Altered DNA methylation at FAAH and ALOX5 genes as a basis for the dysregulated endocannabinoid-eicosanoid network in Alzheimer's disease

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Uncontrolled, chronic, and persistent inflammation is a fundamental underlying hallmark of Alzheimer's disease (AD) and suppressing inflammation has been shown to reduce AD pathological hallmarks as well as cognitive and behavioral deficits in AD models. Accordingly nonsteroidal anti-inflammatory drugs (NSAIDs) have well-documented protective effects when initiated early and taken over prolonged periods. However, an issue with NSAIDs has been their lack of efficacy in AD clinical studies. Epigenetic mechanisms may also play a role in AD pathogenesis; several reports have shown that aging and AD are associated with epigenetic dysregulation at various levels, including abnormal DNA methylation and histone modifications. In search for new potential targets, we have studied the gene expression status and the epigenetic regulation of the endocannabinoid system (ECS) components as well as lipoxygenase (LOX) isoforms in peripheral blood mononuclear cells (PBMCs) of subjects with late-onset AD (LOAD) and age-matched controls (CT). We observed that fatty acid amide hydrolase (FAAH), an enzyme that terminates the signaling of the anti-inflammatory endocannabinoid anandamide (AEA), is up-regulated in PBMCs of LOAD subjects (2.30±0.48) compared to healthy CT (1.00±0.14; $p<0.05$) without changes in the mRNA levels of any other ECS elements (D'Addario et al., 2012). Consistently, we also demonstrated in LOAD subjects an increase in FAAH protein levels (CT: 0.75±0.04; LOAD: 1.11±0.15; $p<0.05$) and activity (pmol/min per mg protein CT: 103.80±8.73; LOAD: 125.10±4.00; $p<0.05$). Moreover, in a separate investigation (Di Francesco et al., 2013), we observed in LOAD subjects a significant increase in ALOX5 gene expression (CT: 1.13±0.16; LOAD: 2.79±0.58; $p<0.05$), that was paralleled by increased 5-LOX protein (CT: 0.71±0.09; LOAD: 1.29±0.12; $p=0.0024$) and plasma levels of the 5-LOX end-product leukotriene (LT) B_4 (CT: 1294.3±212.2; LOAD: 2139.5±320; $p<0.05$). We thus hypothesized that increased AEA hydrolysis by FAAH could contribute to the inflammatory process that occurs in AD, for instance by releasing the arachidonic acid (AA) pool for neuroinflammatory LTB_4. We also provided evidence that ALOX5 and FAAH genes share common epigenetic signatures. According to this, by comparing DNA methylation of FAAH and ALOX5 promoters, we found a direct correlation between these two genes (Spearman $r=0.5148$; $p=0.0119$). Moreover, LTB_4 levels were directly correlated to FAAH mRNA levels (Spearman $r=0.5255$; $p<0.05$), and inversely correlated to FAAH DNA methylation (Spearman $r=-0.5140$; $p<0.05$), suggesting that a parallel increase of FAAH and 5-LOX expression in AD patients could evoke a sustained inflammatory condition, thus reinforcing neurodegeneration. Finally, when data were stratified according to functional and cognitive tests (obtained using the Mini Mental Status Exam, MMSE), a significantly lower level of DNA methylation at ALOX5 and FAAH promoters was observed in LOAD subjects with lower levels of MMSE and severe AD compared to the rest of the samples. In conclusion, we document that epigenetic mechanisms substantially contribute to the control of genes involved in AA metabolism in the course of AD. Although the beneficial effects produced by FAAH or 5-LOX inhibition against neuropathology of AD remain to be determined, our results suggest that these enzymes would be a promising therapeutic target for the prevention and treatment of AD.

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