Neuroepigenomics in Alzheimer's disease

Chapter 13

Impact paragraph

In this section, the scientific and societal impact of the research described in this thesis will be discussed.

Scientific impact

In spite of the enormous intellectual and financial investments into sporadic Alzheimer's disease (sAD) research over the last century, there is still considerable debate regarding the underlying causes of the disease and the precise mechanisms behind disease progression. Curing sAD when the disease is already in a more advanced stage is unlikely, as the damage done to the patient's brain seems irreversible at this point. Moreover, current diagnostic tools fall short in detecting the development of sAD in a timely manner that could improve treatment outcome. While early detection and intervention appear to be key in treating sAD, at this moment, even when detected at an early stage, available treatment options solely provide symptomatic relief rather than disease-modifying benefits; hence, gradual development of the full-blown disease phenotype is still inevitable. Thus, there is an unmet scientific need to develop a better understanding of early sAD-associated molecular mechanisms, which in turn could aid in the establishment of therapeutic interventions and diagnostic alternatives.

In recent years, the role of epigenetic mechanisms in sAD has received increasing attention, as they are thought to provide a mechanistic link between environmental exposures and both the development and course of the disease. In fact, the identification of sAD-associated epigenetic profiles is anticipated to provide us with novel cues towards affected molecular mechanisms and interacting environmental factors that could explain the complex underpinnings of the disease. Within this context, efforts aimed at interrogating brain regions with early manifestation of disease processes are of particular importance, as it is thought that the observed molecular changes in these brain areas are more relevant for sAD's etiology and pathophysiology. The scientific interest in epigenetic mechanisms is furthermore instigated by their clinical potential, as they could serve as biomarkers (when targeting *e.g.* blood), allowing earlier detection of sAD and/or provide opportunities for more detailed disease stratification. Their reversible nature furthermore makes them a realistic target for future preventive treatment strategies and pharmacological interventions.

Despite the growing number of epigenetic studies in sAD (reviewed in **Chapter 2**), the field of neuroepigenomics remains a relatively nascent area of investigation. Recent advances in microarray and sequencing technologies show that both whole genome-scale and targeted studies on the epigenome across much larger sample collections are now conceivable. It is important to acknowledge, however, that a plain exhaustive search for contributing factors, as has been employed in

genetic studies of sAD, is unlikely to be fruitful for neuroepigenomic research. In reality, studies aiming to identify epigenetic mechanisms in complex diseases such as sAD need to consider several important issues related to study design, methodological limitations, tissue/cell type-specificity of epigenetic marks, and inference on causality, among others (reviewed in **Chapter 2**). As such, a more systematic approach towards understanding the role of epigenetic mechanisms in sAD is essential. While pushing this field forward, it will be crucial to remain cautious and scrutinize each of these issues thoroughly; next to extensively profiling brain tissues and/or cells derived from independent patient cohorts – if one wishes to provide conclusive insights into the underlying mechanisms of the disease.

The research described in the first part of this thesis builds upon this notion and highlights the scientific impact of the presented studies. More specifically, **Chapter 3** describes an approach for targeted epigenetic profiling allowing one to distinguish between different epigenetic marks, *i.e.* DNA methylation and hydroxymethylation. Many of the existing methods used to interrogate the epigenome in sAD have been unable to specifically discriminate between these two closely related, but functionally distinct, epigenetic marks. As such, previous data obtained from these studies could be confounded and epigenetic effects related to sAD could have been overlooked and/or over- or underestimated. Thus, the work presented in this chapter represents an elegant solution towards overcoming methodological limitations as currently encountered in neuroepigenetic studies. The incorporation of such standardized protocols is therefore crucial for future neuroepigenomic research, even outside the field of sAD, as they will attribute further meaning and validity to the experimental outcomes.

Chapter 4 describes how a second challenge in the field, *i.e.* that of cellular heterogeneity, is being tackled, hence contributing to directing future neuroepigenomic research into more fruitful avenues. It is well-known that epigenetic profiles vary substantially between different cell types, even in the healthy human brain. Hence, a potential problem arises when heterogeneous bulk tissue samples, such as those from the brain, are being used for epigenomic profiling. Epigenetic marks in one cell type may oppose or dilute those in another, potentially obscuring important cell type-specific changes when analyzed all together. This issue is intensified when heterogeneous samples from healthy individuals are compared to those derived from sAD patients at different stages of the disease, which is common practice. Aside from sampling-induced variation between the tissues, differences in cellular composition *e.g.* as a result from neurodegeneration and increased immune activation as observed in sAD can significantly affect the cellular proportions of different brain samples. Overall, such variation can tremendously

affect epigenetic data, resulting in experimental differences that in reality are not attributable to the disease, or, alternatively, masking actual differences. A practical solution to this issue, however, would be to profile individual cells (or cell types) isolated from these heterogeneous tissue samples. For this reason, **Chapter 4** describes a novel approach relying on a combination of laser capture microdissection (LCM) and limiting dilution bisulfite pyrosequencing (LDBSP) that allows for targeted methylation profiling in individually isolated cells. This novel approach or similar strategies using other isolation techniques in combination with LDBSP will be increasingly valuable for future neuroepigenomic studies, even outside the scope of sAD.

Chapter 5 represents the first large-scale epigenetic analysis performed in the brainstem of sAD to date, targeting both the dorsal raphe nuclei (DRN) and locus coeruleus (LC). Here, the state-of-the-art techniques described in the previous chapters are being combined in an effort to obtain more in-depth knowledge on the exact contribution of epigenetic mechanisms in these brain regions affected early in sAD. The scientific impact of this work is therefore threefold. First, this study is among the first of its kind to examine different epigenetic marks in the sAD brain simultaneously, including both DNA methylation and hydroxymethylation. Second, in contrast with previous studies that targeted (primarily cortical) brain regions affected in more advanced stages of sAD, the work in this chapter aimed at identifying potential disease-specific epigenetic marks in the brainstem, which are indicative of the more incipient stages of sAD. Third, the bulk tissue analysis in the DRN described here was complemented by a targeted cell-specific validation study were individual cells isolated from this brainstem region were analyzed. While replication of the obtained findings in independent patient cohorts remains necessary, the identified genes presented here could serve as pillars for future mechanistic studies into sAD. Moreover, this is the first study to date demonstrating that epigenetic signatures within the DRN are strongly dependent upon the cell type analyzed. Overall, the conclusion we can draw from this work is that future studies will need to implement single cell(type) analyses next to targeting heterogeneous bulk tissue samples.

In the second part of the thesis, starting from **Chapter 6**, the focus moves away from epigenetic profiling and, instead, insights into the potential of human induced pluripotent stem cell (iPSC)-based models for sAD research are being offered. An overview of the studies to date, exploring the use of iPSC-based models for sAD research, is presented, which includes but is not limited to neuroepigenomic studies. Furthermore, opportunities, challenges and considerations related to their use are being addressed. Despite the fact that further detailed characterization and validation of iPSC-based models remains necessary, overall, they are projected to

significantly advance our current understanding of many disease processes and to revolutionize approaches for the identification of therapeutics for sAD. In fact, the recent availability of iPSC-based models, cellular reprogramming techniques and directed neural differentiation protocols, which are reviewed in **Chapter 7**, are anticipated to overcome the persistent translational gap between preclinical and clinical studies into sAD, emphasizing their scientific impact. Furthermore, it is anticipated that the establishment of robust iPSC-based *in vitro* models will contribute to reducing the need for animal experimentation.

Chapter 8 describes an exploratory approach on the characterization of a protocol to differentiate iPSCs cortical forebrain cells, with the ultimate aim to establish an sAD-relevant *in vitro* model. Even though the findings should be regarded as preliminary and the iPSC-derived neural cells are in need of further characterization and validation, the availability of such disease-relevant cultures offers appealing opportunities for future sAD studies. In this regard, iPSC-derived neural cells can be applied for modelling complex gene-environment interactions, by *e.g.* exposing cells derived from healthy individuals and sAD patients with different genetic backgrounds to environmental insults. This would allow more detailed studies on the cellular and molecular responses to these insults, even at an epigenetic level, for example. Furthermore, within this framework, the use of epigenome editing tools in these cultures could broaden our understanding on their causal involvement into sAD. All in all, also the iPSC 'adds' to neuroepigenomic research into sAD.

Anticipated societal impact

At this stage, it is important to acknowledge that the research described in this thesis is very fundamental and exploratory in nature. Therefore, the studies presented here will likely not have a direct impact on society in the short run, but mainly offer novel insights into methodological advances and the disease itself that are important for other scientists in the field. In spite of that, and while replication of the epigenetic analysis performed in the brainstem (**Chapter 5**) is crucial, the identified genes could be further investigated for their mechanistic and functional roles in sAD, and, as such, might turn out to be targets for treatment strategies in future studies. All in all, the work presented in this thesis, ranging from systematic studies on the contribution of epigenetic mechanisms in sAD (**Chapters 2**, **3**, **4** and **5**) to the development of clinically relevant human disease models (**Chapters 6**, **7** and **8**), serve as pillars that could maneuver future studies towards novel directions, aiding in the generation of new knowledge, and eventually, new applications that may benefit the clinic and, as such, society. In essence, expanding our current understanding on factors contributing to disease causation and progression are crucial if one wish to develop a cure for sAD. As such, it will furthermore be crucial to embed the generated data from this thesis within larger efforts that

aim to combine multiple molecular layers, including genomics, epigenomics, transcriptomics, proteomics, metabolomics and other data modalities, in order to deepen our understanding of the mechanisms underlying sAD. It is anticipated that such endeavors will allow for better knowledge-driven development of therapeutics that could directly impact on society.

Having that said, it is important to recognize that advances in the management of other common diseases and the improvement in general health, have resulted in an increasingly elderly population so that the prevalence of sAD is expected to increase significantly in the upcoming years. Given the lack of treatments and (early) diagnostics for sAD, the disease is currently acknowledged as one of the most costly to society, with an immense socioeconomic burden. In fact, patients require the support of multiple stakeholders across the healthcare system and social care sectors, as well as from their direct family members and friends, hence affecting not only the patient, but society as a whole. This also means that the cost of care is not only captured by the healthcare systems but also in the personal sector, impacting the quality of life of patients, caregivers and patients' relatives. I therefore strongly believe that even the smallest achievements in fundamental research contribute 'to the greater good' and thus, the work presented in this thesis might hopefully contribute, in the long-term, to alleviating this social and economic burden. Of course, this will not happen in a matter of days, but the pillars that are set by fundamental research, like described in the present thesis, might inspire other researchers and, as such, contribute to the development of novel ideas that could eventually lead to revolutionary scientific breakthroughs.