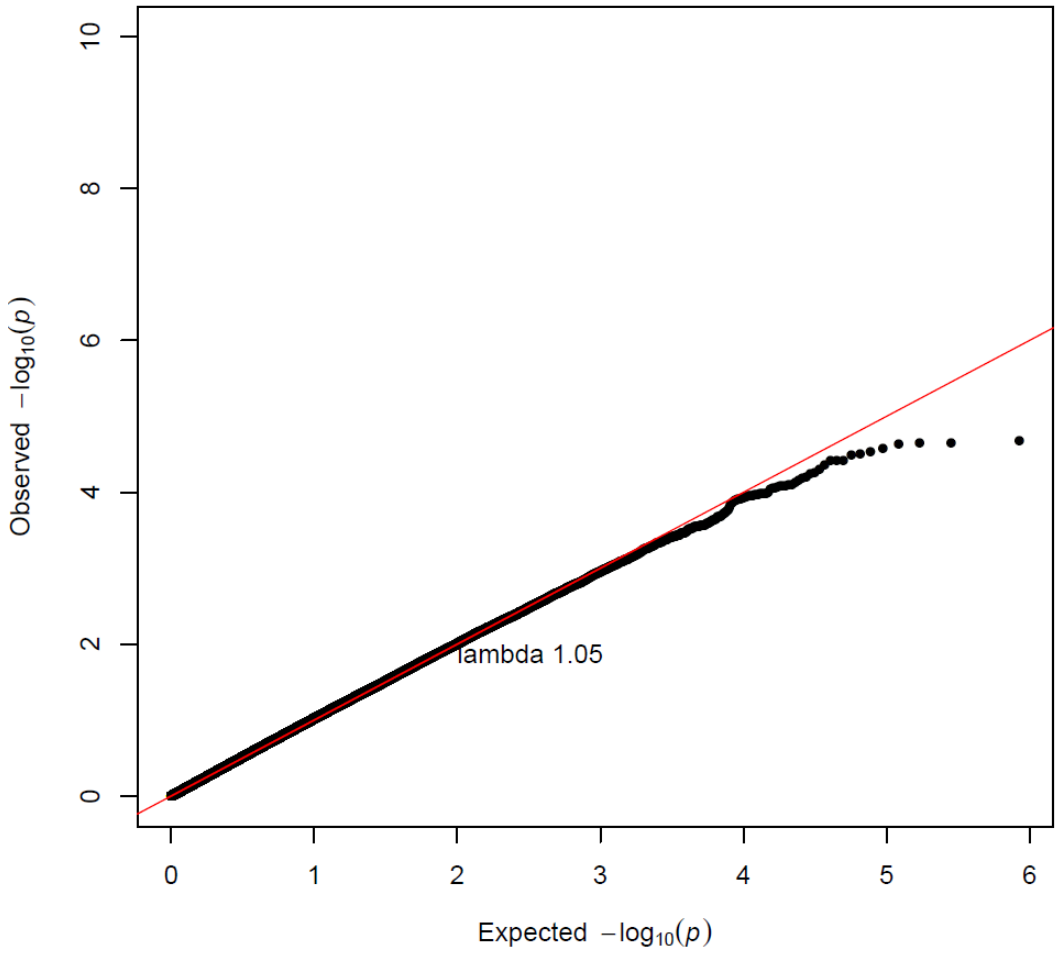
Supplementary Figure 1.
Multidimensional scaling (MDS) plots used to investigate population structure in the MPIP Panic Cohort I (discovery sample).



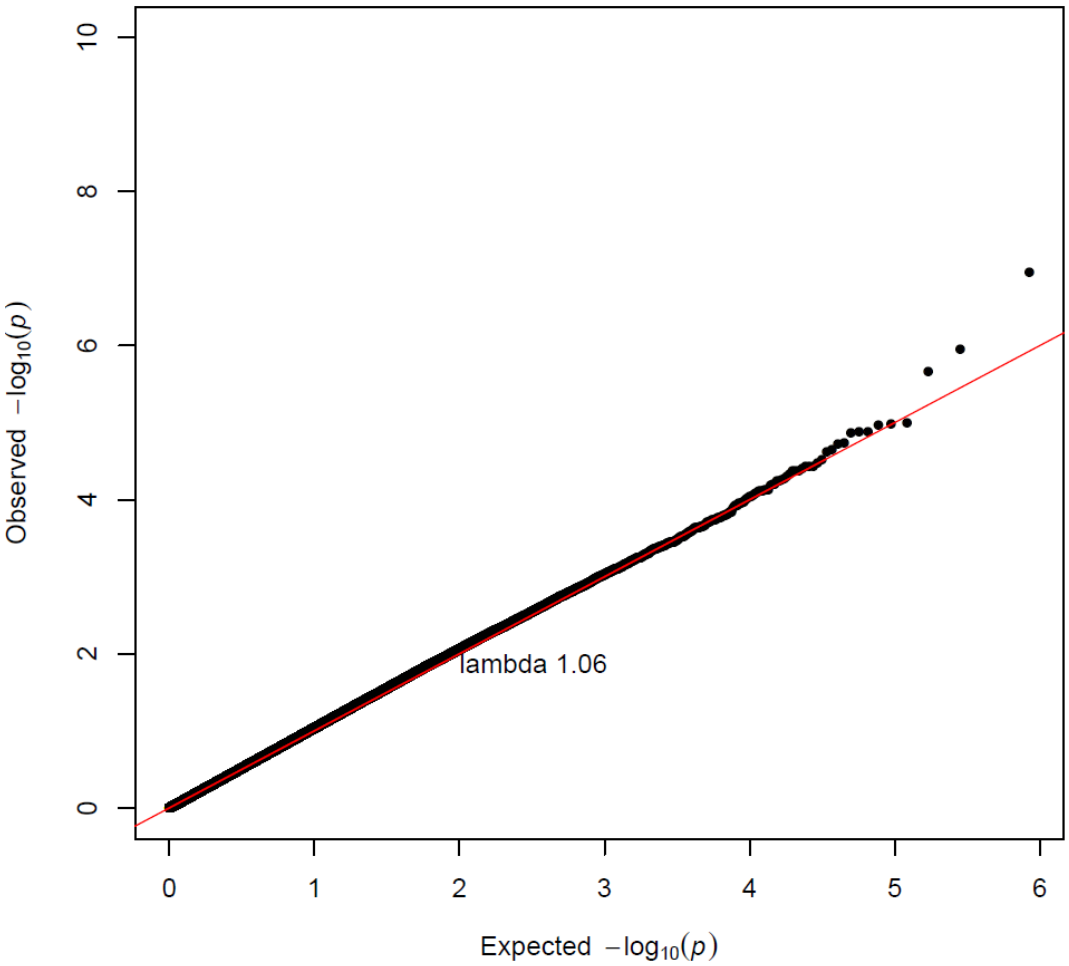
Supplementary Figure 2.
Multidimensional scaling (MDS) plots used to investigate population structure in the MPIP Panic Cohort II (replication sample).



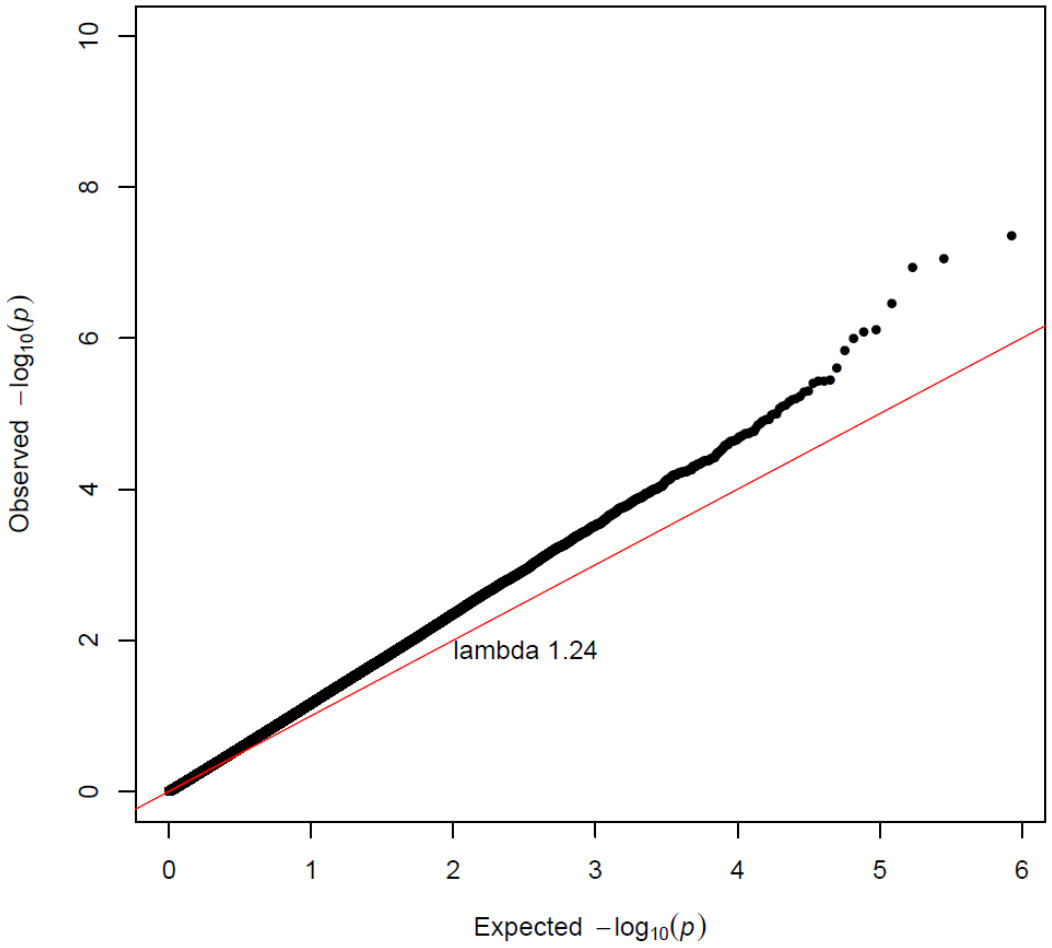
Supplementary Figure 3. Correlation plot for the MPIP Panic Cohort I-MDD data (N=699) after normalization, before batch correction, with the surrogate variables.



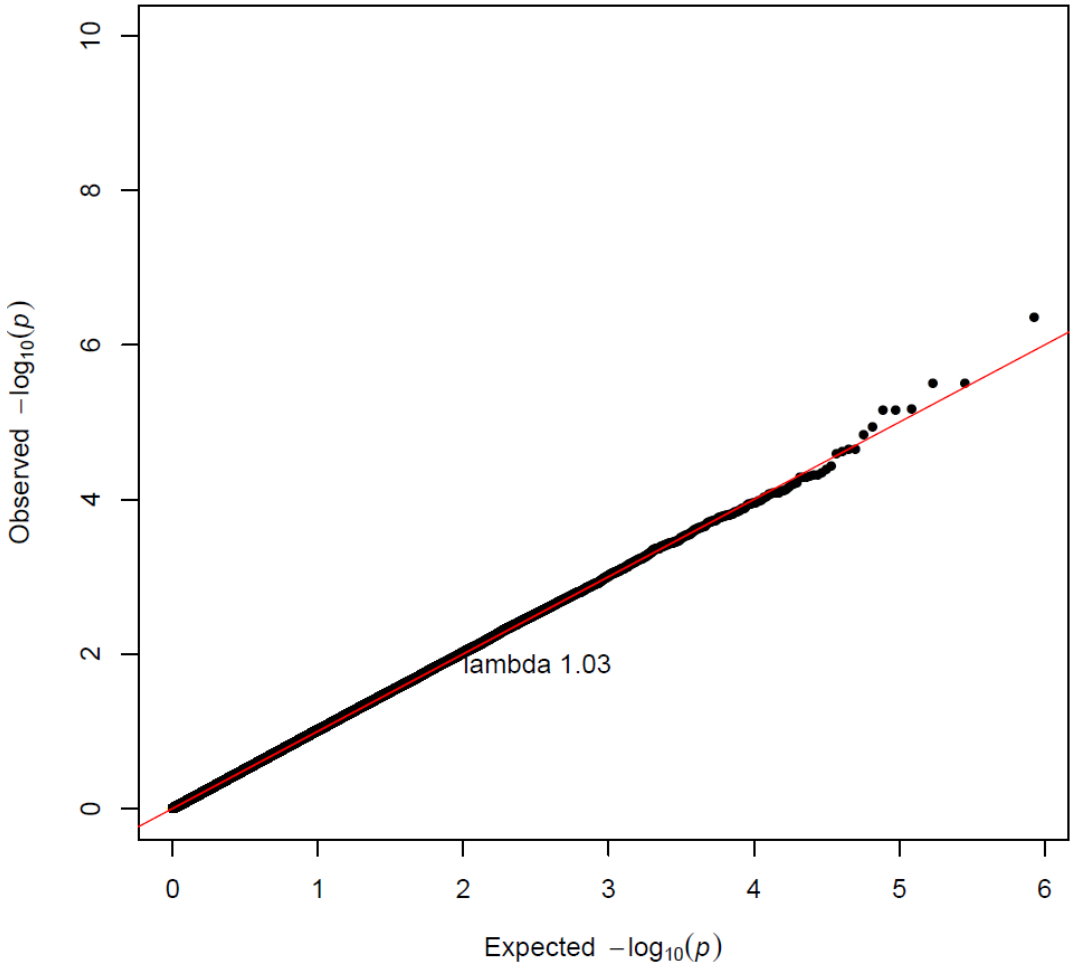
Supplementary Figure 4. QQ-plots of p-values for the MPIP Panic Cohort I (discovery sample).
Theoretical vs observed distributions for all the 424,834 p-values from the case-control analysis, males only.



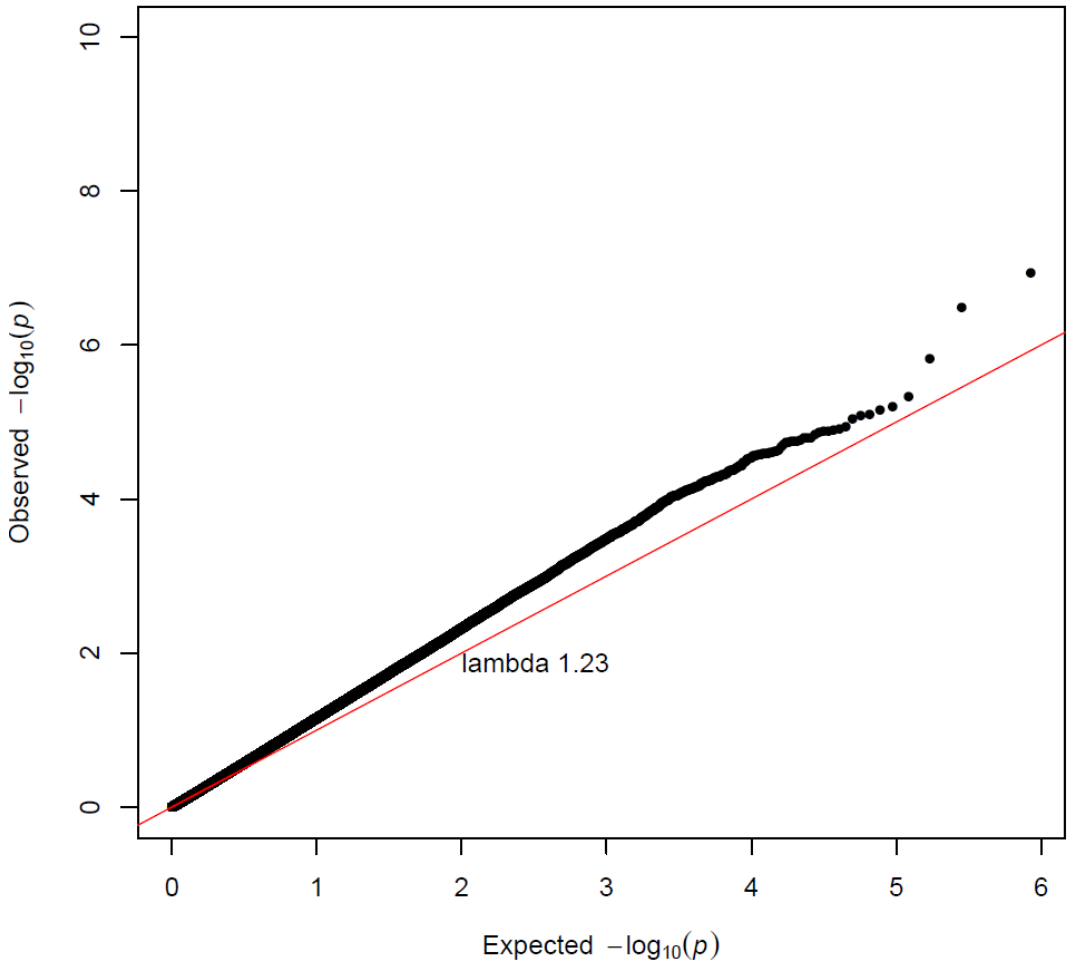
Supplementary Figure 5. QQ-plots of p-values for the MPIP Panic Cohort I (discovery sample).
Theoretical vs observed distributions for all the 424,834 p-values from the case-control analysis, females only.



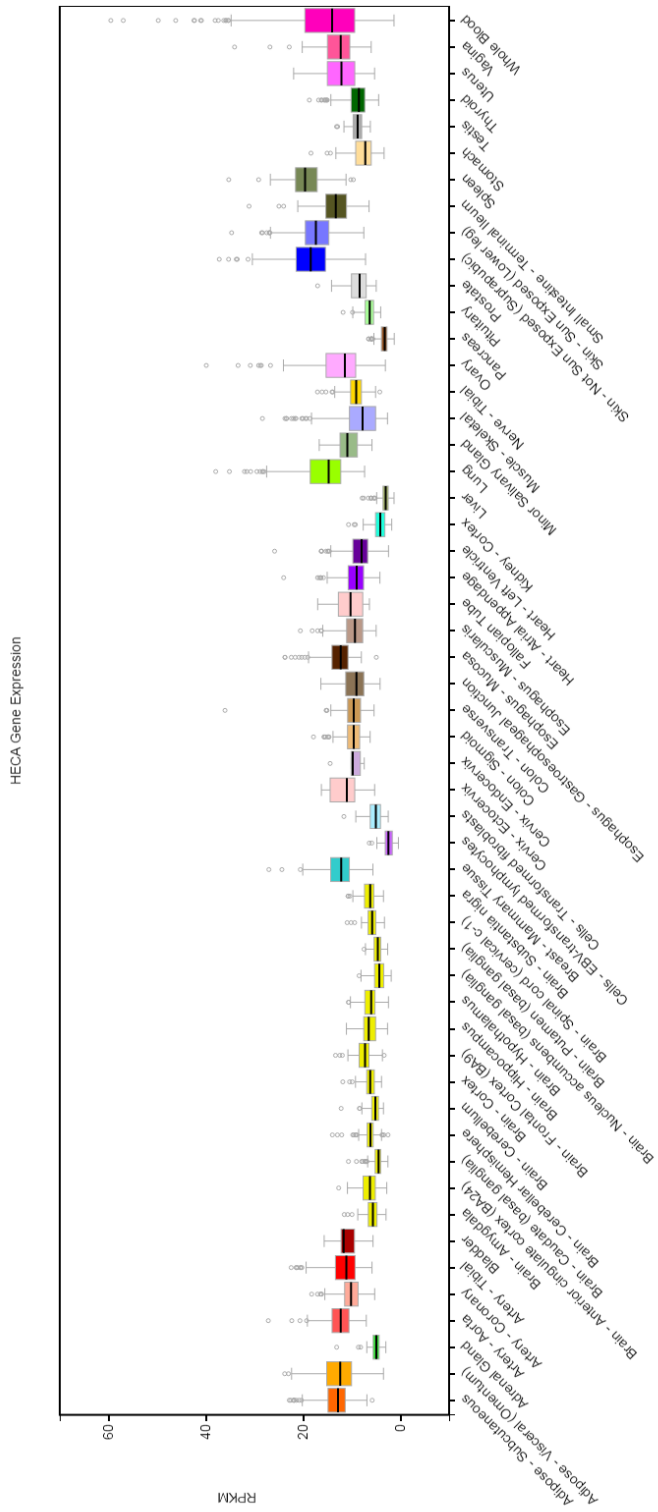
Supplementary Figure 6. QQ-plots of p-values for the MPIP Panic Cohort II (replication sample).
Theoretical vs observed distributions for all 425,119 p-values from the case-control analysis, whole sample.



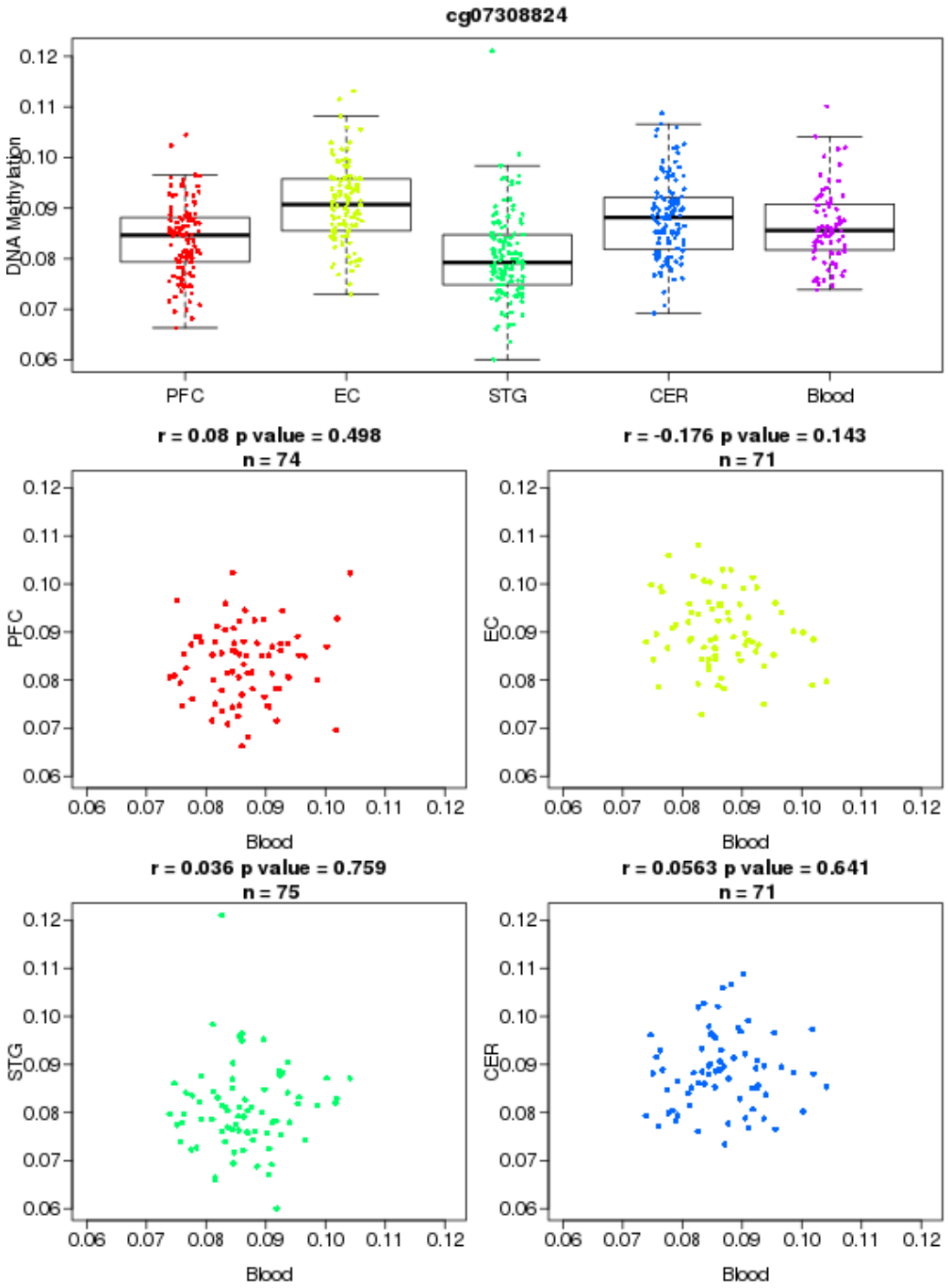
Supplementary Figure 7. QQ-plots of p-values for the MPIP Panic Cohort II (replication sample).
Theoretical vs observed distributions for all 425,119 p-values from the case-control analysis, males only.



Supplementary Figure 8. QQ-plots of p-values for the MPIP Panic Cohort II (replication sample).
Theoretical vs observed distributions for all 425,119 p-values from the case-control analysis, females only.



Supplementary Figure 9. *HECA* Gene Expression in Tissues
Expression values are shown in RPKM (Reads Per Kilobase of transcript per Million mapped reads). Box plots are shown as median and 25th and 75th percentiles; points are displayed as outliers if they are above or below 1.5 times the interquartile range. Data Source: GTEx Analysis Release V6p (dbGaP Accession phs000424.v6.p1) (http://www.gtexportal.org/home/)



Supplementary Figure 10. Correlation of *HECA* methylation levels at the cg07308824 locus in whole blood and four brain regions in a linear regression model. The top panel is a boxplot with the DNA methylation levels per tissue type. Upper left panel: prefrontal cortex (PFC); lower left panel: superior temporal gyrus (STG); upper right panel: entorhinal cortex (EC); lower right panel: cerebellum (CER) (http://epigenetics.iop.kcl.ac.uk/bloodbrain/).

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Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass meine Dissertation selbständig und ohne unerlaubte Hilfsmittel angefertigt worden ist. Die vorliegende Dissertation wurde weder ganz, noch teilweise bei einer anderen Prüfungskommission vorgelegt. Ich habe noch zu keinem früheren Zeitpunkt versucht, eine Dissertation einzureichen oder an einer Doktorprüfung teilzunehmen.

München, den 28. Juni 2017

Stella Iurato

Author Contributions

I have been the main responsible for this project, from sample selection (after recruiting), case-control matching, experimental plan, to experimental execution and analysis. After project completion, I have written the published manuscript (Iurato S et al. 2017).

Tania Carrillo-Roa contributed in the troubleshooting part and gave input in the analytical part.

Janine Arloth contributed with the quality control and analysis of the MPIP Dexamethasone study. Results of her study have been previously published (Arloth J et al. 2015).

Darina Czamara gave feedback regarding the statistical analysis.

Laura Diener-Hölz supported the experimental part as lab technician.

Jennifer Lange contributed to the recruitment of the patients.

Bertram Müller-Myhsok was part of my thesis advisory committee and gave feedback in particular on the statistical analysis.

Elisabeth B. Binder is the managing director of the institute and director of the department of Translational Research in Psychiatry. She was part of my thesis advisory committee and co-supervised my PhD work together with Angelika Erhardt. She consistently contributed to the writing process of the published manuscript (Iurato S et al. 2017).

Angelika Erhardt is the head of the outpatient clinic for psychiatry and psychotherapy. In this function, she was the responsible for the patients and controls recruitment and characterization. She was part of my thesis advisory committee and supervised my PhD work. She consistently contributed to the writing process of the published manuscript (Iurato S et al. 2017).

All the authors proofread the published manuscript (Iurato S et al. 2017).

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