SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

3220.

GÁL ZSÓFIA

Experimentális és klinikai farmakológia

című program

Programvezető: Dr. Szökő Éva, egyetemi tanár

Témavezető: Dr. Bagdy György, egyetemi tanár
Dr. Petschner Péter, egyetemi adjunktus

THE ROLE OF BLOOD-BRAIN BARRIER INTEGRITY IN DEPRESSION

PhD thesis

Zsófia Gál

Pharmaceutical Sciences Doctoral School

Semmelweis University

Supervisors:	György Bagdy, PhD, DSc Péter Petschner, PhD
Official reviewers:	Erzsébet Melinda Tóth, PhD Andrea Szabó-Vereczkei, PhD
Head of the Complex Examination Committee:	Éva Szökő, PhD, DSc
Members of the Complex Examination Committee:	Eszter Ducza, PhD Zoltán Zádori, PhD

Budapest
2025

Table of Contents

List of abbreviations

AD - Alzheimer's disease

ADHD - attention-deficit/hyperactivity disorder

AJ - adherens junction

ASD - autism spectrum disorder

ATE - adult traumatic events

BBB - blood-brain barrier

BD - bipolar disorder

BSI - Brief Symptom Inventory

CNS - central nervous system

CRP - C-reactive protein

CSF - cerebrospinal fluid

CYP450 - cytochrome P450

DSM - Diagnostic and Statistical Manual of Mental Disorders

ECs - endothelial cells

GABA - gamma-aminobutyric acid

GR - glucocorticoid receptor

GRCh37 - Genome Reference Consortium Human Build 37

GWAS - genome-wide association study

GWEIS - genome-wide by environment interaction study

HPA-axis - hypothalamic–pituitary–adrenal axis

HWE - Hardy-Weinberg equilibrium

ICD - International Classification of Diseases

IgLONs - immunoglobulin-like cell adhesion molecules

IFN-γ - interferon γ

IL-6 - interleukin-6

IL-17α - interleukin-17α

IL-23α - interleukin-23 subunit α

JAM - junctional adhesion molecule

MAF - minor allele frequency

MDD - major depressive disorder

MR - mineralocorticoid receptor

MSCs - mesenchymal stem cells

NM - NewMood Study

NSAID - nonsteroidal anti-inflammatory drug

NVU - neurovascular unit

PC - principal component

PCA - principal component analysis

PD - Parkinson's disease

PHQ9 - Patient Health Questionnaire-9

PTSD - post-traumatic stress disorder

QC - quality control

RLE - recent negative life events

SCZ - schizophrenia

SMCs - smooth muscle cells

SNP - single nucleotide polymorphism

SSRI - selective serotonin reuptake inhibitor

SNRI - selective noradrenaline reuptake inhibitor

TGF-β - transforming growth factor β

TJ - tight junction

TJAP1 – tight junction associated protein 1

TNFα - tumor necrosis factor α

UKB - UK Biobank

ZO - zonula occludens

1. Introduction

1.1 Major depressive disorder (MDD)

1.1.1. Diagnosis of major depressive disorder

Based on estimations, over 300 million people, 4.4% of the World's population suffer from major depressive disorder (MDD) (1). Nowadays, two major diagnostic manuals help healthcare professionals formulate the diagnosis: the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) (2), and the International Classification of Diseases, eleventh revision (ICD-11) (3). Diagnosis criteria of MDD consist of the presence of at least one of the two major symptoms - depressed mood or hopelessness most of the day and diminished interest or pleasure in activities - during a 2-week period, along with at least four additional symptoms (2, 3). These additional symptoms could be related 1) to psychomotor functions: psychomotor agitation or -retardation, 2) to cognitive functions and thoughts: feelings of worthlessness or excessive/ inappropriate guilt, diminished ability to concentrate/ think/ decision making or recurrent thoughts of death, 3) and to physical alterations: significant weight change, insomnia or hypersomnia, and loss of energy (2, 3). In addition to the listed symptoms, significant distress preventing normal functioning, and the exclusion of the possibility that the symptoms are caused by any substance or medication, are required for MDD diagnosis. Since psychotic disorders could also be manifested in depressive episodes, patients with a history of mania or hypomania are excluded, similarly if schizophrenia (SCZ) or other psychotic disorders better explain the persistent symptoms. Depression severity varies on a wide scale and it is determined based on the number and intensity of the present symptoms and could be classified as mild, moderate, or severe (2). Age and sex stratifies depression as 1.5 to 3 times more women than men are affected by MDD, and the prevalence is higher in 18-29 years old than in above 60 years old individuals (2). Although, the differences in the prevalence in men and women could not necessarily reflect the frequency of the disorder, but could be derived from diagnosis criteria (4-7).

1.1.2. Biological background of depression

Considering the effects of the first clinically advantageous antidepressants, which increased the concentrations of serotonin and noradrenaline in synapses, theories of imbalances in monoamine systems, emerged to explain MDD's biological basis (8). The

time gap between the biological changes caused by antidepressants and the manifestation of their therapeutic effects, as well as their limited effectiveness, suggested that depression could, however, not be explained solely based on neurotransmitter imbalances (8). The heterogeneity of depressive symptoms also suggests the conjunction of various biological mechanisms behind the development of depression. With years of research, numerous biological and environmental theories of its pathophysiology were revealed, such as alteration in synaptic plasticity, structural and functional brain remodelling, hypothalamic-pituitary-adrenal (HPA)-axis dysfunction, and inflammatory hypothesis (9). While these hypotheses cannot explain all facets of the biological basis of MDD, they can reveal the complex mechanisms in the development and progression of the disorder (9).

The heritability of MDD is estimated at around 30-40% (7, 10, 11), with higher genetic contribution explained by family- and twin-based studies (12), than by polymorphisms-phenotype association analyses in unrelated individuals (13). Results from heritability studies suggest the contribution of a wide spectrum of genetic- and epigenetic factors with small effects (13), in addition to the influencing role of environmental factors, such as nutrition (14), seasonality (15), or socioeconomic status (16). Moreover, certain personality traits have also been associated with increased vulnerability to developing MDD (17). For example, individuals with neuroticism are more likely to experience increased negative emotions and they may be more prone to develop depressive symptoms (17). Based on those, studies suggest predisposing roles for genetic and biological factors in response to various environmental stimuli, in terms of susceptibility or resilience for the appearance of depressive symptoms (9, 18).

1.2. The role of stress in depression

Among the various environmental factors, stress has a major role in the onset of depressive symptoms in certain individuals, implying that there are biological or genetic factors that increase one's vulnerability to stress, and lead to the appearance of MDD (19). Currently used animal models of depression also support this phenomenon, where invoked stress could usually provoke depressive-like symptoms (20). In response to psychological stress, the same "fight or flight" mechanisms are activated, as in case of physical stress, involving the activation of the HPA-axis and the sympathetic nervous

system (21). The rapid response to stress stimuli is evolutionary advantageous as it promotes the individual's survival, however when the excitatory state becomes constant in chronic stress conditions, this beneficial coping mechanism might become disadvantageous.

Depressive symptoms could be the results of stress effects on neuronal networks via loss of synaptic connections involving excitatory glutamate- and inhibitory GABA neurons (22) or could be derived through neuroinflammatory mechanisms and altered brain homeostasis (23). During normal stress responses, the produced cortisol decreases inflammatory mechanisms, in order to increase the prospect of survival when facing threatening situations (23). However, in case of prolonged stress exposure, overstimulation of the HPA-axis causes constant release of cortisol, which could result in the resistance of glucocorticoid receptor (GR) for this steroid hormone due to a decrease in its sensitivity (19, 23-25). Along with that, the overstimulation of the sympathetic nervous system results in increased production of proinflammatory cytokines (26) partly via the activation of the transcription factor, nuclear factor-κB, resulting in the increased secretion of proinflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) (27). This elevation of inflammatory compounds - which was also observed in the nervous system (28), not just in the peripheral blood flow - provides the basis for the inflammatory theory of depression. Researchers showed an association between elevated concentrations of cortisol and cytokines (TNF-α, IL-6, interferon γ (IFN-γ), and transforming growth factor β (TGF-β)) in sera of patients with depression when compared to controls (29, 30). The increased concentration of proinflammatory mediators was particularly associated with treatment-resistant cases of depression, where multiple attempts of pharmaceutical therapies with currently available antidepressants could not achieve recovery (31).

Animal studies, where chronic stress exposures were used for the initiation of depressive-like behaviour, revealed blood-brain barrier (BBB) hyperpermeability, which was caused and maintained by elevated concentrations of proinflammatory cytokines (TNFα, IL-17α, and IL-23α) (32). Additionally, in chronically stressed mice with increased permeability of the BBB, the cerebrovascular volume was also decreased (33). Based on these results, the BBB has become a target of interest behind stress-associated depression (34).

1.3. The potential role of the blood-brain barrier (BBB) in depression

Due to its exceptional requirements, the central nervous system (CNS) establishes a unique microenvironment, which is maintained by various barriers, such as the BBB, the blood-cerebrospinal fluid (CSF) barrier, and the arachnoid barrier, connecting and separating surrounding tissues and fluids (35). Among them, the BBB - formed predominantly by endothelial cells (ECs) of brain capillaries - possesses the largest surface area (36). Owing to the particular role of the endothelial cells in the BBB, their morphological-, structural- and surface-forming characteristics differ from other ECs of the human body (35). With astrocytes, pericytes, microglia, neurons, fibroblasts, mesenchymal and smooth muscle cells (SMCs), they form region-specific neurovascular units (NVU) in the CNS (37).

The molecular features of the NVU comply with the biological function of the BBB, which is sealing the paracellular routes, hampering unsupervised diffusion, and controlling the transport across the barrier. Endothelial cells express: 1) tight junction (TJ) molecules (like claudin-1, -3, -5, -12), occludin, and junctional adhesion molecules (JAMs), 2) adherens junction (AJ) molecules, e.g. cadherin–catenin complexes, 3) and other accessory associated proteins, including zonula occludens-1, -2, -3 (ZO-1, ZO-2, ZO-3), and cingulin (35). Besides the junctional molecules, efflux transporters, such as P-glycoprotein, and drug-metabolizing enzymes (e.g. CYP450) also contribute to the maintenance of normal brain homeostasis (35).

During or after stress, the physiological homeostasis of the CNS could be impaired as a result of alterations in the structure and function of the BBB. The negative effect of stress on the integrity of the BBB is a widely observed phenomenon in case of psychiatric disorders, such as autism spectrum disorder (ASD), SCZ, or MDD (38). In rodents, it was shown that stress could influence the permeability of the BBB by affecting the expression of its most abundant tight junction protein (39), claudin-5 (33, 40-44). The integrity change of the BBB after experiencing stress is usually accompanied by elevated inflammatory activity, and in the initiation of neuroinflammatory processes in the CNS (45), as well as in the elevated concentration of proinflammatory molecules in the periphery (41-43). In addition to the use of human post-mortem samples from individuals diagnosed with MDD (41-43), most of the studies reported results from rodents. Researchers showed that chronic stress effects in stress susceptible mice were

accompanied by decreased claudin-5 protein level at the BBB, elevated IL-6 proinflammatory cytokine level in the periphery and in the *nucleus accumbens*, and by the appearance of depressive-like behaviour (41, 43). It was also suggested that IL-6 could cross the more permeable BBB, which was not possible in stress-resilient animals with intact BBB due to the molecular size of this cytokine (41, 43). Considering that another proinflammatory cytokine, TNF-α reduced the mRNA level of *Cldn5 in vitro* in mice- and in human brain endothelial cells (46), the disruption of the BBB might be caused by inflammation. In support, there are glucocorticoid-response elements in the promoter region of the *CLDN5* gene in mice (46) and in humans (47) as well, which implies that stress might also have a direct effect on the expression of its protein through cortisol. Altogether, these results suggest that the BBB has a central role in stress- and inflammation-associated depression.

1.4. Genetic studies

Studying the biological background of MDD in humans is challenging because of the wide spectra of symptoms and the lack of available non-invasive study protocols. The use of genetic data however provides the opportunity to reveal the heritable part of depression's pathophysiology and the potential susceptibility factors contributing to the development of this disorder. In order to elucidate the exact mechanisms of action of currently used antidepressants, genes encoding proteins of the monoamine systems (e.g. *MAOA*, *COMT*, *SLC6A2*, *SLC6A4*) were constant candidates of genetics studies of depression (11). Yet, the crucial role of these genes in the aetiology of MDD remains unconfirmed. With new theories, the focus of the research broadened, emphasising the different biological and genetic backgrounds of distinct subtypes of the disorder (13).

While numerous studies aimed to reveal genes and their polymorphisms that contribute to the development of depressive symptoms there were no studies looking for the connection among stress effects, inflammation, and the BBB in humans. Considering the previously reported results from rodents and the knowledge gap in terms of stress effects, BBB- and inflammation-related processes, our aims were to investigate these factors using genomic approaches in the etiopathology of stress-associated depression.

2. Objectives

Based on the above, our aims were to translate previous results from animal experiments to human genetics (48), and to reveal the contribution of BBB- and inflammation-related factors in stress-associated depression (49).

In the first study, our goal was to translate results from previous animal studies (41, 43) - , in which *Cldn5* downregulation, elevated concentration of IL-6, together with its infiltration to the area of the *nucleus accumbens* (in the ventral striatum of the basal ganglia (50)) were detected along with the appearance of depressive-like symptoms in stress susceptible mice, after experiencing stress effects, - to human genetics. In order to model this, we selected two functional polymorphisms (rs885985 from *CLDN5* and rs1800795 from *IL6*) and investigated whether the 3-way interaction among the single nucleotide polymorphisms (SNPs) and recent stress contribute to depressive symptoms. Based on results from animal experiments and the functionality of the selected SNPs, our hypothesis was that the minor alleles of SNPs on *CLDN5* (G) and on *IL6* (C) contribute to depressive symptoms after experiencing recent stress. According to the above, we performed:

1. 3-way interaction analysis to investigate, whether *CLDN5* and *IL6* polymorphisms had combined effects on depressive symptoms in interaction with recent stress in the whole UK Biobank cohort;
2. main effect analyses for rs885985 of *CLDN5* and rs1800795 of *IL6*, separately on current depressive symptoms;
3. epistasis (rs885985 of *CLDN5* x rs1800795 of *IL6*) analysis to exclude a significant effect of the interaction between polymorphisms without a stressor;
4. gene-environment interaction analyses of rs885985 of *CLDN5* and rs1800795 of *IL6* with recent stress to show that both *IL6* and *CLDN5* polymorphisms are required to mediate significant effects of recent stress on depression symptoms;
5. 3-way interaction tests with a distal stressor (childhood adversities) and the corresponding depression phenotype (lifetime depression) to investigate the temporal differences in stress exposure;
6. sex-stratified analyses to identify potential sex-specific effects;
7. validation analyses of significant results on the independent NewMood (NM) cohort to confirm findings.

In the second study, we aimed to demonstrate the contributing role of genes involved in maintaining the functionality of the BBB and regulating inflammatory processes in stress-associated depression. To achieve this, we decided to:

1. conduct genome-wide by environment interaction analysis (GWEIS) on depressive symptoms in interaction with stress during adulthood in the UK Biobank cohort;
2. conduct the GWEIS in male- and female subcohorts, separately;
3. identify BBB-related genes and their expected enrichments among the significant gene-level results of the GWEIS;
4. identify inflammation-related genes and their expected enrichments among the significant gene-level results of the GWEIS;
5. compare the number of BBB-related significant genes with genes previously associated with neuroticism, based on the consideration that this trait was connected to elevated stress sensitivity;
6. replicate significant BBB- and inflammation-related results in the independent NM cohort.

3. Methods

3.1. Populations and genetic samples

3.1.1. UK Biobank

UK Biobank (application no. 1602) data collection was conducted in accordance with the Declaration of Helsinki and approved by North West Centre for Research Ethics Committee (11/NW/0382) (51, 52). The genotyping procedures for 488,000 participants, aged between 39 and 72 years, was performed using UKB Axiom Array and Affymetrix UK BiLEVE Axiom Array (53).

3.1.2. NewMood

Data collection (in accordance with the Declaration of Helsinki) under the NewMood Study [New Molecules in Mood Disorders, LHSM-CT-2004-503474, Sixth Framework Program of the European Union (54)] was performed in Budapest, Hungary [approved by the Scientific and Research Ethics Committee of the Medical Research Council (ad.225/KO/2005; ad.323-60/2005-1018EKU and ad.226/KO/2005; ad.323-61/2005-1018 EKU)] and in Manchester, United Kingdom [approved by the North Manchester Local Research Ethics Committee (REC reference no.: 05/Q1406/26)]. Genotyping was performed on 1,820 volunteers aged between 18 and 60 years.

3.2. Genetic quality control procedures

Both cohorts, used in this study underwent standardized quality control (QC) steps (55), a priori to the principal component analysis (PCA) and the statistical analyses. This comprised excluding participants of high relatedness, mismatching sex or extreme heterozygosity, filtering for minor allele frequency ($MAF > 0.01$), Hardy-Weinberg equilibrium tests ($HWE > 0.00001$), excluding participant with > 0.01 missingness per markers rate, and calculation of linkage disequilibria. Ten principal components (PCs) were derived from each cohort after the conduction of QC steps. In the first study, the usage of the preselected functional SNPs of *CLDN5* (rs885985) and *IL6* (rs1800795) limited the number of participant to n = 277,501 in UKB and to n = 1,638 in the NM cohorts. After the QC steps on all polymorphisms (positioned based on GRCh37/hg19 genome assembly), 6,258,585 SNPs remained in the UKB, and 3,474,630 SNPs in the

NM cohorts. SNPs that passed QC were used in the GWEIS. Genetic data were derived from blood samples in the UKB, and from saliva samples in case of the NM study.

3.3. Phenotypes

3.3.1. UK Biobank

In the first study, in order to model and approximate accurately the results of previous animal studies on depressive-like phenotype after experiencing stress with the highest possible number of participants, we utilized self-reported answers (Table 1.) on questionnaire on proximal stress factors [UKB Field ID 6145 ("Illness, injury, bereavement, stress in last 2 years")] and a derived score of current depressive symptoms (56) by taking the sum of Field ID 2050 ("Frequency of depressed mood in last 2 weeks"), Field ID 2060 ("Frequency of unenthusiasm/disinterest in last 2 weeks"), Field ID 2070 ("Frequency of tenseness/restlessness in last 2 weeks"), and Field ID 2080 ("Frequency of tiredness/lethargy in last 2 weeks"). Lifetime depression status was determined based on ICD-10 disease codes of F32 ("Depressive episode", Field ID 130894) or F33 ("Major depressive disorder, recurrent", Field ID 130896). Questionnaire on childhood adversities (Field IDs 20487, 20488, 20489, 20490 and 20491) was used to include early life stress in the analyses.

Table 1. Phenotypes of the UK Biobank used in the first study

Field code	Name	Assessment method	Assessment time
2050	Frequency of depressed mood in last 2 weeks	Self-reported answers (Prefer not to answer, Do not know, Nearly every day, More than half the days, Several days, Not at all)	Initial assessment visit (2006-2010)
2060	Frequency of unenthusiasm/disinterest in last 2 weeks		
2070	Frequency of tenseness/restlessness in last 2 weeks		
2080	Frequency of tiredness/lethargy in last 2 weeks		
6145	Illness, injury, bereavement, stress in last 2 years	Self-reported answers (Prefer not to answer, None of the above, Financial difficulties, Marital separation/ divorce, Death of a spouse or partner, Death of a close relative, Serious illness, injury or assault to yourself	
130894	Date F32 first reported (depressive episode)	Diagnostic code from primary care, hospital admissions and other registries	1941-2022
130896	Date F33 first reported (recurrent depressive disorder)		1952-2022
20487	Felt hated by family member as a child	Self-reported answers (Prefer not to answer, Never true, Rarely true, Sometimes true, Often, Very often true)	Online mental health self-assessment questionnaires (2016)
20488	Physically abused by family as a child		
20489	Felt loved as a child		
20490	Sexually molested as a child		
20491	Someone to take to doctor when needed as a child		

As for revealing the importance of blood-brain barrier (BBB) and inflammation-related genes in stress-associated depression, a depression phenotype, closer to the wide variety of MDD diagnosis was used based on Patient Health Questionnaire (PHQ9) (57). Contrary to the first study, where our aim was the translation of depressive-like phenotype of mice to humans, during the second study, we intended to use a phenotype that modelled human depression with more complexity and consequently the derived results could provide a more precise basis for human drug target research. PHQ9 depression values were derived as a mean value from “Depression in the last 2 weeks” questionnaire (Data Fields: 20507, 20508, 20510, 20511, 20513, 20514, 20517, 20518, 20519). Along with

PHQ9 depression, traumatic events during adulthood (age > 16) were assessed during the "Online follow-up" phase of UKB study (Data Fields: 20521, 20522, 20523, 20524, 20525) and were used in the GWEIS as mean values of stress factors (Table 2.). The usage of PHQ9 depression reduced the number to participants to n = 109,360, however an improved approximation to stress-associated depression could be reached with this model.

Table 2. Phenotypes of the UK Biobank used in the second study

Field code	Name	Assessment method	Assessment time
20507	Recent feelings of inadequacy	Self-reported answers (Prefer not to answer, Do not know, Nearly every day, More than half the days, Several days, Not at all)	Online mental health self-assessment questionnaires (2016)
20508	Recent trouble concentrating on things		
20510	Recent feelings of depression		
20511	Recent poor appetite or overeating		
20513	Recent thoughts of suicide or self-harm		
20514	Recent lack of interest or pleasure in doing things		
20517	Trouble falling or staying asleep, or sleeping too much		
20518	Recent changes in speed/amount of moving or speaking		
20519	Recent feelings of tiredness or low energy		
20521	Belittlement by partner or ex-partner as an adult	Self-reported answers (Prefer not to answer, Never true, Rarely true, Sometimes true, Often, Very often true)	
20522	Been in a confiding relationship as an adult		
20523	Physical violence by partner or ex-partner as an adult		
20524	Sexual interference by partner or ex-partner without consent as an adult		
20525	Able to pay rent/mortgage as an adult		

3.3.2. NewMood

For replication purposes, the available depression questionnaire in the NM cohort of depressive symptoms, based on the "Brief Symptom Inventory" (BSI) ("thoughts of ending your life", "feeling lonely", "feeling blue", "feeling no interest in things", "feeling hopeless about the future", "feelings of worthlessness") (58), plus 4 items ("poor appetite", "trouble falling asleep", "thoughts of death or dying", "feelings of guilt"); and "The List of Threatening Experiences" questionnaire (59) for assessing recent negative life events in the previous 1 year were used. Two items from the background questions

asking about early parental loss and the reduced form of Childhood Trauma Questionnaire (60) were used to determine early stress adversities. Lifetime depression status was assessed self-reportedly. Item scores divided by answered questions were used for current depressive symptoms and childhood stressors. All answers were self-reported, along with basic sociodemographic factors, such as age and sex. In the first part of the research work, available information on rs885985 of *CLDN5* and rs1800795 of *IL6* reduced the number of participants to n = 1,638. During GWEIS, phenotypic information limited the usage of the whole NM dataset, as n = 1,753 participants provided information on current depressive symptoms and recent stressors.

3.4. Polymorphisms selection in order to model the interaction effects of *CLDN5* and *IL6* in stress-associated depression

Selection of the polymorphisms were based on functionality and previous literature data. Rs885985 in *CLDN5* gene is located on the reverse strand of chromosome 22, at position 19,511,925 (GRCh37/hg19) and has two common variations: alleles A and G (61). Functionality of rs885985 is manifested in coding for two types of open reading frames: A allele encodes for a STOP codon, while G allele encodes for a glutamine (62). The frequencies of the alleles are high (MAF of G = 44% in European reference population (63)), and in physiological conditions a 218 amino acids-long claudin-5 protein is translated, regardless of the genotype. Although, during *in vitro* conditions, a longer claudin-5 isoform, with 303 amino-acids could be translated due to the presence of G allele, which - contrary to the shorter isoform, encoded by the A allele - could not be transported and be built into the cell membrane (62). These findings suggest the possibility of altered translation of *CLDN5* gene in pathological conditions, and distinct capabilities for building into the membrane of epithelial cells, and by that, this polymorphism could even influence the permeability of the BBB.

Functional polymorphism, promoter region variant rs1800795 of *IL6* gene is located at 22,766,645 position on chromosome 7 (GRCh37/hg19), encoding G and C alleles (61), with MAF of C = 42% in European reference population (63). Its clinical significance is exhibited in being a risk factor in various conditions and diseases (64-66), among them stress-associated depression (67), too, and its minor allele (C) was associated with higher level of IL-6 plasma concentration (68, 69).

3.5. Statistical analyses

Gene-environment interaction analyses were applied, using Plink 2.0 (70, 71) and R (72) software. During the first study, - in order to reveal their potential contributions to stress-associated depression in humans - alleles in rs885985 of *CLDN5* and rs1800795 of *IL6* were examined additively (with covariates age, sex, genotyping array (in UKB) and the first 10 PCs), in different arrangements such as in main effect-, epistasis- and interaction analyses with stress, separately and simultaneously, too. These analyses were also conducted in male- and in female subjects, in order to reveal any sex differences. Bonferroni method for correction for multiple testing were applied (0.05/ [12 tests * 3 type of division * 2 cohort] = 0.0007), utilizing p = 0.0007 as significance threshold. Significant results were replicated in the independent NM cohort.

For the second study, genome-wide by environment interaction analyses (GWEIS) were applied to the whole set of SNPs in additive models. Analyses were also conducted in male- and female subgroups, as well as in NM cohort for replication purposes. Age, sex genotyping array (in UKB), the first 10 PCs and the above listed stress factors were incorporated in linear regression analyses of depression phenotypes. Summary statistics from GWEIS were further analysed with MAGMA implemented in FUMA software with SNP-wise mean model (73) in order to derive gene-level results. As correction for statistical biases, conventional Bonferroni correction in gene-level analyses ($p < 2.59 * 10^{-6}$; calculating based on 19,296 genes included in the study) and standard GWAS significance threshold ($p < 5 * 10^{-8}$) during the SNP-level analyses were applied in order to reveal significant results.

Chi-square statistics were applied to the gene-level GWEIS results, in order to compare the observed and expected numbers of BBB- and inflammation-related genes among the significant results in order to measure the difference between the observed and expected ratios of BBB- and inflammation-related genes.

Additional t-statistics on age distribution and visualization of the results were prepared with “tidyverse” (74), “jtools” (75), “ggplot2” (76), “wesanderson” (77), “CMplot” (78) and “interactions” (79) R packages.

3.6. Genes connected to blood-brain barrier (BBB)

In order to identify genes from the GWEIS results, that could be connected to BBB, we used gene expression enrichment data from cells, taking part in BBB formation, based on human post-mortem midbrain samples of control subjects (80). In this study, differentially expressed genes were determined for cells of the BBB (endothelial cells (ECs)), ependymal cells, pericytes, smooth muscle cells (SMCs), mesenchymal stem cells (MSCs), fibroblasts and astrocytes), using the examined cells as the basis of the comparison and for the determination of positive enrichment of a given gene (80). Based on this expression data, altogether 1,364 genes were considered as BBB-related in our gene-level results. We used the ratio of BBB-related genes among all genes included in the GWEIS (1,364 BBB-related genes/ 19,296 genes involved in the GWEIS) as baseline, to determine the enrichment of BBB-related genes among the significant gene-level results, by chi-square statistics.

3.7. Genes connected to inflammatory processes

Genes from 162 previously collected MSigDB C2 curated gene sets of inflammatory factors (81), along with genes from inflammation-related Hallmark gene sets (82, 83) were considered as inflammation-related genes in our analyses. Based on that, altogether 4,420 genes were determined as inflammation-related from the gene-level GWEIS results. The enrichment of inflammation-related genes among the significant GWEIS results were calculated by chi-square statistics using 4,420/19,296 as baseline ratio.

3.8 Genes previously associated with neuroticism

We compared significant gene-level results to neuroticism-related genes (84, 85), which were previously associated with this phenotype. Neuroticism is a personality trait, used to measure emotional stability (86). Its high score has been identified as a risk factor for depression, because it is characterized by elevated stress experiences and responses (87). Based on the referenced association studies and meta-analysis (84, 85), altogether 467 genes were considered neuroticism-related in our gene-level results. We compared these neuroticism-related genes to the significant genes in the GWEIS in order to reveal the overlapping candidates that play role in the development of stress-associated depression and neuroticism, too.

4. Results

4.1. Translating previous results from animal studies onto the human genetic level using rs885985 of *CLDN5* and rs180795 of *IL6*

4.1.1. Descriptive statistics

Minor allele frequencies (MAF) of rs885985 (*CLDN5*; MAF (G) = 0.43) and rs1800795 (*IL6*; MAF (C) = 0.43) in both cohorts were in accordance with MAF values in 1000Genomes European reference population (63). The average score of current depressive symptoms and recent negative life events in both cohorts were relatively low, without inconsistences between male- and female subgroups (Table 3.). UKB and NM cohorts significantly differed in age ($t = 92.82$; $p < 2.2 * 10^{-16}$).

Table 3. Characteristics of continuous variables in the two cohorts. RLE - number of recent negative life events during previous 2 years in UKB and during previous 1 year in NM cohorts; min - minimum value of the given variable; max - maximum value of the given variable; mean - average score of the given variable; SE - standard error of mean; current depression refers to a 4-item based depressive symptoms score in the UKB and to depressive symptoms score based on BSI questionnaire in the NM cohort

UK Biobank								
			whole cohort		**male subjects**		**female subjects**	
	min	**max**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
age	39	72	56.88	0.02	57.14	0.02	56.66	0.02
current depression	1	4	1.40	0.00	1.36	0.00	1.43	0.00
RLE	0	6	0.57	0.00	0.54	0.00	0.60	0.00
NewMood								
			whole cohort		**males**		**females**	
	min	**max**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
age	18	60	30.88	0.39	32.86	0.63	29.93	0.49
current depression	0	4	0.56	0.02	0.50	0.05	0.58	0.03
RLE	0	8	1.09	0.04	0.99	0.08	1.14	0.05

4.1.2. Significant 3-way interaction results among rs885985 of *CLDN5*, rs1800795 of *IL6* and recent stress on current depressive symptoms in UK Biobank

During 3-way interaction analyses, highly significant associations ($\beta = 0.0093$; $p = 0.0003$) were revealed among the investigated polymorphisms (rs885985 of *CLDN5* and rs1800795 of *IL6*) and recent stress on current depressive symptoms in the UKB cohort (Table 4.). Stratifying the cohort by sex, provided distinct results for male- ($\beta = 0.0141$; $P = 0.0002$) and female ($\beta = 0.0055$; $p = 0.1208$) subgroups.

Table 4. Results of 3-way interaction analyses of rs885985 in *CLDN5* and rs1800795 in *IL6* on current depression symptoms in interaction with recent negative life events in the UK Biobank. n - number of participants included in the analysis; β– regression coefficient; SE – standard error of β; t – T-statistics; p - asymptotic p-value. Results surviving correction for multiple testing ($p < 0.0007$) are marked in bold.

group	*n*	*β*	*SE*	*t*	*p*
whole cohort	**277501**	**0.0093**	**0.0026**	**3.5922**	**0.0003**
male subjects	**128752**	**0.0141**	**0.0038**	**3.6905**	**0.0002**
female subjects	148749	0.0055	0.0035	1.5514	0.1208

Based on the outcome of the 3-way interaction analysis with recent stress, minor allele carrier status on rs885985 of *CLDN5* (G) and on rs1800795 of *IL6* (C) represented risk factors for stress-associated depression. As expected, β values were small, however, the increased effect size in men and the disappeared significance in women suggested sex-differences in the role of the investigated SNPs in stress-associated depression. Correlation between current depressive symptoms and recent stress were significant without considering genetics ($R = 0.16$; $p < 0.001$), although including rs885985 of *CLDN5* and rs1800795 of *IL6* into the analyses resulted in better-fit linear models in the whole cohort ($R = 0.18$; $p < 0.001$) and in men ($R = 0.21$; $p < 0.001$) (Figure 1).

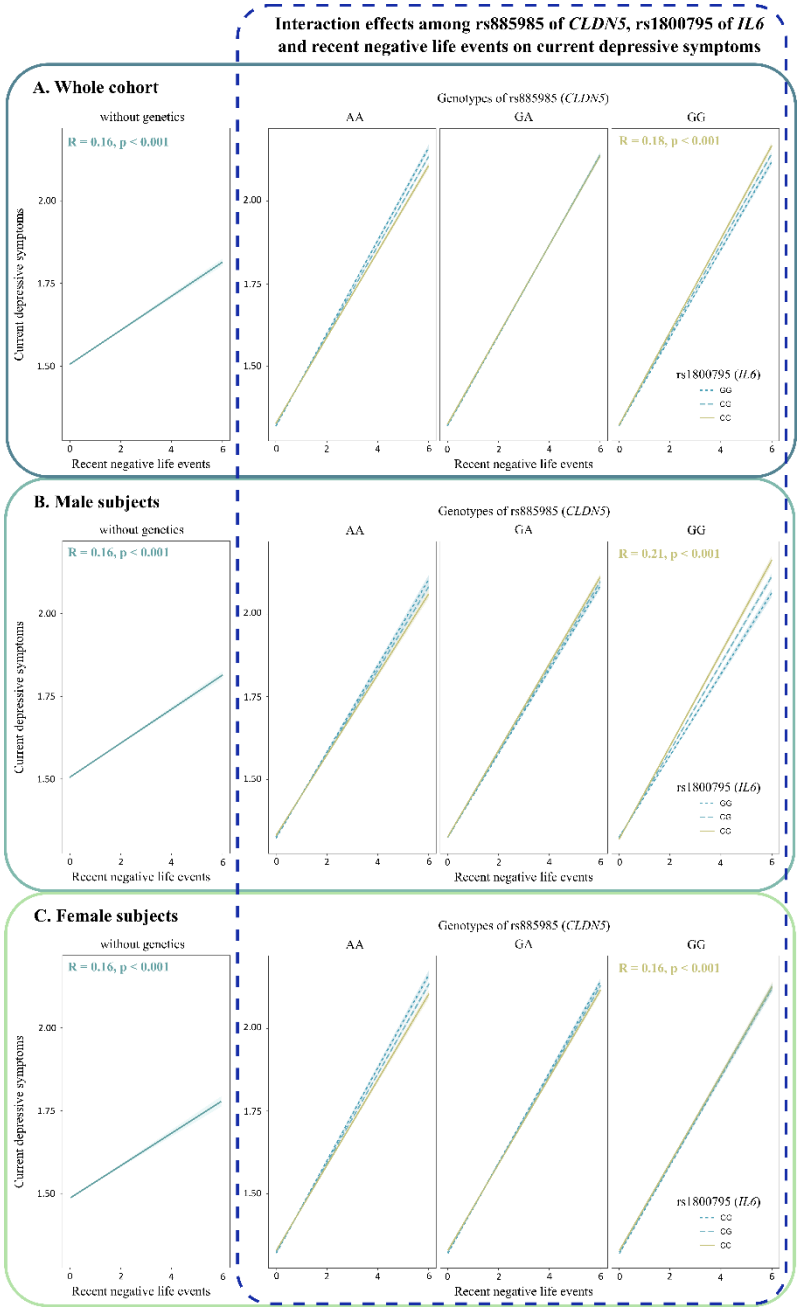


Figure 1. Linear regression models of the combined effect of *CLDN5* and *IL6* polymorphisms on current depressive symptoms in interaction with recent stress. Results from the whole UKB cohort showed better-fit models when including polymorphisms of *CLDN5* and *IL6* into the analyses, than without genetic variables. Regression lines were derived from correlation between current depressive symptoms and recent negative life events (left side of the figure) and from 3-way interactions with alleles of rs885985 in *CLDN5* and of rs1800795 of *IL6* (right side of the figure, framed with a dashed line). From top to bottom: A. results of the whole UKB cohort; B. results in male subjects of the UKB; C. results in female subjects of the UKB.

4.1.3. Further characterisation of the potential contributing role of rs885985 of *CLDN5* and rs1800795 of *IL6* in recent stress-associated depression

Additional analyses with the preselected SNPs (Table 5.) reinforced the importance of the combined contributing role of rs885985 (*CLDN5*) and rs1800795 (*IL6*), as none of the results from other analyses survived correction for multiple testing ($p < 0.0007$). These results indicated that minor allele carrier status solely on *CLDN5* or on *IL6* polymorphisms with or without considering the interacting effect of stress, did not represent risk factors for current depressive symptoms.

Table 5. Results of analyses with rs885985 of *CLDN5* and rs1800795 of *IL6* on current depressive symptoms as outcome variable. *CLDN5* - rs885985 polymorphism of *CLDN5*; *IL6* - rs1800795 polymorphism of *IL6*; RLE - number of recent negative life events during previous 2 years; n - number of participants included in the analysis; β– regression coefficient; SE – standard error of β; t – T-statistics; p - asymptotic p-value. None of the results survived correction for multiple testing ($p < 0.0007$).

whole UK Biobank cohort					
	n	β	*SE*	*t*	*p*
CLDN5 on current depression	277501	-0.0013	0.0014	-0.9231	0.3559
CLDN5 on current depression x RLE	277501	0.0010	0.0014	0.7168	0.4735
IL6 on current depression	277501	0.0033	0.0014	2.3563	0.0185
IL6 on current depression x RLE	277501	-0.0008	0.0014	-0.5658	0.5715
epistasis with *CLDN5* and *IL6*	277501	0.0010	0.0020	0.4976	0.6188
male subgroup of UK Biobank					
	n	β	*SE*	*t*	*p*
CLDN5 on current depression	128752	-0.0013	0.0020	-0.6335	0.5264
CLDN5 on current depression x RLE	128752	0.0034	0.0020	1.7087	0.0875
IL6 on current depression	128752	0.0030	0.0020	1.4745	0.1404
IL6 on current depression x RLE	128752	0.0023	0.0020	1.1724	0.2410
epistasis with *CLDN5* and *IL6*	128752	-0.0012	0.0029	-0.4335	0.6646
female subgroup of UK Biobank					
	n	β	*SE*	*t*	*p*
CLDN5 on current depression	148749	-0.0013	0.0020	-0.6774	0.4981
CLDN5 on current depression x RLE	148749	-0.0009	0.0019	-0.4639	0.6427
IL6 on current depression	148749	0.0036	0.0020	1.8471	0.0647
IL6 on current depression x RLE	148749	-0.0033	0.0019	-1.7119	0.0869
epistasis with *CLDN5* and *IL6*	148749	0.0029	0.0028	1.0348	0.3008

4.1.4. Distal stress did not appear to have role in the contributing effects of rs885985 of *CLDN5* and rs1800795 of *IL6* on depression

In order to reveal the potential effects of distal stress, 3-way interaction analyses were conducted with childhood adversities on lifetime depression status with the preselected polymorphisms of *CLDN5* and *IL6*. Based on the non-significant results, alleles on rs885985 of *CLDN5* and rs1800795 of *IL6* did not contribute to develop depression after experiencing adversities during childhood (Table 6.), confirming the distinct role of timing in case of stress exposure.

Table 6. Results of 3-way interaction analyses of rs885985 in *CLDN5* and rs1800795 in *IL6* on current depression symptoms in interaction with childhood adversities in the UK Biobank. . n - number of participants included in the analysis; OR– odds ratio; SE – standard error of OR; Z – Wald Z-score; p - asymptotic p-value. None of the results survived correction for multiple testing ($p < 0.0007$).

group	*n*	*OR*	*SE*	*Z*	*p*
whole cohort	277 501	1.0083	0.0385	0.2148	0.8299
males	128 752	0.8740	0.0724	-1.8608	0.0628
females	148 749	1.0701	0.0456	1.4871	0.1370

4.1.5. Replication of significant findings in the independent NewMood cohort

Replication of significant results from the UKB, on 1,638 participant of the NM cohort resulted in trend level significance of 3-way interactions on current depressive symptoms with the same direction of effect ($\beta = 0.0553$ (SE = 0.0333); $t = 1.6593$; $p = 0.0972$) among rs885985 (*CLDN5*), rs1800795 (*IL6*) and recent stressors.

4.2. Contribution of blood-brain barrier (BBB)- and inflammation-related genes to stress-associated depression

4.2.1. Descriptive statistics and SNP-level results

In order to determine the involvement of BBB- and inflammation-related genes in human stress-associated depression, at first genome-wide by environment interaction analyses (GWEIS) were conducted on PHQ9 depression scores in interaction with adult traumatic events (ATE) scores. Contrary to our first study, where we aimed to utilize data from the highest possible number of participants with the most accurate approximation of rodent's depressive-like phenotype in humans and conducted the analyses on a 4-item depression score, in this study, we applied the analyses on PHQ9 depression, which was considered to be more close to the wide variety of symptoms in MDD diagnosis. Correlation between the two types of current depressive scores was at moderate level (Pearson's R = 0.50) though, the usage of PHQ9 allowed proceeding towards prospective biomarker and drug target research in human depression. Altogether 109,360 participants of the UKB cohort provided information on current depression status and on adult traumatic events score through filling out PHQ9 and the accompanying stress events questionnaires (Table 7.).

Table 7. Characteristics of continuous variables in the two cohorts. PHQ9 depression - depression score derived from Patient Health Questionnaire; ATE stress - number of stress events during adulthood (age > 16); current depression - depression score derived using BSI questionnaire; RLE - number of recent negative life events during previous year; min - minimum value of the given variable; max - maximum value of the given variable; mean - average of the given variable; SE - standard error of mean

UK Biobank								
			all participants		**male subjects**		**female subjects**	
	min	**max**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
age	39	72	56.19	0.02	56.84	0.04	55.66	0.03
PHQ9 depression	1	4	1.31	0.00	1.27	0.00	1.34	0.00
ATE stress	0	4	0.40	0.00	0.34	0.00	0.48	0.00
NewMood								
			all participants		**male subjects**		**female subjects**	
	min	**max**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
age	18	60	32.56	0.25	34.15	0.45	31.92	0.30
current depression	0	4	0.84	0.02	0.68	0.04	0.91	0.03
RLE	0	8	1.21	0.03	1.07	0.05	1.26	0.04

In total 788 SNPs remained significant after correction for multiple testing ($p < 5 * 10^{-8}$) in the GWEIS on depressive symptoms in interaction with adult stressors in the whole UKB cohort. The most significant rs117435652 ($\beta = 0.0636$; $p = 4.40*10^{-15}$) was considered as an intron variant (61) in *LRRC4C* (Leucine Rich Repeat Containing 4C) gene on chromosome 11, which encodes for a postsynaptic adhesion molecule and plays a role in axon growth (88).

Conducting the GWEIS in male subjects resulted in 412 significant SNPs, with an intergenic polymorphism, rs74297459 on the 7th chromosome as the most significant result ($\beta = 0.0265$; $p = 2.15*10^{-12}$), while in female subjects 631 SNPs remained significant with lead SNP rs76262850 ($\beta = 0.0763$; $p = 1.43*10^{-11}$) mapped as a regulatory region variant on chromosome 11 (61).

4.2.2. Significant gene-level results on PHQ9 depression in interaction with stressors during adulthood

At gene-level, after correction for multiple testing, 63 genes remained significant ($p < 2.591 * 10^{-6}$) in the whole cohort (Figure 2.). Sex-stratified analyses resulted in 44 significant genes in male subjects and 45 significant genes in female subjects.

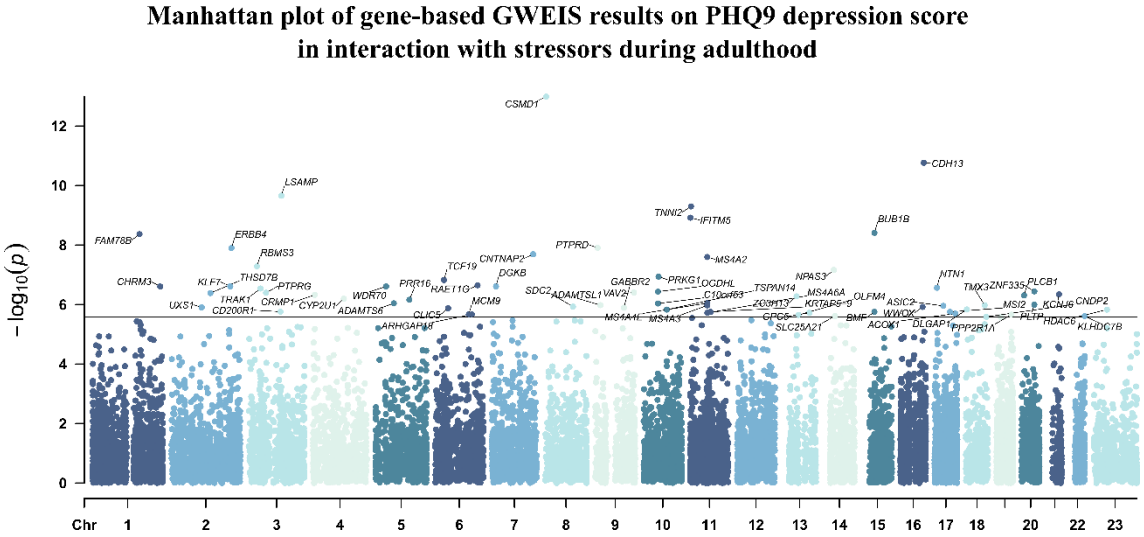


Figure 2. Manhattan plot of gene-based GWEIS results on PHQ9 depression score in interaction with stressors during adulthood (49). The x-axis represents the chromosomal location of genes across the genome. The y-axis represents the $-\log_{10}$ of the gene-based p-values, converted from the interaction between each genetic variant in a gene and adult traumatic events score on PHQ9 depression values in the UKB cohort. The horizontal line indicates the gene-level genome-wide significance threshold ($p = 2.591 \times 10^{-6}$). Points above this line denote genes with significant interaction effects (n = 63). The most significant gene *CSMD1* (Z stat = 7.3461; $p = 1.02 \times 10^{-13}$) was found on chromosome 8.

4.2.2.1. Blood-brain barrier (BBB)-related genes among the significant results of GWEIS in the whole cohort

From 63 significant genes, altogether 17 genes were considered as BBB-related (Figure 3.), based on enrichment results from gene expression data in cells of the BBB from human midbrain samples (80). With that, a 3.82-times enrichment (Pearson's $\chi 2$ (1, n = 19,296) = 38.04; p = $6.94*10^{-10}$) could be detected among the significant results, compared to the expected ratio of the BBB-related genes from all genes included in the GWEIS.

Manhattan plot of gene-based GWEIS results of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood in the whole UK Biobank cohort

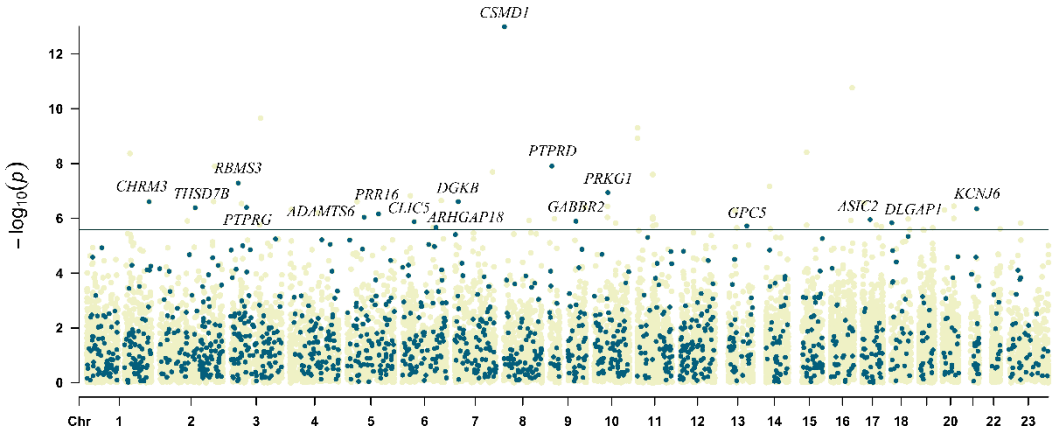


Figure 3. Manhattan plot of gene-based GWEIS results of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood in the whole UK Biobank cohort (49). The x-axis represents the chromosomal locations of the genes, while on the y-axis $-\log_{10}$ p values of the given genes can be found. The horizontal line indicates the gene-level significance threshold (p = $2.591 * 10^{-6}$). BBB-related genes, highly expressed in cells of the BBB in human post-mortem midbrain samples (80) are marked in blue. Symbols of significant BBB-related genes are written in the Manhattan-plot. From 63 significant genes, 17 can be connected to BBB. The most significant gene was *CSMD1* (Z-score = 7.3461; p = $1.02 * 10^{-13}$).

The most significant gene was *CSMD1* (Z-score = 7.3461; p = $1.02*10^{-13}$), which was considered as a BBB-related gene with enrichment in ependymal cells (80). BBB-relatedness of a subset of genes could be reinforced by available results from scientific literature (Table 8.).

Table 8. List of significant BBB-related genes from GWEIS on PHQ9 depression in interaction with stressors during adulthood in the whole UK Biobank. Gene - name of the gene; Chr - chromosome where the gene was positioned; ZSTAT - Z-score (converted from the gene-based p-value); p - p-value of the gene. In the column named "Connection with BBB", additionally to the cell type of BBB, where the given gene was enriched, relevant results from currently available literature data - in terms of the connection between the given gene and the blood-brain barrier - were referenced.

Gene	Chr	ZSTAT	P	Protein name	Connection with BBB
CSMD1	8	7.3461	$1.02 * 10^{-13}$	CUB and sushi domain-containing protein 1	Highly expressed in ependymal- (80) and endothelial cells (89) of the BBB; *CSMD1* ↓ C4A, C3 complement factors ↑ BBB permeability ↑ (90)
PTPRD	9	5.5748	$1.24 * 10^{-8}$	Protein Tyrosine Phosphatase Receptor Type D	Expressed in ependymal- (80) and smooth muscle cells (89) of the BBB; BACE1 (expressed in BBB endothelial cells (91)) ↑ PTPRD ↑ STAT3 phosphorylation ↓ (92) glioma ↑ (93)
RBMS3	3	5.3203	$5.18 * 10^{-8}$	RNA Binding Motif Single Stranded Interacting Protein 3	Expressed in fibroblasts (89), endothelial cells, pericytes and SMCs (80)
PRKG1	10	5.1736	$1.15 * 10^{-7}$	Protein Kinase CGMP-Dependent 1	Expressed in ependymal cells, pericytes and SMCs (89) of BBB (80); part of the inflammation-related gene sets (81)
DGKB	7	5.0301	$2.45 * 10^{-7}$	Diacylglycerol Kinase Beta	Expressed in BBB astrocytes and pericytes (80, 89)
CHRM3	1	5.0282	$2.48 * 10^{-7}$	Cholinergic Receptor Muscarinic 3	Expressed in BBB ependymal cells, MSCs (80) and fibroblasts (89)
PTPRG	3	4.9339	$4.03 * 10^{-7}$	Receptor-type tyrosine-protein phosphatase gamma	Highly expressed in BBB endothelial- (89), ependymal cells, fibroblasts, pericytes and SMCs (80)
THSD7B	2	4.9297	$4.12 * 10^{-7}$	Thrombospondin type-1 domain-containing protein 7B	Expressed in fibroblasts, pericytes (89) and SMCs (80)
KCNJ6	21	4.9117	$4.51 * 10^{-7}$	Potassium Inwardly Rectifying Channel Subfamily J Member 6	Expressed in BBB endothelial cells (80)
PRR16	5	4.8267	$6.94 * 10^{-7}$	Proline Rich 16	Expressed in fibroblasts, pericytes and SMCs (80)
ADAMTS6	5	4.7719	$9.12 * 10^{-7}$	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 6	Expressed in endothelial cells (89) and SMCs (80)
ASIC2	17	4.7319	$1.11 * 10^{-6}$	Acid Sensing Ion Channel Subunit 2	Expressed in MSCs (80)
GABBR2	9	4.7032	$1.28 * 10^{-6}$	Gamma-Aminobutyric Acid Type B Receptor Subunit 2	Expressed in astrocytes (80)
CLIC5	6	4.6948	$1.33 * 10^{-6}$	Chloride intracellular channel protein 5	Expressed in BBB endothelial cells (80, 89); enriched in BBB endothelial cells in mouse (in GFAP+ cells) (94)
DLGAP1	18	4.6757	$1.46 * 10^{-6}$	Disks large-associated protein 1	Expressed in astrocytes (80) and fibroblasts (89)
GPC5	13	4.6224	$1.90 * 10^{-6}$	Glypican 5	Expressed in astrocytes (80) and pericytes (89)
ARHGAP18	6	4.5952	$2.16 * 10^{-6}$	Rho GTPase-activating protein 18	Expressed in brain endothelial-, vascular- (95) and ependymal cells (80)

4.2.2.2. Blood-brain barrier (BBB)-related genes in male subjects among the significant results of GWEIS

In case of male subjects, based on gene expression data (80) 13 genes from 44 significant ones (Figure 4.) showed 4.18-times enrichment compared to the expected ratio of BBB-related genes among all genes included in the analyses (Pearson's $\chi 2$ (1, n = 19,296) = 33.84; p = $5.99*10^{-9}$).

Manhattan plot of gene-based GWEIS results of male subjects of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood

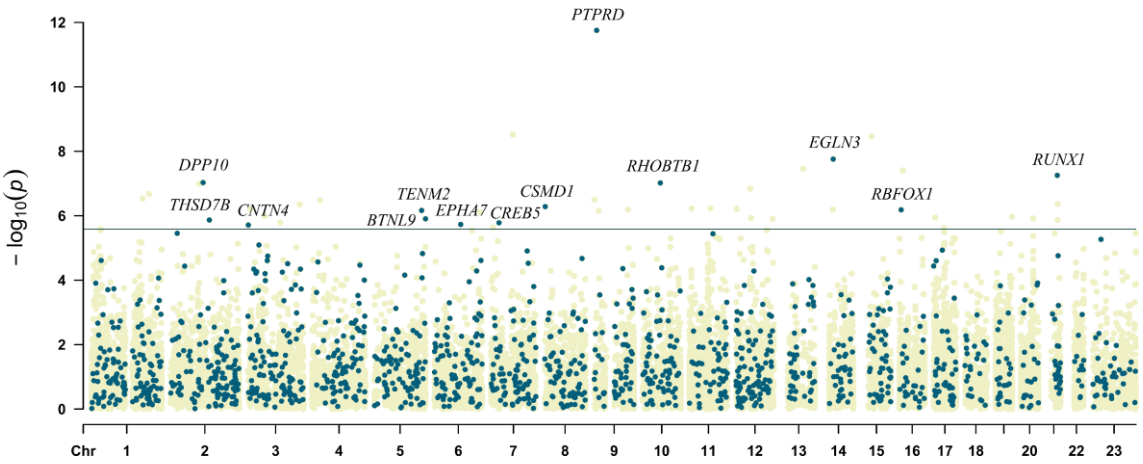


Figure 4. Manhattan plot of gene-based GWEIS results of male subjects of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood (49). The x-axis represents the chromosomal locations of the genes, while on the y-axis -$\log_{10}$ p values of the given genes can be found. The horizontal line indicates the gene-level significance threshold (p = 2.591 * 10^{-6}). BBB-related genes, highly expressed in cells of the BBB in human post-mortem midbrain samples (80) are marked in blue. Symbols of significant BBB-related genes are written in the Manhattan-plot. From 44 genes, that survived correction for multiple testing in the GWEIS, 13 genes can be considered as BBB-related. The most significant gene was *PTPRD* (Z-score = 6.9549; p = $1.76*10^{-12}$) on chromosome 9.

The most significant gene of the GWEIS in male subjects was *PTPRD* (Z-score = 6.9549; p = $1.76*10^{-12}$). This gene encodes for Protein Tyrosine Phosphatase Receptor Type D which was enriched in ependymal cells of the BBB (80). In addition to its role in the regulation of various cellular processes as a signalling molecule, results from the scientific literature showed its contribution in gliomagenesis, too (93). BBB-related

expression data and relevant functions of 13 significant BBB-related genes in men are collected in Table 9.

Table 9. List of significant BBB-related genes in men from GWEIS on PHQ9 depression in interaction with stressors during adulthood. Gene - name of the gene; Chr - chromosome where the gene was positioned; ZSTAT - Z-score (converted from the gene-based p-value); p - p-value of the gene. In the column named "Connection with BBB", additionally to the cell type of BBB, where the given gene was enriched, relevant results from currently available literature data - in terms of the connection between the given gene and the blood-brain barrier - were referenced.

Gene	Chr	ZSTAT	P	Protein name	Connection with BBB
PTPRD	9	6.9549	$1.76 * 10^{-12}$	Protein Tyrosine Phosphatase Receptor Type D	Expressed in ependymal- (80) and smooth muscle cells (89) of the BBB; BACE1 (expressed in BBB endothelial cells (91)) ↑ PTPRD ↑ STAT3 phosphorylation ↓ (92) glioma ↑ (93)
EGLN3	14	5.5146	$1.75 * 10^{-8}$	Egl-9 Family Hypoxia Inducible Factor 3	Expressed in astrocytes (80); via the upregulation of CLDN1 have a role in glia limitans formation after BBB disruption (96)
RUNX1	21	5.3066	$5.58 * 10^{-8}$	RUNX Family Transcription Factor 1	Expressed in fibroblasts (89) and SMCs (80); *RUNX1* ↑ (binds) *CLDN5*, *OCLN*, *ZO1* ↓ (97, 98)
DPP10	2	5.2112	$9.38 * 10^{-8}$	Dipeptidyl Peptidase Like 10	Expressed in astrocytes and ependymal cells (80)
RHOBTB1	10	5.2067	$9.61 * 10^{-8}$	Rho Related BTB Domain Containing 1	Expressed in endothelial-, ependymal cells and MSCs (80)
CSMD1	8	4.8834	$5.21 * 10^{-7}$	CUB and sushi domain-containing protein 1	Highly expressed in ependymal- (80) and endothelial cells (89) of the BBB; *CSMD1* ↓ C4A, C3 complement factors ↑ BBB permeability ↑ (90)
RBFOX1	16	4.8382	$6.55 * 10^{-7}$	RNA Binding Fox-1 Homolog 1	Expressed in ependymal cells (80) and fibroblasts (89); miR-132 ↑ RBFox-1 ↓ *CLDN1*, *TJAP1* ↓ (99)
TENM2	5	4.8291	$6.86 * 10^{-7}$	Teneurin Transmembrane Protein 2	Expressed in astrocytes (80) and fibroblasts (89)
BTNL9	5	4.7087	$1.25 * 10^{-6}$	Butyrophilin Like 9	Expressed in endothelial cells (89) and MSCs (80)
THSD7B	2	4.6911	$1.36 * 10^{-6}$	Thrombospondin type-1 domain-containing protein 7B	Expressed in fibroblasts, pericytes (89) and SMCs (80)
CREB5	7	4.6499	$1.66 * 10^{-6}$	CAMP Responsive Element Binding Protein 5	Expressed in fibroblasts, ependymal- (80) and endothelial cells (89)
EPHA7	6	4.6261	$1.86 * 10^{-6}$	EPH Receptor A7	Expressed in fibroblasts (89), ependymal cells and MSCs (80)
CNTN4	3	4.6167	$1.95 * 10^{-6}$	Contactin 4	Expressed in perciytes (89), fibroblasts and MSCs (80)

4.2.2.3. Blood-brain barrier (BBB)-related genes in female subjects among the significant results of GWEIS

In female subjects, based on gene expression data of cells forming the BBB from human midbrain samples (80) 14 genes from 45 significant ones (Figure 5.) showed 4.40-times enrichment compared to the expected ratio of BBB-related genes among all genes, included in the analyses (Pearson's $\chi 2$ (1, n = 19,296) = 39.60; p = $3.12*10^{-10}$).

Manhattan plot of gene-based GWEIS results of female subjects of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood

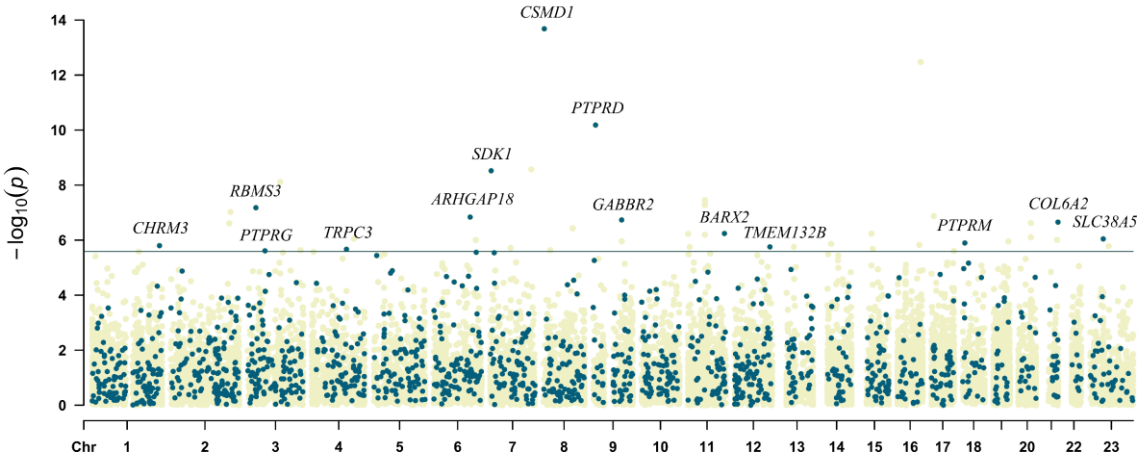


Figure 5. Manhattan plot of gene-based GWEIS results of female subjects of blood-brain barrier related genes on PHQ9 depression score in interaction with stressors during adulthood (49). The x-axis represents the chromosomal locations of the genes, while on the y-axis $-\log_{10}$ p values of the given genes can be found. The horizontal line indicates the gene-level significance threshold (p = 2.591 * 10^{-6}). BBB-related genes, highly expressed in cells of the BBB in human post-mortem midbrain samples (80) are marked in blue. Symbols of significant BBB-related genes are written in the Manhattan-plot. The most significant BBB-related gene was *CSMD1* (Z-score = 7.5556; p = $2.08*10^{-14}$).

Similarly to the results from the whole cohort, *CSMD1* gene showed the highest significance (Z-score = 7.5556; $p = 2.08*10^{-14}$) in female subjects, among all genes, and among the BBB-related genes, too. BBB-related expression data and relevant functions of 14 significant BBB-related genes in women are collected in Table 10.

Table 10. List of significant BBB-related genes in women from GWEIS on PHQ9 depression in interaction with stressors during adulthood. Gene - name of the gene; Chr - chromosome where the gene was positioned; ZSTAT - Z-score (converted from the gene-based p-value); p - p-value of the gene. In the column named "Connection with BBB", additionally to the cell type of BBB, where the given gene was enriched, relevant results from currently available literature data in terms of the connection between the given gene and the blood-brain barrier were referenced.

Gene	Chr	ZSTAT	P	Protein name	Connection with BBB
CSMD1	8	7.5556	$2.08 * 10^{-14}$	CUB and sushi domain-containing protein 1	Highly expressed in ependymal- (80) and endothelial cells (89) of the BBB; *CSMD1* ↓ C4A, C3 complement factors ↑ BBB permeability ↑ (90)
PTPRD	9	6.4255	$6.57 * 10^{-11}$	Protein Tyrosine Phosphatase Receptor Type D	Expressed in ependymal- (80) and smooth muscle cells (89) of the BBB; BACE1 (expressed in BBB endothelial cells (91)) ↑ PTPRD ↑ STAT3 phosphorylation ↓ (92) glioma ↑ (93)
SDK1	7	5.8161	$3.01 * 10^{-9}$	Sidekick Cell Adhesion Molecule 1	Expressed in SMCs (89), fibroblasts, endothelial- and ependymal cells (80)
RBMS3	3	5.2747	$6.65 * 10^{-8}$	RNA Binding Motif Single Stranded Interacting Protein 3	Expressed in fibroblasts (89), endothelial cells, pericytes and SMCs (80)
ARHGAP18	6	5.1288	$1.46 * 10^{-7}$	Rho GTPase-activating protein 18	Expressed in brain endothelial-, vascular- (95) and ependymal cells (80)
GABBR2	9	5.0840	$1.85 * 10^{-7}$	Gamma-Aminobutyric Acid Type B Receptor Subunit 2	Expressed in astrocytes (80)
COL6A2	21	5.0492	$2.22 * 10^{-7}$	Collagen Type VI Alpha 2 Chain	Expressed in human BBB fibroblasts, pericytes, SMCs (89) and MSCs (80); and by primary brain capillary endothelial cells in mice (100)
BARX2	11	4.8649	$5.73 * 10^{-7}$	BARX Homeobox 2	Expressed in ependymal cells (80)
SLC38A5	X	4.7739	$9.03 * 10^{-7}$	Sodium-coupled neutral amino acid transporter 5	Expressed in endothelial cells (80, 89); enriched in mouse BBB cells (101); amino acid transporter in the BBB (102, 103)
PTPRM	18	4.7054	$1.27 * 10^{-6}$	Protein Tyrosine Phosphatase Receptor Type M	Expressed in fibroblasts (80) and endothelial cells (89)
CHRM3	1	4.6592	$1.59 * 10^{-6}$	Cholinergic Receptor Muscarinic 3	Expressed in BBB ependymal cells, MSCs (80) and fibroblasts (89)
TMEM132B	12	4.6385	$1.75 * 10^{-6}$	Transmembrane Protein 132B	Expressed in ependymal cells (80)
TRPC3	4	4.5962	$2.15 * 10^{-6}$	Transient Receptor Potential Cation Channel Subfamily C Member 3	Expressed in fibroblasts, pericytes (89), SMCs and MSCs (80)
PTPRG	3	4.9339	$4.03 * 10^{-7}$	Receptor-type tyrosine-protein phosphatase gamma	Highly expressed in BBB endothelial- (89), ependymal cells, fibroblasts, pericytes and SMCs (80)

4.2.3. Inflammation-related genes in stress-associated depression

Inflammation-related genes were determined based on previously collected MSigDB C2 curated genes sets (81) and inflammation-related Hallmark gene sets (82, 83).

With 23 inflammation-related genes from the 63 significant results in the whole UKB cohort (Figure 6.), a 1.59-times enrichment could be determined with Pearson's $\chi 2$ (1, n = 19,296) = 6.60; p = 0.0102.

Manhattan plot of gene-based GWEIS results of inflammation-related genes on PHQ9 depression score in interaction with stressors during adulthood in the whole UK Biobank cohort

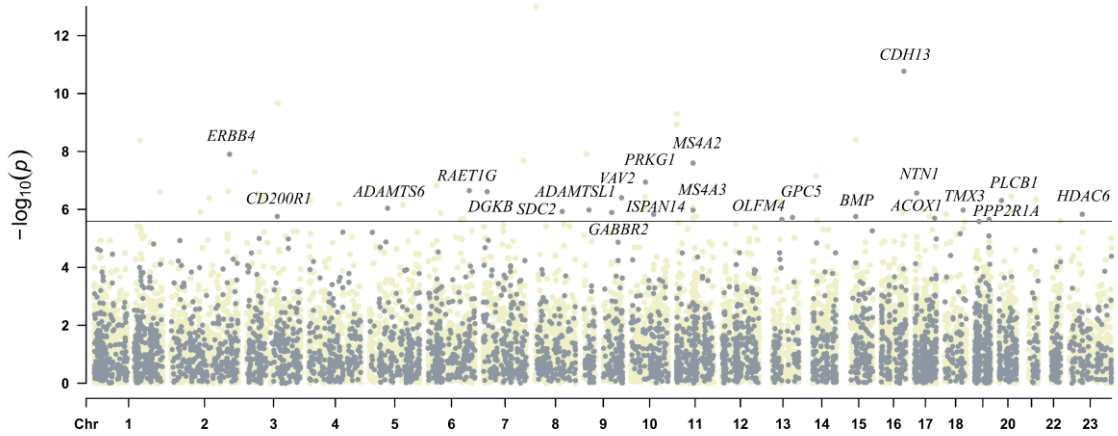


Figure 6. Manhattan plot of gene-based GWEIS results of inflammation-related genes on PHQ9 depression score in interaction with stressors during adulthood in the whole UK Biobank cohort (49). The x-axis represents the chromosomal locations of the genes, while on the y-axis -$\log_{10}$ p values of the given genes can be found. The horizontal line indicates the gene-level significance threshold ($p = 2.591 * 10^{-6}$). Inflammation-related genes, determined based on MSigDB C2 curated genes sets (81) and Hallmark gene sets (82, 83), are marked in grey. Symbols of significant inflammation-related genes are written in the Manhattan-plot. The most significant inflammation-related gene was *CDH13* (Z-score = 6.6273; $p = 1.71*10^{-11}$) on chromosome 16.

Among significant inflammation-related results, 5 genes (*PRKG1*, *DGKB*, *ADAMTS6*, *GABBR2*, and *GPC5*) were also part of the BBB-related genes.

The results of the 23 significant inflammation-related genes from the GWEIS on depressive symptoms in interaction with stressors during adulthood are listed in Table 11.

Table 11. List of significant inflammation-related genes from GWEIS on PHQ9 depression in interaction with stressors during adulthood. Gene - name of the gene; Chr - chromosome where the gene was positioned; ZSTAT - Z-score (converted from the gene-based p-value); p - p-value of the gene. In the column named "Connection with inflammatory processes or with BBB", available literature data in terms of the gene's connection with inflammation or with the BBB were referenced. Genes that were shown to be expressed in cells of the BBB are underlined.

Gene	Chr	ZSTAT	P	Protein name	Connection with inflammatory processes or with BBB
CDH13	16	6.6273	$1.71 * 10^{-11}$	Cadherin 13 (T- or H-cadherin)	Highly expressed in BBB endothelial cells (89); part of the complement system (82); T-cadherin ↑ VE-cadherin ↓ endothelial permeability disruption (104)
ERBB4	2	5.5728	$1.25 * 10^{-8}$	Erb-B2 Receptor Tyrosine Kinase 4	Expressed in SMCs of the BBB (89); ERBB4 ↑ BBB integrity (claudin-5, occludin) ↑ (105); part of the MAPK signalling pathway (81, 83); promotes cell death in pro-inflammatory macrophages (106)
MS4A2	11	5.4496	$2.52 * 10^{-8}$	High affinity immunoglobulin epsilon receptor subunit beta	Encodes β subunit of high affinity IgE receptor (107) involved in innate immune system (83)
PRKG1	10	5.1736	$1.15 * 10^{-7}$	Protein Kinase CGMP-Dependent 1	Expressed in ependymal cells, pericytes and SMCs (89) of BBB (80); part of the adaptive immune system (83)
RAET1G	6	5.0472	$2.24 * 10^{-7}$	Retinoic Acid Early Transcript 1G	Part of the gene set of natural killer cell mediated cytotoxicity (83) and MHC class I related proteins (108)
DGKB	7	5.0301	$2.45 * 10^{-7}$	Diacylglycerol Kinase Beta	Expressed in BBB astrocytes and pericytes (80, 89); part of haemostasis gene set (83)
NTN1	17	5.0095	$2.73 * 10^{-7}$	Netrin-1	Expressed in SMCs of BBB (89); its protein product upregulates tight junction and adherent junction molecules in the BBB, restoring it and reducing the negative effect of inflammation (109); KLF2 ↑ NTN1 ↑ IL-6 ↓ BBB permeability (occludin ↑) ↓ (110)
VAV2	9	4.9373	$3.96 * 10^{-7}$	Guanine nucleotide exchange factor VAV2	Part of the VEGF signalling in the BBB; VEGF ↑ cAMP ↑ Rac1-Vav2 ↑ endothelial barrier integrity (111); part of T-receptor-, and chemokine signalling gene sets (83)
PLCB1	20	4.8946	$4.92 * 10^{-7}$	Phospholipase C Beta 1	Expressed in BBB endothelial cells (89); inflammation ↑ DHHC21 ↑ PLCβ1 palmityolation ↑ endothelial barrier dysfunction, microvascular leakage, leucocyte adhesion ↑ (112); part of "IL-8- and CXCR2-mediated signaling events" gene set (83)
ADAMTS6	5	4.7719	$9.12 * 10^{-7}$	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 6	Expressed in endothelial cells (89) and SMCs (80); part of ECM regulators gene set (83)
ADAMTSL1	9	4.7439	$1.05 * 10^{-6}$	ADAMTS Like 1	Expressed in fibroblasts (89); part of ECM regulators gene set (83)

The table continues on the next page

Table 11. continued

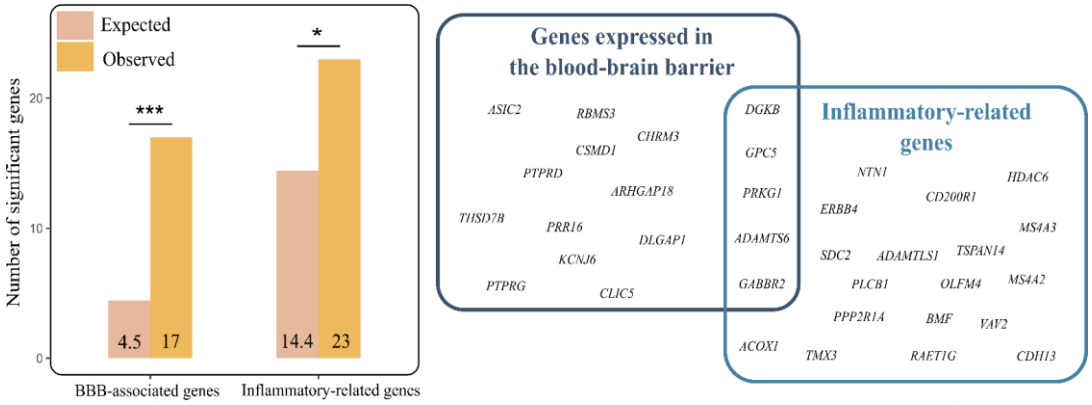
Gene	Chr	ZSTAT	P	Protein name	Connection with inflammatory processes or with BBB
TMX3	18	4.7426	$1.05 * 10^{-6}$	Thioredoxin Related Transmembrane Protein 3	Part of haemostasis gene set (83)
MS4A3	11	4.7405	$1.07 * 10^{-6}$	Membrane Spanning 4-Domains A3	Expressed by granulocyte-monocyte progenitors (113); part of innate immune system gene set (83)
SDC2	8	4.7205	$1.18 * 10^{-6}$	Syndecan-2	Expressed in fibroblasts (89); part of haemostasis- and ECM-related gene sets (83); inflammation elevated the epithelial production of Sdc-2 (114); regulates monocyte-derived macrophages (115)
GABBR2	9	4.7032	$1.28 * 10^{-6}$	Gamma-Aminobutyric Acid Type B Receptor Subunit 2	Expressed in astrocytes (80); part of genes, involved in G alpha signalling (83), inflammation-related gene set (81)
TSPAN14	10	4.6739	$1.48 * 10^{-6}$	Tetraspanin 14	Expressed in endothelial cells (89); part of innate immune system (83)
HDAC6	X	4.6733	$1.48 * 10^{-6}$	Histone Deacetylase 6	HDAC6 expression could contribute to BBB impairment (116); its inhibition prevents inflammatory processes and preserves barrier integrity (117); regulates autophagy and NLRP3 inflammasome (118)
CD200R1	3	4.6400	$1.74 * 10^{-6}$	CD200 Receptor 1	CD200 and its receptor (CD200R) is an important regulator of inflammation and the consequent BBB disruption (119) they mediate inflammation transmission from periphery to CNS (120); involved in the adaptive immune system (83)
BMF	15	4.6376	$1.76 * 10^{-6}$	Bcl2 Modifying Factor	IL-10 may downregulate BMF (121); involved in apoptosis (83)
GPC5	13	4.6224	$1.90 * 10^{-6}$	Glypican 5	Expressed in astrocytes (80) and pericytes (89); part of ECM regulators gene set (83)
ACOX1	17	4.6102	$2.01 * 10^{-6}$	Acyl-CoA Oxidase 1	Part of "Mechanism of Gene Regulation by Peroxisome Proliferators via PPARa(alpha)" gene set (83)
PPP2R1A	19	4.5915	$2.20 * 10^{-6}$	Protein Phosphatase 2 Scaffold Subunit Aalpha	Part of the TGFβ- and T cell signalling gene set (83)
OLFM4	13	4.5891	$2.23 * 10^{-6}$	Olfactomedin 4	Part of the innate immune system gene set (83); marker gene for a subset of neutrophil cells (122)

Contrary to the analysis with BBB-related genes, in case of inflammation-related genes, no significant enrichment were found in male- (Pearson's $\chi 2$ (1, n = 19.296) = 0.011; p = 0.977)) or in female (Pearson's $\chi 2$ (1, n = 19.296) = 0.06; p = 0.806)) subjects, separately.

4.2.4. Significant genes associated both with blood-brain barrier (BBB) and inflammation

Results from GWEIS conducted in the whole UKB cohort revealed significant enrichments in the numbers of BBB- (n = 17) and inflammation-related (n = 23) genes among the significant results (n = 63), compared to the expected ratios (expected number of significant BBB-related genes = 4.5; expected number of significant inflammation-related genes = 14.4). The results also uncovered 5 genes, which were expressed in cells of the BBB in midbrain region and were also part of the inflammatory gene sets: *DGKB*, *GPC5*, *PRKG1*, *ADAMTS6*, and *GABBR2* (Figure 7.).

The expected and observed numbers of BBB- and inflammatory-related genes among the significant GWEIS results



Gene-level GWEIS results		
	all genes	**significant genes**
Number of genes in the GWEIS	19,296	63
BBB-associated genes	1364	17
Inflammatory-related genes	4420	23

Figure 7. The expected and observed numbers of BBB- and inflammation-related genes among the significant GWIES results on PHQ9 depression in interaction with stressors during adulthood in the UK Biobank cohort. Altogether 19,296 genes were included in the GWEIS, and 63 genes remained significant after correction for multiple testing ($p < 2.591 * 10^{-6}$). BBB-related genes were determined using gene expression data from human post-mortem midbrain samples while inflammation-related genes were defined based on MSigDB gene sets. Based on the expected and observed ratio of BBB-associated genes, a significant (***: $p = 6.94 * 10^{-10}$) 3.82-times enrichment could be detected. In case of inflammation-related genes, a significant (*: p = 0.0102) 1.59-times enrichment was calculated. On the right side of the figure the Venn-diagram shows the observed significant genes which were considered as BBB- (n = 17), inflammation-related (n = 23) or both (n = 5).

4.2.5. Neuroticism-related genes among the significant results

Four neuroticism-related genes remained significant after correction for multiple testing in the whole cohort and in women: *CDH13*, *LSAMP*, *ERBB4* and *CNTNAP2*. In men, additionally to *LSAMP*, three different genes were considered as neuroticism-related among the significant results: *RBFOX1*, *TENM2*, *FHIT*.

4.2.6. Replication of significant findings

Due to the different characteristics of the two cohorts in number of participants, in age distribution and in the number of available SNPs, replications of significant BBB- and inflammation-related genes were conducted only in the whole cohort and were based on sign tests, where the same positive sign of Z-score were considered as replication. Based on that, we could replicate 13 genes - among them 4 (*CSMD1*, *ADAMTS6*, *DLGAP1*, and *GPC5*) showed nominal significance ($p < 0.05$) - from 17 significant BBB-related genes. In case of the 23 inflammation-related genes from GWEIS, 15 could be replicated in the NM cohort, with 4 nominally significant results (*ADAMTS6*, *ADAMTSL1*, *TSPAN14*, *GPC5*).

5. Discussion

First, we showed that rs885985 of *CLDN5* and rs1800795 of *IL6* played a role in mediating the effects of recent stress on current depressive symptoms in humans (48). Based on the previously published function of rs885985 in *CLDN5*, rs1800795 in *IL6*, and our results, it is possible that minor allele carrier status on *CLDN5* SNP contributes to the decreased expression of claudin-5 protein in the BBB and that minor allele carriers of *IL6* SNP are more prone to express elevated level of IL-6 proinflammatory cytokine, after experiencing recent stress. These circumstances might promote a more permeable BBB and the accompanied elevated level of proinflammatory cytokine, which could infiltrate the CNS in humans, too.

Second, we provided further evidence for the involvement of the BBB and inflammation in stress-associated depression (49). By comparing the observed number of BBB- and inflammation-related genes to the expected ratio among the significant gene-level results of GWEIS on depressive symptoms, we could show significant enrichments regarding BBB- and inflammation-related genes. The high number of BBB- and inflammation-related genes among the significant GWEIS results indicated that after experiencing stress effects, alteration of the tightly regulated microenvironment of the CNS could result in the appearance of depressive symptoms.

Based on our results, restoration of the disrupted integrity of the BBB together with reducing the elevated level of inflammation might be an important target during pharmaceutical interventions in depression.

5.1. *CLDN5* and *IL6* together have a potential role in the development of stress-induced depressive symptoms

After chronic stress effects, stress-susceptible mice developed depressive-like symptoms, accompanied by reduced claudin-5 expression in the *nucleus accumbens* and passage of IL-6 proinflammatory cytokine from the periphery to the CNS (41, 43). We showed that there was a genetic basis of this phenomenon in humans. The significant results of the 3-way interaction analyses with rs885985 of *CLDN5*, rs1800795 of *IL6*, and recent stress on current depressive symptoms ($\beta = 0.0093$; $p = 0.0003$) indicated that minor allele carriers on these polymorphisms were vulnerable to developing stress-associated

depression. Significant results were reinforced by trend-level significant replication in the NewMood cohort (β = 0.0553; p = 0.0972).

Analyses with the selected polymorphisms separately did not result in significant findings, suggesting the necessity of their combined effects in the association. Our results also indicated sex differences, as the effect size found in the whole cohort increased in men (β = 0.0141; p = 0.0002), and the significance disappeared, with a decreased effect size in women (β = 0.0055; p = 0.1208).

CLDN5 gene encodes for claudin-5, a transmembrane protein, which is expressed in various tissues in the brain, in the lung, and in the kidneys (123), and its protein has a major role in the formation of endothelial barriers (124). Previous studies on post-mortem human tissues already revealed the reduced level of claudin-5 in individuals with depression diagnosis (41, 43), and it was also shown that claudin-5 expression was suppressed by inflammatory factors, such as TNF-α (46, 125, 126), IL-1 (127) and by vascular endothelial growth factor (VEGF) (128). In addition to that, we could provide a genetic basis to the background of this mechanism showing the roles of minor alleles of rs885985 in *CLDN5* and rs1800795 in *IL6* as potential stress susceptibility factors in depression. As hydrocortisone treatment has barrier tightening effects via increasing claudin-5 and occludin levels (125), moreover in the promoter region of *CLDN5* gene there are glucocorticoid-response elements (46, 47), our results suggest that the negative effects of proinflammatory cytokines on the integrity of the BBB might be manifested in case of the assumed glucocorticoid resistance in depression invoked by constant stress stimuli (19, 23-25).

5.2. GWEIS results further support the involvement of blood-brain barrier (BBB)- and inflammation-related factors in stress-associated depression

Results of the GWEIS on current depressive symptoms in interaction with stressors during adulthood revealed that 26.98% of the significant genes could be connected to BBB, and 36.51% of them to inflammation. Compared to the expected ratio, these results indicated a 3.82-times enrichment in BBB-related and a 1.59-times enrichment in inflammation-related genes with both being significant using chi-square tests.

Eight of the significant genes (*CSMD1*, *CDH13*, *ERBB4*, *RBMS3*, *PRR16*, *ASIC2*, *GPC5*, *OLFM4*) were previously associated with depression without stress effects in a large

meta-analysis with more than 800,000 individuals (10). Five significant genes were considered both BBB- and inflammation-related: *DGKB*, *GPC5*, *PRKG1*, *ADAMTS6*, and *GABBR2*. In our study, 4 genes remained significant during the analyses in the whole cohort and in men and in women, separately. These were *CSMD1*, *PTPRD*, *LSAMP*, and *NPAS3*, indicating the strength and sex-independence of these associations.

5.2.1. Common genes of GWEIS conducted in the whole cohort, and separately in men and women

CSMD1 gene encodes for CUB and sushi domain-containing protein 1, which is a transmembrane protein (129), highly expressed in neurons (130), in ependymal- (80) and, in endothelial cells (89) of the BBB. It shows enrichment in the brain and in testicular tissues (131). This gene showed the highest significance in the GWEIS in the whole cohort (Z-score = 7.3461; $p = 1.02*10^{-13}$) and in women (Z-score = 7.5556; $p = 2.08*10^{-14}$). Its protein has a role in cell signalling, complement inhibition (90) and in the determination of the dopamine/ serotonin ratio in the CSF (132). In addition to MDD (10, 133), it has been previously associated with SCZ (129), age-related hearing loss (134), cannabis dependence (135) ASD (136) and was also suggested as a pleiotropic gene of bipolar disorder (BD), SCZ, attention-deficit/hyperactivity disorder (ADHD), MDD and ASD (137). *CSDM1* promotes the degradation of C3b and C4b complement factors (90, 138). The resulting elevated circulating C4 protein level contributed to BBB disruption in SCZ (139) and knocking-out of this gene resulted in the appearance of anxiety- and depressive-like phenotypes (140). The epigenetic pattern of *CSMD1* was shown to be associated with high maternal cortisol level (141), which indicated its vulnerability to stress during foetal development.

PTPRD (Protein Tyrosine Phosphatase Receptor Type D) gene was shown to be expressed in ependymal cells of the BBB (80) and it was the most significant hit in the male subgroup in our GWEIS (Z-score = 6.9549; $p = 1.76*10^{-12}$). *PTPRD* encodes for receptor-type protein tyrosine phosphatase membrane protein, which is considered a synaptic specifier cell adhesion molecule (142) and has a role in bidirectional induction of pre- and postsynaptic differentiation (143). Its protein has a role in the IL-6/JAK/STAT3 signal transduction pathway and it is a substrate of β-secretase 1 (BACE1) proteinase in the BBB (92). Furthermore, it was associated with elevated β-amyloids

production in Alzheimer's disease (AD) (91). *PTPRD* has previously been associated with anxiety (144), mood instability (145), and vulnerability (146).

Although *LSAMP* was not found to be enriched in cells of the BBB in midbrain samples (80), this gene was highly expressed in pericytes of the BBB in temporal lobe samples (89) and was expressed by BBB astrocytes (147). It encodes for Limbic System Associated Membrane Protein, a cell membrane lipid anchor with immunoglobulin domains (148), which has significant role in neuronal growth and axon targeting mediation (149). *LSAMP* is part of the IgLON family, which has a role in maintaining the integrity of the BBB (150). It was revealed in mice that *Lsamp* deficiency led to alteration in neurotransmitter regulation via increased activity of serotonergic system and imbalanced GABA-A (gamma-aminobutyric acid type A) receptor activity (151) and that fluoxetine treatment increased the expression of this gene (152). Based on the scientific literature, *LSAMP* might play a role in completed suicide among men (153) and was found to be highly expressed in limbic region related to stress and arousal (149). SNPs in *LSAMP* were associated with MDD, panic disorder (154, 155), and neuroticism (85).

The fourth gene, which remained significant in GWEIS on the whole cohort, in men and, in women, was *NPAS3*, encoding for Neuronal PAS Domain Protein 3. This transcription factor was found to be expressed in fibroblasts of the BBB in human temporal lobe (89), and it was involved in neurogenesis (156), metabolism, and as a regulator of circadian rhythm (157). *NPAS3* is a pleiotropy candidate in BP, MDD, and SCZ (158) and is associated with AD, PD (159), and with abnormal brain morphology in SCZ (160).

In view of the significant results of *CSMD1*, *PTPRD*, *LSAMP*, and *NPAS3* in sex-stratified analyses, in addition to their biological roles and previously revealed associations with mental disorders, these 4 genes might serve as biomarkers or drug targets in the treatment of stress-associated depression irrespective of stress.

5.2.2. Inflammation-related genes in the GWEIS

Considering inflammation in stress-associated depression, *CDH13* was the most significant inflammation-related gene in the GWEIS in the whole cohort (Z-score = 6.6273; $p = 1.71*10^{-11}$) and in women (Z-score = 7.1851; $p = 3.36*10^{-13}$). This gene was previously associated with neuroticism (84) ADHD, SCZ (161), and MDD (10, 161, 162). *CDH13* gene encodes for T- or H-cadherin, a lipid anchor transmembrane protein, which,

through regulation of VE-cadherin, has a key role in preserving the integrity of endothelial barriers (104). It is part of the complement system (82) and might be a potential candidate for obesity and vascular diseases (163). This gene has also a role in axon guidance, dendritic arborisation (164), and negative regulation of inhibitory synapses in the hippocampus (165). Mice with *Cdh13* deficiency showed impairment in memory, and learning (165) and had significantly reduced level of corticosterone, even after maternal stress separation (166). *CDH13* appeared to be expressed distinctly throughout the lifespan in humans with a lower expression rate in the developing nervous system than in the CNS of adults (164, 167). These results suggest that *CDH13* mediates altered stress-sensitivity during childhood and adulthood.

Another potential candidate from significant inflammation-related genes in stress-associated depression is *ERBB4*, which survived correction for multiple testing in the whole UKB cohort (Z-score = 5.5728; $p = 1.25*10^{-8}$) and in female subjects (Z-score = 5.2100; $p = 9.44*10^{-8}$). *ERBB4* encodes for Erb-B2 Receptor Tyrosine Kinase 4, a cell surface receptor for neuregulin, taking part in mitogenesis and cell differentiation (106). This gene has been associated with cyclothymic temperament (168) and with neuroticism (85). It is part of the MAPK signalling pathway (81, 83) and it has a role in promoting death of pro-inflammatory macrophages (106) and in protecting colonic epithelial cells against tumor necrosis factor-induced apoptosis (169). Its protective role was also manifested in preserving the integrity of the BBB by increasing the expression of claudin-5 and occludin after subarachnoid haemorrhage in rats (105).

5.2.3. Neuroticism-related genes

Animal studies, which elucidated the important role of BBB in depression, have also revealed that the depressive-like phenotype appeared only in a subset of rodents, which were considered vulnerable to stress (41, 43). Based on that, along with genetic factors, in humans, personality traits could also contribute to the development of depression acting through alterations in stress sensitivity or resilience (17). Such a trait could be neuroticism, which is characterized by elevated stress sensitivity and a tendency towards more frequent experience of negative emotions (170), thus, individuals with this trait might experience amplified stress and be more vulnerable to developing depressive symptoms. The comparison of our significant results with previously published neuroticism-associated genes (84, 85) revealed 4 potential candidates (*CDH13*, *ERBB4*,

LSAMP, *CNTNAP2*) contributing to this personality trait, as well as stress-associated depression. These 4 genes have also been shown to be expressed at the BBB, although not in the midbrain region (80), but in the temporal lobe (89), which further emphasised the important role of BBB in stress-related conditions, such as neuroticism or stress-associated depression.

5.3 Genetic findings support distinct biological mechanisms in men and in women in terms of the role of blood-brain barrier (BBB) and inflammation in stress-associated depression

With the results of the two studies, we could provide further evidence for the involvement of the BBB and inflammation in stress-associated depression. In the first study, we demonstrated the combined effect of the minor alleles of polymorphisms in *CLDN5* (rs885985) and in *IL6* (rs1800795) on stress-associated depression, and with that, we could translate results from animal studies into human genetics (48); during the second study, we provided a view of candidate BBB- and inflammation-related genes in stress-associated depression by conducting GWEIS (49).

In accordance with previously published results on depression biology, aetiology, and genetics (171), our studies highlighted sex differences. It is known, that not just the prevalence and the pattern of symptoms of MDD show divergence between men and women (2), but stress responses are manifested differently, too (172). Similarly, the BBB also exhibits variations between men and women (173). Female-related sex hormones, oestrogen, and progesterone have barrier-strengthening properties by increasing claudin-5, occludin, and reducing TNF-α and IL-6 cytokines (39, 42, 173-175). Correspondingly, in men, it was shown that testosterone influenced the integrity of the BBB, suggesting dose-dependent mechanisms, where lack of testosterone and excessive level of this hormone equally resulted in increased permeability (42).

Based on our results, *CLDN5* expression and its regulation also exhibited sex differences. During the first study, stratification by sex provided distinct results, where the interacting effects of rs885985 of *CLDN5* and rs1800795 of *IL6* on stress-associated depression were only detected in men, but not in women. Although, *CLDN5* gene did not survive correction for multiple testing in the second study, as a gene-level outcome, one target of interest in men was the *RUNX1* gene (Z-score in men = 5.3066; $p = 5.58*10^{-8}$), encoding

for RUNX Family Transcription Factor 1. This gene did not remain significant in the whole cohort, or in female subjects. *RUNX1* is expressed at the BBB in fibroblasts (89) and SMCs (80) and as a transcriptional regulator of *CLDN5*, it has an indirect effect on the integrity of the BBB (97). Based on previous studies, it has also been revealed that TNF-α could modulate *RUNX1* (176). It was shown, that this gene was a shared susceptibility factor in post-traumatic stress disorder (PTSD) and MDD (177), which could reinforce its stress susceptibility, too. Another example of the probable sex-specific altered regulation of *CLDN5*, in regard to our gene-level results, is *KLF5*. This gene encodes for Krueppel-like factor 5, a transcription factor, which not only has an effect on *CLDN5* expression and through this on BBB permeability but possesses anti- and proinflammatory roles as well, in different conditions (178). This gene only survived correction for multiple testing in the subcohort of male subjects (Z-score in men = 5.3903; $p = 3.52*10^{-8}$).

In addition, there are potential candidates for female subjects, too. *NR3C2* gene encodes for Nuclear Receptor Subfamily 3 Group C Member 2, a mineralocorticoid receptor (MR), which remained significant only in women (Z-score in women = 4.7794; $p = 8.79*10^{-7}$). Mineralocorticoid receptors are less abundant in the CNS, than glucocorticoid receptors (GR), however, MR shows a greater affinity for glucocorticoids than GR (179). As previous results in mice indicated greater stress responses in female animals (42), significant results of *NR3C2* in women might be a promising candidate for analysing the human genetic aspects of vulnerability to stress. Another candidate of female-specific genes is *NTN1*, encoding for Netrin-1. Based on the gene-level GWEIS results, it survived correction for multiple testing not just in women (Z-score in women = 5.1451; $p = 1.34*10^{-7}$), but in the whole cohort, too (Z-score in the whole cohort = 5.0095; $p = 2.73*10^{-7}$). Although, in expression data from midbrain BBB samples (80), it did not show enrichment, based on experiments with mice it had a role in maintaining BBB integrity and reducing the effects of cytokines on the barrier (109, 110). This protein has been implicated as being a necessary factor for axon guidance, and regulation of tight junctions of the BBB (109, 110). Netrin-1 signalling pathway was significantly associated with MDD without stress effects (180, 181). Our results imply that *NTN1* gene has a role in stress-associated depression, too.

5.4. Limitations

Researching the biological background of human depression lacks a wide variety of applicable methods and, thus, possesses several limitations, which could be applied to our studies as well. First, as MDD is a heterogenetic disorder, influenced by numerous factors (11), results derived from SNPs usually reveal small effects. Consequently, during the 3-way interaction analyses, we selected polymorphisms with known functionalities in order to alleviate the interpretation of the results, however, by conducting simple models, we could not rule out the effects of confounding factors and this method was unable to explain the overall complexity of depression's pathophysiology.

In both studies, we conducted replication analyses on the NM cohort, in order to reproduce the results derived from the UKB population. Nonetheless, the number of participants significantly differed in the two cohorts and the statistical power was consequently lower in the NM population. Taken together, the differences between the two cohorts restrain us from the confirmation of the universality of our results beyond doubt, albeit, they provide a solid foundation on a field mired by unreplicable findings. As the number of participants significantly differed in the two cohorts, the statistical power was similarly lesser in the NM population. It is also known that the physiology and integrity of the BBB change over the lifespan (182) and with significantly distinct average ages between the two cohorts, the contribution of BBB-related genes might be altered, too.

The determination of genes related to BBB or inflammatory processes relied on previous publications. However, gene expression data from cells of the BBB provided an advantage in our study, their enrichments were determined based on a comparison between the selected cells (and without comparing the expression pattern to another type of cells) in one brain area. The transcriptomic pattern of the BBB differs among brain regions (34), which also limits our results.

Finally, as inflammation-related genes were derived from previously collected gene sets of inflammatory mechanisms (81) and from the repository of the Molecular Signatures Database (82, 83), further replication would be needed in order to strengthen these results that extend this selection.

6. Conclusions

In conclusion, both the hypothesis-driven candidate gene approach and the GWEIS results confirmed the important role of BBB in stress-associated depression. First, we could reveal the combined effects of polymorphisms in *CLDN5* and *IL6* in stress-associated depression in humans, and by that, we could provide a genetic basis to the previously described alteration in BBB permeability accompanied by elevated inflammation in rodents. These results further supported the theory on the altered integrity of the BBB and the presence of inflammation in depression, as a consequence of stress.

Moreover, we could specify additional promising candidates of genes related to BBB- and inflammatory processes by discovering a 3.82-times enrichment of BBB-related and a 1.59-times enrichment of inflammation-related genes among the significant GWEIS results. The outcome of the sex-specific analyses conducted in both approaches pointed to partially different etiopathology in men and in women.

These results could provide a basis for further research aiming to reveal the biological background of stress-associated depression.

7. Summary

Previous results from animal studies highlighted the important role of the blood-brain barrier (BBB) and inflammatory factors in stress-associated depression. Our aims were to prove these pathomechanisms, specifically, the role of BBB and inflammation in stress-associated depression with two different genomic approaches in humans. In the first study a hypothesis-driven, candidate gene approach was used to translate evidences to humans from rodent studies, which indicated that reduced expression of tight junction protein claudin-5 after chronic stress, might weaken the BBB and allow interleukin-6 to infiltrate the central nervous system from the periphery, inducing depressive symptoms. Three-way interaction of functional polymorphisms rs885985 of *CLDN5* gene and rs1800795 of *IL6* gene, and recent stressful life events were tested on current depressive symptoms. Further characterizations were also conducted in order to reveal potential sex differences and to uncover the possible individual effects of the polymorphisms. The 3-way interaction including recent stress yielded highly significant results on current depressive symptoms, which was more pronounced in men and could be replicated on trend level in an independent cohort. None of any other interactions, including childhood stressors and lifetime depression as an outcome, yielded significance.

In the second study, a genome-wide by environment interaction analysis (GWEIS) was conducted on 6.26 M genetic variants covering 19,296 genes on PHQ9 depression phenotype in interaction with adult traumatic events scores. Among the 63 genes that remained significant after correction for multiple testing, 17 were associated with BBB, 23 with inflammatory processes, and 4 with neuroticism. Altogether, 4 genes remained significant in the whole cohort, in men, and in women, separately: *CSMD1*, *PTPRD*, *NPAS3*, and *LSAMP*. Compared to all genes, the enrichment of significant BBB-associated genes was 3.82, and that of inflammation-associated genes was 1.59, with both being statistically different from the expected ratio.

In conclusion, both studies confirmed a strong role of BBB and inflammation in stress-associated depression, and the identified risk genes and their encoded proteins could serve as biomarkers or new drug targets to promote BBB integrity to prevent or decrease stress- and inflammation-associated depressive symptoms.

8. References

1. World Health O. Depression and other common mental disorders: global health estimates. Geneva: World Health Organization; 2017 2017. Contract No.: WHO/MSD/MER/2017.2.
2. American Psychiatric Association D-TF. Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed. Arlington, VA, US: American Psychiatric Publishing, Inc.; 2013. xliv, 947-xliv, p.
3. Organization WH. International Classification of Diseases, 11th Revision (ICD-11) 2024 [Available from: https://icd.who.int/browse/2024-01/mms/en#1563440232.
4. Østergaard SD, Seidler Z, Rice S. The ICD-11 opens the door for overdue improved identification of depression in men. World Psychiatry. 2023;22(3):480-1.
5. Rice S, Seidler Z, Kealy D, Ogrodniczuk J, Zajac I, Oliffe J. Men's Depression, Externalizing, and DSM-5-TR: Primary Signs and Symptoms or Co-occurring Symptoms? Harv Rev Psychiatry. 2022;30(5):317-22.
6. Rice SM, Fallon BJ, Aucote HM, Möller-Leimkühler AM. Development and preliminary validation of the male depression risk scale: furthering the assessment of depression in men. J Affect Disord. 2013;151(3):950-8.
7. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry. 2000;157(10):1552-62.
8. Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. Lancet Psychiatry. 2017;4(5):409-18.
9. Cui L, Li S, Wang S, Wu X, Liu Y, Yu W, Wang Y, Tang Y, Xia M, Li B. Major depressive disorder: hypothesis, mechanism, prevention and treatment. Signal Transduct Target Ther. 2024;9(1):30.
10. Howard DM, Adams MJ, Clarke TK, Hafferty JD, Gibson J, Shirali M, Coleman JRI, Hagenaars SP, Ward J, Wigmore EM, Alloza C, Shen X, Barbu MC, Xu EY, Whalley HC, Marioni RE, Porteous DJ, Davies G, Deary IJ, Hemani G, Berger K, Teismann H, Rawal R, Arolt V, Baune BT, Dannlowski U, Domschke K, Tian C, Hinds DA, Trzaskowski M, Byrne EM, Ripke S, Smith DJ, Sullivan PF, Wray NR, Breen G, Lewis CM, McIntosh AM. Genome-wide meta-analysis of depression identifies 102

independent variants and highlights the importance of the prefrontal brain regions. Nat Neurosci. 2019;22(3):343-52.

11. Gonda X, Petschner P, Eszlari N, Baksa D, Edes A, Antal P, Juhasz G, Bagdy G. Genetic variants in major depressive disorder: From pathophysiology to therapy. Pharmacol Ther. 2019;194:22-43.

12. McGuffin P, Katz R, Rutherford J. Nature, nurture and depression: a twin study. Psychol Med. 1991;21(2):329-35.

13. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, Schatzberg AF. Major depressive disorder. Nat Rev Dis Primers. 2016;2:16065.

14. Lang UE, Beglinger C, Schweinfurth N, Walter M, Borgwardt S. Nutritional aspects of depression. Cell Physiol Biochem. 2015;37(3):1029-43.

15. Wirz-Justice A. Seasonality in affective disorders. Gen Comp Endocrinol. 2018;258:244-9.

16. Hao Y, Farah MJ. Heterogeneity of depression across the socioeconomic spectrum. Proc Natl Acad Sci U S A. 2023;120(16):e2222069120.

17. Klein DN, Kotov R, Bufferd SJ. Personality and depression: explanatory models and review of the evidence. Annu Rev Clin Psychol. 2011;7:269-95.

18. Beurel E, Toups M, Nemeroff CB. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. Neuron. 2020;107(2):234-56.

19. Gałecka M, Bliźniewska-Kowalska K, Maes M, Su KP, Gałecki P. Update on the neurodevelopmental theory of depression: is there any 'unconscious code'? Pharmacol Rep. 2021;73(2):346-56.

20. Harro J. Animal models of depression: pros and cons. Cell Tissue Res. 2019;377(1):5-20.

21. Kunz-Ebrecht SR, Mohamed-Ali V, Feldman PJ, Kirschbaum C, Steptoe A. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun. 2003;17(5):373-83.

22. Duman RS, Sanacora G, Krystal JH. Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. Neuron. 2019;102(1):75-90.

23. Hassamal S. Chronic stress, neuroinflammation, and depression: an overview of pathophysiological mechanisms and emerging anti-inflammatories. Front Psychiatry. 2023;14:1130989.
24. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65(9):732-41.
25. Zefferino R, Di Gioia S, Conese M. Molecular links between endocrine, nervous and immune system during chronic stress. Brain Behav. 2021;11(2):e01960.
26. Champaneri S, Wand GS, Malhotra SS, Casagrande SS, Golden SH. Biological basis of depression in adults with diabetes. Curr Diab Rep. 2010;10(6):396-405.
27. Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology. 2008;33(6):693-710.
28. Zhang JC, Yao W, Hashimoto K. Brain-derived Neurotrophic Factor (BDNF)-TrkB Signaling in Inflammation-related Depression and Potential Therapeutic Targets. Curr Neuropharmacol. 2016;14(7):721-31.
29. Jia Y, Liu L, Sheng C, Cheng Z, Cui L, Li M, Zhao Y, Shi T, Yau TO, Li F, Chen L. Increased Serum Levels of Cortisol and Inflammatory Cytokines in People With Depression. J Nerv Ment Dis. 2019;207(4):271-6.
30. Hodes GE, Pfau ML, Leboeuf M, Golden SA, Christoffel DJ, Bregman D, Rebusi N, Heshmati M, Aleyasin H, Warren BL, Lebonté B, Horn S, Lapidus KA, Stelzhammer V, Wong EH, Bahn S, Krishnan V, Bolaños-Guzman CA, Murrough JW, Merad M, Russo SJ. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A. 2014;111(45):16136-41.
31. Kiraly DD, Horn SR, Van Dam NT, Costi S, Schwartz J, Kim-Schulze S, Patel M, Hodes GE, Russo SJ, Merad M, Iosifescu DV, Charney DS, Murrough JW. Altered peripheral immune profiles in treatment-resistant depression: response to ketamine and prediction of treatment outcome. Transl Psychiatry. 2017;7(3):e1065.
32. Cheng Y, Desse S, Martinez A, Worthen RJ, Jope RS, Beurel E. TNFα disrupts blood brain barrier integrity to maintain prolonged depressive-like behavior in mice. Brain Behav Immun. 2018;69:556-67.

33. Lee S, Kang BM, Kim JH, Min J, Kim HS, Ryu H, Park H, Bae S, Oh D, Choi M, Suh M. Real-time in vivo two-photon imaging study reveals decreased cerebro-vascular volume and increased blood-brain barrier permeability in chronically stressed mice. Sci Rep. 2018;8(1):13064.

34. Gal Z, Huse RJ, Gonda X, Kumar S, Juhasz G, Bagdy G, Petschner P. [Anxiety and depression - the role of blood-brain barrier integrity]. Neuropsychopharmacol Hung. 2019;21(1):19-25.

35. Kadry H, Noorani B, Cucullo L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids Barriers CNS. 2020;17(1):69.

36. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis. 2010;37(1):13-25.

37. Schaeffer S, Iadecola C. Revisiting the neurovascular unit. Nat Neurosci. 2021;24(9):1198-209.

38. Kealy J, Greene C, Campbell M. Blood-brain barrier regulation in psychiatric disorders. Neurosci Lett. 2020;726:133664.

39. Greene C, Hanley N, Campbell M. Claudin-5: gatekeeper of neurological function. Fluids Barriers CNS. 2019;16(1):3.

40. Geng S, Yang L, Cheng F, Zhang Z, Li J, Liu W, Li Y, Chen Y, Bao Y, Chen L, Fei Z, Li X, Hou J, Lin Y, Liu Z, Zhang S, Wang H, Zhang Q, Wang H, Wang X, Zhang J. Gut Microbiota Are Associated With Psychological Stress-Induced Defections in Intestinal and Blood-Brain Barriers. Front Microbiol. 2019;10:3067.

41. Menard C, Pfau ML, Hodes GE, Kana V, Wang VX, Bouchard S, Takahashi A, Flanigan ME, Aleyasin H, LeClair KB, Janssen WG, Labonté B, Parise EM, Lorsch ZS, Golden SA, Heshmati M, Tamminga C, Turecki G, Campbell M, Fayad ZA, Tang CY, Merad M, Russo SJ. Social stress induces neurovascular pathology promoting depression. Nat Neurosci. 2017;20(12):1752-60.

42. Dion-Albert L, Bandeira Binder L, Daigle B, Hong-Minh A, Lebel M, Menard C. Sex differences in the blood-brain barrier: Implications for mental health. Front Neuroendocrinol. 2022;65:100989.

43. Dion-Albert L, Cadoret A, Doney E, Kaufmann FN, Dudek KA, Daigle B, Parise LF, Cathomas F, Samba N, Hudson N, Lebel M, Campbell M, Turecki G, Mechawar N,

Menard C. Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. Nat Commun. 2022;13(1):164.

44. Sántha P, Veszelka S, Hoyk Z, Mészáros M, Walter FR, Tóth AE, Kiss L, Kincses A, Oláh Z, Seprényi G, Rákhely G, Dér A, Pákáski M, Kálmán J, Kittel Á, Deli MA. Restraint Stress-Induced Morphological Changes at the Blood-Brain Barrier in Adult Rats. Front Mol Neurosci. 2015;8:88.

45. Krueger JM. The role of cytokines in sleep regulation. Curr Pharm Des. 2008;14(32):3408-16.

46. Burek M, Förster CY. Cloning and characterization of the murine claudin-5 promoter. Mol Cell Endocrinol. 2009;298(1-2):19-24.

47. Felinski EA, Cox AE, Phillips BE, Antonetti DA. Glucocorticoids induce transactivation of tight junction genes occludin and claudin-5 in retinal endothelial cells via a novel cis-element. Exp Eye Res. 2008;86(6):867-78.

48. Gal Z, Torok D, Gonda X, Eszlari N, Anderson IM, Deakin B, Juhasz G, Bagdy G, Petschner P. Inflammation and Blood-Brain Barrier in Depression: Interaction of CLDN5 and IL6 Gene Variants in Stress-Induced Depression. Int J Neuropsychopharmacol. 2023;26(3):189-97.

49. Gal Z, Torok D, Gonda X, Eszlari N, Anderson IM, Deakin B, Petschner P, Juhasz G, Bagdy G. New Evidence for the Role of the Blood-Brain Barrier and Inflammation in Stress-Associated Depression: A Gene-Environment Analysis Covering 19,296 Genes in 109,360 Humans. Int J Mol Sci. 2024;25(20).

50. Salgado S, Kaplitt MG. The Nucleus Accumbens: A Comprehensive Review. Stereotact Funct Neurosurg. 2015;93(2):75-93.

51. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, McVean G, Leslie S, Donnelly P, Marchini J. Genome-wide genetic data on ~500,000 UK Biobank participants. bioRxiv. 2017:166298.

52. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562(7726):203-9.

53. Welsh S, Peakman T, Sheard S, Almond R. Comparison of DNA quantification methodology used in the DNA extraction protocol for the UK Biobank cohort. BMC Genomics. 2017;18(1):26.

54. Deakin JF, Harro J, Anderson IM. NewMood: a productive European model of collaboration for translational research in depression. Eur Neuropsychopharmacol. 2011;21(1):1-2.

55. Eszlari N, Millinghoffer A, Petschner P, Gonda X, Baksa D, Pulay AJ, Réthelyi JM, Breen G, Deakin JFW, Antal P, Bagdy G, Juhasz G. Genome-wide association analysis reveals KCTD12 and miR-383-binding genes in the background of rumination. Transl Psychiatry. 2019;9(1):119.

56. Hullam G, Antal P, Petschner P, Gonda X, Bagdy G, Deakin B, Juhasz G. The UKB envirome of depression: from interactions to synergistic effects. Sci Rep. 2019;9(1):9723.

57. Kroenke K, Spitzer RL, Williams JB, Löwe B. The Patient Health Questionnaire Somatic, Anxiety, and Depressive Symptom Scales: a systematic review. Gen Hosp Psychiatry. 2010;32(4):345-59.

58. Derogatis LR. BSI, Brief Symptom Inventory: Administration, Scoring & Procedures Manual: National Computer Systems; 1993.

59. Brugha T, Bebbington P, Tennant C, Hurry J. The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. Psychol Med. 1985;15(1):189-94.

60. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, Sapareto E, Ruggiero J. Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry. 1994;151(8):1132-6.

61. Cunningham F, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Girón CG, Gordon L, Hourlier T, Hunt SE, Janacek SH, Johnson N, Juettemann T, Kähäri AK, Keenan S, Martin FJ, Maurel T, McLaren W, Murphy DN, Nag R, Overduin B, Parker A, Patricio M, Perry E, Pignatelli M, Riat HS, Sheppard D, Taylor K, Thormann A, Vullo A, Wilder SP, Zadissa A, Aken BL, Birney E, Harrow J, Kinsella R, Muffato M, Ruffier M, Searle SM, Spudich G, Trevanion SJ, Yates A, Zerbino DR, Flicek P. Ensembl 2015. Nucleic Acids Res. 2015;43(Database issue):D662-9.

62. Cornely RM, Schlingmann B, Shepherd WS, Chandler JD, Neujahr DC, Koval M. Two common human CLDN5 alleles encode different open reading frames but produce one protein isoform. Ann N Y Acad Sci. 2017;1397(1):119-29.
63. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
64. Fernández-Real JM, Broch M, Vendrell J, Richart C, Ricart W. Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. J Clin Endocrinol Metab. 2000;85(3):1334-9.
65. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102(7):1369-76.
66. Chen Y, Pawlikowska L, Yao JS, Shen F, Zhai W, Achrol AS, Lawton MT, Kwok PY, Yang GY, Young WL. Interleukin-6 involvement in brain arteriovenous malformations. Ann Neurol. 2006;59(1):72-80.
67. Kovacs D, Eszlari N, Petschner P, Pap D, Vas S, Kovacs P, Gonda X, Bagdy G, Juhasz G. Interleukin-6 promoter polymorphism interacts with pain and life stress influencing depression phenotypes. J Neural Transm (Vienna). 2016;123(5):541-8.
68. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD, Humphries SE. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol. 2001;21(9):1458-63.
69. Albani D, Batelli S, Polito L, Prato F, Pesaresi M, Gajo GB, De Angeli S, Zanardo A, Galimberti D, Scarpini E, Gallucci M, Forloni G. Interleukin-6 plasma level increases with age in an Italian elderly population ("The Treviso Longeva"-Trelong-study) with a sex-specific contribution of rs1800795 polymorphism. Age (Dordr). 2009;31(2):155-62.
70. Purcell SC, Christopher. PLINK 2.0. 2023.
71. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7.

72. Team RC. R: A Language and Environment for Statistical Computing. 4.3.0 ed. Vienna, Austria: R Foundation for Statistical Computing; 2022.
73. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. Nat Commun. 2017;8(1):1826.
74. Wickham H AM, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. “Welcome to the tidyverse.”. Journal of Open Source Software. 2019;4(43).
75. Long JA. "jtools: Analysis and Presentation of Social Scientific Data.". Journal of Open Source Software. 2024;9(101).
76. Wickham H. ggplot2: Elegant Graphics for Data Analysis: Springer-Verlag New York; 2016.
77. Karthik Ram HW, Clark Richards, Aaron Baggett. wesanderson: A Wes Anderson Palette Generator. 0.3.7 ed: R package; 2018.
78. LiLin-Yin. Circle Manhattan Plot. 4.5.1 ed: R package; 2024.
79. Long JA. interactions: Comprehensive, User-Friendly Toolkit for Probing Interactions. 1.2.0 ed: R package version; 2024.
80. Puvogel S, Alsema A, Kracht L, Webster MJ, Weickert CS, Sommer IEC, Eggen BJL. Single-nucleus RNA sequencing of midbrain blood-brain barrier cells in schizophrenia reveals subtle transcriptional changes with overall preservation of cellular proportions and phenotypes. Mol Psychiatry. 2022;27(11):4731-40.
81. de Kluiver H, Jansen R, Milaneschi Y, Penninx B. Involvement of inflammatory gene expression pathways in depressed patients with hyperphagia. Transl Psychiatry. 2019;9(1):193.
82. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417-25.
83. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-50.

84. Belonogova NM, Zorkoltseva IV, Tsepilov YA, Axenovich TI. Gene-based association analysis identifies 190 genes affecting neuroticism. Sci Rep. 2021;11(1):2484.

85. Nagel M, Jansen PR, Stringer S, Watanabe K, de Leeuw CA, Bryois J, Savage JE, Hammerschlag AR, Skene NG, Muñoz-Manchado AB, White T, Tiemeier H, Linnarsson S, Hjerling-Leffler J, Polderman TJC, Sullivan PF, van der Sluis S, Posthuma D. Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. Nat Genet. 2018;50(7):920-7.

86. Xia J, He Q, Li Y, Xie D, Zhu S, Chen J, Shen Y, Zhang N, Wei Y, Chen C, Shen J, Zhang Y, Gao C, Li Y, Ding J, Shen W, Wang Q, Cao M, Liu T, Zhang J, Duan H, Bao C, Ma P, Zhou C, Luo Y, Zhang F, Liu Y, Li Y, Jin G, Zhang Y, Liang W, Chen Y, Zhao C, Li H, Chen Y, Shi S, Kendler KS, Flint J, Wang X. The relationship between neuroticism, major depressive disorder and comorbid disorders in Chinese women. J Affect Disord. 2011;135(1-3):100-5.

87. Alnaes R, Torgersen S. Personality and personality disorders predict development and relapses of major depression. Acta Psychiatr Scand. 1997;95(4):336-42.

88. Choi Y, Park H, Jung H, Kweon H, Kim S, Lee SY, Han H, Cho Y, Kim S, Sim WS, Kim J, Bae Y, Kim E. NGL-1/LRRC4C Deletion Moderately Suppresses Hippocampal Excitatory Synapse Development and Function in an Input-Independent Manner. Front Mol Neurosci. 2019;12:119.

89. Garcia FJ, Sun N, Lee H, Godlewski B, Mathys H, Galani K, Zhou B, Jiang X, Ng AP, Mantero J, Tsai LH, Bennett DA, Sahin M, Kellis M, Heiman M. Single-cell dissection of the human brain vasculature. Nature. 2022;603(7903):893-9.

90. Escudero-Esparza A, Kalchishkova N, Kurbasic E, Jiang WG, Blom AM. The novel complement inhibitor human CUB and Sushi multiple domains 1 (CSMD1) protein promotes factor I-mediated degradation of C4b and C3b and inhibits the membrane attack complex assembly. Faseb j. 2013;27(12):5083-93.

91. Devraj K, Poznanovic S, Spahn C, Schwall G, Harter PN, Mittelbronn M, Antoniello K, Paganetti P, Muhs A, Heilemann M, Hawkins RA, Schrattenholz A, Liebner S. BACE-1 is expressed in the blood-brain barrier endothelium and is upregulated in a murine model of Alzheimer's disease. J Cereb Blood Flow Metab. 2016;36(7):1281-94.

92. Taylor HA, Simmons KJ, Clavane EM, Trevelyan CJ, Brown JM, Przemyłska L, Watt NT, Matthews LC, Meakin PJ. PTPRD and DCC Are Novel BACE1 Substrates Differentially Expressed in Alzheimer's Disease: A Data Mining and Bioinformatics Study. Int J Mol Sci. 2022;23(9).

93. Ortiz B, Fabius AW, Wu WH, Pedraza A, Brennan CW, Schultz N, Pitter KL, Bromberg JF, Huse JT, Holland EC, Chan TA. Loss of the tyrosine phosphatase PTPRD leads to aberrant STAT3 activation and promotes gliomagenesis. Proc Natl Acad Sci U S A. 2014;111(22):8149-54.

94. Daneman R, Zhou L, Agalliu D, Cahoy JD, Kaushal A, Barres BA. The mouse blood-brain barrier transcriptome: a new resource for understanding the development and function of brain endothelial cells. PLoS One. 2010;5(10):e13741.

95. Lee SJ, Kwon S, Gatti JR, Korcari E, Gresser TE, Felix PC, Keep SG, Pasquale KC, Bai T, Blanchett-Anderson SA, Wu NW, Obeng-Nyarko C, Senagbe KM, Young KC, Maripudi S, Yalavarthi BC, Korcari D, Liu AY, Schaffler BC, Keep RF, Wang MM. Large-scale identification of human cerebrovascular proteins: Inter-tissue and intracerebral vascular protein diversity. PLoS One. 2017;12(11):e0188540.

96. Duan M, Ru X, Zhou J, Li Y, Guo P, Kang W, Li W, Chen Z, Feng H, Chen Y. Endothelial EGLN3-PKM2 signaling induces the formation of acute astrocytic barrier to alleviate immune cell infiltration after subarachnoid hemorrhage. Fluids Barriers CNS. 2024;21(1):42.

97. Ren S, Wu G, Huang Y, Wang L, Li Y, Zhang Y. MiR-18a Aggravates Intracranial Hemorrhage by Regulating RUNX1-Occludin/ZO-1 Axis to Increase BBB Permeability. J Stroke Cerebrovasc Dis. 2021;30(8):105878.

98. Miao YS, Zhao YY, Zhao LN, Wang P, Liu YH, Ma J, Xue YX. MiR-18a increased the permeability of BTB via RUNX1 mediated down-regulation of ZO-1, occludin and claudin-5. Cell Signal. 2015;27(1):156-67.

99. Yan H, Kanki H, Matsumura S, Kawano T, Nishiyama K, Sugiyama S, Takemori H, Mochizuki H, Sasaki T. MiRNA-132/212 regulates tight junction stabilization in blood-brain barrier after stroke. Cell Death Discov. 2021;7(1):380.

100. Thomsen MS, Birkelund S, Burkhart A, Stensballe A, Moos T. Synthesis and deposition of basement membrane proteins by primary brain capillary endothelial cells in a murine model of the blood-brain barrier. J Neurochem. 2017;140(5):741-54.

101. Munji RN, Soung AL, Weiner GA, Sohet F, Semple BD, Trivedi A, Gimlin K, Kotoda M, Korai M, Aydin S, Batugal A, Cabangcala AC, Schupp PG, Oldham MC, Hashimoto T, Noble-Haeusslein LJ, Daneman R. Profiling the mouse brain endothelial transcriptome in health and disease models reveals a core blood-brain barrier dysfunction module. Nat Neurosci. 2019;22(11):1892-902.

102. Saverimuttu SCC, Kramarz B, Rodríguez-López M, Garmiri P, Attrill H, Thurlow KE, Makris M, de Miranda Pinheiro S, Orchard S, Lovering RC. Gene Ontology curation of the blood-brain barrier to improve the analysis of Alzheimer's and other neurological diseases. Database (Oxford). 2021;2021.

103. Radzishevsky I, Odeh M, Bodner O, Zubedat S, Shaulov L, Litvak M, Esaki K, Yoshikawa T, Agranovich B, Li WH, Radzishevsky A, Gottlieb E, Avital A, Wolosker H. Impairment of serine transport across the blood-brain barrier by deletion of Slc38a5 causes developmental delay and motor dysfunction. Proc Natl Acad Sci U S A. 2023;120(42):e2302780120.

104. Semina EV, Rubina KA, Sysoeva VY, Rutkevich PN, Kashirina NM, Tkachuk VA. Novel mechanism regulating endothelial permeability via T-cadherin-dependent VE-cadherin phosphorylation and clathrin-mediated endocytosis. Mol Cell Biochem. 2014;387(1-2):39-53.

105. Qian H, Dou Z, Ruan W, He P, Zhang JH, Yan F. ErbB4 Preserves Blood-Brain Barrier Integrity via the YAP/PIK3CB Pathway After Subarachnoid Hemorrhage in Rats. Front Neurosci. 2018;12:492.

106. Schumacher MA, Hedl M, Abraham C, Bernard JK, Lozano PR, Hsieh JJ, Almohazey D, Bucar EB, Punit S, Dempsey PJ, Frey MR. ErbB4 signaling stimulates pro-inflammatory macrophage apoptosis and limits colonic inflammation. Cell Death Dis. 2017;8(2):e2622.

107. Pavón-Romero GF, Pérez-Rubio G, Ramírez-Jiménez F, Ambrocio-Ortiz E, Bañuelos-Ortiz E, Alvarado-Franco N, Xochipa-Ruiz KE, Hernández-Juárez E, Flores-García BA, Camarena Á E, Terán LM, Falfán-Valencia R. MS4A2-rs573790 Is Associated With Aspirin-Exacerbated Respiratory Disease: Replicative Study Using a Candidate Gene Strategy. Front Genet. 2018;9:363.

108. Wittenbrink M, Spreu J, Steinle A. Differential NKG2D binding to highly related human NKG2D ligands ULBP2 and RAET1G is determined by a single amino acid in the alpha2 domain. Eur J Immunol. 2009;39(6):1642-51.

109. Podjaski C, Alvarez JI, Bourbonniere L, Larouche S, Terouz S, Bin JM, Lécuyer MA, Saint-Laurent O, Larochelle C, Darlington PJ, Arbour N, Antel JP, Kennedy TE, Prat A. Netrin 1 regulates blood-brain barrier function and neuroinflammation. Brain. 2015;138(Pt 6):1598-612.

110. Li Y, Liu C, Chen Z, Lin H, Li X. Netrin-1 protects blood-brain barrier (BBB) integrity after cerebral ischemia-reperfusion by activating the Kruppel-like factor 2 (KLF2)/occludin pathway. J Biochem Mol Toxicol. 2024;38(1):e23623.

111. Schlegel N, Waschke J. cAMP with other signaling cues converges on Rac1 to stabilize the endothelial barrier- a signaling pathway compromised in inflammation. Cell Tissue Res. 2014;355(3):587-96.

112. Beard RS, Jr., Yang X, Meegan JE, Overstreet JW, Yang CG, Elliott JA, Reynolds JJ, Cha BJ, Pivetti CD, Mitchell DA, Wu MH, Deschenes RJ, Yuan SY. Palmitoyl acyltransferase DHHC21 mediates endothelial dysfunction in systemic inflammatory response syndrome. Nat Commun. 2016;7:12823.

113. Liu Z, Gu Y, Chakarov S, Bleriot C, Kwok I, Chen X, Shin A, Huang W, Dress RJ, Dutertre CA, Schlitzer A, Chen J, Ng LG, Wang H, Liu Z, Su B, Ginhoux F. Fate Mapping via Ms4a3-Expression History Traces Monocyte-Derived Cells. Cell. 2019;178(6):1509-25.e19.

114. Corti F, Ristori E, Rivera-Molina F, Toomre D, Zhang J, Mihailovic J, Zhuang ZW, Simons M. Syndecan-2 selectively regulates VEGF-induced vascular permeability. Nat Cardiovasc Res. 2022;1(5):518-28.

115. Choi S, Chung H, Hong H, Kim SY, Kim SE, Seoh JY, Moon CM, Yang EG, Oh ES. Inflammatory hypoxia induces syndecan-2 expression through IL-1β-mediated FOXO3a activation in colonic epithelia. Faseb j. 2017;31(4):1516-30.

116. Peng C, Wang Y, Hu Z, Chen C. Selective HDAC6 inhibition protects against blood-brain barrier dysfunction after intracerebral hemorrhage. CNS Neurosci Ther. 2024;30(3):e14429.

117. Lee JY, Ma HW, Kim JH, Park IS, Son M, Ryu KH, Shin J, Kim SW, Cheon JH. Novel Histone Deacetylase 6 Inhibitor Confers Anti-inflammatory Effects and Enhances Gut Barrier Function. Gut Liver. 2023;17(5):766-76.
118. Chang P, Li H, Hu H, Li Y, Wang T. The Role of HDAC6 in Autophagy and NLRP3 Inflammasome. Front Immunol. 2021;12:763831.
119. Denieffe S, Kelly RJ, McDonald C, Lyons A, Lynch MA. Classical activation of microglia in CD200-deficient mice is a consequence of blood brain barrier permeability and infiltration of peripheral cells. Brain Behav Immun. 2013;34:86-97.
120. Xie X, Luo X, Liu N, Li X, Lou F, Zheng Y, Ren Y. Monocytes, microglia, and CD200-CD200R1 signaling are essential in the transmission of inflammation from the periphery to the central nervous system. J Neurochem. 2017;141(2):222-35.
121. Yang H, Chen SC. The effect of interleukin-10 on apoptosis in macrophages stimulated by oxLDL. Eur J Pharmacol. 2011;657(1-3):126-30.
122. Alder MN, Mallela J, Opoka AM, Lahni P, Hildeman DA, Wong HR. Olfactomedin 4 marks a subset of neutrophils in mice. Innate Immun. 2019;25(1):22-33.
123. Morita K, Sasaki H, Furuse M, Tsukita S. Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. J Cell Biol. 1999;147(1):185-94.
124. Fontijn RD, Volger OL, Fledderus JO, Reijerkerk A, de Vries HE, Horrevoets AJ. SOX-18 controls endothelial-specific claudin-5 gene expression and barrier function. Am J Physiol Heart Circ Physiol. 2008;294(2):H891-900.
125. Förster C, Burek M, Romero IA, Weksler B, Couraud PO, Drenckhahn D. Differential effects of hydrocortisone and TNFalpha on tight junction proteins in an in vitro model of the human blood-brain barrier. J Physiol. 2008;586(7):1937-49.
126. Aslam M, Ahmad N, Srivastava R, Hemmer B. TNF-alpha induced NFκB signaling and p65 (RelA) overexpression repress Cldn5 promoter in mouse brain endothelial cells. Cytokine. 2012;57(2):269-75.
127. Williams MR, Kataoka N, Sakurai Y, Powers CM, Eskin SG, McIntire LV. Gene expression of endothelial cells due to interleukin-1 beta stimulation and neutrophil transmigration. Endothelium. 2008;15(1):73-84.
128. Argaw AT, Gurfein BT, Zhang Y, Zameer A, John GR. VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. Proc Natl Acad Sci U S A. 2009;106(6):1977-82.

129. Martins-Silva T, Salatino-Oliveira A, Genro JP, Meyer FDT, Li Y, Rohde LA, Hutz MH, Tovo-Rodrigues L. Host genetics influences the relationship between the gut microbiome and psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2021;106:110153.

130. Baum ML, Wilton DK, Fox RG, Carey A, Hsu YH, Hu R, Jäntti HJ, Fahey JB, Muthukumar AK, Salla N, Crotty W, Scott-Hewitt N, Bien E, Sabatini DA, Lanser TB, Frouin A, Gergits F, Håvik B, Gialeli C, Nacu E, Lage K, Blom AM, Eggan K, McCarroll SA, Johnson MB, Stevens B. CSMD1 regulates brain complement activity and circuit development. Brain Behav Immun. 2024;119:317-32.

131. Battle A, Brown CD, Engelhardt BE, Montgomery SB. Genetic effects on gene expression across human tissues. Nature. 2017;550(7675):204-13.

132. Luykx JJ, Bakker SC, Lentjes E, Neeleman M, Strengman E, Mentink L, DeYoung J, de Jong S, Sul JH, Eskin E, van Eijk K, van Setten J, Buizer-Voskamp JE, Cantor RM, Lu A, van Amerongen M, van Dongen EP, Keijzers P, Kappen T, Borgdorff P, Bruins P, Derks EM, Kahn RS, Ophoff RA. Genome-wide association study of monoamine metabolite levels in human cerebrospinal fluid. Mol Psychiatry. 2014;19(2):228-34.

133. Zhang Y, Li M, Wang Q, Hsu JS, Deng W, Ma X, Ni P, Zhao L, Tian Y, Sham PC, Li T. A joint study of whole exome sequencing and structural MRI analysis in major depressive disorder. Psychol Med. 2020;50(3):384-95.

134. Vuckovic D, Mezzavilla M, Cocca M, Morgan A, Brumat M, Catamo E, Concas MP, Biino G, Franzè A, Ambrosetti U, Pirastu M, Gasparini P, Girotto G. Whole-genome sequencing reveals new insights into age-related hearing loss: cumulative effects, pleiotropy and the role of selection. Eur J Hum Genet. 2018;26(8):1167-79.

135. Sherva R, Wang Q, Kranzler H, Zhao H, Koesterer R, Herman A, Farrer LA, Gelernter J. Genome-wide Association Study of Cannabis Dependence Severity, Novel Risk Variants, and Shared Genetic Risks. JAMA Psychiatry. 2016;73(5):472-80.

136. Cukier HN, Dueker ND, Slifer SH, Lee JM, Whitehead PL, Lalanne E, Leyva N, Konidari I, Gentry RC, Hulme WF, Booven DV, Mayo V, Hofmann NK, Schmidt MA, Martin ER, Haines JL, Cuccaro ML, Gilbert JR, Pericak-Vance MA. Exome sequencing of extended families with autism reveals genes shared across neurodevelopmental and neuropsychiatric disorders. Mol Autism. 2014;5(1):1.

137. Lotan A, Fenckova M, Bralten J, Alttoa A, Dixson L, Williams RW, van der Voet M. Neuroinformatic analyses of common and distinct genetic components associated with major neuropsychiatric disorders. Front Neurosci. 2014;8:331.
138. Kraus DM, Elliott GS, Chute H, Horan T, Pfenninger KH, Sanford SD, Foster S, Scully S, Welcher AA, Holers VM. CSMD1 is a novel multiple domain complement-regulatory protein highly expressed in the central nervous system and epithelial tissues. J Immunol. 2006;176(7):4419-30.
139. Kalinowski A, Liliental J, Anker LA, Linkovski O, Culbertson C, Hall JN, Pattni R, Sabatti C, Noordsy D, Hallmayer JF, Mellins ED, Ballon JS, O'Hara R, Levinson DF, Urban AE. Increased activation product of complement 4 protein in plasma of individuals with schizophrenia. Transl Psychiatry. 2021;11(1):486.
140. Steen VM, Nepal C, Ersland KM, Holdhus R, Nævdal M, Ratvik SM, Skrede S, Håvik B. Neuropsychological deficits in mice depleted of the schizophrenia susceptibility gene CSMD1. PLoS One. 2013;8(11):e79501.
141. Sharma R, Frasch MG, Zelgert C, Zimmermann P, Fabre B, Wilson R, Waldenberger M, MacDonald JW, Bammler TK, Lobmaier SM, Antonelli MC. Maternal-fetal stress and DNA methylation signatures in neonatal saliva: an epigenome-wide association study. Clin Epigenetics. 2022;14(1):87.
142. Uhl GR, Martinez MJ. PTPRD: neurobiology, genetics, and initial pharmacology of a pleiotropic contributor to brain phenotypes. Ann N Y Acad Sci. 2019;1451(1):112-29.
143. Yamagata A, Yoshida T, Sato Y, Goto-Ito S, Uemura T, Maeda A, Shiroshima T, Iwasawa-Okamoto S, Mori H, Mishina M, Fukai S. Mechanisms of splicing-dependent trans-synaptic adhesion by PTPδ-IL1RAPL1/IL-1RAcP for synaptic differentiation. Nat Commun. 2015;6:6926.
144. Li W, Chen R, Feng L, Dang X, Liu J, Chen T, Yang J, Su X, Lv L, Li T, Zhang Z, Luo XJ. Genome-wide meta-analysis, functional genomics and integrative analyses implicate new risk genes and therapeutic targets for anxiety disorders. Nat Hum Behav. 2024;8(2):361-79.
145. Ward J, Strawbridge RJ, Bailey MES, Graham N, Ferguson A, Lyall DM, Cullen B, Pidgeon LM, Cavanagh J, Mackay DF, Pell JP, O'Donovan M, Escott-Price V, Smith DJ. Genome-wide analysis in UK Biobank identifies four loci associated with mood

instability and genetic correlation with major depressive disorder, anxiety disorder and schizophrenia. Transl Psychiatry. 2017;7(11):1264.

146. Kim SE, Kim HN, Yun YJ, Heo SG, Cho J, Kwon MJ, Chang Y, Ryu S, Shin H, Shin C, Cho NH, Sung YA, Kim HL. Meta-analysis of genome-wide SNP- and pathway-based associations for facets of neuroticism. J Hum Genet. 2017;62(10):903-9.

147. Kubick N, Flournoy PCH, Enciu AM, Manda G, Mickael ME. Drugs Modulating CD4+ T Cells Blood-Brain Barrier Interaction in Alzheimer's Disease. Pharmaceutics. 2020;12(9).

148. Jagomäe T, Singh K, Philips MA, Jayaram M, Seppa K, Tekko T, Gilbert SF, Vasar E, Lilleväli K. Alternative Promoter Use Governs the Expression of IgLON Cell Adhesion Molecules in Histogenetic Fields of the Embryonic Mouse Brain. Int J Mol Sci. 2021;22(13).

149. Philips MA, Lilleväli K, Heinla I, Luuk H, Hundahl CA, Kongi K, Vanaveski T, Tekko T, Innos J, Vasar E. Lsamp is implicated in the regulation of emotional and social behavior by use of alternative promoters in the brain. Brain Struct Funct. 2015;220(3):1381-93.

150. Kubick N, Brösamle D, Mickael ME. Molecular Evolution and Functional Divergence of the IgLON Family. Evol Bioinform Online. 2018;14:1176934318775081.

151. Bregin A, Mazitov T, Aug I, Philips MA, Innos J, Vasar E. Increased sensitivity to psychostimulants and GABAergic drugs in Lsamp-deficient mice. Pharmacol Biochem Behav. 2019;183:87-97.

152. Carboni L, Pischedda F, Piccoli G, Lauria M, Musazzi L, Popoli M, Mathé AA, Domenici E. Depression-Associated Gene Negr1-Fgfr2 Pathway Is Altered by Antidepressant Treatment. Cells. 2020;9(8).

153. Must A, Tasa G, Lang A, Vasar E, Kõks S, Maron E, Väli M. Association of limbic system-associated membrane protein (LSAMP) to male completed suicide. BMC Med Genet. 2008;9:34.

154. Koido K, Traks T, Balõtšev R, Eller T, Must A, Koks S, Maron E, Tõru I, Shlik J, Vasar V, Vasar E. Associations between LSAMP gene polymorphisms and major depressive disorder and panic disorder. Transl Psychiatry. 2012;2(8):e152.

155. Chen X, Long F, Cai B, Chen X, Chen G. A novel relationship for schizophrenia, bipolar and major depressive disorder Part 3: Evidence from chromosome 3 high density association screen. J Comp Neurol. 2018;526(1):59-79.
156. Reif A, Schmitt A, Fritzen S, Lesch KP. Neurogenesis and schizophrenia: dividing neurons in a divided mind? Eur Arch Psychiatry Clin Neurosci. 2007;257(5):290-9.
157. Sha L, MacIntyre L, Machell JA, Kelly MP, Porteous DJ, Brandon NJ, Muir WJ, Blackwood DH, Watson DG, Clapcote SJ, Pickard BS. Transcriptional regulation of neurodevelopmental and metabolic pathways by NPAS3. Mol Psychiatry. 2012;17(3):267-79.
158. Huang J, Perlis RH, Lee PH, Rush AJ, Fava M, Sachs GS, Lieberman J, Hamilton SP, Sullivan P, Sklar P, Purcell S, Smoller JW. Cross-disorder genomewide analysis of schizophrenia, bipolar disorder, and depression. Am J Psychiatry. 2010;167(10):1254-63.
159. Santiago JA, Quinn JP, Potashkin JA. Co-Expression Network Analysis Identifies Molecular Determinants of Loneliness Associated with Neuropsychiatric and Neurodegenerative Diseases. Int J Mol Sci. 2023;24(6).
160. Gupta CN, Chen J, Liu J, Damaraju E, Wright C, Perrone-Bizzozero NI, Pearlson G, Luo L, Michael AM, Turner JA, Calhoun VD. Genetic markers of white matter integrity in schizophrenia revealed by parallel ICA. Front Hum Neurosci. 2015;9:100.
161. Hawi Z, Tong J, Dark C, Yates H, Johnson B, Bellgrove MA. The role of cadherin genes in five major psychiatric disorders: A literature update. Am J Med Genet B Neuropsychiatr Genet. 2018;177(2):168-80.
162. Edwards AC, Aliev F, Bierut LJ, Bucholz KK, Edenberg H, Hesselbrock V, Kramer J, Kuperman S, Nurnberger JI, Jr., Schuckit MA, Porjesz B, Dick DM. Genome-wide association study of comorbid depressive syndrome and alcohol dependence. Psychiatr Genet. 2012;22(1):31-41.
163. Teng MS, Wu S, Hsu LA, Chou HH, Ko YL. Differential Associations between CDH13 Genotypes, Adiponectin Levels, and Circulating Levels of Cellular Adhesive Molecules. Mediators Inflamm. 2015;2015:635751.
164. Rivero O, Sich S, Popp S, Schmitt A, Franke B, Lesch KP. Impact of the ADHD-susceptibility gene CDH13 on development and function of brain networks. Eur Neuropsychopharmacol. 2013;23(6):492-507.

165. Rivero O, Selten MM, Sich S, Popp S, Bacmeister L, Amendola E, Negwer M, Schubert D, Proft F, Kiser D, Schmitt AG, Gross C, Kolk SM, Strekalova T, van den Hove D, Resink TJ, Nadif Kasri N, Lesch KP. Cadherin-13, a risk gene for ADHD and comorbid disorders, impacts GABAergic function in hippocampus and cognition. Transl Psychiatry. 2015;5(10):e655.

166. Kiser DP, Popp S, Schmitt-Böhrer AG, Strekalova T, van den Hove DL, Lesch KP, Rivero O. Early-life stress impairs developmental programming in Cadherin 13 (CDH13)-deficient mice. Prog Neuropsychopharmacol Biol Psychiatry. 2019;89:158-68.

167. Takeuchi T, Misaki A, Liang SB, Tachibana A, Hayashi N, Sonobe H, Ohtsuki Y. Expression of T-cadherin (CDH13, H-Cadherin) in human brain and its characteristics as a negative growth regulator of epidermal growth factor in neuroblastoma cells. J Neurochem. 2000;74(4):1489-97.

168. Gonda X, Eszlari N, Torok D, Gal Z, Bokor J, Millinghoffer A, Baksa D, Petschner P, Antal P, Breen G, Juhasz G, Bagdy G. Genetic underpinnings of affective temperaments: a pilot GWAS investigation identifies a new genome-wide significant SNP for anxious temperament in ADGRB3 gene. Transl Psychiatry. 2021;11(1):337.

169. Frey MR, Edelblum KL, Mullane MT, Liang D, Polk DB. The ErbB4 growth factor receptor is required for colon epithelial cell survival in the presence of TNF. Gastroenterology. 2009;136(1):217-26.

170. Barlow DH, Ellard KK, Sauer-Zavala S, Bullis JR, Carl JR. The Origins of Neuroticism. Perspect Psychol Sci. 2014;9(5):481-96.

171. Seney ML, Glausier J, Sibille E. Large-Scale Transcriptomics Studies Provide Insight Into Sex Differences in Depression. Biol Psychiatry. 2022;91(1):14-24.

172. Powers SI, Laurent HK, Gunlicks-Stoessel M, Balaban S, Bent E. Depression and anxiety predict sex-specific cortisol responses to interpersonal stress. Psychoneuroendocrinology. 2016;69:172-9.

173. Weber CM, Clyne AM. Sex differences in the blood-brain barrier and neurodegenerative diseases. APL Bioeng. 2021;5(1):011509.

174. Ishrat T, Sayeed I, Atif F, Hua F, Stein DG. Progesterone and allopregnanolone attenuate blood-brain barrier dysfunction following permanent focal ischemia by regulating the expression of matrix metalloproteinases. Exp Neurol. 2010;226(1):183-90.

175. Jiang C, Wang J, Li X, Liu C, Chen N, Hao Y. Progesterone exerts neuroprotective effects by inhibiting inflammatory response after stroke. Inflamm Res. 2009;58(9):619-24.

176. Whitmore HAB, Amarnani D, O'Hare M, Delgado-Tirado S, Gonzalez-Buendia L, An M, Pedron J, Bushweller JH, Arboleda-Velasquez JF, Kim LA. TNF-α signaling regulates RUNX1 function in endothelial cells. Faseb j. 2021;35(2):e21155.

177. Wuchty S, Myers AJ, Ramirez-Restrepo M, Huentelman M, Richolt R, Gould F, Harvey PD, Michopolous V, Steven JS, Wingo AP, Lori A, Maples-Keller JL, Rothbaum AO, Jovanovic T, Rothbaum BO, Ressler KJ, Nemeroff CB. Integration of peripheral transcriptomics, genomics, and interactomics following trauma identifies causal genes for symptoms of post-traumatic stress and major depression. Mol Psychiatry. 2021;26(7):3077-92.

178. Shen S, Wang J, Zhao Q, Hu Q. The protective effects of butorphanol tartrate against homocysteine-induced blood-brain barrier dysfunction. Bioengineered. 2022;13(3):7209-20.

179. Parker N, Vidal-Pineiro D, French L, Shin J, Adams HHH, Brodaty H, Cox SR, Deary IJ, Fjell AM, Frenzel S, Grabe H, Hosten N, Ikram MA, Jiang J, Knol MJ, Mazoyer B, Mishra A, Sachdev PS, Salum G, Satizabal CL, Schmidt H, Schmidt R, Seshadri S, Schumann G, Völzke H, Walhovd KB, Wen W, Wittfeld K, Yang Q, Debette S, Pausova Z, Paus T. Corticosteroids and Regional Variations in Thickness of the Human Cerebral Cortex across the Lifespan. Cereb Cortex. 2020;30(2):575-86.

180. Barbu MC, Zeng Y, Shen X, Cox SR, Clarke TK, Gibson J, Adams MJ, Johnstone M, Haley CS, Lawrie SM, Deary IJ, McIntosh AM, Whalley HC. Association of Whole-Genome and NETRIN1 Signaling Pathway-Derived Polygenic Risk Scores for Major Depressive Disorder and White Matter Microstructure in the UK Biobank. Biol Psychiatry Cogn Neurosci Neuroimaging. 2019;4(1):91-100.

181. Zeng Y, Navarro P, Fernandez-Pujals AM, Hall LS, Clarke TK, Thomson PA, Smith BH, Hocking LJ, Padmanabhan S, Hayward C, MacIntyre DJ, Wray NR, Deary IJ, Porteous DJ, Haley CS, McIntosh AM. A Combined Pathway and Regional Heritability Analysis Indicates NETRIN1 Pathway Is Associated With Major Depressive Disorder. Biol Psychiatry. 2017;81(4):336-46.

182. Erdő F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood-brain barrier: A review. J Cereb Blood Flow Metab. 2017;37(1):4-24.

9. Bibliography of the candidate's publications

9.1. Publications related to the thesis

Gal Z, Torok D, Gonda X, Eszlari N, Anderson IM, Deakin B, Petschner P, Juhasz G, Bagdy G. New Evidence for the Role of the Blood-Brain Barrier and Inflammation in Stress-Associated Depression: A Gene-Environment Analysis Covering 19,296 Genes in 109,360 Humans. International Journal of Molecular Sciences. 2024;25(20):11332.
expected IF: 4.9

Gal Z, Torok D, Gonda X, Eszlari N, Anderson IM, Deakin B, Juhasz G, Bagdy G, Petschner P. Inflammation and Blood-Brain Barrier in Depression: Interaction of *CLDN5* and *IL6* Gene Variants in Stress-Induced Depression. International Journal of Neuropsychopharmacology. 2022;26(3):189-97.
IF: 4.5

Gal Z, Huse RJ, Gonda X, Kumar S, Juhasz G, Bagdy G, Petschner P. [Anxiety and depression - the role of blood-brain barrier integrity]. Neuropsychopharmacol Hung. 2019;21(1):19-25.

9.2. Publications not related to the thesis

Eszlari N, Hullam G, **Gal Z**, Torok D, Nagy T, Millinghoffer A, Baksa D, Gonda X, Antal P, Bagdy G, Juhasz G. Olfactory genes affect major depression in highly educated, emotionally stable, lean women: a bridge between animal models and precision medicine. Transl Psychiatry. 2024;14(1):182.
expected IF: 5.8

Gezsi A, Van der Auwera S, Mäkinen H, Eszlari N, Hullam G, Nagy T, Bonk S, González-Colom R, Gonda X, Garvert L, Paajanen T, **Gal Z**, Kirchner K, Millinghoffer A, Schmidt CO, Bolgar B, Roca J, Cano I, Kuokkanen M, Antal P, Juhasz G. Unique genetic and risk-factor profiles in clusters of major depressive disorder-related multimorbidity trajectories. Nat Commun. 2024;15(1):7190.
expected IF: 14.7

González-Colom R, Mitra K, Vela E, Gezsi A, Paajanen T, **Gál Z**, Hullam G, Mäkinen H, Nagy T, Kuokkanen M, Piera-Jiménez J, Roca J, Antal P, Juhasz G, Cano I. Multicentric Assessment of a Multimorbidity-Adjusted Disability Score to Stratify Depression-Related Risks Using Temporal Disease Maps: Instrument Validation Study. J Med Internet Res. 2024;26:e53162.
expected IF: 5.8

Kristof Z, **Gal Z**, Torok D, Eszlari N, Sutori S, Sperlagh B, Anderson IM, Deakin B, Bagdy G, Juhasz G, Gonda X. Embers of the Past: Early Childhood Traumas Interact with Variation in P2RX7 Gene Implicated in Neuroinflammation on Markers of Current Suicide Risk. International Journal of Molecular Sciences. 2024;25(2):865.
expected IF: 4.9

Hullam G, **Gal Z**, Gonda X, Nagy T, Gezsi A, Cano I, Van der Auwera S, Koukkanen M, Antal P, Juhasz G. A sound mind in a sound body: a novel concept unravelling heterogeneity of depression. Neuropsychopharmacol Hung. 2023;25(4):183-93.

Kristof Z, **Gal Z**, Torok D, Eszlari N, Sutori S, Erdelyi-Hamza B, Petschner P, Sperlagh B, Anderson IM, Deakin JFW, Bagdy G, Juhasz G, Gonda X. Variation along P2RX7 interacts with early traumas on severity of anxiety suggesting a role for neuroinflammation. Sci Rep. 2023;13(1):7757.
IF: 3.8

Bokor J, Sutori S, Torok D, **Gal Z**, Eszlari N, Gyorik D, Baksa D, Petschner P, Serafini G, Pompili M, Anderson IM, Deakin B, Bagdy G, Juhasz G, Gonda X. Inflamed Mind: Multiple Genetic Variants of IL6 Influence Suicide Risk Phenotypes in Interaction With Early and Recent Adversities in a Linkage Disequilibrium-Based Clumping Analysis. Front Psychiatry. 2021;12:746206.
IF: 5.435

Gonda X, Eszlari N, Torok D, **Gal Z**, Bokor J, Millinghoffer A, Baksa D, Petschner P, Antal P, Breen G, Juhasz G, Bagdy G. Genetic underpinnings of affective temperaments: a pilot GWAS investigation identifies a new genome-wide significant SNP for anxious temperament in ADGRB3 gene. Transl Psychiatry. 2021;11(1):337.
IF: 7.989

Gyorik D, Eszlari N, **Gal Z**, Torok D, Baksa D, Kristof Z, Sutori S, Petschner P, Juhasz G, Bagdy G, Gonda X. Every Night and Every Morn: Effect of Variation in CLOCK Gene on Depression Depends on Exposure to Early and Recent Stress. Front Psychiatry. 2021;12:687487.
IF: 5.435

Kristof Z, Eszlari N, Sutori S, **Gal Z**, Torok D, Baksa D, Petschner P, Sperlagh B, Anderson IM, Deakin JFW, Juhasz G, Bagdy G, Gonda X. P2RX7 gene variation mediates the effect of childhood adversity and recent stress on the severity of depressive symptoms. PLoS One. 2021;16(6):e0252766.
IF: 3.752

Bokor J, Krause S, Torok D, Eszlari N, Sutori S, **Gal Z**, Petschner P, Anderson IM, Deakin B, Bagdy G, Juhasz G, Gonda X. "Out, out, brief candle! Life's but a walking shadow": 5-HTTLPR Is Associated With Current Suicidal Ideation but Not With Previous Suicide Attempts and Interacts With Recent Relationship Problems. Front Psychiatry. 2020;11:567.
IF: 4.157

Baksa D, Gecse K, Kumar S, Toth Z, **Gal Z**, Gonda X, Juhasz G. Circadian Variation of Migraine Attack Onset: A Review of Clinical Studies. Biomed Res Int. 2019;2019:4616417.
IF: 2.276

Gonda X, Petschner P, Eszlari N, Sutori S, **Gal Z**, Koncz S, Anderson IM, Deakin B, Juhasz G, Bagdy G. Effects of Different Stressors Are Modulated by Different

Neurobiological Systems: The Role of GABA-A Versus CB1 Receptor Gene Variants in Anxiety and Depression. Front Cell Neurosci. 2019;13:138.
IF: 3.921

Kumar S, **Gal Z**, Gonda X, Huse RJ, Juhasz G, Bagdy G, Petschner P. Transcriptomic changes following chronic administration of selective serotonin reuptake inhibitors: a review of animal studies. Neuropsychopharmacol Hung. 2019;21(1):26-35.

10. Acknowledgement

I am deeply grateful to the Department of Pharmacodynamics for providing the resources and support that made this research possible. I would like to express my gratitude to my supervisors, György Bagdy and Péter Petschner for their invaluable guidance, insightful feedback, and unwavering support throughout this research process. I am also grateful to Gabriella Juhász, Xénia Gonda, Nóra Eszlári, and Dóra Török for their insightful advice, patience, and encouragement, which helped me overcome numerous challenges.

This dissertation has been conducted using the UK Biobank Resource under Application Number 1602. The studies described in this dissertation were funded by the Hungarian National Research, Development, and Innovation Office 2019-2.1.7-ERA-NET-2020-00005 under the frame of ERA PerMed (ERAPERMED2019-108); the Hungarian National Research, Development, and Innovation Office (K 143391, K 139330, PD 146014, and PD 134449 grants); the Hungarian Brain Research Programs (2017-1.2.1-NKP-2017-00002; KTIA_NAP_13-1-2013-000; KTIA_NAP_13-2-2015-0001; NAP2022-I-4/2022); and the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, under the TKP2021-EGA funding scheme (2020-4.1.1.-TKP2020; TKP2021-EGA-25 and TKP2021-EGA-02). Nora Eszlari is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Peter Petschner was an international research fellow of the Japan Society for the Promotion of Science (Postdoctoral Fellowships for Research in Japan, standard program, P20809).