

therapy; this patient also showed no serious long-term neurological sequelae. The aberrant lymphocyte subsets correlated well with the neurological symptoms.

2. Case report

A previously healthy 11-month-old boy with normal developmental milestone achievements presented with ataxia and abnormal eye movement. Episodes of upward eye deviation lasting about one second started 3 weeks before admission; thereafter, myoclonic movement of the extremities and trunk ataxia appeared. These symptoms worsened gradually.

On admission, he was awake and alert but irritable. He exhibited rapid and random eye movement and myoclonus of the extremities characterized by irregular flickering muscle contractions; these contractions occurred spontaneously and were easily provoked by rapid passive body movement. He showed trunk ataxia and sat without support with difficulty. His muscle tone was hypotonic and his deep tendon reflex was normal. MRI brain scans found no abnormalities. His urinary vanillylmandelic acid and homovanillic acid levels were elevated (16.6 $\mu\text{g}/\text{mg}$ Cr [normal range: 1.2–4.9] and 26.3 $\mu\text{g}/\text{mg}$ Cr [normal range: 1.6–5.5], respectively). Abdominal computed tomographic scanning revealed a 3-cm diameter mass in the paraaortic area on the left renal hilus (Fig. 1). Iodine-123-metaiodobenzylguanidine scintigraphy showed this region accumulated isotopes. Immunophenotype analyses revealed total lymphocyte count of 10,600/ μl with high B-cell frequency (25.3% [normal range: 9.5–18.9]), low T-cell frequency (38.8% [normal range: 65.0–81.4]), and a normal CD4/CD8 ratio (1.74 [normal range: 1.0–2.2]) in the peripheral blood (PB), and total cell count of 3/ μl with high B-cell frequency (9.1% [normal range: 0.4–1.3]) in the cerebrospinal fluid (CSF). The CSF/PB B-cell ratio was also high (0.36 [normal range: 0.02–0.06]).

Stage I NBoma-associated OMS was diagnosed. The total neurological score (calculated by the assessment

scale of Sugie et al. [9]), was six before OMS onset and 19 at diagnosis (Table 1).

Four weeks after OMS onset, the patient received 2 mg/kg/day PSL orally. One week later, the opsoclonus and ataxia were partially improved, he could stand with support, and the total neurological score improved to 11 (Table 1). The PSL was tapered and the tumor was removed surgically. Pathology confirmed MYCN non-amplified NBoma. No chemotherapy was provided. After tumor resection, the myoclonus disappeared, and the total neurological score improved to six. However, 7 weeks later, an episode of upper respiratory tract infection aggravated all OMS symptoms, and the PB CD4-positive cell (19.4%) frequency dropped further resulted in reductions of T cell frequency (31.0%) and CD4/CD8 ratio (0.92).

Oral DEX pulse therapy (three consecutive days of 20 mg/m²/day DEX every 28 days) was commenced and continued for 6 months. Three weeks after the first course, all OMS symptoms improved, and the patient could walk with support. After four courses, the myoclonus disappeared almost completely, he could stand without support, walk a few steps alone. After completing six courses, all OMS symptoms had subsided, he could walk alone. No adverse effects of treatment were observed. Babbling started at 15 months (after the first course) and he could speak several words at two years of age. The PB T-cell and CD4-positive cell frequencies and CD4/CD8 ratios rose with every DEX pulse therapy cycle.

At 2 years and 8 months of age, on the occasion of recurrence of mild OMS symptoms followed by respiratory infection, the patient received three courses of DEX pulse therapy. At the age of 3 years and 8 months, his development was evaluated using Kyoto Scale of Psychological Development (KSPD) which is a standardized developmental test for Japanese children [10]. His total developmental quotient (DQ) was 76, and the details showed postural-motor 83, cognitive-adaptive 79, and language-social 76. Although it cannot be defined as distinct development retardation, his DQ level was regarded as borderline development, especially showing minor decline in language development.

At present he shows no typical presentation suggesting autism or attention deficit hyperactivity disorder (AD/HD). NBoma remission has been maintained. PB lymphocyte markers returned to almost normal.

3. Discussion

Here, a patient with NBoma-associated OMS was treated by six courses of oral high-dose DEX pulse therapy that completely resolved all OMS symptoms and prevented long-term serious neurological impairments with no adverse treatment effects. In two previous reports on NBoma-associated OMS treated with the



Fig. 1. Abdominal computed tomographic scan of the patients. A mass that was 3 cm in diameter was observed in the paraaortic area on the left renal hilus (arrowhead).

Table 1
Effect of DEX pulse therapy on neurological score* and peripheral blood lymphocyte immunophenotype.

Age Status	10 m Before onset	11 m Before PSL	11 m 1 week after PSL	1 y 0 m 4 weeks after surgery	1 y 2 m Before DEX pulse	1 y 3 m After 1st course of DEX pulse	1 y 5 m After 4th course of DEX pulse	2 y 0 m 4 months after 6th course of DEX pulse	3 y 3 m 1.5 years after six courses of DEX pulse
<i>Neurological signs</i>	(0, none; 1, mild; 2, moderate; 3, severe)								
Opsoclonus	0	3	1	0	1	0	0	0	0
Ataxia	0	3	2	1	2	1	1	0	0
Myoclonus	0	3	3	0	2	1	0	0	0
Irritability	0	1	0	0	0	0	0	0	0
<i>Motor functions</i>	(0, possible; 1, possible with difficulties; 2, impossible)								
Rolling	0	0	0	0	0	0	0	0	0
Sitting	0	1	0	0	0	0	0	0	0
Crawling	0	1	0	0	0	0	0	0	0
Standing with support	0	1	0	0	0	0	0	0	0
Walk with support	2	2	1	1	1	0	0	0	0
Standing without support	2	2	2	2	2	2	0	0	0
Walk alone	2	2	2	2	2	2	1	0	0
Total (0–36)	6	19	11	6	10	6	2	0	0
<i>Immunophenotype</i>	(normal range)								
CD20 (9.5–18.9%)	ND	25.3	ND	ND	24.3	24.4	30.4	28.5	24.6
CD3 (65.0–81.4%)	ND	38.8	ND	ND	31.0	38.3	46.9	42.8	57.6
CD4 (32.6–44.2%)	ND	29.5	ND	ND	19.4	24.8	28.8	25.9	35.7
CD8 (20.0–33.4%)	ND	17.4	ND	ND	21.0	19.7	20.2	13.0	16.2
CD4/CD8 (1.0–2.2)	ND	1.74	ND	ND	0.92	1.26	1.43	1.99	2.20

* Calculated by the assessment scales of Sugie et al. [9]; ND: not determined.

same high dose DEX pulse regimen as our patient's [7,8], half of patients attained complete, sustained OMS remission and one fifth had no neurological sequelae, without serious adverse reactions despite treatments exceeding 1 year. Thus, DEX pulse therapy has several advantages over other treatment regimens for OMS: DEX is cheap, only minor side effects (e.g., dysphoria, weight gain, and reversible glucosuria) have been reported [7,8], and out-patient treatment is possible.

As with other OMS patients [3,6], the OMS in our patient was associated with low PB CD4-positive T-cell frequencies, low PB CD4/CD8 ratios, higher CSF B-cell frequency, and high CSF/PB B-cell ratio, and DEX pulse therapy gradually reversed these aberrant values and improved the neurological symptoms. The analyzed cell number in CSF for immunophenotyping in our case was more than thousand, which was a reasonable amount as in previous reports [4,11]. It is reported that glucocorticoid treatment restores the impaired suppressive function of regulatory T cells (both CD4 and CD25-positive cells) in patients with multiple sclerosis [12] that is autoimmune central nervous system (CNS) disease as well as OMS. In our patient immune dysregulation attributed to underlying reduced CD4-positive T-cell frequency might be ameliorated by the DEX pulse therapy. The evidences supporting an important role of CSF B-cell expansion in the pathogenesis of OMS have been accumulated, such as intrathecal autoantibody production, neurological severity correlating with the degree of CSF B-cell expansion and promising therapeutic effects of B-cell depletion with rituximab [11]. The expanding B lineage cells can survive in the inflammatory CNS environment for many years [13], which might be associated with remitting relapsing manner of OMS. Our patient who showed greater B-cell expansion than has been reported previously [11] may have had severe OMS. The alterations of lymphocyte subsets observed in our patient may be responsible for the development of OMS and these could serve as DEX pulse treatment markers.

He has a considerably good prognosis in the motor development; however, we need further careful follow-up for his verbal development to determine the efficacy of this promising treatment.

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自閉症の原因診断と病態に基づいた 治療の可能性について*

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Key Words : autism, genetic disorder, synaptic function, metabolic disorder, biomarker

はじめに

自閉症あるいは広い意味で自閉症スペクトラム障害(ASD)は社会性の障害, コミュニケーションの障害, 興味や行動の範囲が限定的であるなどの症状を示す疾患であるが, その原因は多岐にわたっている。診断基準にあてはめて, 自閉症と診断がつくことは単なるスタートラインに着いたにすぎない。なぜならば, さらに絞り込んで病因を突き止め, 今後の治療に繋げることが重要な課題となるからである。自閉症の治療については, 原因如何にかかわらず, 療育方法, 対応方法は非常に重要な部分を占めるのは言うまでもない。また, 症状に合わせた薬物療法も必要である。しかし, 病態に即した治療は, 原因を突き止めることにより, はじめてその開発が可能となっていくものである。数は少ないものの原因療法の可能な自閉症も報告されてきている¹⁾。今回ここでは, 自閉症の原因として注目されている疾患, 特に現在原因療法がアプローチされている疾患について紹介する。

自閉症には, 症候性(syndromic), 非症候性(nonsyndromic)と分類する方法がある。一般的

に, 症候性の場合は, 顔貌や身体的特徴などを手掛かりとして診断が絞りこまれ, 確定していくことが多い。特異的顔貌や先天性心疾患などの身体所見が明瞭な場合は, 出生後早い時期に染色体検査ですでに特異的診断が確定し, それぞれの疾患特異的に必要な治療計画を立てられている。自閉症状を持つ頻度が高い疾患では, その症状出現を予測しての対応が治療計画に盛り込まれていくこととなる。臨床的身体所見が乏しく自閉症の診断が先になされ後に原因疾患が確定した場合は, 自閉症は診断名としてではなくその疾患に併存する一つの症状として認識されていくこととなる。

ASDの原因疾患と遺伝子

自閉症の原因の多くは多因子遺伝と考えられている。すなわち, common genetic variants of weak allele(自閉症リスク遺伝子の変異の)蓄積で起こる。しかし, それだけではなくrare genetic variants of strong allele(自閉症遺伝子の機能異常)それ単独で自閉症をひき起こすことも知られている²⁾。自閉症の原因は不明な部分が多いが, 近年における分子生物学の進歩に伴い, おそらくその15~20%は特定できるとされる。自閉症の原因の中で, 染色体レベルの異常を示す疾患群は5%以下, コピー数変異(CNV)として検出

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されるものが5~10%, 単一遺伝子疾患の判明するものが5%未満とされている³⁾. CNVの解釈にも課題は残っているものの, 身体所見が明瞭でない自閉症でもCNVを含む染色体検査で異常の認められることがあり, 今後積極的な検査がなされるとこの割合は増加していくものと推測される. そして, 自閉症に限らず, 多くの単一遺伝子疾患において, 遺伝子異常の部位の多様性と症状にも広がり認められ, 遺伝子異常が認められても無症状であることも珍しくないことも判明してきている. このことから, 自閉性障害のみに限定して考えるよりは, スペクトラム, あるいは幅広い表現型(broad phenotype)を考慮していく必要性ももうなずけるのである.

ASDに関与する遺伝子は100以上あり, さらに増え続けているとBetancurは紹介している⁴⁾. 症候性の疾患が圧倒的に多い. その一つ一つの疾患における自閉症状を示す割合が, 決して多くないものも含まれている. その中で, ①ASDがhallmarks(顕著な特徴)となる疾患, ②ASDが一般によく認められる疾患, ③ASDは頻繁ではないが繰り返し報告される疾患の3郡に分けている. その分け方を参考にして, それぞれの疾患における自閉症状の認められる割合, ASDと診断された症例におけるその疾患の占める割合, その疾患の発症頻度⁵⁾⁶⁾を表1にまとめた.

ASDとシナプスの障害

ASDの原因の中で, 頻度が多いとされる疾患は脆弱X症候群, 結節性硬化症, Angelman症候群, SHANK3/22q deletion syndrome, Prader-Willi症候群があげられる. これらの遺伝子がコードするタンパクの機能に共通していることは, シナプスにおける機能にかかわっているということである.

脆弱X症候群は精神遅滞の原因として最も頻度の高い(2.6~8.7%⁷⁾, 日本人では0.8~2.4%⁸⁾疾患として知られている. その半数以上で自閉症状[自閉性障害30%, pervasive developmental disorder-not otherwise specified(PDD-NOS)30%]を示し, ASDの1~3%に本症が認められる. 欧米では男性4,000人に1人(女性8,000人)に1人とされるが, 日本ではそれより少なく1万

人に1人とされる⁷⁾⁸⁾. 身体所見として特徴的顔貌, 巨大睾丸, 関節過伸展があるが, 小児期には典型的な特徴に乏しく, 臨床兆候だけでは診断できない非特異的な症例についても, 今後の調査が進めばこの頻度は増える可能性がある.

この疾患はFMR1遺伝子のCGGのリピート数が200以上となると遺伝子の発現が抑制されることにより発症する. FMR1遺伝子にコードされるタンパク質のFMRPはmRNAの転写を調節しており, シナプスの構造や調節にかかわっている. 脆弱X症候群ではシナプスにおける代謝型グルタミン酸受容体(mGluR1/5)の機能が促進されていることが示され⁹⁾, この病態から脆弱X症候群の治療として, このmGluR1/5を拮抗する薬物が動物実験を経て, アメリカでは治験段階に入っている¹⁾. また, 脆弱X症候群で増加しているタンパクの1つにmatrix metalloproteinase 9(MMP-9)がある. ミノマイシンにMMC-9の異常活性を抑制する作用があり, 同様に治験されている¹⁾.

そのほかにシナプスの形成と機能にかかわっている遺伝子にはneuroigin 3/neuroigin 4, neuroligin 1, CNTNAP2, PAK2/PAK3, MeCP2などがあげられる.

染色体15q11-q13領域は欠失や重複が起りやすい部位であり, Angelman症候群, Prader-Willi症候群を含んでいる. この領域に含まれるcytoplasmic FMR1-interacting protein 1(Cyfi1)はFMRPと結合してシナプスでmRNAの翻訳を調節している.

22q13欠失症候群は重度の表出言語の遅れ, 筋緊張低下, 自閉症様行動特性, 過成長, 軽度の顔貌異常を特徴とする. しかし, 遺伝子解析スクリーニングで偶然診断されるケースもあり¹⁰⁾, 外表奇形に乏しく診断未定の例が存在する可能性がある. この領域に含まれるSHANK3遺伝子の異常が原因と考えられている. そして, このSHANK3遺伝子はシナプスのグルタミン酸受容体と細胞骨格の間をつなぐ足場タンパクをコードしている.

結節性硬化症は神経皮膚症候群の一疾患で知的障害, てんかん, 白斑などに全身性の過誤腫, 腫瘍性病変を特徴とする. 発症にかかわるTSC1遺伝子とTSC2遺伝子はシナプスにおける情報伝

表1 ASDの原因遺伝疾患, 他の身体症状を伴う疾患群 (syndromic ASD)

疾患	遺伝子	その疾患の ASD症状頻度	ASDにおける その疾患の頻度	疾患の頻度 (10万人につき)
・ ASDが主な臨床症状の一つとなる疾患				
単一遺伝子 脆弱X症候群	<i>FMR1</i>	男60%, 女20%	1~3%	25人/男, 12.5人/女, 10人/日本人
単一遺伝子 レット症候群	<i>MECP2</i>		女子の1.3%	7~10人
単一遺伝子 結節性硬化症	<i>TSC1, TSC2</i>	16~60%	1.1~1.3%	10人
単一遺伝子 Timothy症候群	<i>CACNA1C</i>	60~80%		稀
単一遺伝子	<i>ADSL</i>	~50%		稀
単一遺伝子 Smith-Lemli-Opitz症候群	<i>DHCR7</i>	50~75%		2.5~5人, 日本人稀
単一遺伝子	<i>CNTNAP2</i>			
単一遺伝子 SHANK3/22q欠失症候群	<i>SHANK3</i>	44%~しばしば	1.0~1.3%	
単一遺伝子 Angelman症候群	<i>UBE3A</i>	50~63%	1~3%	5~10人
・ ASDの症状がよく認められる疾患				
単一遺伝子 脳クレアチン欠損症	<i>SLC6A8</i> ほか	しばしば		
単一遺伝子 Cornelia de Lange症候群	<i>NIPBL, SMC1A</i>	47~67%		10人
単一遺伝子 CHARGE症候群	<i>CHD7</i>	68%		12人
単一遺伝子 Cohen症候群	<i>VPS13B</i>	49%		1人
単一遺伝子 Joubert症候群と関連症候群	<i>AHI1</i> ほか	13~36%		
単一遺伝子 Myotonic dystrophy type1	<i>DMPK</i>	35~50%		
単一遺伝子 Potocki-Lupski症候群	<i>RAI1</i>	しばしば		
単一遺伝子 Smith-Magenis症候群	<i>RAI1</i>	しばしば90%		4~6人
単一遺伝子 X-linked female-limited epilepsy and ID	<i>PCDH19</i>	22~38%		
単一遺伝子 Xq28欠失症候群	<i>MECP2</i>			
隣接遺伝子 5pモノソミー (Cri du Chat syndrome)	5p欠失	39%		
隣接遺伝子 Williams症候群	7q11.23欠失	50%		
隣接遺伝子 7q11.23重複症候群	7q11.23重複	40%		
隣接遺伝子 8p23.1欠失	8q23.1欠失	57%		
隣接遺伝子 WAGR症候群	11p13欠失	52%		
隣接遺伝子 Prader-Willi症候群	15q11-q13	23%		4~6人
隣接遺伝子 16p11.2微小欠失と微小重複	16p11.2欠失・重複		1%	
隣接遺伝子 17q12欠失	17q12欠失	66%		
隣接遺伝子 22q11欠失症候群 (velocardiofacial/ DiGeorge syndrome)	22q11欠失	14~50%		
・ ASDが高頻度ではないがよく報告されている疾患				
単一遺伝子 Duchenne型とBecker型筋ジストロフィー症	<i>DMD</i>	19%		
単一遺伝子 PTEN関連症候群	<i>PTEN</i>	15%	大頭症の4.7%	0.5人?
単一遺伝子 Cardio-facio-cutaneous症候群	<i>KARS, BRAF</i> ほか	23%		
単一遺伝子 Noonan症候群	<i>PTPN11</i>	8%		40~100人, 10人 (日本人)
隣接遺伝子 1q21.1微小重複症候群		~10%		発達遅滞+ID+先天奇形0.2%
隣接遺伝子 2q37欠失症候群		24%		
隣接遺伝子 3q29微小欠失		26%		
隣接遺伝子 9qサブテロメア欠失症候群				
隣接遺伝子 11q欠失症候群		13%		
隣接遺伝子 15q24微小欠失症候群		22%		
・ ASDのリスクの認められる染色体異数体				
染色体異常 ダウン症候群	21トリソミー	5~15%		126人
染色体異常 ターナー症候群	45, X	女3.3%		女子で50~100人
染色体異常 クライネフェルター症候群	47, XXY	11~48%		男子で166人
染色体異常 XYY症候群	47, XYY	19%		
染色体異常 45, X/46, XYモザイク症	45, X/46, XY	7%		

達経路上にある mammalian target of rapamycin (mTOR) の活性を調節している。

PTEN (the phosphatase and tensin homologue gene) hamartoma tumor syndrome のなかでも Bannayan-Rikey-Ruvalcaba 症候群 (BRRS) は大頭症、脂肪腫、血管腫、陰茎色素沈着、消化管の過誤腫性ポリポーシスを主徴とし、ASD を伴うことが報告されている。大頭症を伴う自閉症の 4.7% に PTEN 遺伝子の異常が検出されており¹⁰⁾、3SD 以上の大頭症の自閉症ではスクリーニングの必要があるであろう。この PTEN 遺伝子も TSC1 や TSC2 同様に mTOR の活性調節にかかわっている。

それぞれの疾患が自閉症に占める割合は多くてもそれぞれ約 1% 程度しかないが、シグナルパスウェイから考えると共通した治療法が導き出される可能性がある。現実の治療への応用までにはまだまだ課題は多いと思われるが、このように遺伝子の異常が判明すると、その遺伝子の機能を手掛かりとして治療へと発展させていくことの可能性が出てくる。

ASD と代謝異常症

ASD の原因として代謝異常症は非常に稀である。しかし、よく知られているように、フェニルケトン尿症は、新生児期にスクリーニングされて早期治療が始まり、治療開始以前にみられた症状は、自閉症状を含めて治療開始後には改善し認められなくなっている。このように、治療が可能である疾患が含まれているため、代謝異常に起因する自閉症を発見し診断することは予後を考えると重要である。自閉症状を示す症例報告のあった代謝異常症とその参考症状、バイオマーカー、治療法を表 2 に示した。

これらの代謝異常の中で現在最も注目されているのは、脳クレアチン欠損症である(表 3)。脳内クレアチンは脳内の ATP の貯蔵と再生に重要な役割を担っている。脳クレアチン欠損症候群 (cerebral creatine deficiency syndrome ; CCDSs) には現在 3 つの遺伝子の異常が知られている。クレアチン合成障害として、① guanidinoacetate methyltransferase (GAMT) 欠損症、② arginine : glycine aminotransferase deficiency (AGAT) 欠損症の 2 種類、クレアチンの uptake の障害である、③ creatine

transporter (SLC6A8) 欠損症 (X 連鎖性のクレアチン症候群) である。症状には幅があり、非特異的知的障害、てんかん、錐体外路症状、自閉症などさまざまである。一般的に CCDSs の症状は、知的障害が主体であるが、GAMT 欠損症では、てんかんが 93%、行動異常 (自閉行動や自傷) が 80%、錐体路/錐体外路症状が 45% に認められる。発症は 3 カ月から 3 歳である。AGAT 欠損症の報告はまだ少なく 5 例のみであり行動異常はない¹¹⁾。SLC6A8 欠損症は軽度から重度の知的障害や言語の遅れ、てんかん (<10%) と、自閉症様の行動異常が認められる。発症は 2 歳から 66 歳と幅が広い。女子のヘテロの約 50% に学習や行動の問題がみられる。GAMT 欠損症と AGAT 欠損症では経口 creatine monohydrate で脳内クレアチンを増加させる治療がなされている。さらに、GAMT 欠損症では食事のアルギニン制限とオルニチンのサプリメント使用が併用される。SLC6A8 欠損症でも経口 creatine monohydrate が試みられているが現在のところ効果は認められていない。L-アルギニン補充療の効果の報告では効果なしと効果あるとで分かれている¹²⁾。早期に治療を開始した場合の効果の違いについてはまだ不明であり、今後症例の蓄積が必要である。診断の手掛かりとなる尿所見については、GAA (guanidinoacetate) 濃度は GAMT 欠損症で高く、AGAT 欠損症で低く、SLC6A8 欠損症で正常である。クレアチン濃度は GAMT 欠損症と AGAT 欠損症で低く、SLC6A8 欠損症で高い (ただし女子では正常あるいは高値) クレアチン/クレアチニン は GAMT 欠損症と AGAT 欠損症で正常、SLC6A8 欠損症の男児で高い (女子では正常あるいは高値) である。血清の GAA は GAMT 欠損症で正常の 20~30 倍、AGAT 欠損症で低く、SLC6A8 欠損症では正常である。血清クレアチンは GAMT 欠損症で低い。確定診断では GAMT 酵素活性、AGAT 酵素活性を測定する。SLC6A8 欠損症ではクレアチン吸収試験と遺伝子診断を行う。頻度については、SLC6A8 欠損症は非症候性知的障害の男児の 2.1%¹³⁾、IQ 70 以下の全体的遅れのある男子の 2.2%¹⁴⁾ との報告がある。男児の知的障害で高頻度であることを報告した Lion-François¹⁵⁾ らは、スポット尿でクレアチン/クレアチニンと GAA/クレアチニンを測定し、異常値を示した症例には脳 MRS (H-MRS) と SLC6A8

表 2 ASDと代謝異常疾患

疾患	遺伝子	ASD症状割合	参考症状	バイオマーカー	治療
フェニールケトン尿症	<i>PAH</i>	治療前5.7% 治療後0%	小頭症, てんかん	血中フェニルアラニン高値, BH4代謝正常	治療ミルク, 食事療法
スミス-Lemli-オピッツ症候群	<i>DHCR7</i>	50~86%	多発奇形, 発育障害	低コレステロール血症	高コレステロール食, 胆汁酸
脳クレアチン欠損症					
アルギニン:グリシンアミノトランスフェラーゼ欠損症	<i>GATM</i> (AGAT)	16%	てんかん高率, 錐体外路症状, 失調	尿中GAA高値, GAMT酵素活性	経口クレアチン水和物, アルギニン制限食, 経口オルニチン
グアニジンアミノアセテートトランスフェラーゼ欠損症	<i>GAMT</i>		行動異常なし, まだ報告が少ない	尿中GAA低値, クレアチン低値, AGAT酵素活性	経口クレアチン水和物
クレアチン輸送担体欠損症	<i>SLC6A8</i>	しばしば	てんかん10%以下, 行動異常	尿クレアチン/クレアチニン高値	Lアルギニン補充療法 (効果はまだ不定)
アデニロコハク酸リアーゼ欠損症	<i>ADSL</i>	50%	筋緊張低下, てんかん	尿・髄液S-AdoとSAICArの検出	
コハク酸アルデヒド脱水素(SSADH)欠損症(4-ヒドロキシ酪酸尿症)	<i>ALDH5A1</i>	12%	筋緊張低下, けいれん, 多動, 小脳失調, 睡眠障害	尿中4-hydroxybutyric acid増加, 培養リンパ芽球SSADH活性低下	Vigavatorin(確証なし)
ビオチナーゼ欠損症	<i>BDT</i>	症例報告	筋緊張低下, けいれん, 皮膚炎	酵素活性	ビオチン投与
L2ヒドロキシグルタル酸尿症	<i>L2HGDH</i>		小脳・錐体外路症状, 大頭症, 基底核・歯状核を含む白質脳症	L-2-HGAの増加	脳腫瘍併発観察
オルニチントランスカルバミラーゼ欠損症	<i>OTC</i>		反復嘔吐, 意識障害, 男児重症, 女児軽症	アンモニア高値, 血漿シトルエイン低値など	高アンモニア血症対策
Sanfilippo病 A 型	<i>SGSH</i>		多動, 多毛, 粗毛, 顔貌	尿中ヘパラン硫酸の排泄	酵素補充量の計画中
カルシナーゼ欠損症	<i>CNSN</i>		発達遅滞	尿中カルノシン増加	
シトリン欠損症	<i>SLC25A</i>		タンパク嗜癖, 低身長, けいれん, 遷延性黄疸, 反復昏睡	高ガラクトース血症, 複数アミノ酸高値	
ヒスチジン血症	<i>HAL</i>		けいれん, 言語障害, 無症状例がほとんど	高ヒスチジン血症	

BH4 : tetrahydrobiopterin, GAMT : guanidinoacetate methyltransferase, AGAT : L-arginine : glycine amidinotransferase, *SLC6A8* : *SLC6A8*-related creatine transporter, GAA : guanidinoacetate, S-Ado : succinyladenosine, SAICAr : succinylaminoimidazole carboxamide ribotide, SSADH : succinic semialdehyde dehydrogenase, L-2-HGA : L-2-hydroxyglutaric acidia

かGAMTの遺伝子検査で確定診断を行っている。症例は軽度から中等度精神遅滞の118名(男114例, 女74例)のうち男子のみの5例に異常が発見され, その5例の知的障害は重度で, 5例中3例に自閉症状が認められている。したがって, 頻度は全体の2.7%, 男児の4.4%であった。家族性は2例で5.4%, 非家族性は3例で1.9%という報告であった。

他の報告によると, 原因不明とされていた精神遅滞の944例(3歳から76歳)中7例(0.74%)において尿検査で先天代謝異常が検出されたとの

報告がある¹⁶⁾。7例のうち自閉的行動を合併していた5例の内訳は, 脳クレアチン欠損症3例, adenylosuccinate lyase欠損症1例, フェニールケトン尿症(スクリーニング以前に出生)1例であった。このように身体的所見が非特異的で乏しく, 従来原因不明と考えられていた症例の中にも代謝異常症を念頭に調べてみると原因の見つかる症例もあり, 特に脳クレアチン欠損症がその中でも頻度が多い可能性がある。

表3 脳クレアチン欠損症候群の症状と尿所見

欠損	ASDの頻度	症状			尿所見		
		知的障害 程度	てんかん 頻度	錐体外路 症状	GAA濃度	クレアチン 濃度	クレアチン/ クレアチニン 比
GAMT	16%	軽～重	93%	無～重	高	低	正
AGAT		軽～中	20%	無	低	低	正
SLC6A8	しばしば	軽～重	<10%	無	男 正	高	高
					女 正	正～高	正～高

GAMT : guanidinoacetate methyltransferase, AGAT : L-arginine : glycine amidinotransferase, SLC6A8 : SLC6A8-related creatine transporter, GAA : guanidinoacetate

まとめ

自閉症スペクトラム障害(ASD)の原因検索の必要性を述べた。しかし、ASDの患者で、血液や尿検査をして異常の見つかることは非常に少ない。最も検出される検査は、染色体、アレイCGH, MLPA法などの臨床遺伝的検査であろう。しかし、検査依頼方法やコストのほかに、倫理的な面その後の対処法も含めてさまざまな配慮が必要であり、どこにおいても検査を行うわけにはいかないのが現状である。もう少し簡便にスクリーニングできる方法が開発されることを切望する。

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Case report

Muscle glycogen storage disease 0 presenting recurrent syncope with weakness and myalgia

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Abstract

Muscle glycogen storage disease 0 (GSD0) is caused by glycogen depletion in skeletal and cardiac muscles due to deficiency of glycogen synthase 1 (GYS1), which is encoded by the *GYS1* gene. Only two families with this disease have been identified. We report a new muscle GSD0 patient, a Japanese girl, who had been suffering from recurrent attacks of exertional syncope accompanied by muscle weakness and pain since age 5 years until she died of cardiac arrest at age 12. Muscle biopsy at age 11 years showed glycogen depletion in all muscle fibers. Her loss of consciousness was gradual and lasted for hours, suggesting that the syncope may not be simply caused by cardiac event but probably also contributed by metabolic distress.

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Keywords: Glycogen storage disease; Glycogen synthase; Glycogen; Syncope; Sudden death

1. Introduction

Glycogen is a high molecular mass polysaccharide that serves as a repository of glucose for use in times of metabolic need. It is stored in liver, cardiac and skeletal muscles, and broken down to glucose to produce ATP as energy as needed. For the synthesis of glycogen, at least two proteins, glycogenin (GYG) and glycogen synthase (GYS), are known to be essential. GYG is involved in the initiation reactions of glycogen synthesis: the covalent attachment of a glucose residue to GYG is followed by elongation to

form an oligosaccharide chain [1]. GYS catalyzes the addition of glucose monomers to the growing glycogen molecule through the formation of alpha-1,4-glycoside linkages [2].

Defect in either GYG or GYS can cause glycogen depletion. Recently, muscle glycogen deficiency due to a mutation in a gene encoding muscle GYG, *GYG1*, was reported [3] and named as glycogen storage disease type XV. In contrast, glycogen depletion caused by the *GYS* gene mutation is called glycogen storage disease type 0 (GSD0). GSD0 was first reported in 1990 in patients with type 2 diabetes who had a defect in glycogen synthesis in liver, which was caused by a defect in liver GYS, *GYS2*, and the disease was named as liver GSD0 (or also called GSD0a) [4,5].

The disease of muscle GYS, *GYS1*, was first described in 2007 in three siblings and named muscle GSD0, which is

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also called GSD0b [6]. One of the patients initially manifested exercise intolerance, epilepsy and long QT syndrome since the age of 4 years, then died of sudden cardiac arrest after exertion when he was 10.5-year-old. The other two siblings were then genetically confirmed as muscle GSD0 with mutations in *GYS1* and cardiac involvement was also found in both. The second muscle GSD0 family was reported in 2009 [7]. The 8-year-old boy had been healthy before collapsing during a bout of exercise, resulting in death. Post-mortem examinations and studies verified the diagnosis of muscle GSD0. He had a female sibling who died at 6 days of age of undetermined cause. Here we report the first muscle GSD0 patient in Asia with some distinct clinical manifestations from other reported cases.

2. Case report

An 11-year-old Japanese girl with repeated episodes of post-exercise loss of consciousness, weakness, and myalgia since age 5 years, was admitted to the hospital. She was the first child of unrelated healthy parents. She was born uneventfully and was normal in psychomotor development. At age 2 years, she developed the first episode of generalized tonic-clonic seizure while she was sleeping. At age 4 years, she had the second episode of generalized tonic-clonic seizure when she was under general anesthesia for tonsillectomy, whose cause was thought to be hypoglycemia due to prolonged fasting. In both episodes, seizure was followed by strong limb pain. At age 5 years, she suffered from the first episode of syncope while climbing up stairs. She recovered after a few hours. One year later, she had the second syncope attack after running 50 m, which was accompanied by subsequent limb muscle weakness and myalgia. Since then, similar episodes were repeated several times a year. For each bout, she first developed leg muscle weakness immediately after exercise, making her squat down, and gradually lost the consciousness. She recovered her consciousness after a few hours but always experienced strong myalgia in legs which lasted for several hours. Blood glucose level was not decreased during these attacks.

On admission, general physical examination revealed no abnormal finding. On neurological examination, she had mild proximal dominant muscle weakness and mildly limited dorsiflexion of both ankle joints. T1-weighted images of skeletal muscle MRI showed high signal intensities in gluteal and flexor muscles of the thigh, which were assessed to be fatty degeneration (Fig. 1). Systemic investigations including electrocardiography, echocardiography, stress cardiac catheterization, stress myocardial scintigraphy, brain imaging, electroencephalography, and screening tests for metabolic diseases revealed no abnormality except for a mild ischemic finding on exercise electrocardiography. Ischemic and non-ischemic forearm exercise tests [8] showed the lack of lactate elevation, raising a possibility of glycogen storage disease. A few months later, resting electrocardiography, 24-h holter monitoring and resting echocardiography were re-evaluated and again revealed normal findings.

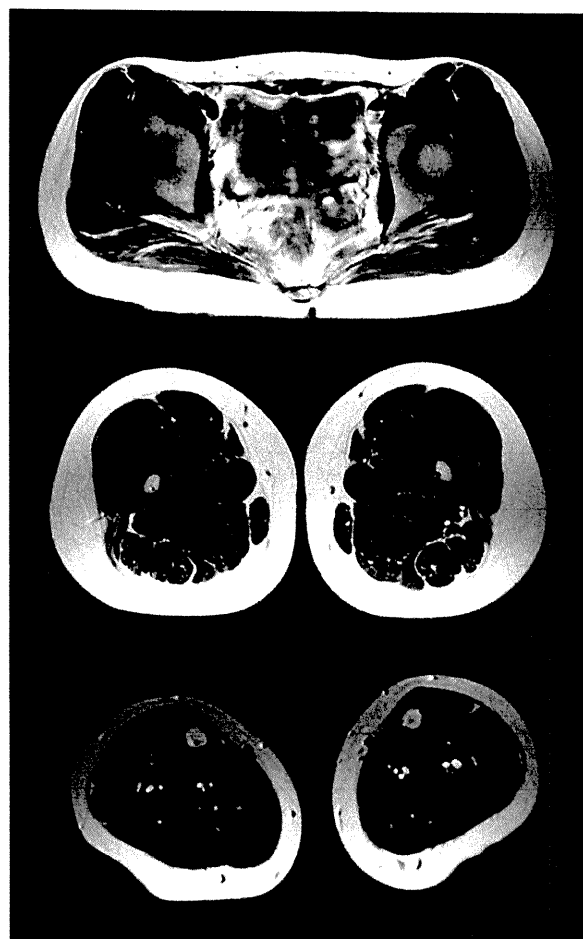


Fig. 1. Muscle MRI, T2WI, axial. It shows high intensity in gluteus maximus and biceps femoris muscles.

3. Histological analysis of skeletal muscle

Muscle biopsy was performed from biceps brachii. Serial frozen sections were stained with hematoxylin and eosin, modified Gomori trichrome, and a battery of histochemical methods. The most striking finding was depletion of glycogen in all muscle fibers but not in the interstitium on periodic acid-schiff (PAS) staining (Fig. 2A). Phosphorylase activity was also deficient in all fibers (Fig. 2B). Mitochondria especially at the periphery of muscle fibers were prominent on modified Gomori trichrome (Fig. 2D). ATPase staining revealed type 2 fiber atrophy. Electron microscopic analysis showed mitochondrial proliferation at the periphery of muscle fibers with no notable intramitochondrial inclusions (Fig. 2E).

4. Biochemical and molecular analysis

Both the activity of *GYS1* and the amount of glycogen in the skeletal muscle were markedly reduced (Table 1). On western blotting, *GYS1* in the patient's skeletal muscle was undetectable (Fig. 2F). The *GYS1* gene sequence analysis revealed compound heterozygous mutation of

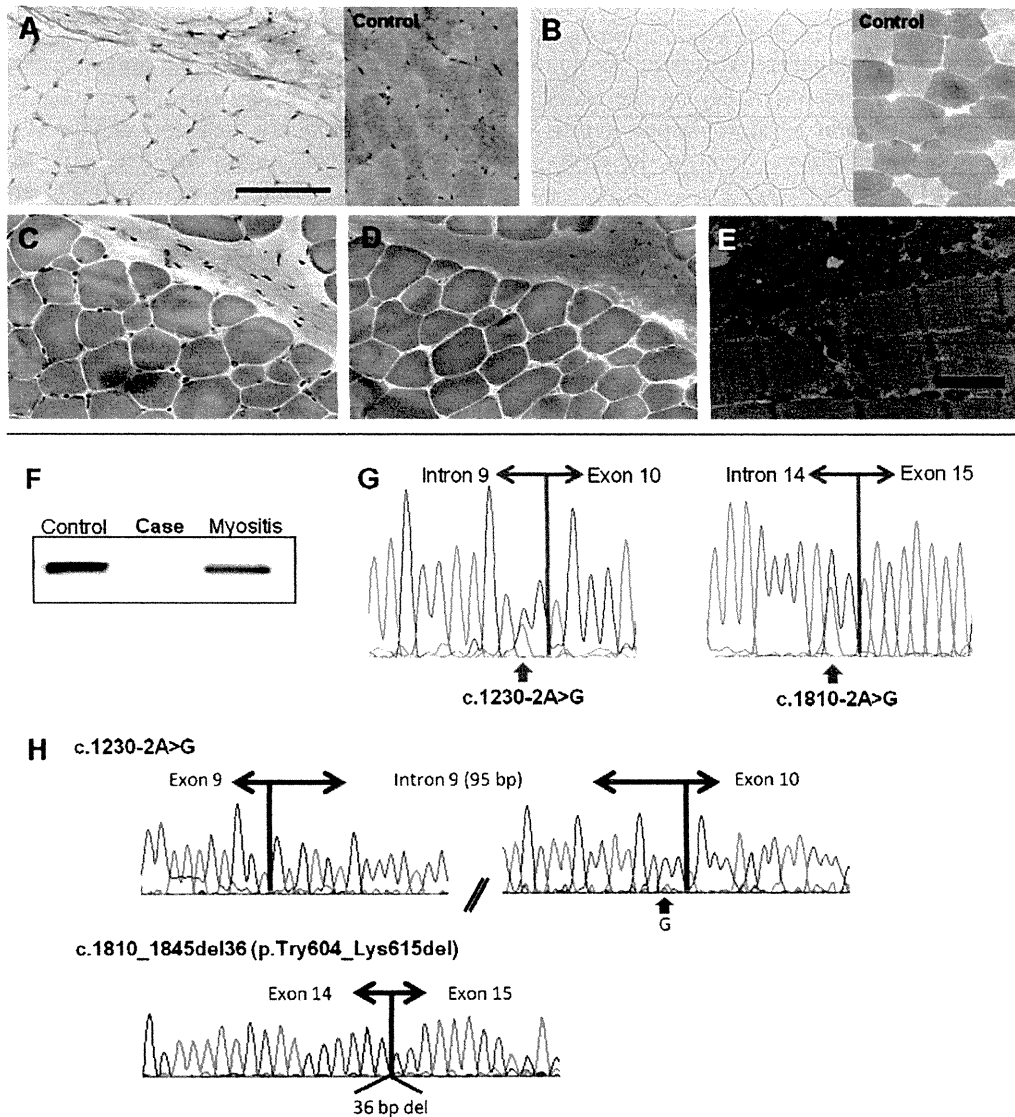


Fig. 2. Histological, genetic and protein analyses. Periodic acid-schiff (PAS) staining shows marked depletion of glycogen in muscle fibers but not in the interstitium (A). Phosphorylase activity is also deficient in all fibers (B). Hematoxylin and eosin staining shows mild fiber size variation (C). On modified Gomori trichrome, mitochondria are prominent especially at the margin of each muscle fiber (D). On electron microscopy (EM), mitochondria are increased in number at the periphery of muscle fibers (E). Bars represent 100 μ m for histochemistry and 7 μ m for EM. On western blotting using anti-GYS1 antibody (Abcam), GYS1 protein is absent in skeletal muscle from the patient (F). Sequence analysis for the *GYS1* gene reveals a compound heterozygous mutation of c.1230-2A > G and c.1810-2A > G (G). cDNA analysis showed insertion of intron 9 between exon 9 and 10 and 36-bp deletion from the beginning of exon 15 (H).

Table 1
Analyses of enzymatic activity and glycogen content. The activity of GYS and glycogen content in skeletal muscle were markedly reduced.

	Glycogen synthase (mol/min/mg)	UDPG-pyrophosphorylase (nmol/min/mg)	Glycogen contents (% of wet weight)
Patient	<i>0.9</i>	30.5	<i>0.03</i>
Control	42.0 \pm 11.2	31.2 \pm 3.5	0.94 \pm 0.55

Italicized values: lower than control range.

c.1230-2A > G in intron 9 and c.1810-2A > G in intron 14 (Fig. 2G). cDNA analysis confirmed the insertion of the full-length intron 9 between exons 9 and 10 and a 36-bp deletion in the beginning of exon 15 (Fig. 2H).

5. Clinical course after diagnosis

Upon the diagnosis of GSD0, exercise was strictly limited to avoid syncope resulted from glucose depletion. In

addition, oral intake of cornstarch (2 g/kg, every 6 h) was started to maintain blood sugar level. Her condition had been stable for 1 year after diagnosis. However, at age 12 years, she was found lying unconsciously on the stairs at her school. She had persistent asystole despite ambulance resuscitation. The blood glucose level in the emergency room was above 100 mg/dl.

6. Discussion

We identified the first Asian patient with muscle GSD0, who manifested recurrent episodes of syncope with subsequent muscle weakness and myalgia, and eventually developed cardiac arrest.

Findings in our patient seem to be similar to previous reports, but some differences indicated the possibility of another pathogenesis of the disease. Our patient repeatedly suffered from episodes of syncope. In contrast to two earlier reports, those patients never had syncope, although the last attack led to sudden death [6,7]. In support of this notion, most muscle glycogen synthase knock-out mice died soon after birth due to impaired cardiac function [8]. However, the pattern of loss of consciousness in our patient cannot be explained by simple cardiac dysfunction, as she lost her consciousness gradually after exercise and took hours to regain, which is different from typical cardiac syncope, usually showing sudden loss of consciousness and rapid recovery. Alternatively, defective glycogen synthesis in brain may be related to syncope, as GYS1 is also expressed in brain, albeit not so much as in cardiac and skeletal muscles. Another possibility may be intermittent arrhythmia. However, electrocardiogram during the episode was never obtained. Further studies are necessary to answer this question.

On muscle pathology and electron microscopy, we found profound deficiency of glycogen in all muscle fibers accompanied by mitochondrial proliferation, which is similar to previous reports. The mitochondrial proliferation may reflect a compensatory mechanism for supplying ATP to glycogen-depleted muscles. Interestingly, phosphorylase activity on histochemistry seemed deficient. This is consistent with the fact that endogenous glycogen is used as a substrate of phosphorylase on histochemistry. Previous reports described the reduced number of type 2 fibers. In our patient, type 2 fiber atrophy, but not type 2 fiber deficiency, was seen. Although type 2 fiber atrophy is a nonspecific finding, this picture might also reflect the dysfunction of glycogen-dependent muscle fibers.

7. Conclusion

We identified the first Asian patient with muscle GSD0. In our patient, recurrent episodes of syncope and eventual sudden death may not be simply explained by cardiac dysfunction. Further studies are necessary to elucidate the mechanism of syncope in muscle GSD0 and to establish appropriate guideline of management for these patients to prevent sudden death.

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CONFIRMATION OF THE EFFICACY OF VITAMIN B₆ SUPPLEMENTATION FOR MCARDLE DISEASE BY FOLLOW-UP MUSCLE BIOPSY

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ABSTRACT: No effective treatment for McArdle disease exists. We report a Japanese patient with McArdle disease who was treated with vitamin B₆ supplementation (60–90 mg/day). After treatment, increased muscle phosphorylase activity was confirmed by follow-up muscle biopsy (3.8 times higher than pretreatment levels). Increased lactate levels were seen on the forearm exercise test, and regular work activities could be resumed. Vitamin B₆ supplementation can enhance residual phosphorylase activity and improve insufficient anaerobic glycolysis of skeletal muscle.

Muscle Nerve 45: 436–440, 2012

McArdle disease is a rare metabolic myopathy caused by a deficiency in muscle phosphorylase, which has an important role in anaerobic glycolysis of skeletal muscle. Clinical features of McArdle disease include muscle cramps, myalgia, exercise intolerance, fatigue, and slowly progressive weakness, although the type and amount of exercise needed to precipitate these symptoms varies from patient to patient and from day to day. Muscle necrosis and myoglobinuria caused by an inadequate energy supply to skeletal muscle during exercise occur in about half of patients, and half of them develop acute renal failure.¹ A diagnosis of McArdle disease is suspected based on patient history and elevation of serum creatine kinase (CK) levels.¹

The forearm exercise test, during which serum ammonia and lactate levels are measured, is a simple, sensitive, and specific test for disorders of muscle glycolysis. In McArdle disease, patients fail to produce lactate during this test.²

Several groups have reported the use of vitamin B₆ treatment in McArdle disease. However, a repeat muscle biopsy in the same patient after treatment has not been performed; thus, the efficacy of this treatment is not well documented. To

date, there is no conclusive evidence of significant benefits from nutritional or pharmacological treatments in McArdle disease.³

We report an adult Japanese patient who was treated with oral vitamin B₆ supplements (60–90 mg/day) for >2 years. Efficacy of treatment was evaluated using manual muscle testing (MMT: –4 = paralysis; –3.5 = paralysis–severe weakness; –3 = severe weakness; –2.5 = severe–moderate weakness; –2 = moderate weakness; –1.5 = moderate–mild weakness; –1 = mild weakness; –0.5 = mild weakness–normal power; 0 = normal power), the forearm exercise test, and a follow-up muscle biopsy; we also measured serum CK levels.

CASE REPORT

In March 2008, a 41-year-old Japanese man was brought to our emergency room with severe myalgia and brown urine after being injured in a fight with his brother. He indicated that, since childhood, he had experienced muscle cramps and myalgia after exercising. His parents were consanguineous, and he had a history of hypertension and subarachnoid hemorrhage. Blood chemistry showed markedly elevated serum CK level (420,950 IU/L), but vitamin B₆ levels were within the normal range (pyridoxine <0.3 ng/ml, pyridoxamine <0.2 ng/ml, pyridoxal 6.5 ng/ml). He developed severe rhabdomyolysis and acute renal failure, but hemodialysis in the intensive care unit greatly improved his renal function.

In April 2008, his height and body weight were 167 cm and 54 kg, respectively, and neurological examination showed moderate weakness of proximal muscles in the upper and lower limbs (MMT = –2). The forearm exercise test revealed virtually no increase in serum lactate level, although increases in lactate levels after the forearm exercise test were five- or sixfold higher than baseline levels in healthy subjects (Fig. 1).² An electromyogram was normal. Computed tomography scans of all his extremities indicated slight atrophy of the proximal muscles. Muscle biopsy of his left biceps

Abbreviations: CK, creatine kinase; H&E, hematoxylin and eosin; MMT, manual muscle testing; PAS, periodic acid–Schiff; PLP, pyridoxal 5'-phosphate

Key words: anaerobic glycolysis; follow-up muscle biopsy; McArdle disease; muscle phosphorylase; vitamin B₆ supplementation

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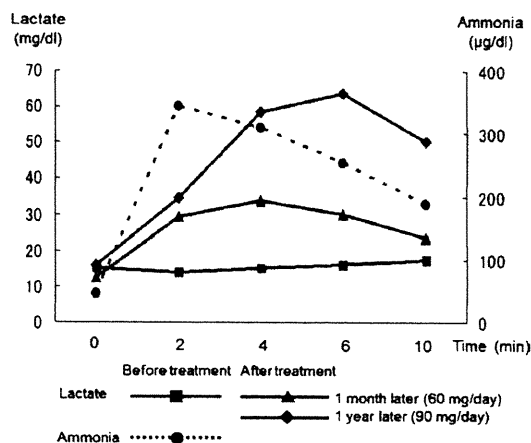


FIGURE 1. Forearm exercise test. For this test, rhythmic (1-Hz) handgrip exercise at maximal voluntary contraction was performed for 2 minutes. Results showed no increase in serum lactate levels before treatment with vitamin B₆ was started. An increase in serum lactate levels was seen 1 month after starting oral vitamin B₆ supplementation at 60 mg/day (1.1 mg/kg/day). Moreover, lactate markedly increased 1 year after treatment with 90 mg/day (1.6 mg/kg/day). Plasma ammonia concentrations were only measured before treatment with vitamin B₆. Time = 0: before exercise. Normal values (at rest): lactate 5.0–20.0 mg/dl; ammonia 9–45 µg/dl.

brachii, which was performed 40 days after the severe rhabdomyolysis, showed variation of muscle fiber size and frequent internal nuclei on hematoxylin and eosin (H&E) staining (Fig. 2A). When muscle phosphorylase activity is preserved, muscle fibers are stained brown or violet with phosphorylase because of their reaction to the iodine–potassium iodide solution used for phosphorylase staining. The higher the activity of phosphorylase, the deeper violet the muscle fibers are stained. However, his muscle fibers did not show phosphorylase staining (Fig. 2B). Periodic acid–Schiff (PAS) staining revealed many glycogen deposits under the sarcolemma of muscle fibers. Under histochemical staining for ATPase activity at pH 4.4, the proportion of type 1 and type 2 fibers was 44% and 56%, respectively. Muscle phosphorylase activity was 3.8 nmol/min/mg protein [control: 58.9 ± 17.5 nmol/min/mg protein (mean ± SD)]. He was found to be homozygous for a single-codon deletion at codon 708/709 in exon 17, which is the most common mutation of muscle phosphorylase among Japanese patients with McArdle disease.⁴

In August 2008, treatment with oral vitamin B₆ supplements (60 mg/day, 1.1 mg/kg/day) was started and, 1 month later, the forearm exercise test showed an increase in lactate levels (Fig. 1). Neurological examination revealed muscle strength improvement (MMT = –0.5). Serum CK levels were normal (146 IU/L) (Fig. 3). In November 2008, we increased the dosage of vitamin B₆ from 60 to 90 mg/day (1.6 mg/kg/day) because serum

CK had increased due to more severe physical stress associated with his job. Serum CK levels normalized 1 month after administration of 90 mg/day of vitamin B₆. Subsequently, his muscle weakness gradually improved.

In July 2009, 1 year after starting treatment, the patient's lactate levels markedly increased on the forearm exercise test (Fig. 1). In October 2009, a follow-up muscle biopsy of his right biceps brachii was performed with his informed consent. The fiber size variation was minimized, and 70–80% of the muscle fibers were stained brown with phosphorylase (Fig. 2C and D). The proportions of type 1 and type 2 fibers in the posttreatment sample were 31% and 69%, respectively. Little accumulation of glycogen was observed in the muscle fibers by PAS staining. Muscle phosphorylase activity was 14.4 nmol/min/mg protein, which was 3.8 times higher than before treatment.

The patient's serum CK levels ranged from 120 to 2,093 IU/L (mean 576 IU/L), depending on his physical activities (Fig. 3). However, his clinical condition was stable regardless of the heavy labor he performed during his daily work as a fish dealer. Furthermore, there had been no adverse effects caused by vitamin B₆, including sensory neuropathy.^{5–7}

DISCUSSION

Oral vitamin B₆ supplementation (60–90 mg/day) in this patient led to improvements in both muscle weakness and inadequate anaerobic glycolysis; a follow-up muscle biopsy confirmed the presence of increased muscle phosphorylase activity after treatment. Our patient has continued to be engaged in his work for 2 years and 2 months. Although the phosphorylase activity after vitamin B₆ treatment is not completely normal, it is sufficient for him to maintain his regular work activities.

McArdle disease is transmitted as an autosomal recessive trait. The gene for muscle phosphorylase is localized on chromosome 11q13. Deficiency of this enzyme results in inability to metabolize skeletal muscle glycogen during anaerobic metabolism, followed by clinical symptoms such as muscle weakness. There is almost no detectable muscle phosphorylase activity in the majority of affected individuals,^{1,4} but some residual activity (i.e., up to 10% of normal values) has been observed in some cases.¹ Our patient had 6.5% of normal muscle phosphorylase activity before treatment, 40 days after the severe rhabdomyolysis in March 2008. The residual activity may have been caused by several regenerating muscle fibers expressing the fetal isoform of muscle phosphorylase.^{8,9} In addition, the difference in the proportion of muscle fiber types before and after vitamin B₆ treatment might have been caused by regeneration of skeletal

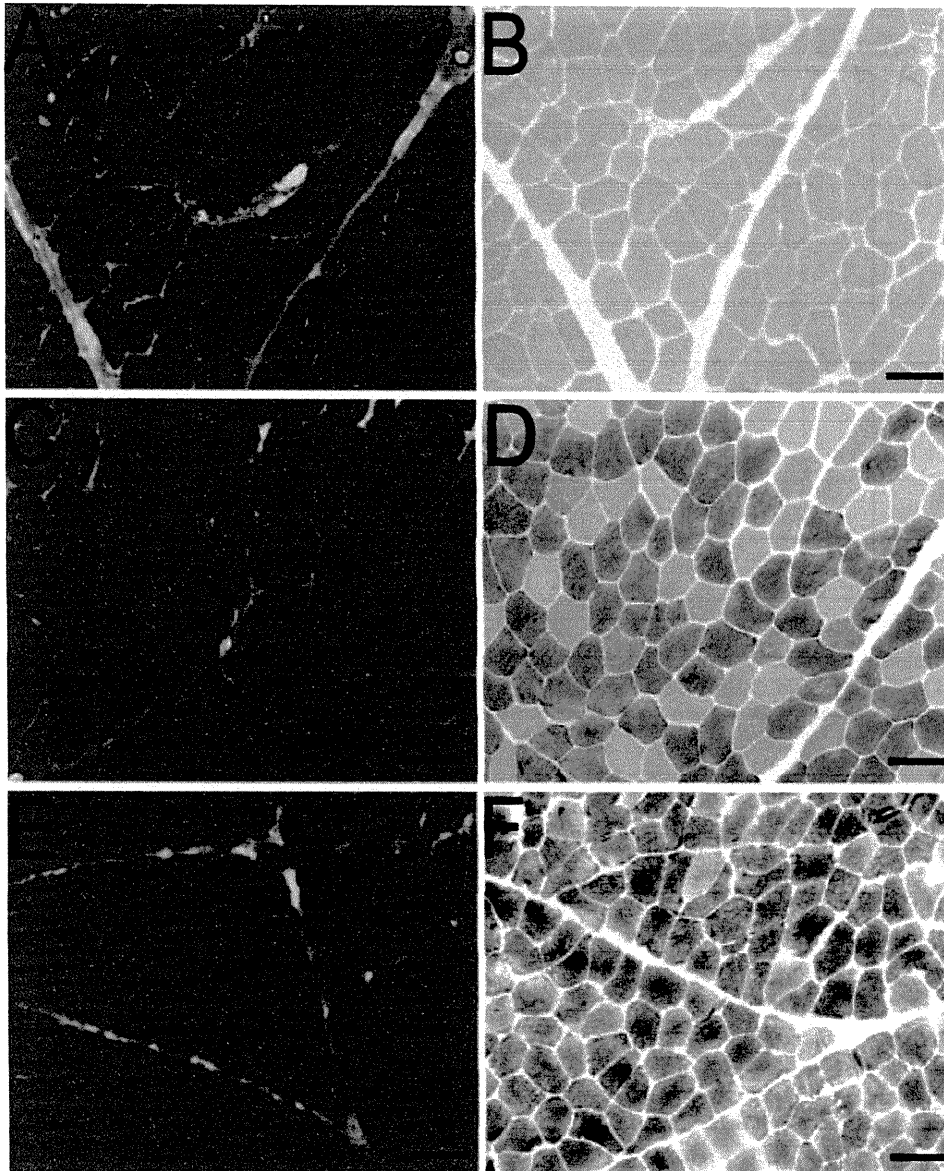


FIGURE 2. Muscle biopsy samples from the patient and a control subject. **(A, B)** Before treatment with vitamin B₆: **(A)** a sample stained with H&E; and **(B)** a sample stained with phosphorylase. Samples showed variation in muscle fiber size and frequent internal nuclei. No muscle fibers were stained with phosphorylase. **(C, D)** After treatment with vitamin B₆: **(C)** a sample stained with H&E; and **(D)** a sample stained with phosphorylase. The muscle fibers stained with phosphorylase increased markedly and muscle fiber size was almost uniform. **(E, F)** Control: **(E)** a sample stained with H&E; and **(F)** a sample stained with phosphorylase. Muscle biopsy samples before and after treatment were stained at the same time as the control sample. Bars = 100 μ m.

muscle fibers, especially type 2C fibers. However, we believe that the increased muscle phosphorylase activity of the follow-up muscle biopsy at 1 year and 3 months after treatment was due to treatment with vitamin B₆ because enough time had passed since the episode of severe rhabdomyolysis, and there had been only mildly increased CK levels during treatment.

Lactate increased more dramatically on the forearm exercise test after treatment with higher doses of vitamin B₆ (90 mg/day, 1.6 mg/kg/day) than with lower doses (60 mg/day, 1.1 mg/kg/day). On the other hand, we decreased the dosage

of vitamin B₆ from 90 to 60 mg/day in April 2009, because we believed that the toxicity of vitamin B₆ (90 mg/day) resulted in transient exacerbation of muscle weakness and a reduction in regular work activities in this period. However, we determined that the worsening was due to more severe physical work, and we returned the dosage to 90 mg/day in May 2009. Except for this episode, he has been in good condition under treatment with 90 mg/day of vitamin B₆. Thus, these results suggest that the effects of vitamin B₆ may depend on the dosage.

Many trial treatments other than oral vitamin B₆ supplementation have been used for McArdle

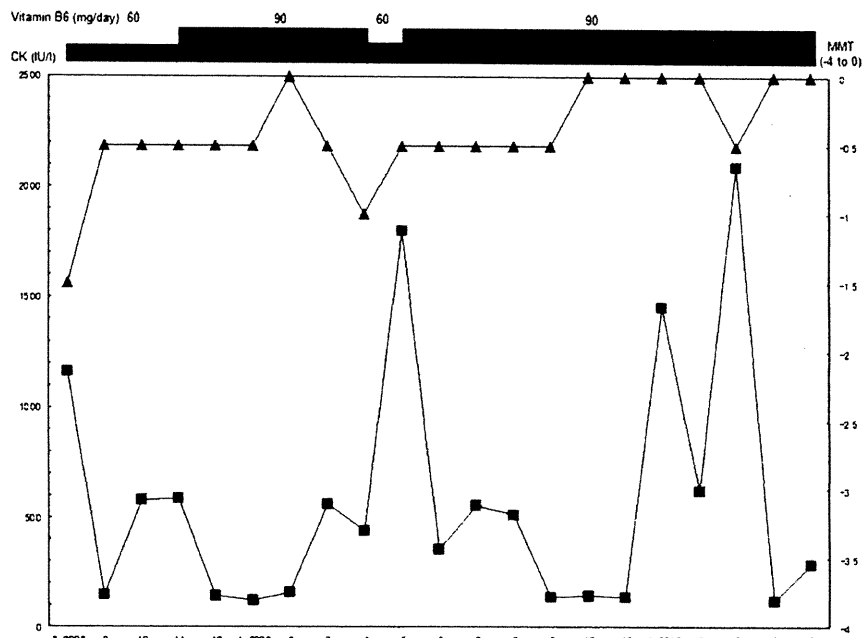


FIGURE 3. Clinical course of the patient. Muscle strength was evaluated by mean MMT of the neck flexors, deltoid muscles, and iliopsoas muscles. The dosage of vitamin B₆ was 60 mg/day from August 2008 to November 2008, 90 mg/day from November 2008 to April 2009, 60 mg/day from April 2009 to May 2009, and 90 mg/day from May 2009 onward. Squares: CK; triangles: MMT. Normal value of CK: 55–290 IU/L.

disease, such as high oral doses of ribose, a fat-rich diet, glucagon, verapamil, a high-protein diet, branched-chain amino acid supplementation, dantrolene sodium, low- or high-dose creatine, oral sucrose, intravenous gentamicin, a ketogenic diet, a high-carbohydrate diet, and ramipril. However, there has been no definitive evidence of any significant benefit from these treatments.³ On the other hand, the withdrawal of vitamin B₆ supplementation from a patient after 2 years of daily administration resulted in decreased exercise tolerance and increased muscle cramps,¹⁰ which suggested the efficacy of therapy with vitamin B₆ supplements. In addition, a Japanese patient with a very mild case of McArdle disease was treated with vitamin B₆ supplementation (90 mg/day) for 3 months, and the forearm exercise test showed improved glycogenolysis, as in our patient.¹¹

In normal individuals, skeletal muscle contains at least 80% of the total body pool of vitamin B₆, bound as pyridoxal 5'-phosphate (PLP) to muscle phosphorylase. One molecule of PLP covalently bound to a lysine residue of each muscle phosphorylase subunit is essential for enzyme activity.^{12,13} The decreased phosphorylase in McArdle disease substantially diminishes PLP in skeletal muscle.^{12,13} The action of vitamin B₆ supplementation may require the presence of some residual muscle phosphorylase, as in our patient, and probably would not be seen in patients with null mutations, including the R50X mutation, which is most common among Caucasians.^{1,14,15}

As noted earlier, most patients lack detectable muscle phosphorylase, as detected by sodium dodecylsulfate–polyacrylamide gel electrophoresis, immunoblot, and enzyme-linked immunosorbent assay.^{1,4} This may result from rapid decay of unstable proteins. Thus, we hypothesize that vitamin B₆ supplementation can restore some stability to the mutant enzyme and enhance the residual phosphorylase activity in skeletal muscle of patients, followed by improvement in insufficient anaerobic glycolysis of skeletal muscle. However, other mechanisms are also possible.

Our study suggests that supplementation of vitamin B₆ may be an effective therapy for McArdle disease, especially for patients who have some residual muscle phosphorylase activity, although further studies, including a double-blind, placebo-controlled study, are necessary to draw firm conclusions about the effects of vitamin B₆ supplementation.

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RELAPSED ACUTE MYELOGENOUS LEUKEMIA OF BRACHIAL PLEXUS AFTER MARROW TRANSPLANT

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ABSTRACT: We present a detailed description of brachial plexus infiltration by acute myelogenous leukemia (AML) in the setting of a remission bone marrow biopsy, without evidence of leukemia by flow cytometric analysis. This case illustrates the possibility of dormant leukemic cells in the peripheral nervous system (PNS) in a patient in apparent clinical remission. In patients with an unexplained brachial plexopathy and a history of AML, leukemic infiltrate of the PNS must be considered.

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Metastatic tumors to the brachial plexus are a relatively rare disease entity. Involvement of the brachial plexus by metastatic tumors occurs in most instances via direct extension of the tumor or by means of lymphatic or hematogenous spread.¹ Primary tumors with reported metastases to this region of the peripheral nervous system most frequently include carcinomas of the breast and lung, lymphomas, and melanoma.² Although involvement of peripheral nerves by a leukemic infiltrate has been reported rarely, this is a detailed description of brachial plexus pathology by a leukemic infiltrate based on immunohistochemical studies. We describe a patient who had a peripheral nervous system (PNS) relapse of acute myelogenous leukemia (AML) manifested by brachial plexopathy. Of particular interest is that the patient had received a gender-mismatched bone marrow transplant 6 years earlier. The relapse occurred in the

setting of a remission bone marrow biopsy with a normal female donor karyotype and with no evidence of leukemia by flow cytometric analysis. A normal complete blood count (CBC) had been present on multiple tests over 6 years.

CASE REPORT

History and Neurological Examination. A 33-year-old man was diagnosed with AML when he presented with a hemoglobin of 8.9 g/dl, hematocrit of 26%, leukocytosis [55,000 white blood cells (WBC)/ μ l], and thrombocytopenia (121,000 platelets/ μ l). Peripheral blood smear evaluation revealed an abnormal white cell differential with 91% blasts. A bone marrow aspirate and biopsy showed AML with maturation, based on the World Health Organization (WHO) classification.³ Flow cytometric analysis of the blasts revealed immunophenotypic features indicative of myeloblasts (CD34, CD117, CD33, HLA-DR, CD15, and CD13 positive). Cytogenetic analysis of the bone marrow revealed a trisomy 8 karyotype.

The patient went into remission after chemotherapy, which consisted of daunorubicin and cytarabine (Ara-C), but 1 year later he had a relapse followed by leukemic meningitis. He received intrathecal Ara-C and high-dose intravenous Ara-C (2 g/m²) and later underwent a gender-mismatched allogeneic bone marrow transplant. His chemotherapeutic regimen for the transplant consisted of ¹³¹I monoclonal antibody and fludarabine in addition to low-dose total body radiation. He again went into remission, but his course was complicated by graft-versus-host disease

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CBC, complete blood count; CLL, chronic lymphocytic leukemia; CNS, central nervous system; CSF, cerebral spinal fluid; EMG, **Key words:** brachial plexus, myelogenous leukemia, peripheral nerve metastasis, transplant

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SHORT COMMUNICATION

Concomitant microduplications of *MECP2* and *ATRX* in male patients with severe mental retardation

Shozo Honda¹, Shigeko Satomura², Shin Hayashi^{1,3}, Issei Imoto^{1,4}, Eiji Nakagawa^{5,6}, Yu-ichi Goto^{5,6} and Johji Inazawa^{1,3,7}, and the Japanese Mental Retardation Consortium⁸

Investigations of chromosomal rearrangements in patients with mental retardation (MR) are particularly informative in the search for genes involved in MR. Here we report a family with concomitant duplications of methyl CpG binding protein 2 (*MECP2*) at Xq28 and *ATRX* (the causative gene for X-linked alpha thalassemia/mental retardation) at Xq21.1 detected by array-comparative genomic hybridization. The alterations were observed in a 25-year-old man who inherited them from his mother, who showed a normal phenotype and completely skewed X-chromosome inactivation, and also in his cousin, a 32-year-old man. The proband and his cousin showed severe MR, muscular hypotonia, recurrent respiratory infections and various other features characteristic of *MECP2* duplication syndrome. However, the proband also had cerebellar atrophy never reported before in *MECP2* duplication syndrome, suggesting that his phenotypes were modified through the *ATRX* duplication in an additive or epistatic manner.

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Keywords: array CGH; *ATRX*; duplication; *MECP2*; X-linked mental retardation

Duplication at Xq28 involving methyl CpG binding protein 2 (*MECP2*) has been detected at high frequency (1–2%) in males with unexplained X-linked mental retardation (XLMR).^{1,2} *MECP2* duplication syndrome is now recognized as a clinical entity showing severe MR, muscular hypotonia, absence of speech, a history of recurrent infection and mild dysmorphic features.³ In the course of a program to screen possible patients with XLMR for copy-number aberrations by array-comparative genomic hybridization (aCGH) using a bacterial artificial chromosome (BAC)-based X-tiling array (MCG X-tiling array),^{2,4} we detected an ~0.4-Mb duplication at Xq28 involving *MECP2* together with an ~0.3-Mb duplication at Xq21.1 that included *ATRX*, the causative gene for ATR-X (X-linked alpha thalassemia/mental retardation) syndrome, in a 25-year-old man and his cousin, a 32-year-old man (Figure 1a).

The proband (III-1, Figure 1b) was born at 41 weeks after an uneventful pregnancy as the first child of non-consanguineous healthy parents. At birth, his weight and occipital–frontal circumference (OFC) were 3280 g (± 0 s.d.) and 33.5 cm (0.3 s.d.), respectively. He was developmentally retarded: first smiling at 3 months, holding up his head at 5 months, rolling over at 7 months, sitting by himself at 12 months and crawling at 13 months. At 25 years, his height, weight and OFC were 160.8 cm (-1.7 s.d.), 50 kg (-1.2 s.d.) and 56.3 cm

(-0.9 s.d.), respectively. The proband exhibited hypertelorism, microcephaly and synophrys (Figure 1c). At 28 years, magnetic resonance imaging (MRI) showed cerebral atrophy, cerebellar atrophy and a thin corpus callosum (Figure 1d). He could walk and communicate until he was 14 years old, but became unable to do either of this after developing epilepsy. At the age of 4 years and 10 months, his total Developmental Quotient was 22, calculated by using the Kyoto Scale of Psychological Development. A blood investigation showed that his IgA level was low. The HbH inclusion body that is detected frequently in patients with *ATRX* mutation was not found by brilliant cresyl staining. His younger brother (III-2) had intrapartum asphyxia and two maternal uncles (II-3, II-4) died immediately after birth.

The cousin of the proband (III-3) was born in 41 weeks after an uneventful pregnancy to non-consanguineous healthy parents by normal delivery. At birth, his weight and OFC were 2850 g (-1.2 s.d.) and 37 cm ($+2.4$ s.d.), respectively. He was characterized by macrocephaly. He had started smiling at 2–3 months, holding up his head at 4 months, sitting by himself at 12 months and walking at 40 months. At 32 years of age, his height, weight and OFC were in the normal range (164.5 cm, -1.1 s.d.; 57 kg, -0.5 s.d.; 59.4 cm, $+1.8$ s.d.). Information on his Developmental Quotient was unavailable. A blood investigation showed that his IgA level was low. He had been affected

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⁸See the Appendix for details regarding the members of the Consortium.

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