2015

Changes in Cerebral Cortical Aquaporin-1 Expression in Multiple Sclerosis

Thao Pham
Portland State University

Changes in Cerebral Cortical Aquaporin-1 Expression in Multiple Sclerosis

By Thao Pham
Dr. Woltjer, Thesis Advisor

Portland State University

Honors Thesis 2015

Abstract

According to the National Multiple Sclerosis (MS) Society, MS, a demyelinating brain disease, affects approximately 2.3 million people worldwide. MS affects African-Americans, Asians and Hispanics, but predominantly Caucasians of northern European ancestry. Women are three times more likely to be diagnosed with this disease, and individuals from populations with low vitamin D levels are more likely as well to develop the disease. Currently, there is no cure for MS, although some moderately effective treatments are available to slow some forms of disease progression. However, our understanding of the cause of MS, which would greatly facilitate treatment, remains very incomplete. One clue may be found in the presence of circulating antibodies to a brain protein, aquaporin-4, in the serum of patient with a form of MS, neuromyelitis optica, which affects chiefly the spinal cord and optic nerves. In this study, immunohistochemistry was used to determine abnormalities of this and a related protein, aquaporin 1, in brain tissues of deceased patients who were diagnosed with MS in comparison to control patients with normal brain tissues. Although MS affects mostly white matter, we found increased expression of aquaporin-1 in gray matter in the vicinity of demyelinated plaques in patients with MS compared to unaffected areas of brain in both MS and control patients. In contrast, expression of aquaporin-4 was relatively unchanged in these areas. We determined that increased aquaporin-1 expression was due to a change in the phenotype of astrocytes, a support cell of the brain, in the cerebral cortex in and near demyelinated areas in MS. This change in astrocytes may underlie some of the brain dysfunction observed in patients with MS.

I. Introduction

Neuromyelitis optica (NO) is a specific type of multiple sclerosis (MS) that targets the spinal cord and the optic nerve, resulting in impaired vision (Lennon, 2004). It is common to misdiagnose NO as multiple sclerosis; however, specific treatments for NO exist and show some efficacy (2004). In general, MS results from damaged or loss of myelin due to an immune attack on oligodendroglia, the myelin-producing cells of the brain (Okahara, 2014) that are found chiefly in the white matter, the major component of the deeper (noncortical) regions of brain tissue. This damage occurs in a patchy distribution, with demyelinated lesions commonly referred to as "plaques." Hence, MS has been primarily considered a white matter disease. However, especially the deeper layers of the cerebral cortex contain lightly myelinated axons, and MS can affect these regions as well. Moreover, the inflammatory cells (macrophages and lymphocytes) that are found in demyelinated white matter plaques of MS may also extend into the cortex and be associated with loss of myelin there.

Aquaporin channels found in a variety of sites in the body, and serve as specialized structures that transport water across a membrane. Aquaporin-4 is the major protein in the central nervous system that permits water flow, and is found mainly in processes of astrocytes. Aquaporins regulate both normal water flow and in response to stress or injuries (Binder, 2013). As a result, a change in aquaporin-4 pathology is likely to result from various brain diseases. Aquaporin-1 plays an important role in water homeostasis in the brain after a traumatic brain injury; these can also function as a CO_2 transporter as well (Young, 2010). A role for aquaporin-1 and -4 in the development of MS and especially NO has been suggested by recent evidence that autoreactive antibodies

to both of these proteins are found in the serum of patients with demyelinating diseases (Tzartos, 2013). Based on these observations, we undertook a study of changes in aquaporin-1 and -4 expression in the brain tissue of patients with MS. We found the most striking changes to occur in aquaporin-1 in affected cortical regions in and near multiple sclerosis plaques. This discovery suggests that changes in aquaporin-expressing astrocytes in gray matter are a previously unrecognized feature of MS that may contribute to tissue dysfunction and symptoms in the disease.

II. Literature Review

Multiple sclerosis (MS) is an inflammatory disease that affects more than 2.1 million people worldwide, most commonly appearing in individuals ranging from ages 20-40 years old. While some scientists try to pin this disease on toxins, viruses and even food, very little is known about the causes of multiple sclerosis. However, patterns such as age, gender, and environmental factors can increase an individual's chances of developing the disease. Treatment often includes antiviral drugs such as beta-interferon, which is effective in decreasing the number of attacks and slowing down progression of the disease. Many patients, however, do not endure therapy since the drugs typically prescribed for MS have severe side effects (NMSS, n.d.).

Currently, multiple sclerosis is classified as a white matter disease. The bundles of axons that make up the white matter in the brain are normally covered in myelin. This white insulating fatty layer of myelin is destroyed in individuals with multiple sclerosis, causing axons to be left exposed and less capable of electrical signal conduction. This

characteristic of multiple sclerosis results in a loss of senses and problems with motor coordination (Wegner, 2009).

While researchers initially looked more at the effects of multiple sclerosis on white matter and how parts of the brain work together, focus has recently shifted to the effects on the gray matter and how this disease affects thinking and learning over time. Little research has been done on the impact of gray matter in multiple sclerosis due to the ease of studying white matter, where lesions are most commonly found and are obvious as areas of white matter discoloration. Scientists are able to observe degradation in myelin of multiple sclerosis patients by examining samples under a microscope and identifying pathologic features such as demyelination, the presence of inflammatory cells, and, in severe disease, axonal damage. However, there is growing appreciation for involvement of gray matter as well, that over time may cause functional losses manifest as loss of cognitive function (Binder, 2013).

I am interested in exploring this topic because since working in a neuropathology lab, I get the opportunity to observe different pathology of various brain diseases. By looking at the pathology of an individual's brain, one can make a fairly good prediction about a patient's symptoms and vice versa. By determining whether or not MS has an effect on the gray matter cortical region of the brain, new treatments can be discovered to improve cognitive outcomes in patients with long-standing disease. In addition, I am interested in promoting a broader consideration of the effects of MS on both white and gray matter in the brain and the implications of discoveries in this area on understanding the root causes and consequences of MS.

III. Methods

Brain tissue from patients who had died with MS was obtained by the Oregon Brain Bank after consent was obtained from the next of kin to the deceased. Tissues were fixed for at least 10 days in neutral buffered formalin, dissected, and demyelinated plaques were grossly identified. Affected areas were embedded into paraffin cassette blocks. The blocks were then cut 7 micrometer thick, applied to a microscope slide, and left overnight to dry. Using a modification of the Abcam immunohistochemistry protocol. 20 different, variably affected brain regions from different patients were stained using immunohistochemistry for aquaporin-1 and -4, as described in more detail below.

On the first day, the slides were placed into a 60^0 Celsius oven for one hour to melt the paraffin, and then put into 6 washes of xylenes, and 2 washes of ethanol to remove the rest of the paraffin and permit tissue proteins to be exposed to solutions subsequently. The slides were then placed into 95% formic acid solution for five minutes to further enhance tissue protein exposure, then transferred to running water for another five minutes to wash off remaining formic acid. Afterwards, the slides were set into citric acid buffer that was already warmed and heated water bath for 45 minutes so that tissue antigens could be recovered. The slides were taken out to cool for then minutes, then dipped ten times in cold water before placing in phosphate buffer saline (PBS) for five minutes. They were then transferred into a 3% milk PBS solution for ten minutes to allow for blocking to prevent non-specific antibody binding. Antibodies dilutions were made in 3% milk PBS solutions; anti-aquaporin-1 was diluted 1:500 and anti-aquaporin-4 was diluted to 1:1000 according to previous determinations of optimal binding and signal

generation. The dilutions were applied on each designated slide and allowed to incubate in 0^0 C overnight for the antibodies to reach equilibrium binding to proteins in the tissues.

The next day the slides were taken out, washed in PBS, and then placed back into 3% milk in PBS blocking solution. Anti-rabbit secondary antibody solution was prepared in 3% milk in PBS. Secondary antibody solutions were made at a concentration of 1:200 dilution of the manufacturer's stock solution. The secondary antibody was applied on the slides and left to incubate in room temperature for one hour. After the incubation, the slides were first taken out and washed in water, and then in a methanol wash for 10 minutes. The methanol wash was prepared using 8 parts anhydrous methanol, 1 part hydrogen peroxide, and 1 part distilled, deionized water to quench endogenous tissue peroxidases. The slides were then briefly put into water for rehydration and 2 washes of PBS each for two minutes. Meanwhile, a developing solution was made using the standard developer for diaminobenzidine (DAB) brown staining. 50-microliters drops of each A and B solutions in the Vector Laboraties Elite developer kit were used per 5 milliliters of PBS. The developer was then applied on the slides and left to incubate at room temperature for one hour. Afterwards, the slides were taken out and washed with PBS. The brown stain was then made using DAB tablets dissolved in PBS; for every tablet used, 20mL of PBS was used to dissolve it, and 40 microliter of hydrogen peroxide was used to activate the enzymatic activity. The brown developer solution was then applied to the slides and left to incubate for 10 minutes while precipitate formed in areas of aquaporin content. The slides were then washed in water and then put into an automatic stainer to counter stain-using hemotoxylin & eosin. Coverslips were applied on

the slides using glue and finally, each slide was evaluated under the microscope while observations were made and recorded with a Leica microscope camera.

The same cases had a replicated set of slides cut and stained in hematoxalin and eosin (H&E). They were first baked in a 60-degree oven and then depariffinized and then went into an automatic stainer for counter-staining and then were stained with Luxol fast blue (LFB) myelin stain. These slides were then cover slipped and ultimately used to complement the observations that resulted from the immunohistochemistry stains and to compare these with the presence of demyelinating lesions.

IV. Results

H&E/LFB-stained sections were examined to identify the presence of demyelinated plaques, inflammatory cells, and other morphologic features of MS brain tissue. Subsequently, immunohistochemically stained sections were evaluated and the presence of aquaporin-1 and -4 were correlated with other tissue features. We found that normal white matter contained abundant aquaporin-1 and -4 and that these were only modestly decreased in areas of MS plaques (Figure 1). Normal cortex also contained abundant aquaporin-4 and this was not significantly decreased in gray matter affected by MS lesions (Figure 2). The most striking disease-associated changes were found in cerebral cortical expression of aquaporin-1, which was markedly increased in gray matter affected by MS, but essentially absent in normal cortex. Similarly, in control brains of individuals without multiple sclerosis, the cortical region of the brain showed little to no aquaporin-1 protein.

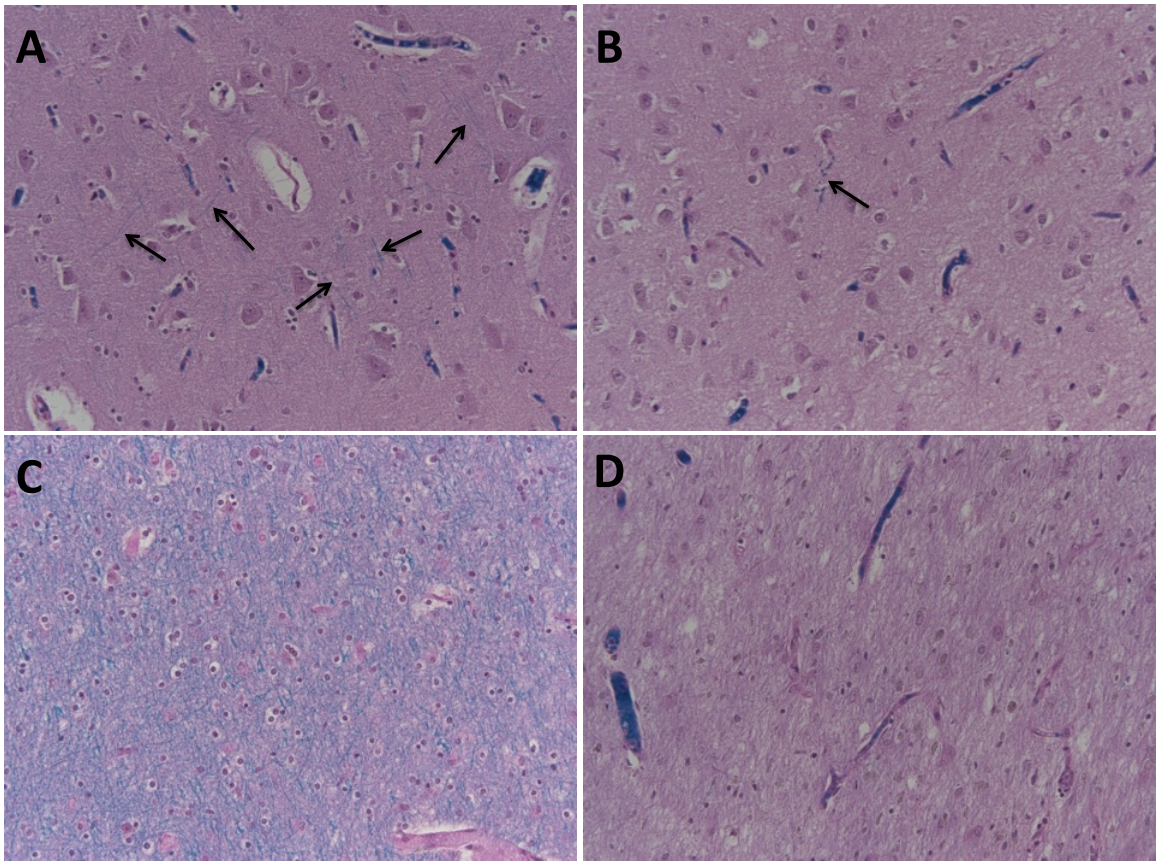

Figure 1. Representative hematoxalin- and eosin-stained sections of human brain tissue affected by multiple sclerosis, with luxol fast blue stain for myelin. (A) Normal cerebral cortex from an area unaffected by MS, showing lightly myelinated axons stained blue (arrows) in a background of intact neurons and glial cells. Larger dark blue structures are blood vessels that contain red blood cells that also are stained by this technique. (B) Cerebral cortex in the area of a demyelinated plaque, with scant myelinated axons with a beaded degenerating morphology (arrow). (C) Normal subcortical white matter, which is more richly and diffusely myelinated. (D) White matter in a demyelinated MS plaque, showing an absence of axonal myelin; residual blood vessels are the only blue-staining structures.

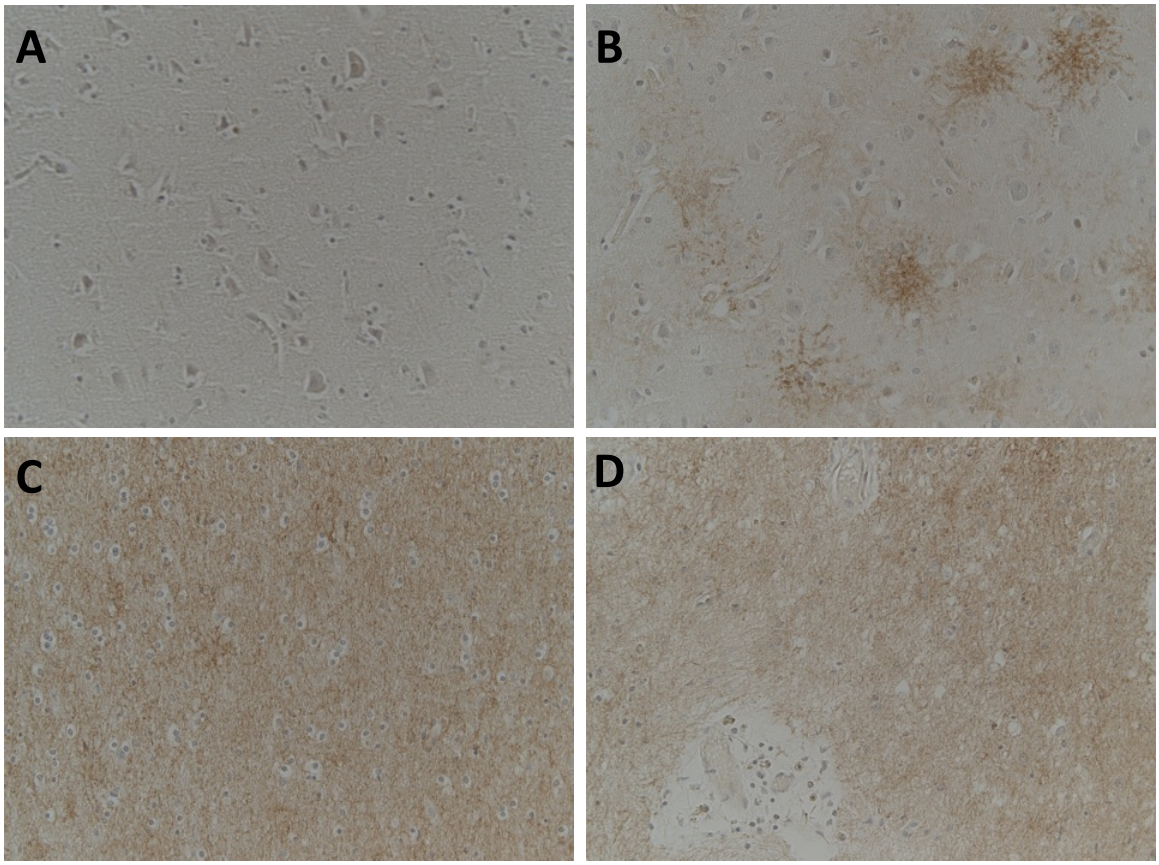

Figure 2. Aquaporin-1 expression in MS as determined by immunohistochemical staining of brain tissue sections with development of signal as brown color and hematoxalin (light blue) nuclear counterstain. Representative areas are depicted. (A) Normal cortex showing neurons and glial cells without significant aquaporin-1 expression. (B) Cortex in an area of demyelination, with astrocyte expression of abnormally high levels of aquaporin-1 in their processes. (C) Normal white matter, with abundant aquaporin-1 expression in glial cells. (D) Demyelinated white matter in an MS plaque, with modest depletion of aquaporin-1 along with other normal white matter components.

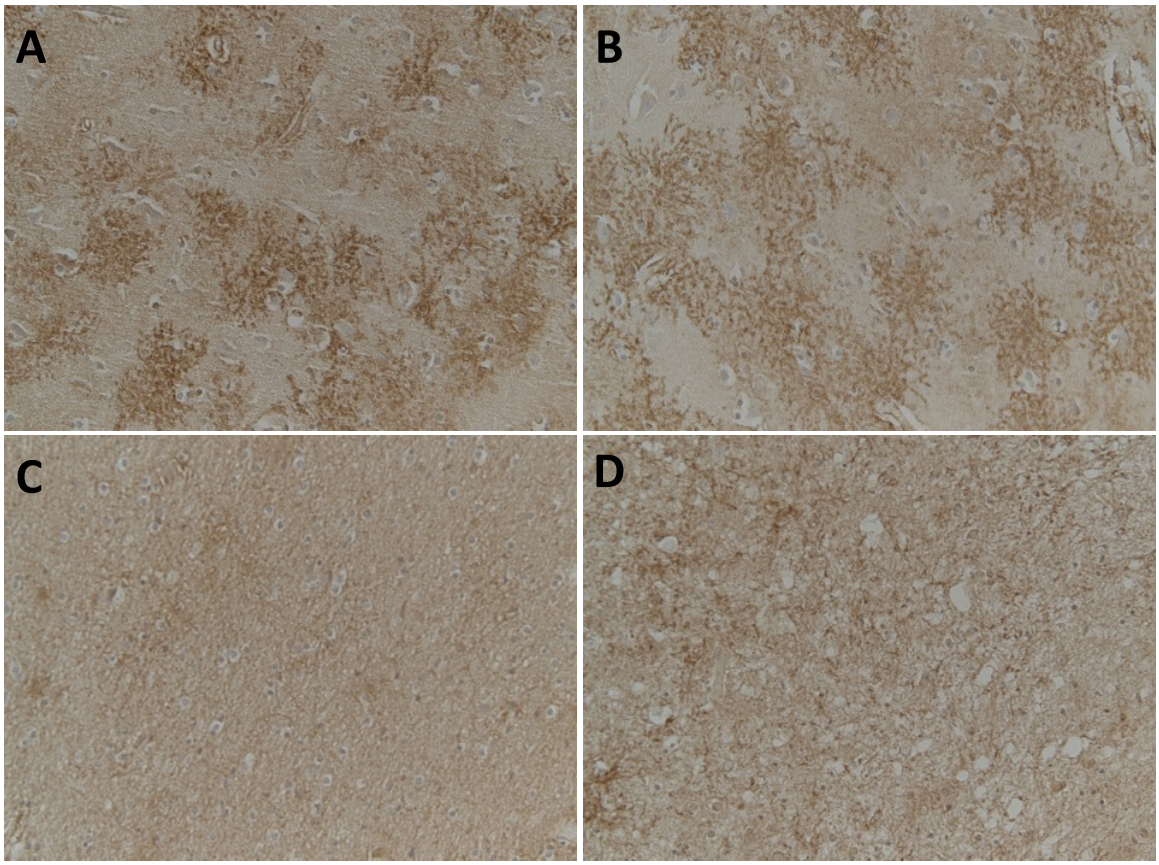

Figure 3. Aquaporin-4 expression in MS as determined by immunohistochemical staining of brain tissue sections with development of signal as brown color and hematoxalin (light blue) nuclear counterstain. Representative areas are depicted. (A) Normal cortex showing aquaporin-4 expression by astrocytes in their processes. (B) Cortex in an area of demyelination, with relatively normal distribution of astrocyte expression of abnormally high levels of aquaporin-4. (C) Normal white matter, with abundant aquaporin-4 expression in glial cells. (D) Demyelinated white matter in an MS plaque, with a subtly more course pattern of aquaporin-4 expression.

V. Discussion

Based on my research, the assumption that multiple sclerosis only affects the white matter in the brain conflicts with the findings that there are pathological changes in the aquaporin-1 protein distribution in the gray matter cortical region in areas near white matter plaques and especially in areas in which tissue damage including inflammatory lesions is adjacent or extends to involve the cerebral cortex. In comparison with control

brains, individuals affected with multiple sclerosis showed increased expression of aquaporin-1 proteins in these affected cortical region of their brains. This is the gray matter of the brain, and could be an explanation to why some individuals with MS demonstrates demented symptoms such as obsession, compulsion, interpersonal sensitivity, anger–hostility, phobic anxiety, paranoid ideation, psychoticism (Sarısoy, n.d.). Although there were changes of aquaporin-1 in the cortical region of the brain, this is not a specific marker for multiple sclerosis based on our observation of similar changes in this protein in other brain diseases (data not shown). However, its expression is a convenient way to identify and map affected cerebral cortical tissue in MS. As noted previously, the presence of autoantibodies against aquaporin-1 or -4, currently serve as a potential helpful marker for chronic demyelinated diseases such as NO (Tzartos, 2013).

An interesting application of this finding could be to compare the IHC stains of aquaporin-1 & 4 with MRI images to determine the scanning parameters. Those scanning parameters, when precisely determined, could be used to optimize scans in living individuals for a more precise diagnosis of multiple sclerosis. More fundamentally, we have identified a novel change in astrocyte phenotype that correlates with cortical damage in MS. Studies of the functional consequences of this change may inform us about additional and unanticipated ways in which MS affected the brain, and point to new disease therapies.

Cited sources

Binder, D. K. Aquaporin-4 water channels and synaptic plasticity in the hippocampus.*Neurochemistry international*, 702-711. Retrieved May 16, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/23684954

Home. (n.d.). *National Multiple Sclerosis Society*. Retrieved May 24, 2014, from http://www.nationalmssociety.org/

Lennon, V., Wingerchuk, D., Kryzer, T., Pittock, S., Lucchinetti, C., Fujihara, K., ... Weinshenker, B. (2004). A serum autoantibody marker of neuromyelitis optica: Distinction from multiple sclerosis. *The Lancet,* 2106-2112.

Okahara, K. Ceramide galactosyltransferase expression is regulated positively by Nkx2.2 and negatively by OLIG2. *glycobiology*.

Sarısoy, G., Terzi, M., Gümüş, K., & Pazvantoğlu, O. (n.d.). Psychiatric symptoms in patients with multiple sclerosis. *General Hospital Psychiatry,* 134-140.

Tzartos, J., Stergiou, C., Kilidireas, K., Zisimopoulou, P., Thomaidis, T., Tzartos, S., & Kira, J. (2013). Anti-Aquaporin-1 Autoantibodies in Patients with Neuromyelitis Optica Spectrum Disorders. *PLoS ONE,* E74773-E74773.

Wegner, C., & Stadelmann, C. (2009). Gray matter pathology and multiple sclerosis.*Current Neurology and Neuroscience Reports*. doi:10.1007/s11910-009-0058-x

Young, H. F. Aquaporin-1–mediated cerebral edema following traumatic brain injury: effects of acidosis and corticosteroid administration. *Journal of Neurosurgery*, 1095-1104.