HUMAN TRANSCRIPTOMIC STUDIES IN MIGRAINE

Ph.D Thesis Booklet

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Introduction

Migraine is a debilitating neurological disorder that significantly impacts the quality of life globally. Migraine attacks usually last between 4 and 72 hours and are characterized by a throbbing headache of up to severe intensity. People with migraine often show sensitization to light (photophobia) and sound (phonophobia). Furthermore, patients may experience nausea and vomiting. Migraine is broadly classified into two subtypes, which are migraine without aura (MO) and migraine with aura (MA). MO contributes to majority of migraine cases compared to MA, whereas MA can include transient neurological symptoms such as visual disturbances, speech impairments, or sensory alterations. The disease is considered a neurovascular disorder that causes pain and hypersensitivity due to the convergence of peripheral and central nervous system dysfunctions. Many factors play a role, such as the vasoactive neuropeptide calcitonin gene-related peptide (CGRP), ion channel dysfunctions, oxidative stress, dysfunctions in mitochondrial phosphorylation, and impairments in glucose metabolism. Extensive research has been conducted, however, migraine pathophysiology is still poorly understood, especially its molecular mechanisms regarding stress sensitivity.

In migraine, stress can be considered a major trigger for attacks, with about 84% of patients reporting it as a trigger. The autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis are involved in the body's stress response, which results in hormonal and neuronal alterations that are connected to migraine. Migraine onset has been linked to both

high and low stress levels (“let-down” effect), which indicates that maladaptive stress reactions are a key component of the disease's pathophysiology. An intravenous citalopram neuroendocrine challenge offers a standardized and safe method of triggering the HPA axis and investigating biological reactions to stress. Citalopram has demonstrated in previous studies to be an appropriate experimental stressor, which provides the possibility to identify stress-related molecular signatures that may underlie migraine vulnerability by comparing the citalopram-evoked transcriptomic responses of MO patients to those of healthy controls.

Transcriptomic approaches, which analyze RNA expression across the genome, provide insights into active gene expression states capable of bridging genetic predisposition and phenotypic manifestation. However, possibly due to methodological flaws, mixed migraine subtypes, and confounding clinical factors, transcriptomic studies on migraine have been unable to detect replicable gene-level alterations. Carefully controlled studies on meticulously phenotyped MO patients may provide reliable transcriptomic signatures, which may promote the development of both therapeutics and a mechanistic understanding.

This study addressed this gap by systematically investigating transcriptomic changes both under the condition of being stress and migraine-free (general interictal) and following acute neuroendocrine stress in migraineurs.

Objectives

The primary objectives were to investigate the transcriptomic profile of our migraine cohort in the interictal state without any interventions and after an acute neuroendocrine challenge:

1. Are there significantly different and consistently replicated genes and pathways in MO compared to healthy controls in a well-controlled population without serious co-morbidities and medication intake?

2. Are there migraine-associated drugs or potential drug candidates capable of binding migraine-relevant genes or acting on pathways with the potential to advance targeted migraine therapy?

3. Can a stress-mimicking neuroendocrine challenge in interictal MO patients reveal an altered stress response and associated genes and pathways, highlighting stress-dependent mechanisms behind MO?

Methods

Participants

52 Participants were enrolled (22 MO, 30 healthy controls), diagnosed by neurologists according to the International Classification of Headache Disorders (ICHD-3) criteria. Recruited individuals underwent rigorous phenotyping, including headache diaries, the Migraine Disability Assessment (MIDAS), the Zung Self-Rating Depression Scale (SDS; depression symptoms), and the State–Trait Anxiety Inventory (STAI; state and trait anxiety) and structured psychiatric

interviews (MINI). We adhered to strict exclusion criteria by excluding, e.g., other chronic diseases and regular medication intake.

Study Design:

A placebo-controlled, double-blind, randomized crossover design was used. Subjects attended two sampling days, where they received 7.5 mg intravenous citalopram or placebo (saline) in a counterbalanced order, with an average washout period of 30 days. Each infusion was administered over a 10-minute period starting after baseline sampling (0 min, pre-infusion). RNA sequencing (RNA-Seq) samples were collected at baseline and 70 min post-infusion. Cortisol was measured at baseline (pre-infusion), 30 min, and 70 min post-infusion (see Figure 1).

General interictal Study:

The general interictal study aimed to capture an interictal profile, without any intervention. On each sampling day, RNA-Seq samples were collected only at baseline. These paired samples were collected approximately four weeks apart to reflect the migraine-free interictal state.

Neuroendocrine Challenge Study:

To capture the stress-induced transcriptomic responses in MO and controls, baseline and 70-min RNA samples were used. Subjects either received citalopram or placebo infusions in a randomized design. Comparisons were made between baseline and post-challenge (0 vs. 70 min) within each condition, and between citalopram and placebo responses across MO and controls. Cortisol was measured at 0, 30, and 70 min.

Comparisons were made between citalopram and placebo conditions in MO and controls.

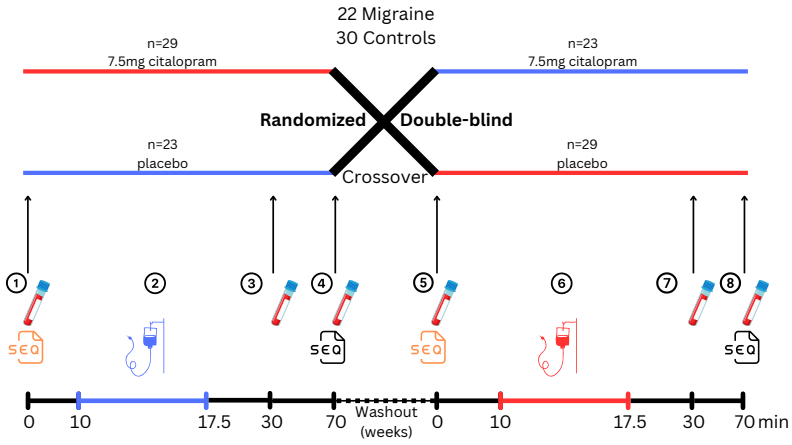

Figure 1 Study Design of Both Experiments

Cortisol Measurement:

Quantification of cortisol was performed from plasma (EDTA tubes, ELISA kits) to assess the neuroendocrine response. Mann-Whitney U tests and linear models controlling for covariates were used to compare differences before and after infusion across groups (MO vs. controls) and conditions (citalopram vs. placebo). The main outcome was the difference in cortisol increase (Δcortisol) between conditions, reflecting HPA-axis reactivity.

RNA Sequencing and Preprocessing:

RNA was extracted from blood samples (PAXgene tubes), libraries were prepared (NEBNext Ultra), and sequencing was performed (Illumina NextSeq 500, ~20M reads/sample). The quality control processes included Trimmomatic, FastQC,

HISAT2 alignment to GRCh38, and StringTie quantification. The R package edgeR was utilized for differential expression analysis, adjusting where possible for age, sex, smoking, allergy status, and study design. We applied multiple testing correction (Benjamini–Hochberg FDR). A gene was considered differentially expressed (DEG in the general interictal study if, at both time points, it showed the same direction of change with FDR ≤ 0.05. The neuroendocrine study used a suggestive significance threshold of $p \leq 0.005$.

Gene Set Enrichment and Leading Edge Analysis

Analyses of pathway enrichment were conducted using GSEA (R package fgsea, using Molecular Signatures Database (MSigDB) C5). In the general interictal study, pathways needed to be significant at both time points with an FDR of ≤ 0.05. The neuroendocrine challenge study yielded a significance value of $p \leq 0.005$. Leading-edge genes (LEGs) that drive pathway enrichment were defined by fgsea. In the general interictal study, we listed the top 5 LEGs (LEG5) based on their logarithmic (base 2) fold change (log2FC) ≤ -2 and those with a log2FC ≤ -0.5 (LEG69).

In Silico Drug Binding (AutoDock Vina)

Using AutoDock Vina, binding simulations were performed for 54 migraine-relevant small molecules against the protein structures of DEGs and LEGs, which were predicted by AlphaFold2. Random sampling was used to normalize binding energies. Bonferroni-corrected p-values $\leq 1.36 \times 10^{-5}$ were used to define significant interactions.

Machine Learning Drug–Target Prediction

A neural network model called HyperAttentionDTI used protein sequences and SMILES strings to predict drug–protein binding scores. More than 11,000 DrugBank compounds were predicted. Log2FC was integrated with predicted binding across gene sets to calculate pathway-level scores. To identify potential treatments for MO, Monte Carlo p-value integration (threshold ≤ 0.0005) and specificity filters were used.

Results

Descriptive Statistics

30 healthy controls (16 female, 14 male) and 22 MO patients (17 female, 5 male) constituted the study population. Although the age groups were matched, MO patients scored higher on STAI and SDS and reported higher levels of stress as a trigger. History of allergies and smoking were also recorded for adjustment in transcriptomic analyses.

General Interictal Transcriptomic Findings

After correction, no transcriptome-wide significant differentially expressed genes were found at the single-gene level. However, across analyses, CYP26B1 and CORIN demonstrated robust and constant downregulation, based on their log2FC values, which was nominally significant at both time points.

Pathway analysis revealed 88 Gene Ontology Biological Process pathways in MO, which were consistently downregulated when compared to controls. These included vascular control, oxidative stress response, hormone signaling,

metabolism (carbohydrate, lipid, and amino acid), and immune responses (antigen presentation, interferon signaling, cytokine pathways). A baseline suppression of immune-metabolic resilience in MO is reflected by this pattern.

The LEG5 comprised CYP26B1, CORIN, PRTN3, CCL23, CTSG. An additional 69 moderately affected genes (LEG69) were identified using LEG analysis, all of these genes displayed negative expression shifts.

Drug Binding Predictions

Current migraine medications showed limited affinity for the identified gene products, according to computational binding analyses. In contrast, a number of new substances were predicted to bind strongly. It is predicted that Probucol, Fasoracetam, Ginsenosides, and Rovafovir will interact with CORIN, one of the cutting-edge genes.
Other medications (such as buclizine) were ptredicted to interact at the pathway level with processes related to cell death and lipid metabolism. These results point to potential mechanism-based repurposing avenues for migraine treatment.

Neuroendocrine Challenge Findings

When compared to controls, cortisol analyses showed that MO patients had a delayed but elevated cortisol response to citalopram infusion. At 70 minutes, MO patients showed a trend toward higher cortisol increases, indicating altered HPA-axis dynamics, whereas early time points (0 and 30 minutes) showed no differences.
In stress-related transcriptomic responses, suggestive gene-level changes in immune regulators (e.g., IKBKGP1, NKRF)

implicated NF-κB signaling. Following stress, pathway analysis showed upregulation of protein synthesis, glycosylation, and carbohydrate metabolism pathways in MO, whereas controls showed downregulation of these pathways. Interestingly, altered glycosylation indicates immune and endothelial vulnerability. This illustrates how acute stress causes an exaggerated and possibly maladaptive transcriptomic response, even though MO patients are characterized by a baseline molecular suppression.

Conclusions

In this thesis, we investigated MO using two transcriptomic approaches: the general interictal state and acute neuroendocrine responses were characterized integrating gene-level, pathway-level, and drug-binding insights, which provides a thorough understanding of how migraine vulnerability is influenced by maladaptive stress reactivity and chronic molecular suppression. All of the data point to a systems model where attack susceptibility in MO is increased by a dysregulated stress response and a low-resilience interictal set point.

Key Findings from the Interictal Transcriptome

The general interictal study provided 88 significantly replicated downregulated biological pathways in MO across two time points, encompassing immune, metabolic, oxidative-stress, vascular, and hormonal programs. This pattern implies a baseline state of diminished physiological resilience, where the immune and metabolic systems function at a consistently reduced capacity, characterizing stress-free interictal MO. Two genes

were identified, CYP26B1 and CORIN, which were consistently downregulated. A disruption in retinoid signaling, which is necessary for immune regulation, neurovascular balance, and cellular differentiation, is implied by the downregulation of CYP26B1. The downregulation of CORIN indicates impaired natriuretic peptide activation, affecting endothelial function and the regulation of vascular tone. Taken together, both genes are consistent with compromised vascular homeostasis and decreased neuroimmune plasticity, which lowers the threshold for initiating an attack.

Drug Binding Insights

Utilizing computational analyses, the data indicated that current migraine therapies may not engage effectively with the molecular targets most disrupted in MO. At the gene level, compounds that do not have a common use in migraine, such as Probucol**,** Fasoracetam, and Ginsenosides, showed predicted values for binding strongly to CORIN. We hypothesize that these drugs can be tested to restore natriuretic-peptide–mediated vascular control.

At the pathway level, an independent screen identified several migraine-associated drug candidates such as buclizine, showing significant binding to replicated pathways (e.g., positive regulation of cell death, response to lipid, receptor tyrosine kinase signaling). These results imply that mechanism-based medication repurposing may fill in the gaps in existing therapeutic approaches and help migraineurs.

Stress-Induced Transcriptomic Reactions

In the neuroendocrine challenge, compared with controls, citalopram caused a delayed but increased cortisol response in

MO, suggesting changed HPA dynamics. At the transcriptomic level, suggestive significant changes were observed in genes regulating NF-κB signaling (IKBKGP1, NKRF), which is central to immune and inflammatory processes. These results indicated that immune system activation triggered by stress in MO may be blunted or dysregulated. After stress, pathway analysis revealed suggestive significant positive enrichments in carbohydrate metabolism, protein synthesis, and glycosylation processes were observed in MO patients, in contrast, controls showed adaptive downregulation. These alterations suggest dysregulated stress transduction, a late, heightened change that modifies immune signaling, cell-surface glycans, and endothelial characteristics, presumably urging a vulnerable system toward attack.

Integrative Interpretation

The findings support a model in which MO patients show, in a stress-free interictal state, a systemic underactivity in immune, metabolic, oxidative-stress, hormonal, and vascular systems, consistent with a chronic low-resilience set point. The stress challenge transforms the suppressed state, with a delayed yet heightened cortisol response, followed by a rapid, amplified activation that can overwhelm homeostatic mechanisms. The observed pathway alterations in glycosylation and metabolic processes, together with suggestive shifts in NF-κB regulators (e.g., IKBKGP1, NKRF), may represent a tipping point where physiological compensation fails, which opens the door for migraine onset. This dual pattern, where there is a baseline suppression as seen in the general interictal experiment, followed by exaggerated reactivity during stress, underscores the complex gene–environment interplay in migraine and is anchored by consistently reduced expression of CYP26B1 and CORIN. From a clinical standpoint, these insights suggest opportunities

for: 1) Patient stratification to determine which patients are most vulnerable to stress-induced attacks, using pathway and gene signatures for that purpose. 2) Novel therapeutic targeting of glycosylation regulation, retinoid metabolism (CYP26B1), and natriuretic peptide signaling (CORIN). 3) Mechanism-based drug repurposing, pointing out that while CORIN-focused candidates (probucol, fasoracetam, ginsenosides, and rovafovir) surfaced from in-silico screens, many existing migraine medications demonstrated limited engagement with these signals. 4) Preventive strategies meant to strengthen vascular and metabolic resistance prior to stress exposure. Taken together, this work reinterprets MO as a disorder at the systems level caused by the interaction of maladaptive stress responses and chronic molecular suppression. This viewpoint creates new opportunities for targeted treatment plans, preventive care, and precision diagnostics based on the biology of MO.

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