AQP4 Association with Amyloid Deposition and Astrocyte Pathology in the Tg-ArcSwe Mouse Model of Alzheimer’s Disease

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Abstract. Amyloid-β deposition in senile plaques is one of the main pathological changes in Alzheimer’s disease (AD). We previously reported that aquaporin-4 (AQP4) is redistributed within the astrocytes in cerebral amyloid angiopathy in the tg-ArcSwe mouse model of AD, suggesting that AQP4 may participate in amyloid-β deposition. However, the role of AQP4 in plaque formation is not currently clear. The objective of the current study was to explore the AQP4 distribution within plaques in the tg-ArcSwe mice in more depth by the combined application of immunofluorescence cytochemistry and immunogold electron microscopy. In addition, the astrocyte marker, glial fibrillary acidic protein (GFAP), was studied in association with AQP4. We demonstrated a robust upregulation of AQP4 expression in areas of plaques. Compared to GFAP, AQP4 appeared predominantly at later stages of plaque formation, in older mice, and within the processes of astrocytes. In combination with GFAP, AQP4 differentiated plaques into three progression stages under light microscopy. This suggests that AQP4 expression was associated with amyloid deposition and astrocyte pathology in the Tg-ArcSwe mouse model of AD. This provides novel proof for the involvement of AQP4 in the process of amyloid deposition in AD.

Keywords: Alzheimer’s disease, aquaporin 4, astrocyte, glial fibrillary acidic protein, senile plaques

INTRODUCTION

Aggregation of the amyloid-β peptide (Aβ) in the brain parenchyma is known as senile plaque (SP) formation, which is a hallmark of Alzheimer’s disease (AD) [1, 2]. The major and more toxic component of the SPs is Aβ1–42 (Aβ42), a product of the amyloid-β protein precursor (AβPP). Overproduction or low excretion of Aβ42 from the brain leads to its extracellular aggregation, which appears related to the development of SPs [3]. Despite considerable research, it is still unclear how SPs are formed and degraded and what role they play in AD pathogenesis.

Reactive astroglia (astrocytosis) are reported in and around the plaque area in AD, suggesting a role of astroglia in the clearance and degradation of Aβ [4, 5]. However, the specific roles that astroglia play in Aβ deposition are still unknown. Aquaporin-4 (AQP4) is the main water channel protein in the brain, where it is highly concentrated in the astrocytic membrane facing microvessels (known as the astrocytic endfoot) and the pia mater (the glia limitans) [6, 7]. AQP4 is implicated in brain edema, astrocyte

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migration toward a lesion, and glial scar formation [8, 9]. Although recent studies have demonstrated abnormal AQP4 expression in AD patients and in animal models of AD [10–15], the results have been contradictory. Moreover, the details of AQP4 expression in relation to astrogial pathology during SP formation has not been studied, and thus the precise role of AQP4 in Aβ deposition remains unknown.

We hypothesized that AQP4 associated with astrogial pathology might participate in SP formation and progression of degeneration. Herein, we investigate AQP4 distribution patterns throughout the amyloid deposition area under light and electron microscopy in the transgenic tg-ArcSwe model of AD. This is a promising AD model for studying intraneuronal amyloid accumulation and amyloid plaque formation as the biochemical structure of the amyloid deposits resembles that of the AD brain [16, 17]. In this model, we further explored how another astrogial marker, glial fibrillary acidic protein (GFAP), was related to AQP4 during the progression of amyloid plaque formation.

**MATERIAL AND METHODS**

**Animals and tissue processing**

Transgenic AD mice (tg-ArcSwe mice), which carry human APP mutated to include the APP KM670/671NL (Swedish) and E693G (Arctic) AD mutations, and age-matched non-transgenic C57BL/6 controls (WT) were used to study AQP4 and GFAP expression associated with Aβ deposition in this study. The expression cassette used to generate transgenic mice and the procedures of genotyping have been described previously [16, 18, 19]. Double transgenic mice (AD/syn-KO) by crossbreeding tg-ArcSwe mice and α-syntrophin (α-syn) null mice (syn-KO) bred on C57BL/6 background [20] were also used to elucidate the impact of α-syntrophin knockout on AQP4 expression and amyloid deposition. For light microscopy studies, four age groups (4-, 8-, 12-, and 16-month-old [mo]) of tg-ArcSwe mice and age-matched WT littermates were used. Five to ten animals were included in each age group. For electron microscopy studies, two age groups (4 and 16 mo) were used. Animals (3–5 pairs) were included in each group. For immunofluorescence and electron microscopy, animals were deeply anesthetized with Equithesin (0.02 ml/g), then transcardially perfused with 4% formaldehyde, followed by post-fixation in the same fixation overnight, and stored in 1/10 paraformaldehyde at 4°C until use. All animal experiments were performed in accordance with the National Institutes of Health Guide for the care and use of laboratory animals and approved by the Biological Research Ethics Committee in Norway.

**Electron microscopy**

Pieces of cerebral cortex (1.0 × 0.5 × 0.5 mm³) were dissected from 500 µm thick free-floating sections under microscopy (Olympus SZX12) and embedded in Lowicryl HM20 as described [13, 14]. Cryoprotection and cryosubstitution were then performed. Cryoprotection was undertaken by immersing the tissues in phosphate buffered glucose, followed by increasing concentrations (10%, 20%, 30%) of glycerol before inserting the tissue specimens into liquid propane at −190°C in a liquid nitrogen cooled unit (KF80, Reichert, Vienna, Austria). Cryosubstitution was done in 0.5% uranyl acetate in anhydrous methanol at −90°C for 24 h in a cryosubstitution unit (AFS, Reichert). The temperature was gradually increased to −45°C, and Lowicryl HM20 was stepwise substituted for methanol. The specimens were polymerized under UV light for 48 h at −45°C. Ultrathin sections (90 nm) were cut and transferred onto formvar-coated single-hole grids. Post-embedding immunogold labeling was carried out as described [14]. Briefly, the sections were incubated in 50 mM glycine in TBST, followed by 2% human serum albumin. The primary antibodies (rabbit anti-AQP4, Sigma; rabbit anti-GFAP, Dako Cytomation; 6E10, mouse anti-Aβ, Nordic Biosite) in human serum albumin were applied to the sections for 2 h. The sections were then rinsed twice in Tris-buffered saline/Tween-20 (TBST) prior to incubation with goat anti-rabbit or anti-mouse Fab fragments coupled to gold particles for 1 h. Double labeling was achieved by incubating sections with a mixture of monoclonal and polyclonal antibodies as primary antibodies. For enhancing contrast, uranyl acetate (Fluorochem) and lead citrate were used successively. Micrographs were obtained digitally by a transmission electron microscope (Technai 12).

**Immunofluorescence**

Twenty-five µm thick free-floating sections were cut on a vibratome (Leica). After treating sections with 90% formic acid, the sections were incubated in a pre-incubation solution (10% normal
donkey serum, 1% bovine serum albumin, 0.5% Triton x 100 in 0.01 M phosphate-buffered saline) for 1 h at room temperature. Sections were then stained in primary antibody combinations (rabbit anti-AQP4, Millipore, 1:1000; 6E10, mouse anti-AB, Nordic Biosite, 1:2000; chicken anti-GFAP, Nordic Biosite, 1:1000) in the primary incubation solution (3% normal donkey serum, 1% bovine serum albumin, 0.5% Triton X 100, 0.05% sodium acid in 0.01 M phosphate-buffered saline) at 4°C overnight. After washing, the sections were incubated in secondary antibody combinations (donkey anti-rabbit Alex488; donkey anti-chicken cy3; donkey anti-mouse Alex488; donkey anti-chicken cy5) at a dilution of 1:1000. Sections were mounted on Superfrost-plus slides (Thermo Scientific) with ProLong Gold antifade reagent with DAPI (Invitrogen) and stored at −20°C in the dark. The relationships among AQP4, 6E10, and GFAP were also examined by immunofluorescence in the four AD age groups (n = 3/group). The primary antibody combination contained rabbit polyclonal anti-AQP4 (1:800), mouse monoclonal 6E10 (1:2000), and chicken polyclonal anti-GFAP (1:500). The secondary antibody mixtures were donkey anti-rabbit cy3 (1:1000), donkey anti-mouse Alex488 (1:1000), and donkey anti-chicken cy5 (1:1000). The procedure was as detailed above. Images were captured under a Zeiss LSM5 PASCAL confocal microscope.

Quantitative image analysis

For semi-quantification, all the sections for comparison were immunostained at one experiment under the same condition as described before. Ten images (400x) were randomly taken from the parietal cortex, temporal cortex, and CA1 region of hippocampus from each stained section under the same settings (including exposure time). All the images were transformed into eight-bit format and inverted in ImageJ 1.46 (NIH, USA). The optical density (OD) was then uncalibrated, scales were removed, and the measurements of area, integrated density, and limit to threshold were also set. All the minimal threshold was set at 2.71, two maximal threshold was set at 0.03 and 0.06 for each image. 0.05 mm² area was used for all the images. The integrated density (IntDen) of each area was obtained as the relative fluorescence intensity. One-way ANOVA was used for comparison of AQP4, GFAP and Aβ42 fluorescence levels between different groups. Correlations between AQP4 and 6E10 were assessed using Spearman correlation and regression analysis. Statistical significance was set at p < 0.05.

RESULTS

Aβ deposition is associated with AQP4 upregulation

Aβ deposition was examined in the four age groups (4, 8, 12, and 16 months of age) of the tg-ArcSwe mice. In the 4 mo mice, Aβ appeared as sparse intracellular aggregates, which were most often found in layers IV-V of the cerebral cortex (Fig. 1A). The intracellular aggregation reached a peak in number in the 8 mo group, and extracellular deposition forming SPs of small to moderate size started to distribute among the intracellular aggregates at this age (Fig. 1C). Aβ deposition in 12 mo AD mice (data not shown) resembled that in the 8 mo group. Within the 16 mo group, numerous mature SPs of small to large size spread throughout the cerebral cortex and hippocampus, whereas intracellular amyloid deposition was sparse.

Robust enhancement of AQP4 immunoreactivity was found in association with amyloid deposition within SPs from cerebral cortex and hippocampus of tg-ArcSwe mice, compared with age-matched WT group mice (Fig. 1B, C, E) Two patterns of AQP4 immunosignal distribution could be recognized by AQP4 and amyloid double labeling under light microscopy. In pattern I, AQP4 immunosignals appeared exclusively in the periphery of the SPs, seldom in the center. Whereas, in pattern II, AQP4 immunoreactivity distributed throughout the amyloid deposition area. Despite no direct corresponding relationship between the AQP4 pattern and SP types, pattern I predominated in all age groups (Fig. 1E).

The relative AQP4 fluorescence intensity (AQP4 IntDen) in the cerebral cortex of AD mice was significantly higher than that of WT at both 8 and 16 months of age by comparison of the semi-quantitative results (p value, stepwise = 0.016; p < 0.001; Fig. 2A). Significant positive correlations between AQP4 fluorescence intensity (AD-AQP4-IntDen) and Aβ42 expression (AD-6E10-IntDen) were found in the cerebral cortex of AD group (p < 0.001) (Fig. 2B).

Electron microscopy shows AQP4 patterns within SP types

SPs could be easily recognized by accumulation of long compact or short thin fibers and necrotic
Fig. 1. AQP4 and 6E10 double labeling in cortex and hippocampus of tg-ArcSwe and wild-type (WT) mice. A) Intraneuronal Aβ (asterisk) could be seen from 4 months of age (mo) in the tg-ArcSwe (AD) mice. AQP4 remained linearly distributed within the astrocytic endfeet (arrowhead). B) One senile plaque (SP) attached to the pia (asterisk) with AQP4 upregulation (arrow). AQP4 was highly concentrated next to the microvessels or pia (arrowhead) in the regions without SP in the transgenic mice. C) SP (asterisk) in CA1 of the hippocampus (CA) in 8 mo transgenic mice with upregulated AQP4 (arrow) surrounding the SP deposition area. D) In age-matched WT control for (C), AQP4 linearly distributed next to the microvessels (arrowhead) in 8WT mice, showing a similar expression pattern in 4 mo WT mice. E) Two large SPs (asterisk) tended to fuse with each other in the 16 mo transgenic mice. Arrow demonstrated Pattern I of AQP4, double arrows demonstrated AQP4 Pattern II. F) Age-matched WT control for (E), arrowhead showed linear distribution of AQP4 along the microvessels. Scale bars, 20 μm.
neurons (dystrophic neurites) under electron microscope (Fig. 3A, D). Two ultrastructural types of SPs are reported here: one type (SP-I) had a compact core with heavy Aβ labeling and high electron density, surrounded by a bundle of fibroid astrogliosis, dystrophic neurites, and other cellular components (Fig. 3A-C). AQP4 gold particles were absent from the dense core (Fig. 3C), concentrated instead to the surrounding astroglia (Fig. 3B). The other type (SP-II) included ruptured gliosis or other cellular constituents crossing scattered dystrophic neurites (Fig. 3D-F). In SP-II, AQP4 particles dispersed in (Fig. 3F) and around the area (Fig. 3E). In the areas without SP formation, the AQP4 gold particles remained linearly distributed along the endfeet facing the microvessels (Fig. 3H) and were sparse in the astrocytic membrane facing the parenchyma (Fig. 3I). Thus, immunogold electron microscopy could relate the AQP4 pattern I (the main pattern) to the SP-I type, and the AQP4 pattern II to the SP-II type.

AQP4, GFAP, and Aβ distributions in different age groups

AQP4 was distributed differently than GFAP in the SPs from different age groups. GFAP-labeled astrogliosis manifested as hypertrophic star-like cell bodies and an increased number of fine processes, and the former seemed the main type in 8 mo AD mice (Fig. 4A). Stepwise, the proportion of hypertrophic astrocytes in GFAP-labeled astrogliosis became slightly smaller in 12 mo mice (Fig. 4B). The hypertrophic astrocytes were fewer, and granular or intact fine processes predominated in 16 mo mice (Fig. 4C). In contrast, the AQP4 that associated with SPs did not demonstrate a star-like astrocyte morphology. Instead, the immunostaining robustly surrounded the SPs, forming a granular structure, with occasional polar distribution along microvessels. When comparing the distribution patterns of these two markers, we found that the distribution of AQP4 highly overlapped with GFAP in 8 mo transgenic mice, whereas AQP4 was more widely distributed than GFAP in 16 mo mice (Fig. 4A-C).

The fluorescence levels of the two glial markers and Aβ42 were further compared. Although not statistically different, GFAP levels tended to decrease from 8 mo to 16 mo mice, whereas AQP4 and Aβ42 density were closely related to each other, which was already illustrated in Fig. 1H. AQP4 and Aβ42 both showed an upward trend in the 8 mo to 16 mo mice, although there was some fluctuation in levels between 8 mo and 12 mo mice (Fig. 5A, B).

Fine structure of subcellular distribution of AQP4 and GFAP

Two types of astrocytic ultrastructures could be identified under the electron microscope in the cerebral cortex of 16 mo AD mice. The predominant type had a regular shape, small to moderate size, and similar electron dense to the other cell types (Fig. 6A, B). This perhaps corresponded to the numerous GFAP-labeled astrocytic processes observed under light
double immunogold electron microscopy with AQP4 and Aβ42 antibodies in 16-month-old transgenic mice. A-C) Solid Aβ bundle forming a large, dense core in the center (asterisk) of senile plaque (SP)-type I, with gliosis (arrow) and dystrophic neurites (arrowhead) in the periphery in the tg-ArcSwe (AD) mice. AQP4 particles concentrated in the periphery of this type of SP. B and C indicated, respectively, high magnification of the core and the periphery region in SP-type I. D-F) Aβ fibers and gliosis (arrow) spread out over dystrophic neurites (arrowhead) in SP-type II, AQP4 particles interspersed in the area. E and F indicate, respectively, high magnification of the core and periphery region in SP-type II. Twenty μm gold particles represented AQP4 signals, and 10 μm particles correspond to Aβ42. G-J) AQP4 is highly concentrated to the astrocytic endfeet facing microvessels (H), with low levels in the parenchyma non-endfoot region (I) in areas not affected by SPs formation. inpla, regions in the center of SPs; peripla, regions in the periphery of SPs; outpla, regions without SP formation; V, lumen of microvessel; Ast, astrocyte; End, endothelium. Scale bar in A, D, and G is 5 μm. Scale bar in B, C, E, F, H, and I is 200 nm.

microscope (Fig. 4C), and mainly presented in type I SPs under electron microscopy (Fig. 3A). This type of astrocytes readily upregulated AQP4 labeling as soon as amyloid fibers appeared (Fig. 6C). The other type of astrocytes appeared infrequently in 16 mo AD mice, relatively more often seen in type II SP under electron microscopy. They were rich in mitochondria and microvesicles, with much larger size and hyperelectron dense than the former type (Fig. 6D-H). They did not increase AQP4 labeling as readily as the former type (Fig. 6E) unless they had a heavy amyloid burden (Fig. 6F). The latter type of astrocytes always spread out processes to surround the dystrophic neurites or centers of SPs, with numerous small astrocytes in the periphery (Fig. 6G, H).

AQP4 and GFAP distribution in SPs of different sizes and progression stages

AQP4 showed a different distribution than GFAP in relation to the different sizes of SPs. Triple labeling of AQP4, Aβ42, and GFAP demonstrated that AQP4 expression appeared in SP-like structures only when the diameters of the SPs were
Fig. 4. AQP4 and GFAP distribution in plaques of the same type and size at different ages. A) The distribution of AQP4 and GFAP mostly overlapped in 8-month-old (mo) mice. Arrow and arrowhead indicate the small astrocytes labeled by GFAP and AQP4 separately, and the double arrow and double arrowhead point to hypertrophic astrocytes labeled by GFAP and AQP4. B) AQP4 (arrowhead and double arrowhead) still matched GFAP (arrow and double arrow) in most areas of 12mo group, but in some regions, AQP4 signals were present (asterisk) without GFAP labeling (double asterisk). C) In 16 mo, AQP4 had robust staining in most areas (arrowhead and asterisk), but GFAP had very weak (arrow) or absent labeling (asterisk).

Fig. 5. The fluorescent levels of AQP4, GFAP, and Aβ42 at the different ages. The AQP4, Aβ42, and GFAP started at significantly lower levels in 4 mo AD mice compared to 8–16 mo mice (asterisk, all p < 0.05). AQP4 and Aβ42 increased with age although an inversion between 8 mo and 12 mo was detected prior to peaking at 16 mo. GFAP rose rapidly to a peak at 8 mo before steadily decreasing at 12 mo and 16 mo. AD, tg-ArcSwe mice; WT, wild type non-transgenic mice. Scale bar, 20 μm. Error bars, ±2SEM.
Fig. 6. Two types of astrocytic ultrastructure in the cortex of 16-month-old AD mice. A-C indicated the main type of astrocytes mainly presented in type I SPs. A) GFAP particles spread throughout the cell body and processes. B) AQP4 gold signals were rare in the astrocytic process facing the neurons in the absence of amyloid deposition. C) Elevated AQP4 gold particles in neuropil was accompanied by amyloid fibers (arrow) aggregation. D-I indicated the second type of astrocytes more often seen in type II SP. D) The second type of astrocyte was stained by GFAP gold particles and contained numerous mitochondria (asterisk) and microvesicles (arrowhead). They also had variable processes surrounding the dystrophic neurite (DN) and amyloid fibers (arrow) (G, H). E, G) AQP4 signals were rarely found in the latter type of astrocyte when the amyloid fibers (arrows) were near but not taken inside the astrocyte. F, H) AQP4 gold particles distributed throughout the latter type of astrocyte after phagocytosing the amyloid fibers (arrows) which separated the astrocyte into compartments. E and F are magnifications of G and H, respectively. Large (20 μm) and small (10 μm) gold particles represented AQP4 and Aβ42, respectively, in C and F. Ast, astrocyte; P, process; DN, dystrophic neurite. Scale bar, 500 nm.

larger than approximately 20 μm, whereas GFAP immunostaining was increased even in intracellular amyloid deposits (Fig. 7A). AQP4 coincided with GFAP staining in AQP4 Pattern I. In Pattern II, however, AQP4 distributed throughout the SP region, but GFAP appeared only in the periphery of SPs (Fig. 7C). In AD/syn-KO mice, Aβ deposition was much lower than that in the AD mice of the same age, whereas there was no linear AQP4 distribution along the microvessels. AQP4 and GFAP immunostaining in SPs of similar size in the AD/syn-KO mice was greater than in the AD mice, but overall, AQP4 and GFAP immunostaining was lower in the AD/syn-KO mice compared to AD mice of the same age (Fig. 7D). This implied that AQP4 and GFAP might follow a different time course in SP formation, and perivascular AQP4 does not solely account for the progression of SPs.
Fig. 7. AQP4 and GFAP distribution in different stages of SP formation in 12-month-old (mo) AD mice. A) GFAP labeled numerous hypertrophic and astrocytes (arrows) around intracellular Aβ aggregates (arrowhead) and SPs that were smaller than 20 μm of diameter (asterisk, Stage I). AQP4 was linearly distribution along the vessels, with weak AQP4 immunostaining appearing in SPs of approximately 20 μm diameter (double asterisk). B) Granular AQP4 (double arrow) staining seemed to coincide with GFAP (arrow) in relatively larger SPs (Stage II). C) In Stage III SPs, AQP4 (double arrow) immunostaining was evident throughout the Aβ deposition area. GFAP was detected in the periphery of SPs (arrow) but never in the center of plaques (double asterisk). D) SPs of relatively small size dominated in 12 mo AD/syn-KO compared to 12 mo AD mice, very weak AQP4 linear distribution (arrowhead), but SP shaped AQP4 (double arrows) distribution and relatively strong GFAP signals (arrow) were detected. E) GFAP, AQP4, and Aβ42 distribution in syn-KO mice. Scale bar, 20 μm.
According to AQP4 and GFAP labeling, SPs were divided into three stages: AQP4-GFAP+ (Stage I), AQP4+GFAP+ (Stage II), and AQP4+GFAP- (Stage III). After counting the number of SPs in these subgroups at each age, we found the percentage of Stage I (AQP4-GFAP+) SPs was higher in 16 mo and 12 mo AD mice than that in 8 mo. Stage II (AQP4+GFAP+) SPs decreased from 8 months to 16 months in the AD mice, and Stage III (AQP4+GFAP-) SPs predominated in the 16 mo AD mice (Fig. 8). This indicated that the occurrence of one or both of the markers of astrocytes might represent different stages of SP deposition. AQP4-GFAP+ (Stage I) may indicate the earliest stage in the SP progression; AQP4+GFAP+ (Stage II) and AQP4+GFAP- (Stage III) may indicate the intermediate and mature stages of SPs, respectively.

DISCUSSION

The aquaporin protein family assembles in cell membranes as homotetramers. Each monomer has six membrane spanning domains forming a water pore that facilitates water transport in both directions [21, 22]. AQP4 is the predominant water channel protein in brain and is highly confined to the specialized astrocyte membrane abutting on cerebral microvessels and pia, forming the astrocyte polarization [6]. In addition to reports of abnormal AQP4 expression in the brains of AD patients and in mouse models [10–13], we previously demonstrated that development of amyloid deposits around microvessels is associated with a loss of astrocyte polarization [14]. However, AQP4 expression and astrocytic pathology in relation to amyloid plaque formation has not been previously studied.

The APP transgenic mouse model tg-ArcSwe is unique because of its prominent phenotype with age-dependent intracellular Aβ aggregation occurring months before the onset of extracellular plaque deposition [16, 17]. Intraneuronal Aβ was also observed in postmortem human AD brains [23]. In the present study, we demonstrated in the tg-ArcSwe mouse line that AQP4 expression was not altered in relation to intracellular amyloid aggregation, but its expression level and distribution in astrocytic processes increased in SP deposition areas in the cortex, hippocampus, and the pia mater. Previous studies have mainly focused on the cerebral cortex, especially the frontal cortex [13, 15]. In the current study, the results in the hippocampus and pia were similar to the cortex. AQP4 appeared in other astrocytic processes in the stratum radiatum layer of CA1 region, in addition to its high concentration in endfeet in contact with microvessels in WT mice. In the AD mice, the AQP4 upregulation in this brain region was restricted to plaque deposition.

Semi-quantification of the fluorescence intensity showed that AQP4 expression was much higher in AD mice at 8 and 16 months of age compared to age-matched WT mice. This is consistent with our previous western blotting results [14] and other reports [13, 15]. The positive relationship between AQP4 and Aβ42 fluorescence intensity imply that AQP4 expression is associated with Aβ deposition. Two patterns of AQP4 distribution in relation to the amyloid plaques were reported here, which is consistent with the light microscopy finding by Hoshi et al. [15]. We extended these findings by performing quantification and electron microscopy in addition to immunofluorescence cytochemistry. Pattern I of AQP4 distribution in which main part of

![Fig. 8. Three stages of senile plaques (SPs) in AD mice at different ages. A) Numbers of SPs labeled by AQP4 and GFAP in three age groups. B) Percentage of the three stages of SPs in three age groups. AQP4-GFAP+ indicated Stage I, AQP4+GFAP+ indicated Stage II, and AQP4+GFAP- indicated Stage III.](image-url)
AQP4 signals located in the periphery of the SPs was found to be the main pattern, twice as common as the evenly distributed AQP4 pattern. Although no direct relationship between AQP4 distribution patterns and SP types under light microscope, electron microscopy could easily distinguish primitive and classical plaques and relate them to the two AQP4 patterns. A previous study demonstrated that the two plaque types may form via distinct processes in Tg-ArcSwe mice [24]. Heparan sulfate was found to selectively facilitate amyloid core formation [25], whereas pathologic chaperones such as ApoE and antichymotrypsin favor both compact and diffuse plaque deposition [26]. Further studies on the functional differences of AQP4 distribution patterns are needed.

The reactive astrocytosis in AD was originally characterized by overexpression of the intermediate filament protein GFAP [4, 5]. In our study, AQP4 expression around the SPs mimicked the pattern of astrocytosis. Previous studies reported that the absence of AQP4 in astrocyte endfeet leads to weaker astrocyte migration toward the nidus and decreased hypertrophy of astrocytes during astrogliosis [8, 9]. Further, AQP4 gene knockout reduced Aβ₁₋₄₂ induced astrocyte activation and apoptosis in vitro [27]. These reports imply that AQP4 may mediate the astrocyte response to Aβ. A definite relationship between AQP4 and astrocytosis and the routine marker GFAP had not been illustrated by previous studies. Here we explored the question by triple labeling immunofluorescence of AQP4, GFAP, and 6E10 combined with immunogold electron microscopy. The expression of AQP4 and GFAP was compared in the same types of SPs at different mouse ages and at different stages of plaque formation. We showed that AQP4 was differently distributed than GFAP in that it associated with later stages of SPs and with older ages of mice. This finding suggests that the traditional cytoskeleton molecule GFAP may participate in early functional Aβ phagocytosis, whereas AQP4 in the fine processes plays the main role in the later stages of SP formation. This is in accordance with previous studies showing that astrogliosis occurred before SP formation [28] and implies that GFAP may facilitate early astrocytosis around intracellular amyloid deposition without AQP4 participation.

Even in relatively large and mature SPs, the two astrocytic markers did not necessarily correspond, and it was problematic to differentiate the SP types merely on basis of the morphology under the light microscope. According to AQP4 and GFAP immunostaining, we grouped the SPs or astrocytes into three subtypes. GFAP+AQP4+ SPs were the earliest plaques, occurring mostly in the youngest mice. GFAP+AQP4+SPs had the highest overlap of AQP4 and GFAP, representing the SPs in middle-aged AD mice, and GFAP-AQP4+ plaques occurred frequently in old AD mice, suggesting that these were mature SPs. The subgroups here are not contrary to the previous classification in which primitive plaques (neurite plaques) and cored plaques (classical plaques) were defined [29]. The GFAP+AQP4- SPs approximately corresponded to primitive plaques, and GFAP-AQP4+ and GFAP-AQP4+ were subgroups of cored plaques. Thus, with the combination of AQP4 and GFAP labeling, we could also identity the time course of the SP formation, especially the progression of classical plaques.

The role of astroglia in Aβ deposition and metabolism is still controversial. On the one hand, the reactive astrocytes in AD were suggested to facilitate the clearance and degradation of Aβ [30, 31]. However, other AD pathology may affect astrogliosis, changing them into Aβ producers. β-site AβPP cleaving enzyme 1 (BACE 1), normally confined to neurons, was detected in activated astrocytes surrounding Aβ plaques in several transgenic models [32]. However, this distinction is yet unclear and requires more in-depth study.

High concentrations of AQP4 on the astrocytic endfeet depend on the dystrophin associated protein complex (DAPC), of which α-syntrophin is a member [33]. We crossed syntrophin knockout mice with Tg-ArcSwe AD mice to produce AD-syn-KO mice. We primarily found that the plaques formed in AD-syn-KO mice were much smaller than those that formed in pure AD mice of the same age, and SP-like AQP4 and GFAP levels were much lower than that in AD mice of the same age. This is support for a role of AQP4 polar distribution in astrogliosis during Aβ extracellular deposition. However, this finding will need to be confirmed by quantitative analysis and a larger sample, particularly since this is contrary with a report that showed deletion of AQP4 increased Aβ accumulation with exacerbated cognitive deficits in APP/PS1 mice [34]. Such a contradiction in findings between research groups has previously occurred in relation to GFAP function in the process of amyloid plaque formation. One recent study showed that knockout of GFAP alone or of GFAP and another astrocytic marker, vimentin, but did not alter the development of plaque load [35]. In contrast, the study by Kraft demonstrated that deletion of GFAP and vimentin...
accelerates plaque pathogenesis in APP/PS1 mice [36]. Thus, astrocytosis may be a double-edged sword in the pathogenesis of amyloid deposition. The time-related or dose-related mechanism could be a further target to be studied.

Taken together, we conclude AQP4 expression is associated with amyloid deposition and astrocytosis in the Tg-ArcSwe mouse model of AD. This may provide novel proof for AQP4 participation in the process of amyloid deposition.

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Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/16-0957r1).

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