Peptides From the Variable Domain of Immunoglobulin G as Biomarkers in Chronic Inflammatory Demyelinating Polyradiculoneuropathy

Joris Godelaine, PharmD, Yamini Chitale, MSc, Bart De Moor, PhD, Chantal Mathieu, MD, PhD, Lina Ancheva, PhD, Philip Van Damme, MD, PhD, Kristl G. Claeyys, MD, PhD, Xavier Bossuyt, MD, PhD, Sebastien Carpentier, PhD, and Koen Poesen, PharmD, PhD

Neurol Neuroimmunol Neuroinflamm 2023;10:e200162. doi:10.1212/NXI.0000000000200162

Abstract

Background and Objectives
Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a clinically heterogeneous immune-mediated disease. Diagnostic biomarkers for CIDP are currently lacking. Peptides derived from the variable domain of circulating immunoglobulin G (IgG) have earlier been shown to be shared among patients with the same immunologic disease. Because humoral immune factors are hypothesized to be involved in the pathogenesis of CIDP, we evaluated IgG variable domain–derived peptides as diagnostic biomarkers in CIDP (primary objective) and whether IgG-derived peptides could cluster objective clinical entities in CIDP (secondary objective).

Methods
IgG-derived peptides were determined in prospectively collected sera of patients with CIDP and neurologic controls by means of mass spectrometry. Peptides of interest were selected through statistical analysis in a discovery cohort followed by sequence determination and confirmation. Diagnostic performance was evaluated for individual selected peptides and for a multipepptide model incorporating selected peptides, followed by performance reassessment in a validation cohort. Clustering of patients with CIDP based on IgG-derived peptides was evaluated through unsupervised sparse principal component analysis followed by k-means clustering.

Results
Sixteen peptides originating from the IgG variable domain were selected as candidate biomarkers in a discovery cohort of 44 patients with CIDP and 29 neurologic controls. For all 16 peptides, univariate logistic regressions and ROC curve analysis demonstrated increasing peptide abundances to associate with increased odds for CIDP (area under the curves [AUCs] ranging from 64.6% to 79.6%). When including age and sex in the logistic regression models, this remained the case for 13/16 peptides. A model composed of 5/16 selected peptides showed strong discriminating performance between patients with CIDP and controls (AUC 91.5%; 95% CI 84.6%–98.4%; p < 0.001). In the validation cohort containing 45 patients and 43 controls, 2/16 peptides demonstrated increasing abundances to associate with increased odds for CIDP, while the five-peptide model demonstrated an AUC of 61.2% (95% CI 49.3%–73.2%; p = 0.064). Peptide-based patient clusters did not associate with clinical features.
Glossary

AUC = area under the curve; CDR = complementarity-determining region; CIDP = chronic inflammatory demyelinating polyradiculoneuropathy; DDA = data-dependent acquisition; FR = framework region; IgG = immunoglobulin G; INCAT = Inflammatory Neuropathy Cause and Treatment; IVIg = IV immunoglobulin; MRC = Medical Research Council; MS = mass spectrometric; PLS-DA = partial least-squares discriminant analysis; POI = peptides of interest; sPCA = sparse principal component analysis; VIP = variable importance in projection.

Discussion

IgG variable domain–derived peptides showed a valid source for diagnostic biomarkers in CIDP, albeit with challenges toward replication. Our proof-of-concept findings warrant further study of IgG-derived peptides as biomarkers in more homogeneous cohorts of patients with CIDP and controls.

Classification of Evidence

This study provides Class III evidence that the pattern of serum IgG-derived peptide clusters may help differentiate between patients with CIDP and those with other peripheral neuropathies.

Introduction

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a neuroinflammatory disorder demonstrating autoimmune responses against peripheral nerves. With a reported prevalence of 0.7–10.3 cases per 100,000 people, it is a rare disease that shows considerable variation in clinical phenotype and therapy response among patients. Due to this large clinical heterogeneity, CIDP is often misdiagnosed or underdiagnosed despite diagnostic criteria being available. While highly specific paranodal antibodies have been described in small subsets of patients initially diagnosed with CIDP, biomarkers applicable to the broader population of patients that can aid in diagnosing CIDP are still lacking. Nevertheless, both discovery of paranodal antibodies and earlier reports indicating antibody responses directed against Schwann cells or compact myelin in up to 40% of patients with CIDP suggest circulating antibodies as a candidate source for future discovery of novel CIDP biomarkers. Findings of immunoglobulin G (IgG) deposits on sural nerves or a CSF-restricted monoclonal IgG band in CIDP reported by some further suggest antibodies may provide novel CIDP biomarkers.

Human IgG antibodies are composed of 2 light and 2 heavy chains. Light chains consist of 1 variable and 1 constant domain while heavy chains have 1 variable and 3 constant domains. The variable parts together with the first constant domain form the antigen-binding fragment, wherein 3 complementarity-determining regions (CDRs) embedded in framework regions (FRs) form a groove that fits the epitope of an antigen. As such, CDRs determine antigen specificity of the immunoglobulin.

A relatively novel approach in biomarker discovery for immune-mediated diseases is mass spectrometric (MS) analysis of peptides derived from circulating IgG. Such peptides might be specifically or more abundantly present in patients compared with that in controls and hence may provide interesting diagnostic biomarkers. This MS-based approach allows unbiased study of circulating antibodies independent of the targeted antigen. Although biological processes such as somatic recombination and hypermutation produce a greatly diverse human antibody repertoire, thereby rendering it theoretically less likely to find common sequences in IgG variable domains among different individuals, multiple groups have already shown overlapping variable domain–derived peptide sequences in patients with the same neuroimmunologic disorder. Moreover, a variable domain–derived peptide panel already illustrated being capable of differentiating patients with lung cancer from controls, thereby demonstrating the biomarker potential of such peptides.

Because the humoral immune response is considered an important mediator in CIDP’s pathogenesis, studying IgG-derived peptides may provide novel insights regarding biomarker research in CIDP. Therefore, the aim of this proof-of-concept study was to investigate the IgG-derived peptide profile through LC-MS/MS analysis to determine (1) whether sera of patients with CIDP contain clusters of IgG-derived peptides capable of differentiating patients with CIDP from controls and (2) whether individual variable domain–derived peptides in these clusters or a panel thereof demonstrate potential as diagnostic biomarker in CIDP (primary objectives). We also investigated whether IgG-derived peptide profiles could cluster patients in the CIDP population and whether these clusters differed in clinical features (secondary objective).

Methods

Study Population

Adult patients were prospectively recruited at University Hospitals Leuven Neuromuscular Reference Center on written
informed consent. Sera of patients with CIDP or related neurologic controls (Guillain-Barre syndrome, IgM antitymulin-associated glycoprotein-related neuropathy, monoclonal gammopathy of undetermined significance-related neuropathy, multifocal motor neuropathy, Charcot-Marie-Tooth 1A neuropathy, diabetic peripheral neuropathy, and amyotrophic lateral sclerosis) were collected between April 2014 and July 2022, aliquoted and stored at -80°C until use. Sampling occurred before initiating immunomodulatory therapy or when patients were already receiving therapy during recruitment, at trough levels (i.e., before next administration of therapy). Diagnoses were established by trained neurologists. For CIDP diagnosis, patients had to fulfill probable or definite CIDP criteria according to 2010 EFNS/PNS diagnostic criteria.3 Patients with CIDP were seen at our reference center for diagnostic workup or as required by Belgian legislature for reimbursement of IV immunoglobulin (IVIg) therapy for confirmation of diagnosis or related periodic follow-up. Relevant clinical data were obtained through electronic medical records and included demographics (age, sex, disease duration, therapy, and CIDP phenotype) and disability during sampling (through the Inflammatory Neuropathy Cause and Treatment [INCAT] scale and an 80-point Medical Research Council [MRC] sum score).

**Study Design**

We first investigated in a discovery cohort (1) whether IgG-derived peptide clusters differentiated patients with CIDP from controls and (2) whether individual IgG variable domain-derived peptides showing promise as diagnostic CIDP biomarkers were present (further termed peptides of interest [POI]) (primary objectives). Next, because amino acid sequences were not obtained for all POI during the discovery phase, targeted MS analysis was performed to obtain higher-quality MS/MS spectra of POI, thereby allowing elucidation of their sequences. Elucidated sequences were further validated against synthetically constructed peptides (Thermo Scientific). Next, diagnostic performance of individual POI and of a multipeptide model incorporating POI was evaluated in the discovery cohort and subsequently reassessed in an independent validation cohort (Figure 1). We also investigated whether IgG-derived peptide profiles allowed establishing objective clusters of patients with CIDP within the discovery cohort and whether such clusters related to clinical characteristics (secondary objective).

**IgG Isolation and In-Solution Digestion**

IgG was isolated from serum using Magne Protein G magnetic beads (Promega) following manufacturer’s instructions with minor modifications. Twenty microliters of beads were incubated with 500 μL of diluted serum (1:250 with TBS1X) for 1 hour followed by washing the beads 3 times with 2M ammonium acetate/TBS1X and 2 times with TBS1X. Next, IgG was eluted over 30 minutes using 50 μL of 0.2% formic acid. The elution fraction was subsequently neutralized in vials containing 1M Tris pH 8. After adding 8M urea, disulfide bonds were reduced using 70 mM DTT at 37°C for 1 hour following alkylation with 220 mM iodoacetamide for 30 minutes in the dark. Next, digestion at 37°C was performed overnight using Pierce Lys-C (Thermo Scientific) followed by a 4-hour digestion with Pierce Chymotrypsin (Thermo Scientific).
All steps were performed while shaking at room temperature unless specified otherwise. Last, digested samples were desalted using C18 spin columns (Thermo Scientific) according to manufacturer’s instructions, followed by drying through SpeedVac and storage at −80°C until LC-MS/MS analysis.

**LC-MS/MS Analyses**

Desalted peptides were dissolved in 50 μL of 0.1% formic acid/5% acetonitrile and 5 μL injected and separated on an Ultimate 3000 UPLC system (Dionex, Thermo Scientific) coupled online to an Orbitrap Elite Velos Pro ETD mass spectrometer (Thermo Scientific) operating in data-dependent acquisition (DDA) mode. For further settings, we refer to eMethods (links.lww.com/NXI/A898). Samples were randomized before measurement and each sample analyzed once. Before each sample, a blank to monitor system background and reduce carryover was run. Every third sample, an additional 2-hour wash step was performed. Quality control samples (BSA control (discovery cohort) or designated patient sample (validation cohort)) were analyzed at regular intervals during LC-MS/MS runs. Replicates of a designated patient sample were also used to evaluate reproducibility of LC-MS/MS measurement of POI (eMethods).

To obtain amino acid sequences for POI not sequenced in the discovery phase, targeted MS analysis was performed on discovery cohort samples demonstrating highest abundance for POI using an Orbitrap Q Exactive mass spectrometer (Thermo Scientific), and possible sequences elucidated in this manner were further confirmed through analyzing synthetic peptides (eMethods, links.lww.com/NXI/A898; eFigure 1, links.lww.com/NXI/A897). These synthetic peptides were later also spiked in QC samples of the validation cohort to carryover was run. Every third sample, an additional 2-hour wash step was performed. Quality control samples (BSA control (discovery cohort) or designated patient sample (validation cohort)) were analyzed at regular intervals during LC-MS/MS runs. Replicates of a designated patient sample were also used to evaluate reproducibility of LC-MS/MS measurement of POI during further data analysis.

**MS Data Analyses**

Raw data files were imported into Progenesis v4.2 software (Nonlinear Dynamics) for alignment, label-free quantification, and normalization of the different sample runs. The software automatically selects the run with greatest similarity to all other runs as reference alignment. Peaks (features) with charge state 2–5 were retained. Tandem mass spectra were extracted by Progenesis and processed through Mascot (version 2.2.06; Matrix Sciences) against an in-house database (NCBI Homo sapiens database supplemented with IgG-related sequences obtained from the international ImMunoGeneTics information system (IMGT) database; 204906 accessions) using the following search parameters: peptide mass tolerance of 12 ppm; fragment mass tolerance of 0.5 Da; maximum 4 missed cleavages; Lys-C + chymotrypsin (FYWKL |) as enzyme; oxidation of M and deamidation of N/Q as variable modifications; and carbamidomethylation of cysteines as fixed modification. De novo sequencing was also performed using the same search parameters using PEAKS Studio 5.1 (Bioinformatics solutions Inc.). Search results from both Mascot and PEAKS were used and a peptide database generated containing both peptide information (m/z, Mascot-derived sequence, PEAKS-derived sequence,...) and peptide abundances for each sample included. Amino acid sequences were also aligned to V-, D-, J-, or C-region germline sequences derived from the IMGT database using the IgBlast algorithm.18,19 Peptides with sufficient match (bit score ≥12.5 and alignment score ≥70%) to the V-region database were assigned a position on the immunoglobulin molecule.13

**Selection of Peptides of Interest**

The discovery cohort peptide database was exported from Progenesis and subjected to statistical analysis. First, partial least-squares discriminant analysis (PLS-DA) using the NIPALS algorithm was performed to test our hypothesis that IgG-derived peptide clusters correlated to disease status (dependent variable: CIDP or control; independent variables: peptide abundances). Then, to select individual POI, PLS-DA results were used to retain only peptides significantly contributing to differentiating CIDP from controls, based on variable importance in projection (VIP) scores and X-loadings. Variables with a VIP score greater than 1 were considered to have a significant effect on classification of sample categories.20,21 Next, only peptides showing upregulation in CIDP by univariate analysis were further retained, applying unpaired t tests or Mann-Whitney U tests based on normality of data. Further selection of peptides was then performed based on standardized abundances observed in patients with CIDP. In detail, for each thus far retained peptide and each sample, we expressed an inter-z score indicating how many SDs the abundance of that sample differed from the mean abundance observed in samples of controls (i.e., inter-z score of +1 indicates an abundance of 1 SD above the mean observed in controls). Only peptides for which in at least 10% of CIDP samples inter-z scores of ≥+3 were observed were retained.21,22 The 10% cutoff was chosen in line with findings regarding paranodal antibodies, which are observed in approximately 1%–10% of patients initially diagnosed with CIDP. Last, a final selection was performed based on peptide sequence. Only peptides for whom the amino acid sequence could be determined through targeted MS analysis and confirmed against synthetic peptides (eMethods, links.lww.com/NXI/A898; eFigure 1, links.lww.com/NXI/A897) and that originated from the IgG variable domain were retained as POI.

**Statistical Analyses**

Continuous demographics were compared through unpaired t test or Mann-Whitney U test based on normality of data, while categorical demographics were compared through Fisher exact tests. The Pearson or Spearman correlation was used to investigate correlations of peptide abundances with clinical data such as disability scales.

Univariate and multivariable (including demographics) logistic regressions were performed to evaluate whether POI could differentiate patients with CIDP from controls in the discovery cohort. Univariate ROC curve analysis was also performed to report diagnostic performance of peptides in terms of area under the curves (AUCs). POI were additionally incorporated into a multipeptide model by means of stepwise multiple logistic
regression using a normalized dataset (details on model construction available in eMethods, links.lww.com/NXI/A898). To validate POI, their diagnostic performance was reevaluated in an independent validation cohort using similar methodologies (logistic regression, multipeptide model). Clustering of patients with CIDP based on IgG-derived peptides in the discovery cohort and correspondence to clinical traits (secondary objective) was investigated by means of sparse principal component analysis (sPCA) applied to the complete peptide dataset followed by k-means clustering into 3 patient clusters.23

Statistical analyses were performed using Statistica 14 (TIBCO), Graphpad Prism V9.0 (GraphPad software), and R (version 4.2.2). p Values of 0.05 were considered statistically significant.

Standard Protocol Approvals, Registrations and Patient Consents
Written informed consent was obtained from all participants included in the study. Ethical approval was granted by Ethical Committee Research UZ/KU Leuven (S6226S).
Data Availability
Data not published within this article are available at University Hospitals Leuven or will be shared in an anonymized manner on request from any qualified investigator, subject to local and European regulations.

Results
Clinical Characteristics of the Study Population
The discovery cohort consisted of 44 patients with CIDP and 29 controls with related neurologic disorders, while the validation cohort comprised 45 patients with CIDP and 43 controls (Table 1). In both cohorts, the CIDP group displayed a higher proportion of male participants, reflecting CIDP’s known sex ratio,24 and of patients treated with immunomodulatory therapy during sampling (IVIg, steroids, or immunosuppressive drugs). In the discovery cohort, patients with CIDP were older compared with controls (\(p = 0.029\)). When comparing only CIDP groups, patients of the validation cohort demonstrated longer disease duration and a shorter time since last IVIg treatment (\(p = 0.043\) and \(p = 0.005\), respectively).

Patients with CIDP displayed no antibodies against contactin-1, neurofascin-155, or neurofascin-186. Seropositivity against contactin-associated protein 1 was not assessed.

Peptide Clusters in the Discovery Cohort
A total of 130,310 peptide features were detected in the discovery cohort. PLS-DA performed on these features could group patients with CIDP thereby separating them from controls, hinting at the presence of specific IgG-derived peptides (clusters) in sera of patients with CIDP (Figure 2). The distinction between patients with CIDP and controls related to a small portion of peptide features because extracted components explained a small percentage of the variance observed in our dataset (Figure 2). However, extracted components could explain 86.2% of the variance in the dependent variable (CIDP vs controls), thereby demonstrating a strong correlation with patient classification.

Selected Peptides of Interest
Peptides measured in the discovery cohort were subjected to PLS-DA as mentioned earlier, followed by filtering through univariate statistical analysis and further selection based on required inter-z scores of \(\geq +3\) in at least 10% of patients with CIDP. Peptides thus selected were subsequently subjected to targeted MS analysis for elucidation of amino acid sequences after which sequences were confirmed against synthetically constructed peptides. In total, 16 peptides derived from the IgG variable domain were retained as potential POI. All selected peptides demonstrated positive inter-z scores in patients with multiple CIDP, with inter-z score profiles of the 4 most promising peptides shown in Figure 3. Inter-z score profiles of other POI are shown in eFigure 2 (links.lww.com/NXI/A897). Characteristics of POI are listed in Table 2 with additional details in eTable 1 (links.lww.com/NXI/A899). Abundance of selected POI showed no correlation with age or disease duration and did not differ between untreated patients and patients receiving immunomodulatory therapy during sampling nor between IVIg-treated and non–IVIg-treated patients. Of interest, the abundance of peptide TISRDNAQNSLY correlated with the MRC sum score in patients with CIDP (Spearman \(\rho = -0.43; p = 0.003\)) (eFigure 3). Replicate measurement of a designated patient sample indicated POI could be measured with acceptable
reproducibility with an average intrarun and interrun CV of 12.4% and 15.3%, respectively.

**Diagnostic Performance of Individual Selected Peptides of Interest**

The 16 retained peptides were further evaluated in the discovery cohort through univariate and multivariable logistic regression models and by univariate ROC curve analysis. In univariate models, increasing peptide abundances associated with increased odds of CIDP for all peptides, with ROC curve analyses demonstrating Youden index–based AUCs ranging from 64.6% to 79.6% (Table 3). On including age and sex as covariates, logistic regression models demonstrated for 13/16 peptides that increasing peptide abundance associated with increased odds of CIDP diagnosis (Table 3; full multivariable models available in eTable 2, links.lww.com/NXI/A899).

When evaluating diagnostic performance of POI through their inter-z score profiles, the 4 most promising POI taken together could identify 37/44 (84.9%) cases with CIDP at a fixed specificity of 100% per peptide (eFigure 4, links.lww.com/NXI/A897).

**Diagnostic Performance of a Multipeptide Model**

Stepwise multiple logistic regression performed on the POI resulted in a multipeptide model composed of 5 POI, namely peptides 3, 8, 12, 14, and 16 (Table 2; eTable 3, links.lww.com/NXI/A899). When applied to the discovery cohort, this model demonstrated a Youden index–based AUC of 91.5% (95% CI 84.6%–98.5%; p < 0.001) (eFigure 5, links.lww.com/NXI/A897).

**Validation of Individual POI and the Multipeptide Model**

To validate POI, statistical analyses regarding diagnostic performance were repeated with peptide abundances of POI measured in an independent validation cohort. In univariate logistic regression, peptide SPSFQGQVTISADK remained a significant independent variable for CIDP, although it now demonstrated diminished discriminating performance in ROC curve analysis (Table 4). In multivariable models, this
was seen for peptide IVLTQSPATL (Table 4; full multivariable models available in eTable 4, links.lww.com/NXI/A899). Diminished diagnostic performances for most peptides were also apparent in inter-z score profiles of the validation cohort (eFigure 6, links.lww.com/NXI/A897) while the earlier constructed multipeptide model now displayed a Youden index–based AUC of 61.2% (95% CI 49.3%–73.2%; p = 0.064).

Because POI were selected using a discovery cohort including only 1 patient with anti-MAG neuropathy, we hypothesized that selected peptides might not be best suited to differentiate CIDP from anti-MAG neuropathy. This was confirmed as validation cohort peptide performances improved on omitting this control group in the associated statistical analyses (e.g., up to a total of 5 peptides could be confirmed as significant independent variables for CIDP; eTable 5, links.lww.com/NXI/A899).

**Discussion**

In this proof-of-concept study, we used LC-MS/MS analysis to investigate IgG-derived peptides in sera of patients with CIDP and related neurologic controls in 2 independent cohorts. In our discovery cohort, PLS-DA showed that peptide clusters could differentiate patients with CIDP from controls, possibly indicating an at least partly shared humoral immune response among patients with CIDP. Examination of individual peptides within these clusters yielded 16 peptides from the IgG variable domain demonstrating diagnostic performance for CIDP as evidenced by logistic regression models and ROC curve analysis. A multipeptide model incorporating 5 POI yielded an AUC of 91.5%, further illustrating selected peptides to display strong discriminating performance between patients with CIDP and neurologic controls in the discovery cohort.

**Table 2 Characteristics of Selected Peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>m/z</th>
<th>Charge</th>
<th>Protein description (accession number</th>
<th>origin) CDR/FR*</th>
<th>Blast bit score</th>
<th>IMGT % identified</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TISRDNAQNSLY</td>
<td>691.339</td>
<td>2</td>
<td>P01782</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>27.7</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>SPSFQGQVTISADK</td>
<td>732.870</td>
<td>2</td>
<td>A0A0C4DH38</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>31.2</td>
<td>100%</td>
</tr>
<tr>
<td>3</td>
<td>LTHTDDYLQVQSGAEVK</td>
<td>635.313</td>
<td>3</td>
<td>A0A0G2MJ13</td>
<td>Ig heavy chain variable region</td>
<td>FR1</td>
<td>36.6</td>
<td>84.2%</td>
</tr>
<tr>
<td>4</td>
<td>LQRRGQPPRLLY</td>
<td>560.329</td>
<td>3</td>
<td>A0A0C4DH68</td>
<td>Ig kappa variable region</td>
<td>FR2</td>
<td>33.5</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>DASTLESGVPSRF</td>
<td>683.342</td>
<td>2</td>
<td>A0A0B4J2D9</td>
<td>Ig kappa variable region</td>
<td>CDR2-FR3</td>
<td>27.7</td>
<td>92.3%</td>
</tr>
<tr>
<td>6</td>
<td>LQMNSLRADDTAVY</td>
<td>799.379</td>
<td>2</td>
<td>P02TE1</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>29.6</td>
<td>92.9%</td>
</tr>
<tr>
<td>7</td>
<td>IVLTQSPATL</td>
<td>521.810</td>
<td>2</td>
<td>A0A0C4DH25</td>
<td>Ig kappa variable region</td>
<td>FR1</td>
<td>22.3</td>
<td>100%</td>
</tr>
<tr>
<td>8</td>
<td>LQMNSLRPETAVY</td>
<td>818.911</td>
<td>2</td>
<td>P02TE1</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>28.9</td>
<td>85.7%</td>
</tr>
<tr>
<td>9</td>
<td>LQMNSLRVDETALY</td>
<td>827.412</td>
<td>2</td>
<td>A0A0C4DH32</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>29.6</td>
<td>92.9%</td>
</tr>
<tr>
<td>10</td>
<td>EVLLVESGGGLVK</td>
<td>650.380</td>
<td>2</td>
<td>A0A0B4J1D0</td>
<td>Ig heavy chain variable region</td>
<td>FR1</td>
<td>25.8</td>
<td>92.30%</td>
</tr>
<tr>
<td>11</td>
<td>VRQAPGGRLEWSY</td>
<td>539.954</td>
<td>3</td>
<td>P01763</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>33.1</td>
<td>92.9%</td>
</tr>
<tr>
<td>12</td>
<td>LQWWGSLK</td>
<td>416.240</td>
<td>2</td>
<td>A0A0J9YX1</td>
<td>Ig heavy chain variable region</td>
<td>FR3</td>
<td>17.3</td>
<td>85.7%</td>
</tr>
<tr>
<td>13</td>
<td>DVBLTQSPSPL</td>
<td>486.274</td>
<td>2</td>
<td>P06310</td>
<td>Ig kappa variable region</td>
<td>FR1</td>
<td>20.4</td>
<td>88.9%</td>
</tr>
<tr>
<td>14</td>
<td>DVQVESGGGLVQPGRSL</td>
<td>905.984</td>
<td>2</td>
<td>P01782</td>
<td>Ig heavy chain variable region</td>
<td>FR1</td>
<td>37.4</td>
<td>94.4%</td>
</tr>
<tr>
<td>15</td>
<td>VGLVTQSPATL</td>
<td>543.314</td>
<td>2</td>
<td>P04433</td>
<td>Ig kappa variable region</td>
<td>FR1</td>
<td>20.8</td>
<td>100%</td>
</tr>
<tr>
<td>16</td>
<td>AELMTQSPATL</td>
<td>630.829</td>
<td>2</td>
<td>P01624</td>
<td>Ig kappa variable region</td>
<td>FR1</td>
<td>23.9</td>
<td>90.9%</td>
</tr>
</tbody>
</table>

Abbreviations: CDR = complementarity-determining region; FR = framework region; Ig = immunoglobulin; IMGT = ImMunoGeneTics. Underlined amino acids were aligned to a CDR. Bold text indicates statistically significant p values. The position of the sequence in the Ig structure is indicated.

Clustering of Patients With CIDP Based on IgG-Derived Peptides

sPCA was used to reduce dimensionality of the discovery cohort peptide dataset to 5 components consisting of 8 peptides each. K-means clustering was then performed on these components resulting in 3 clusters of 22, 16, and 6 patients with CIDP, respectively. Established clusters demonstrated no differences in age, sex, disease duration, CIDP phenotype, INCAT score, MRC sum score, number of patients treated with immunomodulatory therapy, or time since IVIg treatment (eTable 6, links.lww.com/NXI/A899).
Of interest, most peptides selected in our study originated from FRs, although we initially hypothesized that rather peptides derived from the hypervariable CDRs would be unraveled as candidate biomarkers. Similar observations were made in an earlier study in which it was hypothesized that because FR peptides carry fewer mutations relative to the germline compared with CDR peptides, they are more likely to be present in several antibody clones, therefore displaying a higher abundance that, in turn favors their detection by LC-MS/MS analysis. An alternative explanation is that hypermutated CDRs are not as likely to be shared between multiple patients as previously put forward. This has been illustrated by studies that, e.g., found only few CDR-derived peptides to be shared in only a small number of patients with MS. Moreover, because we focused on selecting peptide markers present in multiple patients with CIDP, our workflow may have precluded the selection of CDR peptides shared by only a few patients. The large heterogeneity observed in CIDP could also further explain the presence of fewer CDR peptides shared among patients.

In an independent validation cohort, we could validate the diagnostic performance of selected peptides SPSFQGQVTISADK and IVLTQSPATL, albeit with reduced performance than was observed in the discovery cohort. A similar finding was noted for the multipeptide model, which displayed a decreased AUC in the validation cohort. However, the finding that diagnostic performance improved for multiple POI on omitting the anti-MAG neuropathy control group possibly not only indicates IgG-derived peptides may prove valuable to differentiate CIDP from most differential diagnoses included in this study but also illustrates the composition of discovery and validation cohorts should ideally be matched. Furthermore, the challenge encountered in validating our selected POI may be related to differences between cohorts. CIDP is characterized by a large heterogeneity, and peptides or peptide panels suitable for diagnosing 1 cohort of patients with CIDP may not be as useful for other cohorts. This is in line with earlier biomarker research in CIDP because initial findings regarding pathogenic antibodies directed against, e.g., myelin components in CIDP could often not be replicated by later studies. Patients with CIDP from the validation cohort also demonstrated increased disease duration and a larger spread in disease duration when compared with those from the discovery cohort. It is possible that these patients, who have been experiencing the disease and subsequently been treated with immunomodulatory therapy for a longer period of time, display

### Table 3 Logistic Regression Models of Selected Peptides in the Discovery Cohort

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Univariate model</th>
<th>Multivariable model</th>
<th>p Value</th>
<th>95% CI</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.212 (1.067–1.418)</td>
<td>1.186 (1.048–1.393)</td>
<td>&lt;0.001</td>
<td>0.707 (0.003)</td>
<td>1.070 (0.731)</td>
<td>1.094 (1.024–1.185)</td>
<td>0.016</td>
</tr>
<tr>
<td>2</td>
<td>1.112 (1.044–1.200)</td>
<td>1.054 (1.002–1.189)</td>
<td>&lt;0.001</td>
<td>0.731 (0.001)</td>
<td>1.070 (0.701)</td>
<td>1.445 (1.044–2.189)</td>
<td>0.053</td>
</tr>
<tr>
<td>3</td>
<td>1.920 (1.358–2.903)</td>
<td>1.921 (1.313–3.052)</td>
<td>&lt;0.001</td>
<td>0.75 (0.001)</td>
<td>1.070 (0.701)</td>
<td>1.445 (1.044–2.189)</td>
<td>0.053</td>
</tr>
<tr>
<td>4</td>
<td>1.533 (1.110–2.345)</td>
<td>1.487 (1.062–2.325)</td>
<td>&lt;0.001</td>
<td>0.646 (0.036)</td>
<td>1.070 (0.701)</td>
<td>1.445 (1.044–2.189)</td>
<td>0.053</td>
</tr>
<tr>
<td>5</td>
<td>1.735 (1.287–2.504)</td>
<td>1.679 (1.337–2.811)</td>
<td>&lt;0.001</td>
<td>0.777 (0.001)</td>
<td>1.070 (0.701)</td>
<td>1.445 (1.044–2.189)</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Abbreviations: AUC = area under the curve; CI = confidence interval; OR = odds ratio. Bold text indicates statistically significant p values.

For identity of the peptides, we refer to Table 2 of the main manuscript. Odds ratios are reported per 10,000 increase in normalized abundances unless otherwise specified.

Odds ratios reported per 1,000 increase in normalized abundance (lower abundant peptides).

Full multivariable logistic regression models are available in eTable 2 (links.lww.com/NXI/A899).

Neurology.org/NN
Furthermore, no differences in results were observed when logistic regression models were constructed with or without IVlg treatment as additional independent variable (results not shown). Last, inter-z score profiles of POI did also not differ between IVlg-treated and non–IVlg-treated patients with CIDP (results not shown), which further argues against selected POI originating from IVlg. However, the possibility that immunomodulatory therapies may conversely result in decreased levels of certain IgG-derived peptides cannot be excluded because proposed mechanisms of action of, e.g., IVlg therapy include provision of anti-idiotypic antibodies targeting autoantibodies and a suppression of autoantibody production.28–30 Indeed, IVlg therapy already demonstrated to decrease circulating autoantibody levels in various autoimmune diseases.31,32 Hence, it may also result in decreased levels of peptides derived from circulating (auto)antibodies. Because our validation cohort included a higher proportion of patients treated with immunomodulatory therapies while displaying more widespread between individuals that originate from B cells which have undergone less cycles of somatic hypermutation might be less abundantly present in these patients. Similar findings have been reported in studies on HIV-1 in which highly unique broadly neutralizing antibodies required years and multiple rounds of somatic hypermutation to develop in infected individuals.26,27

Another factor possibly affecting our results is the treatment of our patients with immunomodulatory therapies. Especially IVlg therapy may pose a significant confounding factor because observed IgG-derived peptides may have theoretically been derived (partly) from IgGs present within IVlg. However, all IVlg-treated patients were sampled at trough levels. Moreover, none of the POI were specifically upregulated in IVlg-treated patients compared with those in non–IVlg-treated patients. Furthermore, no difference in results was observed when reduced disease activity and hence reduced levels of certain IgG-derived peptides. Moreover, the biological process of affinity maturation, in which somatic hypermutations can lead to the production of higher avidity antibodies, may lead to patients displaying more unique, higher-avidity antibodies on more extensive disease duration and hence prolonged antigen exposure. In turn, antibodies more widespread between individuals that originate from B cells which have undergone less cycles of somatic hypermutation might be less abundantly present in these patients. Similar findings have been reported in studies on HIV-1 in which highly unique broadly neutralizing antibodies required years and multiple rounds of somatic hypermutation to develop in infected individuals.26,27

### Table 4 Logistic Regression Models of Selected Peptides in the Validation Cohort

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Univariate model</th>
<th>Multivariable model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>1</td>
<td>0.997</td>
<td>0.970–1.002</td>
</tr>
<tr>
<td>2</td>
<td>1.017</td>
<td>1.003–1.033</td>
</tr>
<tr>
<td>3</td>
<td>1.031</td>
<td>0.994–1.072</td>
</tr>
<tr>
<td>4</td>
<td>1.049</td>
<td>0.960–1.154</td>
</tr>
<tr>
<td>5</td>
<td>1.015</td>
<td>0.957–1.081</td>
</tr>
<tr>
<td>6</td>
<td>1.070</td>
<td>0.980–1.179</td>
</tr>
<tr>
<td>7</td>
<td>1.132</td>
<td>0.948–1.376</td>
</tr>
<tr>
<td>8</td>
<td>1.010</td>
<td>0.953–1.072</td>
</tr>
<tr>
<td>9</td>
<td>0.987</td>
<td>0.913–1.059</td>
</tr>
<tr>
<td>10</td>
<td>0.944</td>
<td>0.863–1.012</td>
</tr>
<tr>
<td>11</td>
<td>1.054</td>
<td>0.929–1.209</td>
</tr>
<tr>
<td>12b</td>
<td>1.003</td>
<td>0.940–1.073</td>
</tr>
<tr>
<td>13b</td>
<td>1.008</td>
<td>0.994–1.024</td>
</tr>
<tr>
<td>14</td>
<td>1.056</td>
<td>0.990–1.161</td>
</tr>
<tr>
<td>15b</td>
<td>1.070</td>
<td>0.501–2.414</td>
</tr>
<tr>
<td>16b</td>
<td>0.891</td>
<td>0.584–1.277</td>
</tr>
</tbody>
</table>

Abbreviations: AUC = area under the curve; CI = confidence interval; OR = odds ratio. Bold text indicates statistically significant p values.

b For identity of the peptides, we refer to Table 2 of the main manuscript. Odds ratios are reported per 1,000 increase in normalized abundances unless otherwise specified.

c Odds ratios reported per 1,000 increase in normalized abundance (lower abundant peptides).

d Full multivariable logistic regression models are available in eTable 4 (links.lww.com/NXI/A899).
Likewise, ideally, neurologic controls should have been untreated and age-matched and sex-matched to patients with CIDP, but the limited number of samples from patients with these disorders available precluded us from doing so.

We also evaluated whether patients with CIDP of the discovery cohort could be clustered based on their IgG-derived peptide profile. Three clusters could be identified of 22, 16, and 6 patients, respectively. No difference in demographics could be determined between these clusters, although we cannot exclude the possibility that established clusters might differ in other features such as electrophysiologic parameters. Due to the clinical nature of our cohort, however, electrophysiologic parameters were not available for most patients during sampling (often during follow-up, i.e., postdiagnosis) because nerve conduction studies were mostly performed during diagnostic workup. The relatively small size of the established patient clusters might have also precluded us from establishing meaningful clinical associations. Patient clusters did also not relate to CIDP subtype, although it has been hypothesized that different CIDP variants might demonstrate different immunopathologic mechanisms. Theoretically, the IgG-derived peptide repertoire might therefore also differ between subtypes. The relatively limited number of CIDP variants included in our cohort may have precluded discovery of peptide profiles related to specific variants. Likewise, it is possible that certain peptides could have shown increased diagnostic performance when considering CIDP subtypes as distinct entities in our analysis, but the relatively limited number of CIDP variants precluded us from doing so. A follow-up study specifically focused on larger numbers of patients with typical CIDP and CIDP variants might reveal specific peptide profiles per variant and thereby further support the hypothesis of different variants displaying distinct immunopathologic mechanisms.

The major limitation in this study is the use of a clinical cohort of patients resulting in significant variability in demographics both within and between cohorts, e.g., in disease duration, sex, or number of patients treated with immunomodulatory therapy. This also resulted in a significant portion of patients already receiving therapy during sampling. Ideally, only newly diagnosed treatment-naive patients and age-matched and sex-matched controls would have been included, but as discussed earlier, these were only available to us in very limited numbers. Nevertheless, despite this limitation, we demonstrated the antibody repertoire to be an interesting source of potential CIDP biomarkers. Hence, we encourage future prospective studies that might achieve greater success in finding and validating IgG-derived peptide biomarkers for CIDP by using well-characterized treatment-naive cohorts. Another remark can be made regarding using DDA for LC-MS/MS analysis of the validation cohort as opposed to using targeted MS, which would have offered increased sensitivity and specificity to measure POI. However, because CIDP is a highly variable disease and we were forced to use heterogeneous cohorts as discussed earlier, we acknowledged the risk of being unable to validate selected peptides. Hence, by opting for DDA also for analyzing the validation cohort, we generated a second peptide dataset, which can be additionally analyzed in later studies to identify other potentially interesting peptides. To circumvent the possibility of lower abundant POI not being identified in DDA (because only the top N most abundant peptides eluting at a given time point are selected for fragmentation in this mode), we spiked validation cohort QC samples with an excess of synthetic version of POI, which ensured reliable identification of our targets in this cohort. A last limitation inherent to the use of bottom-up LC-MS/MS analysis is that information regarding identity of the "parent IgGs", from which selected POI were derived, is lost. Nevertheless, it would be interesting for future studies to elucidate the identity of these parent IgGs and investigate whether POI retained in this study relate to earlier described autoantibodies in CIDP such as glycolipid antibodies.

In conclusion, current study indicates differences to exist in serum IgG-derived peptides between patients with CIDP and controls, thereby providing proof-of-concept of IgG-derived peptides as potentially interesting source of CIDP biomarkers. Further prospective studies using well-defined cohorts of treatment-naive patients in an early disease stage are likely required to obtain validated IgG-derived peptide biomarkers for CIDP.

Acknowledgment
The authors thank all patients who contributed samples for use in this study and Mr. Kusay Arat from the KU Leuven SyBioMa mass spectrometry facility for his excellent technical assistance.

Study Funding
This work was funded by the Catholic University of Leuven (KU Leuven) through C2-project Industrial Research Fund (IOF) financing (3M190242). J Godelaine has a PhD fellowship of the Research Foundation—Flanders (FWO) (1191420N). K Poesen is a senior clinical investigator (18B2622N) of FWO and holds a Clinical Research fund of the University Hospitals Leuven. P Van Damme holds a senior clinical investigatorship of the Research Foundation—Flanders and is supported by the ALS Liga België and the KU Leuven funds "een hart voor ALS," "Laevensfonds voor ALS Onderzoek," and the "Valéry Perrier Race against ALS Fund." B De Moor is a fellow of SIAM, IEEE, and IFAC, and, among others, funded by EWI (FAIR: Flanders AI Research Program) and by the European Commission (EU Research Council under the European Union’s Horizon 2020 research and innovation program (ERC Adv. Grant) under grant 885682).

Disclosure
J. Godelaine reports no disclosures relevant to the manuscript; Y. Chitale reports no disclosures relevant to the manuscript; B. De Moor reports no disclosures relevant to the manuscript; C. Mathieu reports no disclosures relevant to the manuscript; L. Ancheva reports no disclosures relevant to the manuscript; P. Van Damme is chairholder of the Emil von Behring Research Council under the European Union’s Horizon 2020 research and innovation program (ERC Adv. Grant) under grant 885682).
Chair for Neuromuscular and Neurodegenerative Disorders by CSL Behring and is a member of the European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD) and of the European Reference Network for Rare Neurologic Diseases (ERN-RND); K.G. Claeyts is chairholder of the Emil von Behring Chair for Neuromuscular and Neurodegenerative Disorders by CSL Behring, is a member of the European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD) and of the European Reference Network for Rare Neurologic Diseases (ERN-RND), and has received advisory board honoraria, research grants unrelated to the current study, and travel reimbursement from Alynamyl, Amicus, ArgenX, Biogen, CSL Behring, Ipsen, and Sanofi-Genzyme; X. Bossuyt reports no disclosures relevant to the manuscript; S. Carpentier reports no disclosures relevant to the manuscript; K. Poesen reports no disclosures relevant to the manuscript. Go to Neurology.org/NN for full disclosures.

Publication History

Received by Neurology: Neuroimmunology & Neuroinflammation March 27, 2023. Accepted in final form July 24, 2023. Submitted and externally peer reviewed. The handling editor was Associate Editor Marinos C. Dalakas, MD, FAAN.

Appendix (continued)

Names Location Contribution

Philip Van Damme, MD, PhD

Department of Neurology, University Hospitals Leuven, Belgium; Department of Neurosciences, Experimental Neurology, Laboratory of Neurobiology, Leuven Brain Institute, VIB KU Leuven Center for Brain and Disease Research, Leuven, Belgium

Drafting/revision of the article for content, including medical writing for content

Kristi G. Claeyts, MD, PhD

Department of Neurology, University Hospitals Leuven, Belgium; Department of Neurosciences, Laboratory for Muscle Diseases and Neuropathies, Leuven Brain Institute, KU Leuven, Leuven, Belgium

Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data

Xavier Bossuyt, MD, PhD

Laboratory Medicine, University Hospitals Leuven, Belgium; Department of Microbiology, Immunology and Transplantation, Clinical and Diagnostic Immunology, KU Leuven

Drafting/revision of the article for content, including medical writing for content

Sebastian Carpentier, PhD

Division of Crop Biotechnics, Tropical Crop Improvement Laboratory, Department of Biosystems, KU Leuven, Belgium

Drafting/revision of the article for content, including medical writing for content; study concept or design

Koen Poesen, PharmD, PhD

Department of Neurosciences, Laboratory for Molecular Neurobiomarker Research, Leuven Brain Institute, KU Leuven, Belgium; Laboratory Medicine, University Hospitals Leuven, Belgium

Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data

References


Appendix Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joris Godeleine, PharmD</td>
<td>Department of Neurosciences, Laboratory for Molecular Neurobiomarker Research, Leuven Brain Institute, KU Leuven; Laboratory Medicine, University Hospitals Leuven, Belgium</td>
<td>Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data</td>
</tr>
<tr>
<td>Bart De Moor, PhD</td>
<td>STADIUS Center for Dynamical Systems, Signal Processing, and Data Analytics, Department of Electrical Engineering (ESAT), KU Leuven, Belgium</td>
<td>Drafting/revision of the article for content, including medical writing for content</td>
</tr>
<tr>
<td>Chantal Mathieu, MD, PhD</td>
<td>Department of Endocrinology, University Hospitals Leuven; Department of Chronic Diseases and Metabolism, Clinical and Experimental Endocrinology, KU Leuven, Belgium</td>
<td>Drafting/revision of the article for content, including medical writing for content</td>
</tr>
<tr>
<td>Lina Ancheva, PhD</td>
<td>Department of Microbiology, Immunology and Transplantation, Clinical and Diagnostic Immunology, KU Leuven, Belgium</td>
<td>Drafting/revision of the article for content, including medical writing for content</td>
</tr>
</tbody>
</table>


30. Dalakas MC. IgG4-mediated neurologic autoimmune: understanding the pathogenicity of IgG4, ineffectiveness of IV Ig, and long-lasting benefits of anti-B cell therapies. Neurol Neuroimmunol Neuroinflamm. 2022;9(1)e1116. doi:10.1212/NXI.0000000000001116


Peptides From the Variable Domain of Immunoglobulin G as Biomarkers in Chronic Inflammatory Demyelinating Polyradiculoneuropathy
Joris Godelaine, Yamini Chitale, Bart De Moor, et al.
*Neurol Neuroimmunol Neuroinflamm* 2023;10;
DOI 10.1212/NXI.00000000000200162

This information is current as of August 28, 2023

<table>
<thead>
<tr>
<th>Updated Information &amp; Services</th>
<th>including high resolution figures, can be found at: <a href="http://nn.neurology.org/content/10/6/e200162.full.html">http://nn.neurology.org/content/10/6/e200162.full.html</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>This article cites 34 articles, 7 of which you can access for free at: <a href="http://nn.neurology.org/content/10/6/e200162.full.html##ref-list-1">http://nn.neurology.org/content/10/6/e200162.full.html##ref-list-1</a></td>
</tr>
<tr>
<td>Subspecialty Collections</td>
<td>This article, along with others on similar topics, appears in the following collection(s):</td>
</tr>
<tr>
<td></td>
<td><em>Autoimmune diseases</em> <a href="http://nn.neurology.org/cgi/collection/autoimmune_diseases">http://nn.neurology.org/cgi/collection/autoimmune_diseases</a></td>
</tr>
<tr>
<td></td>
<td><em>Chronic inflammatory demyelinating polyneuropathy</em> <a href="http://nn.neurology.org/cgi/collection/chronic_inflammatory_demyelinating_polyneuropathy">http://nn.neurology.org/cgi/collection/chronic_inflammatory_demyelinating_polyneuropathy</a></td>
</tr>
<tr>
<td></td>
<td><em>Class III</em> <a href="http://nn.neurology.org/cgi/collection/class_iii">http://nn.neurology.org/cgi/collection/class_iii</a></td>
</tr>
<tr>
<td></td>
<td><em>Peripheral neuropathy</em> <a href="http://nn.neurology.org/cgi/collection/peripheral_neuropathy">http://nn.neurology.org/cgi/collection/peripheral_neuropathy</a></td>
</tr>
<tr>
<td>Permissions &amp; Licensing</td>
<td>Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://nn.neurology.org/misc/about.xhtml#permissions">http://nn.neurology.org/misc/about.xhtml#permissions</a></td>
</tr>
<tr>
<td>Reprints</td>
<td>Information about ordering reprints can be found online: <a href="http://nn.neurology.org/misc/addir.xhtml#reprintsus">http://nn.neurology.org/misc/addir.xhtml#reprintsus</a></td>
</tr>
</tbody>
</table>

*Neurol Neuroimmunol Neuroinflamm* is an official journal of the American Academy of Neurology. Published since April 2014, it is an open-access, online-only, continuous publication journal. Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2332-7812.