では、有意差はみられなかったが、健常者と比較し、脳血管性認知症患者およびアルツハイマー型認知症患者において抗TPM4抗体価が高値となる傾向がみられた。広範な大脳深部白質病変を有する高齢者およびアルツハイマー型認知症患者においては抗TPM4抗体が大脳微小血管の消失をもたらす原因の一つとなっている可能性もあり、今後、抗TPM4抗体の血管新生阻害作用に関して、in vivoにおいてその効果を確認する必要があると考えられる。また、その他の神経疾患を含めたさらに多数例での抗TPM4抗体の測定と大脳白質病変との関連性を検討する必要もあると考えられる。これらの検討により、抗TPM4抗体の、大脳白質病変との関連性が確立したものとなれば、大脳白質病変の血清バイオマーカーとなり、抗体価が高値の症例に対して、抗免疫療法などによる大脳白質病変の進展予防、ひいては認知症や寝たきりの予防につながる可能性も考慮される。

今回、抗TPM4抗体以外にも広範な大脳白質病変との関連性が予想される5つの抗血管内皮細胞抗体を同定した。これら自己抗体の認識抗原蛋白は、コラーゲン様ペプチドのリシル残基のヒドロキシル化を触媒する蛋白、小胞体内腔に存在するカルシウム結合性蛋白、脂肪滴に関連する蛋白、ATP依存性にユビキチン化蛋白のデグラデーションに関連する蛋白、解糖系酵素であった。現在までに、このうちのprocollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), reticulocalbin-1, phosphoglycerate mutase 1 (PGAM1)に対する自己抗体につきELISA解析を行ったが、抗体価と大脳白質病変の程度に相関性は見られなかった。今後、残りのPirilipin 2と26S protease regulatory subunit 8につきELISA解析を行う予定である。

一方、本研究において、60歳以上の大脳白質病変非合併患者・健常者に特異的と思われる２種類の抗血管内皮細胞抗体を同定した。これらの自己抗体が血管内皮細胞に保護的に作用している可能性もあり、今後さらなる検討が必要と考えられた。

また今回の研究成果として、晩期発症型アルツハイマー型認知症患者の血清中に比較的多く認められる抗血管内皮細胞抗体を同定したことがあげられる。これまでアルツハイマー型認知症の発症機序において脳実質内におけるアミロイドβ蛋白質（Aβ）の過剰な蓄積と神経細胞死が重要とされてきたが、同時にBBBの機能障害や大脳還流障害といった血管障害因子も重要であるとされている(Jack C, Lancet Neurol, 2004)。これらは、単に認知機能の低下を促進させるだけでなく、最近の研究では発症早期よりみられることが確かめられ、アルツハイマー型認知症の病態機序における重要な役割を担っている可能性も示唆される。一方、アルツハイマー型認知症の病態において炎症機序や自己免疫機序の存在を指摘する報告もある。自己抗体という観点からは、これまでアルツハイマー型認知症患者においていくつかの報告があり(Kellner A et al., Ann Neurol. 2009) 、最近では同年代の健常者と比較すると、アルツハイマ

一型認知症患者の剖検脳ではニューロンに免疫グロブリンの沈着が有意に多くみられることが報告されている（Levin et al., Brain research. 2010）。

今回、晩期発症型アルツハイマー型認知症患者の血清中に特異的に存在する抗血管内皮細胞抗体の認識抗原蛋白の一つとしてMitochondrial import receptor subunit TOM40 homolog (Tom40)を同定した。Tom40はミトコンドリア外膜に存在し、ミトコンドリア内への蛋白の選択輸送に関与するチャンネルを形成していることが知られている。Tom40をコードする遺伝子TOMM40は19番染色体上のAPOE遺伝子近傍に位置し、最近のゲノムワイド関連解析の結果、TOMM40のリスクアリルはコントロールと比較し晩期発症型アルツハイマー型認知症の発症が２倍になる（Potkin et al., PLoS ONE, 2009）といった報告やTOMM40のイントロン変異（poly-T insertion）を持つ患者は、晩期発症型アルツハイマー型認知症の発症が約7年早くなる（Rosese et al., Pharmacogenomics J, 2010）といった報告もある。一方、アルツハイマー型認知症患者の脳内においては、エネルギー代謝障害と低還流状態がおきているとされている。ミトコンドリア機能障害及び血管障害はアルツハイマー型認知症の病態において極めて重要な役割を担っているものの、その原因に関しては十分解明されていない。健常者にはなくアルツハイマー型認知症患者の脳内のみで、アミロイド前駆体蛋白がミトコンドリアの蛋白輸送に関連するチャンネルに沈着しているといった報告や（Devi L et al., J Neurosci. 2006）。アミロイド前駆体蛋白が、Tom40と結合し480kDaの安定した複合体を形成する、もしくはアミロイド前駆体蛋白がTom40とミトコンドリア内膜の蛋白であるTim23と結合し、620kDaのさらに大きな複合体を形成することによりミトコンドリア内への蛋白輸送が障害されミトコンドリア機能障害が生じるといった報告もある（Anandatheerthavarada HK et al., J Cell Biol. 2003）。一方、Aβのミトコンドリア内への輸送がTom40を介して行われ、神経毒性を生じるといった報告もある（Hansson Petersen CA et al., Proc Natl Acad Sci. USA, 2008）。これらの報告が示すようにTom40はアルツハイマー型認知症の病態機序、特にミトコンドリア機能障害の原因を考える上で極めて重要な分子であることが予想される。

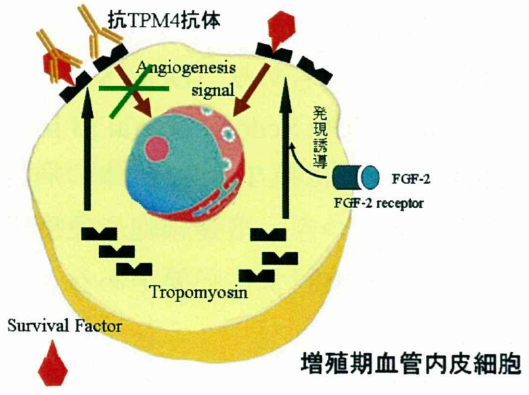
今回、抗Tom40抗体はアルツハイマー型認知症患者の認知機能障害と有意な関連性が認められた。抗Tom40抗体の病的意義に関しては不明であるが、Tom40の局在性を考慮すると、抗体が病態に直接的な影響を及ぼすのは困難と思われる。しかし、自己抗体が細胞内に選択的に取り込まれ、細胞内抗原と結合することを指摘した報告もあり（Deng SX et al., Int Immunol. 2000; Seddiki N et al., J Immunol. 2001）、今後は抗Tom40抗体の病態に及ぼす影響につき、培養細胞や免疫動物などを用いて検討する必要があると考えられた。

また同じく今回アルツハイマー形認知症患者より新たに同定したProtein

disulfide isomerase（PDI）に関しては、蛋白のジスルフィド結合を触媒することにより蛋白のフォールディングを促進することが知られている。最近アルツハイマー型認知症患者の神経原線維変化中に、このPDI陽性の封入体が存在することが報告された（Honjo et al., Brain Res, 2010）。今後、これら自己抗体とアルツハイマー型認知症の病態との関連性を検討することにより、同疾患の病態機序の解明ならびに新たな診断マーカーの確立と治療法の開発につながる可能性も考えられた。

大脳微小血管内皮細胞障害は、認知機能障害と歩行障害などの運動機能障害をきたす原因となりうる大脳白質病変と密接に関連し、その血清学的診断マーカーの確立や治療法の開発は極めて重要と考えられる。今回同定した抗血管内皮細胞抗体以外にも、新たなバイオマーカーとなり得る抗血管内皮細胞抗体が存在する可能性があり、今後は、今回用いた二次元免疫ブロット法に変わる新たな自己抗体の検出法の開発も重要と考える。

図９）抗TPM4抗体の血管新生阻害作用機序の仮説



（Donate F, et al. Current Cancer Drug Targets 2004より一部改変）

E. 結論

1. 高齢者は若年者に比較し、多くの抗血管内皮細胞抗体を有する
2. 広範な大脳白質病変と関連することが予想される抗血管内皮細胞抗体が存在する。
3. 抗TPM4抗体高値は、大脳深部白質の癒合性病変の出現に関連する独立した危険因子である。
4. TPM4は大脳微小血管内皮細胞の細胞膜表面にも発現している。
5. 抗TPM4抗体は、大脳微小血管内皮細胞のangiogenesisを抑制することにより大脳白質病変の進展に関っている可能性がある。
6. 抗Tom40抗体は、アルツハイマー型認知症患者の認知機能障害と関連する可能性がある。

F. 健康危険情報

なし

G. 研究発表

1, 論文発表

- Kimura A, Sakurai T, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Tanaka R, Takemura M, Seishima M, Inuzuka T: High prevalence of autoantibodies against phosphoglycerate mutase 1 in patients with autoimmune central nervous system diseases. J Neuroimmunol. 219, 105-108, 2010.
- Kimura A, Sakurai T, Koumura A, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Yoshino H, Yuasa T, Inuzuka T: Motor-dominant chronic inflammatory demyelinating polyneuropathy. J Neurol. 257, 621-629, 2010.
- Kimura A, Kanoh Y, Sakurai T, Koumura A, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Takemura M, Seishima M, Inuzuka T: Antibodies in Patients With Neuropsychiatric Systemic Lupus Erythematosus. Neurology. 74, 1372-1379, 2010.
- 櫻井岳郎, 田中優司, 木村暁夫, 保住功, 犬塚　貴：胃腸炎後に発症した抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺の１例 神経内科 2009;71:514-516.
- 木村暁夫, 犬塚　貴:膠原病に伴う神経・筋障害－診断と治療の進歩　膠原病における新たな抗神経抗体の検索. 日本内科学会雑誌, 99, 115-120, 2010.
- Hayashi Y, Sakurai T, Tanaka Y, Yamada M, Koumura A, Kimura A, Hozumi I, Inuzuka T: Markedly ring-enhanced optic nerves due to metastasis of signet-ring cell gastric carcinoma. Internal medicine. 49, 517, 2010.
- Hozumi I, Koumura A, Kimura A, Hasegawa T, Honda A, Hayashi Y, Hashimoto K, Yamada M, Sakurai T, Tanaka Y, Satoh M, Inuzuka T: High levels of copper, zinc, iron and magnesium, but not calcium, in the cerebrospinal fluid of patients with Fahr's disease. Case Rep Neurol. 2, 46-51, 2010.
- Hayashi Y, Kimura A, Watanabe M, Yamada M, Sakurai T, Tanaka Y, Hozumi I, Inuzuka T: Serial monitoring of basal metabolic rate for therapeutic evaluation in an Issacs' syndrome patient with chronic fluctuating symptoms. Internal medicine. 49, 475-477, 2010.
- 櫻井岳郎, 木村暁夫, 山田恵, 林祐一, 田中優司, 保住功, 犬塚貴：特発性中脳水道狭窄症による閉塞性水頭症に対するV-Pシャント術の1年後から急速に進行するparkinsonismを呈した1例. BRAIN and NERVE. 62, 527-531, 2010.
- Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J: Antibodies to the $GABA_B$ receptor in limbic encephalitis with seizures: case series and characterization of the antigen. Lancet Neurol. 9, 67-76, 2010.
- Sakurai T, Kimura A, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Inuzuka T : Identification of

antibodies as biological markers in serum from multiple sclerosis patients by immunoproteomic approach. J Neuroimmunol. 233, 175-180, 2011.

- Hozumi I, Hasegawa T, Honda A, Ozawa K, Hayashi Y, Hashimoto K, Yamada M, Koumura A, Sakurai T, Kimura A, Tanaka Y, Satoh M, Inuzuka T: Patterns of levels of biological metals in CSF differ among neurodegenerative diseases. J Neurol Sci. 303, 95-99, 2011.
- Tanaka Y, Kato T, Nishida H, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T. Is there delayed gastric emptying of patients with early-stage, untreated Parkinson's disease? An analysis using the (13)C-acetate breath test. J Neurol. 258, 421-426, 2011.
- Tanaka Y, Hayashi Y, Kato J, Yamada M, Koumura A, Sakurai T, Kimura A, Hozumi I, Hatano Y, Hirose Y, Takami T, Nakamura H, Kasahara S, Tsurumi H, Moriwaki H, Inuzuka T. Diffuse skeletal muscles uptake of [18F] fluorodeoxyglucose on positron emission tomography in primary muscle peripheral T-cell lymphoma. Intern Med. 50, 2021-2024, 2011.
- Tanaka Y, Yoshikura N, Harada N, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Moriwaki H, Inuzuka T. Neuromyelitis optica in Japanese sisters. Intern Med. 50, 2829-2832, 2011.
- Kimura A, Sakurai T, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Takemura M, Seishima M, Inuzuka T: Elevated anti-heat shock protein 60 antibody titer is related to white matter hyperintensities. J Stroke Cerebrovasc Dis. 21, 305-309, 2012.
- Kimura A, Sakurai T, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Ohtaki H, Chousa M, Takemura M, Seishima M, Inuzuka T. Antibodies Against the Tom40 Subunit of the Translocase of the Outer Mitochondrial Membrane Complex and Cognitive Impairment in Alzheimer's Disease. J Alzheimers Dis. 29, 373-377, 2012.
- Tanaka Y, Yoshikura N, Harada N, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Inuzuka T. Late-onset patients with sporadic amyotrophic lateral sclerosis in Japan have a higher progression rate of ALSFRS-R at the time of diagnosis. Intern Med. 51, 579-584, 2012.
- Tanaka Y, Kato T, Nishida H, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T. Is there delayed gastric emptying in patients with multiple system atrophy? An analysis using the (13)C-acetate breath test. J Neurol. 2012 in press
- Yamaguchi Y, Hayashi A, Campagnoni CW, Kimura A, Inuzuka T, Baba H. L-MPZ, a novel isoform of myelin P0, is produced by stop codon readthrough. J Biol Chem. 2012 in press.

2, 学会発表

- 木村暁夫、櫻井岳郎、山田　恵、林祐一、田中優司、保住　功、犬塚　貴. 抗 Hsp60 抗体と大脳白質病変に関する研究. 第 50 回日本神経学会総会. 2009, 5, 20. 仙台
- 櫻井岳郎、木村暁夫、山田　恵、林祐一、田中優司、保住　功、犬塚　貴. プロテオミクス解析を用いた通常型多発性硬化症における疾患特異的抗神経抗体の検索. 第 50 回日本神経学会総会. 2009, 5, 22. 仙台
- 木村暁夫、櫻井岳郎、山田　恵、林祐一、田中優司、保住　功、犬塚　貴. 多発性硬化症患者における抗 Phosphoglycerate mutase 1 抗体の特異性に関する検討. 第 21 回日本神経免疫学会学術集会. 2009, 3, 13. 大阪
- 櫻井岳郎、木村暁夫、山田　恵、林祐一、田中優司、保住　功、犬塚　貴. プロテオミクス解析を用いた通常型多発性硬化症における疾患特異的抗神経抗体の検索. 第 21 回日本神経免疫学会学術集会. 2009, 3, 13. 大阪
- Kimura A, Sakurai T, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Inuzuka T: Identification of Antibodies Against Brain Antigen in Sera of Patients with Neuropsychiatric Systemic lupus erythematosus. The 61th American Academy of Neurology. Seattle, USA, 2009
- Tanaka Y, Nishida H, Yamada M, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Inuzuka T : Is there difference in gastric emptying of Parkinson's disease patients under long-term l-dopa therapy between with and without motor fluctuations? : An analysis using the 13C-acetate breath test. The 61th American Academy of Neurology. Seattle, USA, 2009
- 木村暁夫、櫻井岳郎、山田　恵、香村彰宏、林　祐一、田中優司、保住　功、犬塚　貴. Motor-dominant CIDP 患者の臨床的検討. 第 51 回日本神経学会総会. 2010, 5, 21. 東京
- 櫻井岳郎、木村暁夫、山田　恵、香村彰宏、林　祐一、田中優司、保住　功、犬塚　貴. 通常型多発性硬化症における抗 Stress 70 protein 抗体の特異性に関する検討. 第 51 回日本神経学会総会. 2010, 5, 22. 東京
- Tanaka Y, Kato T, Nishida H, Yamada M, Kohmura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T: Is there the delayed gastric emptying of untreated patients with early-stage Parkinson's disease? : An analysis using the 13C-acetate breath test. The 62th American Academy of Neurology. Toronto, USA, 2010
- 木村暁夫, 櫻井岳郎, 吉倉延亮, 山田恵, 林　祐一, 田中優司, 保住　功, 大瀧博文, 帖佐光洋, 犬塚　貴. 多発性筋炎患者における抗MYBPC2抗体および抗 PK M1/M2 抗体の同定と特異性の検討. 第 23 回日本神経免疫学会学術集会. 2011, 9, 15. 東京
- 木村暁夫, 櫻井岳郎, 山田　恵, 香村彰宏, 林　祐一, 田中優司, 保住　功, 犬塚　貴. プロテオーム解析を用い

た大脳白質病変に関連する抗血管内皮細胞抗体の同定. 第 52 回日本神経学会学術大会. 2011, 5, 18. 名古屋

- Kimura A, Sakurai T, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Ohtaki H, Chousa M, Takemura M, Seishima M, and Inuzuka T. Anti-Endothelial Cell Antibodies in the Elderly with Extended White Matter Hyperintensity. The 63th American Academy of Neurology. Honolulu, USA, 2011
- Tanaka Y, Kato T, Nishida H, Yamada M, Kohmura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Nagaki M, Moriwaki H, Inuzuka T: Is there the delayed gastric emptying of Patients with Multiple System Atophy? An analysis using the 13C-acetate breath test. The 63th American Academy of Neurology. Honolulu, USA, 2011

H. 知的財産権の出願・登録状況

1. 特許取得

   なし

2. 実用新案登録

   なし

3. その他

   なし

Ⅱ　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kimura A, Sakurai T, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Tanaka R, Takemura M, Seishima M, Inuzuka T	High prevalence of autoantibodies against phosphoglycerate mutase 1 in patients with autoimmune central nervous system diseases	Journal of Neuroimmunology	219	105-108	2010
Kimura A, Sakurai T, Koumura A, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Yoshino H, Yuasa T, Inuzuka T	Motor-dominant chronic inflammatory demyelinating polyneuropathy	Journal of Neurology	257	621-629	2010
Kimura A, Kanoh Y, Sakurai T, Koumura A, Yamada M, Hayashi Y, Tanaka Y, Hozumi I, Takemura M, Seishima M, Inuzuka T	Antibodies in patients with neuropsychiatric systemic lupus erythematosus	Neurology	74	1372-1379	2010
櫻井岳郎, 田中優司, 木村暁夫, 保住　功, 犬塚　貴	胃腸炎後に発症した抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺の１例	神経内科	71:	514-516	2009
木村暁夫, 犬塚　貴	膠原病に伴う神経・筋障害－診断と治療の進歩 膠原病における新たな抗神経抗体の検索	日本内科学会雑誌	99	115-120	2010
Hayashi Y, Sakurai T, Tanaka Y, Yamada M, Koumura A, Kimura A, Hozumi I, Inuzuka T	Markedly ring-enhanced optic nerves due to metastasis of signet-ring cell gastric carcinoma	Internal medicine	49	517	2010
Hozumi I, Koumura A, Kimura A, Hasegawa T, Honda A, Hayashi Y, Hashimoto K, Yamada M, Sakurai T, Tanaka Y, Satoh M, Inuzuka T	High levels of copper, zinc, iron and magnesium, but not calcium, in the cerebrospinal fluid of patients with Fahr's disease.	Case Reports in Neurology	2	46-51	2010

Hayashi Y, Kimura A, Watanabe N, Yamada M, Sakurai T, Tanaka Y, Hozumi I, Inuzuka T	Serial monitoring of basal metabolic rate for therapeutic evaluation in an Issacs' syndrome patient with chronic fluctuating symptoms	Internal medicine	49	475-477	2010
櫻井岳郎, 木村暁夫, 山田恵, 林祐一, 田中優司, 保住功, 犬塚貴	特発性中脳水道狭窄症による閉塞性水頭症に対するV-Pシャント術の1年後から急速に進行するparkinsonismを呈した1例	BRAIN and NERVE	62	527-531	2010
Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J	Antibodies to the GABAB receptor in limbic encephalitis with seizures: case series and characterization of the antigen	Lancet Neurol	9	67-76	2010
Sakurai T, Kimura A, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Inuzuka T	Identification of antibodies as biological markers in serum from multiple sclerosis patients by immunoproteomic approach	Journal of Neuroimmunology	233	175-180	2011
Hozumi I, Hasegawa T, Honda A, Ozawa K, Hayashi Y, Hashimoto K, Yamada M, Koumura A, Sakurai T, Kimura A, Tanaka Y, Satoh M, Inuzuka T	Patterns of levels of biological metals in CSF differ among neurodegenerative diseases	Journal of the Neurological Sciences	303	95-99	2011
Tanaka Y, Kato T, Nishida H, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T	Is there delayed gastric emptying of patients with early-stage, untreated Parkinson's disease? An analysis using the (13) C-acetate breath test	Journal of Neurology	258	421-426	2011

Tanaka Y, Hayashi Y, Kato J, Yamada M, Koumura A, Sakurai T, Kimura A, Hozumi I, Hatano Y, Hirose Y, Takami T, Nakamura H, Kasahara S, Tsurumi H, Moriwaki H, Inuzuka T	Diffuse skeletal muscles uptake of [18F] fluorodeoxyglucose on positron emission tomography in primary muscle peripheral T-cell lymphoma	Internal Medecine	50	2021-2024	2011
Tanaka Y, Yoshikura N, Harada N, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Moriwaki H, Inuzuka T	Neuromyelitis optica in Japanese sisters	Internal Medecine	50	2829-2832	2011
Kimura A, Sakurai T, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Takemura M, Seishima M, Inuzuka T	Elevated anti-heat shock protein 60 antibody titer is related to white matter hyperintensities	Journal of Stroke and Cerebrovascular Diseases	21	305-509	2012
Kimura A, Sakurai T, Yamada M, Koumura A, Hayashi Y, Tanaka Y, Hozumi I, Ohtaki H, Chousa M, Takemura M, Seishima M, Inuzuka T	Antibodies Against the Tom40 Subunit of the Translocase of the Outer Mitochondrial Membrane Complex and Cognitive Impairment in Alzheimer's Disease	Journal of Alzheimers Disease	29	373-377	2012
Tanaka Y, Yoshikura N, Harada N, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Inuzuka T	Late-onset patients with sporadic amyotrophic lateral sclerosis in Japan have a higher progression rate of ALSFRS-R at the time of diagnosis	Internal Medecine	51	579-584	2012
Tanaka Y, Kato T, Nishida H, Yamada M, Koumura A, Sakurai T, Hayashi Y, Kimura A, Hozumi I, Araki H, Murase M, Nagaki M, Moriwaki H, Inuzuka T	Is there delayed gastric emptying in patients with multiple system atrophy? An analysis using the (13)C-acetate breath test	Journal of Neurology		In press	2012
Yamaguchi Y, Hayashi A, Campagnoni CW, Kimura A, Inuzuka T, Baba H	L-MPZ, a novel isoform of myelin P0, is produced by stop codon readthrough	Journal of Biological Chemistry		In press	2012

Ⅲ　研究成果の刊行物・別刷り

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High prevalence of autoantibodies against phosphoglycerate mutase 1 in patients with autoimmune central nervous system diseases

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ABSTRACT

We identified the autoantibody against phosphoglycerate mutase 1 (PGAM1), which is a glycolytic enzyme, in sera from multiple sclerosis (MS) patients by proteomics-based analysis. We further searched this autoantibody in sera from patients with other neurological diseases. The prevalence of the anti-PGAM1 antibody is much higher in patients with MS and neuromyelitis optica (NMO) than in those with other neurological diseases and in healthy controls. It was reported that the anti-PGAM1 antibody is frequently detected in patients with autoimmune hepatitis (AIH). Results of our study suggest that the anti-PGAM1 antibody is not only a marker of AIH but also a nonspecific marker of central nervous system autoimmune diseases.

1. Introduction

Over the past few years, compelling data on the roles of B cells as sensors, coordinators and regulators of the immune response have strengthened the view that B cells and autoantibodies are fundamental factors for activating T cells and/or mediating tissue injury in several autoimmune-mediated diseases of the central nervous system (CNS) (Dalakas, 2008; Hasler and Zouali, 2006). In this study, we identified the autoantibody against phosphoglycerate mutase 1 (PGAM1) in sera from multiple sclerosis (MS) patients by proteomics-based analysis. Phosphoglycerate mutase is a glycolytic enzyme that catalyzes the interconversion of 3- and 2-phosphoglycerate with 2, 3-bisphosphoglycerate as the primer of the reaction (Fothergill-Gilmore and Watson, 1989). In mammalian tissues, there are two types of phosphoglycerate mutase: type M (also known as PGAM2) in muscles and type B (also known as PGAM1) in other tissues (Omenn and Cheung, 1974; Zhang et al., 2001). However, there are few reports on the anti-PGAM1 antibody (Lu et al., 2008; Zephir et al., 2006) and the specificity of this autoantibody is not clearly understood. To evaluate the specificity of this autoantibody, we assessed the prevalence of this autoantibody in sera from patients with various neurological diseases and healthy controls.

2. Patients and methods

2.1. Patients

Serum samples were obtained from patients with MS [$n=21$; male:female = 9:12; age range, 31–75; mean age, 49], neuromyelitis optica (NMO) [$n=13$; male:female = 2:11; age range, 26–79; mean age, 49], multiple cerebral infarctions (MCI) [$n=20$; male:female = 9:11; age range, 53–83; mean age, 71], infectious meningoencephalitis (IME) [$n=19$; male:female = 14:5; age range, 15–71; mean age, 45], and Parkinson's disease (PD) [$n=21$; male:female = 11:10; age range, 50–85; mean age, 68], and from healthy controls [$n=17$; male:female = 7:10; age range, 25–74; mean age, 44]. All the MS patients were diagnosed with clinically definite MS according to the criteria of Poser et al. (1983). All the NMO patients satisfied the 2006 revision to the Wingerchuk diagnostic criteria (Wingerchuk et al., 2006).

2.2. Preparation of tissue proteins

Under ether anesthesia, adult Wistar rats were sacrificed. Their cerebrums were immediately removed and frozen in dry-ice powder. The frozen brain tissue was homogenized in lysis buffer (7 M urea, 2 M thiourea, 0.4% CHAPS, 0.1% DTT, 0.5% Triton X-100, and 0.2% SDS) and centrifuged at 100,000 × *g* for 40 min. The obtained supernatant was used in all experiments.

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2.3. Screening for autoantibodies against protein sample in sera from MS patients

We examined autoantibodies against a prepared protein sample in sera from five MS patients and five healthy controls by one-dimensional electrophoresis (1DE) and immunoblotting. The extracted proteins were applied at 20 μg/well to 4–20% polyacrylamide gel for western blotting. The proteins were separated by SDS-PAGE and separated proteins were blotted onto polyvinylidine difluoride (PVDF) membranes at 0.8 mA/cm^2 for 1 h using a semidry blotting apparatus (Trans-Blot SD semidry transfer cell, Bio-Rad Laboratories). Subsequently, the membranes were incubated in blocking solution overnight in a cold room, and then reacted with the sera from MS patients and healthy controls (diluted at 1:1500) for 1 h at room temperature, followed by washing. Then the membranes were incubated with HRP-conjugated anti-human Ig (A + G + M) antibodies (Zymed) (diluted at 1:2000) for 1 h at room temperature and reacted with the ECL-Plus Western blotting detection system (GE Healthcare).

2.4. Two-dimensional electrophoresis (2DE) and immunoblotting

A sample was loaded onto an immobilized and rehydrated dry strip (pH 3–10, nonlinear 18 cm long, GE Healthcare). Up to 100 μg of extracted proteins was applied to the dry strip for western blotting. Isoelectric focusing was carried out at 20 °C for 85,000 Vh at a maximum of 8000 V using a horizontal electrophoresis system (Coolphorestar IPG-IEF Type-PX, Anatech). This IPG strip was transferred to 12.5% polyacrylamide gel. The second-dimension run was carried out vertically using an electrophoresis apparatus (Coolphorestar SDS-PAGE Dual-200 K, Anatech) at 30 mA/gel. After the electrophoresis, the SDS-PAGE gels were stained with SyproRuby (Bio-Rad Laboratories) or used for protein transfer onto PVDF membranes (Toda and Kimura, 1997). Separated proteins were electrophoretically transferred to a PVDF membrane at a constant voltage of 32 V for 3 h using a buffer transfer tank with cool equipment (Toda et al., 2000). Subsequently, this membrane was incubated in a blocking solution (5% skim milk in 1× TBST and 1× TBS containing 0.1% Tween 20) overnight in a cold room, and then reacted with serum from a patient diluted (1:1500) with 1% skim milk in 1× TBST for 1 h at room temperature. The PVDF membrane was washed five times with 1× TBST and reacted with peroxidase-conjugated goat anti-human Ig (A + G + M) antibodies (Zymed) diluted (1:2000) with 1% skim milk in 1× TBST for 1 h at room temperature. After six washes, the membrane was incubated with the WB detection reagent (ECL-Plus, GE Healthcare) for 5 min and then scanned using a variable-mode imager (Typhoon 9400, GE Healthcare). The antibody-reactive protein spots were matched with the protein spots stained with SyproRuby (Bio-Rad Laboratories) using image analysis software (Adobe Photoshop 6.0).

2.5. In-gel digestion and mass spectrometry

Proteins were detected by staining with SyproRuby (Bio-Rad Laboratories). For mass spectrometric identification, the target protein spot on the SyproRuby-stained 2D electrophoresis gel was excised using FluoroPhoreStar 3000 (Anatech). In-gel digestion was performed in according with a standard protocol (Toda and Kimura, 1997) with minor modifications. Briefly, gel pieces were dehydrated and the dried gel pieces were rehydrated in 5 μl of 100 mM ammonium bicarbonate containing 10 μg/ml trypsin (Promega) for 3 h at 37 °C. After digestion, tryptic peptides were extracted twice with 50 μl of 66% acetonitrile in 0.1% trifluoroacetic acid (TFA) in a sonicator. The extracted peptides were dried, redissolved in 0.1% TFA, and injected onto a MonoCap 0.1 mm × 250 mm monolithic C18 column (Kyoto Monotech) with Prominence Nano (Shimadzu). The column eluent was spotted every 15 s onto a μFocus MALDI plate (Shimadzu GLC) with α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) as a matrix using AccuSpot (Shimadzu). Buffer A consisted of 5% acetonitrile and 0.1% (v/v) TFA and buffer B consisted of 90% acetonitrile and 0.1% (v/v) TFA. The separation gradient was 5–60% buffer B over 30 min at a flow rate of 1 μl/min. The digests were

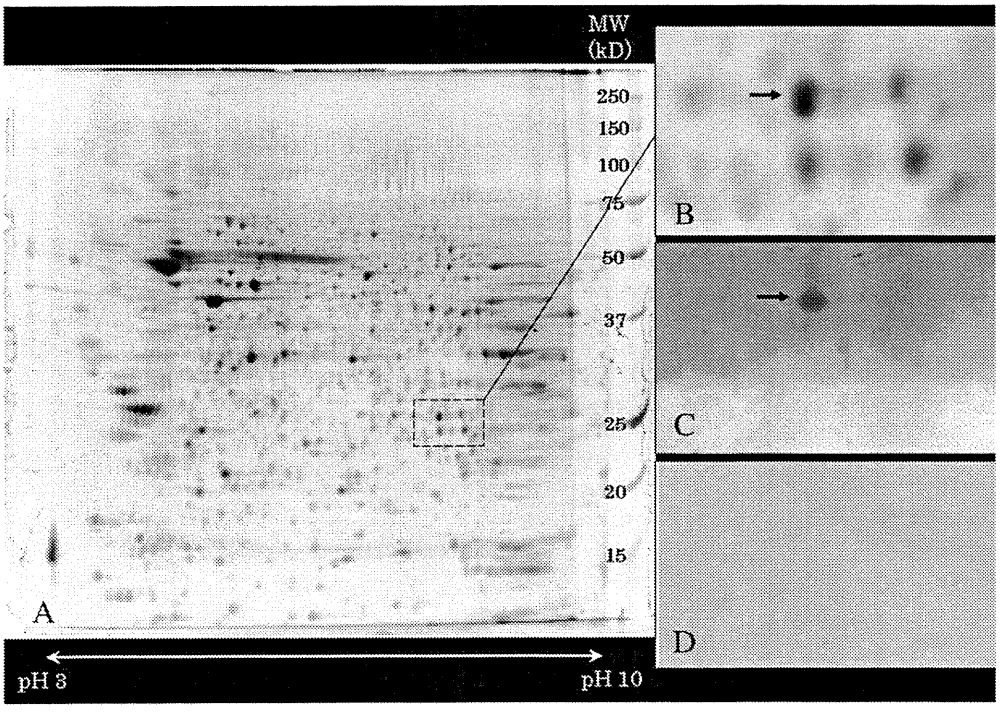

Fig. 1. Autoantibodies visualized by two-dimensional electrophoresis (2DE) and immunoblotting in sera from patients with multiple sclerosis (MS) and from healthy controls. (A, B) Total protein extracts of homogenized rat brain tissue were separated by 2DE, followed by SyproRuby staining. Arrow indicates the protein spot matching the immunoreactive spot detected by western blotting of MS patients' sera. Subsequently, this spot was identified as phosphoglycerate mutase 1 (PGAM1) by MALDI TOF-MS; (C) 1:1500-diluted MS patient's sera; arrow indicates the protein spot (PGAM1) recognized in sera from MS patients; and (D) 1:1500-diluted sera from healthy controls.

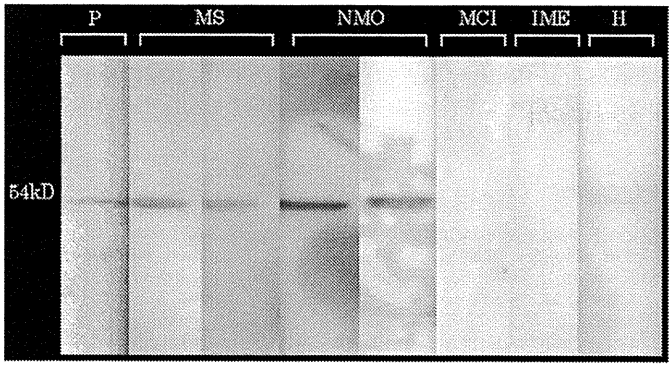

Fig. 2. Immunoblotting of human phosphoglycerate mutase 1 (PGAM1) full-length recombinant protein with GST. The recombinant protein (0.28 μg) was applied to each well. P, 1:300-diluted anti-PGAM1 monoclonal antibody (Abnova); MS, 1:1500-diluted sera from patients with multiple sclerosis; NMO, 1:1500-diluted sera from patients with neuromyelitis optica; MCI, 1:1500-diluted sera from patients with multiple cerebral infarctions; IME, 1:1500-diluted sera from patients with infectious meningoencephalitis; H, 1:1500-diluted sera from healthy controls.

analyzed on a MALDI TOF/TOF instrument, AXIMA Performance (Shimadzu). By utilizing information on the *x–y* positions of spotted samples on AccuSpot, autoexperiments using AXIMA Performance were performed to analyze the samples on the plates. Every auto-experiment and protein identification were performed using an integrated software, Kompact Ver.2.8. Protein identification was carried out using the MS/MS ion search database, Mascot (http://www.matrixscience.com/; Matrix Science Ltd.).

2.6. Immunoreactivity of sera from patients with various neurological diseases against human PGAM1 full-length recombinant protein

We examined the anti-PGAM1 antibodies in sera from 21 MS, 13 NMO, 21 PD, 20 MCI, to 19 IME patients, and 17 healthy controls by 1DE immunoblotting using the commercially available human PGAM1 full-length recombinant protein with GST (Abnova). Immunoblotting was carried out as described in Section 2.3. The screening dilution of sera from all patients and healthy controls was 1:1500.

2.7. Statistical analysis

We used the chi-square test with Yates' continuity correction to assess the difference in the prevalence of the anti-PGAM1 antibody between groups. Differences were considered significant at $P<0.05$.

3. Results

3.1. Screening and identification of target antigen of MS patients' autoantibodies (Fig. 1)

We examined the target antigen that reacted selectively with MS patients' sera. We detected by 1DE immunoblotting an approximately 30 kDa band corresponding to a protein that reacted with antibodies in sera from two out of five MS patients, but not with sera from five healthy controls. The same sample was subjected to 2DE, and one spot (observed MW/p*I*: 26,000/6.9) with a similar molecular weight reacted with the sera from these two MS patients but not with the sera from the healthy controls. We analyzed this spot by MALDI TOF-MS. This protein spot was identified as PGAM1 (accession number, P25113; score/coverage identification (%), 660/40; number of matched peptides, 11; theoretical MW/p*I*, 28,948/6.67).

3.2. Immunoreactivity of sera from patients with various neurological diseases against human PGAM1 full-length recombinant protein (Figs. 2 and 3)

To investigate whether the anti-PGAM 1 antibody is specific for MS, we examined this autoantibody in sera from patients with various neurological diseases (21 MS patients, 13 NMO patients, 21 PD patients, 20 MCI patients, and 19 IME patients) and 17 healthy controls by 1DE immunoblotting using the human PGAM1 full-length recombinant protein with GST (Figs. 2 and 3). As a result, the positivity rates were 81% (17 of 21) in MS patients, 77% (10 of 13) in NMO patients, 24% (5 of 21) in PD patients, 30% (6 of 20) in MCI patients, 32% (6 of 19) in IME patients, and 24% (4 of 17) in healthy controls. Statistically, the prevalence of the anti-PGAM1 antibody was significantly higher in patients with MS than in patients with PD ($P<0.001$), MCI ($P<0.003$), and IME ($P<0.005$), and in healthy controls ($P<0.002$). The prevalence of the anti-PGAM1 antibody was also significantly higher in patients with NMO than in patients with PD ($P<0.008$), MCI ($P<0.03$), and IME ($P<0.04$), and in healthy controls ($P<0.02$). These findings indicate that the anti-PGAM1 antibody has a stronger correlation with MS and NMO than with PD, MCI, IME, and being healthy.

4. Discussion

We identified PGAM1 as the target antigen of autoantibodies in sera from the MS patients by proteomics-based analysis. Western blotting analysis using the human PGAM1 recombinant protein

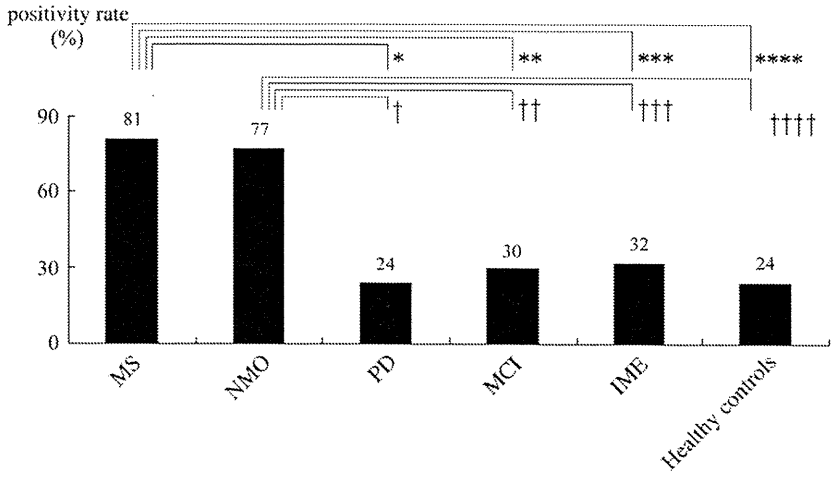

Fig. 3. Prevalence of antibodies against human phosphoglycerate mutase 1 (PGAM1) full-length recombinant protein. MS, patients with multiple sclerosis; NMO, patients with neuromyelitis optica; PD, patients with Parkinson's disease; MCI, patients with multiple cerebral infarctions; IME, patients with infectious meningoencephalitis. *$P<0.001$, **$P<0.003$, ***$P<0.005$, ****$P<0.002$, †$P<0.008$, ††$P<0.03$, †††$P<0.04$, and ††††$P<0.02$.

showed that the prevalence of the anti-PGAM1 antibody is much higher in not only patients with MS, but also those with patients with NMO, than in those with other neurological diseases and in healthy controls. To the best of our knowledge, this is the first study that elucidated the relationships between the anti-PGAM1 antibody and CNS autoimmune diseases. Lu et al. (2008) reported that the prevalence of the anti-PGAM1 antibody is much higher in patients with autoimmune hepatitis (AIH) than in those with other hepatic diseases and in healthy subjects. AIH is a rare liver disease and is characterized by hypergammaglobulinemia even in the absence of cirrhosis, characteristic autoantibodies, and a favorable response to immunosuppressive treatment (Zachou et al., 2004; Zolfino et al., 2002). Although the etiology of this disease is as yet unknown, the presence of several circulating autoantibodies such as the anti-nuclear antibody, anti-smooth muscle antibody, anti-liver kidney microsome type 1 antibody, and anti-liver cytosol type 1 antibody, which are serological markers for diagnostic criteria (Alvarez et al., 1999), suggests the important role of humoral mechanisms in AIH. There are several reports on MS patients with the complication of AIH (Pulicken et al., 2006; Takahashi et al., 2008; Ferrò et al., 2008). de Seze et al. (2005) reported that the prevalence of AIH seems to be about tenfold higher in patients with MS than in the general population. The anti-PGAM1 antibody can be generated in an immunological background common to both autoimmune CNS diseases and AIH.

Phosphoglycerate mutase is a glycolytic enzyme that catalyzes the interconversion of 3- and 2-phosphoglycerate with 2, 3-bisphosphoglycerate as the primer of the reaction (Fothergill-Gilmore and Watson, 1989). In mammalian tissues, PGAM exists in three isozymes, composed of homodimeric and heterodimeric combinations of two different subunits, type M (muscle form, PGAM2) and type B (brain form, PGAM1). The homodimer MM form is mainly expressed in the muscle; the BB form in the brain, kidney and liver; and the heterodimer MB form in the heart (Omenn and Cheung, 1974; Zhang et al., 2001). A previous study showed that PGAM1 is induced after hypoxia, which would occur in patients with cerebral infarction (Takahashi et al., 1998). In this study, the positivity rate of the anti-PGAM1 antibody in patients with MCI is not significantly higher than those in patients with other neurological diseases and in healthy controls. This finding suggests that an immunological background is important for production of the anti-PGAM 1 antibody.

In conclusion, the results of this study suggest that the anti-PGAM1 antibody is not only a marker of AIH but also a nonspecific marker of CNS autoimmune diseases. However, further studies are required to assess the presence of the anti-PGAM1 antibody in a large cohort of patients, including those with other autoimmune-mediated diseases, and controls.

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References

Alvarez, F., Berg, P.A., Bianchi, F.B., Bianchi, L., Burroughs, A.K., Cancado, E.L., Chapman, R.W., Cooksley, W.G., Czaja, A.J., Desmet, V.J., Donaldson, P.T., Eddleston, A.L., Fainboim, L., Heathcote, J., Homberg, J.C., Hoofnagle, J.H., Kakumu, S., Krawitt, E.L., Mackay, I.R., MacSween, R.N., Maddrey, W.C., Manns, M.P., McFarlane, I.G., Meyerzum Büschenfelde, K.H., Mieli-Vergani, G., Nakanuma, Y., Nishioka, M., Penner, E., Porta, G., Portmann, B.C., Reed, W.D., Rodes, J., Schalm, S.W., Scheuer, P.J., Schrumpf, E., Seki, T., Toda, G., Tsuji, T., Tygstrup, N., Vergani, D., Zeniya, M., 1999. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. J. Hepatol. 31, 929–938.

Dalakas, M.C., 2008. B cells as therapeutic targets in autoimmune neurological disorders. Nat. Clin. Pract. Neurol. 4, 557–567.

de Seze, J., Fajardy, I., Delalande, S., Stojkovic, T., Godet, E., Vermersch, P., 2005. Autoimmune hepatitis and multiple sclerosis: a coincidental association? Mult. Scler. 11, 691–693.

Ferrò, M.T., Franciotta, D., Riccardi, T., D'Adda, E., Mainardi, E., Montanelli, A., 2008. A case of multiple sclerosis with atypical onset associated with autoimmune hepatitis and silent celiac disease. Neurol. Sci. 29, 29–31.

Fothergill-Gilmore, L.A., Watson, H.C., 1989. The phosphoglycerate mutase. Adv. Enzymol. Relat. Areas Mol. Biol. 62, 227–313.

Hasler, P., Zouali, M., 2006. B lymphocytes as therapeutic targets in systemic lupus erythematosus. Expert Opin. Ther. Targets 10, 803–815.

Lu, F., Xia, Q., Ma, Y., Yuan, G., Yan, H., Qian, L., Hu, M., Wang, M., Lu, H., Wang, H., Liu, B., Xue, Y., Wang, H., Li, M., Shen, B., Guo, N., 2008. Serum proteomic-based analysis for the identification of a potential serological marker for autoimmune hepatitis. Biochem. Biophys. Res. Commun. 367, 284–290.

Omenn, G.S., Cheung, S.C.Y., 1974. Phosphoglycerate mutase isoenzyme: marker for tissue differentiation in man. Am. J. Hum. Genet. 26, 393–399.

Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., Johnson, K.P., Sibley, W.A., Silberberg, D.H., Tourtellotte, W.W., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann. Neurol. 13, 227–231.

Pulicken, M., Koteish, A., DeBusk, K., Calabresi, P.A., 2006. Unmasking of autoimmune hepatitis in a patient with MS following interferon beta therapy. Neurology 66, 1954–1955.

Takahashi, A., Kanno, Y., Takahashi, Y., Sakamoto, N., Monoe, K., Saito, H., Abe, K., Yokokawa, J., Irisawa, A., Ohira, H., 2008. Development of autoimmune hepatitis type 1 after pulsed methylprednisolone therapy for multiple sclerosis: a case report. World J. Gastroenterol. 14, 5474–5477.

Takahashi, Y., Takahashi, S., Yoshimi, T., Miura, T., 1998. Hypoxia-induced expression of phosphoglycerate mutase B in fibroblasts. Eur. J. Biochem. 254, 497–504.

Toda, T., Kimura, N., 1997. Standardization of protocol for Immobiline 2-D PAGE and construction of 2-D PAGE protein database on World Wide Web home page. Jpn. J. Electrophoresis. 41, 13–20.

Toda, T., Sugimoto, M., Omori, A., Matsuzaki, T., Furuichi, Y., Kimura, N., 2000. Proteomic analysis of Epstein-Barr virus-transformed human B-lymphoblastoid cell lines before and after immortalization. Electrophoresis 21, 1814–1822.

Wingerchuk, D.M., Lennon, V.A., Pittock, S.J., Lucchinetti, C.F., Weinshenker, B.G., 2006. Revised diagnostic criteria for neuromyelitis optica. Neurology 66, 1485–1489.

Zachou, K., Rigopoulou, E., Dalekos, G.N., 2004. Autoantibodies and autoantigens in autoimmune hepatitis: important tools in clinical practice and to study pathogenesis of the disease. J. Autoimmune Dis. 1, 2–13.

Zephir, H., Almeras, L., Behi, E.M., Dussart, P., Seze, d.J., Steibel, J., Trifilieff, E., Dubucquoi, S., Dessaint, J.P., Vermersch, P., Prin, L., Lefranc, D., 2006. Diversified serum IgG response involving non-myelin CNS proteins during experimental autoimmune encephalomyelitis. J. Neuroimmunol. 179, 53–64.

Zhang, J., Yu, L., Fu, Q., Gao, J., Xie, Y., Chen, J., Zhang, P., Liu, Q., Zhao, S., 2001. Mouse phosphoglycerate mutase M and B isozymes: cDNA cloning, enzyme activity assay and mapping. Gene 264, 273–279.

Zolfino, T., Heneghan, M.A., Norris, S., Harrison, P.M., Portmann, B.C., McFarlane, I.G., 2002. Characteristics of autoimmune hepatitis in patients who are not of European Caucasoid ethnic origin. Gut 50, 713–717.

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ORIGINAL COMMUNICATION

Motor-dominant chronic inflammatory demyelinating polyneuropathy

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Abstract We reviewed the clinical, electrophysiological an laboratory findings, plus the therapeutics and evolution of patients with motor-dominant Chronic inflammatory demyelinating polyneuropathy (CIDP) and compared them with those of other CIDP patients. Among 12 consecutive CIDP patients, we identified five patients with motor-dominant CIDP. The five patients with motor-dominant CIDP initially presented with weakness of the upper limbs. Cervical magnetic resonance imaging (MRI) examinations of the patients with motor-dominant CIDP showed that the most affected lesions are the cervical nerve roots and brachial plexus. The clinical course of these patients was relapsing-remitting, and they improved markedly after treatment by intravenous immunoglobulin (IVIg) infusion or plasmapheresis. However, they did not improve in response to corticosteroid therapy during the acute phase of relapses. The relapses frequently occurred within 2 years, but rarely occurred after that. The score in the modified Rankin disability scale (mRDS) at the last follow-up period was statistically lower for the patients with motor-dominant CIDP than for the other CIDP patients ($P < 0.002$). The characteristic clinical features, responsiveness to treatment, and prognosis suggest that motor-dominant CIDP is a distinct subtype of CIDP, with a specific immunological background. Repeated IVIg therapy is required to maintain the motor functions of patients with motor-dominant CIDP. We consider that treatment for recurrence prevention as an alternative to IVIg therapy is very important for patients with motor-dominant CIDP.

Keywords Corticosteroid · IVIg · Motor-dominant CIDP · Pure motor CIDP · Relapsing-remitting

Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a clinically heterogeneous autoimmune disease. There are several subtypes of chronic acquired demyelinating neuropathies that are broadly classified under the umbrella term CIDP [1]. The subtypes of CIDP differ in clinical presentation, electrophysiological and laboratory features, and response to treatment [1]. There are several reports on the cases of patients with motor-dominant CIDP who showed selective motor involvement upon clinical and electrophysiological examination [2–4]. Furthermore, the clinical, pathological, electrophysiological, and prognostic features of patients with motor-dominant CIDP are poorly understood.

In this report, we reviewed the clinical, electrophysiological, laboratory findings, therapeutics and evolution of five patients with motor-dominant CIDP and compare them with those of other CIDP patients to clarify the characteristic features of motor-dominant CIDP.

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Patients and methods

Patients

Between September 2001 and July 2009, among 12 consecutive patients with definite CIDP, we identified five patients with motor-dominant CIDP. The diagnosis of CIDP was made on the basis of the criteria of the Joint Task Force of the European Federation of Neurological Societies (EFNS) and the Peripheral Nerve Society (PNS) [5]. Diagnostic criteria of motor-dominant CIDP included absence of sensory symptoms, except for mild distal paresthesia, and almost normal results in sensory conduction studies. Extensive clinical and laboratory evaluations excluded other causes of neuropathy.

Clinical assessment

Neurological examination was carried out for all 12 CIDP patients enrolled in this study. Measures of motor impairments consisted of a grip strength test and Medical Research Council (MRC) rating scale bilaterally performed on 14 muscle groups. Disability at the last follow-up period was assessed using the modified Rankin disability scale (mRDS) [6].

Electrophysiologic study

Nerve conduction studies were performed using Viking Nicolet electromyography equipment. Motor nerve conduction studies included surface recordings with stimulation of the median (wrist, elbow), ulnar (wrist, elbow), and tibial (ankle, knee) nerves. Sensory nerves were studied by antidromic recordings from the median, ulnar, and sural nerves. We defined the partial conduction block on the basis of the electrodiagnostic criteria of the Joint Task Force of EFNS and PNS [5].

Laboratory investigations

Serum and cerebrospinal fluid (CSF) samples were collected before therapy from the 12 patients with definite CIDP. We examined serum samples for anti-nuclear antibody (ANA), anti-DNA antibody, anti-Sm antibody, anti-SS A/B antibody, proteinase-3 (PR-3)-anti-neutrophil cytoplasmic antibody (ANCA), and myeloperoxidase (MPO)-anti-neutrophil cytoplasmic antibody (ANCA). We performed general CSF examination and determined the CSF IgG and CSF albumin indexes.

Other investigations

Cervical magnetic resonance imaging (MRI) was performed for two of the five patients with motor-dominant CIDP, and thoracolumbar MRI was performed for three of the five patients with motor-dominant CIDP. A sural nerve biopsy was performed at ankle level for two of the five patients with motor-dominant CIDP. Details of the pathological analysis method were as previously reported [7, 8].

Statistical analysis

All statistical analyses were performed using the Mann–Whitney's U test or Fisher's exact probability test, as indicated. P values of < 0.05 were considered significant.

Results

Clinical features

We identified five patients (41.7%) with motor-dominant CIDP (patients 1–5) among 12 patients with definite CIDP. Patient 5 was a case we described earlier [9]. The average age of the patients with motor-dominant CIDP is 54.8 ± 8.67 years (mean ± SD, range 46–66 years), and that of the other CIDP patients (patients 6–12) is 64.1 ± 11.2 years (range 40–72 years). The patients with motor-dominant CIDP included three males (60%) and two females, and the other CIDP patients included three males (42.9%) and four females. Two patients (patients 1 and 2) with motor-dominant CIDP also had diabetes mellitus. All the patients with motor-dominant CIDP initially presented with weakness. The initial regions affected by weakness were bilateral hands (patients 1, 2, and 5), right hand (patient 4), and left upper limb (patient 3). Besides weakness, only one of the patients had bilateral paresthesia of the hands (patient 5). The mean duration from disease onset until admission was 3.6 months (range 2–6 months). The neurological findings upon admission were symmetrical weakness of the extremities, particularly the upper limbs, and generalized areflexia. However, the only sensory deficit was mild paresthesia in the fingers (patients 1, 4, and 5) and no muscle atrophy or cranial nerve involvements were observed (Tables 1, 2).

Electrophysiological findings

All the patients with motor-dominant CIDP showed reduced motor conduction velocities (MCVs). Four of the five patients showed partial motor conduction block in at least one motor nerve (patients 1, 2, 4, and 5). Prolonged distal motor latencies were found in three of the five patients (patients 1, 2, and 3). F-wave abnormalities (prolonged F-wave latencies and decreases in the frequency of F-wave occurrence) were found in four of the four patients (patients 1, 2, 4, and 5). Reduced distal compound muscle

Table 1 Summary of clinical features and laboratory findings of 12 CIDP patients

Patient	Age/ Gender	Type	Complication	Initial symptom	Antibodies	CSF cell count (mm^3)	CSF protein content (mg/dl)	CSF IgG index	CSF albumin index
1	66/F	Motor dominant	Diabetes mellitus	Weakness (Blt. hand)	ANA	2	42	0.58	5.18
2	62/M	Motor dominant	Diabetes mellitus	Weakness (Blt. hand)	ND	1	86	0.49	12.9
3	50/F	Motor dominant	–	Weakness (Lt. upper limb)	ND	1	98	0.61	10.3
4	50/M	Motor dominant	–	Weakness (Rt. hand)	ND	6	113	0.61	17.5
5	46/M	Motor dominant	–	Weakness and parestheisa (Blt. hand)	ND	8	145	0.60	23.2
6	72/F	Sensory motor	Sjögren syndrome	Weakness (Blt. leg)	Anti-SS A	2	27	0.30	3.23
7	72/F	Sensory motor (MADSAM)	–	Dysesthesia (Lt. hand)	ANA	1	58	0.49	7.46
8	69/F	Sensory motor	–	Weakness and pain (Blt. thigh)	ND	0	54	0.52	7.87
9	69/M	Sensory motor (DADS)	–	Paresthesia (Rt. foot)	ND	2	101	0.58	15.3
10	65/M	Sensory motor (DADS)	MGUS	Hypesthesia (Rt. foot)	ND	1	53	0.60	4.00
11	62/F	Sensory motor	Multiple myeloma	Paresthesia (Blt. foot)	ND	3	183	0.44	29.7
12	40/M	Sensory motor (ataxic form)	Diabetes mellitus	Dysesthesia (Blt. hand)	ND	14	577	0.58	72.0

ANA anti-nuclear antibody, *DADS* distal acquired demyelinating symmetric neuropathy, *MADSAM* multifocal acquired demyelinating sensory and motor neuropathy, *MGUS* monoclonal gammopathies of unknown significance, *ND* not detected

Table 2 Medical Research Council (MRC) rating scale of patients with motor-dominant CIDP on admission (Right, Left)

Patient	Duration[a] (month)	Deltoid	Pectoralis major	Biceps	Triceps	Wrist extensors	Wrist flexors	Digits extensors	Digits flexors	Grip strength (kg)
Upper										
1	6	4, 4	4−, 4−	4−, 4−	4, 4	4−, 4−	3, 3	3, 3	3, 3	0, 0
2	3	5, 5	4, 4	4+, 4+	5, 5	5, 5	5, 5	4, 4	5, 5	15, 8
3	3	2, 2	NE	3, 3	3+, 3+	2−, 2−	2−, 2−	2−, 2−	2−, 2−	0, 0
4	4	2, 2	3+, 3+	3+, 3+	3+, 3+	3+, 4	3+, 3+	3+, 3+	3+, 3+	3, 3
5	2	5, 5	NE	3, 3	3, 3	2, 2	2, 2	NE	NE	4, 2

Patient	Iliopsoas	Gluteus	Hamstrings	Quadriceps	Tibialis anterior	Gastrocnemius
Lower						
1	4, 4	4−, 4−	4−, 4−	4+, 5−	4, 4	4, 4
2	4, 4	5, 5	5, 5	5, 5	5, 5	5, 5
3	3+, 3+	5−, 5−	3+, 3+	2−, 2−	2−, 2−	2−, 2−
4	4, 4	4, 4	3+, 3+	4+, 4+	5, 5	5, 5
5	5−, 5−	NE	5−, 5−	5, 5	5, 5	5, 5

NE not examined

[a] Duration: until admission from disease onset

Table 3 Electrophysiological findings of patients with motor-dominant CIDP

Rt. median						Lt. median				
Patient	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	***4.6***	***33.9***	10/***3.1 [69]***	***24.0*** [69]	43.3	4.4	***37.1***	13/7.6 [42]	***42.5*** [63]	45.8
2	***6.0***	***27.3***	12/8.8 [27]	***22.2 [37]***	44.4	***5.8***	***30.7***	9.0/***3.4 [62]***	***23.4 [25]***	42.1
3	***5.1***	***37.7***	8.0/7.2 [10]	NE	46.1	4.3	***41.7***	6.5/7.1 [−9.5]	NE	45.2
4	3.2	***40.2***	14/***6.7 [52]***	***29.9 [6]***	49.0	3.1	***43.3***	14/8.1 [42]	***19.2 [19]***	52.0
5	4.4	***31.8***	8.1/***3.1 [62]***	***34.7*** [68]	50.3	4.2	***31.4***	7.9/4.4 [44]	***32.8 [31]***	49.7

Rt. ulnar						Lt. ulnar				
Patient	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	3.6	***35.4***	5.5/4.3 [22]	***32.7*** [56]	***36.0***	3.8	***31.7***	5.6/2.9 [48]	NE	42.6
2	3.9	***33.1***	4.3/4.0 [21]	***31.5*** [93]	***37.5***	***4.5***	***30.7***	6.9/6.2 [10]	***24.1 [87]***	40.2
3	3.8	50.0	***2.9/2.3*** [21]	NE	55.2	3.3	***37.0***	4.5/4.5 [0]	NE	60.1
4	3.3	***43.8***	10/9.0 [10]	***32.1 [38]***	54.9	3.0	52.1	11/9.2 [16]	***33.0 [31]***	51.9
5	3.1	***37.4***	6.1/4.7 [23]	***33.8*** [68]	44.0	3.0	***38.6***	7.7/5.7 [26]	***34.2*** [93]	42.8

Rt. tibial					Rt. sural	Lt. tibial				Lt. sural
Patient	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.)	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.)	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	5.6	42.9	8.4/7.5	***35.2 [69]***	40.1	5.1	***38.9***	***4.1/4.9***	***29.5 [94]***	***39.4***
2	7.3	***30.2***	***3.9/3.4***	NE	***38.5***	***8.7***	***31.5***	***2.7/1.1***	***27.4 [43]***	42.3
3	5.1	***37.8***	7.1/4.6	NE	46.3	3.8	***38.1***	***3.5/3.4***	NE	46.2
4	3.9	41.2	18/14	***40.0*** [100]	50.0	3.7	41.9	23/20	***40.3 [94]***	52.6
5	4.3	***35.8***	7.9/5.7	***37.6*** [100]	47.8	4.6	***38.2***	16/8.6	***34.3*** [100]	42.7

Bold italic indicates abnormal value

CMAP compound muscular action potential amplitude, *CB* conduction block, *FWCV* F-wave conduction velocity, *MCV* motor conduction velocity, *NE* not examined, *SCV* sensory conduction velocity