歴はない．

現病歴：20歳から走るとよく転ぶようになったが，階段昇降は手すりなしで可能であった．25歳で第1子妊娠時から，夜間頻尿，残尿が歩行障害に加わった．通常の妊娠経過をとり，満期自然分娩で出産した．出産後，出血性膀胱炎が出現し，40℃発熱があった．児は母乳で1年哺育した．この頃，首から下の発汗低下，全身倦怠が出現し，頻尿・残尿・歩行障害は継続した．29歳時，第2子を妊娠，満期自然分娩した．出産後，尿路感染症による高熱があった．その後，手足のしびれ感が加わり，31歳時に痙性歩行が悪化し，階段昇降に手すりが必要となり受診し，HAMと診断された．両下肢痙性著明で痙性歩行，四肢深部反射亢進，両側Babinski反射・Chaddock反射陽性，両下肢異常知覚，下肢振動覚低下，内反尖足2度，第12胸髄以下発汗低下，夜間頻尿，残尿，尿失禁，便秘と下痢，外反股で運動機能障害は4/13段階であった．血清抗HTLV-1抗体(PA)32,768倍，血清可溶性IL2レセプター620(145～519U/m*l*)，髄液細胞数6/μ*l*，総蛋白134.7mg/d*l*，髄液抗HTLV-1抗体(PA)512倍，HTLV-1プロウイルス量715コピー/10^4 PBMCであった．

〔症例3〕35歳，女性．

家族歴：母がHTLV-1抗体陽性．既往歴はない．

生活歴：輸血歴はない．

現病歴：12歳から頻尿，排尿困難，便秘が出現．13歳，右足がでにくく，つまづくようになった．15歳で走れず，17歳から右足痙性が出現し，19歳で階段昇降困難となった．22歳時，転倒が多く，歩行障害悪化のため近医を受診した．両下肢痙性歩行，四肢深部反射亢進，両側Babinski反射・Chaddock反射陽性，Th12以下の発汗低下，Th12以下異常知覚，1日14回の頻尿，残尿，便秘を認め，運動機能障害4/13段階であった．血清・髄液抗HTLV-1抗体陽性，髄液ネオプテリン87.8(正常30pmol/m*l*以下)，末梢血HTLV-1プロウイルス量446コピー/10^4PBMCであった．Lip biopsyで導管にリンパ球浸潤を認め，Sjögren症候群を合併したHAMと診断された．23歳からインターフェロンα300万連日投与後，週3回インターフェロン筋注とアジドチミジン100mg投与を受け，ウイルス量は190コピー/10^4PBMCに減少した．運動機能障害は4/13段階のままであった．26歳で第1子を妊娠してから2本杖歩行となり，運動機能障害は6/13段階で，帝王切開で出産した．28歳で第2子を妊娠し，2本杖歩行であったが，帝王切開で出産後，排尿障害が進行し，自己導尿10回，移動に車椅子使用となった．30歳時，血清可溶性IL2レセプター1,062，ウイルス量は956コピー/10^4PBMCに増加し，メチルプレドニゾロン125mg/日3日間点滴したが，両杖歩行と車椅子生活であった．

〔症例4〕55歳，女性．

家族歴：父母がHTLV-1抗体陽性，血族結婚あり．既往歴はない．輸血歴はない．

現病歴：10歳から走りが遅くなった．15歳で痙性歩行が出現．16歳で転倒するようになり，頻尿が出現した．18歳で駆け足ができなくなり，19歳で階段に手すりを要し，下肢の突っ張りが強くなった．20歳で杖を使用し，内反足がみられ，圧迫排尿であった．29歳時にHAMの診断を受けた．両下肢痙性，一本杖で痙性歩行，下顎反射含め四肢反射亢進，足クローヌス陽性，両側Babinski反射・Chaddock反射陽性，下肢筋力低下，下肢振動覚低下，圧迫排尿，頻尿，便秘，内反尖足．血清・髄液抗HTLV-1抗体陽性，髄液で成人T細胞白血病(ATL)様細胞あり，インターフェロンα300万を30日間筋注した．30歳時，末梢血HTLV-1プロウイルス量487コピー/10^4末梢血リンパ球で，運動機能障害は5/13段階であった．31歳時に第1子を妊娠し，帝王切開で出産．運動機能障害は5/13段階であった．36歳時に第2子妊娠，帝王切開で出産後，両杖歩行で運動機能障害は6/13段階であった．55歳現在，下肢筋力低下が進行し車椅子移乗に介助を要するものの，郵便局長として勤務している．

〔症例5〕42歳，女性．

家族歴：なし．

既往歴：13歳右ぶどう膜炎．輸血歴はない．

現病歴：15歳から痙性歩行で靴の外側がすれ，便秘になった．21歳で四肢反射亢進が出現した．23歳で第1子妊娠中期より夜間頻尿となった．妊娠・出産の経過異常はなかったが，出産後，痙性歩行が増強し，2年後走れなくなった．26歳時，両下腿感覚鈍麻，残尿，頻尿がみられ，近

医に入院した．第3腰髄以下発汗低下，両下肢痙性，痙性歩行，軽度下肢筋力低下，四肢反射亢進．両側Babinski反射・Chaddock反射陽性，両足クローヌス，夜間頻尿，便秘，内反尖足，運動機能障害3/13段階であった．血清・髄液抗HTLV-1抗体陽性，髄液細胞数32/3/μl，髄液ネオプテリン205.1pmol/mlと高値で，HAMと診断された．ビタミンC2g/日の大量療法と筋弛緩剤の治療を受け，歩行，頻尿は改善した．39歳時には一本杖歩行で，末梢血HTLV-1プロウイルス量2,619コピー/10^4PBMCと高値であった．

〔症例6〕27歳，女性．

家族歴・既往歴・輸血歴：なし．

現病歴：18歳時献血で抗HTLV-1抗体陽性を指摘された．20歳になって腎盂腎炎を2回生じ，3回目は尿閉となったため，泌尿器科で導尿して，神経因性膀胱の診断をうけた．近医神経内科を受診し歩行障害はないが，両下肢病的反射陽性のため，キャリア外来を受診した．

受診時現症：皮膚アトピー陽性，甲状腺腫，軽度残尿感，第3腰髄以下発汗低下，両下肢軽度痙性，四肢深部反射亢進，Babinski反射およびChaddock反射両側陽性，両下腿痛覚低下，内反尖足2度，運動機能障害0/13段階であった．血清・髄液抗HTLV-1抗体陽性，末梢血HTLV-1プロウイルス量510コピー/10^4PBMC．26歳時に妊娠し，妊娠9カ月過ぎ，発汗低下部位が第12胸髄以下となり，頻尿，残尿，尿失禁があった．よくつまずくようになり，運動機能障害が2/13段階に悪化した．帝王切開で出産，断乳し人工栄養．出産後も運動機能障害は2/13段階で頻尿，尿失禁，残尿，便秘は続いている．

〔症例7〕39歳，女性．

家族歴：母がHAM，父はHTLV-1キャリアで脊髄小脳変性症．

既往歴：30歳で左三叉神経領域の帯状疱疹．輸血歴はない．

現病歴：20歳時，献血で抗HTLV-1抗体陽性を指摘された．33歳時，妊娠2回も人工流産した．便秘あり．34歳から不妊症治療を産婦人科でうけた．34歳から夜間頻尿がみられた．37歳HTLV-1キャリア外来を受診した．

現症：Th7以下発汗低下，両下肢痙性はなく，両下肢反射亢進，両側Babinski反射およびChaddock反射陽性，内反尖足3度，1日尿10回，夜間尿3回の頻尿，便秘がみられ，運動機能障害は0/13段階であった．血清・髄液抗HTLV-1抗体陽性，髄液細胞数17/μl，末梢血HTLV-1プロウイルス量1,076コピー/10^4PBMCであった．デカドロン2mg筋注後，プレドニン10mg/日1週間，プレドニン5mg/日1週間投与し，乳酸菌カゼイン菌シロタ株飲料800億個を継続飲用した．翌年37歳で妊娠し10カ月後自然分娩した．妊娠中，残尿，頻尿が続いたが，出産後は頻尿および残尿は軽減した．出産4カ月後，発汗低下部位は第12胸髄以下に下がり，夜間頻尿消失，便秘改善，運動機能障害は0/13段階のままであった．四肢反射亢進，両側Babinski反射およびChaddock反射陽性，内反尖足はそのままであった．末梢血HTLV-1プロウイルス量は669コピー/10^4PBMCに減少していた．出産後，凍結母乳にしたが，1カ月後人工栄養に変更した．児に異常はみられていない．

症例1～7の妊娠・出産経過と運動機能・排尿障害の変化を表1に示した．

HAM患者の妊娠・出産

HAMと妊娠出産については，Andoらが妊娠22週と12週にHAMと診断した2例について，出産経過，子宮収縮に異常を認めなかったと報告している[5]．自験7例でも妊娠とその継続に問題なく，児への感染防止目的で帝王切開を選んだ2例をのぞいて分娩も正常に行われ，児に特に問題は生じていない．すなわち，HAM患者も正常に妊娠，出産を経て児を得ることが可能であることを示している．一方で，Mizokamiらは第1子出産後HAMが発症し，第2子妊娠中に症状は改善し，出産後に再度悪化し，さらに無痛性甲状腺炎を発症した例を報告している[6]．また，2005年にHAM患者会が行ったアンケート調査「HAM患者の生活実態調査報告」(村上，松崎)では，出産経験のある女性HAM患者82名が，HAMの発症は13％が第1子出産後，46.3％が第2子出産後，22.0％が第3子出産後であったと回答しており，第2子出産後にHAM発症は多くみられている．紹介した自験例で，症例1は妊娠でHAM症状が出現し，出産後改善なく，症例2～6は妊娠でHAM

表1　HAM患者の妊娠・出産経過と運動機能・排尿障害の変化

No	発症年齢	発汗障害部位	運動機能障害スコア			排尿障害スコア			ウイルス量/10^4PBMC	
			妊娠前	妊娠時	出産後	妊娠前	妊娠時	出産後	妊娠前	妊娠時
1	24	Th10	0	1	2	0	2	3	−	922
2	20	Th1	3	3	4	0	3	3	−	715
3	12	Th12	4	6	6	4	4	5	446	956
4	10	不明	5	5	6	4	4	4	487	975
5	15	L3	1	1	3	0	2	3	−	2,619
6	20	Th12	0	2	2	1	5	6	510	−
7	34	Th7	0	0	0	3	4	0	1,076	669

症状が増悪し，さらに出産後悪化した．症例3，4は症状悪化とともにHTLV-1プロウイルス量も増加している．患者自身へのHTLV-1感染がいつ生じたのかなど考慮すべきことが多いが，妊娠・出産がHAMの病態になんらかの影響を与えており，妊娠出産を契機にHAMが顕性化する可能性を示唆している．一方，症例7は妊娠前にステロイド治療などを行っているが，出産後も症状は改善し，プロウイルス量も減少傾向を示している[7][8]．妊娠をひかえての治療介入には慎重でなければならないが，適切にHAMの活動性をコントロールすることにより，妊娠・出産のHAMへの影響を最小限に抑えることが可能であると思われる．

HTLV-1の母児感染

HAMと妊娠・出産のもう一つの側面は母児間感染である．母乳による児への感染は数万年前からHTLV-1感染がヒトで維持され続けた主な感染ルートであり，断乳により長期母乳の20～30％の感染率が数％に低下することが報告されている[9]．厚生労働省は，2011年(平成23年度)からHTLV-1抗体検査を妊婦健康診査の標準的検査項目に追加し，陽性者に適切な哺育法を指導する体制がスタートした．HAM患者は体内の末梢血中のプロウイルス量が非常に高値で，児への感染が生じやすいと考えられるが，抗体価も高値で，移行中和抗体による感染阻止の効果[10]などもあり，その実態は不明である．自験例では産道感染を心配してHIV感染に準じて帝王切開を選び，断乳し，人工栄養による哺育を選択した例では児への感染は起こっていない．厚生労働科学研究費の板橋研究班で，妊婦キャリアとキャリアから生まれた子供の3年経過観察がすすめられており，母子感染の実態把握が開始されている．

おわりに

HTLV-1関連の患者会「スマイルリボン」[11]の中にHTLV-1関連脊髄症(HAM)患者会「アトムの会」，成人T細胞白血病(ATL)患者会に加え，HTLV-1キャリアママの会「カランコエ」が設立された．HAM患者をはじめ，HTLV-1陽性の若い妊婦が気軽に相談できるような状況になることが期待される．また，症例6のように，発汗障害，軽度の下肢つっぱり感や靴の外側がすりへったり，頻尿，残尿などの軽度の排尿障害をきっかけにHAMの早期診断が可能であり，妊婦検診でのHTLV-1抗体検査の開始により，早期に神経内科を受診し，HAMの長期予後の改善が得られることが期待される．HTLV-1情報に関しては，「HTLV-1情報サービス」(http://htlv1joho.org/)でWeb公開されている．

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PLOS | NEGLECTED TROPICAL DISEASES

CSF CXCL10, CXCL9, and Neopterin as Candidate Prognostic Biomarkers for HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

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Abstract

Background: Human T-lymphotropic virus type 1 (HTLV-1) -associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a rare chronic neuroinflammatory disease. Since the disease course of HAM/TSP varies among patients, there is a dire need for biomarkers capable of predicting the rate of disease progression. However, there have been no studies to date that have compared the prognostic values of multiple potential biomarkers for HAM/TSP.

Methodology/Principal Findings: Peripheral blood and cerebrospinal fluid (CSF) samples from HAM/TSP patients and HTLV-1-infected control subjects were obtained and tested retrospectively for several potential biomarkers, including chemokines and other cytokines, and nine optimal candidates were selected based on receiver operating characteristic (ROC) analysis. Next, we evaluated the relationship between these candidates and the rate of disease progression in HAM/TSP patients, beginning with a first cohort of 30 patients (Training Set) and proceeding to a second cohort of 23 patients (Test Set). We defined "deteriorating HAM/TSP" as distinctly worsening function (≥3 grades on Osame's Motor Disability Score (OMDS)) over four years and "stable HAM/TSP" as unchanged or only slightly worsened function (1 grade on OMDS) over four years, and we compared the levels of the candidate biomarkers in patients divided into these two groups. The CSF levels of chemokine (C-X-C motif) ligand 10 (CXCL10), CXCL9, and neopterin were well-correlated with disease progression, better even than HTLV-1 proviral load in PBMCs. Importantly, these results were validated using the Test Set.

Conclusions/Significance: As the CSF levels of CXCL10, CXCL9, and neopterin were the most strongly correlated with rate of disease progression, they represent the most viable candidates for HAM/TSP prognostic biomarkers. The identification of effective prognostic biomarkers could lead to earlier detection of high-risk patients, more patient-specific treatment options, and more productive clinical trials.

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Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is a human retrovirus associated with persistent infection of T-cells [1]. While the majority of HTLV-1-infected individuals remain asymptomatic, approximately 2.5–5% develop an aggressive T-cell malignancy, termed adult T-cell leukemia (ATL) [2,3] and 0.3–3.8% develop a serious chronic neuroinflammatory disease, termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [4–6]. Aside from Japan, endemic areas for this virus and the associated disorders are mostly located in developing countries in the Caribbean, South America, Africa, the Middle East, and Melanesia [7,8], which may explain why these conditions have remained ill-defined and virtually untreatable for so long [9].

HAM/TSP is characterized by unremitting myelopathic symptoms such as spastic paraparesis, lower limb sensory disturbance, and bladder/bowel dysfunction [10,11]. Although

Author Summary

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a rare neurodegenerative disease caused by infection with human T-lymphotropic virus type 1 (HTLV-1). HTLV-1 infects 10–20 million people worldwide, and, depending on the region, 0.25–3.8% of infected individuals develop HAM/TSP. As the disease progresses, chronic inflammation damages the spinal cord and lower limb and bladder function gradually decline. In the worst cases, even middle-aged patients can become perpetually bedridden. Today, there are treatments that may alleviate the symptoms to a certain degree, but there is no cure that can halt disease progression, and there are no known biomarkers to indicate the level and speed of disease progression. In this study, we successfully identified three promising candidate biomarkers. We believe that the use of these biomarkers could lead to more accurate prognoses and more prudent, patient-specific treatment plans. We not only hope that these biomarkers are sensitive enough to use as selection criteria for clinical trials, but also that measurements of these biomarkers can be used to accurately evaluate drug effectiveness. In short, the biomarkers we identified have the potential to help more effectively treat current HAM/TSP patients and to pave the way for new drugs to potentially cure future HAM/TSP patients.

the symptoms of HAM/TSP have been well documented for quite some time, the rate at which these symptoms progress has only recently become a point of interest. The clinical course of HAM/TSP has classically been described very simply as insidious onset and continuous progression [12], but recent reports have hinted at a more complex, heterogeneous pool of patients with differing clinical needs. Recent studies have shown that although HAM/TSP usually progresses slowly and without remission as per the classical description, there is a subgroup of patients whose conditions decline unusually quickly and who may be unable to walk within two years of onset and another subgroup whose conditions decline unusually slowly and who may only display very mild symptoms [13–15]. It is only logical that these patients should receive treatments tailored to suit their individual needs rather than identically aggressive treatments. Unfortunately, clinicians are currently only able to distinguish between these different groups by observing the way a patient's disease progresses over time, usually years; clinicians often decide to treat the patients immediately and identically rather than wait and allow the disease to progress further. Therein lies the dire need for biomarkers with the power to forecast the rate and extent of disease progression and enable clinicians to make more accurate prognoses and prescribe the most appropriate and effective treatments in a timely manner.

Several candidate prognostic biomarkers with elevated levels in HAM/TSP patients have already been identified in the peripheral blood and cerebrospinal fluid (CSF). In the peripheral blood, such candidates include the HTLV-1 proviral load in peripheral blood mononuclear cells (PBMCs) and serum levels of the soluble IL-2 receptor (sIL-2R) [16,17]. The level of neopterin in the CSF has been reported to be a useful parameter for detecting cell-mediated immune responses in the spinal cord of HAM/TSP patients and the CSF anti-HTLV-1 antibody titer has been shown to be associated both with CSF neopterin levels and the severity of clinical symptoms [18–20]. In addition, several cytokines have been detected in the CSF and/or spinal cord of HAM/TSP patients, including interleukin (IL)-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, and tumor necrosis factor (TNF)-α [21–24]. Some chemokines, such as chemokine (C-X-C motif) ligand (CXCL) 9, CXCL10, and chemokine (C-C motif) ligand (CCL) 5, have been shown to be substantially elevated in both the blood and the CSF with respect to asymptomatic carriers (ACs) or patients with other neurological diseases such as multiple sclerosis [25–28]. This is the first study to compare the adequacies of several of these candidate biomarkers for forecasting the rate of disease progression.

We hypothesized the existence of biomarkers capable of differentiating stable and deteriorating HAM/TSP patients. In this retrospective study, a preliminary experiment was first conducted to select the most promising candidate biomarkers by comparing blood and CSF levels in HAM/TSP patients and control subjects (Figure S1). Four candidate blood markers (sIL-2R, CXCL9, CXCL10, and proviral load) and five candidate CSF markers (CXCL9, CXCL10, neopterin, cell count, and anti-HTLV-1 antibody titer) were selected. To evaluate the relative effectiveness of these candidate biomarkers for predicting rate of disease progression, a classification system was created and HAM/TSP patients were designated as either deteriorating or relatively stable. The levels of candidate biomarkers were then compared between the two patient groups. In the current study, we identified three viable candidates for HAM/TSP prognostic biomarkers that could lead to more accurate prognoses and more prudent, patient-specific treatment plans.

Materials and Methods

Ethical considerations

The study was designed and conducted in accordance with the tenets of the Declaration of Helsinki. The protocol in this study was approved by the Ethics Review Committee of St. Marianna University School of Medicine (No. 1646). Prior to the collection of blood or CSF samples, all subjects gave written informed consent permitting the analysis of their samples for research purposes as part of their clinical care.

Subjects

Between April 2007 and February 2013, we enrolled 53 HAM/TSP patients according to the inclusion and exclusion criteria shown in Table 1, and divided them into two cohorts based on the chronological order of their doctor's visits: a 30-patient Training set and a 23-patient Test set. Demographics and clinical characteristics of the Training set and Test set are shown in Table 2 and Table 3, respectively. Between April 2007 and December 2009, we enrolled 22 HTLV-1-infected ACs as control subjects for blood analysis and eight HTLV-1-infected subjects (seven ACs, one patient with smoldering ATL) as control subjects for CSF analysis according to the inclusion and exclusion criteria shown in Table 1. These two groups were not mutually exclusive; some ACs donated both blood and CSF to this study. Demographics of control subjects as compared to the HAM/TSP patients are shown in Table S1.

Sample preparation

Blood and/or CSF samples were obtained within a one-hour window for each subject. Peripheral blood samples were collected in heparin-containing blood collection tubes and serum-separating tubes. Plasma and PBMCs were obtained from the former tubes and serum was obtained from the latter. PBMCs were isolated with standard procedures using Pancoll® density gradient centrifugation (density 1.077 g/mL; PAN-Biotech GmbH, Aidenbach, Germany). Plasma and serum samples were stored at −80°C until

Table 1. Inclusion and exclusion criteria for this study.

	HAM/TSP	Control for Blood	Control for CSF
Inclusion Criteria	Willing and able to give informed consent		
	HTLV-1 seropositive individuals conformed by CLEIA and Western blot		
	Diagnosed with HAM/TSP as defined by WHO criteria		Choose to provide CSF for the purposes of differential diagnosis
Exclusion Criteria	History of treatment with corticosteroids or other immunomodulating drugs (interferon, cyclosporin, methotrexate, etc.)		
	Diagnosed with an autoimmune disease or other chronic inflammatory disorder aside from HAM/TSP		
	Diagnosed with additional disease affecting gait disturbance (e.g. parkinsonism, rheumatoid arthritis, cervical spondylosis, brain infarction, etc.)		
	History of severe urinary infection, decubitus scars, pneumonia, deep venous thrombosis, or other condition potentially affecting disease course within the last four years	Diagnosed with HAM/TSP as defined by WHO criteria	
	Diagnosed with adult T-cell leukemia (ATL)		

CLEIA = chemiluminescent enzyme immunoassay.
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use. CSF was collected in polypropylene tubes. A small amount of CSF was used for routine laboratory tests, which included total protein, cell count, and IgG level. The remaining CSF was aliquoted into cryotubes and stored at −80°C until undergoing further analysis. All tests in this study were performed on samples from these frozen stocks.

Measurement of blood candidate markers

The serum concentration of sIL-2R was determined using an ELISA (Cell Free N IL-2R; Kyowa Medex Ltd., Tokyo, Japan). HTLV-1 proviral load was measured using real-time PCR, following DNA extraction from PBMCs, as previously described [29–31]. Plasma levels of IL-1β, TNF-α, and IFN-γ were measured using a cytometric bead array (CBA) (BD Biosciences, Franklin Lakes, NJ USA), which was used according to the manufacturer's instructions. Plasma concentrations of CXCL9, CXCL10, CXCL11, and CCL5 were also measured using a CBA (BD Biosciences).

Measurement of CSF candidate markers

CSF cell count was determined using the Fuchs–Rosenthal chamber (Hausser Scientific Company, Horsham PA USA). Total protein and IgG levels in the CSF were measured using a pyrogallol red assay and a turbidimetric immunoassay, respectively. The anti-HTLV-1 antibody titer was determined using the gelatin particle agglutination test (Serodia-HTLV-1; Fujirebio, Tokyo, Japan). CSF concentration of sIL-2R was determined using an ELISA (Cell Free N IL-2R; Kyowa Medex). CSF neopterin level was measured using high-performance liquid chromatography. IFN-γ and six chemokines (CXCL9, CXCL10, CXCL11, CCL3, CCL4, and CCL5) were measured using a CBA (BD Biosciences). The CSF concentrations of three chemokines (CCL17, CCL20, and CCL22) and IL-17A were measured using commercially available ELISA kits (CCL17, CCL20, and CCL22: TECHNE/R&D Systems, Minneapolis, MN USA; IL-17A: Gen-Probe, San Diego, CA USA). All assays were conducted according to the respective manufacturers' instructions.

Table 2. Demographics and clinical characteristics of HAM/TSP patients (Training Set).

	Total n = 30	Stable HAM/TSP n = 14	Deteriorating HAM/TSP n = 11	*p*-value*
Demographics				
Age, y**	58 [37–75]	54.5 [39–75]	62 [53–72]	0.0183†
Female sex	80.0%	64.3%	90.9%	0.1696‡
Clinical characteristics				
Age of onset, y**	48 [20–70]	33 [20–58]	57 [40–70]	0.0021†
Disease duration, y**	12.5 [1–33]	19 [7–33]	9 [1–13]	0.0021†
OMDS**	6 [2–11]	5 [2–9]	8 [5–11]	0.0065†

In the Training set, deteriorating patients were significantly older, experienced disease onset later in life, had been living with the disease for shorter periods, and were more severely disabled (OMDS).
*Stable HAM/TSP vs Deteriorating HAM/TSP.
**Data are expressed as median [range].
†By Mann-Whitney test.
‡By Fisher's exact test.
OMDS = Osame's Motor Disability Score.
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Table 3. Demographics and clinical characteristics of HAM/TSP patients (Test Set).

	Total n=23	Stable HAM/TSP n=11	Deteriorating HAM/TSP n=9	p-value*
Demographics				
Age, y**	58 [22–75]	61 [22–75]	59 [48–68]	0.8491†
Female sex	78.3%	81.8%	77.8%	1.000‡
Clinical characteristics				
Age of onset, y**	43 [12–70]	40 [14–70]	51 [39–63]	0.0184†
Disease duration, y**	9 [2–41]	19 [5–41]	6 [2–14]	0.0148†
OMDS**	5 [2–8]	5 [4–8]	5 [4–8]	0.4526†

In the Test set, deteriorating patients experienced disease onset later in life and had been living with the disease for shorter periods, but there were no significant differences in current age or OMDS.
*Stable HAM/TSP vs Deteriorating HAM/TSP.
**Data are expressed as median [range].
†By Mann-Whitney test.
‡By Fisher's exact test.
OMDS = Osame's Motor Disability Score.
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Classification system based on the natural history of HAM/TSP

The 53 total HAM/TSP patients without any history of HAM/TSP-targeting treatments were interviewed using a questionnaire (Figure S2) to determine the changes in Osame's Motor Disability Score (OMDS) over time (Figure S3). OMDS is a standardized neurological rating scale as a measure of disability [10] (Figure S1). Based on the changes in OMDS, "deteriorating cases" and "stable cases" were identified in both the Training set and Test set patient cohorts. Patients with deteriorating HAM/TSP were defined as those whose OMDS worsened ≥3 grades over four years and patients with stable HAM/TSP were defined as those whose OMDS remained unchanged or worsened 1 grade over four years. Patients whose OMDS worsened 2 grades over four years were excluded from the patient cohort in order to create a larger gap between the deteriorating and stable patient groups.

Statistical analysis

GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA USA) was used to plot graphs and perform statistical analyses. Differences between the two subject groups were tested using the Mann-Whitney U-test. Receiver operating characteristic (ROC) analysis was performed to examine the sensitivity and specificity of individual biomarkers. For the ROC analyses, an area under the ROC curve (AUC) of 1.0 was used to represent a perfect test with 100% sensitivity and 100% specificity, whereas an area of 0.5 was used to represent random discrimination. Spearman's rank correlation test was employed to investigate the correlation between the four CSF markers (CXCL10, CXCL9, neopterin, and cell count) and the proviral load in PBMCs. To compare the four CSF markers between three groups (HTLV-1-infected control, n = 8; stable HAM/TSP, n = 25; and deteriorating HAM/TSP, n = 20), we used the Kruskal–Wallis test followed by Dunn's post-hoc tests. P-values<0.05 were considered statistically significant.

Results

Identification of biomarkers elevated in the blood of HAM/TSP patients

In order to identify candidate blood markers for HAM/TSP, the concentrations of IL-1β, TNF-α, and IFN-γ were measured in plasma samples from four ACs and four HAM/TSP patients. Plasma levels of IL-1β and TNFα were below the detection limits (<2.3 pg/mL and <1.2 pg/mL, respectively) except in one patient with HAM/TSP. Plasma IFN-γ levels showed no significant differences between ACs and HAM/TSP patients (median 10.4 pg/mL and 13.9 pg/mL, respectively). Therefore, these quantities were not measured in additional samples (Figure S1). The proviral DNA load in PBMCs, serum sIL-2R, and plasma levels of the chemokines CXCL9, CXCL10, CXCL11, and CCL5 were also measured in 22 ACs and 30 HAM/TSP patients without any history of immunomodulating treatments, including corticosteroids, IFN-α, and immunosuppressive drugs. The results revealed that serum levels of sIL-2R, plasma levels of CXCL10 and CXCL9, and proviral DNA load in PBMCs were markedly higher in HAM/TSP patients compared to ACs ($p \leq 0.0001$, Figure 1A). These quantities were then compared using ROC analysis to determine which parameters were superior markers for HAM/TSP. From the results of the ROC analysis, we determined that serum sIL-2R and plasma CXCL10 had the highest potential for distinguishing HAM/TSP patients from ACs with high sensitivity and specificity (area under the ROC curve [AUC]>0.9), followed by plasma CXCL9 and HTLV-1 proviral load in PBMCs (0.8<AUC<0.9) (Figure 1B). Thus, four candidate blood biomarkers were selected for further investigation: serum sIL-2R, plasma CXCL10, plasma CXCL9, and HTLV-1 proviral load in PBMCs.

Identification of biomarkers elevated in the CSF of HAM/TSP patients

In order to identify candidate CSF markers for HAM/TSP, elevated levels of various potential markers were screened for in CSF samples from HAM/TSP patients. CSF IL-17A was detectable (>3.0 pg/mL) in only one of eight HAM/TSP patients screened (including six deteriorating-type patients), and the level in this one patient (deteriorating-type) was negligible (4.0 pg/mL). CSF IFN-γ was detectable (>1.8 pg/mL) in only 3 of 10 HAM/TSP patients screened (six deteriorating patients), and the levels in all three were negligible (range 3.3–4.2 pg/mL). Therefore, these cytokines were not measured in additional patients. Total protein, cell count, IgG, neopterin, sIL-2R, and nine chemokines (CXCR3 ligands: CXCL9, CXCL10, and CXCL11; CCR5 ligands: CCL3, CCL4, and CCL5; CCR4 ligands: CCL17 and CCL22; CCR6

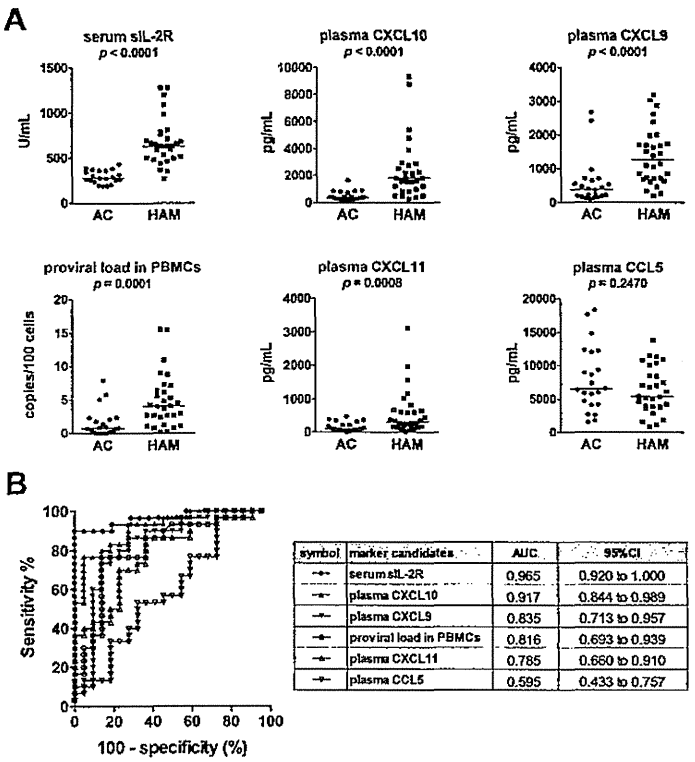

Figure 1. Selection of candidate biomarkers in the blood by comparing HAM/TSP patients and asymptomatic carriers. **(A)** Serum levels of soluble IL-2 receptor (sIL-2R), proviral loads in peripheral blood mononuclear cells (PBMCs), and plasma levels of four chemokines (chemokine (C-X-C motif) ligand (CXCL) 9, CXCL10, CXC11, and chemokine (C-C motif) ligand (CCL) 5) were compared between HAM/TSP patients (HAM; n = 30) and asymptomatic carriers (AC; n = 22). Horizontal bars indicate the median values. The Mann-Whitney *U*-test was used for statistical analysis. **(B)** Receiver operating characteristic (ROC) analysis was employed to assess the sensitivities and specificities of the six markers exhibited in part (A) for discriminating HAM/TSP patients from ACs: greater proximity of the ROC curve to the upper left corner indicates higher sensitivity and specificity of the marker. AUC = area under the ROC curve; 95% CI = 95% confidence interval.
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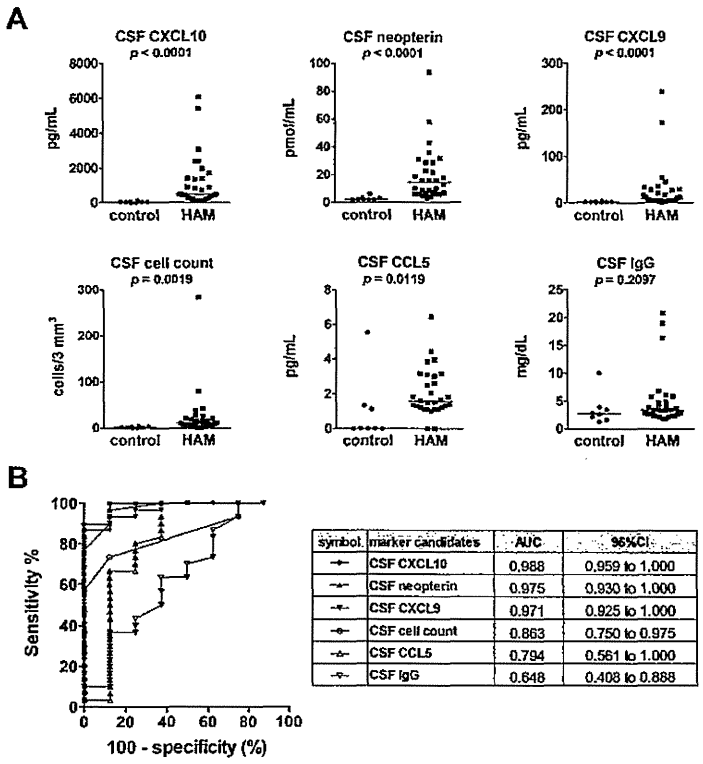
ligand: CCL20) were also measured in the CSF of 30 untreated HAM/TSP patients and in eight HTLV-1-infected control subjects (seven ACs and one patient with smoldering ATL). The results indicated that CSF levels of CXCL10, neopterin, and CXCL9 were remarkably higher in HAM/TSP patients compared to control subjects ($p<0.0001$ overall, Figures 2A and S4) and that CSF levels of cell count and CCL5 were less so but still significantly higher ($p=0.0019$ and $p=0.0119$, respectively; Figure 2A). By contrast, there were no differences in the CSF levels of IgG and total protein between HAM/TSP patients and control subjects, and CSF sIL-2R levels were only detectable in a single HAM/TSP patient (data not shown). ROC analysis showed that the CSF levels of CXCL10, neopterin, CXCL9, and CSF cell count could be used to relatively accurately distinguish HAM/TSP patients from control subjects (AUC>0.8) (Figure 2B). Therefore, these four CSF markers were selected as candidates for further investigation. It should be noted that the sensitivity of CSF cell count was very low (36.7%) when compared to the other three: CXCL10 (83.3%), CXCL9 (86.7%), and neopterin (76.7%) (Figure S5).

Identification of biomarkers correlated with rate of HAM/TSP disease progression

In short, we selected nine markers: eight markers chosen based on the analyses described above and CSF anti-HTLV-1 antibody titer, which is a known diagnostic marker for HAM/TSP. To determine which biomarkers were associated with HAM/TSP disease progression, the levels of these nine markers were compared between the deteriorating and stable HAM/TSP patient groups (see Methods for definitions of deteriorating and stable). The results revealed that all five CSF markers were significantly higher in the deteriorating group compared to the stable group (Figure 3A), but that none of the four blood markers, including proviral load, were significantly different between the two groups. The deteriorating group included three patients with particularly rapidly progressive HAM/TSP, defined as those who had been confined to wheelchairs (OMDS: ≥ grade 6) within two years after the onset of symptoms [13,14] (black circles in Figures 3A and S3B). These rapid progressors exhibited high levels of the CSF markers and high proviral loads. ROC analysis revealed that the levels of the CSF markers (CXCL10, CXCL9, neopterin, and cell count), but not anti-HTLV-1 antibody titer, distinguished clearly between patients with deteriorating HAM/TSP and stable HAM/TSP (AUC>0.8, Figure 3B).

Validation of nine candidate biomarkers using the Test Set

To validate the results obtained using the Training Set, the same nine markers were compared between deteriorating and stable patients using the Test Set (a second cohort of 23 HAM/



symbol	marker candidates	AUC	95%CI
	CSF CXCL10	0.988	0.959 to 1.000
	CSF neopterin	0.975	0.930 to 1.000
	CSF CXCL9	0.971	0.925 to 1.000
	CSF cell count	0.863	0.750 to 0.975
	CSF CCL5	0.794	0.561 to 1.000
	CSF IgG	0.648	0.408 to 0.888

Figure 2. Selection of candidate biomarkers in the cerebrospinal fluid (CSF) by comparing HAM/TSP patients and control subjects. (A) CSF levels of total protein, cell count, IgG, neopterin, sIL-2R, and nine chemokines (CCL3, CCL4, CCL5, CXCL9, CXCL10, CXC11, CCL17, CCL20, and CCL22) were measured and compared between HAM/TSP patients (HAM; n = 30) and HTLV-1-infected control subjects (control; n = eight: seven ACs and one ATL patient). Data is shown for the top six CSF markers ranked according to the significance of the difference between the HAM/TSP patients and the control subjects. Horizontal bars indicate the median values. The Mann-Whitney *U*-test was used for statistical analysis. (B) ROC analysis was employed to assess the sensitivities and specificities of the six markers exhibited in part (A) for discriminating HAM/TSP patients from controls. AUC = area under the ROC curve; 95% CI = 95% confidence interval.
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TSP patients that had not undergone HAM/TSP-targeting treatment). As shown in Figure 4A, the results indicated that the levels of five CSF markers, proviral load in PBMCs, and serum sIL-2R were significantly higher in deteriorating cases than in stable cases. Among them, CSF levels of CXCL10, CXCL9, neopterin, and CSF cell count exhibited particularly high sensitivities and specificities for detecting the deteriorating HAM/TSP cases in the Test set as well as Training set (AUC>0.8, Figures 4B and S1).

Demographic and clinical characteristics of the subjects

The demographics of the HAM/TSP patients versus the control subjects for both the blood tests and CSF analyses were compared and evaluated for statistical significance (Table S1). There were no significant differences in age or gender distribution between the HAM/TSP patients and either control subject group.

Similarly, the demographic and clinical characteristics of stable versus deteriorating HAM/TSP subjects in both the Training and Test sets are shown in Tables 2 and 3, respectively. There were no significant differences in age or gender distribution among either set, but deteriorating patients in both sets were significantly older at disease onset and had been living with the disease for shorter periods of time. Deteriorating patients in the Training set scored higher OMDS values than their stable counterparts ($p<0.01$), but there was no such significant difference in the Test set.

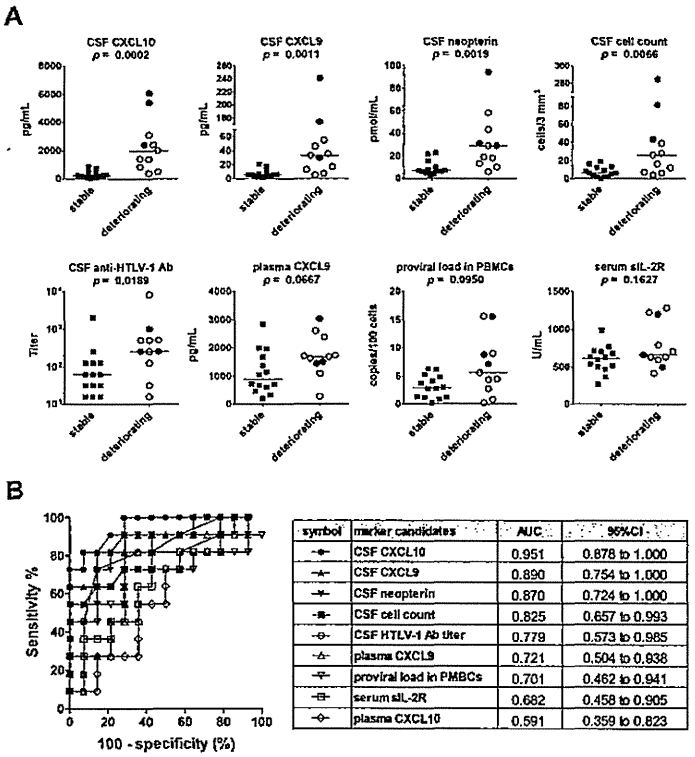
To investigate the potential influence of disease duration as a secondary variable, a new test group was created containing only those patients for whom the disease onset date was 7–13 years prior to the sample collection day. Patients fitting this criterion were selected from the 53 total available from both the Training and Test sets: eight stable patients and ten deteriorating patients; we confirmed that there was no significant difference in disease duration between these two groups. The results remained consistent with our previous findings: CSF CXCL10, CXCL9, and neopterin were all elevated in deteriorating patients with respect to stable patients ($p<0.01$, Figure 5).

Follow-up mini-study on biomarker levels over time

Four stable HAM/TSP patients were left completely untreated and followed for a period of three to five years. Within this time, one patient rose one grade on the OMDS scale, and the other three experienced no change in OMDS grade at all. The levels of CSF CXCL10 and neopterin remained consistently low over time (Figure S6).

Discussion

To date, there have been few well-designed studies that have evaluated the relationship between biomarkers and HAM/TSP disease progression. In a previous retrospective study with 100 untreated HAM/TSP patients, a significant association was



symbol	marker candidates	AUC	95%CI
-●-	CSF CXCL10	0.951	0.878 to 1.000
-▲-	CSF CXCL9	0.890	0.754 to 1.000
-✳-	CSF neopterin	0.870	0.724 to 1.000
-■-	CSF cell count	0.825	0.657 to 0.993
-○-	CSF HTLV-1 Ab titer	0.779	0.573 to 0.985
-△-	plasma CXCL9	0.721	0.504 to 0.938
-▽-	proviral load in PMBCs	0.701	0.462 to 0.941
-⊟-	serum sIL-2R	0.682	0.458 to 0.905
-◇-	plasma CXCL10	0.591	0.359 to 0.823

Figure 3. Identification of biomarkers associated with clinical progression of HAM/TSP. **(A)** Five CSF marker candidates (CXCL10, CXCL9, neopterin, cell count, and anti-HTLV-1 antibody titer) and four blood marker candidates (proviral load in PBMCs, serum sIL-2R, plasma CXCL9, and plasma CXCL10) were compared among a cohort of patients called the Training Set (deteriorating HAM/TSP, n = 11; stable HAM/TSP, n = 14). Data is shown for the top eight CSF markers ranked according to the significance of the difference between the deteriorating and stable subjects. Black circles indicate patients with particularly rapidly progressive HAM/TSP. Horizontal bars indicate the median values. The Mann-Whitney *U*-test was used for statistical analysis. **(B)** ROC analysis was employed to assess the sensitivities and specificities of the nine markers listed above for discriminating deteriorating HAM/TSP patients from stable patients. AUC = area under the ROC curve; 95% CI = 95% confidence interval.
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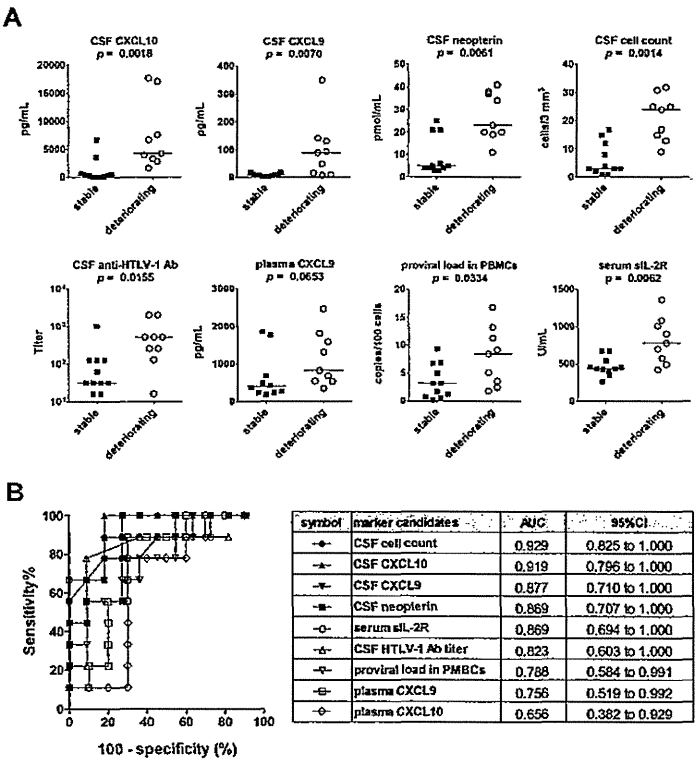
demonstrated to exist between higher HTLV-1 proviral load in PBMCs and poor long-term prognosis; however, the predictive value of high proviral load appeared to be too low to qualify it as a marker for disease progression in clinical practice [32]. Here we conducted a retrospective study to compare for the first time the relationships of PBMC proviral load and several inflammatory biomarker candidates to disease progression in untreated HAM/TSP patients.

In this study, elevated CSF cell count, neopterin concentration, and CSF levels of CXCL9 and CXCL10 were well-correlated with disease progression over the four year period under study, better even than HTLV-1 proviral load in PBMCs (Figures 3 and 4). As CSF pleocytosis, CSF CXCL10, CSF CXCL9, and CSF neopterin are known indicators of inflammation in the central nervous system [33,34], our findings indicate that the rate of HAM/TSP progression is more closely reflected by the amount of inflammatory activity in the spinal cord than by the PBMC proviral load. However, we also found a significant correlation between PBMC proviral load and the levels of the CSF markers identified in this study (Figure S7), indicating that a higher PBMC proviral load does indeed suggest more inflammation in the spinal cord and therefore a poorer long-term prognosis. These findings are consistent with the theory that HAM/TSP is the result of an excess of inflammatory mediators caused by the presence of HTLV-1-infected T-cells [35–37].

The HTLV-1 proviral load in the CSF as well as the ratio of the proviral load in the CSF to that in PBMCs have been reported to be effective for discriminating HAM/TSP patients from ACs or multiple sclerosis patients infected with HTLV-1 [38,39]. Some researchers have suggested that these values might be associated with the rate of disease progression, but there has been only one small cohort study and one case report investigating this point, and so the significance of this experimental evidence is still questionable [40,41]. In addition to statistical validation with multiple, larger cohorts, it would also be beneficial to use precise definitions for progressive versus stable patients, as we have done in this study. Although the volume of CSF available per sample was too limited to measure CSF proviral load in the present study, we plan to incorporate CSF proviral load in a future prospective study and compare its usefulness to that of other biomarker candidates.

From our results, we concluded that of the potential biomarkers under study, CXCL10, CXCL9, and neopterin are the most fit for determining the level of spinal cord inflammation, and thus the most fit for predicting disease progression in HAM/TSP patients. Although the CSF cell count is an easily measurable inflammatory marker, it is not sensitive enough to reliably detect the level of spinal cord inflammation. Numerous patients with CSF cell counts within the normal range exhibited high levels of other inflammatory markers, such as neopterin and CXCL10 (Figure S5). In fact, it has been reported that CSF pleocytosis is present in only approximately 30% of HAM/TSP patients [42]. Furthermore, in



symbol	marker candidates	AUC	95%CI
-●-	CSF cell count	0.929	0.825 to 1.000
-▲-	CSF CXCL10	0.919	0.796 to 1.000
-▼-	CSF CXCL9	0.877	0.710 to 1.000
-■-	CSF neopterin	0.869	0.707 to 1.000
-○-	serum sIL-2R	0.869	0.694 to 1.000
-△-	CSF HTLV-1 Ab titer	0.823	0.603 to 1.000
-▽-	proviral load in PMBCs	0.788	0.584 to 0.991
-⊟-	plasma CXCL9	0.756	0.519 to 0.992
-◇-	plasma CXCL10	0.656	0.382 to 0.929

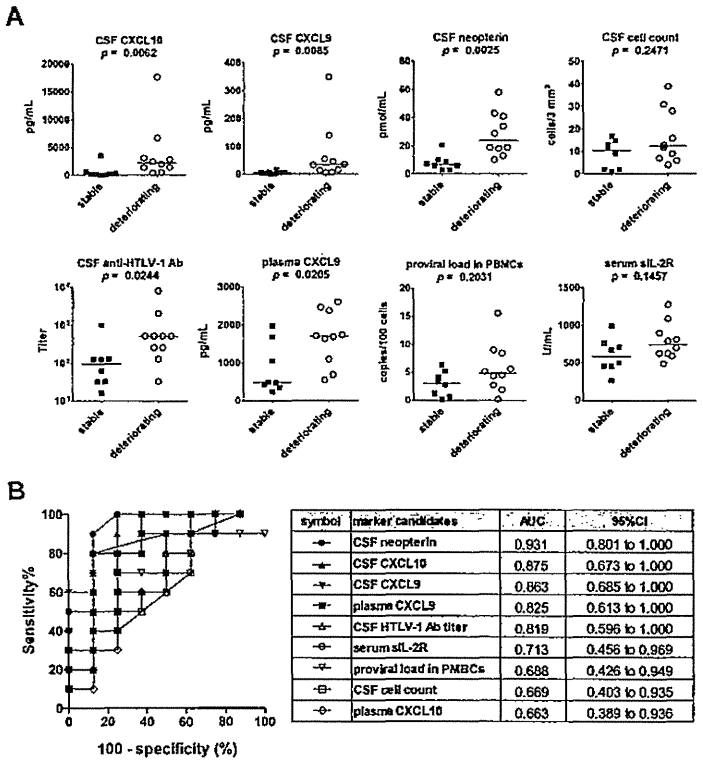
Figure 4. Validation of potential markers using the Test Set. (A) Five CSF marker candidates (CXCL10, CXCL9, neopterin, cell count, and anti-HTLV-1 antibody titer) and four blood marker candidates (proviral load in PBMCs, serum sIL-2R, plasma CXCL9, and plasma CXCL10) were compared among a second cohort of patients called the Test Set (deteriorating HAM/TSP, n = 9; stable HAM/TSP, n = 11). Data is shown for the top eight CSF markers ranked according to the significance of the difference between the deteriorating and stable subjects. Horizontal bars indicate the median values. The Mann-Whitney *U*-test was used for statistical analysis. (B) ROC analysis was employed to assess the sensitivities and specificities of the nine markers listed above for discriminating deteriorating HAM/TSP patients from stable patients. AUC = area under the ROC curve; 95% CI = 95% confidence interval.
doi:10.1371/journal.pntd.0002479.g004

our study, there was no significant difference in CSF cell count between the control subjects and the stable HAM/TSP patients (Figure S8).

We also explored the possibility of combining multiple biomarkers via multiple logistic regression to form a combination more sensitive and specific than individual markers, but the results indicated that there is not much to be gained from combinations (data not shown).

While there were no significant demographic differences between subject groups, the clinical characteristics of stable versus deteriorating HAM/TSP patients of course differed widely (Tables 2, 3, and S2). We confirmed the already well-reported statistic that deteriorating patients experience HAM/TSP onset relatively late in life [12,14,20] ; our data also reflected the short disease duration expected of deteriorating patients, who by definition progress through the disease more rapidly than their stable counterparts. As patients in all groups were of similar age at sample collection, the significant difference in age of onset should not have any impact on our findings. However, it was necessary to consider the possibility that those patients in a later stage of the disease (i.e. those listed with longer disease durations) might possess elevated or diminished biomarker levels regardless of rate of disease progression. We confirmed that this difference in disease duration was not a confounding factor in our selection of candidate biomarkers by comparing stable and deteriorating HAM/TSP patients with similar disease durations (7–13 years), and we were able to obtain results consistent with our earlier findings (Figure 5). Finally, the OMDS values for the stable and deteriorating patient groups in the Test set were perfectly identical, eliminating the need to consider the possibility that the biomarkers could have been elevated according to disease severity regardless of rate of progression.

The main limitation of our retrospective study is that our samples were collected from patients at the end of the four year period during which the extent of progression was analyzed as opposed to the beginning of the four year period, which would have been optimal for directly measuring their prognostic powers. Of course, the patients with severe HAM/TSP symptoms began undergoing treatment soon after sample collection, rendering any observations on disease course after sample collection un-useable for analysis in this study. While this situation is non-ideal, we hypothesize that biomarker levels in a given patient do not substantially change over a few years' time. We were actually able to monitor the biomarker levels of four untreated HAM/TSP patients over 3–5 years, and the levels remained relatively stable in all four subjects over time (Figure S6), supporting our hypothesis. However, these were all stable HAM/TSP patients (hence the lack of treatment), and so we cannot rule out the possibility that biomarker levels in untreated deteriorating patients may dramatically rise, fall, or fluctuate. The results of the analysis of patients with similar disease durations (Figure 5) also support our hypothesis that disease duration is not an important determinant



symbol	marker candidates	AUC	95%CI
	CSF neopterin	0.931	0.801 to 1.000
	CSF CXCL10	0.875	0.673 to 1.000
	CSF CXCL9	0.863	0.685 to 1.000
	plasma CXCL9	0.825	0.613 to 1.000
	CSF HTLV-1 Ab titer	0.819	0.596 to 1.000
	serum sIL-2R	0.713	0.456 to 0.969
	proviral load in PMBCs	0.688	0.426 to 0.949
	CSF cell count	0.669	0.403 to 0.935
	plasma CXCL10	0.663	0.389 to 0.936

Figure 5. Comparison of potential markers in stable and deteriorating HAM/TSP patients with similar disease durations. (A) Five CSF marker candidates (CXCL10, CXCL9, neopterin, cell count, and anti-HTLV-1 antibody titer) and four blood marker candidates (proviral load in PBMCs, serum sIL-2R, plasma CXCL9, and plasma CXCL10) were compared among all patients from both the Training and Test Sets pooled together with similar disease durations (range: 7–13 years; no significant difference in duration between stable (n = 8) and deteriorating (n = 10) groups). Data is shown for the top eight CSF markers ranked according to the significance of the difference between the deteriorating and stable subjects. Horizontal bars indicate the median values. The Mann-Whitney *U*-test was used for statistical analysis. (B) ROC analysis was employed to assess the sensitivities and specificities of the nine markers listed above for discriminating deteriorating HAM/TSP patients from stable patients while controlling for disease duration. AUC = area under the ROC curve; 95% CI = 95% confidence interval.
doi:10.1371/journal.pntd.0002479.g005

of biomarker levels, but it is of course not conclusive. We expect that a prospective study in the future will reveal the answer to this question.

The results of this study indicate that CXCL9 and/or CXCL10 may play a key role in the pathogenesis of HAM/TSP by recruiting more inflammatory cells to the spinal cord lesions. In this study, we measured the levels of the chemokines in the CSF that might play a part in inducing the migration of T-helper (Th) cells. $CD4^+$ Th cells differentiate from naïve T-cells to members of the Th subset (e.g., Th1, Th2, Th17, or Treg cells), and each one expresses its own characteristic chemokine receptors [43]. Usually, Th1 cell express CCR5/CXCR3 receptors, Th2 and Treg cells express CCR4, and Th17 express CCR6. Interestingly, CCR4 ligands (CCL17 and, CCL22) and the CCR6 ligand (CCL20) were not detected in the CSF of HAM/TSP patients. Moreover, of the CCR5 ligands, only CCL5 was elevated, but only slightly, and there was no association with rate of disease progression. Of the CXCR3 ligands, only CXCL9 and CXCL10 were correlated with the rate of disease progression. These results show that the pathology of HAM/TSP is unique among immune disorders in that, unlike other inflammatory disorders such as multiple sclerosis or rheumatoid arthritis that exhibit Th17 as well as Th1 involvement, the chemokine involvement in HAM/TSP is Th1-dominant. In a previous study, cytokines produced by HTLV-I-infected T-cells in HAM/TSP patients were analyzed, and the results showed that IFN-γ was elevated and IL-17 reduced [43,44]. Taken together, the results of these studies indicate that the characteristics of HTLV-1-infected T-cells themselves may be responsible for the Th1-dominant chemokine production observed in HAM/TSP. Also, these results suggest that the CXCR3-ligand (CXCL9 and CXCL10) interactions play an important role in the pathophysiology of HAM/TSP. Recently it was established that these CXCR3-ligand interactions are extremely important for the pathogenesis of several neurological disorders [33]. Therefore, future research on the significance of these interactions in the pathogenic process of HAM/TSP will be important for clarifying the suitability of CXCL9 and CXCL10 as biomarkers or therapeutic targets.

In conclusion, in this retrospective study, we have demonstrated that CSF levels of CXCL10, CXCL9, and neopterin are promising candidate prognostic biomarkers for HAM/TSP. These biomarkers may provide a means for the early identification of patients at increased risk of debilitating disease progression, those that may need anti-inflammatory therapies to limit or prevent this, and for evaluating the efficacy of such therapies. This initial identification of prognostic biomarkers for HAM/TSP should be followed by a future multicenter prospective clinical study.

Supporting Information

Figure S1 Diagram illustrating the biomarker selection process. A total of 26 biomarker candidates including 9 in the blood and 17 in the CSF underwent the following selection processes: 1) pre-screening of the cytokines for presence in HAM/TSP patients, 2) selection for markers elevated in HAM/TSP patients with respect to controls (AUC>0.8), 3) selection for markers elevated in deteriorating HAM/TSP patients with respect to stable patients (AUC>0.8) in a cohort termed the Training Set, 4) validation of the selected markers by evaluating again (AUC>0.8) in a second cohort termed the Test Set. The darkening of an arrow's color represents that marker's failure to meet the selection criteria, and the termination of an arrow indicates that no further testing was conducted for that marker. CYT = cytokine, HTLV-1 PVL = HTLV-1 proviral load, Ab Titer = anti-HTLV-1 antibody titer, AUC = area under the ROC curve.
(TIF)

Figure S2 Questionnaire on the development of motor disability over time as measured using Osame's Motor Disability Score (OMDS). The first and second columns indicate the OMDS numerical value and description, respectively. Doctors interviewed the patients and filled in the table according to the following instructions: in the bottom row, write the ages at which symptoms listed to the left first appeared, and above the age check the box in the row corresponding to the symptom.
(TIF)

Figure S3 Rate of disease progression in HAM/TSP patients without any history of HAM/TSP-targeting treatment. Each line illustrates the change in OMDS over time for an individual patient after disease onset for (**A**) all patients in the Training Set (n = 30) and (**B, left**) only deteriorating patients (n = 11) including three particularly rapidly progressive patients (shown as solid black circles) and (**B, right**) only stable patients (n = 14).
(TIF)

Figure S4 Comparison of CSF levels of nine chemokines in control subjects and HAM/TSP patients. The CSF levels of nine chemokines (CCR5 ligands: CCL3, CCL4, and CCL5; CXCR3 ligands: CXCL9, CXCL10, and CXCL11; CCR4 ligands: CCL17 and CCL22; CCR6 ligand: CCL20) were compared between control subjects (control; n = 8) and HAM/TSP patients (HAM; n = 30). Horizontal bars indicate median values. The Mann-Whitney *U*-test was used for statistical analysis.
(TIF)

Figure S5 Low sensitivity of CSF cell count for detection of HAM/TSP. (**A**) Sensitivities of four potential CSF markers for detection of HAM/TSP. For CSF CXCL10, CXCL9, and neopterin, dotted lines indicate reference values, defined as mean for control subjects +3 standard deviations. For CSF cell count, the dotted line represents the pre-established reference value of 15/3 mm^3. The sensitivity of CSF cell count was much lower than those of the other CSF markers. (**B**) Direct comparison of the sensitivities of CSF cell count and the other three CSF markers. The horizontal dotted lines all represent the reference value for CSF cell count (≤15/3 mm^3), and each vertical dotted line indicates the reference value for each of the other CSF markers. With these lines drawn, one can see in the shaded area the numerous patients with CSF cell counts within the normal range but abnormally high levels of each of the other inflammatory markers, thus directly illustrating the comparatively low sensitivity of CSF cell count.
(TIF)

Figure S6 Changes in levels of CSF markers and OMDS over time in four untreated HAM/TSP patients. The three graphs illustrate the changes over time in CSF CXCL10 (top), neopterin (middle), and OMDS (bottom) for four untreated stable HAM/TSP patients. The patients were observed for 60 months (No. 1), 56 months (No. 2), 49 months (No. 3), and 39 months (No. 4).
(TIF)

Figure S7 Significant positive correlation between the proviral load in PBMCs and four CSF markers. HTLV-1 proviral load in PBMCs was compared with the levels of each of four CSF markers (CXCL10, CXCL9, neopterin, and cell count) in HAM/TSP patients (n = 53). Data analysis was performed using the Spearman's rank correlation test.
(TIF)

Figure S8 Significant higher CSF levels of CXCL10, CXCL9, and neopterin even in stable HAM/TSP compared to controls. The levels of four CSF markers (CXCL10, CXCL9, neopterin, and cell count) were compared among three groups (HTLV-1-infected controls, n = 8; stable HAM/TSP patients, n = 25; and deteriorating HAM/TSP patients, n = 20) assembling patients from both Training and Test Sets combined. The horizontal bar indicates the median value for each group. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post-hoc tests. ns: not significant, * $P<0.05$, *** $P<0.001$.
(TIF)

Table S1 Demographics of HAM/TSP patients and control subjects. There were no significant differences in the demographics of HAM/TSP patients versus control subjects.
(DOCX)

Table S2 Demographics and clinical characteristics of HAM/TSP patients (Training set + Test Set). Among the HAM/TSP patients from the Training and Test Sets pooled together, deteriorating patients experienced disease onset significantly later in life and had lived with the disease for shorter periods.
(DOCX)

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Author Contributions

Conceived and designed the experiments: YY TS SJ SI. Performed the experiments: TS HA NA JY. Analyzed the data: TS AU NA NY HA JY EI TU YH KN TN. Contributed reagents/materials/analysis tools: YY AU YH. Wrote the paper: YY TS ACR.

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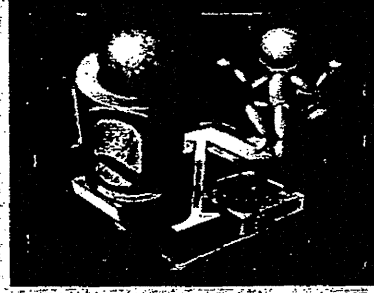
QUESTION & ANSWER
あなたの質問にお答えします

Q シャルコー・マリー・トゥース病とは、どんな病気ですか



小学校中学年の頃から、足関節外果骨折や捻挫を起こしていた男児が、高学年になって、整形外科で「シャルコー・マリー・トゥース病」と診断されました。初めて聞く病名ですが、どんな病気なのでしょう。今後、どのように経過していくのでしょうか。くわしく教えてください。

●北海道　H小学校　C・Y

京都府立医科大学附属
北部医療センター
病院長　**中川 正法**

シャルコー・マリー・トゥース病とは

A 遺伝子異常による末梢神経疾患。多くは20歳頃までに発症します

シャルコー・マリー・トゥース病とは

ご質問にあるシャルコー・マリー・トゥース病（Charcot-Marie-Tooth disease、以下CMTと略）は、遺伝子異常による末梢神経疾患の総称です。一般的に、まれな病気と言われていますが、欧米の疫学調査では2,500人に１人、わが国でも１万人に１人との報告があります。

遺伝子異常の種類にもよりますが、基本的に男女差はありません。CMTは、一般的には０歳～20歳頃までに発症しますが、60歳以降に発症される方もおられます。

末梢神経障害により、手足の筋力や感覚の低下がゆっくりと進んでいきます

主な症状

CMT患者さんの症状は、足や下腿・手・前腕などの四肢遠位部の筋肉が、少しずつゆっくりと進行しながら（緩徐進行性）萎縮し、同部位の感覚が少し鈍くなることがあげられます（次頁写真）。

患者さんの多くは、青年期から中年期にかけて、足・足趾の変形（凹足）や足の筋力低下（スリッパが脱げやすい、段差につまずくなど）があり、特徴的な歩き方（鶏のように、両大腿をやや大げさに挙上し、両趾先を垂れて歩くので、「鶏歩」と言います）で気づかれます。

中には、下肢の筋力低下や変形のために、足首の捻挫や骨折をされることもあります。話をよくうかがうと、「子どもの頃からかけっこで遅いほうだった」「子どもの頃から足が小さかった」など、軽い症状は子どもの頃から出現している方が多いようです。また、症状の重い患者さんでは、幼少期、場合によっては、生まれたときにすでに症状が出ている場合もあります。

CMTの中核症状は、末梢神経障害による筋力低下や感覚低下などですが、中には、目が見えにくい・音が聞こえにくいなどの症状（網膜や聴神経の障害）が合併したり、病気の進行とともに、脊柱の変形を生じたりするなど、多様な症状を呈する患者さんもいます。

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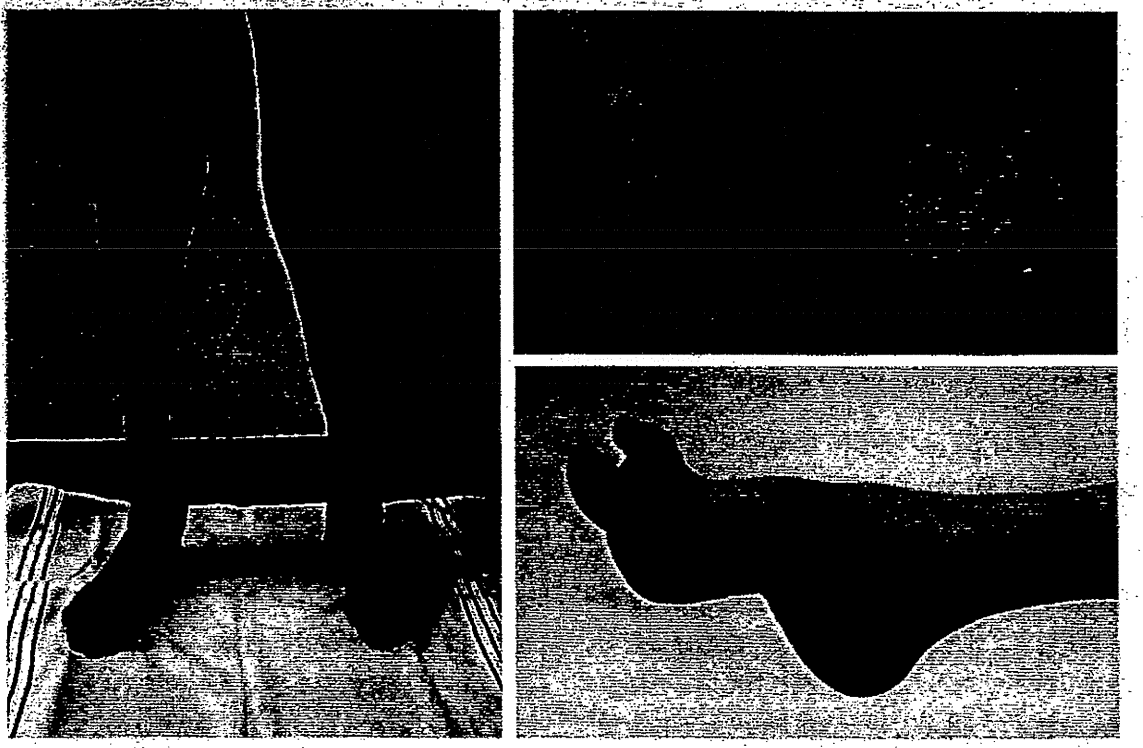
●写真　シャルコー・マリー・トゥース病でみられる四肢遠位部の筋萎縮と凹足



親から子に必ず遺伝するわけではなく、原因となる遺伝子も多数あります

CMTと遺伝子

前述したように、CMTは遺伝子の異常によって生じる疾患です。

遺伝様式には、常染色体優性遺伝（両親のどちらかに症状があって、だいたい50%の確率で子どもに遺伝するもの）や、常染色体劣性遺伝（両親には症状がなくても、子どもに発症することがあるもの）、X染色体劣性遺伝（X染色体上の遺伝子の異常で、男子のみに発症するもの）などがあり、遺伝子が関係していても、親から子どもに必ず遺伝するわけではないことに注意する必要があります。

遺伝性疾患としてのCMTのもう一つの特徴は、「遺伝的多様性」と言われています。「遺伝的多様性」とは、異なる遺伝子の異常によって、同じ症状が出現するということです。つまり、遺伝子Aの異常でも、遺伝子Bの異常でも、区別がつきにくい、同じような手足の筋力低下というCMTに共通した症状が出現するということであり、CMT患者さん同士であっても、原因となる遺伝子が異なっている場合があるということです。

現在までに分かっているだけでも、50個の異なるCMTの原因遺伝子が知られています。その中で、もっとも多いのがPMP22というタンパク質をコードしている遺伝子の異常です。CMTの40%の患者さんは、この遺伝子の異常であることが知られています（CMT1A型といいます）。

シャルコー・マリー・トゥース病の治療と経過

まず、問診と神経学的診察を行ない、必要に応じて各種検査を実施します

診断

CMTの診断は、問診、神経学的診察、電気生理学的検査、遺伝子検査で行なわれます。

問診と神経学的診察でCMTが疑われた場合には、末梢神経の働きを調べる神経伝導検査を行ないます。必要に応じて、針筋電図検査、神経超音

波検査、神経生検（足のくるぶしのところにある腓腹神経の生検です）なども行ないます。神経超音波検査は痛くありませんが、神経伝導検査、針筋電図検査、神経生検は痛みを伴います。

これらの検査を行なった上で異常が見られた場合には、遺伝子検査にて確定診断となります。

遺伝子検査といっても、患者さんの負担としては採血だけです。ＰＭＰ22遺伝子のＦＩＳＨ法という検査は健康保険が適用されますが、ほかの遺伝子検査は、大学の研究室での解析になります。

多くは自力歩行や杖歩行が可能ですが、約20%は車いすを使用

経過

ＣＭＴの経過については、原因となっている遺伝子異常によって異なりますが、一般的には筋力低下、感覚障害がゆるやかに進行していきます。

厚生労働科学研究費補助金難治性疾患克服研究事業ＣＭＴ研究班（ＣＭＴ研究班）の調査では、多くの方は自力歩行または杖歩行が可能ですが、車いすを使用される方は約20%、寝たきりになる方は１%とされています。

ＣＭＴに特異的に効果がある治療法は、まだありません

治療

残念ながら、現時点では、ＣＭＴに特異的に効果があると科学的に証明された治療はありません。

ＣＭＴのモデル動物では、オナプリストンという抗ホルモン剤や、ビタミンB_{12}、クルクミンなどの治療効果が報告されていますが、現時点では、これらの薬剤のヒトでの安全性や臨床効果については、十分検討されていません。

最近、ＣＭＴ１Ａに対するアスコルビン酸投与試験が、わが国と欧米で行なわれました。ＣＭＴ１Ａのモデル動物では有効性が見られたので期待されましたが、いずれの試験でもＣＭＴ１Ａに対するアスコルビン酸の有効性は証明されませんでした。

わが国のアスコルビン酸投与試験では、握力の若干の改善がみられましたが、主要評価項目では投与群と非投与群で有意差がありませんでした。しかし、あきらめることなく、今後も新しい治療法を検討していきたいと思います。

適切なフットケアや理学療法、運動で機能的な改善や筋力を維持できます

とはいえ、何も対応方法がないわけではありません。

患者さんに合った靴や下肢装具など、適切なフットケアを行なうことで、機能的な改善が期待できます。また、理学療法や適度の運動は、筋力と筋の耐性を維持する上で推奨されます。手術療法が機能改善や機能維持に役立つ場合もあります。

ＣＭＴは致死的な疾患ではありませんし、寿命に影響を与える疾患でもありません。ＣＭＴの患者さんの多くは、仕事を続けることが可能であり、杖が必要になることは多いですが、車いすのみの生活になることはまれです。太りすぎには注意してください。

ＣＭＴの研究は進んでいます。現在の状態を維持し、希望ある毎日を送ってください

患者さんとご家族のみなさんへ

原因遺伝子が次々に明らかになり、ＣＭＴ発症のメカニズムについての研究成果の報告も相次いでいます。５年前に、「ＣＭＴ友の会」も発足しました。ＣＭＴについて正しく理解し、今後の研究成果を期待しながら、現在のＡＤＬ（activities of daily living／日常生活動作）を少しでも維持し、希望のある毎日を送っていただければと考えています。

ＣＭＴの詳細については、ＣＭＴ研究班から『シャルコー・マリー・トゥース病診療マニュアル』（金芳堂）が出版されていますので、参考にしてください。

神経疾患治療ノート

Charcot-Marie-Tooth 病

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はじめに

Charcot-Marie-Tooth 病（CMT）は，最も頻度の高い遺伝性ニューロパチーであり世界の患者数は約 260 万人と推定され，わが国でも人口 10 万人対 10.8 人との報告がある[1]．CMT は運動神経伝導速度に基づいて，脱髄型，軸索型，中間型に大別される．一般的に生命的予後はよいが，車椅子使用患者は約 20%，寝たきり患者は 1%とされている．CMT の原因遺伝子は 50 種類以上が特定され，CMT の遺伝子診断は大きく進展している．本稿では，CMT の治療に関する最近の知見を概説する[2]．

CMT に対する薬物治療

遺伝子診断が不十分な時代の CMT 治療研究として，Cronassial 筋注（ガングリオシド製剤），linoleic/γ-linoleic essential fatty acids，vitamin E，coenzyme Q10，modafinil などの使用報告がある．いずれの研究も十分な規模の無作為化比較対照試験 randomized controlled trial（RCT）ではない．

1．CMT1A の薬物療法

最も頻度が高い CMT1A は *PMP22* の重複によって引き起こされる脱髄型 CMT である．

A．アスコルビン酸臨床試験

アスコルビン酸は，後根神経節-Schwann 細胞の培養系における myelination に必須であり，アスコルビン酸欠乏が大腿神経障害を引き起こすことが報告されている．アスコルビン酸が CMT1A モデルマウスに有効であるとの報告があり，国内外で臨床試験が行われた．厚生労働省研究委託費「難治性ニューロパチーの病態に基づく新規治療法の開発」研究班のもとで行われた CMT1A に対するオープン試験（UMIN 試験 ID：UMIN000001535）では，プライマリーエンドポイントである CMT neuropathy score（CMTNS）に有意な改善はなくアスコルビン酸の有効性は確認できなかった[3]．海外でのアスコルビン酸投与試験でもわが国の研究班の結果と同様にアスコルビン酸の有効性は証明されなかった[4]．しかし，Burns らは 12ヵ月間の追加オープン試験（アスコルビン酸 25～37 mg/kg/日）を 7～16 歳の CMT1A 5 例に行い，四肢遠位部筋力の有意な改善を認め，軽症の若年 CMT1A にはアスコルビン酸の大量長期投与が有効である可能性が示された[5]．わが国の臨床試験でも右握力は有意に改善しており，ある程度の効果はあるのではないかと考えられる．現在，非利き手正中神経の運動神経軸索興奮性を測定し，アスコルビン酸 20 mg/kg/日/12ヵ月間投与前後での変化を検討中である．

B．Neurotrophin-3（NT-3）

Sahenk らは，8 例の CMT1A 患者に NT-3 を 150 μg/kg/週 3 回 24 週，またはプラセボ投与を行なった．その結果，NT-3 投与群では末梢神経障害スコア（NIS）が改善し，再生軸索が増加したことを報告した[6]．その後，この結果を再現する報告はなく，また，運動機能の改善はなかったことなどの問題点がある．

C．プロゲステロン拮抗薬

プロゲステロンは Schwann 細胞や神経細胞で産生され，*PMP22*，*MPZ* などの発現を促進し，CMT1A 動物モデルの症状を悪化させること，プロゲステロン拮抗薬であるオナプリステロンが CMT1A 動物モデルに有効であることが報告されている[7]．しかし，オナプリステロンは肝毒性のためヒトに使用することは出来ない．一方，プロゲステロン刺激薬は *PMP22*，*MPZ* の mRNA 発現を増加させる作用があり，ハプロ不全を示す hereditary neuropathy with liability to pressure palsies（HNPP）や nonsense-mediated mRNA decay 関連 *MPZ* 変異 CMT に有効である可能性がある．

最近，培養細胞に *PMP22* を発現させ，その発現を抑制する化合物をオートマチックにスクリーニングするハイスループットな方法が開発されている．

D．Network pharmacology

Network pharmacology という bioinformatics に基づく新しい治療薬開発法が注目されている．このネットワーク薬理学からデザインされた CMT1A の治療法開発が進められている．パリにある Pharnext 社が CMT1A 60 例を対象に PXT3003（バクロフェン，ナルトレキソン，ソルビトールの合剤）の治験を行なっている[8]．

2．点変異による CMT の薬物療法

クルクミン：　クルクミンは秋ウコンやカレー粉に多く含まれている自然の黄色色素である．クルクミンは用量依存的に *pmp22* 点変異マウスの運動機能を改善した．Burns らは，*PMP22* 点変異（Ser72Leu）を有する 15 歳の女性患者にクルクミンを 50 mg/kg/日（1500 mg，250 mg×6 カプセル/分 2）4ヵ月，その後，75 mg/kg/日（2500 mg，250 mg×10 カプセル/分 3）8ヵ月の計 12ヵ月間，経口投与した．安全性に問題はなかったが，評価指標の改善はなかった．しかし，幸福感，満足感に関する自覚的な改善があったと報告している[9]．

モデル動物等を用いた CMT 治療法の開発

CMT のモデル動物による研究も進展しており，脱髄型 CMT では約 25 種類の動物モデルが報告されている（http://www.molgen.ua.ac.be/CMT Mutations）．TrkB と TrkC に対する作動性抗体が TremblerJ マウスの運動機能を改善，間葉系幹細胞が CMT1B を含む脱髄性 CMT の治療として有望，ドキシサイクリンが hNF-L^{P22S}；tTa マウスの症状を改善，Colony-stimulating factor-1 とその受容体が CMT の治療標的分子，histone deacetylase 6（HDAC6）阻害薬が遺伝性軸索障害の治療に有効，c-Jun N-terminal kinase（JNK）が CMT 治療の標的，*MFN1* 発現増加が変異 *MFN2* による軸索障害を

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改善などの報告がある[2]．最近，CMT 患者から iPS 細胞を確立し研究が進められている．

注意すべき薬物

CMT 患者が他の内科疾患等に罹患した場合，必要に応じて使用される薬剤が末梢神経障害を悪化させる場合がある．とくに抗腫瘍薬であるビンクリスチン・シスプラチン・タキソール，HIV 治療薬などが CMT の症状を悪化させる可能性がある(http://www.charcot-marie-tooth.org/med_alert.php)．最近，癌化学治療薬の投与により末梢神経障害が顕在化し，CMT の遺伝子変異が明らかとなった例が報告されている[10]．CMT の臨床症状を示さない潜在的な CMT 患者がいる可能性があり，抗腫瘍薬投与前の神経伝導検査の実施は末梢神経障害の重症化を防ぐ点で推奨される．

炎症性ニューロパチーと CMT

CMT1A を代表とする遺伝性ニューロパチーと chronic inflammatory demyelinating polyneuropathy(CIDP)との合併例の検討から，CMT 患者 250 人に 1 人が CIDP 様の炎症性ニューロパチーを発症すると推定されている．CMT 患者で臨床症状の急性悪化を認めた場合には，CIDP の治療に準じた対応も考慮すべきである[11]．

外科治療

関節変形が進行し，装具を用いても足を適切な位置に保てず歩行に支障が出てきた場合，関節の安定性を図るために筋延長術や骨切り術などの整形外科手術が適応となる場合がある．Leeuwesteijn らは CMT の凹足に対する骨切り術後成績の検討を行い，CMT 33 例の術後平均 57ヵ月の評価で疼痛，歩行障害が有意に改善し，90％の患者が足変形の矯正に満足していたと報告している[12]．内反尖足の外科治療は CMT 患者により安定した歩行をもたらすと考えられるが，その手術適応や外科的治療施行時期についてのより明確な基準が必要とされている．

麻　酔

CMT 患者が手術や出産などのために麻酔を受ける際にも注意が必要である．一般的に，末梢神経障害を増悪させないために脊椎麻酔(脊髄くも膜下麻酔)や硬膜外麻酔は避けるべきであるといわれているが，脊髄くも膜下麻酔や硬膜外麻酔で良好な結果が得られた帝王切開の例，吸入麻酔のみで骨折の観血的整復固定術を行なった例，全静脈麻酔と閉鎖神経ブロックを併用した膀胱腫瘍手術例なども報告されている[13]．

リハビリテーション

「運動のし過ぎはよくないでしょうか？」と CMT 患者または家族から尋ねられることが多い．「過労による筋力低下 overwork weakness」についてはこれまでも論議が多い．CMT 患者に日常生活において手の使用をひかえるようにアドバイスする十分なデータはない．CMT の関節可動域制限の予防のために，発症早期から下腿三頭筋の持続伸張訓練を行う必要がある[14]．日々の生活に運動療法を組み込むことで，疾患の自然経過による進行以上の悪化を抑える効果が期待される．

1．装具療法

装具の使用においては，① 機能障害にあった装具を，② 使用目的と使用時間帯を明確にして，装着することが大切である．短下肢装具の使用は歩行と姿勢の異常を部分的に改善したとの報告がある[15]．

2．ロボット技術

下肢自立支援ロボットでは，レジーナ®(日本ロジックマシン)，ロボットスーツ HAL®(筑波大学：Hybrid Assistive Limb)がある．厚生労働省難治性疾患克服研究事業として，下肢装着型補助ロボット(HAL-HN01)に関する医師主導治験(研究代表者 中島 孝先生)が開始された．

3．日常生活の工夫

CMT に対する有効な薬物療法は未だ開発されていないが，少しでもよい健康状態を維持することは重要である．日常的な運動習慣と食事療法が大切である．CMT 患者は消費カロリー/日が健常者より有意に少なく，メタボリック症候群になりやすい傾向がみられる．少なくとも「現在の体重を維持する」ことが重要である．四肢遠位の冷感，浮腫，外傷，胼胝，潰瘍の形成などに注意が必要である[16]．

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