Background

Hyperphosphorylation of Tau is one of the major hallmarks of Alzheimer’s Disease (AD) pathology. Neurofibrillary tangles (NFTs) are used in clinical staging (Braak & Braak 1997). Yet, the sample reflects the last stage of the disease, while stained histological sections (H/E) aggregations, which appear much earlier and are typical found in neuritl and not neuronal somas. Furthermore, those consist of a large variety of differently hyperphosphorylated Tau (pTau), whereas only a single phosphoform was found to be especially related to AD. However, today’s knowledge about appearance, quantity and localization of pNFTs with different phosphorylation and in different brain regions during disease progression is rather poor. To elucidate the quantitative distribution of different pTau sites in the AD brain, we analyzed total human Tau, pThr231, pSer202/Thr205 Tau, pSer262, pThr178 Tau as well as ThioS positive tangles in four different subcortical brain regions and the hippocampus.

Methods

Systematic random sets of brain sections deriving from human AD cases of three different Braak stages (Braak stages III/IV: Hutter et al. 1997, V/VI) were immunohistochemically labeled for evaluation of different Tau species, intensity and quantified by evaluation of IR area using image analysis software (Axio-Imager Z1 microscope, Image-ProPlus). Sections of aged-matched non AD human subjects served as controls. Samples of humans were evaluated by rater independent automated counting in two mosaic image stripes of a mean of 5 mm² imaged at 20X through white and grey matter per region and subject.

Results

This finding is not new but should be presented here as well since rater independent quantitation is rarely presented. ThioflavinS (similar to Congo red) binds to beta-sheet structures due to physical-chemical properties. Staining is present in mature hyperphosphorylated Tau-positive NFTs and not on NFTs in general. Tangle load increases in both hippocampus and cortex, especially between III/IV and V/VI, whereas the total load is three times greater at end stage in the hippocampus than in the cortex (Fig. 4A). In both regions the increase is significant at all stage vs all groups. A similar result is found for pThr178 (Fig. 4B) and pSer202 (Fig. 4C). Much higher load is found for pThr231 (Fig. 4D) in the scope of 3% surface area at equal levels in both regions. In contrast to pSer202/Thr205 and pSer262, the hippocampal increase is significant for both marks, whereas the cortical increase is not significant but the difference is significant between different patient stages. pSer262 is a 1:200um² Tangle load is not significant with higher load detected in the hippocampus, the outermost pathological event related to Tau protein detected with both antibodies. Images to the all used labelings are presented in Fig. 1, whereas it should be mentioned that this poster just concentration on pTau and does not show other labelings (GFAP, ThioS, plaques, etc.). Some important findings are highlighted, e.g. that the total human Tau detecting antibody H77 does not bend to ThioS positive tangles. Even more impressive is the enormous load of pThr231 positive tangles, which seems to be a hallmark of AD. Representative images for each of the Braak stages are given in Fig. 4 (indicating the early appearance of pThr231 positive neuritl already at stage III/IV, while pThr178 Tau can be clearly seen at stage IV/VI as well as pSer202/Thr205 Tau positive objects.

Conclusions

The study contributes to the understanding of Tau hyperphosphorylation in AD. It proves Tau tangles to be a rather late stage event, while appearance of neuritl tangles starts much earlier and well displays the severe progression of neuronal malfunction and neurodegeneration.

Furthermore it confirms the hippocampus as a more valuable region for pTau accumulation among cortical regions with a rater independent quantitation method, especially for pThr178 and pThr231 hyperphosphorylation.

Hippocampal pThr231 Tau increase is the earliest measurable event with the used set of markers. However, analyses of further potentially early stage C-terminal Tau markers are still outstanding and will be accomplished in future experiments.