THE ROLE OF BLOOD-BRAIN BARRIER INTEGRITY IN DEPRESSION

Ph.D. thesis

Zsófia Gál

Pharmaceutical Sciences Doctoral School
Semmelweis University

Supervisor:	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 Final Examination Committee:
Eszter Ducza, PhD
Zoltán Zádori, PhD

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1. Introduction

Major depressive disorder (MDD) is one of the most prevalent and debilitating mental disorders worldwide, affecting approximately 300 million people. Based on estimations, 1.5 to 3 times more women than men are affected by MDD. Despite the availability of various antidepressants, their efficacies remain limited, as many patients do not respond adequately to treatment. This suggests that the underlying biological mechanisms of depression are complex and multifaceted. Emerging evidence suggests that stress-induced alterations in brain homeostasis - particularly those affecting the blood-brain barrier (BBB) and inflammatory processes - may underlie some forms of depression.

The BBB is a selectively permeable interface formed primarily by endothelial cells connected by tight junction proteins such as claudins, occludin, and junctional adhesion molecules. Its integrity is essential for protecting the central nervous system (CNS) from uncontrolled peripheral effects. Animal studies have demonstrated that exposure to stress can lead to changes in BBB integrity, including reduced expression of tight junction protein claudin-5, increased permeability, and infiltration of proinflammatory cytokines such as interleukin-6 (IL-6) into the CNS. These findings indicate that BBB dysfunction may be a

key factor in stress-associated depression. Given that depression has an estimated heritability of 30-40%, genetic studies provide an opportunity to investigate the biological mechanisms that contribute to stress-related depression 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

In the first study, our goal was to translate results from previous animal studies -, in which *Cldn5* downregulation, elevated concentration of IL-6, together with its infiltration to the CNS 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, we hypothesized 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

The analyses were conducted using the phenotypic and genetic repository of the UK Biobank (UKB). The independent NewMood (NM) cohort served as a replication sample. After genotyping, both cohorts underwent genetic quality control steps, including filtering for minor allele frequency, Hardy–Weinberg equilibrium, and removal of individuals with high relatedness or missing data.

3.2. Phenotypes

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 on a questionnaire on proximal stress factors, and on a 4-item based depression questionnaire. Lifetime depression status was determined based on ICD-10 disease codes of depression. Additionally, a questionnaire on childhood adversities was used to include early life stress into the analyses.

In the second study, in order to reveal the importance of 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). Contrary to the first study, where our aim was the translation of the 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. Along with PHQ9 depression, traumatic events during adulthood were also assessed and included in the analyses.

For replication purposes in both studies, the available depression questionnaire in the NM cohort of depressive symptoms, based on the Brief Symptom Inventory (BSI) and The List of Threatening Experiences questionnaire for assessing recent negative life events in the previous 1 year were used.

3.3. Candidate gene analysis

In the first study, candidate gene analyses were conducted using two SNPs: rs885985 in *CLDN5*, which influences the translation of claudin-5 *in vitro*, and rs1800795 in *IL6*, associated with altered IL-6 plasma levels. A series of linear regression analyses were conducted additively to assess the combined effects of the

two polymorphisms plus stress, their main effects, their epistasis effects, and their interactions with stress. Age, sex, genotyping array, and the first 10 principal components were used as covariates, and Bonferroni method for correction for multiple testing was applied to the results. These analyses were also conducted in male- and in female subjects, in order to reveal any sex differences.

3.4. Genome-wide by environment interaction study (GWEIS)

For the second study, GWEIS was applied to the whole set of SNPs in additive models, using PHQ9 depression score as outcome phenotype and a score derived from stressors during adulthood as environmental interaction. In order to identify genes from the GWEIS results that could be connected to BBB, we used gene expression and enrichment data from cells, taking part in BBB formation, based on human post-mortem midbrain samples. Genes from previously collected curated gene sets of inflammatory factors were considered inflammation-related genes in our analyses. 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.

4. Results

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

The 3-way interaction analysis in the UK Biobank revealed a statistically significant effect ($\beta = 0.0093$, $p = 0.0003$) for the combined interaction between rs885985 (*CLDN5*), rs1800795 (*IL6*), and recent negative life events on current depressive symptoms. Notably, when stratified by sex, the effect remained robust in males ($\beta = 0.0141$, $p = 0.0002$) but did not reach significance in females ($\beta = 0.0055$; $p = 0.1208$). These findings suggest that minor alleles at these loci, in the context of stress, may predispose individuals - especially men - to developing depressive symptoms. Replication of significant results in the NM cohort resulted in trend-level significance with the same direction of effect ($\beta = 0.0553$; $p = 0.0972$). Additional analyses with the preselected SNPs reinforced the importance of the combined contributing role of *CLDN5* and *IL6* polymorphisms, 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.

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

The GWEIS on PHQ9 depression in interaction with stressors during adulthood identified 788 SNPs reaching genome-wide significance ($p < 5 * 10^{-8}$). Gene-level aggregation of these results resulted in 63 significant genes in the whole cohort. Chi-square analyses demonstrated that genes associated with BBB functions were overrepresented among the significant hits - 17 out of 63 genes - with significant enrichment exceeding 3.82-times compared to expectations. In male subjects, 13 out of 44 significant genes were BBB-related, showing a significant 4.18-times enrichment, whereas in female subjects the enrichment of BBB-related genes was 4.40-times, compared to their expected ratios, with 14 out of 45 significant results. Altogether 4 genes remained significant in the whole cohort, in men and in women: *CSMD1*, *PTPRD*, *LSAMP*, and *NPAS3*.

With 23 inflammation-related genes from the 63 significant results in the whole UKB cohort, a 1.59-times enrichment could be calculated. Contrary to the analysis with BBB-related genes, in case of inflammation-related genes, no significant enrichment was found in male- or in female subjects, separately. Notably,

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*), emphasizing the interplay between barrier integrity and inflammatory signalling in modulating depression risk.

The comparison of our significant results with previously published neuroticism-associated genes 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, which further emphasised the important role of BBB in stress-related conditions, such as neuroticism or stress-associated depression.

Due to different the 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 from 17 significant BBB-related genes and 15 genes from 23 significant inflammation-related genes.

5. 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. While the mechanisms underlying these differences require further elucidation, they underscore the necessity of considering sex as a key variable in both research and clinical practice. Limitations of the current work include the reliance on self-reported stress measures and depressive

symptoms, which, although standardized, may introduce reporting biases.

In summary, we could provide evidence for the contributing role of the BBB in stress-associated depression in humans and we revealed candidate BBB- and inflammatory-related genes that could serve as susceptibility factors or potential drug targets in human depression research.

6. Bibliography of the candidate's publications

Publications related to the thesis

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