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TDP-43 in the olfactory bulb in Alzheimer's disease

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The TAR DNA binding protein of 43kDa (TDP-43) has been shown to be associated with neurodegenerative diseases, including frontotemporal lobar degeneration and amyotrophic lateral sclerosis[1]. Subsequently, TDP-43 was found to be present in Alzheimer's disease[2] and has been shown to be associated with key features of Alzheimer's disease including episodic memory loss and hippocampal atrophy[3]. TDP-43 deposition has also been shown to progress in a stereotypic manner across brain regions in Alzheimer's disease, starting with deposition in the amygdala then spreading to entorhinal cortex, then hippocampus proper, before spreading to neocortex[4]. At present, it is unknown whether TDP-43 deposition occurs in the olfactory bulb in Alzheimer's disease, and if deposition in the olfactory bulb occurs early in the progression across brain regions (i.e. before involvement occurs in the hippocampus) or late (i.e. after involvement occurs in the hippocampus). In addition it is unknown how TDP-43 deposition in the olfactory bulb compares to tau and alpha-synuclein deposition in the olfactory bulb. Assessing for TDP-43 deposition in the olfactory bulb is important since anosmia is well known to occur in Alzheimer's disease and could possibly be due to olfactory bulb pathology.

The aims of this study were therefore to determine the frequency of TDP-43 deposition in the olfactory bulb, to determine whether TDP-43 deposition in the olfactory bulb occurs before or after TDP-43 deposition in the hippocampal dentate granule cells, and to compare TDP-43 deposition in the olfactory bulb to tau and alpha-synuclein deposition in the olfactory bulb. We were able to show that deposition is infrequent (16%) and a late occurrence.

In order to address our aims, we selected all cases of Alzheimer's disease from the Mayo Clinic Brain Bank located in Jacksonville, Florida, in which there was available paraffin embedded sections of the olfactory bulb (n=107). In all cases we had taken multiple transverse sections per bulb which was embedded. All 107 cases had undergone detailed neuropathological examination and each case had been assigned a Thal Phase [5] and a Braak neurofibrillary tangle (NFT)[6] stage using thioflavin stained slides. Thal Phase was assigned by retrospectively assessing for the presence of senile plaques in neocortex, hippocampus, basal ganglia and cerebellum. Based on the Thal Phase and Braak NFT Stage all cases fulfilled National Institute on Aging - Alzheimer's Association (NIA-AA) criteria

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for intermediate-high probability Alzheimer's disease [7]. As per routine practice of our laboratory, all 107 cases had also previously undergone TDP-43 immunohistochemistry on hippocampal sections which was reviewed for the presence of TDP-43 immunoreactivity in the dentate granule cells of the hippocampus.

For this study, paraffin embedded sections of olfactory bulb on all 107 cases were immunostained for TDP-43 (polyclonal antibody MC2085 that recognizes a peptide sequence in the 25-kDA C-terminal fragment; Mayo Clinic) with a DAKO-Autostainer (DAKO-Cytomaton, Carpinteria, CA) with 3,3'-diaminobenzidine as the chromogen. This is the same antibody that was used to assess for TDP-43 immunoreactivity in the hippocampus. All TDP-43 stained slides were reviewed by one of the co-authors (KAJ) and 10% were reviewed by both co-authors with 100% interrater reliability. A region was considered TDP-43 positive if there were any TDP-43 immunoreactive neuronal cytoplasmic inclusions, dystrophic neurites, or neuronal intranuclear inclusions, identified at 40X magnification. All 107 cases were also immunostained for tau (CP-13, 1:1000; monoclonal; a gift from Peter Davies, Feinstein Institute for Medical Research) and alpha-synuclein (NACP, 1:3000; rabbit polyclonal; Mayo Clinic). TDP-43, tau and alpha-synuclein deposition were graded on a 4-point scale: absent = no inclusions; mild (1–5 inclusions), moderate (5–20); and severe (more than 20).

Statistical analyses were performed using JMP® 9.0.1 (SAS Institute Inc., Cary, NC). Non-parametric Mann-Whitney U test was used to compare continuous data between groups while Chi-square test was used to compare nominal data. A p-value <0.05 was considered significant. The study was approved by the Mayo Clinic IRB.

Of the 107 cases, 58 (54%) were female. The average age at death was 81 years old (range: 38–99 years). The average Braak NFT stage was 5.6 (range:4–6) and the average Thal β -amyloid phase was 4.8 (range:3–5).

Fifty-six cases (53%) had TDP-43 immunoreactivity in the dentate granule cells of the hippocampus. Sixteen cases (15%) had TDP-43 immunoreactivity in the olfactory bulb. Of these 16, TDP-43 immunoreactivity was mild in all 16 cases; in actuality scant (1–2 inclusions) in 12 (Figure a & b). In all 16, neuronal cytoplasmic inclusions were observed while only three cases had dystrophic neurites. No neuronal intranuclear inclusions were observed. TDP-43 immunoreactivity was present in the dentate gyrus cells of the hippocampus in all 16 cases in which TDP-43 immunoreactivity was observed in the olfactory bulb. On-the-contrary, there were 42 cases in which TDP-43 immunoreactivity was present in the hippocampus but not in the olfactory bulb.

The presence of TDP-43 in the olfactory bulb was associated with the presence of TDP-43 in the hippocampus ($p < 0.0001$). There were no significant differences in age at death (median 83.5 vs 83), Braak NFT stage (5.8 vs 6.0), or Thal Phase (5 vs 5) between those with and without TDP-43 immunoreactivity in olfactory bulb.

All cases (100%) had tau immunoreactivity in the olfactory bulb. Tau deposition was mild in 21 cases (20%), moderate in 31 (29%) and severe in 55 (51%) (Figure c & d). Thirty-seven cases (35%) had alpha-synuclein immunoreactivity in the olfactory bulb. Alpha-synuclein

deposition was mild in nine cases (24%), moderate in 20 (54%) and severe in 8 (7%) (Figure e & f).

In this study we have shown that TDP-43 deposition does occur in the olfactory bulb in Alzheimer's disease with a frequency of 15%. Whenever TDP-43 immunoreactivity was observed in the olfactory bulb we also observed TDP-43 deposition in the hippocampus. On-the-contrary, TDP-43 deposition in the hippocampus was present 37% of the times (40/107 cases) while absent in the olfactory bulb, suggesting that TDP-43 deposition in the olfactory bulb is not an early occurrence. Unlike with tau and alpha-synuclein where deposition in the olfactory bulb was often moderate to severe, TDP-43 deposition was scant.

TDP-43 deposition in the olfactory bulb has only been previously reported in cases of amyotrophic lateral sclerosis [8]. In comparison to our cases of Alzheimer's disease, TDP-43 deposition was also found to be more frequent in the dentate gyrus cells of the hippocampus (49%) compared to the olfactory bulb (31%) in amyotrophic lateral sclerosis. However, while the frequency of TDP-43 deposition in the dentate granule cells of the hippocampus was similar across both diseases (49% vs 53%), deposition in the olfactory bulb in amyotrophic lateral sclerosis was more than double the frequency we observed in Alzheimer's disease (31% vs 15%). Hence, one has to wonder whether TDP-43 deposition in the olfactory bulb in Alzheimer's disease is due to a different underlying mechanism than TDP-43 deposition in the olfactory bulb in amyotrophic lateral sclerosis. In fact, TDP-43 was not found to be present in the olfactory epithelium of the nasal cavity in Alzheimer's disease[9] arguing against a mechanism of spread via the nasal cavity to the olfactory bulb; TDP-43 spread in amyotrophic lateral sclerosis is thought to occur via anterior axonal transport[10].

Other proteins have been shown to be deposited in the olfactory bulb in Alzheimer's disease. In two studies, tau deposition was observed in the olfactory bulb in 100% of Alzheimer's disease cases, and was also found to be associated with higher Braak neurofibrillary tangle stages[11, 12]. We did not find any association between TDP-43 deposition in the olfactory bulb and Braak NFT stages in this study likely because of a limited range in the Braak stage in this study (IV–VI) compared to the other studies that utilized the full range (Braak 0–VI). TDP-43 deposition in the olfactory bulb was also observed to occur less frequently than alpha-synuclein deposition. In this study 35% of the cases had alpha-synuclein deposition in the olfactory bulb, similar to the frequency reported in another study in which 5/15 cases of Alzheimer's disease showed alpha-synuclein deposition in the olfactory bulb[11]. We did not assess for β -amyloid deposition in the olfactory bulb in this study but β -amyloid deposition has been reported to occur in the olfactory bulb in approximately a third of Alzheimer's diseases cases[12]. Hence, in comparison to tau, alpha-synuclein and β -amyloid, TDP-43 is deposited less frequently. In addition to frequency, we also observed a striking difference in burden between protein depositions. While tau and alpha-synuclein deposition were usually moderate to severe, TDP-43 deposition was frequently scant with only 1–2 inclusions observed. The finding of scant deposition limited our ability to see any patterns of where exactly TDP-43 is deposited in the bulb although we did observe deposition in the anterior olfactory nucleus in some cases.

In summary, we have shown that TDP-43 is deposited in the olfactory bulb in Alzheimer's disease, albeit of low frequency. The deposition appears to be a late occurrence compared to TDP-43 deposition in other brain regions such as the amygdala, entorhinal cortex, subiculum which appears to be affected before the dentate granule cells of the hippocampus[4]. The clinical significance, if any, of TDP-43 deposition in the olfactory bulb remains to be determined.

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Figure.

Figure comparing TDP-43 (a & b), tau (c & d), and alpha-synuclein (e & f) deposition in the olfactory bulb and anterior olfactory nucleus (AON) in the same patient in which all three proteins were observed to be present. Note that in comparison to tau and alpha-synuclein deposition that were typically moderate to severe when present, TDP-43 deposition tends to be relatively scant.

Scale bars a, c, e = 100 µm. Scale bars b, d, f = 20 µm.