Approaches of genetic signal detection in polygenic, multifactorial disorders

Ph.D. Thesis

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1. Introduction

Migraine and depression are complex disorders influenced by both genetic and environmental factors. Migraine affects approximately 14-15% of the global population, while depression is one of the leading causes of disability worldwide, impacting over 300 million individuals. Both conditions are more common in women and frequently co-occur, not only with each other but also with other conditions such as anxiety, other psychiatric disorders, and cardiovascular disease.

Extensive research has explored their genetic basis, yet key questions remain. Although genome-wide association studies (GWAS) have advanced our understanding, they explain only a fraction of the heritability estimated from twin studies. For migraine, SNP-based heritability was estimated at 14.6% and 11.2% in large meta-analyses, compared to 36-48% in twin studies. Depression shows a similar pattern: twin studies suggest 37% heritability, while the largest GWAS to date reported only 8.4%. This discrepancy, known as missing heritability, may be influenced by rare variants, structural variants, epigenetics, or gene-environment interactions that are not fully captured by current GWAS approaches. Despite methodological progress, much of the heritable component remains unexplained.

One contributing factor may be how phenotypes are defined. To maximize statistical power, GWAS often prioritize large sample sizes over diagnostic precision, relying on broad or self-reported phenotypes. Comorbidity increases further complexity; previous studies suggested that co-occurring depression can alter genetic associations in migraine. Restricting analyses to individuals with a single, well-defined diagnosis may help unravel more specific genetic contributors.

Another limitation is that genes relevant to disease biology may not meet the stringent thresholds for genome-wide significance and thus remain undetected. Since genes participate in complex biological pathways, integrating prior biological knowledge, such as transcriptomic findings from animal models, can help in identifying relevant signals. Integrating transcriptomic data may help reveal novel pathways, improving diagnostic precision and helping the development of more effective treatments. In both migraine and depression, gene expression studies in animals are discovering molecular mechanisms; however, replication of these findings in humans is missing.

In depression research, one such candidate is the NAD+/SIRT1 pathway. SIRT1, a NAD+-dependent deacetylase, plays a role in metabolism, stress response, and brain function. A recent animal study showed that early life stress (ELS) in male rodents

led to increased adiposity and reduced sociability via decreased SIRT1 expression in the nucleus accumbens. Notably, NAD+ supplementation reversed these effects, suggesting a causal role. However, the relevance of this pathway in humans, particularly in relation to sex differences and early adversity, remains unexplored.

2. Objectives

Based on the above, the following hypotheses were formulated:

Hypothesis 1

A narrower migraine phenotype, by excluding comorbidities, improves the discovery of polygenic signals and leads to a deeper understanding of migraine pathomechanism.

Hypothesis 2

A human transcriptomic findings-driven genomic analysis provides novel insights into migraine pathophysiology.

Hypothesis 3

Rodent model-derived transcriptomic findings allow for genomic signal detection in the NAD+/SIRT1 pathway in humans for stress-induced depression, thereby linking early environmental adversity and obesity to the development of depression.

3. Methods

Study 1: Polygenic Signal Detection in Migraine-First Diagnosis

Data from the UK Biobank (application ID: 71718) were used, focusing on participants with migraine (G43) as their first medical diagnosis. After phenotype filtering, the final dataset included 6,139 migraine-first cases and 193,790 healthy controls with genetic data available. Genome-wide quality control (QC) followed a standard pipeline, excluding SNPs with MAF < 0.01, Hardy-Weinberg equilibrium $p < 1\times10^{-5}$, LD-pruned ($R^2 < 0.2$), and imputation info score < 0.5. Sex discrepancies and heterozygosity outliers were removed. After QC, 6,077,313 SNPs were retained.

GWAS was performed using PLINK2, with covariates: age, sex, 10 genetic principal components, and genotyping chip. Heritability was estimated using SumHer (LDAK v5.2) under multiple conditions: all SNPs, excluding significant loci, and HapMap3 SNPs, with the MHC region excluded (chr6: 25-35 megabase-pairs). Heritability estimates were transformed to the liability scale using sample-based migraine prevalence (3%) and population prevalence (16%).

Robustness was tested through four validations: 1) using an alternative control group (N = 327,986), 2) varying the case-

control ratio, 3) applying a broader migraine phenotype (N_{cases} = 17,679; $N_{controls}$ = 316,446), and 4) comparing four heritability models (GCTA, HDM, LDAK, BLD-LDAK). Post-GWAS analyses were conducted using FUMA for risk locus identification ($p < 5\times10^{-8}$ genome-wide threshold) and MAGMA for gene-set and tissue enrichment based on GTEx expression data.

Study 2: Genetic Validation of Transcriptomic Findings

Consistent with the definition in Study 1, the migraine-first cohort was used. Covariates included age, sex, smoking, allergy history, and estimated intake of vitamin A and retinol equivalents. For replication purposes, a Hungarian migraine cohort (N_{cases} = 172, $N_{controls}$ = 117) with a well-defined migraine diagnosis and healthy controls was analyzed.

From a prior gene-set enrichment transcriptomic study, 69 Leading Edge Genes (LEGs) and vitamin A pathway genes were selected. SNPs (N = 13,650 in LEGs; N = 13,635 in vitamin A genes) within ±10 kilobase-pairs of each gene were annotated using ANNOVAR. Logistic regression for migraine was performed in both cohorts using PLINK2, adjusting for sex, age, 10 PCs, smoking, allergy history, and, where applicable, vitamin A intake.

Study 3: NAD+/SIRT1 Pathway Genetic Risk in Depression

The UK Biobank discovery sample (N = 228,595) was used to conduct GWAS, and a separate target sample (N = 105,654) was used for polygenic risk score (PRS) analyses. Depressive symptoms were quantified using a composite score derived from multiple UK Biobank data fields, capturing core aspects of depression. Early life stress (ELS) was assessed using items from the Childhood Trauma Screener.

Control individuals of the Hungarian migraine cohort (N = 102) served as an independent replication sample, providing both genetic and resting-state functional MRI data. This allowed us to examine whether the genetic risk of the NAD+/SIRT1 pathway and its interaction with ELS influenced not only depressive symptoms but also brain connectivity.

Genetic analyses focused on 3,827 SNPs in the UK Biobank and 1,885 SNPs in the Hungarian cohort, selected within ±10 kilobase-pairs of 20 NAD+/SIRT1-related genes, based on prior pathway definitions. SNP annotation was performed using biomaRt and LDlink. GWAS was conducted on the depression phenotype in the UKB discovery set, and pathway-specific PRS were computed in both the UKB target and Hungarian cohorts using LDpred2. We then tested the interaction between

NAD+/SIRT1 PRS and ELS in predicting depression scores and functional connectivity.

To evaluate the PRS × ELS interaction, we used linear regression to compare a baseline model (main effects only) with a full model including the interaction term. The change in explained variance (ΔR^2) was used to quantify the contribution of the interaction. Mediation analysis conducted using the lavaan package in R (v0.6-12), assessed whether body fat percentage mediated the PRS × ELS effect on depressive symptoms by estimating direct, indirect, and total effects.

For the resting-state fMRI analysis, scans were acquired using two 3T scanners: Philips Achieva (N = 40) and Siemens Prisma (N = 62). Seed-based connectivity maps were generated using the nucleus accumbens (NAc; MNI coordinates: x = 10, y = 12, z = –8) as the seed region. Preprocessing followed a standardized pipeline, and voxel-wise analyses were performed using SPM12, adjusting for head motion, age, sex, and scanner type.

4. Results

Study 1

Using a refined migraine-first phenotype and the Human Default Model (HDM), SNP-based heritability of migraine was estimated at 19.37% (± 0.019 SD) on the liability scale. After removing genome-wide ($p < 10^{-8}$) and suggestively significant ($p < 10^{-5}$) variants, heritability remained high at 18.13% (± 0.019 SD), indicating a strong underlying polygenic component. Restricting analysis to HapMap3 SNPs further increased heritability to 21.31% (± 0.019 SD).

Validation analyses supported these findings. Using all non-migraine individuals as controls slightly reduced heritability to 17.39% (± 0.016 SD). When controls were subsampled to create more balanced case-control ratios, the average estimate was 18.45% (± 0.017 SD). Applying the same pipeline to a broader migraine phenotype (all G43 diagnoses) yielded a lower estimate of 12.92% (± 0.007 SD), likely due to greater phenotypic heterogeneity and later age of onset. The different heritability models produced a comparable result: 23.89% (± 0.021 SD).

The migraine-first GWAS identified 9 independent SNPs surpassing the $p < 10^{-8}$ threshold and one at the conventional $p < 5 \times 10^{-8}$ level. These were located in five genomic regions: *PRDM16*, *FHL5*, *ASTN2*, *STAT6*/*LRP1*, with eight SNPs associated with increased migraine risk (odds ratio > 1) and two

showing protective effects (odds ratio < 1). All genome-wide significant loci had been previously reported in large migraine GWAS/meta-analyses. Among the 54 significantly significant SNPs, several key loci, including *MEF2D*, *TRPM8*, *PHACTR1*, *ITPK1*, *SUGCT*/*C7orf10*, and *ADAMTSL4*, were consistently replicated across previous, and this study.

While no significant gene sets emerged from pathway-level analysis of genome-wide significant SNPs, analysis of suggestively significant SNPs revealed enrichment in seven KEGG pathways, including retinol metabolism and steroid hormone biosynthesis. Transcription factor target enrichment also pointed to STAT1 and PEA3, suggesting potential regulatory mechanisms.

Study 2

Genetic analyses were conducted on genes previously identified through transcriptomic differential expression, referred to as Leading Edge Genes (LEGs). While none of the tested variants survived Bonferroni correction for multiple comparisons ($p \leq 3.66 \times 10^{-6}$), several genes demonstrated clusters of nominally significant SNPs ($p \leq 0.05$), including *CBR3*, *CORIN*, *EFNB2*, *KCNMA1*, and *TEK*. These results, although not reaching correction-level significance, suggest possible gene-level effects

and provide targets for further investigation in migraine susceptibility.

In the targeted analysis of vitamin A-related genes, *LRP1* emerged as a key gene. Eleven SNPs within *LRP1* were significantly associated with migraine in the UK Biobank sample after multiple testing correction. Of these, seven variants were also present and showed nominal significance in the Hungarian replication cohort. Notably, across all analyses, the minor alleles of these *LRP1* SNPs were associated with a protective effect against migraine, implying an elevated risk in carriers of the major allele.

Study 3

In the UK Biobank sample, a significant ($p \leq 0.016$) interaction was observed between NAD+/SIRT1 pathway polygenic risk scores (PRS) and early life stress (ELS) on depression scores, particularly in males (beta = 2.9275, p = 0.0002) The male subgroup also showed the highest percentage increase in explained variance (ΔR^2 = 0.5037%), suggesting a stronger gene-environment interaction effect in this group.

Mediation analyses revealed that while the PRS × ELS interaction had a significant direct effect on depression in both

the total sample and males ($p \leq 0.05$), body fat percentage did not mediate this relationship.

No association was found between the PRS × ELS interaction and nucleus accumbens (NAc) resting-state functional connectivity in the total population. However, a sex-specific pattern emerged: males showed significantly stronger connectivity between the NAc and frontal brain regions, including the middle frontal gyrus (Peak T = 3.967; MNI: 44, 30, 30) and inferior frontal gyrus, triangular part (Peak T = 4.142; MNI: 42, 22, 30), compared to females (pFWE = 0.0139, cluster size = 159).

Connectivity patterns showed differences in sex-separated regression analyses: in males, higher PRS × ELS interaction scores were associated with increased NAc-frontal connectivity ($\beta = 0.3859$, SE = 0.1513, $R^2 = 0.1135$), whereas in females, higher interaction scores were linked to decreased connectivity ($\beta = -0.3850$, SE = 0.1104, $R^2 = 0.1637$). These findings suggest a sex-specific neural mechanism underlying gene-environment interactions in depression.

5. Conclusions

This work demonstrates that refined phenotype definitions and pathway-focused genetic analyses can enhance the detection and biological interpretation of genetic signals in complex disorders.

In migraine, restricting the sample to individuals whose first diagnosis was migraine increased SNP-based heritability to 19.37% (±0.019 SD), exceeding previous estimates. This approach confirmed established migraine risk loci, including *PRDM16*, *FHL5*, *ASTN2*, *STAT6*/*LRP1*, and *SLC24A3*, while also identifying suggestive associations in *CALCB*, a gene critical for CGRP signaling. Integrating findings of a transcriptomic study, *LRP1* emerged as a central gene with eleven significant SNP associations, seven of which replicated independently, highlighting its potential role in migraine pathophysiology.

Extending the pathway-based approach to depression revealed a significant interaction between NAD+/SIRT1 pathway genetic risk and early life stress, explaining 0.5037% of variance in depressive symptoms among males ($p = 0.0002$). Body fat percentage did not mediate this effect. Functional connectivity analysis further revealed sex-specific alterations in nucleus accumbens-prefrontal cortex connectivity linked to this

interaction, consistent with neurobiological mechanisms of reward processing and anhedonia in depression.

Together, these findings show that focusing on a well-defined phenotype and transcriptomic finding-based biological pathways helps improve genetic discovery and understanding of diseases.

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