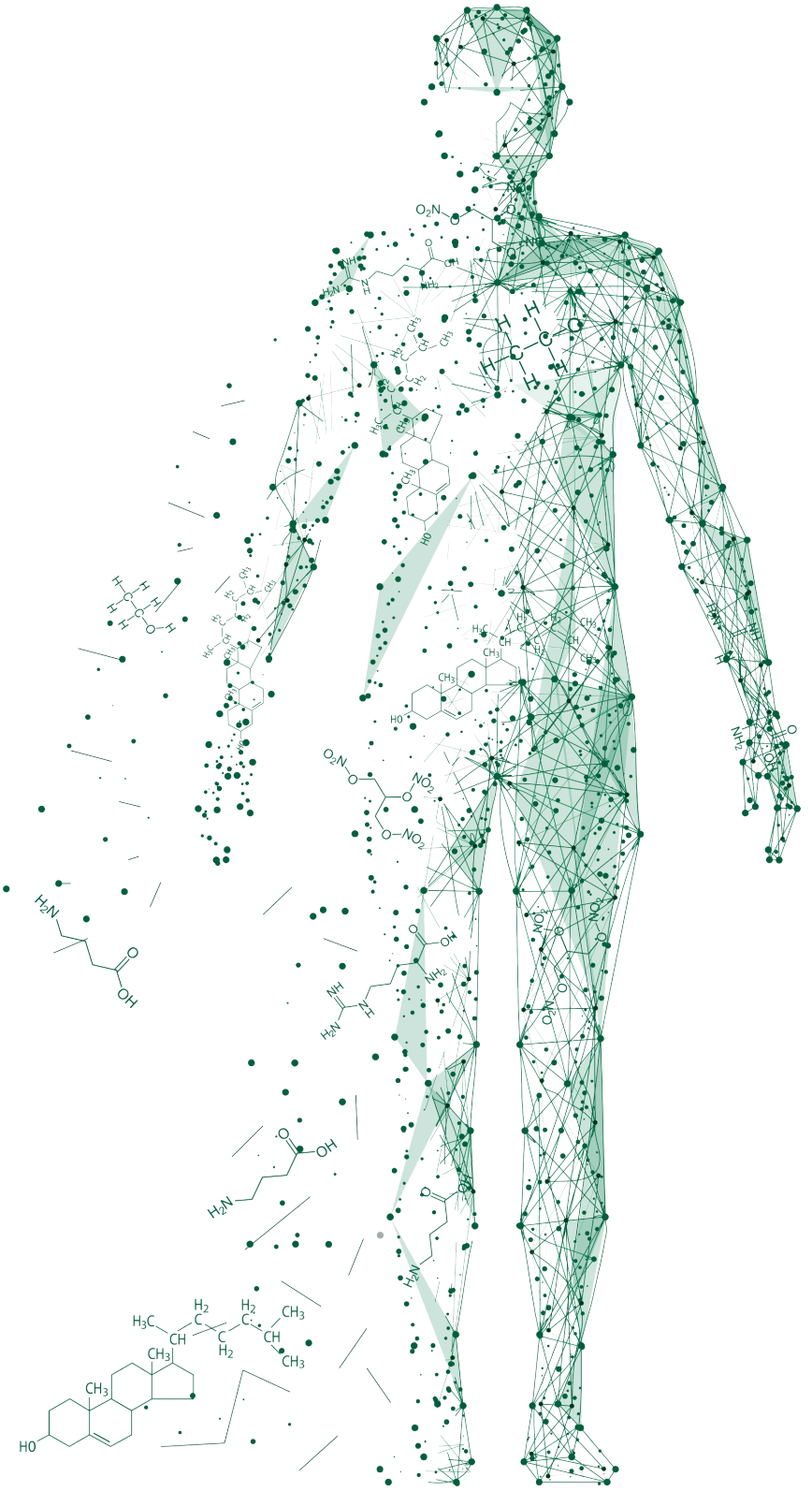
Biochemistry in different phases of the migraine attack

Onderwater, G.L.J.



Appendices

Summary

This thesis is aimed to investigate biochemical changes associated with migraine pathophysiology, outside (interictal) and during different phases of migraine attacks. Discovering metabolites and metabolic pathways associated with migraine will be instrumental to enhance knowledge of molecular biochemical pathways involved in migraine in order to identify potential new acute and prophylactic treatment targets.
The research enclosed in this thesis is divided in two parts. **Part one (Chapters 2 and 3)** describes biofluid investigations in the interictal phase of migraine aimed at the validation of known, and the discovery of new molecules related to migraine pathophysiology. These investigations were performed in plasma and cerebrospinal fluid (CSF) of migraine patients and controls using various mass-spectrometry techniques. **Part two (Chapters 4 – 6)** describes studies into the transition towards the preictal and ictal phases of the migraine attack. In this research several triggers of attacks were investigated as reported by patients themselves, among which alcoholic beverages. In addition, the established pharmacological trigger for attacks, glyceryl trinitrate (GTN), was used to investigate early symptoms of attacks and biochemical changes *in vivo* related to the (pre)ictal phases using ultra-high field proton magnetic resonance spectroscopy (^{1}H-MRS).

Part 1: Migraine biochemical profiling in biofluids
Metabolomics using ultra-performance liquid chromatography mass spectrometry (UPCL-MS) or proton nuclear magnetic resonance (^{1}H-NMR) allows for the identification and quantification of an extensive number of metabolites ('metabolite profiling'). Metabolite profiling that may provide more detailed pathophysiologic insight, that is beyond the traditional biofluid-based measurements, such as (multiplex) enzyme-linked immunosorbent assays (ELISAs) that tend to investigate single or at best a few dozen specifically selected compounds. UPCL-MS and ^{1}H-NMR were used to study the biochemical constituents of migraine patients outside attacks (**Chapters 2 and 3**). In **Chapter 2** we used a ^{1}H-NMR-based metabolomics platform to profile plasma samples from 2,800 migraine patients and 7,353 controls from eight Dutch cohorts. The platform was aimed at the detailed assessment of the concentration of over 200 metabolic measures composed of cholesterol measures, triglycerides, creatine, lipids, fatty acids, apolipoproteins, amino acids, glycolysis-related metabolites, and ketone bodies. This method was applied to identify a plasma metabolomic biomarker signature for migraine. One hypothesis was that systemic (micro)vascular dysfunction but not atherosclerosis is the underlying cause for the association of migraine with cerebro- and cardiovascular disease. A decreased level of apolipoprotein A1 and lowered free cholesterol to total lipid ratio present in small high-density lipoprotein subspecies (HDL) were found to be associated with migraine status. Furthermore, a decreased level of omega-3 fatty acids was associated with migraine, however, only in male migraine patients. On a

larger scale, HDL traits (but no other lipoprotein classes) were found associated with (interictal) migraine status. This extensive metabolic profiling of plasma yielded evidence supporting alterations in HDL metabolism in migraine patients.

In **Chapter 2** we investigated biochemical alterations in plasma of patients. Although blood (plasma or serum) can be collected rather easily, it may be considered more a reflection of the overall metabolic state of a person and less what happens inside the brain. Instead, CSF, which is in direct contact with the brain, may more closely reflects biochemical alterations associated with migraine and its effects on the brain. In **Chapter 3** we aimed to investigate the association of various amines with migraine in CSF and blood. This research builds on the notion that glutamate, glutamine, and gamma-aminobutyric acid (GABA) have been extensively linked to migraine pathophysiology but accurate measurements in body fluids of patients are essentially lacking. Hence, CSF and plasma samples from age- and sex-matched migraine patients and healthy controls were measured using UPCL-MS in order to determine the levels of individual amines, as well as amine pathways and the global amine profile. In CSF, arginine levels were lower in migraine patients (10.4% in migraine with aura and 5.0% in migraine without aura) compared to healthy controls. Of note, the strongest evidence for an association of an amine pathway with migraine was arginine metabolism. Overall global amine profiles were different for migraine patients compared to controls, but showed similarity between patients with the different subtypes of migraine. This seems a strong argument that amines play an important role as a whole, independent on specific migraine characteristics. Arginine is strongly implicated, presumably mediated through nitric oxide signaling. Arginine is linked to the formation of nitric oxide, which is a molecule already known to be involved in migraine attack provocation and pathophysiology.

Part 2: Triggering migraine and biochemical profiling of upcoming attacks

Susceptibility to migraine is thought to be driven by a combination of non-modifiable genetic factors and modifiable internal (e.g. hormonal fluctuations and comorbid diseases) and external (e.g. alcohol and food consumption, and pharmacological drugs) risk modulation factors. Identifying and modifying external risk modulation factors, also called trigger factors, may not only prevent attacks but can also be used for research to deliberately trigger attacks in order to study early attack features in a controlled manner. Trigger research is difficult as it is almost impossible to study this in a controlled and/or blinded fashion, so most trigger research is based on self-reports of patients. For instance, alcoholic beverages are frequently reported by patients to trigger their migraines but undebatable scientific evidence is hard to find. Hence, in **Chapter 4**, we aimed to assess in a large migraine cohort the potential of various alcoholic beverages as a migraine attack trigger. To this end, we conducted a questionnaire study among 2,197 patients with migraine and assessed alcoholic beverage consumption and self-

reported trigger potential, reasons behind alcohol abstinence and time between alcohol consumption and migraine attack onset. Wine, especially red wine, was recognized as the strongest trigger among the alcoholic beverages. However, red wine consistently led to an attack in only 8.8% of participants, with a rapid (<3 hours) time of onset in one-third of patients. In conclusion, red wine and other alcoholic beverages are recognized as a migraine trigger factor by migraine patients with a potential rapid time of onset. The low consistency of provocation in the far majority of patients, however, suggests that alcoholic beverages do not typically act as a singular trigger but its trigger potential may depend on a fluctuating trigger threshold and co-occurrence of other trigger factors.

In **Chapter 5** we investigated the pharmacological compound GTN, which is a well-known trigger of attacks. Headaches provoked by GTN are defined as migraine-like attacks and have been reported to be preceded by nonheadache symptoms comparable to naturally occurring premonitory symptoms. These premonitory symptoms are frequently mislabeled by patients as migraine triggers. We set out to systematically evaluate nonheadache symptoms in migraine patients and healthy controls following GTN infusion. We investigated the incidence of 21 possible premonitory symptoms after GTN infusion in women with migraine without aura and age-matched women without migraine. Migraine-like headaches occurred in 82.4% of migraineurs. Concentration difficulties, yawning, nausea, and photophobia were more frequently reported by those who developed a migraine-like attack (GTN responders) than in healthy controls. Concentration difficulties were exclusively reported by GTN responders. Yawning and nausea were the earliest symptoms reported, followed by photophobia and concentration difficulties that occurred nearer to the onset of migraine-like headache. In conclusion, GTN may induce premonitory symptoms, irrespective of a subsequent migraine-like headache. Yawning, nausea, photophobia and, in particular, concentration difficulties, in that temporal order, seemed most specific for an impending GTN-induced migraine-like headache.

MRS is a non-invasive *in vivo* method to detect biochemical compounds in the brains of humans. In **Chapter 6** MRS was used to investigate the glutamatergic system in the visual cortex in migraine patients without aura and healthy controls following GTN infusion. Enhanced activity of this system has been linked to migraine pathophysiology, however, its role in the onset of migraine attacks remains largely unknown. We assessed glutamate, its major precursor glutamine, and its product GABA, using 7 Tesla proton MRS (7T ^{1}H-MRS) in female migraineurs before and over the course of a provoked attack to detect possible involvement of the glutamatergic system in the onset of attacks. Female migraineurs without aura as well as age- and sex-matched healthy controls were scanned using ^{1}H-MRS three times at fixed time slots (i.e. at baseline and 90 and 270 minutes after the start of GTN infusion). Although we were not able to show changes

for glutamate and glutamine levels, GABA levels increased from baseline to the preictal state. These results showed that high-resolution 7T ^{1}H-MRS is able to reveal changes in the glutamatergic system towards a triggered migraine attack, supporting the hypothesis that migraine attack susceptibility is related to dysregulation of brain excitability.

Finally, **Chapter 7** places the biochemical findings in a broader perspective. The relevance of these biochemical changes in migraine, their role in pathophysiological mechanisms, and future directions for research are discussed.