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Aquaporin 4 Molecular Mimicry and Implications for Neuromyelitis Optica

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Abstract

Neuromyelitis Optica (NMO) is associated with antibodies to aquaporin 4 (AQP4). We hypothesized that antibodies to AQP4 can be triggered by exposure to environmental proteins. We compared human AQP4 to plant and bacterial proteins to investigate the occurrence of significantly similar structures and sequences. High similarity to a known epitope for NMO-IgG, AQP4(207-232), was observed for corn ZmTIP4-1. NMO and non-NMO serum was assessed for reactivity to AQP4(207-232) and the corn peptide. NMO patient serum showed reactivity to both peptides as well as to plant tissue. These findings warrant further investigation into the role of the environment in NMO etiology.

Introduction

Neuromyelitis Optica (NMO, Devic's disease) is a severe relapsing disease that affects gray and white matter in the brain and spinal cord causing inflammatory infiltrates, demyelination, axonal damage and necrosis resulting in sensory loss and paralysis (Jarius, et al., 2008). In three-quarters of cases, NMO is associated with the development of IgG1 antibodies that bind selectively to aquaporin 4 (AQP4) (Dujmovic, et al., 2011; Jarius and Wildemann, 2010; Kim, et al., 2012; Kim, et al., 2010; Lennon, et al., 2005; Petzold, et al., 2010), a water channel belonging to the aquaporin family. AQP4 is the predominant water channel in the CNS and is expressed in astrocytes, ependymocytes and endothelial cells, but

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not in neurons (Nagelhus, et al., 2004; Nesic, et al., 2006). It is highly distributed in the astrocytic foot processes at the blood brain barrier in contact with brain microvessels or subarachnoid space (Nagelhus, et al., 2004). By contributing to regulation of activity-dependent extracellular volume changes that affect solute concentration and electrical activity, it helps to modulate normal neuronal transmission and excitability (Nagelhus, et al., 2004). NMO-IgG1 antibodies are damaging to astrocytes and presumably cause demyelination in the spinal cord and optic nerve (Kinoshita, et al., 2009; Kinoshita, et al., 2010). The reason for the development of these autoantibodies and their precise role in the etiology of this disease is unclear (Roemer, et al., 2007; Verkman, et al., 2011), although it has been recently shown that intracerebral injection of IgG from NMO patients and human complement into mice causes development of pathological features characteristic of NMO (Saadoun, et al., 2010; Verkman, et al., 2011). The pathogenesis of NMO involves binding of IgG1 to AQP4 and complement activation, which leads to loss of AQP4 in lesions through tissue damage (Jarius, et al., 2008; Phuan, et al., 2012). Deposition of immunoglobulins, complement and inflammatory infiltrates cause demyelination and tissue destruction that correlates with regions where AQP4 is expressed. Since IgG1 is produced in peripheral tissues, its access to the extracellular space of the CNS is greater in areas where blood brain barrier permeability is higher or compromised, allowing antibodies to reach their target antigens (Bradl and Lassmann, 2008; Lennon, et al., 2005). AQP4 has 6 membrane spanning α -helices and 2 pre-helices. Several studies have documented the relative reactivity of NMO serum to AQP4 epitopes, and both conformational as well as linear epitopes have been described (Crane, et al., 2011; Graber, et al., 2008; Jarius, et al., 2008; Kampylafka, et al., 2011; Mader, et al., 2010; Petzold, et al., 2010; Tani, et al., 2009). For example, a major epitope for AQP4-IgG has been reported to occur within amino acids 207 to 232 (Crane, et al., 2011; Graber, et al., 2008; Mader, et al., 2010; Tani, et al., 2009). We hypothesize here that pathogenic antibodies to AQP4 may be triggered by exposure to environmental proteins that have similarity to this epitope (loopE:207-232). We compared protein sequences in nature to this selected sequence to determine which may be most likely to cross-react with this epitope.

A recent report suggested that T cells may be important in the pathophysiology of NMO (Kalluri, et al., 2011). NMO T-cell epitopes were characterized and it was demonstrated that a peptide in the N-terminus region of AQP4, namely “22-IMVAFKGVWTQAFWK-36” was likely the core immunogenic T cell epitope (Kalluri, et al., 2011).

Here we describe our bioinformatics comparison of human AQP4 to other proteins in nature to investigate the occurrence of epitopic molecular mimicry. Cross-reactivity of sera from NMO subjects to one of the sequences selected was investigated and is reported here as well.

Methods

Structural neighbor analysis

Structural neighbor searches for primary, secondary and tertiary structure similarities to the reported structure of human AQP4 (Ho, et al., 2009) were done using the National Center for Biotechnology Information (NCBI) Vector Alignment Search Tool (VAST). This tool is a computer algorithm that uses geometric criteria to identify similar protein 3-dimensional structures, including distant homologs that cannot be recognized by sequence comparison alone. VAST was applied comparing AQP4 to every protein in NCBI's Molecular Modeling Database (MMDB) in order to identify similar 3D structures. VAST searches were done using the default parameters. No taxonomical or other restrictions were imposed. As structural data continues to be deposited on MMDB, the results shown in this study reflect the most updated structural data available as of September 2012. The NCBI nonredundant

sequence database is very complete, containing all the organisms represented in GenBank. Patent sequences and environmental samples are omitted (W. Matten, NCBI, personal correspondence, 3/9/2013).

All of the similarity measures for each structural neighbor detected by VAST were listed in a table to facilitate the examination of VAST results. Table 1 includes a four character Protein Data Base (PDB) Number, the PDB chain name and MMDB 3D domain identifier based on geometrical criteria. Table 1 further includes the Aligned Length, the VAST structure-similarity score (related to the number and quality of secondary structure elements superimposed), the VAST p value as a measure of the significance adjusted for the effects of multiple comparisons, the root mean square superposition residual (RMSD), the percent identical residues in the aligned sequence region (%Id), the Loop Hausdorff Metric (a similarity measure that shows how well two secondary structures conform to each other) and the Gapped Score (an algebraic score that uses RMSD, aligned length, and the number of gapped regions in the alignment). A smaller gapped score correlates with greater similarity.

Three dimensional overlays and structural visualization

The sequence alignment was generated with the ClustalW bioinformatics tool (Goujon, et al., 2010; Larkin, et al., 2007). All the structural figures and superimposed structures were produced with PyMOL Molecular Graphics System (2002; DeLano Scientific, San Carlos, CA, USA).

Sequence similarity analysis

Using the human AQP4 protein sequence from amino acids 207-232, we did Basic Local Alignment Search Tool (BLAST) analysis searches at the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The parameters for the searches were set for maximum target sequences at 1000, expect threshold at 1000, word size at 2, Point Accepted Mutation matrix of PAM30 and no adjustments or filters included.

Cross-reactivity study: Patient selection and diagnosis

All subjects were selected from the Multiple Sclerosis Center, at the Sheba Medical Center, Tel Aviv University, Israel. Subjects were selected as part of a larger study investigating protein and protein expression in patients with demyelinating disease and were diagnosed between January 2009 and December 2010. Patients with Multiple Sclerosis (non-NMO disease control group) were diagnosed by the McDonald 2005 criteria (McDonald, et al., 2001; Polman, et al., 2005) and those with NMO were diagnosed by the presence of optic neuritis, myelitis or both, the presence of extensive spinal cord lesion by MRI and having high levels of anti-AQP4 antibodies. Rheumatologic diseases were not excluded in keeping with strong recent evidence that autoimmune conditions like SLE can coexist with NMO and may indicate patient susceptibility to autoimmunity (Jacobi, et al., 2006; Kampylafka, et al., 2012; Mehta, et al., 2008; Nasir, et al., 2009; Polgar, et al., 2011; Wingerchuk and Weinshenker, 2012).

Aliquots of serum collected were transferred to the University of Louisville Department of Neurology for further examination. Written informed consent was obtained from all participants in the study. This study was approved by the Institutional Review Boards of Sheba Medical Center and the University of Louisville.

Cross-reactivity of serum antibodies with human and plant peptides

The cross-reactivity of anti-AQP4 antibodies in human serum with human and plant peptides was determined by ELISA as described previously with modifications (Liu, et al., 2005). Briefly, Nunc Immuno maxiSorp™ plates were coated with 100 µl/well of peptide

(15 µg/ml concentration) at 4°C overnight. After blocking with 10% FCS, 100 µl per well of serum samples of patients with NMO (1:50 dilution) were added and incubated at room temperature for 2 hours. Plates were further incubated for 1 hour with horseradish peroxidase (HRP)-conjugated goat anti-human IgG (1:1,000 dilution), followed by color development with TMB (3, 3', 5, 5'-tetramethylbenzidine) substrate. Results were expressed as optical density (OD) at 450 nm. Peptides used in this study, Human AQP4(207-232) sequence YTGASMNPARSFGPAVIMGNWENHWI and plant ZmTIP4-1(196-221) sequence FTGASMNPARSFGPALATGDWTNHWV, were synthesized commercially by Advanced Automated Peptide Protein Technologies (AAPTEC), Louisville, KY. Statistical analysis was performed using PASW and Excel using Student's t-test as previously reported for ELISA analysis (Liu, et al., 2013) and a p value of 0.05 was considered significant.

In silico validation of peptides Nt-TIPa, TIP and Nod-26

Support Vector Machine (SVM) algorithms have been tested as Bioinformatics tools (Anderson, et al., 2003; Liu, et al., 2006). By using a SVM classificatory binding method, the sequences obtained from BLAST were tested against our experimentally validated sequences from AQP4 and Zm-TIP4-1. Sequences 26 amino acids long, AQP4(207-232) and ZmTIP4-1(196-221), were taken as the positive class values for this analysis. Eleven random 26 amino acid sequences were generated and were given a negative class value. Both groups were weighted based on their side-chains' pKas and charge at physiological pH, for each of their amino acid sequences. By using gist-train-svm (Pavlidis, et al., 2004) with its default settings, a SVM was trained with these positive and negative classes. Following the training using the above parameters, the SVM was tested with corresponding similar sequences of proteins *Nicotiana tabacum* (tobacco, Nt-TIPa), *Solanum lycopersicum* (tomato, TIP), and *Glycine max* (soybean, Nod-26) and six randomly created amino acid sequences.

Immunohistochemistry

Paraffin-embedded sections were de-paraffinized and standard immunohistochemistry procedures were followed as per the ABC kit (Vector Laboratories). Briefly, following rehydration and blocking (30 min) in normal goat serum, sections were incubated in NMO serum (1:100) or buffer alone overnight at 4 degrees C. Sections were washed and incubated in anti-human IgG biotinylated secondary antibody for one hour at room temperature. Sections were washed and developed using ABC reagent for 30 minutes followed by 5 minutes in the diaminobenzidine reagent.

Results

Structurally similar proteins

Proteins showing highly similar secondary and tertiary structures were determined (total of 148 similar structures) and were rank ordered based on the VAST p value. AQP4 and its top 9 structural neighbors are listed in Table 1. All proteins are identified by their unique four character Protein Data Base (PDB) number as well as protein name in Table 1.

The overall structure of 3GD8 (human Aqp4) with the transmembrane alpha helical regions and extracellular and intracellular loops is shown in Figure 1. Structural overlays with 1LDF (E. coli GlpF), 1Z98 (Spinach Sosp2) and 3NK5 (E. coli AqpZ) highlight their overall highly similar structures. The loop E region of interest (AQP4 amino acids 207-232) is shown in Figure 2 along with the superimposition of this region for 3GD8 (human AQP4) with 1LDF (GLPF), 1Z98 (Sosp2) and 3NK5 (AqpZ). From this comparison, it appears that E. coli GLPF is less similar to the human AQP4(207-232) than Sosp2 and AqpZ.

Sequence similarities of AQP4(207-232) with plant and bacterial proteins

The sequences with the highest rankings among statistically significant sequences to human AQP4(207-232) were notably observed in plant aquaporins belonging to the Plasma Intrinsic Protein (PIP), Tonoplastic Intrinsic Protein (TIP) and Nodulin 26-like Membrane Intrinsic Protein (NIP) families. Representative matches are summarized in Table 2. Interestingly, other plant aquaporins such as Small Intrinsic Proteins (SIP) and X Intrinsic Protein (XIP) did not appear in the list of proteins showing similarity to this epitope.

We also investigated the occurrence of mimotopes in nature to the reported T cell epitope of human AQP4 (22-IMVAFKGVWTQAFWK-36). Our bioinformatics analysis and data mining revealed that this sequence is not conserved across species, making other aquaporins in nature unlikely mimotopes. Instead of other aquaporin matches, interestingly, we identified interesting and significant cross-kingdom similarities to Parsnip Yellow Fleck Virus 26 kD coat protein (NP_619734.1) and *Medicago truncatula* Serpin-ZX (AES78897) protein as shown in Figure 3.

Antibodies to AQP4, AQP4(207-232) and ZmTIP4-1 (196-221) in NMO patient serum

Serum collected from 8 confirmed NMO patients, one probable NMO patient, and 9 non-NMO controls was evaluated for anti-AQP4 antibodies (Table 3). All confirmed NMO patients showed positive seroreactivity for anti-AQP4 antibodies. The probable NMO patient showed positive immunoreactivity for anti-AQP4 antibodies. All non-NMO control subjects were seronegative for anti-AQP4 antibodies.

Serum ELISAs for AQP4(207-232 and ZmTIP4-1(196-221) sequences (selected from the top sequence homology candidates shown in Table 2) were done for all 18 serum samples. Higher serum reactivity was observed in NMO patient serum samples against AQP4(207-232) and ZmTIP4-1(196-221) compared to non-NMO control subjects ($p < 0.05$). These results are graphically represented in Figure 4 (top). There was a correlation between reactivity of NMO patient serum to human AQP4 and plant TIP4-1, although there was a stronger reactivity, as expected, to the human AQP4 peptide (Figure 4, bottom).

Based on the ELISA results for AQP4(207-232) and ZmTIP4-1(196-221), we did an in-silico analysis of the other sequences identified in Table 2 to simulate their likelihood to produce similar cross-reactivity. The results of our in silico analysis using support vector machine regression (SVM) algorithms classified *Nicotiana tabacum* (Nt-TIPa), *Solanum lycopersicum* (TIP), and *Glycine max* (Nod-26) as positive (discriminant positive values of 0.53, 0.74, and 0.68 respectively) while randomly generated sequences were classified as negative. The results of the SVM computational analysis suggest that these three sequences are also likely to cross-react with the NMO serum as seen with AQP4(207-232) and ZmTIP4-1(196-221).

To further confirm that NMO serum can cross react with corn aquaporin, we performed immunohistochemistry, where we observed that the serum from NMO patients reacted with corn tissue sections. The localization of the staining appeared to be primarily membranous in corn stem sections (Figure 5).

Discussion

Our results show that several proteins in nature have a remarkable similarity in sequence and structure to human AQP4. We also demonstrate that NMO patient serum cross-reacts with a sequence found in plant aquaporins that was similar to a human epitopic sequence. This reactivity was significantly higher in NMO patient serum when compared to non-NMO controls.

NMO is an autoimmune condition seen in both men and women, although the relapsing or familial forms of this condition are more common in women than men (5:1); the median age of onset is in the late 30's which is later than for MS (Kim, et al., 2012; Kim, et al., 2010). There is a low prevalence of NMO in Caucasians and a high prevalence in Asian, Hispanic and African populations (Jacob, et al., 2007; Mandler, 2006; Nandhagopal, et al., 2010).

Molecular mimicry is a mechanism by which exogenous agents, including plant, bacterial and viral proteins, may trigger immune responses against self or non-self antigens (Agris, et al., 1990; Bahmanyar, et al., 1987; Bullard-Dillard, et al., 1992; Friedland, et al., 2008; Kakalacheva and Lunemann, 2011; Srinivasappa, et al., 1986). This is not surprising given the fact that protein families with similar structural and functional attributes exist across animal and plant kingdoms. AQP4, a protein member of the Major Intrinsic Protein (MIP) superfamily is present in eukaryotes, bacteria and archaea. Five plant aquaporin families, namely PIP, TIP, NIP, SIP and XIP, have been well studied and characterized structurally and functionally (Baiges, et al., 2002; Johnson, et al., 1990; Maurel, 2007). Spinach leaf plasma membranes express two thermally stable members of the aquaporin family, PM28A (SoPIP-2) and PM28C (SoPIP-1), which together constitute 20% of the integral membrane proteins (Johansson, et al., 1998; Kukulski, et al., 2005; Plasencia, et al., 2011). Soybean aquaporins occur in the germinating seeds in addition to root nodules (Dean, et al., 1999; Fleurat-Lessard, et al., 2005; Rivers, et al., 1997). Our observation here that spinach SoPIP-2 and soybean Nodulin-26 are structural neighbors of human AQP4 is of particular interest because this mirrors the dietary demographics of the Asian population, particularly Japanese, in whom NMO constitutes as much as half of demyelinating autoimmune disorders. Asians are major consumers of both soybean and spinach (Lucier, et al., 2004; Schryver, et al., 2007). Plant aquaporins, are highly stable (Plasencia, et al., 2011), further supporting the hypothesis that they may survive harsh food preparation conditions and reach the gastrointestinal (GI) system of humans as intact proteins/peptides where they may be antigenic. In fact human AQP4 itself is a resident water channel in the stomach (Laforenza, 2012). Clearly, the occurrence of plant and bacterial aquaporins in the gut and their role in human health is an area ripe for further investigation. Of particular interest would be conditions such as early infant development, when acid production is low, and in adults that use pharmacological H-2 blockers which result in diminished acidity. It is known that the GI wall is considerably leaky and the immune system continuously samples luminal contents for immunological surveillance; undoubtedly immune reactions to protein in food are not uncommon (Berer, et al., 2011; Fasano, 2011; Ochoa-Reparaz, et al., 2011). For example, neuropathic or ataxic gluten sensitivity is associated with serum antibodies that cross react with synapsin 1, GM1 ganglioside or Purkinje cells (Hadjivassiliou, et al., 2010). Also, ankylosing spondylitis and Crohn's disease have been linked to overgrowth of *Klebsiella* in the gut as a potential causative factor (Rashid and Ebringer, 2012). While the existence of such a mechanism has been suggested for the etiology of NMO (Jarius, et al., 2011) and MS (Kakalacheva and Lunemann, 2011; Varrin-Doyer, et al., 2012), molecular mimicry in the etiology of these diseases has not been documented.

Interestingly, we also noted that a sequence similar to a primary T-cell epitope in NMO occurred in the potentially immunogenic coat protein of the Parsnip Yellow Fleck Virus (PYFV) that infects parsnips, celery, carrots, parsley, cilantro, chervil and dill. This epitope also showed similarity to a sequence present in a serine-protease inhibitor in the legumes *Medicago truncatula*, whose complete genome has been sequenced. It is possible that the etiology of NMO is linked to a combination of exposures to more than one type of sequence, and recent reports suggest that combined with the immunoglobulin response, T cells are an important component of the etiology of NMO.

Our bioinformatics results and initial evaluation of cross-reactivity show that proteins in nature have a remarkable similarity in sequence and structure to human AQP4 and provide a compelling argument for further investigation. Further studies need to be undertaken to fully characterize the possible role of exogenous proteins in the etiology of NMO. It was quite remarkable that we detected cross-reactivity to the plant epitope in our study, and it will be of value to carry out a prospective epidemiological survey to determine the association of diet with incidence of NMO. It is possible that exposure to epitopes that resemble AQP4, from exogenous sources such as plants, plant viruses and bacteria, may play a role in the etiology of NMO. Although to our knowledge this is the first report of NMO serum cross-reacting with plant derived antigens, an association between plant antigens and human autoimmune diseases has been previously suggested for lupus and scleroderma (Agris, et al., 1990; Bullard-Dillard, et al., 1992). Future directions would include rigorous *in vitro* and *in vivo* studies to evaluate the ability and role of these proteins to generate cross-reactive antibodies to AQP4 and consequently contribute to the development, immunity and/or progression of NMO. There is potential for these naturally expressed proteins to be exploited in therapeutic interventions, as well as the development of guidelines for dietary modifications.

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Highlights

- We compared human AQP4 to plant and bacterial proteins to investigate the occurrence of significantly similar structures and sequences.
- High similarity to a known epitope for NMO-IgG, AQP4(207-232), was observed for corn ZmTIP4-1.
- NMO and non-NMO serum was assessed for reactivity to AQP4(207-232) and the corn peptide.
- NMO patient serum showed reactivity to both human and plant peptides as well as plant tissue.
- We conclude NMO patient serum can cross react with plant proteins.

Figure 1.

The overall structure of 3GD8 (human AQP4) is shown in green with the transmembrane alpha helical regions and extracellular and intracellular loops (A), as well as structural overlays with 3NK5 (B) (E. coli AqpZ; magenta); 1LDF (C) (E. coli GLPF; beige), 1Z98 (D) (Spinach Sospip2; blue) to demonstrate their overall highly similar structures.

Figure 2.

Structural neighbor analysis of human AQP4. The upper panel, left shows the entire monomeric structure of AQP4 in green with the peptide region of interest highlighted in gold. Upper panel, center shows a 90 degree rotated view of the same monomer. Upper panel right image shows the human AQP4 loop E region of interest (amino acids 207-232). The lower panel shows the superimposition of this region of interest for 3GD8 (human AQP4) with 1LDF (E. coli GLPF) shown in grey, 1Z98 (Spinach Sopip2) shown in blue and 3NK5 (E. coli AqpZ) shown in magenta. From this comparison, it appears that E. coli GLPF is less similar to the human AQP4 (207-232), structurally, than Sopip2 and AqpZ.

Figure 3.

T cell immunogenic peptide in the N-terminus region of AQP4 representing amino acids 22–36 is non-conserved and was similar to two cross-kingdom proteins as shown, namely, Parsnip Yellow Fleck Virus 26 kD coat protein and Medicago truncatula Serpin-ZX (AES78897) protein.

Figure 4.

Graphical representation of cross-reactivity of NMO and non-NMO serum to peptides from human AQP4 and Zea Mays TIP4-1. Top: Histogram depicting the means and standard errors for antibody titers against human AQP4 and plant TIP4-1 peptides. Y-axis shows O.D. at 450 nm. *, P 0.05; error bars represent standard errors over means. Bottom: Scatter plot showing the relationship between serum reactivity against human AQP4 (O.D.s on X-axis) and plant TIP4-1 (O.D.s on Y-axis). Data is shown for all 9 NMO patients, and regression analysis suggests a relationship amongst the two ($R^2=0.7676$). Slope of 0.6829 (less than 1) suggests overall higher reactivity to human AQP4 for NMO patient serum samples.

Figure 5. Immunohistochemistry shows positive reactivity of NMO serum to corn shoots and leaves (top, 10x). Inset in top panel shows magnified image (40x), indicating that proteins to which the NMO serum reacts are present on plasma membranes. Control experiment (no primary serum) shows lack of signal (bottom, 10x), demonstrating specificity of NMO serum to proteins in the corn tissue.

Table 1

Cross-Kingdom Structural Neighbors

PDB	Protein symbol	Organism	Aligned Length	Score	P Val	RMSD	% Id	LHM	GSP
3GD8	Aqp4	Homo Sapiens	323	n/a	n/a	n/a	100	n/a	n/a
3M9I	Aqp0	Ovis aries	216	14.0	10e-14.9	1.0	48.1	2.4	0.5
1LDF	Glpf	Escherichia coli	213	13.5	10e-12.5	1.8	27.7	5.5	0.9
2F2B	Aqpm	Methanothermobacter marburgensis	208	14.0	10e-14.9	1.5	38.5	4.6	0.7
2W2E	Aqy1	Pichia pastoris	208	13.8	10e-14.1	1.7	32.2	3.7	0.8
3C02	Pfaqp	Plasmodium falciparum	206	13.8	10e-13.2	1.5	24.3	4.6	0.8
1Z98	Sopip2	Spinacia oleracea	205	13.3	10e-11.2	1.4	42.9	5.2	0.7
3NK5	Aqpz	Escherichia coli	203	12.9	10e-12.2	1.8	32.0	NA	NA
3TDP	Hsc	Clostridium difficile	203	11.1	10e-6.2	3.6	10.3	NA	NA
3KLY	Pentameric Formate Channel	Vibrio cholera	163	9.4	10e-4.4	2.9	14.1	10.8	1.8

Human AQP4 (query) along with its top 9 statistically significant structural neighbors, ranked by aligned length, are displayed in table above.

Table 2

Plant Sequences Similar to AQP4(207-232)

Organism	Protein Symbol	Protein Name	Accession Number	Sequence similar to AQP4 LoopE
Homo Sapiens (human)	AQP4	Aquaporin 4	AAH22286	207-YTGASMNPARSFGPAVIMGNWENHWI-232
Zea Mays (maize)	ZmTIP4-1	Tonoplast Intrinsic Protein 4	AAK26772	196-FTGASMNPARSFGPALATGDWTNHWV-221
Nicotiana tabacum (tobacco)	Nt-TIPa	Aquaglyceroporin	CAB40742	188-FSGASMNPARSFGPAFVSGIWDHWV-213
Solanum lycopersicum (tomato)	TIP	Tonoplast Intrinsic Protein	AAU44787	191-FSGGSMNPARSFGPAVVAGDFSQNWV-216
Glycine Max (soybean)	Nod-26	Nodulin-26	NP_001238026	193-FDGASMNPVSEFGPAVVSWTWSNHWV-218
Spinacia oleracea (spinach)	Sopip2	Spinach Aquaporin	AAA99274	216-ITGTGINPARSEGAIVIFNSNKVWDDQWI-244

Table 3

Demographics and clinical characteristics

Subject number	Neurological Diagnosis	AQP4 serology	Age at onset	Sex	Duration of symptoms
1	RR MS	-	31	M	-
2	RR MS	-	31	M	-
3	SP MS	-	45	M	-
4	RR MS	-	36	M	-
5	RR MS	-	45	F	-
6	RR MS	-	39	F	-
7	Not MS	-	49	F	-
8	Probable MS	-	44	F	-
9	Myelitis	-	43	F	-
10	NMO CLL	+	68	M	<5yrs
11	NMO CLL	+	38	F	<5yrs
12	NMO SLE	+	33	F	<5yrs
13	NMO	+	38	F	>5yrs
14	NMO	+	40	F	>5yrs
15	Possible NMO	+	8	F	>5yrs
16	NMO SLE	+	17	F	<5yrs
17	NMO	+	26	F	>5yrs
18	NMO	+	1yr, 8mo	F	<5yrs

NMO, Neuromyelitis Optica; MS, Multiple Sclerosis; SLE, Systemic Lupus Erythematosus; CLL, Chronic Lymphocytic Leukemia; RR, Relapsing Remitting; SP, Secondary Progressive.