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Cerebrospinal fluid proteome of patients with acute Lyme disease

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Abstract

During acute Lyme disease, bacteria can disseminate to the central nervous system (CNS) leading to the development of meningitis and other neurologic symptoms. Here we have analyzed pooled cerebrospinal fluid (CSF) allowing a deep view into the proteome for patients diagnosed with early-disseminated Lyme disease and CSF inflammation. Additionally, we analyzed individual patient samples and quantified differences in protein abundance employing label-free quantitative mass spectrometry based methods. We identified 108 proteins that differ significantly in abundance in patients with acute Lyme disease from controls. Comparison between infected patients and control subjects revealed differences in proteins in the CSF associated with cell death localized to brain synapses and others that likely originate from brain parenchyma.

Keywords

Proteomics; mass spectrometry; Lyme disease; cerebrospinal fluid; Lyme neuroborreliosis

INTRODUCTION

Lyme Disease, caused by *Borrelia burgdorferi sensu stricto*, is the most common tick-borne disease in the United States [1]. Vector-associated bacterial transmission from animal to human occurs as a consequence of an infected arthropod, the *Ixodes* tick, biting and taking a blood meal from a human host. Bacterial transmission occurs via tick salivary secretions after sustained attachment of an infected tick [2]. Exposure to any of four or more distinct *Borrelia burgdorferi ss* genotypes may lead to a disseminated infection [3, 4] and dissemination to the CNS results in neurologic symptoms and clinical findings [2, 5] that commonly include Lyme meningitis, often accompanied by cranial neuritis. Approximately 10–15% of untreated patients will develop neurologic Lyme disease [6].

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Conflict of interest statement

Thomas E. Angel- no conflict, Jon M. Jacobs- no conflict, Robert P. Smith- no conflict, Mark S. Pasternack- no conflict, Susan Elias- no conflict, Marina A. Gritsenko- no conflict, Anil Shukla- no conflict, Edward C. Gilmore- no conflict, Carol McCarthy- no conflict, David G. Camp II- no conflict, Richard D. Smith- no conflict, H. Shaw Warren- no conflict

Cerebrospinal fluid (CSF) is routinely sampled to identify the presence of and monitor indicators of ongoing pathological processes in the CNS. Amongst other tests, clinicians measure total protein concentration, although until recently little has been known regarding the specific protein composition, or the proteome, of CSF in normal or disease states [7, 8]. The intimate contact between the CSF and the brain parenchyma provides the rationale for proteome analysis of CSF as a means for gaining insight into neurological disease processes. Mass spectrometry (MS) and gel-based proteome analyses of CSF have been successfully applied to the characterization of normal subjects [7] and neurological diseases including Alzheimer's disease [9], multiple sclerosis [10], Parkinson's disease [11], chronic fatigue syndrome and neurologic post treatment Lyme disease Syndrome [8](reviewed in [12]).

Presently, there is a lack of knowledge of the broad spectrum of changes in proteins bathing the CNS following early-disseminated Lyme disease. Here we characterize the CSF proteome for patients with acute Lyme neuroborreliosis as compared to the CSF proteome of control patients without evidence of CNS inflammation.

MATERIALS AND METHODS

Patient and control sample description

CSF samples with elevated WBC counts (ie, >5 WBCs/mm3) obtained from patients who underwent lumbar puncture for evaluation of neurologic symptoms associated with Lyme disease between 2006 and 2009 at either Massachusetts General Hospital or Maine Medical Center were provided for this study (see Table 1). All protected health information was removed prior to use, and only fully de-identified CSF aliquots were submitted for proteomic analysis. In this study we analyzed CSF from patients with Lyme disease (n=26) and control CSF samples (n=19). The mean age of Lyme disease patients was 27 (range 4–67), and mean age of controls 44 (3–83) (Supplemental table S1). Seventeen cases were male and 9 were female; among the control group 11 were male and 8 were female. Based upon CDC guidelines (2011), 22 cases met criteria for confirmed Lyme disease. The four other cases were diagnosed as Lyme disease on the basis of supportive clinical and laboratory data, but completion of two tiered laboratory testing was not documented. Recent or concurrent erythema migrans rashes were documented in 8 cases, and cranial neuritis, almost entirely VII th nerve palsies, was noted in 14 (see Table 1). One case did not meet our criteria for CSF pleocytosis, but had clinical signs of meningismus. All patients had positive serology for antibodies to Lyme disease and four (of six tested) had positive CSF/serum index for antibodies to *B. burgdorferi* consistent with intrathecal antibody synthesis. Western blots were interpreted according to CDC criteria[13].

Our comparator control group consisted of CSF from patients who had lumbar punctures performed for a variety of clinical indications. Fever was present in 11/19 control patients. A variety of other diagnoses (postoperative fever, alcohol withdrawal, pneumonia, pyelonephritis) was made in 6 of the 11 cases. Of the 5 remaining cases presenting with fever and headache, 2 had completely negative Lyme blood and/or CSF diagnostic studies and one had a positive serum IgM immunoblot, negative IgG immunoblot, and negative CSF capture EIA studies and was not felt to have central nervous system Lyme disease. Two patients were diagnosed with non-meningitic viral syndromes based on their clinical, laboratory, and epidemiologic features and resolution without therapy for neuroborreliosis.

CSF samples used in this study were transported to the microbiology laboratory, clarified by centrifugation, and the supernatants stored at −70 °C prior to shipping overnight on dry ice to the Pacific Northwest National Laboratory, and stored at −80 °C prior to preparation and analysis.

The study protocol was approved by the institutional review boards of the Massachusetts General Hospital, Maine Medical Center, and Pacific Northwest National Laboratory. The use of excess cerebrospinal fluid, a clinical specimen collected solely for non-research purposes, falls within the guidelines for expedited review promulgated by the Office for Human Research Protections, Department of Health and Human Services following the passage of HIPAA.

BORRELIA culture conditions

The Bb strain B31 from the American Type Culture Collection (ATCC®) was obtained and expanded by incubation in Barbour-Stoenner-Kelly (BSK) media with antibiotics for 7–10 days at 34 °C until log phase was reached. A Petroff-Hausser hemocytometer under dark-field microscopy was used to determine when log phase was reached (70–80% confluency, 70–80% viability, $\approx 10^7$ spirochetes/mL). The cells were expanded, washed by centrifugation, and killed by adding solid urea to each sample to a final concentration of 8 M. Control samples were Hanks added to CSF (1:3.28) with 8 M urea.

Proteomic methods

Our characterization of the CSF proteome was performed in a manner similar to that previously reported [7, 8]. Briefly, pooled CSF samples were subjected to trypsin proteolysis and then fractionated by strong cation exchange (SCX) chromatography. The resultant fractionated peptides were identified, following reversed phase liquid chromatography (LC) separation, by tandem mass spectrometry (MS/MS) for creation of the accurate mass and time tag database. CSF samples from individual patients were analyzed by high resolution LC-MS employing the accurate mass and time tag label free quantification approach [7, 8].

Protein digestion

CSF peptide samples were prepared as previously described [8]. Briefly, CSF proteins were denatured with 8 M urea and disulfide bonds were reduced with 5 mM dithiothreitol at 37 °C for 60 min. Proteins were then digested with porcine Trypsin (Promega, Madison, WI) over night at 37 °C. Tryptic peptide mixtures were cleaned up by solid phase extraction, with a 1-mL SPE C18 column (Discovery DSC-18, Supelco, Bellefonte, PA) as described previously [14]. Final peptide concentrations were determined by BCA assay (Pierce). All tryptic digests were snap frozen in liquid nitrogen and stored at −80 °C until further processing and analysis. Ammonium bicarbonate and acetonitrile were from Fisher Scientific (Fair Lawn, NJ). Sequencing grade, modified trypsin was from Promega (Madison, WI). Bicinchoninic acid (BCA) assay reagents and standards were from Pierce (Rockford, IL). All other reagents were from Sigma-Aldrich (St.Louis, MO). Water was purified using a Barnstead Nanopure Infinity water purification system (Dubuque, IA).

Off-line strong cation exchange (SCX) fractionation

Tryptic peptide mixtures from pooled CSF samples from either 12 control samples or 9 patient samples were fractionated by strong cation exchange (SCX) chromatography to reduce sample complexity. 460 Pg of tryptic peptides from pooled patient samples or 700 μg from control pooled samples were suspended in a final volume of 900 μL in 10 mM ammonium formate, pH 3.0, 25% acetonitrile and fractionated by SCX chromatography as described previously [14]. A total of 35 SCX fractions were collected for the patient pooled sample and 25 fractions from the pooled control sample and lyophilized prior to high resolution, gradient, reversed phase capillary LC-MS/MS analysis.

Reversed phase capillary LC-MS/MS and LC-MS analysis

Peptide fractions were separated by high resolution, reversed phase capillary liquid chromatography as previously described using an automated four-column capillary liquid chromatography system [15] in-line with mass spectrometers modified with electrodynamic ion funnels developed and assembled in-house [16]. SCX fractions were analyzed using an LTQ mass spectrometer and label free quantification of CSF was performed as previously described [8].

Data analysis

LTQ raw data was extracted using Extract_MSn (version 3.0) and analyzed with the SEQUEST algorithm (V27 revision 12) searching the MS/MS data against the human IPI database (Version 3.39) and sequences from the *B. burgdorferi* B31 protein database that contained 1737 protein entries. Database search parameters and FDR analysis was performed as previously described [8]. Analysis of quantification of the differences in protein abundance between control and LM patient CSF samples was performed as previously described [8] and visualized using in-house software DAnTE [17]. Briefly, peptide intensities from the LC-MS analyses were log2 transformed. Peptide abundances were then "rolled up" to the unique protein level employing the Z-rollup method (based on trends observed at the peptide level) implemented in DAnTE. ANOVA and clustering analyses were also performed using DAnTE. Functional enrichment and pathway analysis was performed with Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Right-tailed Fisher's exact test was used to calculate a p-value determining the probability that each biological function and/or disease assigned to our data set is due to chance alone. Annotated tissue expression profile for CSF proteins was retrieved from Ensemble-Biomart (www.Ensembl.org)

RESULTS

We performed an in-depth analysis of the CSF proteome, followed by a quantitative analysis of differences in protein abundance occurring in the CSF of 26 patients with Lyme disease and evidence of CNS dissemination and from 19 control subjects without Lyme disease. For the deepest view of the CSF proteome, we first analyzed an unbiased subset of pooled patient and control samples by LC-MS/MS following fractionation by strong cation exchange (SCX) chromatography. We then analyzed a second set of individual patient and control samples that lacked sufficient sample volume for fractionation, leveraging the accurate mass and time (AMT) tag database initially created from the analysis of the pooled sample sets. This allowed for high throughput label-free quantitative analysis of the differences in CSF protein abundance profiles between patients with disseminated Lyme disease with neurologic symptoms and control subjects.

Analysis of pooled CSF samples for human proteins

In-depth analysis of highly fractionated pooled patient (n=9) and control (n=12) samples resulted in the identification of 5625 peptides covering 1104 proteins in the Lyme patient pooled samples and 4778 peptides corresponding to 906 proteins in the control pooled samples. There were 1458 non-redundant (unique) proteins confidently identified in total, with 552 proteins being identified in both Lyme disease and control samples. A total of 552 proteins were uniquely identified in the Lyme pooled sample and 354 proteins were uniquely identified in control subjects (Figure 1 and Supplementary Table S2). Approximately 60% of the proteins identified CSF samples were unique to the CSF proteome and not present in the plasma proteome [14] (Supplementary Table S2). Collectively 49% of the CSF proteome of Lyme and control subjects were annotated as being expressed in CNS associated tissues (Supplementary Table S2 and Figure 2). A comparison between

proteins identified in previous studies of brain postsynaptic density (PSD) tissue [18], plasma [14], and CSF revealed 111 proteins common to all three proteomes and 102 proteins (7%) were identified in the CSF and brain tissue, but not in blood plasma (Supplementary Table S2 and Figure 3). There were approximately 2 times more plasma proteins uniquely identified in the CSF of Lyme subjects compared to control, consistent with the likelihood of reduced blood-brain barrier integrity.

Pathway analysis for all proteins identified in the pooled CSF samples (employing Ingenuity Pathway Analysis, www.ingenuity.com) led to the identification of enriched canonical signaling associated with acute phase signaling response, complement cascade, coagulation, and both intrinsic and extrinsic prothrombin activation pathways (Supplementary Table S2), consistent with a pro-inflammatory response. Analysis of enrichment of protein function identified in only one of the two pooled samples (disease or control) or common to both was performed (Figure 1). Notable was the presence of 116 proteins (29% of the unique proteins) associated with cell death for the Lyme patients; this functional category was not enriched in the proteins uniquely identified in the control sample or in the proteins common to both pooled samples. These results are consistent with previous reports showing that *Borrelia* induces inflammatory processes resulting in apoptosis of neuronal cells [19, 20].

Analysis of pooled CSF samples for bacterial proteins

In addition to searching for peptides derived from human proteins, the LC-MS/MS data sets were searched for peptides derived from *Borrelia*. We were unable to detect any peptides from *B. burgdorferi.*

Determination of detection limit of bacterial peptides

We spiked a range of *B. burgdorferi* (10^4, 10^5, 10^6, and 10^7 organisms per mL of CSF) into control CSF to determine our nominal level of detection for bacterial proteins in unfractionated CSF. We detected bacterial derived proteins, such as the antigenic outer surface lipoproteins OspA and OspB among others, in CSF spiked with 10^5 or greater Bb organisms per mL.

Analysis of non-fractionated individual CSF samples

Analysis of non-fractionated CSF from a second set of individual Lyme patient samples and control subjects employing a label-free quantitative MS-based approach [21] was performed. Analysis of CSF from 26 Lyme patients and 7 control subjects resulted in the identification of 247 unique proteins (2 or more peptides identified for all proteins) across all samples (Supplementary Table S3).

Comparison of quantified protein abundances between Lyme patient and control samples employing a one way analysis of variance (ANOVA) resulted in 108 proteins being identified as significantly different in abundance ($p \leq 0.05$), where 48 were decreased and 60 increased in Lyme compared to control samples (Figure 4A). Unsupervised hierarchical clustering of the 108 proteins revealed that there were identifiable disease-associated differences in the CSF proteome that allowed for the partial separation of disease and control samples (Figure 4B). Samples organized into two main clusters, one cluster being composed of patients (right, cluster 1) and the second cluster (left, cluster 2) being a mixture of negative control samples and 7 patient samples. Annotation of the protein-tissue expression profile, employing Ensembl-Biomart (www.ensembl.org/biomart), was performed (Supplementary Table S4 and Figure 2). The greatest numbers of significantly different proteins (20/31 annotated proteins) were annotated as being expressed in the superior cervical ganglion and trigeminal ganglion. Additionally, eleven proteins (gene symbols – CD59, CNTN1,CSF1, FBLN1, FCGBP,HRG, LYNX1, NCAM1, NEGR1, PZP,

THY1) expressed in the dorsal root ganglion differed in abundance in Lyme patient CSF samples. These findings were consistent with a previous report of dorsal root ganglion inflammatory lesions in non-human primate studies of neuroborreliosis [20]. Network analysis of proteins that were found to be significantly different by ANOVA ($p \leq 0.05$) in abundance between Lyme patient samples and control subjects and known functional and disease related activities, shown in Figure 5, highlights the quantitative differences identified in the CSF proteome. Notably, there is a reduction in the amyloid precursor protein (gene symbol, APP) in patient samples and this protein is at a highly connected node, participating as a member of a large subset of proteins that have altered abundance levels in diseased versus control state (Figure 5). Many of the proteins that displayed differential abundance are associated with neurological disorders.

The descriptive and discriminating power of the differences in protein abundance can be quantified employing a receiver operating characteristic (ROC) curve analysis by calculating the area under the ROC curve (AUC). The discriminating power of each of the 247 proteins was calculated. Selection of proteins that were significantly different in abundance by ANOVA (p values ≤ 0.05) and that had an AUC ≥ 0.8 resulted in identification of a panel of 13 proteins (Figure 6).

We compared the identified proteins that significantly differed in relative abundance in the Lyme CSF proteome with those previously reported significantly changed for subjects with chronic neurological diseases for which there are data (Parkinson's Disease [11], multiple sclerosis [12], Alzheimer's disease [9], and late (post-treatment) neurological Lyme disease [8] (Supplementary Table S5). There were 53 proteins that uniquely differed in Lyme disease (not identified as changing in any of the chronic diseases above), and of these proteins, 37 were previously identified in the plasma proteome [14].

DISCUSSION

Here we present, for the first time, a detailed analysis of the proteome of the CSF of patients with acute Lyme neuroborreliosis. We found that the proteome reflects increased immune activation, with components of the complement cascade in particular being increased in abundance.

Proteins in the CSF originate from plasma proteins crossing the blood-cerebrospinal fluid barrier as well as proteins from the brain parenchyma and associated tissues crossing the brain-cerebrospinal fluid barrier [22, 23]. CSF functions as a sink for degradation products resulting from both normal and pathologic processes occurring in the brain parenchyma and meninges. Accordingly, differences in CSF proteome composition in patients with Lyme disease presumably reflect increased innate immune activation with a cellular response, proteins from brain tissue itself, and plasma proteins from damage to the blood-brain barrier [24].

Limited data are available on the characteristics of the CSF proteome in meningitis due to different bacterial or viral pathogens. A recent proteome analysis of CSF in patients with pneumococcal meningitis [25] highlighted an array of proteins up-regulated in a disease with a massive inflammatory reaction characterized with a predominantly neutrophil response. We identified many differentially abundant proteins in the Lyme CSF samples that play central roles in innate immunity. These data were consistent with a prior report of increased levels of the marker of innate immune activation, neopterin, in CSF of patients with neurologic Lyme disease [26]. We observed decreased levels of amyloid precursor protein (APP) in LM patient samples compared with normal subjects consistent with previous reports by Mattsson et al. [27]. Amyloid metabolism is sensitive and is markedly affected by inflammation

associated with innate immune activation, as has been reported for several neurodegenerative diseases [28]. Reduction in APP abundance was also observed as a function of patient disease severity for viral encephalitis in the case of HIV infection [29]. Kallikrein 6 (KLK6) is present at reduced levels in Lyme samples and is thought to play a role in removal of α-synuclein and prevent its polymerization, where accumulation is associated with Lewy body formations and Parkinson's disease [30]. Additionally it has been shown to generate amyloid fragments from amyloid precursor protein in cell culture, and may in part contribute to the reduced levels quantified in the CSF of Lyme patients [31].

The small chemokine CXCL13 has been proposed for use as a biomarker of neurologic Lyme disease [32]. CXCL13 is a B lymphocyte chemo-attractant and ligand for the G protein-coupled receptor CXCR5. Increased levels in the CSF are associated with increased numbers of B lymphocytes in the CSF [32]. CXCL13 has been shown to be released by monocytes following exposure to the outer surface proteins from *Borrelia* and recognition by the toll like receptor 2 (TLR2).[33] We did not identify CXCL13 in our broad coverage of the CSF proteome, and CXCL13 was not identified in previous analyses of human plasma or normal CSF proteomes [7, 14]. It is possible that it is below the limit of detection by the methods used [34, 35] and a targeted proteomics approach applying LC-SRM-MS and synthetic, stable isotopically labeled peptides may eventually lead to detection of CXCL13 in patient blood plasma in future studies [36]. In contrast, we did identify proteins such as CD44, which is also associated with TLR2 signaling and plays a role in the presentation of lipoproteins (such as OspC from Bb [37]) to the TLR2 signaling complex. CD44, decreased in abundance in the CSF proteome for Lyme patients (Figure 5), has been associated with inflammation (reviewed in [38]), and has also been reported to play a role as a negative regulator of TLR mediated inflammation [39].

We identified 13 proteins out of 247 possible discriminating proteins with favorable receiver operating characteristic curves. This group of proteins constitutes a potential candidate protein profile for discriminating Lyme from control samples. Several proteins in the panel were associated with the complement cascade: C1QB, C1QC, and VTN. C1QB and C1QC are positive effector molecules for innate immunity, while VTN is a negative effector [40]. Complement activation and increased levels of C1q and C3a in CSF as a consequence of CNS involvement in Lyme disease have been previously reported[41]. Receiver operating characteristic analysis revealed that complement component C1QC shows the greatest discriminating power for all proteins with an AUC of 0.95. Osteopontin (SPP1) was reduced in Lyme patient samples and had an AUC of 0.81. Osteopontin has been shown to act as a cytokine, enhancing the production of interferon gamma and IL-12 [42]. Pathway analysis showed that these proteins are linked to the centrally located and highly connected amyloid precursor protein (Figure 5).

Of particular interest were proteins identified and known to be present in brain tissue. We detected 20 proteins differing in abundance in patients with Lyme that were present in the postsynaptic density (PSD) tissue. These proteins directly or indirectly participate in signal transduction events that underlie the processes of learning and memory [18]. The presence of these proteins in the CSF may reflect restructuring of the synaptic arbor as a consequence of toxic processes such as inflammation coincident with infection.

Comparison of the data set with a recent report describing the CSF proteome of patients with post-treatment Lyme disease[8] revealed 11 proteins in common that differed from controls in abundance: C1QB, C1QC, IGFBP6, SERPINA3, GSN, CD44, CNTN1, CNDP1, AGT, OMG, and ORM2. Several of these proteins are brain-derived and participate in axonal maintenance and growth regulation. The relationship in pathophysiology, if any, between

acute meningitis in Lyme disease and late neurological post-treatment Lyme disease is not clear.

We failed to detect evidence of bacterial peptides in any of our samples. Bacterial load in the CSF is low even when there is clinical evidence of dissemination, with CSF often being both culture and PCR negative [43]. *Ex vivo* spiking experiments required a level of 10^5 organisms per mL for detection.

There are several possible limitations to our study. Formal and complete Lyme disease testing was not available in all control patients, since in many patients epidemiologic and clinical features were not consistent with Lyme disease. CSF pleocytosis is considered to be an important finding in patients with acute Lyme meningitis [44, 45], so the absence of CSF pleocytosis in all of our control patients supports their inclusion for this analysis. The absence of pleocytosis also makes the presence of other acute meningitic processes unlikely. In addition, we are unable to resolve if the findings that we report are specific to meningitis caused by *Borrelia burgdorferi* as compared to other infectious agents that might cause a lymphocytic meningitis. Future studies that compare the proteome in Lyme disease with the proteome in the CSF of other acute infections (i.e., viral or mycobacterial meningitis) that present with a similar, predominantly lymphocytic or monocytic, cellular response will be necessary to determine how much of the response reported here is specific to infection with *Borrelia*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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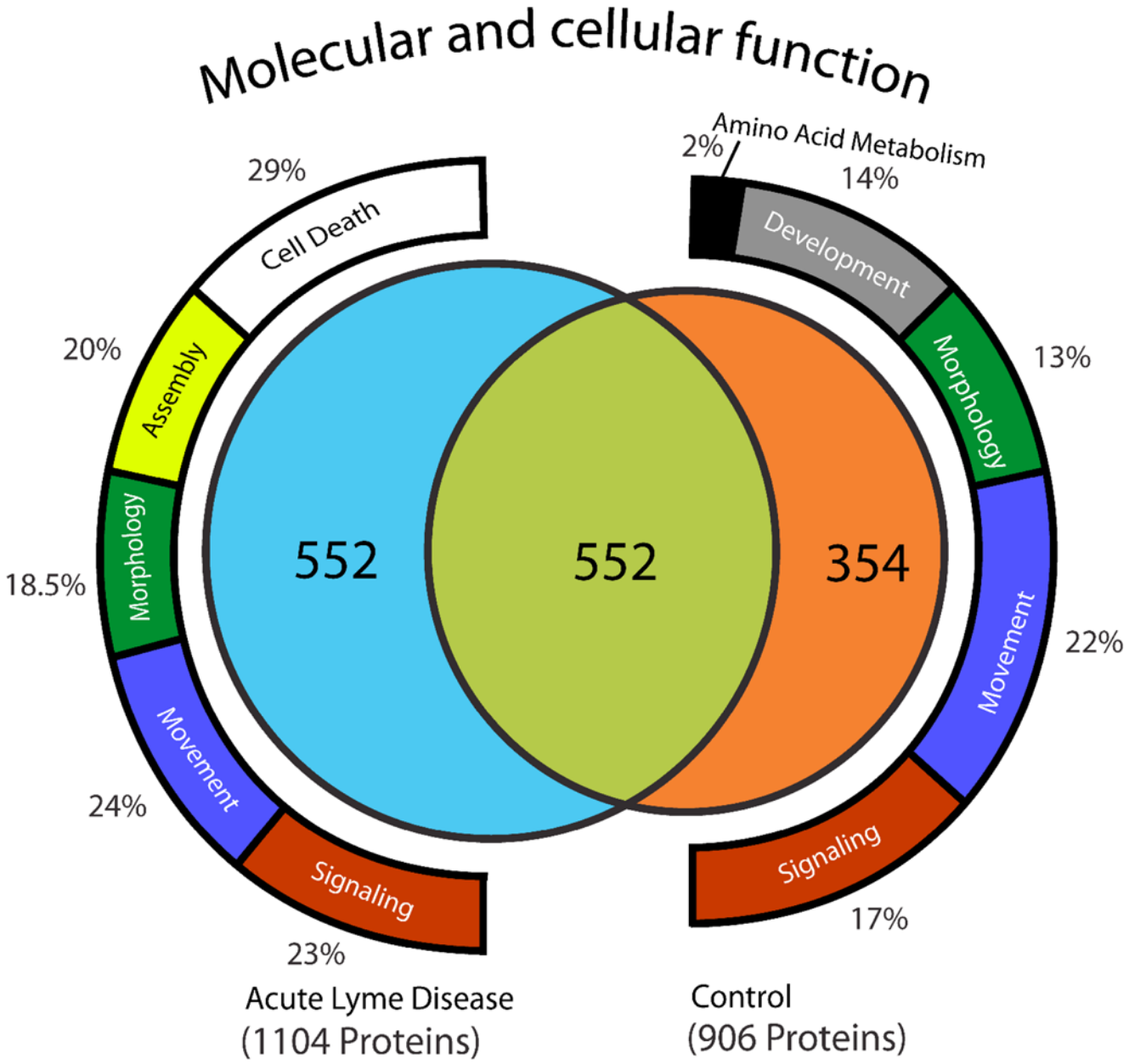

Figure 1.

Cerebrospinal fluid proteome of pooled samples from subjects with acute Lyme disease and non-infected control subjects. Pooled CSF from Lyme disease and control samples were digested, fractionated offline by strong cation exchange, and analyzed by reversed phase LC-MS/MS. We identified peptides attributable to a total of 1458 proteins considering all sample fractions, 906 in the pooled control sample and 1104 in the pooled acute Lyme disease sample. There were 552 proteins common to both samples, 552 were uniquely identified in the acute Lyme disease pooled sample and 354 were uniquely identified in the control pooled sample. Functional analysis for proteins unique to either control or Lyme subject pooled samples was performed employing Ingenuity Pathway tools (www.ingenuity.com) revealing the presence of proteins associated with cell death in the

acute Lyme disease pooled samples. In contrast proteins relating to development and amino acid metabolism were singularly identified in pooled control samples.

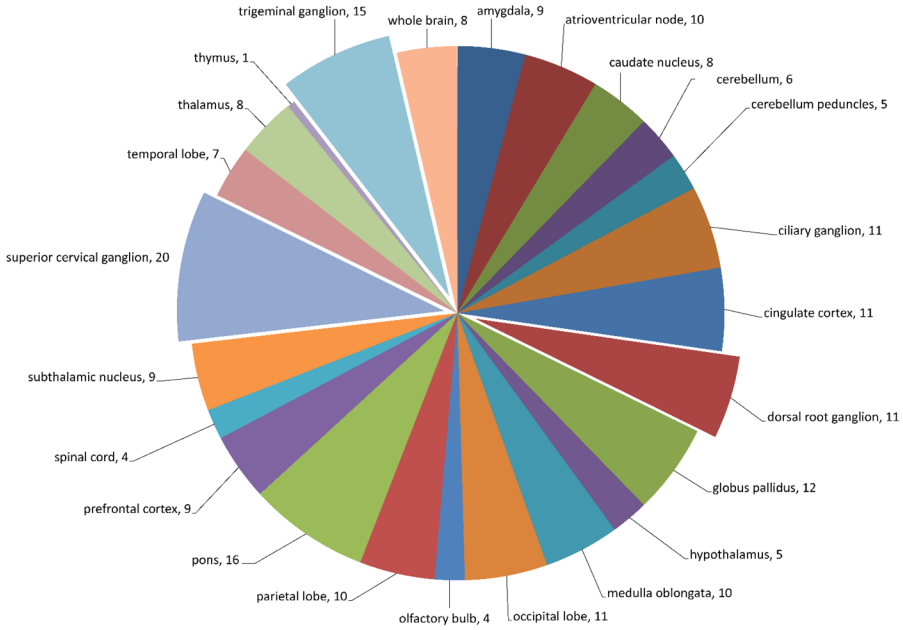

Figure 2.
Distribution pattern for CNS proteins identified in the CSF proteome of Lyme disease and control subject samples.

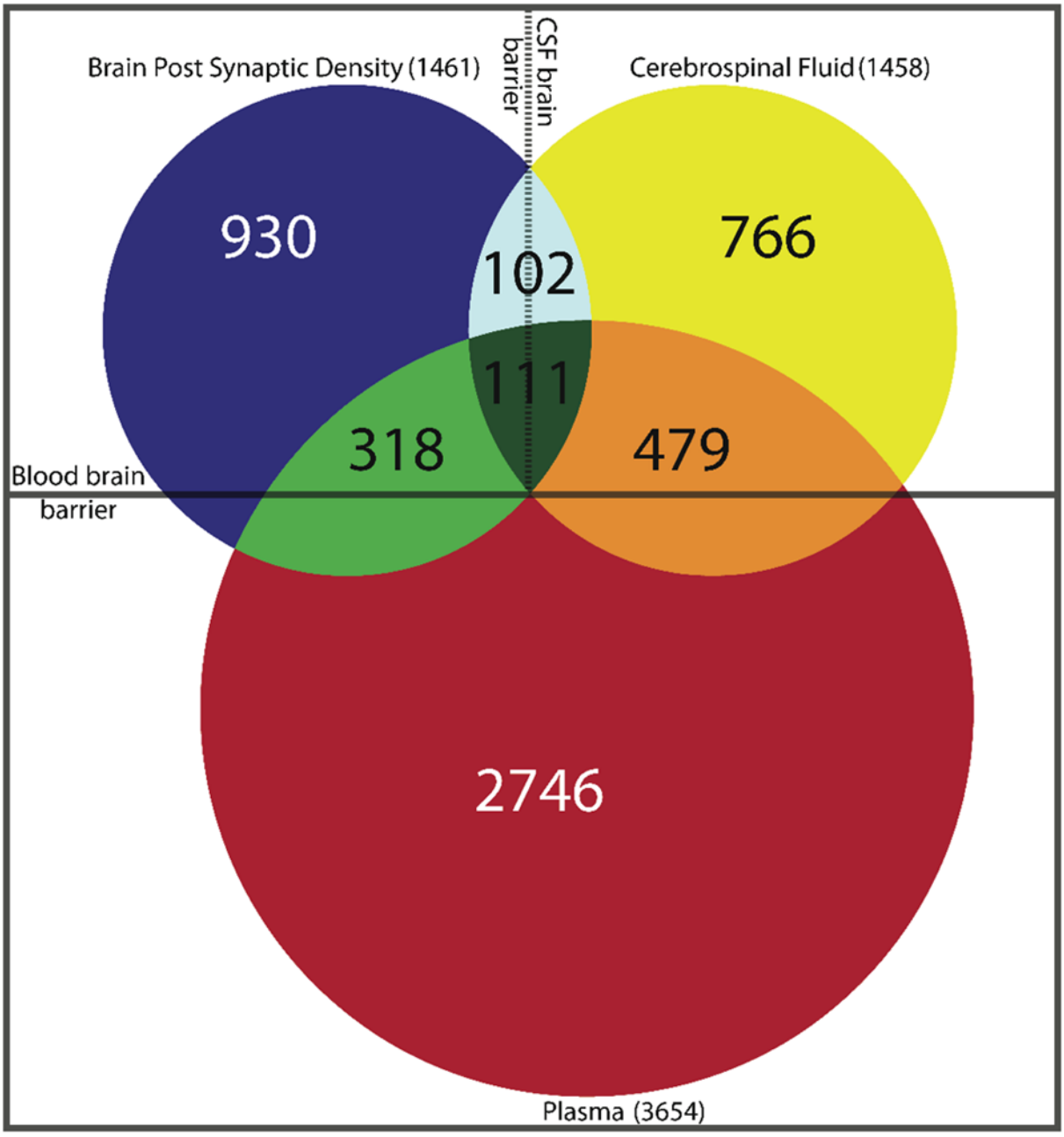

Figure 3.
Venn diagram with CNS compartmentalization. Comparison between the CSF proteome of combined normal subjects and acute Lyme disease patients (yellow circle) with previously reported plasma proteome (red circle) and brain postsynaptic density proteome (blue circle).

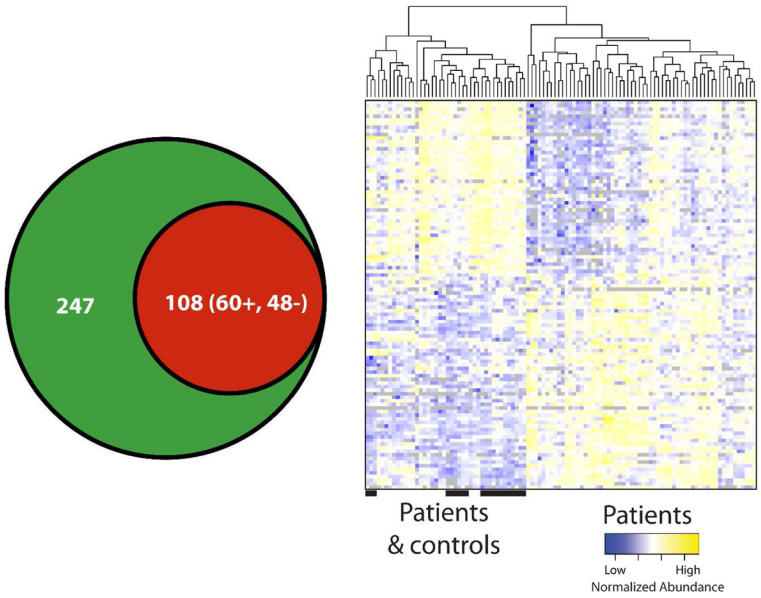

Figure 4.

Analysis of individual patient samples led to the quantification of 247 proteins. An analysis of variance of the protein abundances revealed 108 proteins as being differentially abundant (p value < 0.05) (A) where 60 were increased and 48 were decreased in abundance relative to control. Unsupervised cluster analysis of the subject samples shows that the quantified protein abundances (rows in matrix) allowed for partial segregation of patient and control samples (columns) (B).

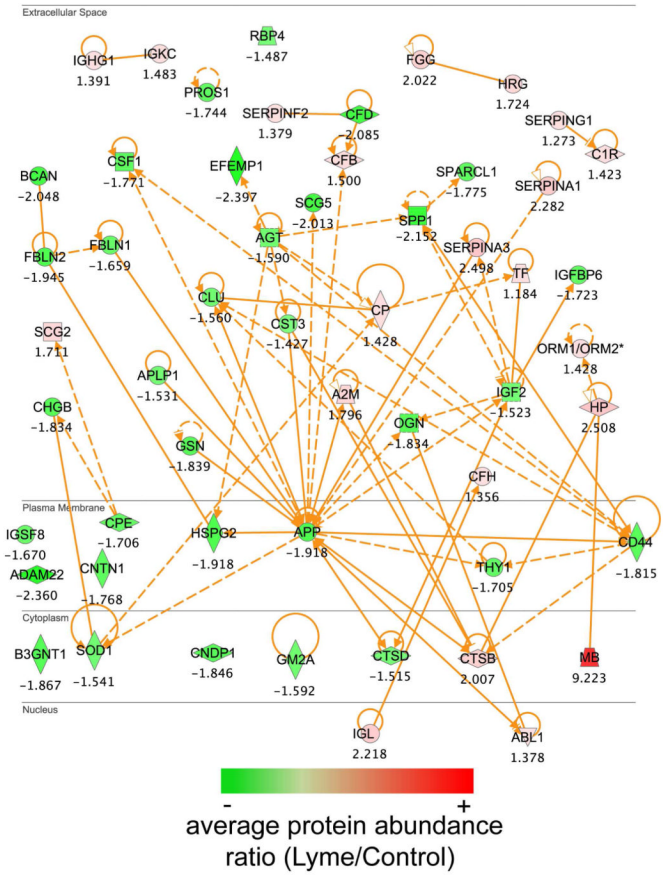

Figure 5.

Network analysis of proteins found to be significantly altered in abundance in the CSF proteome by ANOVA (p value <0.05) following analysis of individual subject samples. Network analysis was performed employing ingenuity pathway tools (www.ingenuity.com) on proteins that were annotated in the Ingenuity database (101 of the 108 proteins). Illustrated are proteins, colored by ratio (Lyme/Control) of average protein abundances, with known functional and disease associated relationships that differed in abundance levels in the CSF.

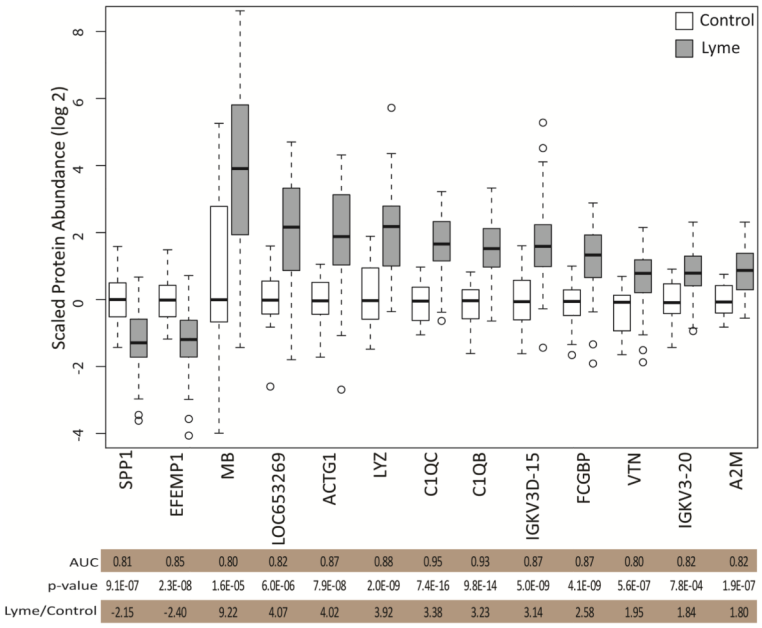

Figure 6.

Proteins with greatest discriminating power for Lyme disease. ROC curve analysis of all proteins identified and quantified in the CSF led to the identification of 13 proteins that had good discriminating power with areas under the curve (AUC) greater than 0.8. Shown are the scaled protein abundance values of the 13 proteins that show the greatest discriminating power in the ROC curve analysis.

Table 1

Clinical and laboratory characteristics of Lyme disease patients.

Pt	Erythema	Cranial	Positive Serology		CSF WBC Differential				Total		Bb Index CSF/Serum	
Number	Migrans	Neuritis	Screen	Western Blot[1]	Total	% Polys	% Lymphs	% Monohistiocytes	Protein	Glucose	Done	Positive
1	no	yes	ELISA	n/a[2]	120	0	87	13	94	49.5	yes	neg
2	yes	no	ELISA	IgG	69	9	76	15	57	47	yes	pos
3	no	no	ELISA	n/a	6.5	22	78	0	35	74	yes	neg
4	no	no	ELISA	IgG	689	8	57	32	185	34	yes	pos
5	no	yes	ELISA	IgG	238	0	88	4	95	48	yes	pos
6	no	no	n/a	IgM	2	3	75	22	19	79	n/a	n/a
7	no	no	ELISA	IgG	495	0	91	0	140	72	n/a	n/a
8	no	no	ELISA	IgG	59	59	100	0	47	58	yes	pos
9	no	no	ELISA	n/a	21	12	78	8	19	71	n/a	n/a
10	yes	no	ELISA	n/a	30	0	85	15	67	114	n/a	n/a
11	yes	no	ELISA	IgM	544	6	85	9	173	29	n/a	n/a
12	yes	yes	ELISA	IgM	316	1	88	11	52	47	n/a	n/a
13	yes	yes	ELISA	IgG,IgM	13	0	79	21	24	55	n/a	n/a
14	yes	yes	ELISA	IgM	37	0	91	9	78	52	n/a	n/a
15	no	no	ELISA	IgG,IgM	56	0	91	9	86	56	n/a	n/a
16	no	yes	ELISA	IgM	146	2	81	17	71	77	n/a	n/a
17	yes	no	ELISA	IgG,IgM	180	1	88	11	135	50	n/a	n/a
18	yes	no	ELISA	IgG	11	55	34	11	22	62	n/a	n/a
19	no	yes	ELISA	IgM	10	10	78	12	30	61	n/a	n/a
20	no	yes	ELISA	IgM	66	3	68	29	25	49	n/a	n/a
21	no	yes	ELISA	IgM	164	1	84	15	55	54	n/a	n/a
22	no	yes	ELISA	IgM	72	3	84	13	53	52	n/a	n/a
23	no	yes	ELISA	IgG,IgM	339	11	83	6	75	51	n/a	n/a
24	no	yes	ELISA	IgM	964	3	93	4	199	38	n/a	n/a
25	no	yes	ELISA	IgG	135	2	89	9	204	83	n/a	n/a
26	no	yes	ELISA	IgM	323	8	74	18	145	49	n/a	n/a

[1] CDC criteria

[2] data not available (test not done)