EPI-AD
Alzheimer's disease-associated (hydroxy)methylomic changes in the brain and blood

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Alzheimer's disease (AD) is the leading cause of age-related dementia characterized by depositions of protein aggregates and neuronal death, resulting in progressive cognitive and non-cognitive deteriorations. Although little is known about the exact underlying molecular mechanisms of this neurodegenerative disease, considerable evidence suggests that epigenetic processes including DNA methylation and hydroxymethylation represent critical factors in its development and course.

Within the EPI-AD project (http://www.epi-ad.eu), we performed an epigenome-wide association study (EWAS), assessing both DNA methylation and hydroxymethylation, using bulk tissues from the dorsal raphe nuclei (DRN), locus coeruleus (LC) and middle temporal gyrus (MTG) derived from AD patients and age- and sex-matched non-demented controls. We then followed up these analyses by exploring cell-type specific methylomic signatures using limiting dilution bisulfite pyrosequencing on DNA derived from laser-captured microdissected serotonergic (5-HT) DRN cells. In parallel, in an independent (longitudinal) cohort, we compared the blood methylome of converters to AD dementia and non-converters at a preclinical stage.

Within both brainstem regions, *i.e.* the DRN and LC, we revealed overlapping Braak stage-associated epigenetic abnormalities in *TNXB*, *ANKRD2* and *MBP*, both at the level of DNA methylation (5-methylcytosine, 5mC) and hydroxymethylation (5-hydroxymethylcytosine, 5hmC). Interestingly, when comparing methylation levels of *TNXB* in individually isolated 5-HT neurons with those of non-5-HT cells in the DRN, we found a significant interaction between cell-type and condition, with opposite AD-associated methylation profiles in 5-HT neurons and non-5-HT cells, the latter of which resembled the EWAS data. Within the MTG, DNA (hydroxy)methylation profiling revealed epigenetic differences close to or overlapping with genes such as *OXT*, *CHRNB1*, *RHBDF2* and *C3* that were associated with Braak staging. Strikingly, by comparing the blood methylome of converters to AD dementia and non-converters at a preclinical stage, we found that DNA methylation in the exact same region of the *OXT* promoter as seen in the MTG was associated with subsequent conversion to AD dementia.

Overall, data on the MTG confirmed previous findings, identifying loci such as *RHBDF2* and *C3* that have previously been associated with AD in various EWAS studies on cortical brain regions. Notably, our brainstem work highlighted several novel epigenetic signatures that we hypothesize to play a pivotal role in early AD development. Furthermore, we show that dysregulation in *TNXB* methylation in the DRN is not only dependent on the disease phenotype, but also on the cell type analyzed, which warrants the need for further cell-type specific neuroepigenetic studies in AD. Finally, we demonstrate that the detection of differential *OXT* methylation at pre-dementia stages holds potential relevance as a novel biomarker and therapeutic target. Current work by EPI-AD consortium is aimed at causality assessment of various AD-associated epigenetic signatures making use of e.g. functional epigenetic editing studies.