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Featured Article

The effects of noncoding aquaporin-4 single-nucleotide polymorphisms on cognition and functional progression of Alzheimer's disease

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Abstract

Introduction: The glymphatic system is a brain-wide perivascular network that facilitates clearance of proteins, including amyloid β, from the brain interstitium through the perivascular exchange of cerebrospinal fluid and interstitial fluid. The astrocytic water channel aquaporin-4 (AQP4) is required for glymphatic system function, and impairment of glymphatic function in the aging brain is associated with altered AQP4 expression and localization. In human cortical tissue, alterations in AQP4 expression and localization are associated with Alzheimer's disease (AD) status and pathology. Although this suggests a potential role for AQP4 in the development or progression of AD, the relationship between of naturally occurring variants in the human *AQP4* gene and cognitive function has not yet been evaluated.

Methods: Using data from several longitudinal aging cohorts, we investigated the association between five *AQP4* single-nucleotide polymorphisms (SNPs) and the rate of cognitive decline in participants with a diagnosis of AD.

Results: None of the five SNPs were associated with different rates of AD diagnosis, age of dementia onset in trial subjects. No association between *AQP4* SNPs with histological measures of AD pathology, including Braak stage or neuritic plaque density was observed. However, *AQP4* SNPs were associated with altered rates of cognitive decline after AD diagnosis, with two SNPS (rs9951307 and rs3875089) associated with slower cognitive decline and two (rs3763040 and rs3763043) associated with more rapid cognitive decline after AD diagnosis.

Discussion: These results provide the first evidence that variations in the *AQP4* gene, whose gene product AQP4 is vital for glymphatic pathway function, may modulate the progression of cognitive decline in AD.

Keywords: Alzheimer's disease; Genetics; Glymphatic system; Aquaporin-4; Amyloid β; Cognitive decline; Cohort study

1. Introduction

Impaired clearance is suggested as a major cause of pathologic accumulation of amyloid β (Aβ) in the brain and subsequent development of sporadic Alzheimer's disease (AD), in contrast to familial AD in which aberrant production of Aβ is the key driver [1–3]. Emerging findings from longitudinal studies utilizing cerebrospinal fluid (CSF)–based or positron emission tomography–based Aβ biomarker measurements suggest that Aβ begins to deposit within the brains of subjects in their 40s and 50s, presumably decades before the onset of clinical symptoms [4]. Recent studies have shown that the rate of Aβ clearance is significantly slowed both with advancing age and with the

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presence of AD [5,6]. However, the mechanism underlying this age-related slowing of Aβ clearance is not yet known.

Recently, a brain-wide perivascular clearance pathway has been described that facilitates the clearance of interstitial solutes and proteins, including Aβ and tau, from the brain [3,7,8]. Termed the "glymphatic" system, this perivascular pathway is dependent upon the astrocytic water channel aquaporin-4 (AQP4) that is localized to perivascular astrocytic endfeet that ensheathe the cerebral vasculature [8]. Genetic deletion of the mouse *Aqp4* gene slows Aβ clearance [8] and accelerates the deposition of Aβ plaques in a transgenic mouse model of AD [9]. In the aging rodent brain, glymphatic function is impaired and interstitial Aβ clearance is slowed, changes that are associated with the loss of perivascular AQP4 localization [10]. In a recently published study carried out in human-autopsy tissue, loss of perivascular AQP4 localization was a strong predictor of AD status and was associated with greater Aβ plaque density and neurofibrillary pathology [11].

Although glymphatic function has only been visualized in human subjects in a single case report [12], these findings suggest that changes in AQP4 expression, localization, or function may alter glymphatic pathway function and contribute to the development of AD or other neurodegenerative conditions. If true, then naturally occurring variants in the human *AQP4* gene may be associated with changes in the development of age-related cognitive decline or AD. Indeed, single-nucleotide polymorphisms (SNPs) in the human *AQP4* gene have been associated with altered clinical outcomes in numerous neurological diseases including sudden infant death syndrome [13], stroke [14], leukoaraiosis [15], and traumatic brain injury (TBI) [16]. However, no studies to date have investigated the role of *AQP4* SNPs in the setting of AD.

We utilized data from seven well-characterized longitudinal cohorts on aging to determine if *AQP4* SNPs were associated with more rapid cognitive or functional decline in older individuals with and without AD. We employed a linear mixed modeling approach to compare the rate of cognitive and functional decline to SNP genotypes using a battery of neuropsychiatric and functional evaluations. Our findings show that *AQP4* SNPs are significantly associated with altered rates of cognitive decline in AD, supporting a role for AQP4 in the development of AD and as a potential therapeutic target in the prevention or treatment of AD.

2. Methods

2.1. Database sources and study population

The protocols for the studies used for analysis were approved by the Institutional Review Board at Oregon Health & Science University, Portland, Oregon, with participants providing written informed consent across all cohorts. We conducted a retrospective cohort study using a number of existing longitudinal natural history studies of cognitive aging available through the Oregon Alzheimer's Disease Center: the Oregon Brain Aging Study (OBAS I and OBAS II; $n = 130$) [17], the Intelligent Systems for Assessment of Aging Changes ($n = 88$) [18], the African-American Dementia and Aging Project ($n = 49$), the Klamath Exceptional Aging Project ($n = 151$) [19], the Oregon Community Brain Donor Program ($n = 86$), Oregon Living Laboratory ($n = 46$), and the Layton Alzheimer's Disease Center's patient registry ($n = 74$) [20], leading to a final starting cohort of 634 subjects.

Different initial entry criteria were present across the seven studies leading to a potential for heterogeneity in the final data set. Although not required for study entry, the data set used for analysis required all subjects to begin their baseline visit as cognitively intact; however, a significant proportion of participants eventually developed cognitive decline and dementia. There were no further restrictions on prior comorbid illnesses beyond individual study criteria.

To appropriately contribute to the longitudinal analysis, all participants were required to have at least one follow-up visit during their study involvement and DNA available for analysis. Although the final visit content varied between studies, a set of predefined cognitive assessments were selected based on their prevalence across the annual and semi-annual assessments of the studies, with patient caregivers participating when applicable. Using the aforementioned cohorts, we derived a comprehensive longitudinal data set including demographic, outcome, and SNP genotype variables.

Of particular interest was whether the *AQP4* SNPs had a larger effect on the rate of decline following a clinical diagnosis of AD. We hypothesized that since individuals with AD have impaired Aβ clearance [6], and AQP4 is important in Aβ clearance [8], changes in AQP4 structure and function due to SNPs may be more evident in individuals with AD than those without AD due to pathologic changes in the brain. Subjects were only considered for categorization as "AD" if their final study visit included a positive diagnosis of dementia. This was deemed a necessary criterion for pathological diagnosis when available so that all AD subjects would end their study involvement as AD. For visits before the last one, subjects were classified as "post-AD" only after two concurrent follow-up visits with a clinical diagnosis of AD based on established criteria [21]. For modeling purposes, this diagnosis was considered persistent for all subsequent time points, even if occasional follow-up visits suggested possible changes in clinical diagnosis. Of the 634 subjects that all entered cognitively intact, 163 had adiagnosis of Alzheimer's dementia and 471 remained cognitively intact at the time of their last evaluation. Frequency of *AQP4* SNPs among non-AD and AD subjects is shown in Supplementary Table 1.

2.2. Neuropathologic analysis

Brain tissue for longitudinal aging study subjects that come to autopsy is maintained within the Oregon Brain Bank. At the time of autopsy, brain tissues from several