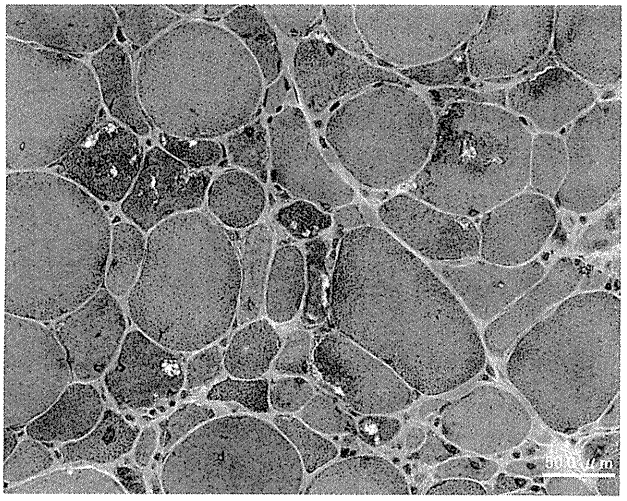

Fig. 1　DMRV の筋病理
萎縮した筋線維が散見され，一部ではまとまって存在している。一方，非萎縮線維は代償性にやや肥大している。萎縮線維を中心に縁取り空胞が認められる。(ゴモリトリクローム変法染色)

Ⅰ．縁取り空胞を伴う遠位型ミオパチー

1．疾患名

本疾患は，1981 年に本邦において世界に先駆けて報告された疾患であり，「縁取り空胞を伴う遠位型ミオパチー(distal myopathy with rimmed vacuoles：DMRV)」と命名された[1-3]。諸外国では，しばしば，埜中ミオパチー(Nonaka myopathy) とも呼ばれる。

DMRV が報告されて 3 年後に，1984 年にイスラエルの Argov ら[4] により，臨床病理学的に極めて類似する筋疾患が，quadriceps sparing myopathy (QSM) として報告された。その後 QSM は，封入体筋炎(inclusion body myositis：IBM) との病理学的類似性から，Askanas ら[5] により遺伝性封入体ミオパチー (hereditary inclusion body myopathy：hIBM) と呼ばれることが提唱され，現在に至っている。

本邦を中心とするアジア諸国では DMRV が，欧米では hIBM が病名として用いられることが多い。あるいは，遺伝性の封入体ミオパチーの中で，常染色体優性遺伝のものを IBM1 としたため，本症は IBM2 とも呼ばれる。しかし，hIBM または IBM2 という病名は，炎症性筋疾患であり明らかに病態の異なる封入体筋炎と同じ略語が用いられており混乱しやすい。そのため，洋の東西を問わず専門家の間では hIBM という病名に対して批判的な意見が強い。混乱を防ぐ目的で統一的な名称を求める動きも強く，最近では，後に述べる原因遺伝子名に基づき GNE myopathy と呼ぶことが提唱されつつある。

2．臨床症状

本邦では血族婚が少ないこともあり，世代をまたぐ家族歴を有する患者はいない。同胞発症もあるが，患者の多くは孤発例である。一般に発症年齢は 15～40 歳で，男女ともに罹患する[1-3]。前脛骨筋が好んでおかされ，スリッパが脱げやすい，あるいは，段差でつまずきやすいなどの垂れ足の症状で異常に気づくことが多い[1-3]。頸部屈筋群，傍脊柱筋，大腿後面の膝屈筋群もおかされやすいが，比較的後期まで大腿四頭筋が保たれる。

筋力低下と筋萎縮は進行し，発症からおよそ 10 年で歩行不能となる。デュシェンヌ型筋ジストロフィーが 4～5 歳頃に発症し，15 歳までに歩行不可能となることを考えると，DMRV の進行はデュシェンヌ型筋ジストロフィーと同程度に早いと考えることもできる。ただし，患者によって重症度や進行速度にかなり差があることがわかってきている。生命予後に関する知見は確立していない。

3．筋病理所見

筋病理では，小角化した萎縮線維とともに，筋線維内に一部が空胞状に白く抜けて，その周りがゴモリトリクローム変法で赤紫色に染まる顆粒状物質で囲まれる構造が認められる。この構造を縁取り空胞(rimmed vacuole) という[1-3] (Fig. 1)。縁取り空胞を電子顕微鏡で観察すると，自己貪食空胞あるいはその類縁構造物であるミエリン様小体 (myeloid body) が集塊をなしている[1-3]。核内および細胞質の一部には，直径 15～20 nm の管状線維性封入体 (tubulofilamentous inclusion) を認める[1-3]。縁取り空胞にも管状線維性封入体にも疾患特性はなく，縁取り空胞が出現する疾患ではほぼ例外なく管状線維性封入体が認められることに注意が必要である。

しばしば，筋線維内に β アミロイドの沈着やリン酸化タウ蛋白質を認め，アルツハイマー病類似の変性過程が存在すると考えられている[1-3,5]。自己貪食空胞は，しばしば β アミロイドの沈着と近接して存在している。ユビキチン・プロテアソーム系の活性化やアポトーシスの関与を示唆するデータもあり，さまざまな変性のプロセスが知られている。

4．原因遺伝子

DMRV/hIBM の原因遺伝子は第 9 染色体上の *GNE* である[6,7]。この *GNE* はシアル酸生合成経路の律速段階

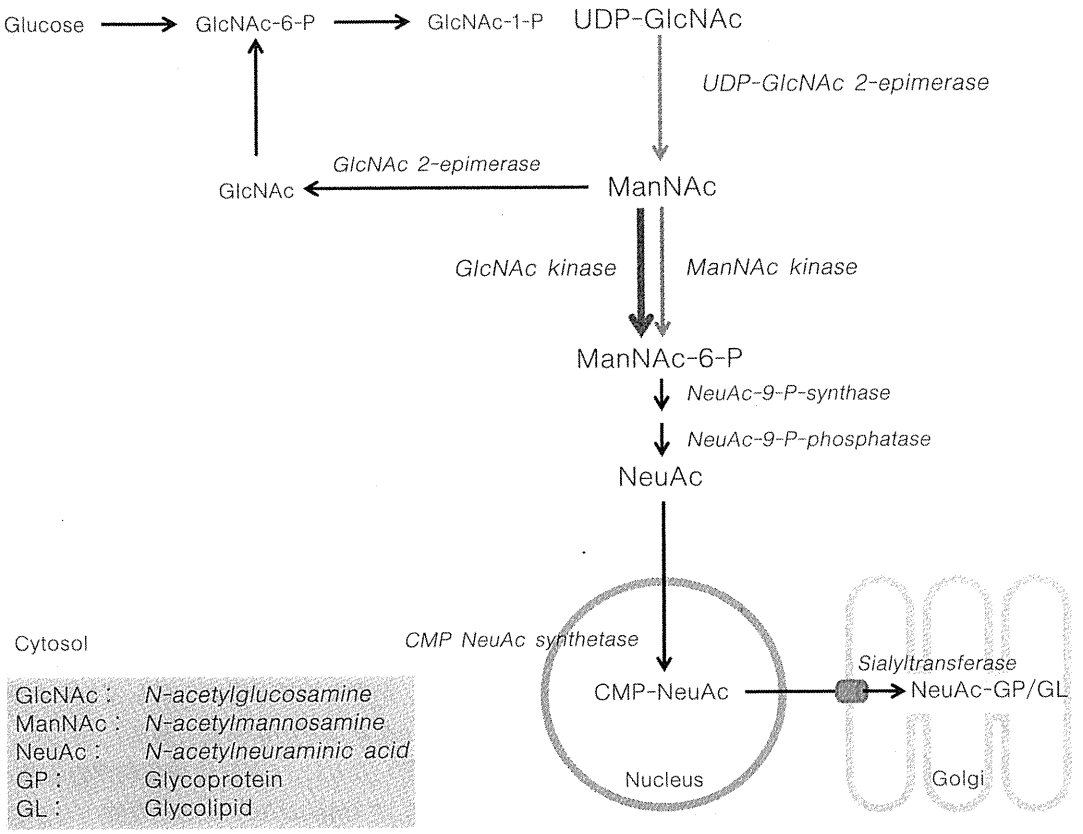

Fig. 2　シアル酸合成経路

シアル酸［ヒトの場合は *N*-アセチルノイラミン酸（NeuAc）］は細胞質でグルコースから生合成される。合成された NeuAc は核に移行して CMP-NeuAc となり，これがゴルジ体において，糖蛋白質または糖脂質の糖鎖合成に用いられる。糖鎖にシアル酸が取り込まれる現象をシアリル化という。DMRV の原因遺伝子 *GNE* がコードする蛋白質は，シアル酸生合成経路律速段階の UDP-*N*-アセチルグルコサミン（UDP-GlcNAc）→ *N*-アセチルマンノサミン（ManNAc）の反応を触媒する UDP-*N*-アセチルグルコサミン 2-エピメラーゼ（UDP-GlcNAc 2-epimerase：GNE）と，その次の反応（ManNAc → ManNAc-6-P）を触媒する酵素 *N*-アセチルマンノサミン・キナーゼ（ManNAc kinase）の 2 つの酵素活性を有している。このうち，後者の反応については，細胞質内に豊富に存在する *N*-アセチルグルコサミン・キナーゼ（GlcNAc kinase）も同じ反応を触媒できることから，恐らく DMRV においては，*N*-アセチルマンノサミン・キナーゼ活性の低下ではなく，UDP-*N*-アセチルグルコサミン 2-エピメラーゼ活性の低下が本質的な原因と考えられる。後述するように，*GNE* 遺伝子に変異があっても ManNAc を投与することでシアル酸合成およびその後のシアリル化を回復できることは，GlcNAc kinase が ManNAc kinase の反応を代償できる事実と合致する。

を触媒する酵素 UDP-*N*-アセチルグルコサミン 2-エピメラーゼ（UDP-GlcNAc 2-epimerase：GNE）と，その次の反応を触媒する酵素 *N*-アセチルマンノサミン・キナーゼ（ManNAc kinase）の 2 つの酵素活性を持つ蛋白質をコードしている（**Fig. 2**）。患者で見出されるほぼすべての変異がミスセンス変異であり，両アレルに null 変異を有する例は 1 例も報告されていない。

日本人では，p.V572L 変異が 1 番多く，日本人患者アレルの半数以上を占めている。次に多いのが，p.D176V 変異で，20%程度のアレル頻度である[7]。韓国でも p.V572L 変異が最も多いが[8]，北京では p.D176V 変異が最も高頻度という（Dr. Yun Yuan, Personal communication）。ユダヤ人患者では，大半の患者が p.M712T を有している[9,10]。これ以外にも世界各地から，多くの共通変異が見出されてきている。

遺伝子型・表現型相関（genotype-phenotype correlation）についてははっきりとその存在を示した論文はないが，p.M712T を有するユダヤ人患者よりも主に p.V572L を有する日本人患者のほうが重症傾向にあるようである（Dr. Zohar Argov, Personal communication）。ただし，同じ変異を有する患者であっても重症度にはかなりばらつきがあることがわかっており，話は単

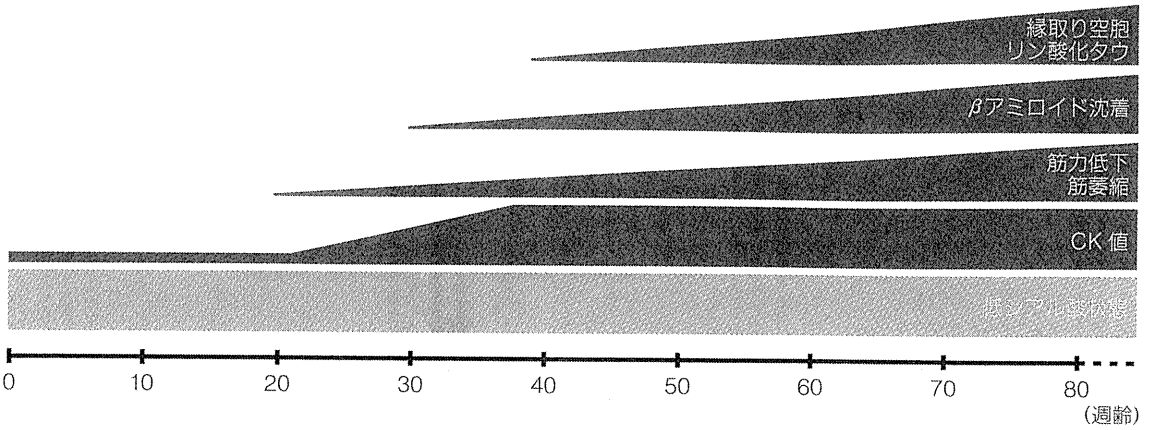

Fig. 3　DMRV モデルマウスの疾患再現性

国立精神・神経医療研究センターで作製したモデルマウスは，20 週齢過ぎから筋力低下と筋萎縮を，30 週齢過ぎからβアミロイド沈着，40 週齢過ぎから縁取り空胞やリン酸化タウを認める。さらに，血中 CK 値が 20 週齢過ぎから軽度上昇する。一方，各種臓器での低シアル酸状態（シアル酸欠乏）は，生下時より持続的に認められる。これらの変化は，臨床的・病理学的・生化学的に DMRV を良好に再現している。

純ではない[7,11]。さらに興味深いことに，*GNE* 遺伝子にホモ接合型の変異を有していながら，60 歳を過ぎても無症状の健常者も存在する[7,10]。このことは，*GNE* 変異の存在のみが DMRV/hIBM 発症の決定的な因子ではないことを意味している。言い換えるならば，もしこのような因子を見出すことができれば，治療法開発の糸口となる可能性がある。

5．疫学

正確な患者数を知ることは困難であるが，国立精神・神経医療研究センターで行っている筋病理診断サービスでの検体数からは，国内で 150〜400 名程度と推計される。これまでに国立精神・神経医療研究センターで遺伝学的診断を確定した例は，既に 150 名を超えている。他施設での診断例を併せると，本邦には少なくとも 200 名を超える患者が存在するものと考えられる。遺伝学的診断が確立している患者に関していえば，本邦の患者数は世界最多である。

Ⅱ．分子病態から治療法開発へ

1．生化学的異常

DMRV における *GNE* 変異は，機能喪失型変異であり，酵素活性が低下している[7]。そのためシアル酸量が減少し，患者細胞ではシアリル化が低下している[12]。シアル酸は細胞表面の糖脂質および糖蛋白質上のオリゴ糖の末端に広範に存在しており，細胞表面の保護や細胞の相互認識など多彩かつ重要な役割を担っていると考えられているが，依然としてどのようにしてシアル酸の低下がミオパチーをきたすかは不明である。

興味深いことに，GNE 代謝産物である *N*-アセチルマンノサミン（ManNAc）や最終産物（シアル酸）である *N*-アセチルノイラミン酸（NeuAc）を患者培養細胞に投与すると，線維芽細胞，骨格筋細胞のいずれにおいても，正常レベルまで細胞内シアル酸量が回復する[13]。このことは，もし，低シアリル化がミオパチーの原因であるならば，既に *in vitro* で治療できていることを意味している。

2．モデルマウスの作製

GNE 遺伝子をノックアウトさせたマウス $Gne^{-/-}$ は胎生致死である[14]。このことは，シアル酸生合成が哺乳類の発生にとって必須であることを意味している。事実，DMRV 患者で見出される変異はほぼすべてミスセンス変異であり，null 変異のホモ接合体は見出されていない。

米国 National Institute of Health のグループは，ユダヤ人患者に認められる p.M712T 変異を有するノックインマウスの作製を行った。しかし，ホモ接合型マウスは，重篤な腎障害を呈し，ほぼすべてが生後 72 時間以内に死亡した[15]。妊娠母胎に ManNAc を投与したところ，12 匹が誕生したが，9 匹が 12 日までに死亡した。いずれのマウスにおいても筋障害を認めなかった。

これに対し，国立精神・神経医療研究センターでは，運よくヒト DMRV を良好に再現するマウスの作製に成功した[16-18](Fig. 3)。われわれは，まず，*GNE* トランスジェニックマウス（h*GNE*D176V-Tg）を作製した。この

マウスは，日本人患者で2番目に多いp.D176V変異を有するヒト*GNE*を高発現するものである[14]。このh*GNE*D176V-Tgマウスと*Gne*$^{-/-}$のヘテロ接合体マウス（*Gne*$^{-/+}$）を掛け合わせて，内在性のマウス*Gne*を欠きp.D176V変異を有するヒト*GNE*のみを発現するDMRVモデルマウス（*Gne*$^{-/-}$h*GNE*D176V-Tg）を作製した[14]。

このDMRVモデルマウスは，生下時には特に異常を認めないものの，20週齢以降に筋力低下と筋萎縮，運動能力低下を示した[17]。また，血中CK値も軽度に上昇していた。30週齢からは，骨格筋内βアミロイド沈着，40週齢以降には縁取り空胞，リン酸化タウを認めるとともに，筋萎縮と筋力低下はさらに進行した（Fig. 3）。シアル酸は血中および脳を除くすべての組織で著しく減少していた。すなわち，臨床的・病理学的・生化学的に，ヒト患者における表現型を良好に再現していたのである。

3．治療法開発研究

まず，DMRVモデルマウスに対して，離乳時からManNAcを3種類の投与量（低用量20 mg/kg/day，中用量200 mg/kg/day，高用量2,000 mg/kg/day）で飲水に混ぜ，連続投与した。その結果，いずれの用量においても，50週齢を越えても，運動能力低下，筋萎縮，筋力低下，縁取り空胞形成，βアミロイド沈着，リン酸化タウ，高CK血症などの所見をいずれも認めず，ほぼ完全にDMRV発症を抑制できた[17]。

そこで，次に，NeuAcならびにシアリル乳糖を低用量20 mg/kg/dayで飲水投与した。その結果，NeuAc，シアリル乳糖においても同様に，ほぼ完全にDMRVを抑制することに成功した[19]。ManNAc，NeuAc，シアリル乳糖の3種類の化合物間で有効性の違いはみられなかった。骨格筋内のシアル酸は正常の70%程度にまで回復していた。また，これら3種類の化合物の長期投与において，肝機能と腎機能への毒性は認めなかった。これらの結果は，低シアル酸状態が確かにミオパチーの原因となっていること，外部からGNE代謝産物を投与することでミオパチーを予防できることを示している[17]。

この結果を受け，後に述べるように，NeuAcを用いた第Ⅰ相試験が日本および米国で実施されている。まだ，NeuAcのヒトへの効果は不明であるが，少なくともモデルマウスにおいては，単純なNeuAcやManNAcの投与では，骨格筋内のシアル酸量を完全に正常化することはできていない。さらに治療効果を高めることを考えると，骨格筋内のシアル酸量をさらに増加させる必要性がある。われわれのグループは，ManNAcを人工的にアセチル化したテトラ-O-アセチルManNAcがより強力なシアル酸増加効果を有し，より高い治療効果を有することを見出している[20]。

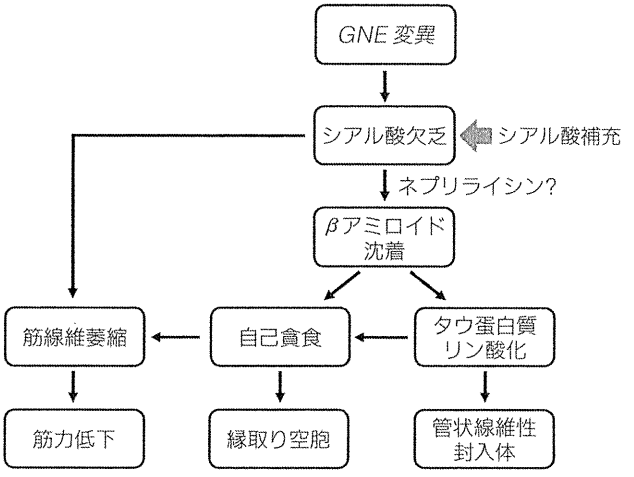

Fig. 4　DMRVの分子病態
これまでに明らかになった事実から，シアル酸欠乏こそが，ミオパチーの原因となっており，筋萎縮，βアミロイド沈着，縁取り空胞形成などの変化は，その下流現象として生じていると考えられる。シアル酸補充によりこのシアル酸欠乏を回復させることができれば，一連の下流現象を止めることができるはずである。シアル酸補充療法はこのような考え方に基づいている。

4．依然として不明な分子病態

われわれは，モデルマウスでの実験において，シアル酸代謝物投与によって骨格筋のシアル酸レベルを上昇させることで，ミオパチー症状を抑制できることを示した[17]。このことは，ミオパチーが低シアル酸状態を原因としていること，すなわち，DMRVはいわば先天的なシアル酸欠乏症であることを意味している（Fig. 4）。

それでは，なぜシアル酸欠乏によってミオパチーをきたすのであろうか。βアミロイド蛋白質の分解に関わる膜金属ペプチダーゼのネプリライシンにおいては，そのシアリル化がペプチダーゼ活性に必要なことから，脱シアリル化によってβアミロイド沈着をきたすと考える研究者もいる[21]。DMRV発症へのネプリライシンの関与の程度についてはさらなる検討を待つ必要があるが，筋変性を引き起こす分子病態の詳細を明らかにすることができれば，新たな治療法開発への道が開けるだけでなく，同様にβアミロイド沈着や縁取り空胞の出現を認める封入体筋炎などの根本的治療法がない疾患に対しても治療法開発への道が開ける可能性がある。

III. 臨床試験

2010年11月から東北大学神経内科の青木正志教授をリーダーとするチームによって，シアル酸製剤の薬物動態を探る第I相試験が6名の患者を対象として，医師主導型治験の形で行われた（ClinicalTrials.gov Identifier：NCT01236898）。途中，2011年3月11日に東北地方太平洋沖地震に見舞われたものの，2011年6月末までに，予定された試験は無事終了した。最大で，1日2,400 mgを5日間投与したが，有害事象はまったく認められなかった。さらに，米国では2011年9月から，シアル酸徐放剤を用いた第I相試験が企業主導で実施されている（ClinicalTrials.gov Identifier：NCT01359319）。本試験においては，最大，1日4,875 mgが7日間投与される。早ければ，2011年12月末には終了し，2012年中にも，第II相試験を開始したいとしている。

IV. これから進むべき道

基礎研究レベルでは，既にシアル酸の有効性を示すことができた。シアル酸欠乏症であるDMRVにシアル酸を補充することは，極めて理にかなった治療法である。加えて，シアル酸は日常的に食事から摂取しているものであり，本質的に毒性が高いとは考えられない。このように比較的安全と予想され，その理論的根拠が明確である治療法であっても，DMRVのような“超”希少疾病で第I相試験にたどり着くのは容易ではなかった。これは，ひとえに希少疾病薬開発が，仮に治験に成功したとしてもその費用を回収するためのマーケットが小さく，製薬会社にとっては極めてリスクの高いプロジェクトであるからである。そのため，希少疾病の治療薬開発に乗り出そうとする製薬企業は，特に日本では，皆無に近いのが現状である。

このような現状を克服しようと，遠位型ミオパチー患者会（http://enigata.com）が立ち上がり，署名集めや政府・関係省庁・国会への要望書提出など精力的な活動を繰り広げてきた。これまでに180万筆を超す署名を集めたが，このことは，日本の一般人口のおよそ70人に1人がDMRVという疾患名を耳にしたことがあることを意味している。DMRVのような“超”希少疾病がこれほど認知されている国は，世界中どこを探しても日本以外にはない。曲がりなりにも第I相試験を行うことができたのは，このような患者会の活動があってこそである。実際，シアル酸製剤開発を引き受けるべく企業を説得したのも患者会であった。今後ともDMRVのような“超”希少疾病の治療薬開発が問題になると予想されるが，遠位型ミオパチー患者会の活動は素晴らしいモデルケースとなるであろう。

今後，実際に「くすり」として世の中に出るまでには，まだまだ多くの困難が待ち受けており，患者・医師・研究者・製薬会社・関係省庁などの関係者が協力していくことが必須である。DMRVのような“超”希少疾病の治療薬開発，すなわち，ウルトラオーファン・ドラッグの開発においては，教科書的な対応をしていてはまったく埒が明かない。関係者は，不可能を可能にするという意識で，現在の各種規制に立ち向かっていくことが必要であろう。

基礎研究レベルでは，単なるシアル酸補充から一歩進んで，シアル酸欠乏により生じている筋変性現象のより深い病態理解と，その病態理解に基づく治療法開発が求められる。このような治療法が開発されれば，シアル酸補充にアドオンの形で投与が行われ，より高い治療効果を期待できると考えられる。また，われわれは骨髄移植や遺伝子治療の可能性も探っている。しかしこのような理論的に根本的な治療が成功しても，進行期の患者において，失われた骨格筋を元に戻すことはそう簡単ではないだろう。DMRVに限らず，筋疾患の根本的治療法開発では，このような失われた筋肉の再生を視野に入れていく必要がある。

文　献

1）Nonaka I, Sunohara N, Ishiura S, Satoyoshi E: Familad distal myopathy with rimmed vacuole and lamellar (myeloid) body formation. J Neurol Sci **51**: 141-155, 1981
2）Nonaka I, Murakami N, Suzuki Y, Kawai M: Distal myopathy with rimmed vacuoles. Neuromuscul Disord **8**: 333-337, 1998
3）Nonaka I: Distal myopathies. Curr Opin Neurol **12**: 493-499, 1999
4）Argov Z, Yarom R: 'Rimmed vacuole myopathy' sparing the quadriceps. A unique disorder in Iranian Jews. J Neurol Sci **64**: 33-43, 1984
5）Askanas V, Engel WK: Sportadic inclusion-body myositis and hereditary inclusion-body myopathies: current concepts of diagnosis and pathogenesis. Curr Opin Rheumatol **10**: 530-542, 1998
6）Eisenberg I, Avidan N, Potikha T, Hochner H, Chen M, et al: The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. Nat Genet

29: 83-87, 2001
7) Nishino I, Noguchi S, Murayama K, Driss A, Sugie K, et al: Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. Neurology **59**: 1689-1693, 2002
8) Kim BJ, Ki CS, Kim JW, Sung DH, Choi YC, et al: Mutation analysis of the GNE gene in Korean patients with distal myopathy with rimmed vacuoles. J Hum Genet **51**: 137-140, 2006
9) Eisenberg I, Grabov-Nardini G, Hochner H, Korner M, Sadeh M, et al: Mutations spectrum of GNE in hereditary inclusion body myopathy sparing the quadriceps. Hum Mutat 21: 99, 2003
10) Argov Z, Eisenberg I, Grav-Nardini G, Sadeh M, Wirguin I, et al: Hereditary inclusion body myopathy: The middle Eastern genetic cluster. Neurology **60**: 1519-1523, 2003
11) Arai A, Tanaka K, Ikeuchi T, Igarashi S, Kobayashi H, et al: A novel mutation in the GNE gene and a linkage disequilibrium in Japanese pedigrees. Ann Neurol **52**: 516-519, 2002
12) Noguchi S, Keira Y, Murayama K, Ogawa M, Fujita M, et al: Reduction of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase activity and sialylation in distal myopathy with rimmed vacuoles. J Biol Chem **279**: 11402-11407, 2004
13) Bardor M, Nguyen DH, Diaz S, Varki A: Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J Biol Chem **280**: 4228-4237, 2005
14) Schwarzkopf M, Knobeloch KP, Rohde E, Hinderlich S, Wiechens N, et al: Sialylation is essential for early development in mice. Porc Natl Acd Sci U S A **99**: 5267-5270, 2002
15) Galeano B, Klootwijk R, Manoli I, Sun M, Ciccone C, et al: Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. J Clin Invest **117**: 1585-1594, 2007
16) Malicdan MC, Noguchi S, Nonaka I, Hayashi YK, Nishino I: A Gne knockout mouse expressing human GNE D176V mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. Hum Mol Genet **16**: 2669-2682, 2007
17) Malicdan MC, Noguchi S, Hayashi YK, Nishino I: Muscle weakness correlates with muscle atrophy and precedes the development of inclusion body or rimmed vacuoles in the mouse model of DMRV/hIBM. Physiol Genomics **35**: 106-115, 2008
18) Malicdan MC, Noguchi S, Nishino I: Recent advances in distal myopathy with rimmed vacuoles (DMRV) or hIBM: treatment perspectives. Curr Opin Neurol **21**: 596-600, 2008
19) Malicdan MC, Noguchi S, Hayashi YK, Nonaka I, Nishino I: Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the DMRV-hIBM mouse model. Nat Med **15**: 690-695, 2009
20) Malicdan MC, Noguchi S, Tokutomi T, Goto YI, Nonaka I, et al: Peracetylated N-acetylmannosamine, a synthetic sugar molecule, effectively rescues muscle phenotype and biochemical defects in a mouse model of sialic acid deficient myopathy. J Biol Chem **287**: 2689-2705, 2012
21) Broccolini A, Gidaro T, De Cristofaro R, Morosetti R, Gliubizzi C, et al: Hyposialylation of neprilysin possibly affects its expression and enzymatic activity in hereditary inclusion-body myopathy muscle. J Neurochem **105**: 971-981, 2008

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2010) **52**, e150–e153 doi: 10.1111/j.1442-200X.2010.03073.x

Patient Report

Case of glycogen storage disease type VI (phosphorylase deficiency) complicated by focal nodular hyperplasia

Atsushi Ogawa,[1] Emi Ogawa,[1] Shigenori Yamamoto,[1] Tokiko Fukuda,[2] Hideo Sugie[2] and Yoichi Kohno[1]

[1]*Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba-shi, Chiba and* [2]*Department of Pediatrics, Jichi Children's Medical Center Tochigi, Shimotsuke-shi, Tochigi, Japan*

Key words focal nodular hyperplasia, glycogen phosphorylase, glycogen storage disease.

Although it is well known that hepatic tumors often develop in patients with glycogen storage disease (GSD) types Ia and III, the formation of these tumors has not been reported in other forms of hepatic GSD. In this report, a patient with GSD type VI (phosphorylase deficiency; OMIM 232700) complicated with a hepatic benign tumor, focal nodular hyperplasia (FNH), is presented. This case indicates that regular check-ups for hepatic tumors are necessary, not only in patients with GSD types Ia or III, but also in patients with other forms of hepatic GSD.

Case Report

A female patient was referred to our hospital when she was 5 years of age for further investigation of hepatomegaly, which had been detected when she had visited a clinic when she was 5 years old. She was born to healthy non-consanguineous parents and had no history of hypoglycemia or nasal bleeding. On physical examination, her height was 101 cm (−1.5 SD) and her bodyweight was 16 kg (−1.0 SD). The liver was firm and palpable 7 cm below the right costal margin, whereas the spleen was not palpable. The results of a fasting blood test collected at that time were as follows: aspartate aminotransferase 37U/L, alanine aminotransferase 24U/L, blood glucose 85 mg/dL, lactate 6.2 mg/dL, uric acid 5.9 mg/dL, total cholesterol 229 mg/dL and triglyceride 88 mg/dL. A plain abdominal computed tomography (CT) scan showed an enlarged liver with a density considerably higher than that of the spleen (CT values: liver, 80; spleen, 42) (Fig. 1). Glucose and galactose loading tests were performed. The serum lactate level was not elevated when glucose was loaded, although it increased to a maximum of 56 mg/dL one hour after loading (normal <35 mg/dL). A glucagon loading test was performed after a 15-h fast, with the serum glucose level increasing from 71 to 128 mg/dL one hour after loading. On the basis of these data, GSD was suspected and accordingly the enzyme activities of hepatic GSD, that is, debranching enzyme, phosphorylase and phosphorylase b kinase, were measured in peripheral blood. The results of all these tests were normal (Table 1). Informed consent for a liver needle biopsy for measurement of enzyme activity was not obtained. Although the enzyme activity of phosphorylase b kinase measured in peripheral blood was normal, a tentative diagnosis of GSD type IX (phosphorylase b kinase deficiency) was made based on the physical, laboratory and radiological findings and the results of the loading tests. Regular check-ups including abdominal CT scans for potential formation of hepatic tumor were performed every year. The patient's growth curve showed that she attained mean values around the time of puberty. The results of blood tests obtained between 5 and 14 years of age were as follows (mean ± SD): uric acid 5.9 ± 0.6 mg/dL, total cholesterol 208 ± 21.0 mg/dL and triglyceride 198 ± 111 mg/dL.

When the patient was 15 years of age, the early phase of a contrast-enhanced abdominal CT scan revealed an enhanced lesion in the liver (Fig. 1). After obtaining informed consent, specimens were obtained by needle biopsy from the tumor and non-tumor part of the liver. Histological findings of the non-tumor specimen showed strong periodic acid-Schiff (PAS) staining in hepatocytes that disappeared following diastase treatment, findings compatible with GSD. Histology of the tumor specimen demonstrated pericellular fibrosis, compatible with the diagnosis of FNH (Fig. 2). Fibrous bands containing bile ductules were not observed in the specimens. Enzyme activities of hepatic GSD were measured using liver tissue from the non-tumor section, which revealed that phosphorylase enzyme activity was 2.3 nmol/min/mg protein, a value corresponding to 24% of normal. The enzyme activity of both debranching enzyme and phosphorylase b kinase was normal (Table 1). Informed consent for gene analysis of phosphorylase (*PGYL*) could not be obtained. We concluded that the patient's diagnosis was GSD VI (phosphorylase deficiency) complicated by FNH. We elected to forego surgical treatment in favor of long-term observation. The size of the tumor has been monitored regularly with ultrasonography. As of now, the tumor does not appear to be enlarging.

Discussion

In this report we present a patient with GSD type VI complicated by FNH. This is the first report of a hepatic tumor complication

Correspondence: Atsushi Ogawa, MD PhD, Department of Pediatrics, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Email: aogawa@faculty.chiba-u.jp

Received 28 February 2009; revised 7 June 2009; accepted 9 July 2009.

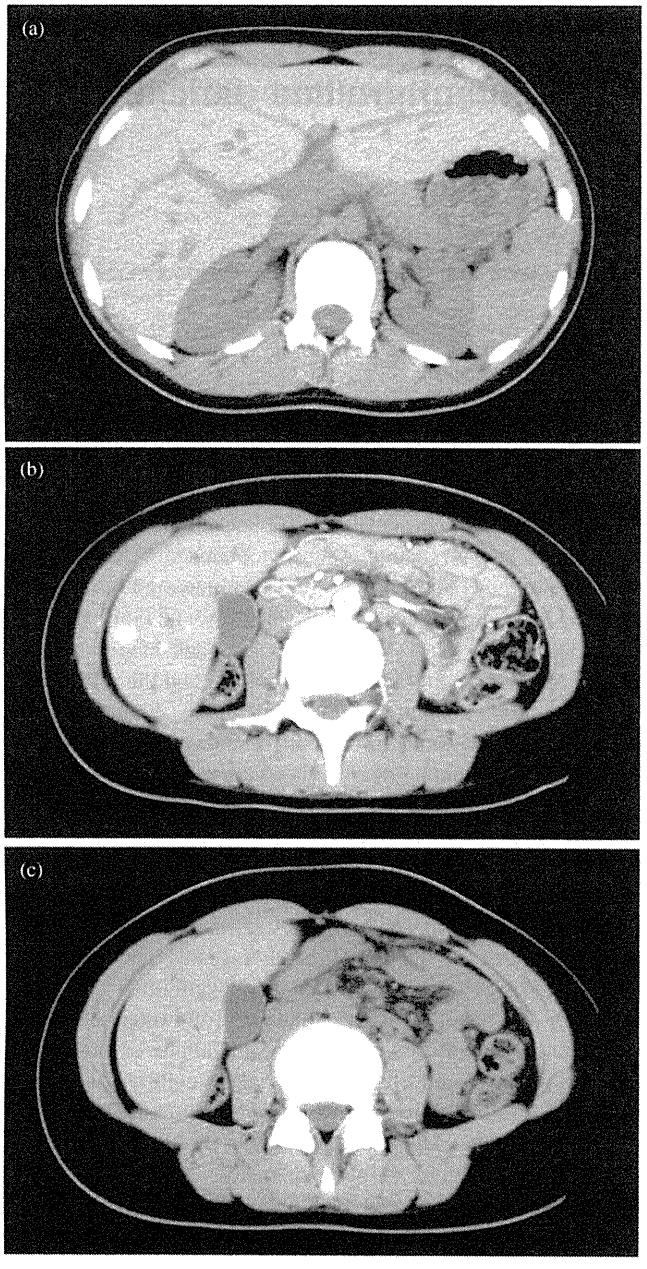

Fig. 1 (a) The findings of a plain abdominal computed tomography (CT) scan at 5 years of age. The CT value of the liver was markedly elevated compared with that of the spleen and kidneys. (b) The findings of the early phase of a contrast-enhanced abdominal CT scan at 15 years of age showing an enhanced lesion. (c) The findings of the same section as (b), without contrast enhancement.

in a patient with GSD type VI, a different hepatic form of GSD than types Ia or III. As hepatic tumors are often found in patients with GSD types Ia and III, regular check-ups for these tumors are performed routinely in these patients. However, this report indicates that regular check-ups for hepatic tumor are also necessary in patients with hepatic forms of GSD other than types Ia or III.

In patients with GSD type Ia, hepatic adenoma is the most common tumor described; however other tumors, including hepatocellular carcinoma (HCC),[1] described in patients with GSD III,[2] hepatoblastomas,[3] and FNH[4] have also been reported.

Hepatic adenomas are a benign tumor, consisting of a nodular proliferation of hepatocytes arranged in cords having no relationship to portal tracts. They often have a pushing border abutting against the surrounding liver. The hepatic adenoma has, on rare occasions, been known to progress to HCC,[1] and this is one of the most important reasons why regular check-ups and follow up after the discovery of an adenoma are necessary in a patient with GSD Ia. FNH is typically a single mass in an otherwise healthy liver characterized by central scarring that radiates between multiple nodules of regenerating parenchyma. Like the hepatic adenoma, it is also a benign tumor parenchyma but the potential for malignant transformation of FNH into HCC has not been demonstrated. However, a case of HCC arising within FNH has been reported recently[5] and this report emphasizes the importance of detecting FNH, even though the FNH itself is benign.

The mechanism of tumor formation in GSD type Ia is considered to occur by the following sequence.[6] Increased amounts of free fatty acids are released from adipose tissue, taken up by the liver and channeled into triglyceride formation. Malonyl-CoA is a key lipogenic intermediate in this process, which, in turn, causes inhibition of carnitine palmitoyltransferase I and limitation of mitochondrial beta-oxidation. This results in fatty acids being more likely to be channeled into extramitochondrial pathways, such as within peroxisomes, leading to an increase in hydrogen peroxide generation. This results in increased generation of free radicals that are capable of inflicting direct DNA damage, which may initiate the development of hepatic tumors. Although the patient reported here was diagnosed with GSD type VI, hypertriglyceridemia was almost always observed during the clinical course of the disease, similar to that seen in cases with type I GSD. We anticipate this would have resulted in increased generation of free radicals by the mechanism described above and could possibly have caused the formation of FNH we observed in the patient.

In our patient we observed a difference in phosphorylase activity between peripheral blood and liver tissue. Three isoforms of phosphorylase exist, that is, liver, brain and muscle. As the liver isoform is expressed in peripheral blood,[7,8] phosphorylase activity in peripheral blood and the liver should be the same. The reason why phosphorylase activity in peripheral blood and liver was different in our patient is not clear, although similar findings have been reported elsewhere.[9] Mutation analysis of the liver glycogen phosphorylase gene (*PYGL*) is necessary for further confirmation of this diagnosis.

In summary, we report a patient with GSD VI complicated with FNH. This case indicates that regular check-ups for hepatic tumors are necessary, not only in patients with GSD types Ia or III, but also in patients with other forms of hepatic GSD.

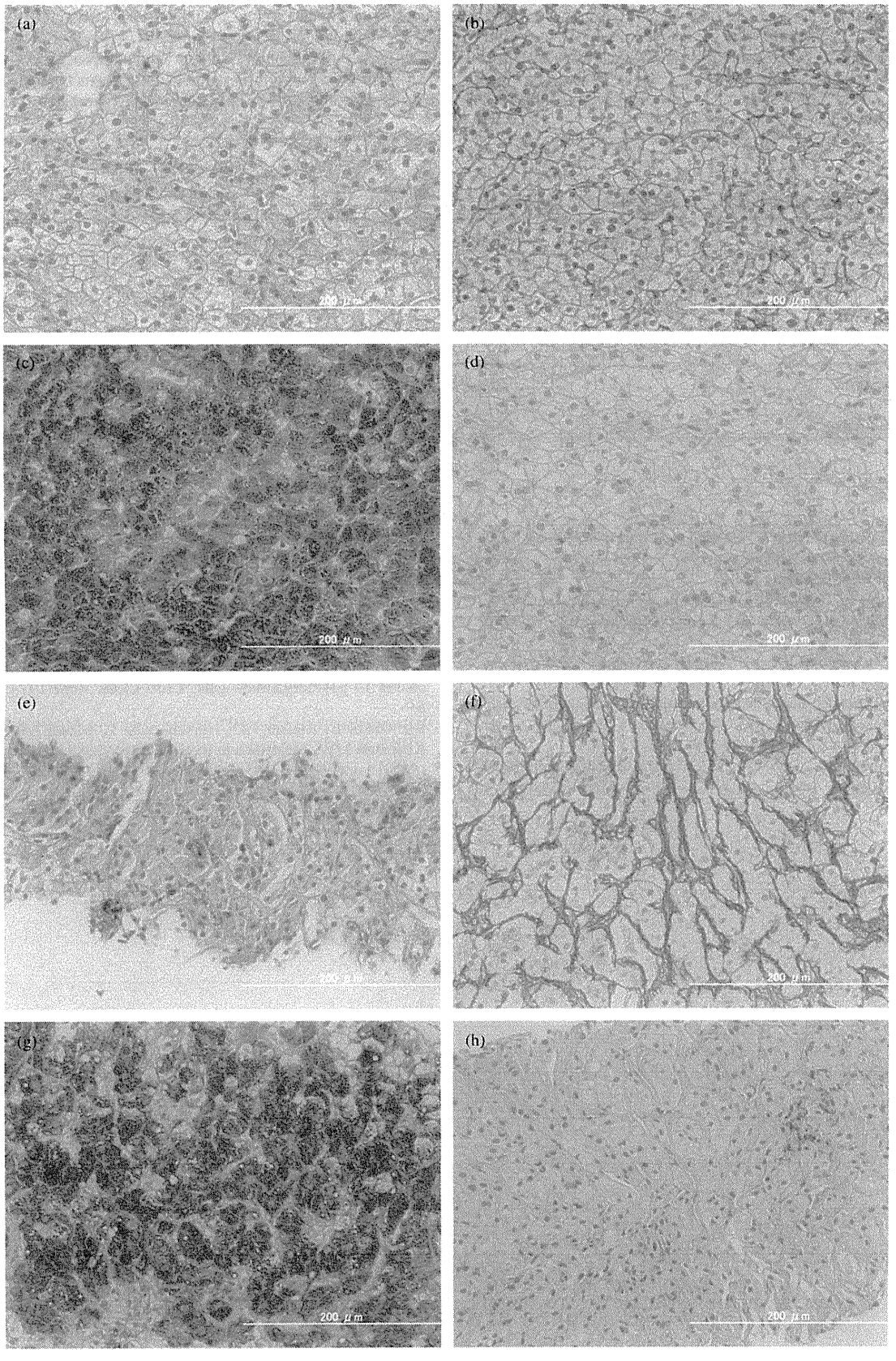

Fig. 2 Histological findings of the liver from (a–d) non-tumor and (e–h) tumor specimens. (a and e) Hematoxylin–eosin (HE) stain, (b and f) silver staining, (c and g) periodic acid-Schiff (PAS) staining and (d and h) PAS staining after diastase treatment. In the non-tumor specimen, the hepatocytes had (a) clear cytoplasm with (b) no fibrosis observed. (c and d) All the hepatocytes were stained strongly by PAS, which disappeared following diastase treatment. (e and f) In tumor specimens, pericellular fibrosis was observed, whereas fibrous bands in which bile ductules were proliferating were not. On the basis of the finding of pericellular fibrosis, a diagnosis of focal nodular hyperplasia was made. The original magnification was ×20.

Table 1 Results of enzyme activity measurements in the patient and controls

Peripheral blood	Patient	Control 1	Control 2	
Debranching enzyme	14.8	24.9	19.1	Nmole glucose/hour/mg
Phosphorylase	6.3	6.1	7.2	Nmole/min/mg
Phosphorylase b kinase	45.8	44.5	42.0	Nmole/min/g Hb
Liver	**Patient**	**Controls**		
Debranching enzyme	243.4	197.4 ± 32.8 (n = 10)		Nmole glucose/hour/mg
Phosphorylase	2.3	9.6 ± 1.7 (n = 10)		Nmole/min/mg
Phosphorylase b kinase	49.6	62.7 ± 11.8 (n = 9)		Nmole/min/mg

References

1 Franco LM, Krishnamurthy V, Bali D *et al.* Hepatocellular carcinoma in glycogen storage disease type Ia: A case series. *J. Inherit. Metab. Dis.* 2005; **28**: 153–62.

2 Demo E, Frush D, Gottfried M *et al.* Glycogen storage disease type III-hepatocellular carcinoma a long-term complication? *J. Hepatol.* 2007; **46**: 492–8.

3 Ito E, Sato Y, Kawauchi K *et al.* Type 1a glycogen storage disease with hepatoblastoma in siblings. *Cancer* 1987; **59**: 1776–80.

4 Takamura M, Mugishima H, Oowada M, Harada K, Uchida T. Type La glycogen storage disease with focal nodular hyperplasia in siblings. *Acta Paediatr. Jpn.* 1995; **37**: 510–3.

5 Petsas T, Tsamandas A, Tsota I *et al.* A case of hepatocellular carcinoma arising within large focal nodular hyperplasia with review of the literature. *World J. Gastroenterol.* 2006; **12**: 6567–71.

6 Lee PJ. Glycogen storage disease type I: Pathophysiology of liver adenomas. *Eur. J. Pediatr.* 2002; **161**(Suppl 1): S46–9.

7 Proux D, Dreyfus JC. Phosphorylase isoenzymes in tissues: Prevalence of the liver type in man. *Clin. Chim. Acta* 1973; **48**: 167–72.

8 Proux D, Vibert M, Meienhofer MC, Dreyfus JC. The isozymes of glycogen phosphorylase in human and rabbit tissues. II. Electrofocusing in polyacrylamide gels. *Clin. Chim. Acta* 1974; **57**: 211–6.

9 Burwinkel B, Bakker HD, Herschkovitz E, Moses SW, Shin YS, Kilimann MW. Mutations in the liver glycogen phosphorylase gene (PYGL) underlying glycogenosis type VI. *Am. J. Hum. Genet.* 1998; **62**: 785–91.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society
Pediatrics International (2011) **53**, 129–130 doi: 10.1111/j.1442-200X.2010.03282.x

Letter to the Editor

Liver biopsy is an important procedure in the diagnosis of glycogen storage disease type IV

Tatsuki Mizuochi,[1] Akihiko Kimura,[1] Hiroshi Nishiura,[1] Yukihiro Inomata,[4] Hideaki Okajima,[4] Hideo Sugie,[6] Hiroshi Mitsubuchi,[5] Minoru Yagi[2] and Masayoshi Kage[3]

Departments of [1]Pediatrics and Child Health, and [2]Pediatric Surgery, and [3]Pathology, Kurume University School of Medicine, Kurume, and Departments of [4]Pediatric Surgery and Transplantation and [5]Pediatrics, Kumamoto University Graduate School of Medical Science, Kumamoto University, Kumamoto, and [6]Department of Pediatrics, Jichi Medical University and Jichi Children's Medical Center, Tochigi, Japan

Glycogen storage disease type IV (GSD IV) is a rare autosomal recessive metabolic disorder characterized by deficient glycogen branching enzyme (GBE) activity. This severe metabolic disease results in abnormal deposition of amylopectin-like glycogen in multiple organs, such as the liver, muscle, heart, and the nervous system.[1,2] This disease most frequently presents in the first few months of life, with hepatosplenomegaly and failure to thrive. This is followed by progressive liver cirrhosis with portal hypertension, ascites, esophageal varices, and death by 5 years of age.[3] Generally, diagnosis of GSD does not require liver biopsy. However it is difficult to diagnose GSD IV when symptoms extend to multiple organs. No specific treatment for this disease exists. Liver transplantation has been proposed as a treatment;[2,4,5] however, this may not improve extrahepatic manifestations in the same patients.[4]

We experienced a case of GSD IV in a 5-month-old boy who was born without complications after 38 weeks of gestation. He had no significant family history and developed normally until the age of 4 months, at which time he experienced high fever, tachypnea, and poor feeding. On admission to our hospital, he had hepatosplenomegaly with elevation of serum transaminases (aspartate aminotransferase [AST], 312 IU/l and alanine aminotransferase [ALT], 108 IU/l); hypotonia; cardiomegaly (cardiothoracic ratio, 67%); elevated white blood count (27 570/μl) and C-reactive protein (9.1 mg/dl). Ultrasonography revealed pericardial effusion and increased myocardial thickness. After admission, the patient rapidly developed signs of cardiomyopathy and respiratory distress accompanied by high fever and petechiae. However, serum creatine phosphokinase concentration was normal. Therefore, his cardiac findings may have been due to a respiratory problem, such as infection, or to a combination of abnormal deposition of amylopectin-like glycogen and infection. He was treated with respiratory therapy, antibiotics, γ-globulin, and a diuretic. However, his condition did not improve, and his liver function sharply deteriorated: AST, 729 IU/l; ALT, 146 IU/l; total bilirubin, 2.6 mg/dl; and prothrombin, 54% (normal range: >60%). At that time we were still unable to make a diagnosis so we carried out an open liver biopsy. We did not evaluate leukocytes.[5] The biopsy specimen showed periodic acid-Schiff-positive cytoplasmic inclusions, largely resistant to diastase digestion (Fig. 1). GBE activity in a sample from the specimen was very low (0.09 μmol Pi/min/mg protein; control, 1.2 ± 0.3), as measured in the laboratory of Dr H. Sugie.

From the above results, especially the histological findings from the biopsy, the patient was diagnosed as having GSD IV. He received a living-donor liver transplant from his mother at Kumamoto University Hospital. After transplantation, his symptoms, including abnormal liver function, cardiomyopathy, dyspnea, hypotonia, and petechiae, rapidly improved except for fever. Histological findings from the liver biopsy specimen, particularly the faintly stained basophilic inclusions in hepatocytes, were very useful and ultimately led to the diagnosis of GSD IV. We therefore consider liver biopsy very important for the diagnosis of this disease. However, it should be kept in mind that enzyme assay in the liver can be very tricky when the liver is cirrhotic.

We carried out living-donor liver transplant with the patient's mother as the donor. After liver transplantation, all disease manifestations except for fever abated. Resorption of extrahepatic deposits of abnormal glycogen has been demonstrated after liver transplantation;[6,7] the mechanism for resorption of deposits in organs apart from the liver remains unknown.

In conclusion, we report a 5-month-old boy with GSD IV, including fever of unknown origin both before and after liver transplantation, and emphasize the importance of liver biopsy in the diagnosis of GSD IV.

Correspondence: Akihiko Kimura, MD PhD, Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Email: hirof@med.kurume-u.ac.jp

Received 21 January 2010; revised 12 April 2010; accepted 8 September 2010.

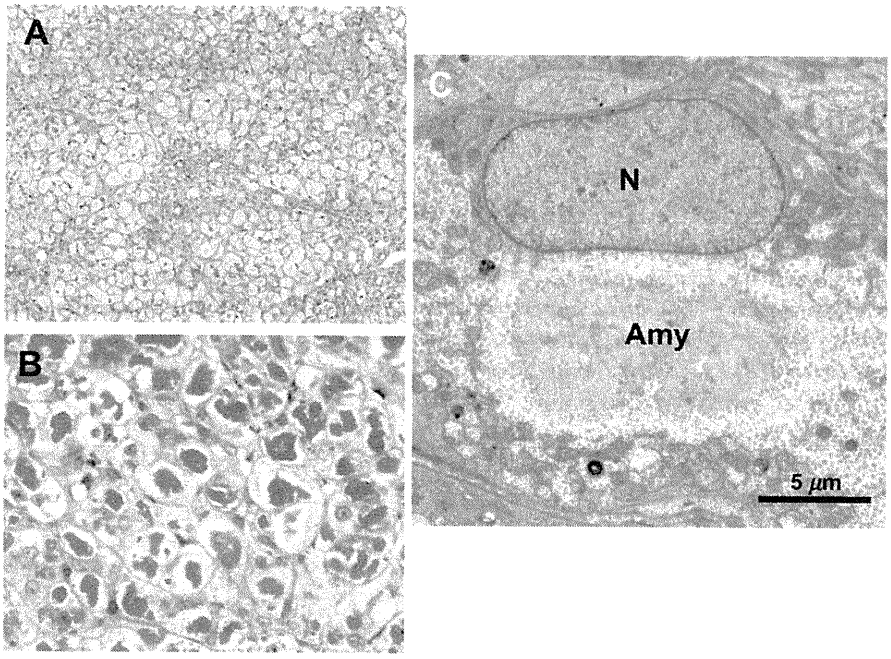

Fig. 1 Liver biopsy specimen from our patient with GSD IV. (a) Hepatocytes are enlarged and contain faintly stained basophilic cytoplasmic inclusions (hematoxylin–eosin stain, ×100). (b) The inclusions are periodic acid-Schiff-positive and diastase-resistant (×400). (c) Ultrastructurally, hepatocytes are occupied by large aggregates consistent with amylopectin (×10 000). N, nucleus; Amy, amylopectin.

References

1 Greene HL, Brown BI, McClenathan DT, Agostini RM Jr, Taylor SR. A new variant of type IV glycogenosis: deficiency of branching enzyme activity without apparent progressive liver disease. *Hepatology* 1988; **8**: 302–6.
2 Sokal EM, Von Hoof F, Alberti D *et al.* Progressive cardiac failure following orthotopic transplantation for type IV glycogenosis. *Eur. J. Pediatr.* 1992; **151**: 200–3.
3 Chen Y-T. Glycogen storage disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic & Molecular Bases of Inherited Disease*. McGraw-Hill, New York, 2001; 1521–51.
4 Rosenthal P, Podesta L, Grier R *et al.* Failure of liver transplantation to diminish cardiac deposits of amylopectin and leukocyte inclusions in type IV glycogen storage disease. *Liver Transpl. Surg.* 1995; **1**: 373–6.
5 Shin YS. Glycogen storage disease: clinical, biochemical, and molecular heterogeneity. *Semin. Pediatr. Neurol.* 2006; **13**: 115–20.
6 Starzl TE, Demetris AJ, Trucco M *et al.* Chimerism after liver transplantation for type IV glycogen storage disease and type I Gaucher's disease. *N. Engl. J. Med.* 1993; **328**: 745–9.
7 Davis MK, Weinstein DA. Liver transplantation in children with glycogen storage disease: controversies and evaluation of the risk/benefit of this procedure. *Pediatr. Transpl.* 2008; **12**: 137–45.

CONFIRMATION OF THE EFFICACY OF VITAMIN B_6 SUPPLEMENTATION FOR MCARDLE DISEASE BY FOLLOW-UP MUSCLE BIOPSY

SHINYA SATO, MD,[1] TAKEKAZU OHI, MD, PhD,[2] ICHIZO NISHINO, MD, PhD,[3] and HIDEO SUGIE, MD, PhD[4]

[1] Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[2] Department of Neurology, Kyoto City Hospital, Kyoto, Japan
[3] Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[4] Department of Pediatrics, Jichi Children's Medical Center Tochigi, Tochigi, Japan

Accepted 29 August 2011

ABSTRACT: No effective treatment for McArdle disease exists. We report a Japanese patient with McArdle disease who was treated with vitamin B_6 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 B_6 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 B_6 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 B_6 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 B_6 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 B_6 supplementation

Correspondence to: S. Sato; e-mail: sato_s@neuro.med.kyusha-u.ac.jp

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.22290

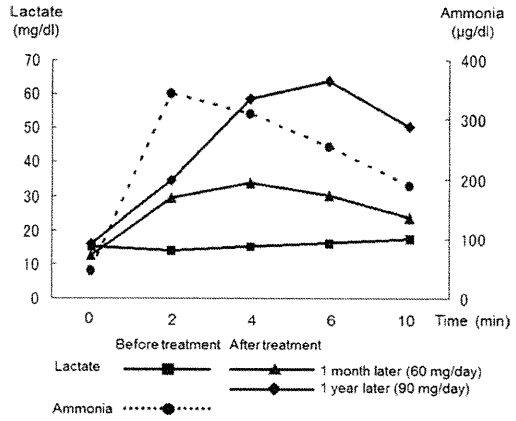

FIGURE 1. Forearm exercise test. For this test, rhythmic (1-Hz) handgrip exercise at maximal voluntary contraction was performed for 2 minutes. Results showed no increase in serum lactate levels before treatment with vitamin B_6 was started. An increase in serum lactate levels was seen 1 month after starting oral vitamin B_6 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 B_6. 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 B_6 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 B_6 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 B_6. 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 B_6, including sensory neuropathy.[5–7]

DISCUSSION

Oral vitamin B_6 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 B_6 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 B_6 treatment might have been caused by regeneration of skeletal

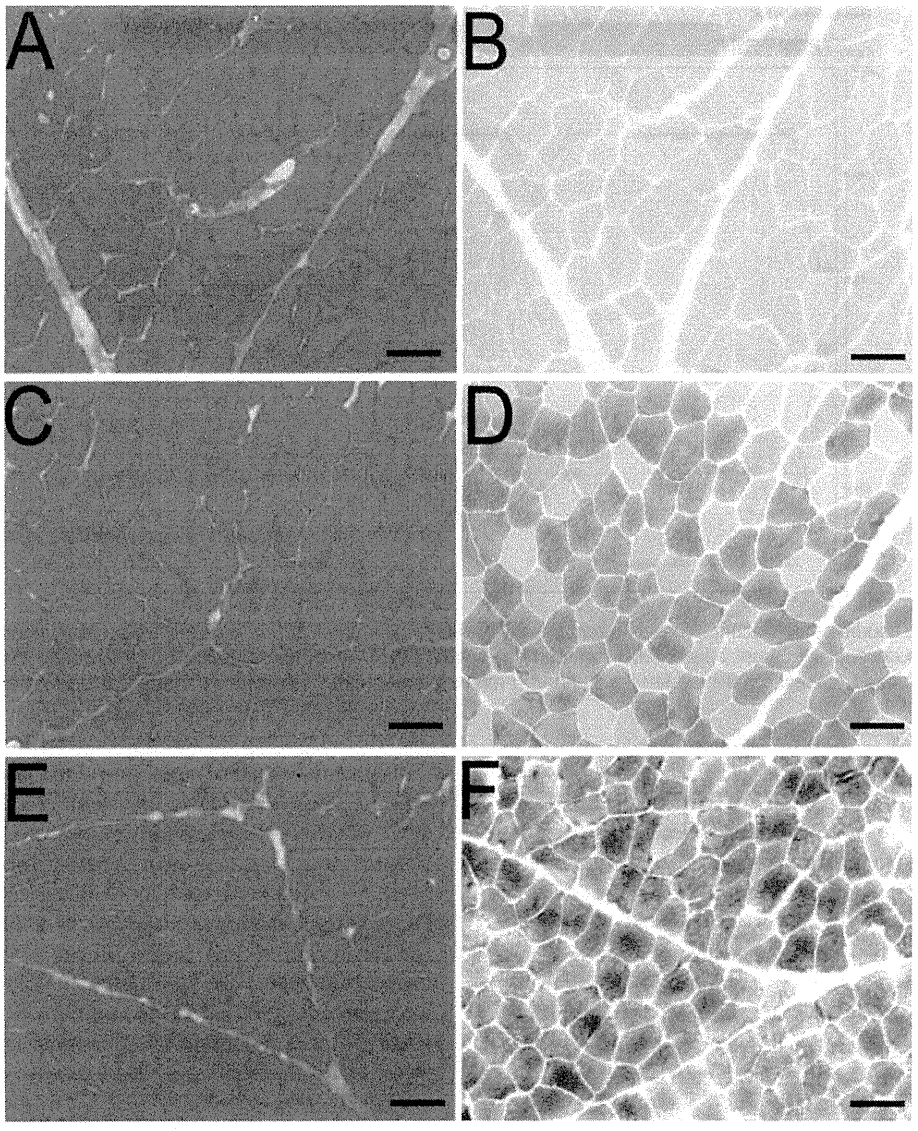

FIGURE 2. Muscle biopsy samples from the patient and a control subject. **(A, B)** Before treatment with vitamin B_6: **(A)** a sample stained with H&E; and **(B)** a sample stained with phosphorylase. Samples showed variation in muscle fiber size and frequent internal nuclei. No muscle fibers were stained with phosphorylase. **(C, D)** After treatment with vitamin B_6: **(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 μm.

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 B_6 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 B_6 (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 B_6 from 90 to 60 mg/day in April 2009, because we believed that the toxicity of vitamin B_6 (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 B_6. Thus, these results suggest that the effects of vitamin B_6 may depend on the dosage.

Many trial treatments other than oral vitamin B_6 supplementation have been used for McArdle

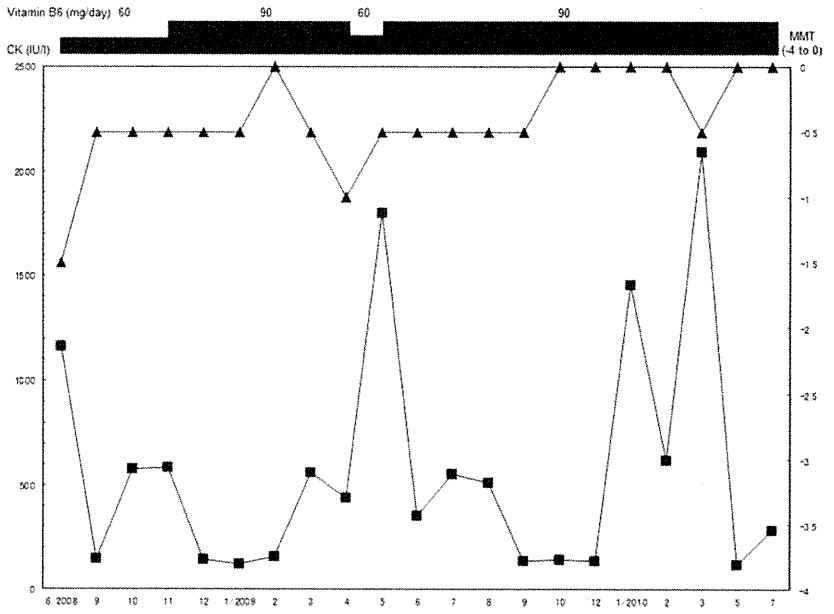

FIGURE 3. Clinical course of the patient. Muscle strength was evaluated by mean MMT of the neck flexors, deltoid muscles, and iliopsoas muscles. The dosage of vitamin B_6 was 60 mg/day from August 2008 to November 2008, 90 mg/day from November 2008 to April 2009, 60 mg/day from April 2009 to May 2009, 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 B_6 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 B_6 supplements. In addition, a Japanese patient with a very mild case of McArdle disease was treated with vitamin B_6 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 B_6, 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 B_6 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 B_6 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 B_6 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 B_6 supplementation.

The authors thank Hiromi Koda at the Department of Pathology in Kurashiki Central Hospital for her technical assistance and histological analysis.

REFERENCES

1. DiMauro S. Phosphorylase deficiency. Vol. 2. In: Engel AG, Franzini-Armstrong C, editors. Myology. 3rd ed. New York: McGraw-Hill; 2004. p 1537–1542.
2. Kazemi-Esfarjani P, Skomorowska E, Jensen TD, Haller RG, Vissing J. A nonischemic forearm exercise test for McArdle disease. Ann Neurol 2002;52:153–159.
3. Quinlivan R, Beynon RJ, Martinuzzi A. Pharmacological and nutritional treatment trials for McArdle disease (glycogen storage disease type V). Cochrane Database Syst Rev 2008;16:1–18.

4. Sugie H, Sugie Y, Ito M, Fukida T, Nonaka I, Igarashi Y. Genetic analysis of Japanese patients with myophosphorylase deficiency (McArdle's disease): single-codon deletion in exon 17 is the predominant mutation. Clin Chim Acta 1995;236:81–86.
5. Schaumburg H, Kaplan J, Windebank A, Vick N, Rasmus S, Pleasure D, Brown MJ. Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. N Engl J Med 1983;309:445–448.
6. Albin RL, Albers JW, Greenberg HS, Townsend JB, Lynn RB, Burke JM Jr, et al. Acute sensory neuropathy–neuronopathy from pyridoxine overdose. Neurology 1987;37:1729–1732.
7. Perry TA, Weerasuriya A, Mouton PR, Holloway HW, Greig NH. Pyridoxine-induced toxicity in rats: a stereological quantification of the sensory neuropathy. Exp Neurol 2004;190:133–144.
8. Dimauro S, Arnold S, Miranda A, Rowland LP. McArdle disease: the mystery of reappering phosphorylase activity in muscle culture—a fetal isoenzyme. Ann Neurol 1978;3:60–66.
9. Mitsumoto H. McArdle disease: phosphorylase activity in regenerating muscle fibers. Neurology 1979;29:258–262.
10. Phoenix J, Hopkins P, Bartram C, Beynon RJ, Quinlivan R, Edwards R. Effect of vitamin B6 supplementation in McArdle's disease: a strategic case study. Neuromuscul Disord 1998;8:210–212.
11. Izumi R, Suzuki N, Kato K, Warita H, Tateyama M, Nakashima I, et al. A case of McArdle disease: efficacy of vitamin B6 on fatigability and impaired glycogenolysis. Intern Med 2010;49:1623–1625.
12. Haller RG, Dempsey WB, Feit H, Cook JD, Knochel JP. Low muscle levels of pyridoxine in McArdle's syndrome. Am J Med 1983;74:217–220.
13. Beynon RJ, Bartram C, Hopkins P, Toescu V, Gibson H, Phoenix J, et al. McArdle's disease: molecular genetics and metabolic consequences of the phenotype. Muscle Nerve 1995;26(suppl 3):S18–S22.
14. Tsujino S, Shanske S, DiMauro S. Molecular genetic heterogeneity of myophosphorylase deficiency (McArdle's disease). N Engl J Med 1993;329:241–245.
15. Quinlivan R, Buckley J, James M, Twist A, Ball S, Duno M, et al. McArdle disease: a clinical review. J Neurol Neurosurg Psychiatry 2010;81:1182–1188.

RELAPSED ACUTE MYELOGENOUS LEUKEMIA OF BRACHIAL PLEXUS AFTER MARROW TRANSPLANT

JENNIFER A. SWEET, MD,[1] ROBERT V. JONES, MD,[2] ALAN SIU, MD,[1] LOUIS DePALMA, MD,[2] and ANTHONY J. CAPUTY, MD[1]

[1] Department of Neurological Surgery, George Washington University School of Medicine, 2150 Pennsylvania Avenue NW, Suite 7-420, Washington, DC 20037, USA

[2] Department of Pathology, George Washington University School of Medicine, Washington, DC, USA

Accepted 29 August 2011

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.

Muscle Nerve **45:** 440–444, 2012

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.

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

Correspondence to: J.A. Sweet; e-mail: sweetj@gwu.edu

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.22292

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)/μl], and thrombocytopenia (121,000 platelets/μl). 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/m^2) and later underwent a gender-mismatched allogeneic bone marrow transplant. His chemotherapeutic regimen for the transplant consisted of ^{131}I 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

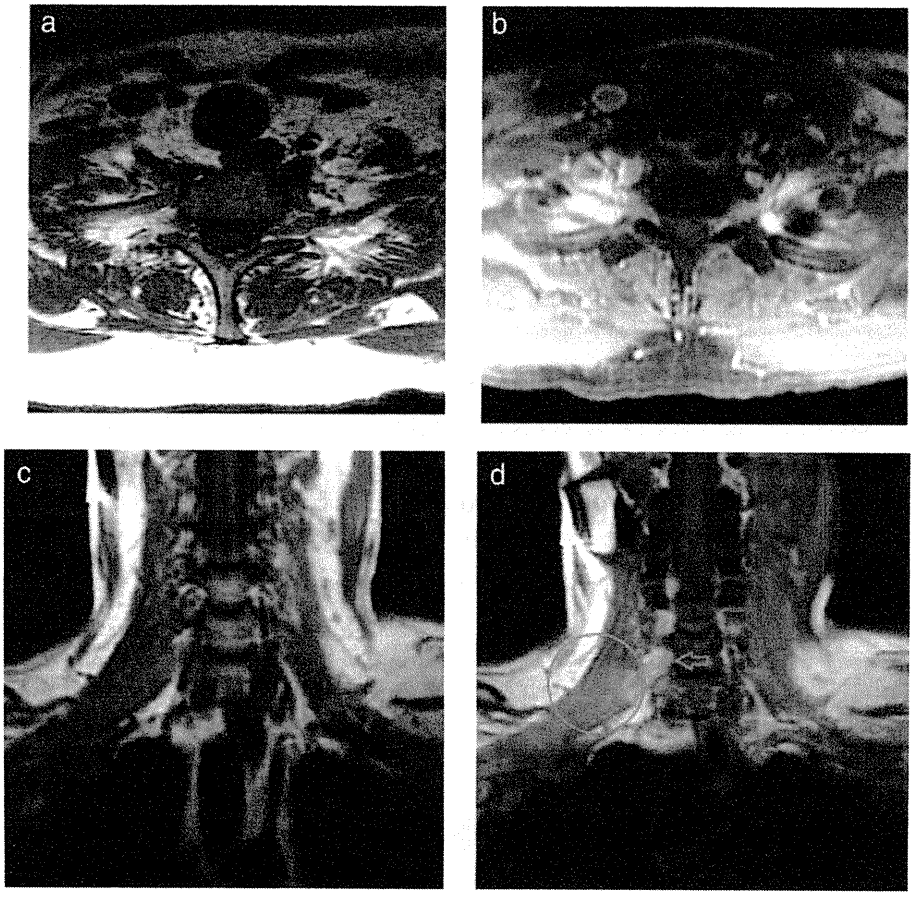

FIGURE 1. MRI of brachial plexus with and without contrast. **(a)** Axial, T1-weighted MRI without contrast, and **(b)** axial, T1-weighted MRI with contrast demonstrate enhancement of the right brachial plexus on the post-contrast image. **(c)** Coronal T1-weighted MRI without contrast, and **(d)** coronal T1-weighted MRI with contrast show enhancement of the right exiting nerve roots (small arrow) in addition to gross enlargement of the scalene muscles surrounding the affected brachial plexus (circle). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(GVHD), for which he was treated with prednisone 50 mg/day for 3 months, and then 50 mg every other day for nearly 1 year. This resulted in aseptic necrosis of the hip, requiring multiple joint replacements. He also experienced treatment-induced hypothyroidism and transfusion-related hemochromatosis.

Six years after his initial diagnosis of AML, he was found once more to have leukemic meningitis. His cerebrospinal fluid (CSF) analysis revealed 2100 WBC/μl with 96% blasts. Flow cytometric analysis revealed an immunophenotype similar to the original presentation with the added finding of CD2 expressed on the blasts. A bone marrow biopsy at this time did not reveal the presence of relapsed leukemia, and cytogenetic analysis was normal, with a female donor karyotype (XX). He then underwent multiple treatments of intrathecal chemotherapy, consisting of methotrexate (12 mg) in three cycles over 1 week, followed by liposomal cytarabine (50 mg) in five cycles over 2 months. There was no evidence of leukemic presence in the CSF analysis on three separate occasions within a 2-month period.

Concomitantly, while the patient was receiving his intrathecal chemotherapy, he developed weakness of the proximal right upper extremity associated with dysethesias and allodynia. This progressed over the 2-month course of his intrathecal liposomal cytarabine, to the point of proximal paralysis of the right upper extremity and severe distal weakness of the right-hand intrinsic muscles and wrist. Electromyography (EMG) was performed and showed evidence of severe upper trunk brachial plexopathy on the right with moderate involvement of the middle and lower trunks. Nerve conduction studies suggested an underlying peripheral neuropathy as well.

Given the temporal relationship between the patient's symptoms and his treatment with intrathecal liposomal cytarabine, the decision was made to stop the treatment in the event that this was the etiology of his weakness. This was further supported by the results of CSF analysis, which

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Neuromuscular Disorders 22 (2012) 389–393

www.elsevier.com/locate/nmd

Case report

Acid phosphatase-positive globular inclusions is a good diagnostic marker for two patients with adult-onset Pompe disease lacking disease specific pathology

Rie S. Tsuburaya [a,b], Kazunari Monma [a], Yasushi Oya [a], Takahiro Nakayama [c], Tokiko Fukuda [d], Hideo Sugie [d], Yukiko K. Hayashi [a], Ikuya Nonaka [a], Ichizo Nishino [a,*]

[a] *Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan*
[b] *Department of Pediatrics, Tohoku University School of Medicine, Miyagi, Japan*
[c] *Department of Neurology, Yokohama Rosai Hospital, Kanagawa, Japan*
[d] *Department of Pediatrics, Jichi Medical University and Jichi Children's Medical Center, Tochigi, Japan*

Received 18 August 2011; received in revised form 19 October 2011; accepted 15 November 2011

Abstract

Diagnosis of adult-onset Pompe disease is sometimes challenging because of its clinical similarities to muscular dystrophy and the paucity of disease-specific vacuolated fibers in the skeletal muscle pathology. We describe two patients with adult-onset Pompe disease whose muscle pathology showed no typical vacuolated fibers but did show unique globular inclusions with acid phosphatase activity. The acid phosphatase-positive globular inclusions may be a useful diagnostic marker for adult-onset Pompe disease even when typical vacuolated fibers are absent.

Keywords: Pompe disease; *GAA*; Globular inclusion; Acid phosphatase

1. Introduction

Pompe disease (glycogen storage disease type 2; acid maltase deficiency; OMIM #232300) is an autosomal recessive disease caused by mutations in the gene encoding acid α-glucosidase (GAA, OMIM #606800), a lysosomal enzyme involved in glycogen degradation [1]. Based on age of onset and clinical severity, which depends on residual GAA activity, the disease can be classified into infantile, childhood-onset, and adult-onset forms.

Most of the infantile and childhood-onset forms exhibit disease-specific skeletal muscle pathology, which shows fibers occupied by huge vacuoles that contain basophilic amorphous materials. However, diagnosis of the adult-onset form is sometimes challenging due to clinical similarities to muscular dystrophy and the paucity of typical vacuolated myofibers. We diagnosed 37 patients with Pompe disease including 11 infantile, 16 childhood-onset, and 10 adult-onset forms in the muscle repository of the National Center of Neurology and Psychiatry (NCNP), Japan, based on a deficiency of GAA enzyme activity assayed using biopsied muscles, as previously described [2]. Among these 37 patients, two unrelated Japanese patients did not have disease-specific vacuolated muscle fibers but did have unique cytoplasmic inclusions. Here, we report the diagnostic utility of acid phosphatase (ACP)-positive globular inclusions for adult-onset Pompe disease.

* Corresponding author. Address: National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 341 2711; fax: +81 42 346 1742.
E-mail address: nishino@ncnp.go.jp (I. Nishino).

0960-8966/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2011.11.003

2. Case report

2.1. Clinical summary

Patient 1: A 44-year-old man had been well until the age of 41 years when he started having difficulty in running. He was admitted to the hospital because of progressive muscle weakness. His parents were first cousins, but there was no family history of neuromuscular disorders. He was clinically suspected to suffer from muscular dystrophy because of slowly progressive muscle weakness and elevated creatine kinase levels of around 800 IU/L (normal, <171 IU/L). On examination, he had grade 4-muscle weakness on medical research council (MRC) scale and marked atrophy in his thighs. He did not have apparent respiratory impairment. Electromyography (EMG) showed myopathic changes with fibrillation and increased polyphasic motor unit potentials (MUPs).

Patient 2: A 62-year-old woman first noticed difficulty in climbing stairs at the age of 35 years, and needed a stick to walk at 45 years. Muscle weakness gradually worsened predominantly in her proximal limbs, and she became wheelchair-bound at 55 years. A muscle biopsy was performed at the age of 61 years. On examination, she had muscle weakness and atrophy predominantly in the proximal upper and lower limbs at the grade 3–4 on MRC scale. Serum CK level was 70 IU/L (normal, <142 IU/L). An EMG showed myopathic changes with increased polyphasic MUPs and myotonic-like repetitive discharges. She had been on non-invasive positive-pressure ventilation since the age of 62 years when the respiratory insufficiency appeared.

2.2. Skeletal muscle pathology

The skeletal muscle pathology from the vastus lateralis of patient 1 and from the biceps brachi of patient 2 showed nonspecific myopathic changes with moderate fiber size variation, mild endomysial fibrosis, and some fiber splitting (Fig. 1A). No necrotic or regenerating fibers were seen. No vacuoles containing amorphous materials were observed. Importantly, both muscles contained red–purple globular inclusions on modified Gomori-trichrome (mGT) stain (Fig. 1A and B). The average percentages of fibers with globular inclusions in the whole mGT-stained section were 0.5% in patient 1 and 2% in patient 2. These inclusions were invariably highlighted by ACP stain but not stained by periodic acid Schiff (PAS) (Fig. 1C). Inclusions were stained only faintly on menadione-linked α-glycerophosphate dehydrogenase (MAG) without substrate (Fig. 3A). Fibers with ACP-positive globular inclusions were also found in 15 of 16 childhood-onset and seven of eight adult-onset patients with disease-specific pathology in varying proportions (0.1–10%). The rate of fibers with inclusions was not significantly different between the childhood-onset and adult-onset forms. Fibers carrying inclusions did not have typical vacuoles with amorphous materials inside. In the infantile cases, more than 90% of the fibers were vacuolated, whereas non-vacuolated fibers with inclusions were hardly recognizable.

Double immunostaining was performed using primary antibodies against a lysosomal marker, lysosomal associated membrane protein-2 (LAMP-2; Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, USA) and an autophagosomal marker, microtubule-associated protein 1 light chain 3 (LC3; Novus Biologicals, Littleton, CO, USA). In fibers with ACP-positive inclusions, immunoreactivity for LAMP-2 and LC3 were accumulated focally in inclusions and surrounding area (Fig. 1D). We also examined another samples from adult-onset patients with typical vacuoles. Fibers with typical vacuoles were entirely positive for LAMP-2 and LC3 (data not shown).

On PAS staining, performed on epon-embedded sections (Epon-PAS) to detect glycogen more sensitively, PAS was negative in globular inclusions but positive in the surrounding area (Fig. 1E).

Electron micrography was performed as previously described using a Tecnai spirit transmission electron microscope (FEI, Hillsboro, OR, USA) [3]. The inclusions consisted of homogeneous electron-dense globules surrounded by increased glycogen particles and autophagic vacuoles (Fig. 1F). The globules contained neither dotted glycogen particles nor a filamentous structure.

2.3. GAA enzymatic analysis and genetic analysis

Presence of globular inclusions led us to suspect Pompe disease, and GAA enzymatic activity analyses revealed 7.5% of normal control activity in patient 1 and 12.3% in patient 2.

Genomic DNA was extracted from peripheral lymphocytes or biopsied muscle using a standard protocol for mutational analysis of *GAA*. All exons and their flanking intronic regions of *GAA* were amplified by PCR and directly sequenced with an ABI PRISM 3100 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Both patients carried the homozygous *GAA* mutation at the last codon of exon 2 (c. 546G > T). RT-PCR and direct sequencing were performed using RNA extracted from biopsied muscles. This novel mutation causes aberrant splicing by skipping exon 2 (Fig. 2). This homozygous c. 546G > T mutation was also found in another patient with the adult-onset form, whose muscle pathology showed typical skeletal muscle pathology with vacuolated fibers.

3. Discussion

ACP-positive globular inclusions were a good diagnostic marker for the two patients with adult-onset Pompe disease lacking typical vacuolated fibers. Among 12,103 muscle biopsies in the NCNP repository from 1979 to 2010, ACP-positive globular inclusions were not reported, except for Pompe disease.

The globular inclusions are most likely the same as "reducing body-like globular inclusions in late-onset Pompe disease" reported by Sharma et al., as the pathological features are