Probing age-dependent cerebrovascular alterations in the Tg2576 mouse model of Alzheimer's disease by magnetic resonance angiography at 17.6 T

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Introduction: Many neurodegenerative diseases including Alzheimer's disease are linked to abnormalities in the vascular system. The deposition of amyloid β (Aβ) peptide in the cerebral vessel walls, known as cerebral amyloid angiopathy (CAA) is frequently observed, leading to blood flow abnormalities in AD brains [1]. Recent studies in transgenic mouse models of AD and CAA demonstrated associations between cerebrovascular abnormalities and Aβ peptide [2]. Here we present the first ultra-high field (17.6 T) MR angiography (MRA) study to monitor age-dependent cerebrovascular alterations in the Tg2576 mouse model of AD. Our results revealed that age-dependent cerebrovascular alterations observed in this study are part of the pathological alterations developed byTg2576 mouse models of AD.

Methods: Tg2576 mouse model of AD (n=8) were used in this study [3]. The transgene is expressed in C57B6/SJL F1 mice, backcrossed to C57B6 breeders. The N2 generation mice of both genders were studied at the ages of 14, 16 and 18 months. Age-matched non-transgenic littermates served as controls (n=8). All measurements were conducted on a vertical wide bore 17.6 T Bruker spectrometer, with a 1 Tm^{-1} actively shielded imaging gradient insert (Bruker). A birdcage radio-frequency (RF) coil (inner diameter 2 cm) was used. Before MR imaging, the mice were initially anesthetized with 2 % isoflurane (Forene, Abott, UK), in air (0.3 L/min) and oxygen (0.3 L/min) and maintained between 1- 1.5 % isoflurane during all procedure. While inside the probe, the respiration rate of the mouse was constantly monitored (Bio-SAM monitoring system. The 3time-of-flight (3D TOF) gradient echo sequence was applied with the following parameters: TE = 1.86 ms; FOV = 17 X 17 X 17 mm; FA = 20°; and TR = 20 ms. A three-dimensional view was obtained by generating maximum-intensity projections (MIP) using Paravision 5 software (Bruker). T2- weigted MR images were acquired using a RARE sequence at 9.4 T as described previously [4]. Coronal images were obtained with a slice thickness of 0.5 mm. A resolution of 78x78 µm was achieved. Histological analyses were performed to detect Aβ and CAA as described previously [4]. The severity of the vascular abnormalities in Tg2576 mice and control mice was evaluated by visual assessment based on the pointing system as described previously [5] as well as by a semi-quantitative analysis, based on calculation of vessel contrast-to-noise ratio (CNR) as described previously [6].

Results and Discussion:

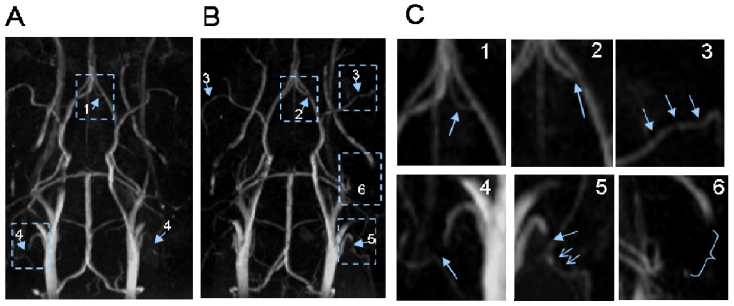

Fig. 1. (A, B) *3D TOF angiograms collected at 17.6 T magnet of 18-month-old transgenic mice showing various level of severities of morphological changes appointed in visual assessment in 3D MIP. The number indicates the appointed score to the level of severity of alterations. The enlarge view of alterations is shown in* ***(C)****.* ***(D)*** *MCA alteration mean score in wild type and transgenic mice with age. Values are mean ± SE (error bars); one-tail student T test; *P <0.05; n = 4*

Blood flow abnormalities were graded on the basis of the number and extents of signal voids detected on the MR angiograms (Fig. 1 A-C). In general, middle cerebral artery (MCA) was one of the most altered vessels on angiograms of Tg2576 mice (Fig. 1D). A marked decrease in signal intensity on angiograms was observed in anterior communicating artery (AComA) in addition to MCA in Tg2576 mouse, but not in control mice (Fig. 2 A & B). Individual slices of MRA data set were used to validate the changes seen in 3D MIP of the Tg2576 mouse brain (Fig 2A (c, e) & B (d, f)). Age-dependent changes in CNR of AComA (Fig 2 C) and MCA (Fig 2D) were investigated. The evaluation of MRA data revealed a significant decrease in CNR of both vessels in 18-month-old Tg2576 mice compared with control mice. Histological staining of Aβ confirmed the presence of CAA and plaques that were attached to vessels in the Tg2576 mouse (Fig. 3 A-C). An age-dependent increase in hypointense regions, corresponding to Aβ plaques, was observed in Tg2576 mouse brain (Fig. 3D). The quantitative analysis of Aβ plaque load in the cortex of Tg2576 mice (between 12 to 18 months of age) revealed an age-dependent increase in plaque load (Fig. 3E). The blood flow alterations in 3D MIP of Tg2576 mice might be caused by many factors such as partial occlusion, slow inflow, local microturbulent flow, increase in the viscosity of blood, increase in the shear stress caused by the vascular wall and vascular resistance, and reduction in vessel width [2]. These alterations may be associated with CAA related elevation of Aβ levels in the vessel wall of Tg2576 mice. The causal factors for blood flow abnormalities occurring in vessels of Tg2576 mice require further investigation. These results show that vascular abnormalities observed in this study are part of the pathological alterations developed by Tg2576 mouse models of AD. MRA studies at ultra-high magnetic field provide a powerful non-invasive tool to test new therapeutic intervention in mouse model of AD *in vivo*.

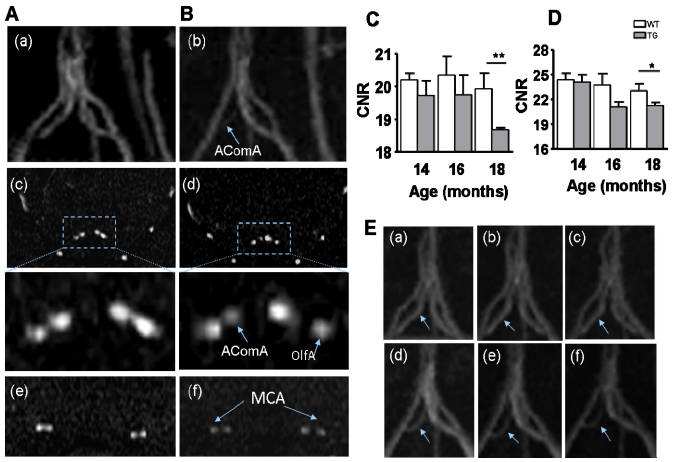

Fig. 2. (A, B) *The slices of 3D MRA data collected at 17.6 T for 18-month-old wild type (a, c) and Tg2576 (b, d) mice. Age-dependent changes in CNR of AComA* ***(C)*** *and MCA* ***(D)*** *in wild type and Tg2576 mice. One-tail student T test was used for statistical evaluation. *P <0.05 and **P <0.01. n = 6-8* ***(E)*** *Age-dependent changes in AComA in a control mouse (a-c) and a Tg2576 (d-f) mouse imaged at the age of 14 (a,d); 16 (b, e) and 18 (c, f) months.*

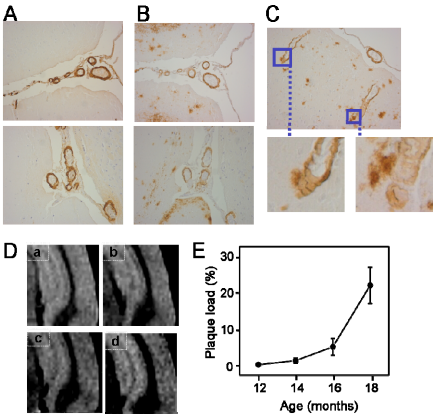

Fig. 3. (A, B) *CAA detected in the brain sections of 23-month-old Tg2576 mouse stained with Aβ40 (A) and Aβ42 (B).* ***(C)*** *Plaques attached to vessels in brain sections stained with Aβ42.* ***(D)*** *In vivo T2–weighted MR images of the brain of a 16 (a, c) and 23 (b, d) month old control (a, b) and Tg 2576 mouse (c, d). (E) Age dependent changes in Aβ plaques load in Tg2576 mouse detected by µMRI.*

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