# ARTERIAL DIAMETER PROGRESSIVELY INCREASES IN MUTANT AD MICE - A COMPARATIVE STUDY TO HUMAN AD CASES

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## BACKGROUND

Changes of gross vascular morphology were reported for different mouse models of Alzheimer's disease (AD) and for human AD cases. Those changes comprise cerebral amyloid angiopathy (CAA) but also increased IR area of vascular markers. The latter was interpreted as vascular sprouting and/or increase of vascular diameter. However, this interpretation is critical within two respects: A) Sprouting or diameter alteration are two totally different mechanisms to increase blood flow as a reaction to lacking nourishment as found in AD. Thus it is important to figure out, which one exactly is responsible for increased vascular marker signal. B) The translationability of findings in mouse models to human cases has to be investigated.

## MATERIALS AND METHODS

Uniform systematic random sets of brain sections deriving a) from AD model $APP_{SL}$ transgenic (Tg) mice at three ages (months 7; 9 ;11) and b) from human AD cases* of three different Braak stages (Braak stages I/II; III/IV; V/VI) were immunohistochemically labeled for detection of amyloid (clone 6E10) and vessels (SMA, Collagen IV) , imaged and quantitatively evaluated in terms of mean vascular diameter and percentage of amyloid related to vascular cross section area using image analysis software (Axio.Imager Z1 microscope, ImageProPlus). Sections of non-transgenic littermate mice (nTg) and non AD human subjects served as controls.

In mice mean vascular cross sectional size was evaluated by manual delineation of smooth muscle actin (SMA, specific to arteries in the brain) labeling of 11 defined arteries in the cerebral cortex at three different lateral levels of the brain. Data are meta-data and derived from Tg and nTg vehicle controls of different studies.

Since SMA antibodies did not label on human sections (formalin over-fixation and/or paraffin embedding), Collagen IV was used, which also labels to venules. This implies that unfortunately no direct comparable data to mean vascular size between mice and men could be collected. Samples of the cingulate cortex were evaluated by counting in stripes of a mean of 3 mm² imaged at 20x through white and grey matter.

In both cases, the mean 6E10 IR area was measured and normalized to the individual vessel size.

*… provided and staged by the Brain Bank of Newcastle

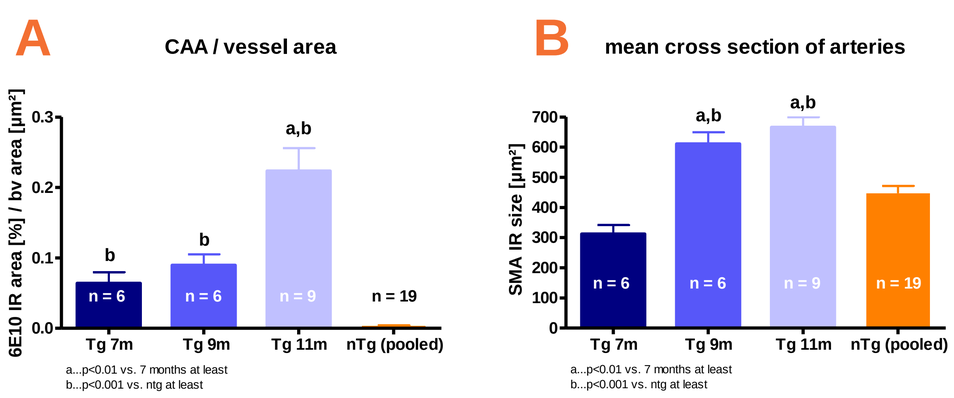


**Figure 1. Mean amyloid load on vasculature and arterial lumen increase with age in $APP_{SL}$ mice. (A)** The relative amyloid load per blood vessel size measured by 6E10 immunoreactivity increases significantly, especially between 8 and 11 months of age. **(B)** Mean cross sectional vascular diameter is significantly altered in AD model mice compared to nTg controls and especially rises between 7 and 9 months of age. Presented data are meta-data from controls of three different studies. Statistical significance is indicated by One-Way ANOVA (Tukey's Multiple Comparison Test). Data are shown as group mean + SEM.

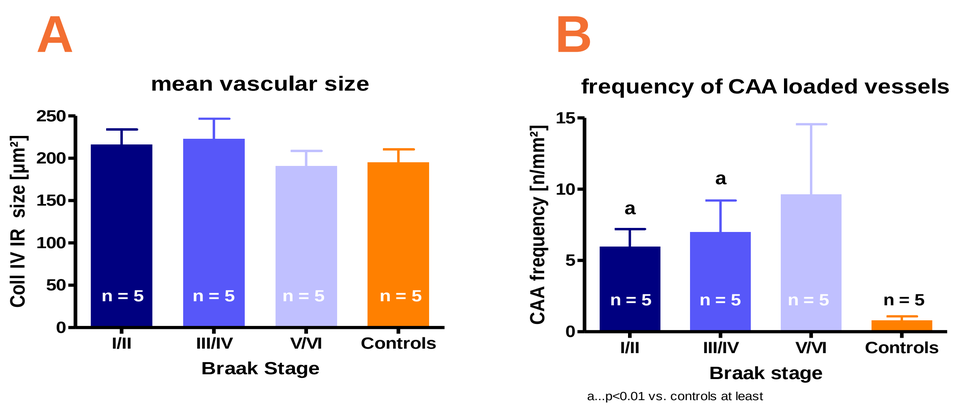


**Figure 3. Mean vascular size is not altered and frequency of CAA vessels increases with Braak stage in human AD. (A)** Mean cross sectional vascular size remained unaltered if measured with Collagen IV labeling also to venules. **(B)** The frequency of CAA vessels was significantly higher in Braak I/II and III/IV AD subjects. At Braak V/VI variance increased which interfered with statistical significance. Data are shown as mean of 2 approximately 3mm² large areas in the cingulate cortex. Statistical significance is indicated by One-Way ANOVA (Tukey's Multiple Comparison Test). Data are shown as group mean + SEM.

## RESULTS

### Mean CAA and vascular diameter and increase with age in $APP_{SL}$ mice

$APP_{SL}$ mice develop an AD like brain pathology, among it an age-dependently increasing amyloid plaque pathology and concomitant gliosis. One hallmark is an age-dependently increasing CAA (Fig. 1A) whereas a strong increase is observable between 9 and 11 months of age .nTg mice feature practically no amyloid load on arteries. Amyloid deposits from the parenchymal side and clogs the intercellular gaps between endothel cells, which results in a band-like labeling pattern. As probable reaction to increased clogging of arteries with amyloid (Fig. 2A) mice react with a significant widening of vascular diameter and lumen, respectively (Fig. 1B) especially between 7 and 9 months of age. Notably this increase influences the percentage of amyloid on vasculature, since 6E10 levels were normalized to individual vessel size.

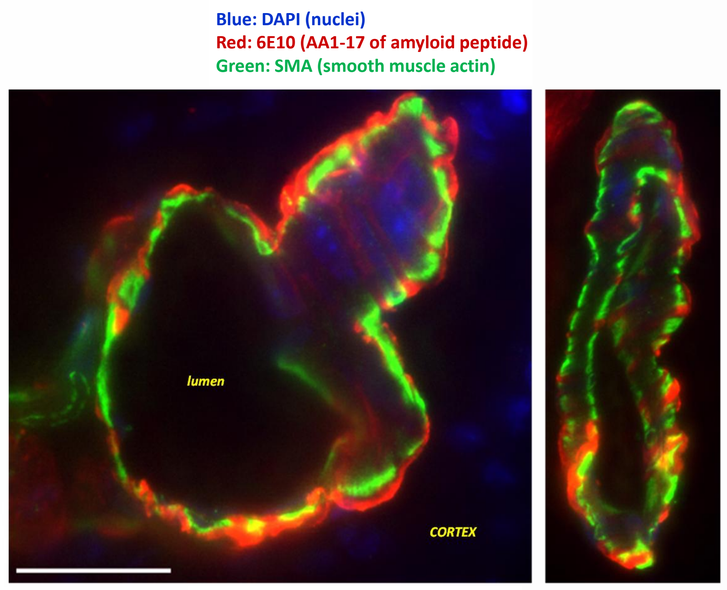


**Figure 2A. CAA in $APP_{SL}$ mice.** Amyloid labeled by 6E10 (red) deposits from the parenchymal side of the brain and clogs the inter-cellular gap between endothel cells that are SMA (green) positive in brain arteries. This leads to a band-like labeling pattern in vasculature with strong CAA. Section thickness 10µm; scale 10 µm; 63x. Age: 9 months.

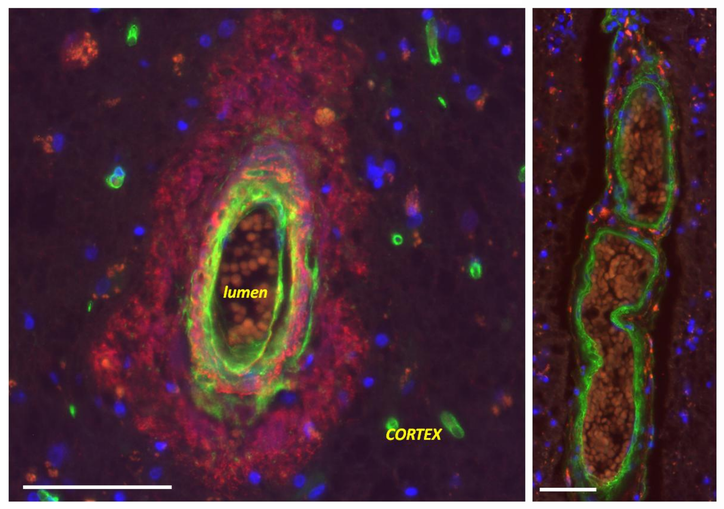


**Figure 2B. CAA in human AD.** Amyloid labeled by 6E10 (red) deposits from the parenchymal side of the brain and clogs the inter-cellular gaps between endothel cells. Collagen IV is labeled green. Note strong amyloid accumulation around CAA vessels at late stage, while the labeling is comparable to APPSL mice at earlier stage. Section thickness 5µm; scale 50 µm; 20x. Braak stage: VI (left) and III (right).

### Total vascular diameter does not alter but frequency of CAA vessels increases with Braak stage in human AD cases

The mean vascular size remained unaltered with Braak stage in humans (Fig. 3A). Notably these data are not comparable to mice, because also containing venules. Since a direct comparison to arterial CAA in mice was thus not possible with Collagen IV, we concentrated on the frequency of 6E10 positive vessels in general. The number of CAA vessels was significantly enhanced in both Braak I/II and III/IV stages, however at equal level (Fig. 3B). At higher stage variance rose. 2 out of 5 subjects only showed very few loaded vessels, while 2 displayed extreme load (Fig. 2B, left) and one intermediate. However, this variance led to lower level significance.

## CONCLUSIONS

The translationability of results is still weak due to technical reasons. A marker specific to human arteries has to be found and vice versa mice need to be investigated with Collagen IV to measure total vascular changes. Investigation of brain regions in humans has to be extended.

The observed effects are still highly interesting. On the one hand there is increase of vascular lumen in $APP_{SL}$ mice paired with increased vascular amyloid plaque size. On the other hand in humans rather the frequency of amyloid deposits rises with Braak stage than the vascular amyloid plaque size.

Further challenging work will clarify alterations of CAA in both mice and men and for sure yield information of high interest in the AD field.

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