Epigenetics in mental and neurodegenerative disorders

8 Summary

The work presented in this thesis collectively examines the involvement of epigenetic mechanisms in mental and neurological disorders, in particular Alzheimer's disease (AD), schizophrenia (SZ) and bipolar disorder (BP). The epigenetic mechanisms under interrogation are primarily DNA modifications, and to a lesser extend microRNAs (miRNAs).

CHAPTER 1 of this dissertation familiarizes the reader with DNA modifications and the status quo of their involvement in the pathology and pathogenesis of these specific neurological disorders. After this concise introduction, **CHAPTER 2** unfolds the timeline of epigenomic technology advancements, starting from the earliest antibody-based methods and following up to contemporary genome-wide arrays as well as sequencing approaches, in the context of AD. While the reported technological progress is highly remarkable, it does not come limitation-free and therefore limitations as well as suggestions to overcome them are also reported. Then, the reader is challenged with the question whether the "state-of-the-art" technology, used for AD research, is actually capitalized optimally for the discovery of novel therapeutic/preventive and diagnostic strategies. The personal, yet rational answer provided, suggests that for a pharmacological option to be effective, it should be administered at the most incipient stages of the disease in order to target the preclinical pathology and therefore prevent the later seemingly unresponsive to treatment phase. Considerable evidence suggests that early dysregulation in the brainstem, more specifically in the raphe nuclei and the locus coeruleus, accounts for the earliest, non-cognitive, symptomatology, indicating a potential causal relationship with the pathogenesis of AD, and thus evidence associating these two nuclei with AD is reviewed. The take-home message from this chapter is that temporal and spatial manifestations of the disease should be aligned and, thus, structures more closely related to the prodromal stages of AD should be examined further. Capitalizing the advanced and nowadays affordable epigenomics technology, the pairing of brainstem pathology with deviant epigenetic regulation could yield novel candidate targets for the development of early biomarkers as well as therapeutic alternatives that could halt or even reverse the deleterious progression of AD.

Following on that hypothesis, **CHAPTER 3**, the first experimental study, sets the dorsal raphe nucleus (DRN) as the cornerstone for interrogating early-stage AD epigenetic signatures. Specific genome-wide patterns of methylated (5mC), hydroxymethylated (5hmC) as well as unmodified (UC) cytosines were investigated by means of the Illumina MethylationEPIC array, in relation to disease progression as characterized by Braak staging. Using an inventive approach, genomic DNA extracted from DRN homogenates was treated in parallel with bisulfite and oxidative bisulfite, allowing for distinction of the true methylation signal from the combined methylation and hydroxymethylation one, and thus permitting the examination of three discrete cytosine states. Differentially modified and unmodified positions as well as regions emerged which were then interrogated at a systems-level by employing a weighted gene co-expression network analysis (WGCNA), correlating co-methylated probes with disease progression in order to create modules of probes with a significant involvement in pathology aggravation. Finally, a pathway analysis was performed for the most significant modules for each cytosine state subset in order to reveal which biological pathways are associated with aggravation of AD pathology. Interestingly, the reported modified and unmodified genomic positions and regions have never been identified in epigenome-wide studies previously, but the genes affected have previously been shown to be involved in the pathophysiology of AD, specifically with respect to beta amyloid pathology. This study represents the first systematic epigenetic analysis, distinguishing among 5mC, 5hmC and UC in the DRN of AD patients making use of EPIC array technology. To this end, a unique structure-specific epigenomic profile in association with Braak stage progression was identified.

CHAPTER 4, while remaining focused on examining epigenetic variation in AD, deviates from the previous line and sets microRNAs (miRNAs) in the centre of the attention. MiR-137 regulates presynaptic vesicle release as well as synaptic strength within the adult hippocampus and its expression is regulated by genetic factors as well as epigenetic modifications. The connecting link between miR-137 and AD has been previously detected in AD-associated downregulated MIR137 expression, centrally as well as peripherally, and, furthermore, in cognitive deficits associated with rare genetic variants of MIR137. Thus, this chapter aimed to investigate the interplay between methylation and expression of MIR137 in relation to AD diagnosis and pathology in brain and blood samples. Making use of Illumina's BeadChip and 450K Beadarray, expression and methylation patterns of MIR137 were profiled, respectively, in brains of AD patients and age-matched controls. Additionally, increasing the clinical relevance of this project, miR-137 blood methylation changes over time were interrogated in a

cohort of healthy aged individuals, some of which converted to AD patients during MIR137 tends to be, it was highly associated with pathological hallmarks of AD. On a side note, it was also demonstrated that MIR137 expression is highly affected by the post mortem interval (PMI) of provided tissue, indicating that the mRNA decay rate of the specific miR-137 is rather fast, baring into consideration that the PMI was less than 4h. Collectively, the reported MIR137 methylation patterns are correlated with AD pathology centrally and there is a tendency towards decreased global methylation of MIR137 with AD diagnosis peripherally. The work presented in this chapter is the first of its kind to examine the methylomic profile of MIR137 in the brain and blood of AD patients, and further advocates that methylation signatures in the blood could potentially serve as disease biomarkers.

The experimental pipeline of **CHAPTER 5** focused on detecting methylomic signatures printed in the cerebellum of patients with major psychosis, as defined by the diagnosis of schizophrenia (SZ) or bipolar disorder (BP), and replicate them in an independent cohort. The widely overlooked cerebellum was the centre of attention in this chapter as psychosis-associated cognitive and emotional dysfunctionalities have been attributed to it. We therefore set to examine methylation changes associated with major psychosis as an umbrella term, and then proceed to identify whether these modifications are more closely related to SZ or BP. Utilizing once more the Illumina technology (EPIC array) we did not manage to identify cytosine methylation differences between controls and psychotic patients. Expanding our analysis to regional methylation analysis we detected two regions at the ZFP57 and PM20D1 loci to be significantly hypermethylated in both SZ and BP. Following up on this observation, we acquired the data from an independent epigenome-wide study and applied the same pipeline on them in order to replicate the aforementioned results. To this end, the region associated with ZFP57 emerged solely as a BP-related methylation mark, hinting that the specific locus is more closely associated with the pathophysiology of BP. Our study represents the first analysis of epigenetic variation associated with major psychosis leveraging the EPIC technology. To this end, it highlights that the BP-afflicted cerebellum is imprinted with distinct methylation signatures, a finding consistent between two independent cohorts.

The final experimental part of this thesis is presented in **CHAPTER 6** which deviates from the epigenetic-centred character of this dissertation while still focusing on AD pathology. This study represents an attempt to provide a therapeutic alternative to the main clinical symptom of AD, memory impairment. A pharmacological approach was followed targeting the brain-derived neurotrophic factor/

tropomyosin-receptor-kinase B (BDNF/TrkB) signalling pathway, which is highly dysregulated in AD. To this end we treated a transgenic mouse model of AD with TB001, a synthetic compound that activates the BDNF/TrkB signalling system, and we compared its efficacy with the most-studied compound with a similar, yet less specific, mechanism of action, 7,8 dihydroxyflavone (DHF). Our findings indicated that TB001 can rescue AD-related memory deficits in vivo upon acute as well as chronic administration. Excitingly, the specific compound outperformed DHF, which exhibited partly memory-restoring properties only after chronic treatment.

Overall, this dissertation approached fundamental complex disease-associated research questions from novel perspectives as well as highly innovative methodologies, and provided more insight into the underling pathological mechanisms. Therefore, it contributed in an intensive and collaborative effort of the scientific community to structure multilevel causal networks of these disorders. Such networks will assist the prediction of personalized resilience or vulnerability for disease pathology, and potentially the identification of disease biomarkers as well as novel targets for therapeutic drug development.