Glial Fibrillary Acidic Protein Immunoglobulin G as Biomarker of Autoimmune Astrocytopathy: Analysis of 102 Patients

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Objective: A novel autoimmune central nervous system (CNS) disorder with glial fibrillary acidic protein (GFAP)-IgG as biomarker was recently characterized. Here, 102 patients with GFAP-IgG positivity are described.

Methods: The 102 included patients had: (1) serum, cerebrospinal fluid (CSF), or both that yielded a characteristic astrocytic pattern of mouse tissue immunostaining; (2) confirmation of IgG reactive with specific GFAP isoforms (α, ε, or κ) by cell-based assays; and (3) clinical data available. Control specimens (n = 865) were evaluated by tissue (n = 542) and cell-based (n = 323) assays.

Results: Median symptom onset age was 44 years (range = 8–103), and 54% were women. The predominant phenotype (83 patients; 81%) was inflammation of meninges, brain, spinal cord, or all 3 (meningoencephalomyelitis). Among patients, highest specificity for those phenotypes was observed for CSF testing (94%), and highest sensitivity was for the GFAPα isoform (100%). Rare GFAP-IgG positivity was encountered in serum controls by tissue-based assay (0.5%) or cell-based assay (1.5%), and in CSF controls by cell-based assay (0.9%). Among patients, striking perivascular radial enhancement was found on brain magnetic resonance imaging in 53%. Although cases frequently mimicked vasculitis, angiography was uniformly negative, and spinal imaging frequently demonstrated longitudinally extensive myelitic lesions. Diverse neoplasms encountered were found prospectively in 22%. Ovarian teratoma was most common and was predicted best when both N-methyl-D-aspartate receptor–IgG and aquaporin-4–IgG coexisted (71%). Six patients with prolonged follow-up had brisk corticosteroid response, but required additional immunosuppression to overcome steroid dependency.

Interpretation: GFAPα-IgG, when detected in CSF, is highly specific for an immunotherapy-responsive autoimmune CNS disorder, sometimes with paraneoplastic cause.

We recently reported detailed antibody characterization for a novel autoimmune central nervous system (CNS) disorder specific for glial fibrillary acidic protein (GFAP).1 GFAP-IgG was defined by a characteristic pattern of IgG binding to mouse brain, observed by indirect immunofluorescence assay (IFA) in all patients. Identification of GFAP as the autoantigen was undertaken using Western blot to detect a 50kDa protein band and mass spectrometry of that product, and recapitulating the IFA pattern by applying patient IgG acid-eluted from a replicate.
band to mouse tissue. Antigen specificity was further confirmed by GFAP-transfected HEK293 cell-based assay (CBA). Patient GFAP-IgGs reacted with mature (α, the intermediate filament protein predominant in adult astrocytes) and immature (δ/ε, predominant in neural progenitor cells and immature astrocytes) GFAP isoforms.¹²

The clinical and radiological phenotype of the first 16 patients included inflammatory meningitis, encephalitis, and myelitis, termed autoimmune GFAP astrocytopathy, which resembled a necrotizing meningoencephalitis accompanied by cerebrospinal fluid (CSF) GFAP autoantibodies previously reported in dogs.³

We now report 102 GFAP-IgG–positive patients, to evaluate specificity of serum and CSF testing, define the clinical and radiological phenotype, and report accompanying neoplasms. We also investigated the neurologic and oncologic predictive values of different GFAP isoform IgG specificities (α, ε, and κ), and the significance of coexisting antibodies. We also report immunotherapy responses and outcomes.

Subjects and Methods

Patients

Mayo Clinic’s institutional review board approved the study. Included patients (102 evaluated from January 1, 2000 until February 28, 2016) had: (1) serum, CSF, or both revealing the characteristic GFAP-IgG pattern of staining by our standard IFA, in which a composite of mouse brain, kidney, and gut was utilized¹; (2) GFAP specificity confirmed by CBAs; and (3) clinical data available. All patients were evaluated serologically in the Mayo Clinic Neuroimmunology Laboratory; clinical evaluations occurred at Mayo Clinic (detailed, n = 38) or elsewhere (limited data, n = 64).

Review of our 20-year clinical laboratory archive revealed 874 patients in whom the characteristic GFAP-IgG tissue IFA pattern had been detected in serum, CSF, or both (approximately 44 cases per year). At the time of writing, 1 new patient per week is identified in our laboratory (compared to 3/wk with N-methyl-D-aspartate receptor [NMDA-R] encephalitis, and 7/wk with aquaporin-4 [AQP4] autoimmunity).

Controls

Control specimens (n = 865 total) were evaluated by IFA (n = 542) or CBA (n = 323 total).

IFA controls were: (1) 205 serums, from 100 Biobank healthy donors, 35 patients with hypergammaglobulinemia by serum protein electrophoresis (no clinical data available), 35 patients with systemic lupus erythematosus without neurological complications, and 35 pediatric patients with miscellaneous nonautoimmune neurological disorders; and (2) 118 CSF specimens, from 26 adult patients with normal pressure hydrocephalus, 50 pediatric patients with miscellaneous nonautoimmune neurological disorders, 29 adult patients with diverse seronegative demyelinating diseases (multiple sclerosis, 17; clinically isolated syndromes, 8; other, 4), all 10 patients diagnosed with NMDA-R encephalitis (2015–2016), and all 3 patients with AQP4-IgG detected in CSF (2015–2016).

Assays
Substrates for tissue-based IFA were 4μm cryosections of adult mouse tissue composite (cerebellum, midbrain, cerebral cortex, striatum and hippocampus, kidney and stomach)¹ and for CBA were stable clones of HEK293 cells transfected with plasmid from OriGene (Rockville, MD), encoding a single GFAP Homo sapiens transcript variant (variant 1 [RG204548; pCMV6-AC-GFAP-z-GFP], variant 2 [RG225707; pCMV6-AC-GFAP-ε-GFP], or variant 3 [RG234093, pCMV6-AC-GFAP-k-GFP]). Cells were plated in 8-well poly-D-lysine–coated chamber slides (Corning, Corning, NY), fixed (4% parafomaldehyde, 15 minutes), and permeabilized (0.2% Triton-X-100, 10 minutes). Normal goat serum (10%) was applied to block nonspecific IgG binding. After exposing to patient serum (1:200 dilution) or CSF (1:4) for 45 minutes at ambient temperature, cells were washed in phosphate-buffered saline (PBS), then exposed to tetramethylrhodamine isothiocyanate–conjugated goat antihuman IgG (1:200) for 45 minutes, washed in PBS, and mounted in Prolong Gold antifade reagent containing 4,6-diamidino-2-phenylindole (Molecular Probes, Eugene, OR). Normal values for tissue IFAs were: serum, <1:120; CSF, <1:2. Coexisting IgG neural autoantibodies were detected as described previously.³

Statistical Methods

Summary statistics were reported as median (range, minimum–maximum) for continuous variables and as frequencies and
percentages for categorical variables. Wilcoxon rank sum test or Fisher exact test were used for comparison as appropriate. Analyses were performed using JMP 8.0 software (SAS Institute, Cary, NC).

Results

Antibody Test Results among Controls

All 105 control CSF specimens were negative by IFA, although 1 of 118 was positive by GFAP-IgG (0.9%, from an AQP4-IgG–positive patient with myelitis). Serums from 2 of 437 controls had the GFAP antibody staining pattern (faint) on tissue IFA (0.5%, 1 healthy, and 1 with polyclonal hypergammaglobulinemia), but both were CBA negative. Three of 205 control serums were GFAP-IgG positive by CBA (1.5%, all healthy adults), but were IFA negative. IgG in those sera bound to GFAPa and GFAPb isoforms, 1 of which additionally bound to GFAPc.

Clinical Findings

The demographics, and clinical, CSF, and serologic findings of the 102 included patients are summarized in Tables 1 and 2 (16 reported previously). The predominant clinical syndrome in 83 patients (81%) was 1 or more of meningitis, encephalitis, and myelitis (meningoencephalomyelitis, or limited forms, referred to from hereon as meningoencephalomyelitis). All but 4 patients of 64 with GFAP-IgG positivity in CSF had this phenotype (specificity = 94%). Of the 19 patients without meningoencephalitis, 15 (79%) had GFAP-IgG detected in serum only, although only 1 of these 15 patients had CSF submitted for evaluation.

In CSF, 88% of patients had marked elevation of white cells (median number = 78/µl, range = 13–550), 83% had elevated protein (median = 80mg/dl, range = 44–205), and 54% had elevated CSF-exclusive oligoclonal band numbers (see Table 2). GFAP-IgG isoform

<table>
<thead>
<tr>
<th>Clinical Syndrome</th>
<th>Patients, No. (%)</th>
<th>Serum GFAP-IgG, No. (%)</th>
<th>CSF GFAP-IgG, No. (%)</th>
<th>Coexisting Serum or CSF AQP4-IgG, No. (%)</th>
<th>Coexisting CSF NMDA-R-IgG, No. (%)</th>
<th>Head MRI Radial Enhancement Pattern, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalitis</td>
<td>43 (42)</td>
<td>18 of 33 (55)</td>
<td>34 of 36 (94)</td>
<td>7 (16)</td>
<td>19 (44)</td>
<td>5 of 7 (71)</td>
</tr>
<tr>
<td>Meningoencephalitis</td>
<td>13 (12.5)</td>
<td>7 of 12 (58)</td>
<td>10 of 11 (91)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>6 of 8 (75)</td>
</tr>
<tr>
<td>Myelitib</td>
<td>11 (10.5)</td>
<td>7 of 8 (88)</td>
<td>5 of 5 (100)</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>1 of 3 (33)</td>
</tr>
<tr>
<td>Ependymitis</td>
<td>8 (8)</td>
<td>5 of 8 (62.5)</td>
<td>4 of 4 (100)</td>
<td>2 (25)</td>
<td>0 (0)</td>
<td>1 of 3 (33)</td>
</tr>
<tr>
<td>Neurorpsa</td>
<td>8 (8)</td>
<td>8 of 8 (100)</td>
<td>1 of 2 (50)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 of 3 (33)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>5 (5)</td>
<td>1 of 2 (50)</td>
<td>4 of 4 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 of 2 (50)</td>
</tr>
<tr>
<td>Ataxia</td>
<td>5 (5)</td>
<td>4 of 6 (66)</td>
<td>1 of 1 (100)</td>
<td>0 (0)</td>
<td>1 (20)</td>
<td>0 of 1 (0)</td>
</tr>
<tr>
<td>Meningoencephalomyelitis</td>
<td>3 (3)</td>
<td>2 of 2 (100)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>1 (33)</td>
<td>2 of 2 (100)</td>
</tr>
<tr>
<td>Encephalopathy in context of brain tumors</td>
<td>2 (2)</td>
<td>1 (50)</td>
<td>1 of 1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 of 2 (0)</td>
</tr>
<tr>
<td>Myasthenia gravis, AChR Ab positive</td>
<td>1 (1)</td>
<td>1 (100)</td>
<td>N/A</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>1 (1)</td>
<td>1 (100)</td>
<td>N/A</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dementia</td>
<td>1 (1)</td>
<td>1 (100)</td>
<td>N/A</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dysautonomia</td>
<td>1 (1)</td>
<td>N/A</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

a Three had 1 each of opsoclonus–myoclonus syndrome, brainstem encephalitis, and optic neuritis.

b Two had a history of optic neuritis (1 had neuromyelitis optica).

c Neurorpsa subtypes: large fiber, 5 (1 had coexisting myelitis, 1 had coexisting ataxia); small fiber, 1; acute inflammatory demyelinating polyneuropathy, 1; and cranial neuropathy, 1.

Ab = antibody; AChR = acetylcholine receptor; AQP4 = aquaporin-4; CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; MRI = magnetic resonance imaging; N/A = not available/applicable; NMDA-R = N-methyl-D-aspartate receptor.
specificities, detected in serum, CSF, or both by CBAs, were: α, all patients; ε, 76 of 94 (81%); and κ, 51 of 94 (54%), all of whom were also GFAP-ε-IgG positive; see Table 2 and Fig 1).

**Detailed Neurological and Imaging Findings in 38 Mayo Clinic Patients**

**NEUROLOGICAL FINDINGS.** Thirty-eight patients evaluated at Mayo Clinic had detailed information available (Supplementary Table and Table 3). Five of these patients, evaluated by 2 of us (A.J.A., B.G.W.), were reported in abstract form prior to the discovery of GFAP-IgG.6 Neuropathological findings included chronic inflammation, with microglia abundant, without evidence of vasculitis.

The most common clinical features among the 38 patients were encephalopathy, seizures, psychiatric symptoms, tremor, meningeal symptoms (including headache), myelopathic symptoms (sensory and mild motor), and blurred vision (due to optic disk edema; Fig 2D4). Eight patients (21%) had 1 or more coexisting autoimmune disorders: type 1 diabetes mellitus, 3; rheumatoid arthritis, 2; myasthenia gravis, 2 (1 had coexisting dysautonomia); alopecia, 1; Grave disease, 1; and hypothyroidism, 1.

**MAGNETIC RESONANCE IMAGING FINDINGS.** Head magnetic resonance images for 32 of 38 Mayo Clinic patients were available for review (see Supplementary Table and Figs 2–4), of which 18 of 32 (56%) had T2 hyperintensities (see Fig 4A, C) and 21 of 32 (66%) had gadolinium enhancement. Abnormalities were most notable on T1-weighted postgadolinium sequences. A striking pattern of linear perivascular radial gadolinium enhancement, extending outward from the ventricles, was observed in 17 patients (53%; see Figs 3A–C, 4E, G). Enhancement was sometimes punctate in appearance (see Fig 3C, D). A similar radial enhancement pattern was noted in the cerebellum in 2 patients (see Fig 2D3). Other enhancement patterns observed less frequently included leptomeningeal (7, 22%), serpentine (6, 19%), and ependymal (3, 9%). Magnetic resonance imaging (MRI) diffusion-weighted sequences were normal in all patients evaluated. Seven patients (18%) had normal imaging (clinical phenotypes were dementia, meningitis, cranial neuropathy, encephalitis with optic neuritis, peripheral neuropathy, dysautonomia, and epilepsy).

Among 8 patients with myelitis and MRI spine images available, 6 had longitudinally extensive myelitic abnormalities (≥3 vertebral segments long, 75%), 1 had a short myelitic lesion, and 1 had normal imaging. Two of 6 with longitudinally extensive lesions had AQP4-IgG coexisting. A further 2 patients with encephalitis, but not myelitis, had longitudinally extensive spinal cord lesions. Linear-appearing central canal enhancement (see Fig 2A2, B2, C2) was noted in 21% of spinal cord magnetic resonance images, but more generalized enhancement patterns were encountered also (punctate or patchy; see Fig 2C2, D2).

MRI abnormalities frequently resolved with corticosteroid treatment (see Fig 4). The remaining MRI abnormalities are summarized in the Supplementary Table 1. The radiological appearance prompted consideration of CNS vasculitis (12 patients, 32%). However, magnetic resonance angiograms (n = 12) and digital subtraction cerebral angiograms (n = 6) were normal in all patients tested.

### Table 2. Laboratory and Serologic Findings of 102 GFAP-IgG–Positive Cases

<table>
<thead>
<tr>
<th>Laboratory and Serologic Findings</th>
<th>Patients (%)</th>
<th>Median [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset, yr</td>
<td>44</td>
<td>8–103</td>
</tr>
<tr>
<td>Female sex</td>
<td>55 (54)</td>
<td></td>
</tr>
<tr>
<td><strong>CSF findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated white cell count, &gt;5/μl</td>
<td>45 of 51 (88)</td>
<td>78.5</td>
</tr>
<tr>
<td>Elevated protein, &gt;35mg/dl</td>
<td>30 of 36 (83)</td>
<td>80</td>
</tr>
<tr>
<td>Hypoglycorrhachia, &lt;40mg/dl</td>
<td>4 of 22 (18)</td>
<td>37</td>
</tr>
<tr>
<td>CSF-exclusive oligoclonal bands, ≥4</td>
<td>13 of 24 (54)</td>
<td></td>
</tr>
<tr>
<td><strong>Serological data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFAP IFA positivity</td>
<td>102 (100)</td>
<td></td>
</tr>
<tr>
<td>Serum IFA positive, titer, end-dilution</td>
<td>56 of 83 (67)</td>
<td>1:7,680 [1:120–491,520]</td>
</tr>
<tr>
<td>CSF IFA positive, titer</td>
<td>64 of 68 (94)</td>
<td>1:128</td>
</tr>
<tr>
<td>GFAPε CBA positive</td>
<td>102 (100)</td>
<td></td>
</tr>
<tr>
<td>GFAPε CBA positive</td>
<td>76 of 94 (81)</td>
<td></td>
</tr>
<tr>
<td>GFAPε CBA positive</td>
<td>51 of 94 (54)</td>
<td></td>
</tr>
</tbody>
</table>

*Lympocyte predominant, 94%; monocytic, 6%.*

CBA = cell-based assay; CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; IFA = immunofluorescence assay.
Sensitivity and Specificity for Meningoencephalomyelitis Is Greater for GFAP-IgG CSF Testing

Among the 102 patients, 49 had both serum and CSF testing performed; 45 (92%) were GFAP-IgG positive in CSF, but only 22 of 49 (45%) were positive in serum ($p < 0.0001$). The frequency of meningoencephalomyelitis was more common among those with CSF positivity (60 of 64, 94%) than among those with serum positivity only (23 of 38, 61%; $p < 0.0001$). However, CSF was not available for testing in 34 of those 38 patients.

Meningoencephalomyelitis Diagnosis Is Independent of IFA GFAP-IgG Titer

Patients with high titers (reciprocal of last dilution scored positive) in CSF (tissue IFA values $> 1:32$, $n = 42$) or
serum (>1:7,680, n = 41) were as likely to have meningoencephalomyelitis, or limited forms, as patients with lower values (CSF ≤ 1:32, n = 11; \( p = 1.000 \); serum ≤ 1:7,680, n = 13; \( p = 0.512 \)).

**GFAP Isoform Specificity of IgG Detected by CBA Does Not Predict Tissue IFA Titer, Neurological Phenotype or Cancer Diagnosis**

IgG reactive with the GFAP\(x\) isoform was detected in serum, CSF, or both in all 102 patients (100%). GFAP\(-IgG\) reactive IgG was additionally detected in 76 of 94 patients tested (81%), and GFAP\(x\)-IgG was detected in 51 of 94 patients tested (54%, all of whom were additionally GFAP\(-IgG\) positive).

Serum GFAP-IgG titers by tissue IFA were similar regardless of GFAP isoform (median titer for patients positive only for GFAP\(x\)-IgG, 1:7,680, range = 1:120–491,520; for those with coexisting GFAP\(-IgG\), 1:7,680, range = 1:120–245,760). Median CSF titers by tissue IFA were also similar (within 1 dilution) regardless of GFAP isoform reactivity (median titer, only GFAP\(x\) positive, 1:64, range = 1:32–1,024; coexisting GFAP\(-IgG\), 1:128, range = 1:4–8,192).

The frequency of meningoencephalomyelitis diagnosis (or a limited form) was independent of the antigen isoform (GFAP\(x\)-IgG only positive, 6 of 7 patients, 86%; GFAP\(-IgG\) also positive, 46 of 48 patients, 96%; \( p = 0.477 \)). The frequency of cancer detection was also independent of the antigen isoform (GFAP\(x\)-IgG only positive, 9 of 25, 36%; GFAP\(-IgG\) also positive, 8 of 27, 30%; GFAP\(-IgG\) and GFAP\(x\)-IgG, 18 of 50, 36%).

**Oncologic Findings**

Thirty-five patients of 102 (34%) had neoplasia, and 66% of tumors were detected within 2 years of symptom onset. Twenty-four neoplasms detected in 22 patients subsequent to neurological presentation (median = 0.5 months, range = 0–60) included: ovarian teratoma, 15 (mature, 13; immature, 1; immature and mature, 1 patient with bilateral disease); adenocarcinoma, 3 (1 each of endometrium, esophagus, and kidney), glioma, 2; and 1 each of head and neck squamous cell carcinoma, multiple myeloma, pleomorphic parotid adenoma, and carcinoma. Eighteen historical neoplasms recorded in 14 patients (median = 72 months, range = 3–192) were: prostate adenocarcinoma, 3; Hodgkin lymphoma, 2; lung carcinoma, 2; colon adenocarcinoma, 2; melanoma, 2; and 1 each of mature ovarian teratoma, ovarian adenocarcinoma, nasopharyngeal carcinoma, chronic lymphocytic leukemia, renal cell carcinoma, breast ductal carcinoma, and urothelial bladder carcinoma. Fifteen GFAP-IgG–seropositive patients (serum, 9; CSF, 4; both, 2) without coexisting antibodies had a cancer detected (15%).

Two Mayo patients with gliomas (Patients 13 and 38; see Supplementary Table) had GFAP-IgG detected prior to brain biopsy (choroid plexus glioma, 1; astrocytoma, 1). Patient 13 had GFAP-IgG positivity in serum (GFAP\(x\)-IgG and GFAP\(-IgG\); IFA titer = 1:122,880), but CSF was not evaluated. Patient 38 had GFAP\(x\)-IgG positivity in CSF only (IFA titer = 1:32). Neoplasia seemed causal of encephalopathic symptoms in both.

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**TABLE 3. Detailed Clinical and Treatment Characteristics of 38 Mayo Clinic GFAP-IgG–Positive Cases**

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Patients, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute onset, &lt;8 weeks</td>
<td>27 (71)</td>
</tr>
<tr>
<td>CNS disorder</td>
<td>33 (87)</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>21 of 37 (57)</td>
</tr>
<tr>
<td>Tremor</td>
<td>15 of 37 (41)</td>
</tr>
<tr>
<td>Headache</td>
<td>14 of 36 (39)</td>
</tr>
<tr>
<td>Myelopathic symptoms/signs</td>
<td>9 of 37 (24)</td>
</tr>
<tr>
<td>Other meningeal symptoms/signs</td>
<td>12 of 37 (32)</td>
</tr>
<tr>
<td>Optic disk edema&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 of 37 (32)</td>
</tr>
<tr>
<td>Ataxia</td>
<td>10 of 35 (29)</td>
</tr>
<tr>
<td>Psychiatric symptoms&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 of 35 (29)</td>
</tr>
<tr>
<td>Autonomic dysfunction&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8 of 34 (24)</td>
</tr>
<tr>
<td>Seizures</td>
<td>7 of 37 (19)</td>
</tr>
<tr>
<td>Eye movement disorder</td>
<td>6 of 37 (16)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 of 37 (16)</td>
</tr>
<tr>
<td>Coexisting autoimmune disorder</td>
<td>8 of 37 (22)</td>
</tr>
<tr>
<td>Acute treatment response and outcome</td>
<td></td>
</tr>
<tr>
<td>Improved with corticosteroids</td>
<td>14 of 16 (87.5)</td>
</tr>
<tr>
<td>Improved with IVIG</td>
<td>3 of 4 (75)</td>
</tr>
<tr>
<td>Improved with PLEX</td>
<td>1 of 2 (50)</td>
</tr>
<tr>
<td>Duration of follow-up, mo [range]</td>
<td>22 [0–174]</td>
</tr>
<tr>
<td>Median modified Rankin score [range]</td>
<td>2 [0–6]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Except where stated.

<sup>b</sup>Normal CSF opening pressure in 6 of 8 cases suggested optic disk edema (papillitis); 2 had mildly elevated opening pressures (280 and 298 mm H₂O, respectively; normal ≤ 200).

<sup>c</sup>Depression, 6; anxiety, 2; insomnia, 2; vivid dreams, 1; catatonia, 1.

<sup>d</sup>Orthostasis, 5; gastrointestinal motility disorder, 3; bladder dysfunction, 2; erectile dysfunction, 1.

CNS = central nervous system; GFAP = glial fibrillary acidic protein; IVIG = intravenous immunoglobulin; PLEX = plasma exchange.
Infectious and Immunodeficiency Accompaniments

Eleven of 38 Mayo Clinic patients (29%) had prodromal influenza-like symptoms immediately preceding neurologic presentation, 5 of whom had symptoms of infection (affecting upper respiratory tract, 2; lower respiratory tract, 1 [pneumococcal pneumonia]; urinary tract, 1; prostate, 1).

Two further patients, evaluated outside Mayo Clinic, with dysregulated T-lymphocyte function developed encephalitis. One patient had chronic human immunodeficiency virus (HIV)/acquired immune deficiency syndrome infection. The other patient with melanoma had received ipilimumab (monoclonal antibody antagonist of cytotoxic T-lymphocyte–associated protein 4).

Coexisting Neural Autoantibodies

Forty-one patients had 1 or more coexisting antibodies detected in serum or CSF (40%). NMDA-R-IgG detected in CSF (22 of 102, 22%) was the most common coexisting antibody, and AQP4-IgG was the next most common, detected in serum or CSF, depending on specimen availability, in 10 patients (10%).

Neurological syndromes were: encephalitis in all with both NMDA-R-IgG and AQP4-IgG coexisting; encephalitis (n = 13), encephalomyelitis (n = 1), and meningoencephalomyelitis (n = 1) in those with NMDA-R-IgG alone coexisting; and encephalomyelitis (n = 2) and neuromyelitis optica (NMO; n = 1) in those with AQP4-IgG alone coexisting.

Among the NMDA-R-IgG–positive patients with MRI of the head available, 4 of 4 (100%) had gadolinium enhancement (radial, 3; leptomeningeal and serpentine, 1).

Teratoma was detected in 5 of 7 patients with both NMDA-R-IgG and AQP4-IgG coexisting (71%), 8 of 15 patients with NMDA-R-IgG alone coexisting (53%), and 2 of 3 patients with AQP4-IgG alone coexisting.
(66%; neither patient had CSF available for NMDA-R–IgG testing). Only 1 of 15 teratoma patients (7%) was NMDA-R–IgG and AQP4-IgG negative.

Other coexisting neural antibodies were reactive with: glutamic acid decarboxylase 65, 7 (median value = 0.17nmol/l, range = 0.04–4.16, normal ≤ 0.02); striated muscle antigens, 5 (median value = 1:480, range = 120–61,440, normal ≤ 120); ganglionic acetylcholine receptor, 4 (median value = 0.09nmol/l, range = 0.04–0.22, normal ≤ 0.02); P/Q-type calcium channel, 3 (median value = 0.08nmol/l, range = 0.03–0.11, normal ≤ 0.02); and voltage-gated potassium channel complex, 2 (0.05 and 0.06nmol/l, normal ≤ 0.02; both patients were Lgi1 and CASPR2 antibody negative).

**Treatment Response and Outcome (Mayo Clinic Patients)**

Treatment responses and outcome are summarized in the Supplementary Table and Table 3. Median follow-up duration for the 38 Mayo Clinic patients was 20 months (range = 0–174). Of 26 patients with meningoencephalomyelitis, or limited forms, 13 had a relapsing course, 7 had a monophasic course, and 6 had progressive disease despite treatment.

Long-term (≥24 months) treatment details were available for 9 Mayo Clinic patients. Three of these had coexisting NMDA-R–IgG detected and were treated with intravenous corticosteroids, 3; oral prednisone, 2; plasma exchange, 1; mycophenolate mofetil, 1; and azathioprine, 1. One patient relapsed during steroid tapering 2 months after symptoms onset. All 3 patients were eventually weaned off of steroids successfully without known subsequent relapse.

The remaining 6 Mayo Clinic patients with GFAP-IgG positivity, without coexisting AQP4-IgG or NMDA-R–IgG (median treatment time = 54 months, range = 24–144), all had encephalitis with or without meningeal or myelitic findings. These patients were treated with
intravenous corticosteroids, 6; oral steroids, 6; mycophenolate mofetil, 5; and azathioprine, 2. Relapses occurred in 3 patients not taking a steroid-sparing drug during steroid dose reduction (median 1.5 relapses, range 1–5, occurring at prednisone doses < 20mg per day) and in 3 patients when steroid-sparing immunotherapy was discontinued. Clinical relapses were frequently accompanied by recurrent gadolinium enhancement on MRI and elevated CSF white cell counts, with further remission on restarting steroids (see Fig 4). All 6 patients were in remission on steroid-sparing immunotherapy at last follow-up; 5 had discontinued prednisone.

Two patients required increases in mycophenolate dosing from 2,000mg/day to 2,500 or 3,000mg/day to overcome steroid dependency.

**Discussion**

GFAP antibody, when detected in CSF, unifies a spectrum of immunotherapy-responsive autoimmune inflammatory CNS disorders, termed autoimmune GFAP astrocytopathy, distinct from infectious meningoencephalitis and idiopathic inflammatory CNS disorders such as multiple sclerosis, vasculitis, and sarcoidosis.

Autoimmune GFAP astrocytopathy most frequently presents with subacute onset of memory loss, confusion (with or without psychiatric symptoms), and 1 or more of meningeal symptoms (headache, photophobia, neck stiffness) and myelopathic symptoms (weakness or numbness in extremities), although outright paralysis (a frequent NMO accompaniment) is rare. Neurological examination revealed cognitive impairment, tremor, and optic disk edema. The high frequency of elevated CSF white cell count (88%) is a helpful marker of this condition but can also occur with infectious and neoplastic causes of meningoencephalitis. The presence of CSF GFAP-IgG helps distinguish from these alternative etiologies and alerts the clinician to an immune-mediated, steroid-responsive disorder.

**FIGURE 4:** Evolution of magnetic resonance imaging abnormalities in Patient 19 with relapsing glial fibrillary acidic protein autoimmune meningoencephalitis (see Supplementary Table). Axial fluid inversion recovery images (A-D) and axial T1 postgadolinium images are shown (E-H). At initial presentation, T2-hyperintensity (A) was accompanied by linear radial enhancement (E). These abnormalities receded after initial steroid treatment (B, F) but became prominent again during dose reduction (C, G), receding again after reinitiation of high-dose steroids (D, H). IVIG = intravenous immunoglobulin; IVMP = intravenous methylprednisolone.
A radiological hallmark of autoimmune GFAP astrocytopathy is the striking radial linear periventricular enhancement. A similar radiological pattern has been reported in patients with lymphomatoid granulomatosis, neurosarcoidosis, and CNS vasculitis, including those lacking cerebral infarcts diagnosed with “angiogram negative primary central nervous system vasculitis.”7–11 This “microvascular vasculitis” subtype is known to be steroid responsive, and has a better prognosis than vasculitis in general.12 The authors speculate that some reported cases of microvascular vasculitis, particularly those accompanied by optic disk edema and radial periventricular enhancement, had autoimmune GFAP astrocytopathy.

The most frequent spinal MRI finding of GFAP autoimmunity was a longitudinally extensive T2-hyperintense lesion, which is also typical of AQP4 autoimmunity. All but 3 patients with myelitis were AQP4-IgG CBA negative. Myelitic T2-hyperintense lesions, subtle and hazy, differed slightly in appearance from those typically encountered in AQP4 autoimmune NMO spectrum disorders (NMOSDs).13 The spinal cord enhancement pattern was sometimes distinctive, thin, and linear along the course of the central canal, corresponding to antigen-enriched regions in rodent cord,1 unlike the patchy or ringlike appearance of parenchymal enhancement typical of NMOSDs.13 Central canal enhancement may also rarely occur in spinal cord sarcoidosis but is usually accompanied by linear dorsal subpial enhancement.14 Patients with GFAP mutations (Alexander disease) may also have central spinal cord T2 hyperintensity.15

Positivity for GFAP antibody (sometimes transiently detectable) has been described by other investigators in various disorders (traumatic brain injury, brain tumors, autism, lead-exposed workers, and diabetes).16–19 In all 14 articles we encountered, the specimen tested was serum (not CSF), and GFAP antibody was detected by one assay only (Western blot or enzyme-linked immunosorbent assay) in all but 1. In this current study, we found GFAP-IgG among 0.5 to 1.5% of controls sera, although never by both tissue and cell-based testing, and just once in control CSF. The phenotypes encountered among those with serum positivity were heterogenous. In contrast, GFAP-IgG detection was highly specific for inflammatory meningoencephalomyelitis when screened for in CSF, by tissue IFA, and then confirmed by CBA. Whereas CSF is the preferred specimen type for GFAP-IgG and NMDA-R-IgG testing, serum is preferred for AQP4-IgG testing.20 We had limited availability of CSF specimens paired with serum, where serum was positive only. Prospective work will further evaluate the sensitivity and specificity of serum and CSF testing.

GFAP isoforms α, ε, and κ have identical head and coiled-coil rod domains, but divergent C-terminal tails, and likely differential functions.2 GFAPα is expressed from early development onward but is most abundant in neural progenitor niches in periventricular regions, hippocampus, and central spinal cord.21 GFAPε expression is most abundant in early fetal development, but rapidly diminishes as a proportion of α and ε GFAP isoforms thereafter.22 Epsilon and κ made sense as the most immunogenic and disease-specific isoforms, because of the predominance of patient IgG staining of mouse tissues and MRI abnormalities in neural progenitor cell-rich brain regions (periventricular and dentate gyrus) and central spinal cord.23 However, the highest clinical sensitivity and specificity for CNS autoimmunity was observed for GFAPε-IgG detected in CSF. Being additionally GFAPα-IgG or GFAPk-IgG positive did not improve neurological or oncological predictive values. The paradox of dominant reactivity with GFAPε in isolation (panastrocytic marker) in contrast to the apparently selective binding of patient IgG to neural progenitor cell-rich locations in adult mouse brain (inferring GFAPδ/ε restriction) suggests epitope obscuring.1

GFAP expressed in neoplasm is plausible as immunogen triggering paraneoplastic neurological autoimmunity (25% of patients). In other patients, a parainfectious cause seemed plausible, although laboratory data supporting a specific organism were lacking. Herpes viruses in particular have been implicated.24 Neurological autoimmunity appeared to occur in the setting of immune dysregulation also (1 patient with HIV infection, and another who received a T-regulatory “checkpoint” inhibitor which has autoimmune encephalitis as a known complication).25

GFAP-IgG was sometimes accompanied by coexisting NMDA-R–IgG (15%), AQP4-IgG (3%), or both (7%). The latter 2 antibodies have previously been shown to coexist frequently.26 GFAP and NMDA-R (and likely AQP4) are expressed in teratomas.27,28 We found the predictive value for teratoma was highest (71%) when GFAP-IgG was accompanied by both NMDA-R–IgG and AQP4-IgG. By comparison, the reported association of teratoma with solely NMDA-R–IgG seropositivity is approximately 50%.29 Unlike NMDA-R encephalitis in general, all of our patients with coexisting NMDA-R and GFAP IgGs had gadolinium enhancement on head MRI scans. Although CSF generally gave high specificity for meningoencephalomyelitis, there were occasional exceptions. GFAP-IgG may also arise as the product of an immune response triggered by GFAP expressed in gliomas. GFAPδ expression is a diagnostic marker of spinal cord astrocytoma.30

GFAP is the fourth glial autoantigen with validated clinical utility, the others being AQP4, myelin-
Oligodendrocyte glycoprotein (MOG), and SOX1. AQP4 and MOG are plasma membrane targets for pathogenic IgG accessing the intrathecal compartment. However, like SOX1 (nuclear location), GFAP is an intracellular (cytoplasmic) protein and not accessible to IgG in intact glial cells. It has been shown, in a mouse model of autoimmune GFAP meningoencephalomyelitis, that cytotoxic T cells specific for peptides derived from GFAP are pathogenic. Unlike patients with a well-characterized cytotoxic T-cell–mediated autoimmune disorder, PCA-1 (anti-Yo) autoimmunity, GFAP-autoimmune patients have inflammatory-appearing MRI scans and CSFs, and are exquisitely steroid responsive. The presence of AQP4 and NMDA-R autoimmunity in some, but not all, also raises the possibility of an additional, yet to be discovered, plasma membrane protein-directed IgG initiating a primary inflammatory event, disrupting astrocytic function, and GFAP autoimmunity occurring as a secondary phenomenon. Future immunohistopathological studies should provide insights into disease mechanisms.

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Author Contributions

Conception and design of the study: V.A.L., S.J.P., and A.M. Acquisition and analysis of data: all authors. Drafting the manuscript or figures: E.P.F, S.R.H., and A.M.

Potential Conflicts of Interest

S.R.H., V.A.L, B.F., and A.M. are all named inventors on a patent application filed by Mayo Foundation relating to GFAP antibody.

References
