Neurobiology of Aging 97 (2021) 1–9

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Aquaporin-4 polymorphisms predict amyloid burden and clinical outcome in the Alzheimer's disease spectrum

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ARTICLE INFO

Article history:
Received 8 September 2019
Received in revised form 5 June 2020
Accepted 10 June 2020
Available online 17 June 2020

Keywords:
Alzheimer's disease
Mild cognitive impairment
Amyloid-β
Aquaporin-4
Glymphatic system

ABSTRACT

Clearance of amyloid-β (Aβ) from the brain is hypothesized to be mediated by the glymphatic system through aquaporin-4 (AQP4) water channels. Genetic variation of *AQP4* may impact water channel function, Aβ clearance, and clinical outcomes. We examined whether single-nucleotide polymorphisms (SNPs) of the *AQP4* gene were related to Aβ neuropathology on [^{18}F]Florbetapir PET in 100 Aβ positive late mild cognitive impairment (LMCI) or Alzheimer's disease (AD) patients and were predictive of clinical outcome in prodromal AD patients. *AQP4* SNP rs72878794 was associated with decreased Aβ uptake, whereas rs151244 was associated with increased Aβ uptake, increased risk of conversion from MCI and LMCI to AD, and an increased 4-year rate of cognitive decline in LMCI. *AQP4* genetic variation was associated with Aβ accumulation, disease stage progression, and cognitive decline. This variation may correspond to changes in glymphatic system functioning and brain Aβ clearance and could be a useful biomarker in predicting disease burden for those on the dementia spectrum.

1. Introduction

One of the neuropathological hallmarks of Alzheimer's disease (AD) is the accumulation of amyloid-β (Aβ) and neuritic plaque formation, which contributes to synaptic damage and neurodegeneration (Hardy and Selkoe, 2002; Harrington, 2012). Brain Aβ deposition has also been shown in patients with mild cognitive impairment (MCI), which may precede AD (Forsberg et al., 2008). Approximately 43% of MCI patients progress to AD after 4–6 years (Hansson et al., 2006), with conversion rates up to 62% in Aβ-positive patients (Frings et al., 2018). MCI patients with high Aβ levels also have greater declines in episodic memory than those with low levels (Lim et al., 2013).

An impaired ability to clear Aβ from the brain could contribute to its accumulation. Growing evidence implicates the glymphatic system as a mechanism for this clearance (Tarasoff-Conway et al., 2015). The glymphatic system is a brain waste disposal system, active during slow-wave sleep (Xie et al., 2013), that clears solutes through interstitial bulk flow. Polarized aquaporin-4 (AQP4) water channels at perivascular astrocytic end feet modulate this process (Iliff et al., 2012). Inefficient glymphatic transport has been demonstrated in mouse models of AD and aging and was associated with AQP4 perivascular localization loss (Kress et al., 2014; Peng et al., 2016). *AQP4* gene deletion resulted in increased Aβ retention and memory deficits in a mouse model of AD (Xu et al., 2015). In a human postmortem study, markers of AQP4 dysfunction were related to AD neuropathology (Zeppenfeld et al., 2017). Therefore, alterations at the *AQP4* gene, and subsequently dysfunction of the AQP4 water channel protein, could play a role in the accumulation of Aβ and AD manifestation.

Previous studies have shown that *AQP4* gene single-nucleotide polymorphisms (SNPs) were related to rates of decline on cognitive measures in AD patients (Burfeind et al., 2017). In older adults, *AQP4* SNPs moderated associations between sleep and Aβ brain retention (Rainey-Smith et al., 2018). However, there is a lack of understanding for the link between *AQP4* genetic expression (SNPs) and Aβ deposition in a group of individuals with well-established Aβ pathology, which is important given evidence that cerebral Aβ

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[1] Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at http://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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https://doi.org/10.1016/j.neurobiolaging.2020.06.007

accumulation could modulate AQP4 astrocytic expression (Hoshi et al., 2012). Associations between *AQP4* genetic variations and risk of disease stage progression also remains unexplored in patients with MCI and AD.

We aimed to determine whether *AQP4* genetic variation was related to Aβ pathology as measured by [^{18}F]Florbetapir PET in a group of late MCI (LMCI) or mild AD patients that were Aβ positive. Prior studies have utilized combined MCI and AD groups of patients to examine neuroimaging outcomes on PET (Johnson et al., 2016; Passamonti et al., 2018). However, for the present study, we sought to use a patient cohort with established amyloid neuropathology indicated by Aβ-positive status to most clearly elucidate the role of *AQP4* SNPs in cortical Aβ accumulation. In addition, we chose a prodromal AD group (LMCI) that is at a very high risk for progression to AD dementia (Jessen et al., 2014) and is classified based on the same threshold for objective memory impairment as those with mild AD. We investigated whether any *AQP4* SNPs that were related to amyloid burden were also predictive of risk of conversion from MCI and LMCI to AD and associated with the magnitude of decline on standardized cognitive measures over a period of 4 years in patients with LMCI.

2. Material and methods

All data used in this study were from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). This ongoing study was launched in 2003 to investigate the contribution of clinical, neuropsychological, and neuroimaging biomarkers, to disease progression in MCI and early AD.

2.1. Study participants

The following groups were included in this study: 100 Aβ-positive patients with LMCI or mild AD, 469 patients with MCI (either Aβ positive [n = 168], or negative [n = 145], or with no amyloid PET imaging [n = 156]), and 244 patients with LMCI (either Aβ positive [n = 62], or negative [n = 27] or with no amyloid PET imaging [n = 155]). We also included a control group of 97 Aβ-negative cognitively normal participants (Table 1). Of the 469 MCI patients, 225 were classified as having early MCI (EMCI). Shared key diagnostic criteria for EMCI and LMCI included subjective memory complaints, Mini-Mental State Examination scores between 24 and 30, and a Clinical Dementia Rating (CDR) of 0.5. The primary difference between the 2 groups was the threshold for abnormal memory functioning defined by scores on the Logical Memory II subscale (Delayed Recall). For EMCI, Logical Memory II subscale (Delayed Recall) scores were between 9 and 11 if participants had 16 or more years of education, 5–9 for 8–15 years of education, and 3–6 for 0–7 years of education. For LMCI, Logical Memory II subscale (Delayed Recall) scores were less than 8 for 16 or more years of education, less than 4 for 8–15 years of education, and less than 2 for 0–7 years of education. The objective abnormal memory functioning threshold for mild AD was the same as LMCI. Further diagnostic criteria for AD was a Mini–Mental State Examination score between 20 and 26, a CDR of 0.5 or 1.0, and NINCDS/ADRDA criteria for probable AD. Key exclusion criteria for all participants included MRI abnormalities that indicated infarction, infection, and generalized focal lesions, and a modified Hachinski ischemic score of 4 or less. Aβ-positive status was based on a composite standardized uptake value ratio (SUVR; reference region: whole cerebellum) cutoff of >1.10 (Johnson et al., 2013; Joshi et al., 2012). All patient groups examined were considered ethnically homogenous as in each case 94% or more were classified as Caucasian. Further information on standardized criteria for cognitively normal, MCI, and AD participants in the ADNI has been previously reported (Petersen et al., 2010).

2.2. Genetic analysis

Genotyping for apolipoprotein *(APOE)* isoforms was performed using blood-based DNA samples as described previously (http://adni.loni.usc.edu/wpcontent/uploads/2010/09/ADNI_GeneralProceduresManual.pdf). All subjects underwent whole genome sequencing and these data provided *AQP4* genotypes. Quality control procedures included minor allele frequency of greater than 5% (Tabangin et al., 2009), Hardy-Weinberg equilibrium ($p < 0.05$) (Hosking et al., 2004), and linkage disequilibrium pruning (Hill and Robertson, 1968). Eighteen *AQP4* SNPs were selected following these procedures. Of these 18 *AQP4* SNPs, 6 were selected that have been implicated by prior publications (in PubMed) as having clinical significance in various diseases states including but not limited to Alzheimer's disease and other neurological diseases. Of the selected SNPs, 2 were intron variants (rs3875089, rs3763040), 1 was a 3 prime UTR variant (rs3763043), and 3 were 2 KB upstream variants (rs2075575, rs151244, rs72878794). Subjects possessing one or 2 copies of the minor allele were classified as "carriers" for the SNP, and those with 2 copies of the major allele as "noncarriers" (Supplementary Methods).

2.3. Neuroimaging analysis

PET imaging of Aβ retention was undertaken using [^{18}F]Florbetapir at baseline and 4 years post-baseline. Image preprocessing was conducted within ADNI and included MRI parcellation and segmentation, MRI-PET co-registration, and uptake value generation. This is described in previous studies (Jagust et al., 2015; Mormino et al., 2008). Within the current investigation, cortical subregional (as defined by Freesurfer) [^{18}F]Florbetapir SUVRs were generated by calculating regional standardized uptake values (combining left and right regions) using cerebellar gray matter uptake as the reference region (Ottoy et al., 2017). Cortical regional

Table 1
Demographic and clinical information at baseline

Demographic & clinical measures	Aβ-positive LMCI or mild AD	Aβ-negative cognitive normal (controls)	Mild cognitive impairment	Late mild cognitive impairment
Number of subjects	100	97	469	244
Age (y), mean (SD)	72.96 (7.60)	72.68 (6.09)	72.40 (7.42)	73.41 (7.32)
Education (y), mean (SD)	16.38 (2.66)	16.90 (2.54)	16 (2.83)	16.07 (2.98)
Gender, no. (%) male	54 (54)	50 (51.5)	227 (59.1)	153 (62.7)
Ethnicity, no (%) Caucasian	94, (94)	91 (93.8)	443 (94.5)	234 (95.9)
APOE ε4 status, no. (%) carriers	76 (76)	22 (22.7)	218 (46.5)	126 (51.6)
Alzheimer's Disease Assessment Scale—13 item score, mean (SD)	24.17 (9.59)	8.94 (4.20)	15.35 (6.65)	17.93 (6.73)
Clinical Dementia Rating Scale score, mean (SD)	0.62 (0.24)	0 (0)	0.50 (0.02)	0.50 (0.03)
Trail Making Test Part B, time to complete (s), mean (SD)	154.57 (82.36)	79.15 (43.73)	108 (59.07)	119.27 (66.20)

Key: SD, standard deviation, Aβ, amyloid-β; LMCI, late mild cognitive impairment; AD, Alzheimer's disease; *APOE*, apolipoprotein, no, number.

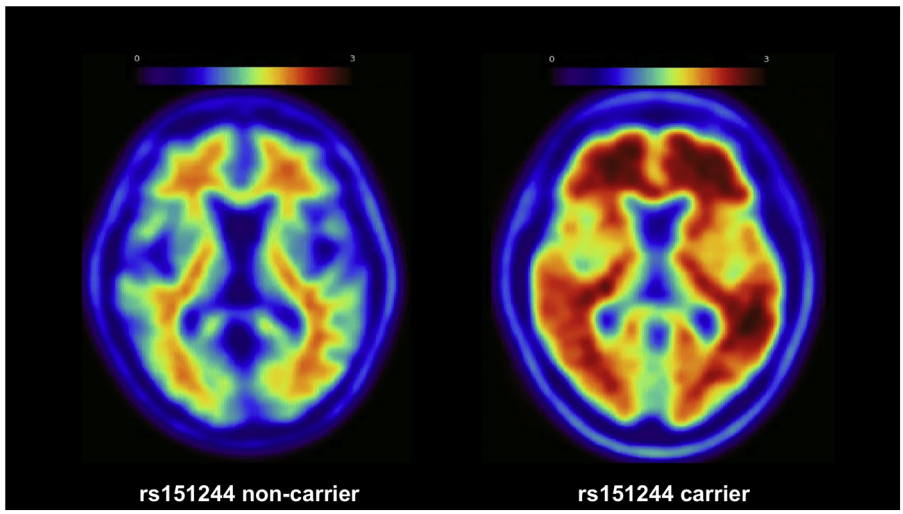

Fig. 1. Composite whole brain images from [^{18}F]Florbetapir PET imaging depicting Aβ uptake for Aβ-positive LMCI patients. The image on the left is from a minor allele noncarrier of the *AQP4* SNP rs151244. The image on the right is from a minor allele carrier of rs151244. The representative images indicate that, relative to the noncarrier, the rs151244 carrier had increased Aβ uptake. Carriers and noncarriers were matched on age, gender, and *APOE* ε4 status. Image details: Axial slice, whole head, non–skull-stripped, z coordinates = 75, mid-striatal transverse section.

SUVRs for the frontal, parietal, and temporal lobes were created by averaging the SUVRs from each brain subregion within their respective lobe (Supplementary Methods). These 3 composite SUVRs were used as the primary Aβ outcome variables for the current investigation (Supplementary Table 1). [^{18}F]Florbetapir PET SUVR images were generated using FSL version 5.0.11 (Supplementary Methods).

2.4. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, Version 25.0) Software (IBM Corp., Armonk, NY). MedCalc Version 18.11.3 (MedCalc Software, Ostend, Belgium), Graphpad Prism Version 7 (Graphpad Software, La Jolla California USA), and SPSS were used to generate graphs.

Three analyses were used to determine the influence of *AQP4* genetic variation on Aβ. First, Pearson chi-square tests were used to determine whether the percentage of minor allele carriers on *AQP4* SNPs was different between Aβ-positive LMCI and AD patients and Aβ-negative cognitive normal participants (controls). Second, in Aβ-positive LMCI and AD patients, multiple linear regression analyses with age, gender, and *APOE* ε4 genotype status as covariates were used to determine associations between minor allele carrier status on *AQP4* SNPs and baseline Aβ accumulation in frontal, parietal, and temporal lobe SUVRs. Finally, for 24 Aβ-positive LMCI patients, multiple linear regression analyses with age, gender, and *APOE* ε4 genotype status as covariates were used to determine associations between minor allele carrier status *AQP4* SNPs and rates of Aβ accumulation over 4 years in frontal, parietal, and temporal lobe SUVRs. SNPs associated with Aβ outcomes in either of these 3 analyses were then investigated for their role in clinical outcomes.

Multivariate Cox proportional hazards regression analyses were used to determine whether minor allele carrier status for *AQP4* SNPs associated with Aβ were predictive of conversion risk from MCI to AD, and from LMCI to AD, which was based on diagnostic classification at baseline compared to the final follow-up visit. Age, gender, and *APOE* ε4 genotype status were selected as covariates. In a subsection of LMCI patients, multiple linear regression analysis with age, gender, education, and *APOE* ε4 genotype status as covariates was used to determine associations between minor allele carrier status on *AQP4* SNPs associated with Aβ and rates of decline over 4 years post-baseline on 3 standardized cognitive measures: the Alzheimer's Disease Assessment Scale–Cognitive Subscale 13 item version (ADAS-Cog 13; global cognition), the CDR scale (dementia severity), and the Trail Making Test Part B test (TMT-B; executive dysfunction). Structural equation modeling was used to determine whether regional [^{18}F]Florbetapir SUVRs associated with *AQP4* SNPs could be also be potential moderators between *AQP4* genetic variation and rates of clinical decline.

Post hoc exploratory analyses were used to determine whether any relationship existed between pathologically or clinically significant *AQP4* SNPs and *APOE* ε4 status. Specifically, Pearson chi-square tests were run in (1) Aβ-positive LMCI and AD patients, (2) Aβ-negative cognitive normal participants (controls), (3) MCI patients, and (4) LMCI patients. This was done to further validate the independent contribution of *AQP4* genetic variation to Aβ neuropathology and cognitive outcomes.

Covariates for all analyses were selected a priori. For all analyses, multiple comparison testing was done using the Benjamini-Hochberg procedure, which controlled the false discovery rate (FDR) at a critical value for an FDR of 0.10. The FDR was controlled at a given level of 10%. The implication is that a rejection of the null hypothesis would occur when the *q*-value of tests was less than or equal to this threshold. (Patel et al., 2016). Given the exploratory nature of the current genetic analysis and approach, a less conservative threshold than the typical 0.05 was adopted in this circumstance. Multiple comparison testing considered *p*-values associated with *AQP4* SNP variables. For all primary regression analyses, significant predictors of Aβ and clinical outcome were only considered if the overall regression model was also statistically significant. $p < 0.05$ was used as the threshold for statistical significance.

3. Results

3.1. Associations between AQP4 SNPs and Aβ neuropathology on PET

In Aβ-positive patients with LMCI or AD (total n = 100, LMCI n = 62, AD n = 38), minor allele carrier status on rs151244 was associated with increased baseline [^{18}F]Florbetapir SUVRs in the parietal (regression model significance = 0.050, β = 0.11, 95% CI: 0.03–0.19, $p = 0.011$; Fig. 1; Table 2) and temporal (regression model

significance = 0.027, β = 0.09, 95% CI: 0.02–0.17, $p = 0.012$; Fig. 1; Table 2) lobes. In addition, for these patients, minor allele carrier status on rs72878794 was associated with decreased baseline [^{18}F] Florbetapir SUVRs in the temporal lobe (regression model significance = 0.025, β = −0.12, 95% CI: −0.21 − −0.03, $p = 0.011$; Fig. 2; Table 2). These associations remained significant following correction for multiple comparisons across the 6 SNPs and 3 cortical areas (Supplementary Table 2). A post hoc analysis determined that only associations between [^{18}F]Florbetapir SUVRs in the temporal lobe and rs151244 (regression model significance = 0.050, β = 0.09, 95% CI: 0.02–0.17, $p = 0.014$; Supplementary Fig. 1) and rs72878794 (regression model significance = 0.046, β = −0.12, 95% CI: −0.21 − −0.03, $p = 0.013$; Supplementary Fig. 2) remained significant after including baseline diagnosis as an additional covariate.

In Aβ-positive patients with LMCI, no associations were found between *AQP4* SNPs and longitudinal rates of Aβ accumulation (Table 2). In addition, the frequency of minor allele carrier status of *AQP4* SNPs was not different between Aβ-positive patients with LMCI or AD compared with Aβ-negative cognitive normal controls (Supplementary Table 3; Fig. 3).

3.2. Association between AQP4 SNPs and risk of progression from MCI to AD

Over a mean period of 59.83 months post-baseline (SD: ±28.15; range: 6–132), 167 of 469 MCI patients subjects converted to AD (35.61%), while 263 remained with a diagnosis of MCI (56.08%) and 39 reverted to a cognitively normal status (8.32%). The mean time until conversion to AD was 36.97 months post-baseline (SD: ±27.98; range 6–132). Over a mean period of 68.85 months post-baseline (SD: ±31.37; range: 6–132), 141 of 244 LMCI patients subjects converted to AD (57.79%), while 91 remained with a diagnosis of MCI (37.3%) and 12 reverted to a cognitively normal status (4.9%). The mean time until conversion to AD was 36.60 months post-baseline (SD: ±29.35; range 6–132).

Minor allele carrier status at rs151244 predicted an increased risk of conversion from MCI to AD (HR = 1.39, 95% CI: 1.02–1.89, $p = 0.036$; Fig. 4A). The increased risk for conversion from MCI to AD was 1.39 times higher for rs151244 carriers. This association remained significant following correction for multiple comparisons (Supplementary Table 4). When including the parietal cortical region SUVR as a covariate, rs151244 was no longer a predictor for conversion risk (HR = 1.40, $p = 0.178$, n = 313). This was also the case when including the temporal cortical region SUVR as a covariate (HR = 1.24, $p = 0.384$, n = 313).

Carrier status for rs151244 was also predictive of an increased risk of conversion from LMCI to AD (HR = 1.59, 95% CI: 1.14–2.22, $p = 0.007$; Fig. 4B). The increased risk of conversion from LMCI to AD was 1.59 times higher for rs151244 carriers. This association remained significant following correction for multiple comparisons (Supplementary Table 5). When including the parietal cortical region SUVR as a covariate, rs151244 was no longer a predictor for conversion risk (HR = 1.21, $p = 0.547$, n = 89). This was also the case when including the temporal cortical region SUVR as a covariate (HR = 1.15, $p = 0.666$, n = 89).

3.3. Association between AQP4 SNPs and rates of cognitive decline

In patients with LMCI, minor allele carrier status on rs151244 was associated with an increased rate of decline on the ADAS-Cog 13 (regression model significance = 0.001, β = 0.21, 95% CI: 0.02–0.41, $p = 0.032$; Table 2) and the TMT-B (regression model significance = 0.002, β = 0.32, 95% CI: 0.05–0.60, $p = 0.022$; Table 2). Of note, higher scores on these measures indicate worse global cognition and greater executive dysfunction, respectively.

Table 2
Associations between *AQP4* SNPs and baseline and longitudinal [^{18}F]Florbetapir and longitudinal clinical outcomes

SNP	Baseline frontal region SUVR β, *p* (regression model *p*)	Baseline parietal region SUVR β, *p* (regression model *p*)	Baseline temporal region SUVR β, *p* (regression model *p*)	4-Year rate of change in the frontal region SUVR β, *p* (regression model *p*)	4-Year rate of change in the parietal region SUVR β, *p* (regression model *p*)	4-Year rate of change in the temporal region SUVR β, *p* (regression model *p*)	4-Year rate of change in the ADAS-Cog 13 β, *p* (regression model *p*)	4-Year rate of change in the CDR β, *p* (regression model *p*)	4-Year rate of change in the TMT-B β, *p* (regression model *p*)
	Cohort: 100 Aβ-positive LMCI or mild AD	Cohort: 100 Aβ-positive LMCI or mild AD	Cohort: 100 Aβ-positive LMCI or mild AD	Cohort: 24 Aβ-positive LMCI	Cohort: 24 Aβ-positive LMCI	Cohort: 24 Aβ-positive LMCI	Cohort: 174 LMCI	Cohort: 178 LMCI	Cohort: 160 LMCI
rs3875089	**−0.05, 0.225 (0.336)**	**−0.05, 0.245 (0.366)**	**−0.04, 0.265 (0.206)**	**−0.01, 0.819 (0.993)**	**−0.02, 0.622 (0.941)**	**−0.002, 0.956 (0.964)**	-	-	-
rs3763040	**0.01, 0.875 (0.543)**	**0.03, 0.553 (0.510)**	**0.02, 0.669 (0.305)**	**−0.03, 0.535 (0.963)**	**−0.03, 0.639 (0.944)**	**−0.05, 0.324 (0.803)**	-	-	-
rs3763043	**−0.01, 0.773 (0.533)**	**−0.01, 0.795 (0.557)**	**−0.01, 0.779 (0.317)**	**−0.03, 0.529 (0.962)**	**−0.03, 0.555 (0.926)**	**−0.04, 0.371 (0.836)**	-	-	-
rs2075575	**−0.05, 0.239 (0.346)**	**−0.04, 0.400 (0.455)**	**−0.04, 0.350 (0.237)**	**−0.03, 0.460 (0.942)**	**−0.03, 0.566 (0.929)**	**−0.04, 0.275 (0.760)**	-	-	-
rs151244	**0.10, 0.020**[a] **(0.073)**	*0.11, 0.011*[a] *(0.050*[a]*)*	*0.09, 0.012*[a] *(0.027*[a]*)*	**−0.01, 0.858 (0.995)**	**−0.003, 0.950 (0.971)**	**−0.004, 0.924 (0.964)**	*0.21, 0.032*[a] *(0.001*[a]*)*	**0.34, 0.055 (<0.001**[a]**)**	*0.32, 0.022*[a] *(0.002*[a]*)*
rs72878794	**−0.13, 0.014**[a] **(0.055)**	**−0.12, 0.018**[a] **(0.072)**	*−0.12, 0.011*[a] *(0.025*[a]*)*	**0.02, 0.600 (0.976)**	**0.03, 0.542 (0.922)**	**0.02, 0.639 (0.935)**	**−0.14, 0.230 (0.002**[a]**)**	**−0.12, 0.588 (<0.001**[a]**)**	**−0.10, 0.576 (0.018**[a]**)**

Italics: Included in final results; Bold: Not included in final results.
Key: SNP, single nucleotide polymorphism; SUVR, standardized uptake value ratio; Aβ, amyloid-β; LMCI, late mild cognitive impairment; AD, Alzheimer's disease; ADAS-Cog 13, Alzheimer's Disease Assessment Scale Cognitive Subscale 13 Item Version; CDR, Clinical Dementia Rating Scale, TMT-B = Trail Making Test Part B; p = p-value.
[a] Significant at $p < 0.05$.

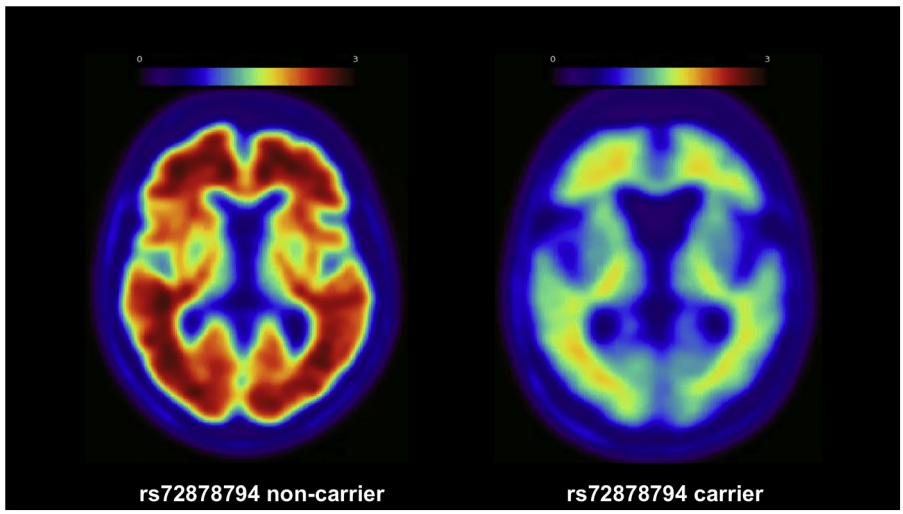

Fig. 2. Composite whole brain images from [^{18}F]Florbetapir PET imaging depicting Aβ uptake for Aβ-positive LMCI patients. The image on the left is from a minor allele noncarrier of rs72878794. The image on the right is from a minor allele carrier of rs72878794. The representative images indicate that, relative to the noncarrier, the rs72878794 carrier had decreased uptake. Carriers and noncarriers were matched on age, gender, and *APOE* ε4 status. Image details: Axial slice, whole head, non–skull-stripped, z coordinates = 75, mid-striatal transverse section.

These associations remained significant following correction for multiple comparisons (Supplementary Table 6). Structural equation modeling was used to examine the potential role of temporal lobe [^{18}F]Florbetapir SUVRs (associated with rs151244 minor allele carrier status) in moderating relationships between rs151244 and rates of cognitive decline. Based on a significant interaction term between rs151244 and temporal lobe SUVRs (estimate = 2.60, standard error = 1.12, critical ratio = 2.31, $p = 0.021$), temporal region amyloid uptake was identified as a moderator of the relationship between rs151244 and an increased rate of decline on the TMT-B. However, no such moderating effect was observed for the ADAS-Cog 13 using this interaction term (estimate = 0.18, standard error = 0.81, critical ratio = 0.22, $p = 0.825$). Of note, final models included age, gender, education, and *APOE* ε4 genotype status as covariates, in addition to rs151244 SNP status, temporal region amyloid SUVRs, and their interaction.

3.4. Association between AQP4 *SNPs and* APOE *ε4 status*

In Aβ-positive patients with LMCI or mild AD (n = 100), Aβ-negative cognitively normal participants (n = 97), MCI patients (n = 469), and LMCI patients (n = 244), the frequency of minor allele carrier status of *AQP4* SNPs rs72878794 and rs151244 was not different between *APOE* ε4 carriers compared to noncarriers (Supplementary Table 7). Thus, no relationship between *AQP4* SNPs and *APOE* ε4 status was established.

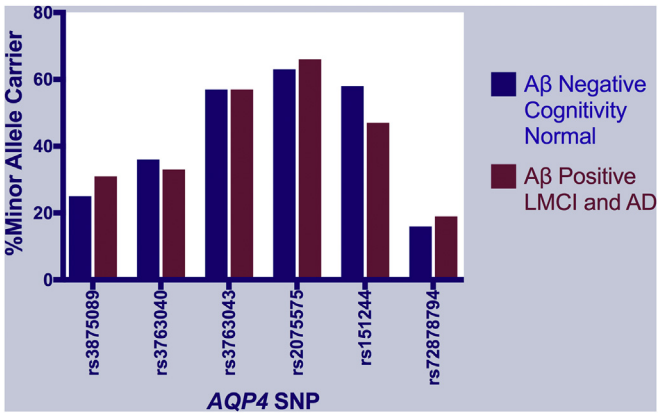

Fig. 3. Frequency distribution depicting percentages of *AQP4* minor allele carriers for Aβ-positive LMCI and AD patients (n = 100) compared to Aβ-negative cognitively normal individuals (n = 97).

4. Discussion

Our findings suggest that 2 *AQP4* SNPs are associated with in vivo brain Aβ pathology in patients on AD spectrum. In patients with LMCI or mild AD with significant amyloid burden, minor allele carrier status on rs72878794 was related to decreased Aβ accumulation in the temporal lobe, whereas rs151244 was related to increased accumulation localized to temporal areas measured by [^{18}F]Florbetapir PET. In regard to the severity of Aβ neuropathology, these 2 SNPs could potentially be genetic protective or risk factors, respectively. We investigated whether these neuropathological associations might also translate into longitudinal clinical deterioration in prodromal AD patients and found that rs151244 was associated with worse cognitive decline, and an increased risk of disease stage progression to AD. The observed influence of *AQP4* genetic variation appeared to be independent of *APOE* ε4 carrier status. To our knowledge, this is the first time that rs72878794 or rs151244 has been implicated in neuropathological and clinical outcomes in MCI or AD.

Prior research indicates that genetic variation in both of these SNPs may exert functional effects on disease phenotype. Specifically, rs72878794 was associated with sudden infant death syndrome (Opdal et al., 2017), and rs151244 with temporal lobe epilepsy (Heuser et al., 2010) and neuromyelitis optica (Mai et al., 2013; Qiu et al., 2015). Two primary isoforms of AQP4 have been identified in the brain with different sites of translation initiation: the M23-AQP4 isoform and the M1-AQP4 isoform, which differ in their structural properties and the ability to form orthogonal arrays of particles which in turn impact AQP4 water permeability. Reductions in the M1 to M23 ratio and associated changes in AQP4 localization in the cortex of AD patients have been observed (Zeppenfeld et al., 2017). Both *AQP4* SNPs from the present study are located in the promotor region around 1,000 bp upstream of exon 1 of AQP4-203 ENST00000440832.7 (M1) (Fig. 5). Promoter regions

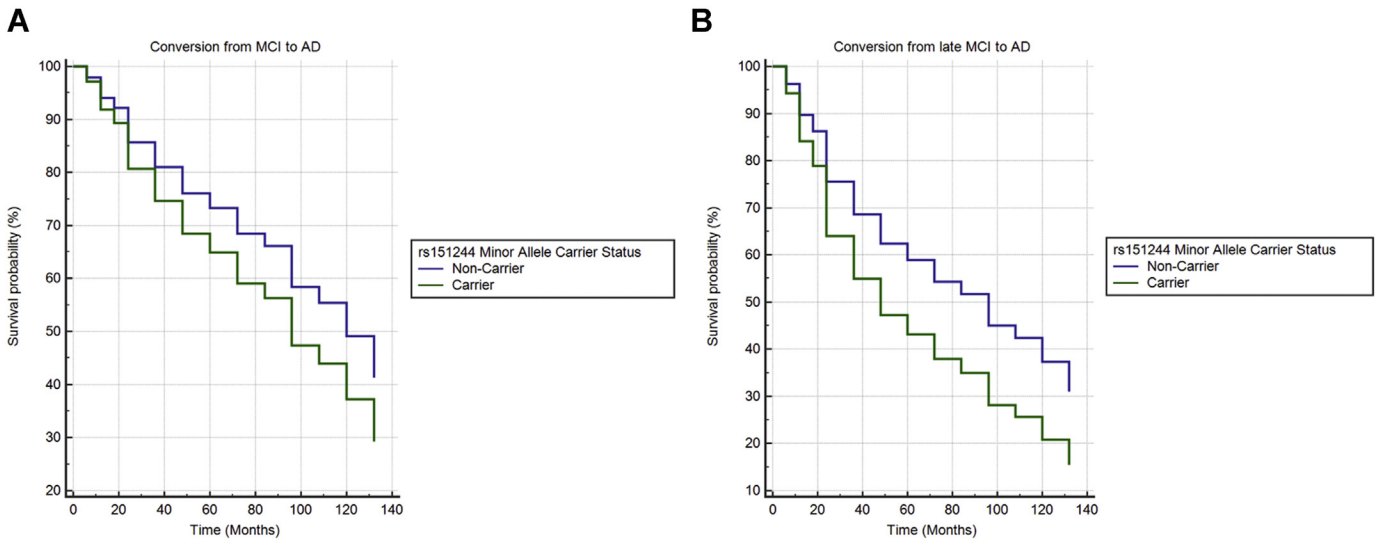

Fig. 4. (A) Cox regression survival curves for conversion from mild cognitive impairment (MCI) to Alzheimer's disease (AD). MCI patients that were minor allele carriers for rs151244 were more likely to convert to AD compared to those that were not minor allele carriers for rs151244. (B) Cox regression survival curves for conversion from late MCI (LMCI) to AD. LMCI patients that were minor allele carriers for rs151244 were more likely to convert to AD compared to LMCI patients that were not minor allele carriers for rs151244.

are acknowledged as functionally significant as they control sites of transcription initiation (Hook-Barnard and Hinton, 2007). The consequences that polymorphisms within promoter regions can have on translation and ultimately clinical phenotype have been demonstrated (Knight et al., 1999; Koslowski et al., 2009). Moreover, the fact that these variations are confined to the promotor region of exon 0 as opposed to exon 1 may have additional significance. Using PCR, a comparatively higher expression of exon 0 containing transcript than exon 1 containing transcript in the human brain has been demonstrated (Umenishi and Verkman, 1998). This relative imbalance may have influenced the current results. Although further investigation using laboratory-based methods is necessary, rs72878794 and rs151244 could play a role in the modulation of M1 and M23 brain expression and impact neurophysiological processes related to the glymphatic system.

Associations between *AQP4* SNPs and [^{18}F]Florbetapir SUVRs, particularly in temporal brain regions, indicate that *AQP4* genetic variation, likely tied to modulation of the glymphatic system, could mediate the response of AQP4 to brain amyloid pathology via brain clearance mechanism functioning. Findings from the present study indicate that this process might occur in either a protective or detrimental manner for patients with advanced symptomatology and amyloid pathology and could result in either an increase or decrease in levels of Aβ clearance and corresponding cortical Aβ reduction or deposition in regions commonly affected in AD. Given that patients in the present study were classified as amyloid positive, potential impacts of rs72878794 on significantly ameliorating levels of Aβ are still uncertain. This is not the first study to demonstrate simultaneous protective and deleterious genetic variations in *AQP4* (Burfeind et al., 2017). It is important to note that this was a correlational study design, and controlled experiments are needed to confirm the directionality of these interrelationships.

When considering mechanistic explanations for these associations, minor allele carrier status at *AQP4* SNP rs151244 could influence the response of the glymphatic system to Aβ by ultimately decreasing perivascular AQP4 localization to astrocytic end feet, which has been previously linked to AD status and pathology (Zeppenfeld et al., 2017) and slowing of Aβ clearance (Kress et al., 2014). Resultant dysfunctional glymphatic system clearance mechanisms could then lead to a worsening of Aβ accumulation in the brain, due to a less efficient pathological protein drainage. Prior

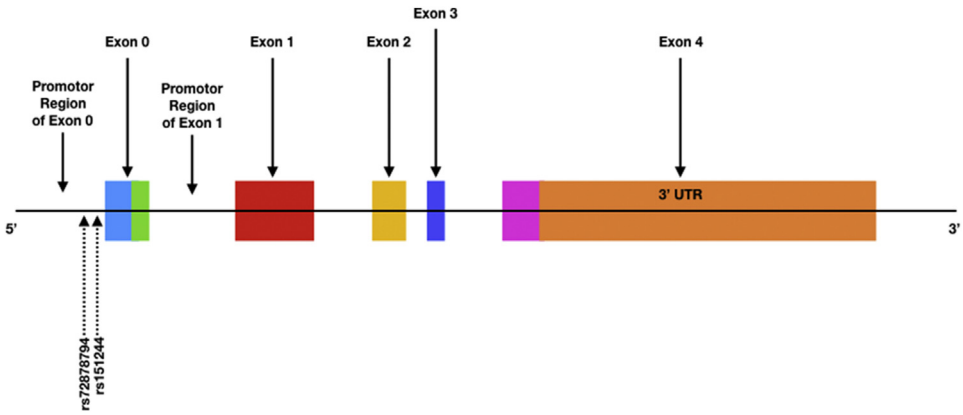

Fig. 5. A schematic of the *AQP4* gene including exons 0–4 and promotor regions. Both rs72878794 and rs151244 were located on the promotor region of exon 0.

research has found that AQP4 expression was highly correlated with Aβ42 plaques. In mature plaques, this expression was minimal internally but more intense toward the plaque boundaries, while in primitive plaques, interior expression was strong (Hoshi et al., 2012). This indicates that AQP4 upregulation may occur in response to more newly formed immature plaques, whereas downregulation might result from mature plaque pathology. Thus, it appears that the population of individuals used in this study with established and later-stage brain amyloidosis might be particularly vulnerable to downregulation of AQP4 expression. Reduced AQP4 expression could then potentially lead to exacerbated impairments in glymphatic system solute clearance. However, it is worth considering the potential role of other AQP4-based aberrant mechanisms such as Ca^{2+} signaling. Genetic alterations at *AQP4* could result in dysregulated signaling, which has been linked to cerebral edema and neuronal apoptosis (Lan et al., 2017). Similar to genetic protective influences in lung cancer triggered by heavy smoking (Blazer and Hernandez, 2006), that of rs72878794 may be stimulated by a significant build-up of cortical Aβ and might employ similar neurophysiological mechanisms as rs151244, but in the opposite direction. These mechanistic explanations are nevertheless speculative, and future work should seek to confirm these. In addition, these effects were noted in a specific cohort of patients with significant clinical symptomatology and neuropathology, and thus, there is also a need to generalize these results across the various stages of the dementia spectrum, including in preclinical stages.

The finding that rs151244 was predictive of an increased risk of phenoconversion from both MCI and LMCI to AD is of clinical significance. This is due to the identification of a new marker to gauge the risk of disease progression in pre-dementia individuals. This adds *AQP4* to a range of genes linked to the risk of conversion from MCI to AD including *SORL1, ACT*, *CHRNA7*, *BDH1*, *ST6GAL1*, *RAB20*, *PDS5B*, *ADARB2*, *SPSB,* and the foremost genetic marker for phenoconversion, the presence of the ε4 allele on the *APOE* gene (Barabash et al., 2009; Lee et al., 2017; Piscopo et al., 2015; Scarabino et al., 2016). In patients with LMCI, rs151244 carrier status was also indicative of greater longitudinal clinical decline on measures of global cognition and executive functioning. While the role of *AQP4* SNPs in cognitive decline in these domains has been established in AD (Burfeind et al., 2017), these findings indicate that this genetic influence on disease prognosis may occur before the onset of AD in patients with a very high risk for developing dementia. In fact, almost 60% of LMCI subjects eventually converted to AD in the present study.

The clinical associations concerning rs151244 are particularly compelling when considering the evidenced role of this SNP in the accumulation of Aβ in the cortex. Evidence from the present study also suggests that amyloid accumulation in the temporal region may have, at least in part, moderated the influence of rs151244 minor allele carrier status on executive function decline. Potential glymphatic system dysfunction associated with this genetic variation likely contributed to a worsening of amyloid pathology and clinical phenotype. This is also consistent with preclinical work showing a link between *AQP4* gene deletion and adverse disease outcomes (Xu et al., 2015) and research demonstrating associations between Aβ neuropathology and cognitive deterioration (Doraiswamy et al., 2012). However, it is important to consider that other biologically based mechanisms that have been tied to the glymphatic system, such as tau pathology (Iliff et al., 2014), may have also driven clinical worsening (Brier et al., 2016), and future studies should focus on moderating effects of various AD-based pathological mechanisms on genetic-clinical correlations. Relationships between *AQP4* variation, Aβ uptake, and clinical outcome indirectly evidence the role of glymphatic system dysfunction in a human model of AD. There remains a need for further research utilizing in vivo biomarkers to confirm this hypothesis.

5. Conclusions

This study has shown that genetic variation at *AQP4* is associated with Aβ uptake as measured by [^{18}F]Florbetapir PET, risk of conversion from MCI to AD, and rates of cognitive decline. Moreover, based on prior research on gene-phenotype associations and sites of gene mapping, the 2 SNPs implicated in this study, rs72878794 and rs151244, are likely to be functionally relevant. An implication of this work is that accurately defining *AQP4* genetically stratified groups might help identify suitable candidates for treatments designed to slow the progression of AD (Citron, 2004). These findings require confirmation in further research studies but are potentially important in the quest of a biomarker to recognize risk of neurodegeneration and track disease progression.

Disclosure statement

Mr. Chandra, Dr. Farrell, Ms. Wilson, Dr. Dervenoulas, and Dr. De Natale report no disclosures. Professor Politis research is supported by Michael J Fox Foundation for Parkinson's Research, Edmond and Lilly Safra Foundation, CHDI Foundation, Glaxo Wellcome R&D, Life Molecular Imaging, Invicro, Curium, Medical Research Council (UK), AVID radiopharmaceuticals, National Institute for Health Research, Alzheimer's Research UK, and European Commission IMI2 fund.

CRediT authorship contribution statement

Avinash Chandra: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Chloe Farrell:** Formal analysis, Writing - review & editing. **Heather Wilson:** Writing - review & editing. **George Dervenoulas:** Writing - review & editing. **Edoardo Rosario De Natale:** Writing - review & editing. **Marios Politis:** Conceptualization, Writing - review & editing, Supervision.

Acknowledgements

Data used for the present study was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; Euroimmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of

Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

The authors report no conflicts of interest. Professor Politis' research is supported by Michael J Fox Foundation for Parkinson's Research, Edmond and Lilly Safra Foundation, CHDI Foundation, Glaxo Wellcome R&D, Life Molecular Imaging, Invicro, Curium, Medical Research Council (UK), AVID radiopharmaceuticals, National Institute for Health Research, Alzheimer's Research UK, and European Commission IMI2 fund.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2020.06.007.

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