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Protein Biomarker Identification in the CSF of Patients With CNS Lymphoma

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Abstract

Purpose—Elucidation of the CSF proteome may yield insights into the pathogenesis of CNS disease. We tested the hypothesis that individual CSF proteins distinguish CNS lymphoma from benign focal brain lesions.

Methods—We used a liquid chromatography/mass spectrometry–based method to differentially quantify and identify several hundred CSF proteins in CNS lymphoma and control patients. We used enzyme-linked immunosorbent assay (ELISA) to confirm one of these markers in an additional validation set of 101 cases.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Results—Approximately 80 CSF proteins were identified and found to be present at significantly different concentrations, both higher and lower, in training and test studies, which were highly concordant. To further validate these observations, we defined in detail the expression of one of these candidate biomarkers, antithrombin III (ATIII). ATIII RNA transcripts were identified within CNS lymphomas, and ATIII protein was localized selectively to tumor neovasculature. Determination of ATIII concentration by ELISA was significantly more accurate (> 75% sensitivity; >98% specificity) than cytology in the identification of cancer. Measurement of CSF ATIII levels was found to potentially enhance the ability to diagnose and predict outcome.

Conclusion—Our findings demonstrate for the first time that proteomic analysis of CSF yields individual biomarkers with greater sensitivity in the identification of cancer than does CSF cytology. We propose that the discovery of CSF protein biomarkers will facilitate early and noninvasive diagnosis in patients with lesions not amenable to brain biopsy, as well as provide improved surrogates of prognosis and treatment response in CNS lymphoma and brain metastasis.

INTRODUCTION

Establishing the etiology of focal brain lesions in patients with unexplained neurologic symptoms is a clinical challenge. Stereotactic brain biopsy is often the test of last resort, especially in patients with a rapidly deteriorating neurologic course.¹ Brain biopsy is, however, associated with 1.2% to 7% risk of hemorrhage and a 10% to 35% risk of failure to achieve definitive histologic diagnosis.^{1,2} A significant number of patients are poor candidates for biopsy because of tumor location in eloquent or deep brain structures, age, and comorbidity. Diagnostic challenge may be particularly difficult if CNS involvement of non-Hodgkin's lymphoma (NHL) is a consideration, as evidenced by radiographic features and lesional response to corticosteroids, inasmuch as these may occur in multiple sclerosis, neurosarcoid, and lymphoma.

Because early diagnosis and treatment of CNS lymphoma attenuates disease progression and neurologic deterioration, new approaches are needed to facilitate diagnosis.^{3,4} Identification of biomarkers that denote the presence of residual disease are also a high priority and may be the basis for new strategies to enhance traditional response criteria for brain tumors. These are based on tumor enhancement on neuroimaging or on malignant CSF cytology.^{5,6}

Although evaluation of the CSF is markedly less invasive than brain biopsy and is standard of care in the evaluation of intracranial processes such as CNS lymphoma or in the staging evaluation of patients with aggressive NHL, cytologic examination is less than 50% sensitive in the diagnosis of lymphoma and other cancers. Therefore, this test rarely substitutes for brain biopsy.⁷ By contrast, even crude analysis of CSF shows it to be abnormal in terms of total protein concentration in most patients with CNS lymphoma and neoplastic meningitis. Although there is evidence that elevation of total CSF protein is associated with adverse prognosis in CNS lymphoma,^{8,9} the specific, prognostically-relevant peptide constituents in CSF have not, until now, been identified.

Surrogate biomarkers measured in the CSF are routinely used to evaluate CNS germ cell tumors and primary CNS lymphoma in AIDS patients. High CSF concentrations of β -human chorionic gonadotropin or alpha-fetoprotein, plus concordant features on magnetic

resonance imaging (MRI), are used to diagnose CNS germ cell tumors and eliminate the need for craniotomy.² Detection of Epstein-Barr virus (EBV) DNA in CSF by polymerase chain reaction (PCR), in addition to neuroimaging, may establish the presence of CNS lymphoma in AIDS patients, obviating the need for histologic confirmation in some cases.¹⁰ Primary CNS lymphoma in the immunocompetent population is not, however, associated with EBV infection.

Recent studies have demonstrated the potential of mass spectrometry (MS) to identify biofluid peptide profiles that might facilitate early diagnosis of cancer. Pilot studies lacking both high-resolution detection and peptide identification have highlighted pitfalls associated with the analysis of clinical blood specimens.^{11–12} In addition, in the majority of these mass spectrometric studies, validation analyses using independent specimens or complementary methodologies have not been performed.

The CSF has several properties that facilitate differential proteomic profiling compared with blood. One, the volume of CSF is, on average, approximately 150 mL, compared with the 5-L blood volume. Two, the CSF compartment is specialized to bathe the CNS and is not exposed to the systemic circulation and to multiple organs. Both features favor the relative over-representation of brain and brain-tumor related proteins in the CSF.

Previously, the most common method for analysis of CSF proteins has been high-resolution two-dimensional (2D) poly-acrylamide gel electrophoresis with MS for identification of excised gel spots.¹³ This process is limited by low sensitivity. In a recent study, high-resolution matrix-assisted laser desorption/ionization (MALDI)/time-of-flight (TOF) MS analysis of tryptic peptides of CSF proteins was applied to discriminate leptomeningeal metastasis from breast cancer patients from control subjects. However, this profile did not exceed the diagnostic accuracy of cytology and identification of peptides was not performed.¹⁴

In the present study, we report an alternate strategy for differential proteomic analysis that also includes large-scale identification of more than 500 proteins to identify the major CSF proteins which distinguish B-cell CNS lymphoma from benign conditions. Further, we demonstrate the reproducibility of this quantitative, 2D liquid chromatography (LC)/MS approach to proteomic profiling in two independent studies involving different control and CNS lymphoma patients. Each study involved CSF collection and its analysis, performed at a 1-year interval. We demonstrate that the CSF proteome contains a wealth of potential diagnostic and prognostic information, and, as an example, we validate in detail one of these candidate biomarkers, CSF antithrombin III (ATIII).

METHODS

CSF from patients with brain tumors and non-neoplastic control conditions were obtained after informed consent in accordance with a University of California, San Francisco Institutional Review Board–approved protocol. CSF was centrifuged within 90 minutes of collection to remove cellular debris and stored at –80°C. Twenty-nine of the 31 CSF specimens used for 2D LC/MS analysis were from lumbar CSF and two specimens

(lymphoma) were from ventricular CSF via an indwelling Ommaya reservoir. All 101 CSF specimens used in cross-validation analysis were from lumbar sac. Exclusion criteria were age less than 18 years, HIV infection, traumatic CSF collection and recent therapeutic intervention (< 1 week). All brain tumor CSF was from patients with active disease.

Gene Expression, Immunohistochemistry, Immunoblot, and Enzyme-Linked Immunosorbent Assay

Gene expression and immunohistochemical analyses were performed as described previously.¹⁵ Quantitative determination of ATIII concentration in CSF (by enzyme-linked immunosorbent assay [ELISA]) was performed using ATIII Bioassay (US Biological, Swampscott, MA).

Sample Preparation

For 2D LC/MS, 3 mL of CSF was used. The abundant proteins albumin, immunoglobulin (Ig) G, IgA, alpha-1-antitrypsin, transferrin, and haptoglobin were depleted using a Multiple Affinity Removal System (Agilent, Palo Alto, CA). The depleted CSF proteome was denatured, reduced, cysteines alkylated and after buffer exchange, digested, desalted, and fractionated into three fractions on a strong cation-exchange (SCX) column as previously described.^{16–20} A total of approximately 20 μ g of processed peptides dissolved in 40 μ L 0.1% formic acid per SCX fraction were injected into the LC/MS system.

LC/MS Differential Quantification and Identification

The proteomic method applied here uses high-resolution LC/MS data for profiling differential quantification without isotopic labeling, combined with extensive protein identification by LC/MS/MS.^{16–20} Molecular ion signal intensities were normalized on a global basis according to each samples' total protein content (after abundant protein depletion). For protein identification, approximately 500 proteins were identified by tandem MS (Model LTQ, Thermo Electron, San Jose, CA) using control CSF samples with an identification criteria score of more than 40 by Mascot software (Matrix Science, London, UK).¹⁷ All profiled molecular ions were additionally constrained when matched against the library via accurate m/z and chromatographic retention times.

Instrumentation: LC/MS

The second dimension of the 2D LC/MS set-up used online reverse-phase capillary high-performance LC coupled with high resolution electrospray ionization (ESI)-TOF (Micromass LCT; Waters Corp, Milford, MA).^{16–20} Samples from each of the cohorts were processed and analyzed in pairs with randomized ordering.

Statistics

Univariate hypothesis tests for each MS component were used for the comparisons of means between control and CNS lymphoma groups. Observations were preprocessed by taking $\log_2$ of their values and filtering out the components with 30% or more observations missing in either test or training sets. Out of an initial total of 5,258 components, 2,783 components

(53%) remained. To test for differential expression, we used empirical Bayes moderated t statistic.²¹ The majority of components had a coefficient of variance (CV) less than 20%.

P values in the training set were adjusted using false discovery rate (FDR) which controls the expected proportion of false findings.²² The components with FDR less than 0.1 in the training set were evaluated in the test set and those with unadjusted P value of less than .01 in the test set and the same direction of association were considered validated (Table 1). Reproducibility between training and validation sets was evaluated by direct comparison of the test statistics in the two studies using Spearman correlation and by testing for enrichment of the small P values in the test set among the components declared to be differentially expressed in the training set.

RESULTS

We used quantitative 2D LC/MS proteomic profiling in two independent studies to identify proteins which are differentially expressed in the CSF in patients with CNS lymphoma. In 2003 to 2004, we confirmed these findings, we repeated the analysis using different patients with CSF collected and analyzed 1 year later. In the validation 2D LC/MS study, the CSF proteome from seven new patients with CNS lymphoma was compared with the CSF proteome of seven new control subjects (Appendix Table A1, online only). Nearly 80 proteins had FDR less than 0.1 in the training set and P value less than .01 in the test set with the concordant direction of association and thus were considered validated in the test set. (Table 1; Fig 1A).

The concordance between training and test set was exceptional as shown by comparing the test statistics from the two data sets (Fig 1B). The Spearman correlation between the two sets of statistics was 0.71, which is comparable to the correlation observed in previous gene expression meta-analyses for lung cancer.²³

The majority of proteins identified have not previously been described in the CSF in CNS lymphoma. A number of proteins detected at the highest relative concentration in the CSF of CNS lymphoma patients are implicated in tumor invasion and in the generation of tumor-associated extracellular matrix, as exemplified by matrix metalloprotease-2, vitronectin, and fibulin-3 (EGF-EMP-1). Notably, members of the complement pathway, including complement inhibitors Factors H, Factor I, and the complement cytolysis inhibitor clusterin, were also expressed at higher levels in CNS lymphoma, suggesting activation of an immune response.

The CSF of CNS lymphoma patients contained lower than control CSF concentrations of several proteins involved in the regulation of normal brain function. Among these were neuronal cell adhesion molecules, as well as cadherin 13, contactin-1 and chromogranin A.

To extend and validate these observations, we investigated the potential utility of candidate CSF biomarkers to facilitate diagnosis and management of patients with established or suspected CNS lymphoma. The first candidate biomarker that we pursued is the serine protease inhibitor ATIII. ATIII is normally synthesized by hepatocytes and has an established role in the regulation of blood coagulation.²⁴ We previously demonstrated

endogenous expression of ATIII RNA transcripts within diagnostic biopsy specimens of primary CNS lymphoma tumors.¹⁵ Intratumoral expression of ATIII RNA was highest in patients with the shortest survival in this cohort (<6 months). This result was confirmed by quantitative real-time PCR (Fig 2). Biopsy specimens of CNS lymphoma used for this microarray analysis were obtained from an independent set of immunocompetent CNS lymphoma patients whose CSF was not analyzed for proteomic content.

Immunohistochemical analysis demonstrated the strongest expression of ATIII protein by the distinct CNS lymphoma neovasculature (Fig 3). ATIII expression was detected in four of five CNS lymphoma tumor specimens, as well as in each of four nodal large B-cell tumors analyzed, but not in the vasculature of reactive germinal centers from tonsils. These findings suggest a potential novel role for ATIII in the modulation of tumor angiogenesis in NHL.

We used immunoblot to confirm the high relative expression of ATIII protein in the CSF of CNS lymphoma patients compared with the CSF of subjects with nonmalignant neurologic conditions (Appendix Fig A1, online only).

To further define the potential clinical utility of ATIII as a CSF biomarker, we used a sandwich ELISA to measure ATIII concentrations in an additional cross-validation set of 101 different CSF specimens not analyzed by the 2D LC/MS. This group consisted of 77 control subjects and 24 patients with primary and secondary CNS involvement by high-grade B-cell non-Hodgkin's lymphoma (Appendix Table A2, online only). The majority of control subjects from whom CSF was collected in this analysis had focal brain lesions with radiographic features similar to CNS lymphoma, such as multiple sclerosis, neurosarcoid, meningeal infection, and cerebrovascular disease. Many of these patients used in the reference set were at risk for CNS dissemination of cancer, including systemic NHL, and underwent lumbar punctures as part of routine staging procedures that were negative for malignant cytology.

CSF concentrations of ATIII were higher in the cohort of CNS lymphoma patients (mean value + 1.68 $\mu\text{g/mL}$) compared with control subjects without brain tumors (mean value + 0.54 $\mu\text{g/mL}$; $n = 77$) ($P < 3.3 \times 10^{-9}$). CSF ATIII concentrations were significantly higher in CNS lymphoma patients, even after normalization for total CSF protein concentration ($P < .015$). The ratio of ventricular: lumbar CSF ATIII concentration was determined in three participants and found to range between 0.42 and 1.07, reflecting the normal CSF protein gradient as well as variations in the dissemination of CNS lymphoma throughout the craniospinal axis (Appendix Fig A2, online only).

The potential utility of ATIII concentration in CSF as a discriminator of CNS lymphoma was evaluated by means of receiver operating characteristic (ROC) curve analysis.²⁵ Area under the ROC curve for the CSF ATIII concentration as a discriminator of CNS lymphoma from benign conditions was 0.912. A cutoff CSF ATIII concentration of 1.2 $\mu\text{g/mL}$ identified CNS lymphoma patients with the highest accuracy (75% sensitivity and 98.7% specificity; Fig 4). By contrast, cytologic evaluation of the CSF in this cohort of 24 lymphoma patients was only 8% sensitive in the detection of CNS malignancy; CSF cytology was positive in 33% of lymphoma specimens subjected to 2D LC/MS analysis.

Elevated CSF ATIII concentration was also detected in other CNS malignancies including metastatic breast and lung cancer patients, in whom the mean CSF ATIII concentration was 2.6 $\mu\text{g/mL}$ ($n = 13$). There did not seem to be an association between the elevation of CSF ATIII and the extent of tumor-associated contrast enhancement on MRI. These results suggest that the pathologic elevation of CSF ATIII, although not specific for CNS lymphoma, might be useful to noninvasively distinguish malignant brain lesions from lesions caused by benign disorders.

The potential value of ATIII as a CSF biomarker is illustrated in two patients with radiographically similar focal brain lesions, each with pathologic contrast-enhancement on MRI; neither lesion was amenable to brain biopsy. Repeat CSF cytologic analyses over several months were nondiagnostic as these patients exhibited progressive neurologic decline while awaiting a diagnosis. Elevated CSF ATIII concentration retrospectively correlated with primary CNS lymphoma as opposed to neurosarcoid (Fig 5).

In light of the RNA expression results, which suggested an association between intratumoral ATIII RNA levels and outcome in CNS lymphoma (Fig 2), we analyzed survival in CNS lymphoma patients in whom CSF ATIII protein concentrations were measured by ELISA. Whereas there was a significant trend between elevated CSF ATIII concentration and shorter survival, this association was strongest in the subset of lymphoma patients at first CNS relapse. Survival of CNS lymphoma patients with elevated CSF ATIII concentrations at first relapse ($>1.4 \mu\text{g/mL}$), was markedly shorter than that of patients with lower CSF ATIII levels (Fig 6; Appendix Table A3, online only). Because there are currently no established biomarkers that stratify risk in NHL patients with relapsed CNS disease, CSF measurement of ATIII and other proteins listed in Table 1 may be useful in clinical decision making for this population of patients.

An essential property of an ideal cancer biomarker is that its differential expression reflects the course of disease and provides insight into disease status with greater sensitivity than methods based on cytology or imaging. Serial CSF ATIII protein measurements agreed with tumor status based on objective criteria (CSF cytology and/or neuroimaging) in each of six CNS lymphoma patients analyzed; two examples are depicted in Figures 7 and 8.

DISCUSSION

We used proteomic technology to perform differential protein quantification and identification within CSF and obtained highly concordant results in two independent studies. We defined the spectrum of CSF proteins which distinguish CNS lymphoma from nonmalignant neurologic conditions in detail not previously achieved.

A variety of recent studies have identified novel properties of ATIII as a regulator of tumor angiogenesis; ectopic expression of ATIII by prostate tumors and cell lines has also been reported.^{26–27} We demonstrate for the first time the selective expression of ATIII protein in NHL with localization of ATIII to tumor cells and especially to neovasculature. We have direct evidence that the CNS lymphoma and its microenvironment are the source of several proteins which we identify to be expressed by concentration at least two-fold higher in CSF.

Gene expression by CNS lymphomas of ATIII, fibulin-3, vitronectin, complement C1q, and clusterin was demonstrated by microarray data; expression of ATIII, fibulin-3, vitronectin and complement factor H within CSF was also confirmed by immunohistochemistry or by immunoblot (data not shown).

The concomitant, low relative CSF expression of normal brain-related proteins such as chromogranin A observed in CNS lymphoma raises the possibility that the biosynthesis of these proteins is reduced in brain tumor patients with space-occupying lesions. Another potential explanation for their decreased CSF concentration could be effects on brain metabolism caused by diffuse lymphomatous infiltration or the administration of high-dose glucocorticoids. A third possibility is that the CSF clearance of these brain-related peptides occurs more rapidly within the tumor environment, perhaps secondary to proteolytic degradation within the leptomeninges in CNS lymphoma.

Prospective measurement of individual CSF proteins such as ATIII may facilitate early, noninvasive diagnosis, risk stratification, and determination of the need and/or timing of therapeutic interventions in patients with indeterminate radiographic or cytologic findings.

ATIII, among other proteins (Table 1), represents the first novel CNS lymphoma CSF biomarker to be identified by LC/MS technology (Appendix Table A4; online only). Further prospective evaluation of ATIII as a CSF biomarker is merited to determine its significance as a surrogate of response and prognosis in CNS lymphoma and other types of tumors in which CSF collection is clinically indicated. In patients without systemic cancer who present with rapid neurologic deterioration and are subjected to brain biopsy to evaluate focal brain lesions, CNS lymphoma must be considered a leading diagnostic possibility,¹ especially for lesions that respond dramatically to glucocorticoids. Although additional CSF biomarkers must be evaluated, determination of CSF ATIII concentration, combined with neuroimaging, may facilitate the assessment of patients with suspected primary CNS lymphoma or support the early detection of occult CNS dissemination of systemic lymphoma or breast or lung cancer.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Fig 1.

(A) Differential expression of CSF proteins that distinguish CNS lymphoma from patients without CNS malignancy. Values are shown as a color scale with positive values represented by red, negative represented by green, and black corresponding to values close to 0. Missing values are shown in white. (B) The concordance between training and test set was exceptional, as shown by comparing the test statistics from the two data sets.

Fig 2.

Intratumoral expression of antithrombin III (ATIII) in diagnostic tumor specimens of primary CNS lymphoma, confirmed by quantitative real-time polymerase chain reaction (n + 20). Intratumoral expression of ATIII RNA was highest in patients with the shortest survival in this cohort (< 6 months).

Fig 3.

Immunohistochemistry demonstrating antithrombin III (ATIII) expression by tumor cells and neovasculature in CNS lymphomas. Magnification = 100 $\times$ (A) and 400 $\times$ (B). (C) von Willebrand Factor expression by all of the vessels. (D) Absent ATIII expression by vessels of healthy brain. compared CSF from eight patients with CNS lymphoma to nine controls without brain tumors. We identified more than 1,000 molecular ions corresponding to 196 CSF proteins expressed at different concentrations (FDR < 0.1), either higher or lower, in CNS lymphoma patients compared with control subjects without CNS malignancy.

Fig 4. CSF antithrombin III (ATIII) concentration distinguishes CNS lymphoma from non-neoplastic CNS conditions. Receiver operating characteristic analysis of ATIII concentration in cross-validation set (n + 101 patients).

Fig 5.

Two patients with focal brain lesions not amenable to biopsy. (A) Diagnosis was ultimately established as neurosarcoid (CSF antithrombin III [ATIII] concentration was 0.5 $\mu\text{g/mL}$). (B) Diagnosis was lymphoma (CSF ATIII level was 2.1 $\mu\text{g/mL}$).

Fig 6.

CSF antithrombin III (ATIII) concentration $> 1.4 \mu\text{g/mL}$ is associated with shorter overall survival in patients with CNS non-Hodgkin's lymphoma at first relapse ($n = 11$; Kaplan-Meier method). Karnofsky performance score < 70 also correlated with short survival ($P < .009$); however, neither elevated total CSF protein nor age > 60 years correlated with shortened survival at first CNS relapse.

Fig 7. Relationship between CNS lymphoma progression and CSF concentration of antithrombin III (ATIII). (A, B) Negative magnetic resonance imaging (MRI), positive CSF cytology. (C) Progression of disease. (D) Response after systemic chemotherapy. CNS lymphoma progression and therapeutic response are reflected by rise and fall in CSF concentrations of ATIII in specimens obtained at time of each MRI. Columns, mean; bars, standard deviation.

Fig 8. CSF antithrombin III (ATIII) concentration reflects persistent disease in patient with refractory CNS lymphoma (A) Remission. (B) Relapse of CNS lymphoma; remission was later achieved after brain irradiation. (C) Two months after irradiation, the patient exhibited new cranial nerve deficits and gait instability; magnetic resonance imaging of brain and spine as well as CSF cytology were negative. Columns, mean; bars, standard deviation.

Table 1

Differentially Expressed CSF Proteins in CNS Lymphoma

GI No.	Protein Description	FDR Training	Fold Change Training	Fold Change Test
112877	Alpha-1-acid glycoprotein 1	0.003	6.49	15.1
231458	Alpha-1-acid glycoprotein 2	0.003	5.63	4.45
112874	Alpha-1-antichymotrypsin	0.003	4.88	3.055
69,990	Alpha-1-B-glycoprotein	0.004	5.26	4.71
112907	Alpha-2-antiplasmin	0.005	2.36	3.01
66932947	Alpha-2-macroglobulin	0.007	4.23	9.9
122801	AMBP protein	0.003	4.65	3.02
113936	Antithrombin-III precursor	0.003	2.85	3.94
113992	Apolipoprotein A-I	0.009	2.4	3.43
114000	Apolipoprotein A-II	0.007	6.75	2.8
114026	Apolipoprotein C-III *	0.014	3.93	5.81
114034	Apolipoprotein D	0.033	2.64	4.23
416733	C4b-binding protein alpha chain *	0.063	3.79	15.06
116117	Ceruloplasmin precursor	0.003	6.93	3.82
84028186	Chitinase-3 like protein 1	0.004	3.63	2.7
116533	Clusterin precursor	0.003	2.71	1.99
20178281	Complement C1q subcomponent	0.007	2.89	2.91
119370332	Complement C3 precursor	0.003	5.37	7.15
179674	Complement C4A precursor	0.008	3.14	4.07
116607	Complement C5 precursor	0.006	2.48	2.94
66347875	Complement component 1	0.089	1.57	2.5
1352108	Complement component C9	0.015	2.36	2.65
67782358	Complement factor B preproprotein	0.08	1.64	2.63
85681919	Complement factor H (H factor 1)	0.003	6.76	4.13
116133	Complement factor I	0.003	3.4	4.21
9973182	EGF-EMP-1 (Fibulin-3) *	0.007	2.55	4.07
4557485	Ferroxidase	0.005	2.85	2.46
74355612	Fibrinogen alpha chain precursor	0.011	6.81	7.88
70906437	Fibrinogen gamma-A chain	0.013	13.1	9.3
1708182	Hemopexin precursor	0.003	4.15	4.37
119964726	Insulin-like growth factor 2 receptor *	0.012	2.32	2.35
31542984	Inter-alpha (globulin) inhibitor H4	0.011	2.73	3.28
116242595	Inter-alpha-trypsin inhibitor	0.006	3.53	3.45
547754	Keratin, type II cytoskeletal 2 *	0.003	3.27	9.34
386853	Kininogen, LMW precursor	0.005	2.84	3.89
116856	Matrix metalloproteinase-2*	0.023	2.87	3.16

GI No.	Protein Description	FDR Training	Fold Change Training	Fold Change Test
20141203	Monocyte differentiation antigen CD14	0.005	4.58	4.16
127857	Neural cell adhesion molecule 1	0.004	4.19	4.97
124106290	Neurexin 3-alpha precursor	0.016	5.22	7.67
27923828	Neurocan core protein precursor	0.048	2.7	3.59
129260	Osteopontin precursor	0.004	3.99	4.28
29789403	PDZ domain containing 8*	0.009	3.03	3.73
21361845	Peptidoglycan recognition protein L	0.005	5.72	4.51
7706387	Plasma glutamate carboxypeptidase*	0.003	3.49	6.86
124096	Plasma protease C1 inhibitor	0.007	3.22	5.05
62298174	Plasma retinol-binding protein	0.009	4.46	2.89
130316	Plasminogen precursor	0.01	2.41	2.95
730305	Prostaglandin-H2 D-isomerase	0.014	7.68	4.56
27754773	Protocadherin 1 isoform 2 precursor	0.035	1.96	3.36
4759166	Secreted phosphoprotein 1*	0.043	2.54	6.78
21359871	SPARC-like 1	0.01	1.99	3.49
24212500	Testican-2 precursor	0.003	3.42	3.52
136464	Transthyretin precursor*	0.003	5.09	6.85
119571467	Vitronectin precursor	0.003	6.74	4.77
141596	Zinc-alpha-2-glycoprotein precursor	0.008	3.06	5.11
41406057	Amyloid beta A4 protein precursor isoform c	0.041	0.42	0.26
41406055	Amyloid beta A4 protein precursor isoform b	0.025	0.43	0.32
28558769	Amyloid-like protein 1 precursor (APLP)	0.039	0.52	0.33
38372935	Brevican isoform 1	0.011	0.42	0.47
1705552	Cadherin-13 precursor	0.035	0.5	0.32
41281561	Calsynenin 1	0.062	0.47	0.34
27894376	Cell adhesion molecule (L1CAM homologue)	0.014	0.48	0.31
180529	Chromogranin A precursor	0.019	0.24	0.33
4502805	Chromogranin A	0.006	0.27	0.33
7706244	Divalent cation tolerant protein CUTA*	0.045	0.48	0.29
2497301	Contactin precursor	0.022	0.47	0.32
23396772	Ectonucleotide pyrophosphatase	0.003	0.21	0.32
51464412	Hypothetical protein XP379250*	0.011	0.42	0.38
61744426	Kallikrein 6 isoform B	0.056	0.51	0.28
51470760	KIAA0830 protein	0.019	0.41	0.33
20141464	Lumican precursor	0.049	0.58	0.31
81158224	Neuronal cell adhesion molecule	0.02	0.45	0.28
3183087	Neuroserpin (Protease inhibitor 12)	0.078	0.56	0.27
129770	Proenkephalin A precursor	0.017	0.38	0.3

GI No.	Protein Description	FDR Training	Fold Change Training	Fold Change Test
2494289	Protein kinase C-binding protein NELL2	0.011	0.47	0.36
19743912	Protein tyrosine phosphatase, receptor type [*]	0.012	0.42	0.3

NOTE. FDR = 0.1 in training set, unadjusted $P = .01$ in test set and same direction of change. Fold change is defined as 2 to the power of the difference between means in CNS lymphoma and control patients.

Abbreviations: GI, gene identification; FDR, false discovery rate.

^{*}Proteins in which only one component peptide was identified.