FACS analysis of granulocytes from our patient demonstrated severely decreased expression of GPI-APs. Functional analysis using *PIGL*-defective CHO cells revealed that the isoforms starting from the downstream methionines showed residual PIGL activity. It has been reported that two well-conserved motifs are essential for PIGL activity (Fig. 5B underlines). Two faint bands corresponding to the sizes of these isoforms were detected by western blot; the bigger band had both motifs but not the N-terminal transmembrane region. These two translation start sites do not fit well with Kozak's rule; therefore, these isoforms were not detected in the wild-type cells.

Severely decreased expression of GPI-APs in granulocytes of the patient suggest that these mutations in *PIGL* are associated with decreased GPI biosynthesis. Previous studies showed that the disruption of *PIGL* caused lethality in Saccharomyces cerevisiae [Watanabe et al., 1999], suggesting that PIGL has been considered as the only enzyme to catalyze the second step of GPI biosynthesis in yeast. Although the disease mechanims remain unknown, it is possible that a truncated protein translated from the allele with c.254_255del or C-terminal protein isoforms shown in our functional analysis using CHO cells might have the minimal residual activity of PIGL.

In conclusion, we identified compound heterozygous deletions in *PIGL* in a patient with distinctive facial appearance, developmental delay, intellectual disability, brachytelephalangy, and hyperphosphatasia. The clinical features were similar to those of HPMRS caused by mutations in *PIGV*, *PIGO*, *PGAP2*, *PGAP3*, and *PIGW*. Our findings will broaden the clinical spectrum of disorders with defects in the GPI biosynthesis pathway.

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＝総　　説＝

知的障害とてんかんを主症状とする新しい疾患
―先天性GPI欠損症―

村上　良子　　木下タロウ

要旨　最近，先天性GPI欠損症が知能障害や乳幼児発症の難治性てんかんの原因疾患として注目を集めている．GPI（glycosyl-phosphatidyl-inositol）は150種以上のタンパク質を細胞膜につなぎとめるアンカーの役割をする糖脂質でその基本骨格は真核生物で保存されている．GPIアンカー型タンパク質の生合成とそのリモデリングに関与する遺伝子は現在までに少なくとも26個あることがわかっている．次世代シークエンサーを使ったエキソーム解析により，最近このうち12個の遺伝子を責任遺伝子とするGPI欠損症が報告されている．変異遺伝子のステップや活性の低下の程度により，症状にはバリエーションがあるが共通症状として知的障害と運動発達障害があり多くはてんかん発作を伴っている．本総説ではこの先天性GPI欠損症について，最近の知見を概説する．

見出し語　GPIアンカー，てんかん，知能障害，高アルカリホスファターゼ血症，ビタミンB6

はじめに

真核生物の細胞表面にはGPI（glycosyl-phosphatidyl-inositol）と呼ばれる糖脂質によって細胞膜に結合するタンパク質のグループ（GPIアンカー型タンパク質）が発現している．GPIの基本構造はホスファチジルイノシトール（PI），グルコサミン（GlcN），3つのマンノース（Man），2つのエタノールアミンリン酸（EtNP）から成り立っており，全ての真核生物でよく保存され，複数のステップにより合成される（図1）．ほ乳類においては現在までに150種以上のGPIアンカー型タンパク質（GPI-APs）が知られており，酵素や受容体，接着因子，補体制御因子など個体発生や神経発達，免疫機能，受精等非常に重要な働きを担っている．GPIが欠損するとこれらのすべてのタンパク質は細胞表面に発現できずに多くは細胞内で破壊されてしまうので，GPI生合成の最初のステップに必要な遺伝子*Piga*のノックアウトマウスは胎生致死になる．

Ⅰ　GPIアンカー型タンパク質（GPI-AP）の生合成とリモデリング（図2）

1. GPIアンカーの生合成

GPI-APは小胞体（ER）で蛋白部分とGPI部分が別々に合成される．GPI生合成の最初のステップは触媒サブユニットPIGAとPIGC，PIGH，PIGP，PIGQ，PIGY，DPM2の7個のタンパク質からなる酵素複合体によって担われている．その後10個のステップを経て完成したGPI-APに付加される．この反応は触媒サブユニットPIGKとPIGS，PIGT，PIGU，GPAA1の5個のタンパク質からなるトランスアミダーゼ複合体によって担われ，この酵素はGPI付加シグナルを持つタンパク質のC末端シグナルペプチドを認識して切断しGPIの末端のエタノールアミンのアミノ基と共有結合させる．このように小胞体でGPI-APに付加されるまでのステップに関わる遺伝子群を*PIG*（PhosphatidylInositol Glycan）genesと呼び，ほぼクローニングされた順にアルファベットの名前がついている．またその後の修飾に関係する遺伝子群をPost GPI Attachment to Proteins（*PGAP*）genesと呼んでいる[1]．

2. GPIアンカー型タンパク質のリモデリング

1）イノシトールの脱アシル化

GPI生合成の初期にPIGWによってGlcN-PIのイノシトールにアシル基が付加される．この反応はGPIの生合成が細胞質側から小胞体内膜側に移行した直後に起こり，その後の効率的なGPI生合成と哺乳動物においてはタンパク質と結合する末端のEtNPの付加に必要である．このアシル基はGPIアンカーがタンパク質に付加された後に小胞体でPGAP1によって除かれる．PGAP1はリパーゼモチーフ（GxSxG）を有するタンパク質で，この遺伝子の欠損細胞では小胞体からゴルジ体へのGPI-APsの輸送が大幅に遅れ，さらにゴルジ体でのリモデリングは受けず，細胞表面にはアシル基の付いた異常構造のまま発現する．そのため細菌由来のリパーゼPIPLC（phosphatidylinositol-specific-phospholipase C）による切断に抵抗性となる[2]．

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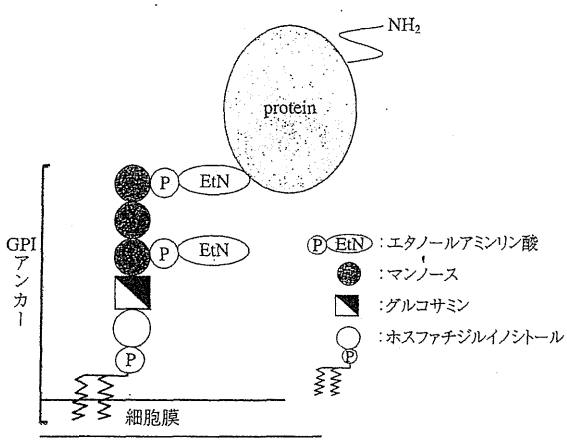

図1　GPIアンカー型タンパク質の構造

GPIの基本構造はフォスファチジルイノシトール（PI），グルコサミン（GlcN），3つのマンノース（Man），2つのエタノールアミンリン酸（EtNP）から成り立っており，すべての真核生物でよく保存されている．

2）GPI糖鎖リモデリング（側鎖EtNPの除去）

GPI生合成過程において3つのManにはそれぞれEtNPが付加される．この反応はフォスファチジルエタノールアミンを基質とし1つ目のManにはPIGNが，2つ目のManにはPIGG/PIGF複合体が，3つ目のManにはPIGO/PIGF複合体が働く．このうち2つ目のManに付加されたEtNPはGPIアンカーがタンパク質に付加された後に小胞体でPGAP5（MPPE1）によって除かれる．PGAP5は金属要求性のリン酸エステラーゼモチーフを有しているタンパク質でGPI-APsはPGAP5に結合して小胞体出口部位へ運ばれさらにカーゴレセプターであるp24ファミリータンパク質と結合して小胞輸送によりゴルジ体に運ばれる．PGAP5の欠損細胞ではPGAP1の欠損細胞と同様ゴルジ体へのGPI-APsの輸送が遅れるが，ゴルジ体での脂質のリモデリングは正常に受けた後，細胞表面にはEtNPが除かれない異常構造のまま発現する．

3）脂肪酸リモデリング

この反応はゴルジ体で行われ，GPI-APsが脂質ラフトに局在するために必須である．まず脂質部分のsn-2位に付加されて

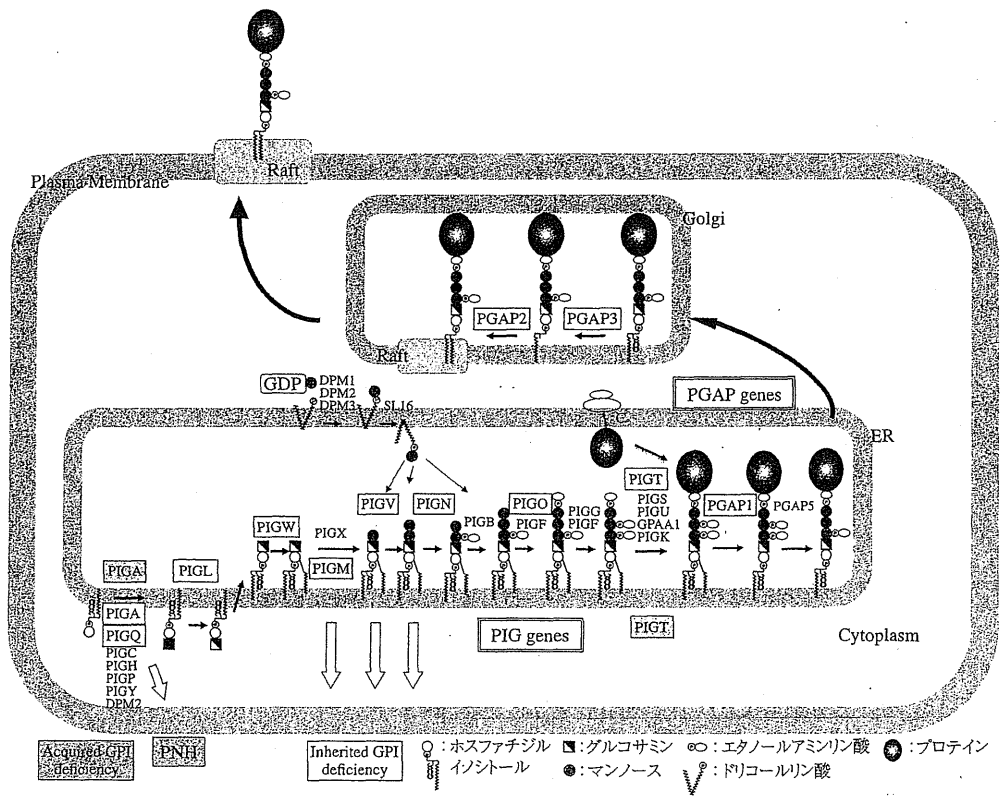

図2　GPIアンカー型タンパク質の生合成とリモデリング

GPIアンカー型タンパク質はタンパク質部分とGPIアンカー部分が小胞体（ER）において別々に合成され，GPIトランスアミダーゼ複合体がGPI付加シグナルを持つタンパク質のC末端シグナルペプチドを認識して切断しGPIアンカーに付加する．その後ERおよびゴルジ体で様々な修飾を受け細胞表面のラフトに局在できるようになる．白抜きの四角で囲んでいるのは現在までに報告のある先天性GPI欠損症，グレーの四角は後天性GPI欠損症（PNH）の原因遺伝子．

いる不飽和脂肪酸（アラキドン酸（C20:4）が主要）がPGAP3により除去され，飽和脂肪酸（ステアリン酸（C18:0））が付加される．この反応にはPGAP2が必須であるが，酵素ではなく制御因子であると考えられており，まだ哺乳動物で働く酵素タンパク質は同定されていない．PGAP3の欠損細胞ではGPI-APsはリモデリングを受けない異常構造のまま細胞表面に発現するが，脂質ラフトに局在できない．PGAP2の欠損細胞ではGPI-APsはsn-2位に脂肪酸が付加されないリゾ体のまま細胞表面に運ばれるが未知のリパーゼによって切断遊離され，その結果細胞表面のGPI-APsは著減する．

Ⅱ　後天性GPI欠損症である発作性夜間ヘモグロビン尿症との相違点と共通点

発作性夜間ヘモグロビン尿症（paroxysmal nocturnal hemoglobinuria;PNH）は，溶血性貧血，骨髄不全，深部静脈血栓を3主徴とする血液疾患である．溶血発作のエピソードと末梢血のフローサイトメトリーで顆粒球および赤血球表面のGPI-APsであるCD59やDAFの発現が低下あるいは欠損している細胞集団を確認することで診断される．後天的に1個あるいは数個の造血幹細胞の*PIGA*遺伝子に突然変異が起こってGPI欠損細胞となり，クローナルに増殖することによって発症する疾患なので正常細胞とGPI欠損細胞が混在していることが特徴である．血球分化にはGPI-APsは必須ではないので，クローナルに増殖している細胞の多くは完全欠損の細胞である．この異常クローンの増殖機序については未だ完全解明には至っていない．数あるGPI生合成遺伝子のうちほとんどの患者において責任遺伝子は*PIGA*である．その理由は*PIGA*のみがX染色体上の遺伝子であり，女性も発生初期のX染色体不活化後には1回の体細胞突然変異でGPI欠損細胞になるためと考えられ，実際にPNHの男女比は等しい．最近，次世代シークエンサーを用いた解析によって*PIGT*を責任遺伝子とするPNHが見つかったが，遺伝的に1本のアレルに変異があるところに，造血幹細胞において体細胞突然変異が起こり*PIGT*周辺領域の欠損が起こって発症したものであった[3]．このように今後まれではあるが他の遺伝子を責任遺伝子とするPNHが見つかる可能性がある．

一方先天性GPI欠損症（inherited GPI deficiency;IGD）においては，完全欠損は致死になるのですべて部分欠損症である．劣性遺伝形式をとり，患者は変異遺伝子のホモ接合体あるいは，複合ヘテロ接合体である．理論的にはPNHと同様1本のX染色体の変異で発症するPIGA欠損症の割合が高いと考えられる．報告例ではヘテロの女性は無症候性であり，男性のみ罹患する[4]．

Ⅲ　現在までに報告されている先天性GPI欠損症（IGD）

1. 最初に発見されたPIGM欠損症

イギリスの研究者との共同研究で解析した症例は，2家系3症例とも顆粒球ではすべてのGPI-APsが著明に減少していたが赤血球では軽微であったため溶血発作はみられず，てんかん発作と門脈血栓症が主症状であった．線維芽細胞のGPI-APsも著減していたことからIGDが疑われた[5]．患者のBリンパ芽球の解析により*PIGM*が責任遺伝子であることがわかった．PIGMは最初のマンノースを付加する酵素であるが，2家系ともに*PIGM*のプロモーターの転写因子Sp1の結合部位の1塩基の変異が原因でその結合が阻害され，ホモの変異を持つ患者では著しく*PIGM*のプロモーター活性が低下していた．Sp1はヒストンのアセチル化に関わる転写因子をリクルートする分子であるので，ヒストン脱アセチル酵素（HDAC）インヒビターであるNa butyrateの投与によって強制的にヒストンのアセチル化を起こすことによりPIGMの発現が回復しBリンパ芽球のGPI-APsの発現が完全に回復した．そこで患者にNa butyrateを投与したところ，難治のけいれん発作が治まった[6]．これらの患者には最近見つかっているIGD症例と異なり異常顔貌や奇形，知能障害などがみられない．おそらく胎生期は他の転写因子によりPIGMの発現が促進されSp1結合部位の異常による基礎的な発現低下が代償されていたのだと考えられる．一方，以下に述べる他のIGD症例に比較して血球や線維芽細胞におけるGPI-APsの低下は著明なので，血管上皮細胞のGPI-APsの発現低下等による局所の補体活性の亢進が血栓症を引き起こす原因になっている可能性がある．

2. Hyperphosphatasia mental retardation syndrome（Mabry syndrome）

古くから高アルカリホスファターゼ（alkaline phosphatase;ALP）血症と知能障害をきたす劣性遺伝の疾患が知られておりヨーロッパでは多くの患者が登録されていた．2010年にドイツの研究者が次世代シークエンサーによるエキソーム解析により，兄弟例に*PIGV*のcoding領域の遺伝子変異を発見し，我々との共同研究によりこの変異のためPIGVの活性が低下していることを示し責任遺伝子であることを証明した[7][8]．その後も同じグループとの共同研究により，同様の症状を示すPIGO欠損症[9]，PGAP2欠損症[10][11]，PGAP3欠損症[12]を報告した．また我々のスクリーニングにより国内初のGPI欠損症であるPIGO欠損症[13][14]，新規のPIGW欠損症[15]が見つかっている．PIGV欠損症はヨーロッパに多くすでに10家系以上が報告されているが，1つのアレルに共通の変異を持つ創始者効果のためと考えられる．これらの欠損症では共通症状として知的障害と運動発達障害，けいれん発作，顔貌異常が見られ，重症例では手指の末節骨や爪の低形成，難聴，心臓奇形，ヒルシュスプルング病，鎖肛等腸管の奇形，他の臓器の奇形；小脳萎縮，大脳萎縮・白質変性・髄鞘化の遅延などが見られる（図3）．このうちPGAP3欠損症以外の欠損症の症状については細胞表面のGPI-APsの発現の低下によって起こり，低下の程度によって表現型が異なるが責任遺伝子による差はあまりないと考えられる．一方PGAP3欠損症では，細胞表面の低下は顕著ではないと考えられGPI-APが脂質ラフトに局在できないために，シグナル伝達等の機能が阻害さ

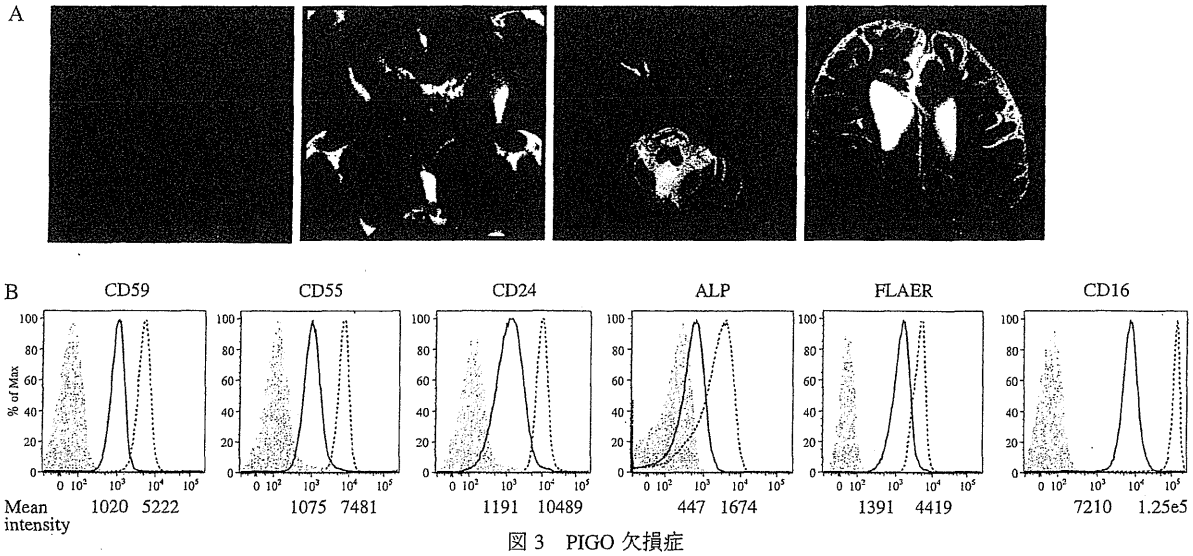

図3 PIGO欠損症

A：患者は精神発達遅滞，難治性てんかん，高アルカリフォスファターゼ血症（5959 IU/L），異常顔貌，手指末節骨の欠損（第5指），爪の低形成，ヒルシュスプルング病，ファロー四徴症をきたしていた．4歳時の手指のX線写真，4カ月時の頭部MRI（T2強調画像）では脳幹部における高シグナルが特徴的で，進行性に小脳萎縮と大脳の白質変性が見られた（共に9歳時の画像）．

B：顆粒球のFACS解析－GPIアンカー型蛋白質の発現の低下が見られた．

点線：正常コントロール，実線：患者，数値：平均蛍光強度（タンパク質発現量を示す）

（文献13）より改変）

れて起こっている可能性がある．知的障害，運動発達障害と軽いけいれん発作，時に小頭症が主症状で，PGAP3の活性がほとんどない重症例であっても発達障害は重度であるが他の臓器の奇形は伴わず手指の異常もない[12]．

3. 高ALP血症の機序

責任遺伝子により機序が異なる．

1）小胞体からの分泌—PIGV，PIGO，PIGW欠損症の場合（図4）

前述したようにPIGKを触媒コンポーネントとするGPIトランスアミダーゼ複合体はGPI付加シグナルを持つ前駆タンパク質のC末端のシグナルペプチドを認識しそのωサイトで切断して酵素－基質中間体を形成し，完成したGPIアンカーに付加する．その切断のためにはトランスアミダーゼの活性化が必要でそれはGPIアンカーの複合体への結合によって起こると考えられている．その結合には少なくともマンノース1つとそれにエタノールアミンが付いていることが必要である．すなわち*PIGM*のステップより前の初期の生合成遺伝子の欠損で蓄積するGPIアンカー中間体はマンノースが付いていないので複合体に結合できず，GPI-APであるALPのシグナルペプチドの切断が起こらないためそのまま細胞内で破壊され（小胞体関連分解によると考えられる），効率よく分泌されない．一方*PIGO*等*PIGV*以降の遺伝子の欠損ではシグナルペプチドの切断が効率よく起こるが，GPIアンカーが完成されていないので付加できずそのまま分泌経路に乗って分泌され，高ALP血症になる[16]．ただしPIGN欠損症では1つ目のマンノースにEtNPが付かないが，以降の生合成は最終ステップまでゆっくり進み効率は悪いものの，シグナルペプチド切断とGPIへの付加が起こるので高ALP血症を呈さない．しかし，細胞表面のGPI-APsの発現は低下し，アンカーの構造もEtNP側鎖を欠いている．またPIGWはPIGMの前のステップでイノシトールにアシル基を付加する酵素であるが，変異によりアシル基がつかない場合，その後の反応はゆっくり進むがPIGOが担う末端のEtNPを付加する反応が起こらない．このとき蓄積するのは，PIGO欠損症で蓄積するGPI中間体のアシル基のない構造であるため，シグナルペプチド切断と分泌が起こり，高ALP血症を呈する．

逆にGPIトランスアミダーゼ複合体の必須成分であるPIGTの欠損症では切断は全く起こらず，すべてが細胞内で破壊されるため低ALP血症をきたす[17]．

2）細胞表面からの遊離—PGAP2とPGAP3欠損症の場合（図5）

前述したようにPGAP2はゴルジ体での脂質部分のリモデリング反応の際にPGAP3で除かれた不飽和脂肪酸の代わりにsn-2の位置に飽和脂肪酸を付加する働きをするタンパク質であるので，PGAP2欠損症ではGPI-APは脂肪酸を1本しか持たないリゾ体のまま細胞表面に運ばれて血清中に遊離され，細胞表面のGPI-APsの低下と高ALP血症を起こす[10)11)]．したがってPGAP2欠損症は上記のPIGV欠損症等GPI生合成遺伝子の欠損症と同様の表現型を示す．細胞表面からの遊離のメカニズムは明らかになっていないが，培養液中ではホスホリパーゼD（PLD）で切断された構造で存在するので細胞膜上あるいは遊離してからPLDによって切断されると考えら

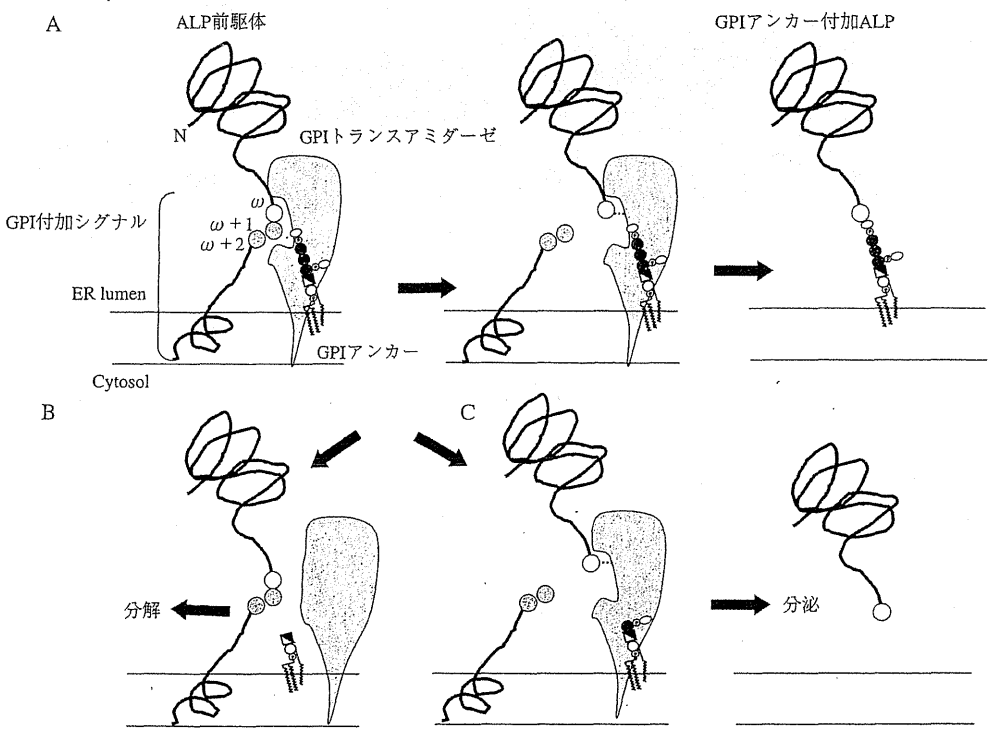

図4 高ALP血症の機序

GPIトランスアミダーゼ複合体はGPI付加シグナルを持つタンパク質のC末端のシグナルペプチドを認識しそのωサイト（切断を受けるアミノ酸）で切断して酵素ー基質中間体を形成し，完成したGPIアンカーに付加する（A）．初期の生合成遺伝子やGPIトランスアミダーゼの欠損ではGPIアンカー型タンパク質であるALPのシグナルペプチドの切断が起こらないためそのまま細胞内で分解される（B）が，生合成の後期ステップの遺伝子の欠損ではシグナルペプチドの切断が効率よく起こるが，GPIアンカーが完成されていないので付加できずそのまま分泌経路に乗って分泌され，高ALP血症になる（C）．

れている．一方PGAP3欠損症ではGPI-APsはリモデリングを受けない不飽和脂肪酸を含んだ脂質部分のまま細胞表面に運ばれるので脂質ラフトに局在できない．実際PGAP3欠損症やPgap3ノックアウトマウスでは高ALP血症になることは観察されるが培養細胞では確認できず，機序は明らかになっていない．GPI局在が異常であるために生体ではリパーゼやプロテアーゼに切断されやすくなっている可能性もある．

4. 高ALP血症をきたさない先天性GPI欠損症（IGD）

1）GPI生合成遺伝子の欠損症

前述したPIGM欠損症を除くと現在までにPIGA，PIGQ，PIGL，PIGN，PIGT[4)17)~24)]欠損症が報告されている．これらはすべてGPIアンカーの生合成に関わる遺伝子なので患者の症状は細胞表面のGPI-APsの発現低下によって起こっていると考えられる．すなわちMabry syndromeと共通な症状として知的障害と運動発達障害，けいれん発作が見られ，重症例では顔貌異常，多臓器の奇形，小脳萎縮，大脳萎縮・白質変性・髄鞘化の遅延などが見られる．手指の奇形は今のところ報告されていない．PIGT欠損症では前述したように低ALP血症を来し，腸管骨の短縮や頭蓋骨の早期癒合などの骨の異常を呈する．このうちPIGN欠損症は完全欠損であっても1つ目のマンノースにEtNPが付かない異常構造のまま細胞表面に少量のGPI-APが発現する．今後症例数が増加してPIGN欠損症に特徴的な症状が明らかになるようであれば，1つ目のマンノースについたEtNPの機能的意義が解明される可能性がある．

2）PGAP1欠損症

前述のようにPGAP1欠損細胞ではGPI-APsは細胞表面にはイノシトールのアシル基が残り，脂質のリモデリングを受けない異常構造のまま発現するが，発現の低下は見られない．PGAP1ノックアウトマウスでは耳頭症（otocephaly）あるいは全前脳胞症（holoprosencephaly）といった頭部の形成異常を来たし生直後に死亡するが，まれに形成異常を示さず育つものは雄性不妊を呈する．形成異常はWntやNodalシグナルの異常，雄性不妊は細胞膜からのGPI-APsの遊離の障害が関係していると考えられている[25)26)]．一方最近PAGP1欠損症の1家系が見つかり，患者ではほとんどPGAP1の活性が認められないにもかかわらず，症状は知的障害と軽症のてんかんを呈し，顔貌は正常で奇形等も見られなかった[27)]．マウスの表現型は系統によって差が見られているので，今後症例数が増えれば特徴的な症状が明らかになると考えられる．

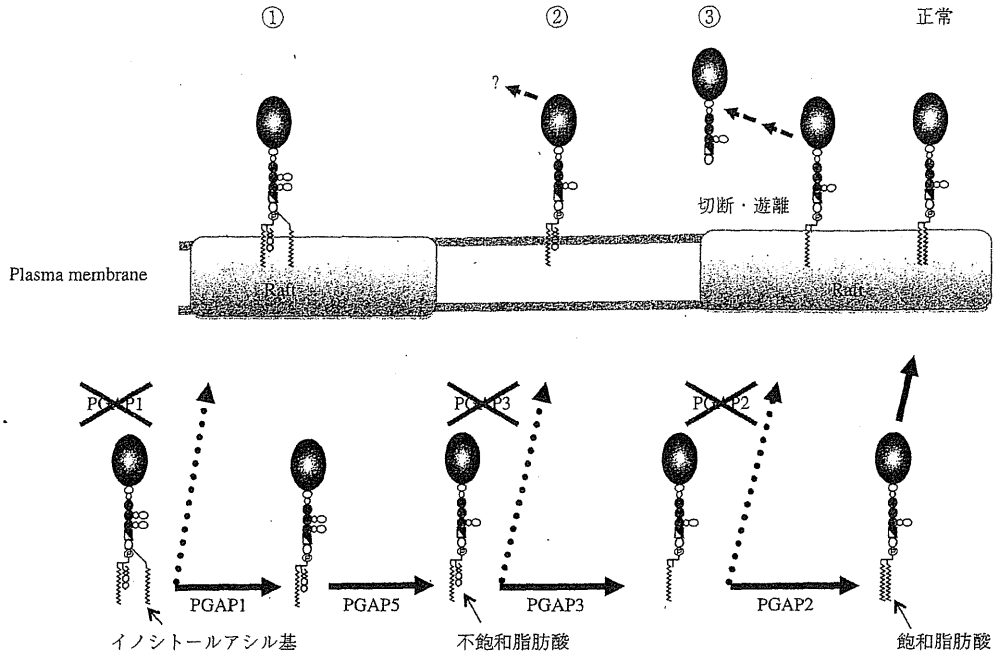

図5 GPI-APsのリモデリング

小胞体でタンパク質付加後にイノシトールのアシル基がPGAP1によって除かれる．PGAP1の欠損細胞ではGPI-APsはゴルジ体でのリモデリングは受けず，細胞表面にはアシル基の付いた異常構造のまま発現する（①）．ゴルジ体では，脂質部分のsn-2位に付加されている不飽和脂肪酸がPGAP3により除去され，PGAP2が関係する反応で飽和脂肪酸が付加される．PGAP3の欠損細胞ではGPI-APsはリモデリングを受けない異常構造のまま細胞表面に発現するが，脂質ラフトに局在できない（②）．PGAP2の欠損細胞ではGPI-APsはsn-2位に脂肪酸が付加されないリゾ体のまま細胞表面に運ばれるが未知のリパーゼによって切断遊離され，その結果細胞表面のGPI-APsは著減する（③）．

Ⅳ 症状の特徴（表1）と検査所見

GPI生合成遺伝子の欠損症ではその症状は活性の低下の程度，すなわちGPIアンカーの量に依存すると考えている．各ステップの遺伝子欠損CHO細胞に変異遺伝子を導入して正常遺伝子と比べてどの程度GPI-APsの発現を回復できるかをみる機能解析の結果は，重症度とよく相関する．最も軽症例でも知的障害は必発である．重症度が増すに従って，運動発達障害，てんかん，テント状の口などの異常顔貌，難聴，Hirschsprung病などの腸の奇形，心奇形等多臓器の奇形，時に魚鱗癬などの皮膚症状，目の異常等が見られる．乳児早期発症のてんかん性脳症である大田原症候群や早期ミオクロニー脳症，West症候群を呈する場合もある[15)20)21)]．また重症例では脳MRIで脳幹部や中脳に拡散強調やT2強調画像で高信号をきたすことが特徴である．また小脳萎縮や大脳白質変性が生後も進行する（図3A）[13)20)]．

検査所見

1）血清ALP値

高ALP血症を伴う発達障害はIGDである可能性が高い．アイソザイムは骨型，肝型共に上昇する．高ALP血症の有無は変異遺伝子のステップによることは先に述べた．このグループの患者では手指，足趾の末節骨や爪の低形成が特徴的であるが，高ALP血症が比較的軽度なPGAP3欠損症とPIGW欠損症では見られていない．一方トランスアミダーゼのコンポーネントであるPIGT欠損症ではALPが細胞内で破壊されてしまうため低ALP血症をきたし，共通の症状に加えて骨の形成異常を示す．

2）顆粒球のフローサイトメトリー（図3B）

末梢血のフローサイトメトリーで顆粒球に発現するGPI-APであるCD16の発現量の低下がIGD診断の決め手になる．重症の場合にはCD24やFLAER（GPI-APに結合するエロリジン毒素の細胞溶解能欠失変異体を蛍光ラベルしたもの）染色性等も顆粒球では低下するが，リンパ球や赤血球のGPI-APsの低下は見られない．

Ⅴ てんかん発作の発症機序と治療

てんかんの発症には神経細胞に発現するGPI-APであるALPの欠損が関係している場合があると考えられる．ALPのアイソザイムのうち組織非特異的（tissue non-specific）ALP（TNAP）の遺伝子異常は低ALP血症を来し骨の形成異常とてんかん発作を主症状とする．TNAPノックアウトマウスにおけるてんかん発作の機序が詳細に報告されている[28)]．すなわ

表1　先天性 GPI 欠損症の報告例と症状のまとめ

Affected Gene		PIGA (11)[a]	PIGQ (1)	PIGL (6)	PIGW (1)	PIGM (3)	PIGV (15)	PIGN (9)	PIGO (6)	PIGT (4)	PGAP1 (2)	PGAP2 (6)	PGAP3 (5)
MIM		300868	605754	280000	610275	610293	239300	614080	614749	610272	611655	614207	611801
Clinical diagnosis		Ohtahara, West syndrome, MCAHS2	Ohtahara syndrome	CHIME syndrome	West syndrome HPMRS		HPMRS1	MCAHS1	EOEE HPMRS2	MCAHS3	ID	HPMRS3	HPMRS
Age at assessment		Died (5/11)	Died at 2Y		7M	Died (1/3)	7M-17Y	Died (6/9)	0-15Y	Died at 2Y (1/4)	4Y, 2Y	3.5-28Y	4-17Y
Neurological disorder		(11/11)	(1/1)	(4/4)	(1/1)	(3/3)	(14/14)	(9/9)	(6/6)	(4/4)	(2/2)	(6/6)	(5/5)
Global developmental delay	HP:0001263[b]	Severe (9/11)	(1/1)	(4/4)	(1/1)	(1/3)	(14/14)	(9/9)	(6/6)	(4/4)	(2/2)	(6/6)	(5/5)
Motor delay	HP:0001270	Severe (9/11)	(1/1)	(4/4)	(1/1)	(1/3)	(9/9)	(9/9)	(6/6)	(4/4)	(2/2)	(5/5)	Severe (5/5)
Delayed speech	HP:0000750	No speech (11/11)		(4/4)			(9/9)	(9/9)	(6/6)	(4/4)	(2/2)	(5/6)	None (5/5)
Microcephaly	HP:0000252	(2/6) <3rd centile		(0/6)	(0/1)		(0/9)	(0/9)	(1/6) −5.5 SD	(0/4)	(2/2)	(1/6) −4.5 SD	(3/5) −3 SD
Muscular hypotonia	HP:0001252	(8/11)			(1/1)		(9/12)	(9/9)	(6/6)	(4/4)	(2/2)	(5/5)	(5/5)
Seizures	HP:0001250	(11/11)	(1/1)	(4/4)	(1/1)	(3/3)	(11/12)	(9/9)	(4/6)	(4/4)	(1/2)	(3/6)	(4/5)
Type		Myoclonic (5/11)			Tonic spasm	Absence		Complex partial	Tonic clonic, Partial seizure, Vitamin B6 dependent	Generalized toinic clonic, Myoclonic Absence seizure	Absence	Absence Myoclonic Tonic-clonic	Generalized toinic clonic, Myoclonic
Tremor	HP:0001337	(1/3)						(7/9)			(0/2)		
Nystagmus	HP:0000639							(7/9)		(4/4)	(0/2)		
Hearing impairment	HP:0000365	(2/3)			(0/1)		(3/10)		(1/6)		(0/2)	(1/6)	(0/5)
Eye abnormality	HP:0000504	Cortical blind (3/3)		Coloboma (4/4)	(0/1)	(0/3)		Cortical blind (1/2)		Cortical blind (4/4)	(0/2)		
Abnormal facial features		(9/11)		(4/4)	(1/1)	(0/3)	(14/14)	(9/9)	(6/6)	(4/4)	(2/2)	(2/6)	(5/5)
Hypertelorism	HP:0000316	(5/7)		(4/4)	(1/1)			(1/9)	(4/6)			(1/6)	(5/5)
Long palpebral fissures	HP:0000637	(5/7)							(4/6)			(1/6)	
Broad nasal bridge	HP:0000431	(5/7)		(4/4)	(1/1)			(1/9)	(4/6)	(4/4)	(2/2)	(2/6)	(5/5)
Broad nasal tip	HP:0000455	(2/2)			(1/1)				(4/6)			(1/6)	(5/5)
Tented upper lip	HP:0010804	(5/7)			(1/1)			(3/9)	(6/6)			(2/6)	(5/5)
Micrognathia	HP:0000347	(2/6)						(4/9)					
Cleft palate	HP:0000175			(2/4)	(0/1)		(1/10)	(0/9)	(1/3)			(1/6)	(2/5)
Abnormal skeletal features					(0/1)	(0/3)				(4/4)	(0/2)		(0/5)
Craniosynostosis	HP:0001363							(2/7)	(1/6)	(2/4)			
Short arms	HP:0009824									(4/4)			
Scoliosis	HP:0002650						(1/5)			(2/4)			
Reduced mineralisation	HP:0004348									(4/4)			
Delayed bone age	HP:0003799						(1/5)			(4/4)			
Brachytelephalangy	HP:0009882	(0/11)		(0/4)	(0/1)		(13/14)	(1/9)	(4/6)			(1/6)	(0/5)
Teeth abnormality	HP:0000164	(6/11)		(4/4)	(0/1)					(2/2)			
Joint contracture	HP:0003121	(8/11)			(0/1)								
Other organ anomalies					(0/1)	(0/3)				(4/4)	(0/2)		(0/5)
Anorectal abnormalities	(anal stenosis) HP:0002025			(0/4)			(6/12)	(2/9)	(3/6)			(1/6)	
Aganglionic megacolon	HP:0002251			(0/4)			(2/14)	(0/9)	(2/6)			(1/6)	
Heart defect	HP:0001631	ASD (2/11) PDA (1/11)		VSD (1/4), TOF (1/4), TGA (1/4)			ASD (1/14)	ASD (2/7), PDA (2/7), PFO (3/7), PS (1/7)	TOF (1/6) ASD, PS (1/6)	PDA (1/4)		ASD (1/6)	
Vesicoureteral reflex or Anomalies in Urinary tract	HP:0000079	(2/4)		(1/1)			(2/5)	(4/9)		(4/4)			
Skin abnormalities					(0/1)	(0/3)					(0/2)		
Deep plantar groove	HP:0001869							(6/7)					
Skin psoriasis, Ichthyosis	HP:0008064	(3/11)		(4/4)									
Clinical Test													
Decreased expression of GPI-APs on patients' cell		(8/8) PMN		(1/1) B lymphoblast, Fibroblast	(1/1) PMN	(2/2) PMN	(2/2) PMN	(2/2) Fibroblast (2/2) PMN	(2/2) PMN	(2/2) PMN	(0/2) B lymphoblast PIPLC resistant		(1/2) PMN
Hyperphoshatasia	HP:0003155	Fluctuating mild elevation (4/9)			(1/1)	(0/3)	(14/14)	(0/2)	(4/6)	(4/4) Hypophosphatasia	(0/2)	(4/4)	(5/5)
MRI abnormality						(0/3)				(3/4)	CT, Brain atrophy		
Thin corpus callosum	HP:0002079	(6/11)						(1/4)					(1/5)
White matter immaturity	HP:0002500	(9/11)	(1/1)		(1/1)			(2/4)	(1/1)				
Restricted diffusion pattern					(1/1)				(1/1)				
Cerebellar atrophy	HP:0001272	(3/11)						(1/4)	(1/1)	(2/4)			
Others		Hepatosplenomegaly (4/4), Iron overload (4/4)				Portal vein thrombosis (2/3)		Large ears (5/7)			Large ears (2/2)		
Reference		4), 18), 19), 20)	21)	22)	15)	5), 6)	7), 8)	23), 24)	9), 13), 14)	17)	27)	10), 11)	12)

MCAHS: multiple congenital anomalies-hypotonia-seizures syndrome, CHIME: colobomas of the eye, heart defect, ichthyosiform dermatosis, mental retardation, and ear defects, HPMRS: hyperphosphatasia mental retardation syndrome, ID: intellectual disability, ASD: atrial septum defect, PDA: patent ductus arteriosus, VSD: ventricular septal defect, TOF: tetralogy of Fallot, TGA: Transposition of great arteries, PS: pulmonary stenosis, PMN: polymorphonuclear leukocyte

a) Numbers in parentheses: number of affected indivisuals, b) Human Phenotype Ontology ID

表2 先天性GPI欠損症の臨床症状

多くは知的障害，運動発達障害，てんかん発作を伴う．（時に家族性に見られる）
新生児期，乳児期早期発症の難治性てんかん（大田原症候群・West症候群）
顔貌異常（両眼解離・幅の広い鼻梁・長い眼裂・テント状の口・口唇，口蓋裂）
手指，足趾の異常（末節骨の短縮・爪の欠損，低形成）
その他の奇形（肛門，直腸の異常・Hirschsprung病・水腎症・心奇形など）
難聴・眼，視力の異常
皮膚の異常（魚鱗癬など）
筋緊張の低下，四肢の短縮，関節拘縮
高アルカリフォスファターゼ血症
低アルカリフォスファターゼ血症

ちALPは細胞表面で，ピリドキサールリン酸を脱リン酸化して細胞内に取り込める形のピリドキサールにし，細胞内に入ったピリドキサールは再びリン酸化されてピリドキサールリン酸となり，抑制性ニューロンにおいてγ-アミノ酪酸（GABA）合成酵素の補酵素として働く．細胞膜上にALPが発現しないと細胞内のピリドキサールリン酸が不足しGABA合成が抑制される結果けいれん発作がおこると考えられる．実際，細胞内のピリドキサールを補うために，患者にビタミンB6（ピリドキシン）の投与を行ったところけいれん発作が消失した[13]．一方ビタミンB6が効かない症例もあり，ALP以外のGPI-APsも多く神経細胞には発現しているので，それらの発現低下がてんかん発作に関与していると考えられる．

Ⅵ 治療の可能性

多くの症例は，大脳の白質変性や小脳萎縮が進行し，発語の消失など退行を示す．将来的には生後すぐに血液検査でスクリーニングして診断し，GPIアンカー生合成を促進する薬を投与すれば，けいれん発作等，神経症状の進行を止めることができる可能性がある．前述したように患者の遺伝子異常はほとんどが部分欠損症であり，変異を持ったcDNAでも強いプロモーターで発現させると，多くの場合正常のcDNAと同様にGPI-APsの発現を回復させることができる．今後化合物のライブラリーを用いてハイスループットにGPIアンカー生合成を促進できる化合物をスクリーニングする系を作りたいと考えている．また症状の原因となっているGPI-APが同定できれば，補充療法も可能になる．前述したビタミンB6による治療もその一つであり，葉酸受容体がGPI-APであることから，フォリン酸の投与が奏功する可能性もある．

Ⅶ 今後の展望

表2にあげた患者を対象に海外，国内の多くの症例を集積し，詳細な症状の観察と検査所見をもとに疾患概念を確立させると共に診断基準，国内外共通の患者データーベースを作成することを目標としている．

GPI生合成遺伝子の欠損症では，変異による活性低下の程度によって症状にバリエーションがある．GPIアンカーの量が制限されているときには，それに付加されるタンパク質の優先度はタンパク質のC末端のGPI付加シグナルの配列に依るとされている．すなわち少しの活性の低下で発現が減少するタンパク質と下がりにくいタンパク質がある．活性がどこまで下がるとどのような症状がでるのか，その症状はどのGPI-APsの低下に起因するのか，そのタンパク質の生理的な機能は何なのか？ iPS細胞を使った細胞レベルの解析やモデルマウスの解析により神経症状の機序の解明を目指したいと考えている．

著者の利益相反：本論文発表内容に関連して開示すべき事項なし．

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Inherited GPI deficiencies: a new disease with intellectual disability and epilepsy

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Glycosylphosphatidylinositol (GPI) is a glycolipid, which anchors 150 or more types of proteins to the cell surface. There are at least 26 genes involved in the biosynthesis and transport of GPI-anchored proteins (GPI-APs). Many inherited GPI deficiencies (IGDs) have been recently found using whole-exome sequencing. Patients with IGD have only a partial deficiency because complete GPI deficiency causes embryonic death. The major symptoms of IGDs include intellectual disability, epilepsy, coarse facial features, and multiple organ anomalies. These symptoms vary in severity depending upon the degree of the defect and/or position in the pathway of the affected gene. We clarified a mechanism of hyperphosphatasia, which is characterized by elevated release of tissue-nonspecific alkaline phosphatase. Hyperphosphatasia is observed in some patients with IGDs, such as hyperphosphatasia mental retardation syndrome or Mabry syndrome, caused by mutations in genes in the later stage of GPI biosynthesis. The possibility of IGD should be considered in patients with seizures and intellectual disability. The presence of hyperphosphatasia is strong evidence of IGD. Flow cytometric analysis of GPI-APs on granulocytes is also useful for the detection of IGD.

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Original article

Prognostic factors for acute encephalopathy with bright tree appearance

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Abstract

Objective: To determine the prognostic factors for encephalopathy with bright tree appearance (BTA) in the acute phase through retrospective case evaluation. *Methods:* We recruited 10 children with encephalopathy who presented with BTA and classified them into 2 groups. Six patients with evident regression and severe psychomotor developmental delay after encephalopathy were included in the severe group, while the remaining 4 patients with mild mental retardation were included in the mild group. We retrospectively analyzed their clinical symptoms, laboratory data, and magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) findings. *Results:* Patients in the severe group developed subsequent complications such as epilepsy and severe motor impairment. Univariate analysis revealed that higher maximum lactate dehydrogenase (LDH) levels ($p = 0.055$) were a weak predictor of poor outcome. Maximum creatinine levels were significantly higher ($p < 0.05$) and minimal platelet counts were significantly lower ($p < 0.05$) in the severe group than in the mild group. Acute renal failure was not observed in any patient throughout the study. MRS of the BTA lesion during the BTA period showed elevated lactate levels in 5 children in the severe group and 1 child in the mild group. MRI performed during the chronic phase revealed severe brain atrophy in all patients in the severe group. *Conclusions:* Higher creatinine and LDH levels and lower platelet counts in the acute phase correlated with poor prognosis. Increased lactate levels in the BTA lesion during the BTA period on MRS may predict severe physical and mental disability.

Keywords: Acute encephalopathy; Acute encephalopathy with biphasic seizures and late reduced diffusion; Bright tree appearance; Prognostic factors; Creatinine; Magnetic resonance spectroscopy; Lactate peak

1. Introduction

Acute encephalopathy with bright tree appearance (BTA), originally described by Shiomi et al. [1], is the most frequently diagnosed form of encephalopathy in Japanese pediatric emergency medicine. Two recent case

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studies reported acute infantile encephalopathy predominantly affecting the frontal lobes and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) in non-Japanese patients; therefore, it seems that this clinical entity is not limited to the Japanese population [2,3]. Acute encephalopathy is typically characterized by a biphasic clinical course. It usually begins with status epilepticus and a mild symptomatic period of 2–3 days, followed by a cluster of seizures accompanied by a decreased level of consciousness. During this period, diffusion-weighted images obtained by magnetic resonance imaging (MRI) show strong signal intensities in the subcortical regions, referred to as BTA [1]. Few atypical types of acute encephalopathy with BTA have been described, including cases with altered consciousness but no status epilepticus or cases with a monophasic clinical course [4,5]. No standard treatment has been described for this entity, although glutamatergic excitotoxicity was proposed as its main pathomechanism [6]. Sequelae may include mild to severe motor and intellectual disability and epilepsy. The prognostic factors for acute encephalopathies, including one case with BTA, have been reported as a decrease in platelet count; an increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), and creatinine (Cr) levels; and abnormal blood sugar levels and clotting times [5,7,8]. Here we retrospectively examined the prognostic values of several serum markers, changes in these markers during the acute stage, and the findings of magnetic resonance spectroscopy (MRS) of the BTA lesion during the BTA period.

2. Methods

The participants included 10 patients with acute encephalopathy and BTA who were admitted to Osaka University Hospital from 2003 to 2012. Eight of the patients presented with fever and status epilepticus, which continued for over 30 min on day 0. MRS confirmed the presence of BTA from the 3rd to the 9th day of the illness. The other 2 patients did not present an obvious convulsive event; however, 1 of them presented with loss of consciousness, and BTA was confirmed in this patient on the 6th day. The other patient was transferred to the emergency room with loss of consciousness and respiratory arrest, and BTA was confirmed on the 8th day. For the 10 participants, we retrospectively examined the clinical history, clinical features, changes in different serum marker levels, and brain MRI findings.

Evaluation of clinical history included the patient's underlying condition, any past history of febrile convulsions, neurological status before encephalopathy, type of infectious disease, existence of a biphasic course, length of the latency period between fever onset and encephalopathic episode onset, duration of primary status epilepticus, specific medication used to cease convulsions and sedate, use of any prescribed antiepileptic agents during the acute phase, and history of specific treatment for acute encephalopathy. Neurological outcome was evaluated 1 year after the onset of encephalopathy. The intelligence or development quotient was not accurately estimated by developmental tests in most patients. Therefore, the severity of cognitive impairment was estimated and classified as follows: normal or mild if the patient could utter some meaningful words (for patients aged 12–24 months) or have a simple conversation (for patients aged >24 months), moderate if a patient could utter a few meaningful words (for patients aged >24 months), and severe if a patient could not utter meaningful words. For patients aged >12 months, the severity of motor impairment (MI) was estimated and classified as follows: normal or mild if the patient could walk without support, moderate if the patient could sit without support but not walk without support, and severe if the patient could not sit without support. The neurological status of patient 6, who presented with an underlying disease before encephalopathy, was not evaluated according to these criteria because the patient was aged <12 months. This patient could babble and hold the head upright but could not turn over unassisted before encephalopathy. We estimated that the patient had moderate mental retardation (MR) and MI. We divided patients into 2 groups according to the severity of encephalopathy, namely severe and mild groups, on the basis of the patient's neurological status at 1 year after the onset of encephalopathy. The mild group included patients with mild cognitive and/or mild motor impairment. The severe group included patients with a more severe neurological impairment. Therefore, 6 patients were included in the severe group and 4 patients in the mild group.

Blood samples were obtained during convulsions, immediately after the end of convulsions, or immediately after arrival at the hospital for patients without convulsions. We investigated the maximum and minimum values and any changes observed during the first 20 days in several serum marker levels. These markers included blood cell counts and AST, ALT, LDH, CK, blood urea nitrogen (BUN), Cr, sodium, potassium, chloride, total protein content, albumin, and blood sugar levels.

Brain imaging findings for the acute phase and chronic phase were examined >7 months later by performing MRI using a 1.5T Signa HD (GE Healthcare, Milwaukee, WI) system with a standard head coil. MRS [point-resolved spectroscopy sequence (PRESS): repetition time/echo time, 1800/136] was subsequently performed on BTA detection, and the region of interest was marked on the BTA lesion. Model information and condition of acquisition was not confirmed in patients 2 and 6. On MRS, we examined the peak of lactate and

Table 1
Clinical features of the patients.

Patient	Age	Sex	Underlying disease	History of FS	Neurological status before encephalopathy	Initial symptom	Infection	Biphasic clinical course	Antiepileptic drugs for the acute phase	Therapy for the acute phase	Outcome
1	6 y	M	No	Yes	Normal	Impaired consciousness	Influenza A	No	MDL, PB	mPSL	Epilepsy, moderate MI, severe MR
2	5 y	M	Klinefelter syndrome	Yes	Epilepsy Mild MI Mild MR	Status epilepticus	Influenza A	No	DZP, PB	mPSL, IVIG	Epilepsy, severe MI severe MR
3	4 y	M	Psychomotor retardation	Yes	Mild MI Mild MR	Status epilepticus	Unknown	No	MDL, thiopental, thiamilal, PB	mPSL, IVIG	Epilepsy, severe MI, severe MR
4	2 y	M	Chromosomal abnormality	No	Moderate MI Moderate MR	Status epilepticus	Influenza A	No	DZP, MDL, LDC, PB, PHT	mPSL, IVIG	Epilepsy, severe MI, severe MR
5	1 y	F	No	No	Normal	Status epilepticus	HHV6	Yes	MDL, thiopental, LDC, PB	mPSL	Epilepsy, moderate MI, severe MR
6	6 m	M	Kabuki syndrome	No	Moderate MI Moderate MR	Status epilepticus	Unknown	No	DZP, MDL, thiopental, PB, PHT	mPSL, IVIG	Epilepsy, severe MI, severe MR
7	4 y	F	Costello syndrome	Yes	Epilepsy Mild MR	Status epilepticus	Influenza A	Yes	DZP, MDL, PB	mPSL	Epilepsy Mild MR
8	2 y	M	No	No	Normal	Impaired consciousness	Adenovirus type3	Yes	MDL, PB	mPSL	Mild MR
9	2 y	F	No	No	Normal	Status epilepticus	Unknown	No	DZP, MDL, PB	mPSL, IVIG	Mild MR
10	1 y	M	Cerebral Palsy	Yes	Mild MI Mild MR	Status epilepticus	Influenza A	No	DZP, MDL, thiamilal, PB	mPSL	Mild MI Mild MR

DZP: diazepam, MDL: midazolam, LDC: lidocaine, PB: phenobarbital, PHT: phenytoin, mPSL: methylprednisolone, IVIG: intravenous immunoglobulin, MR: mental retardation, MI: motor impairment, FS: febrile seizures, M: male, F: female, y: year, m: month.
Patients 1–6 belong to the severe group, while patients 7–10 belonged to the mild group.

the ratio of N-acetyl aspartic acid and creatine + phosphocreatine (NAA/Cr).

Statistical analyses were performed using JMP 8.0 statistical software (SAS Institute Inc., Cary, NC, USA) and the Mann–Whitney U test was used to test for significant differences between the severe and mild groups. Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Clinical features

The total observation period was 7 months to 9 years. The latency period from fever onset to seizures or state of altered consciousness was <24 h in all patients. The age of onset ranged from 6 months to 6 years (median, 3 years) in the severe group and from 1 year to 4 years (median, 3 years) in the mild group. No significant difference in age was observed between the 2 groups ($p = 0.587$). Six of the 10 patients presented some underlying condition. The duration of status epilepticus ranged from 40 to 60 min (median, 50 min) in the severe group and 40–50 min (median, 45 min) in the mild group. No significant difference in duration was observed between the 2 groups ($p = 0.362$). Thiopental or thiamilal was required to cease convulsions in addition to the initially administered diazepam (DZP) or midazolam (MDL) in 5 patients in the severe group; DZP alone was sufficient for 2 patients in the mild group. Two patients without evident convulsions developed respiratory or cardiopulmonary arrest on arrival, and resuscitation was performed in the emergency room. These patients were placed on respirators and received continuous infusions of MDL during the acute phase. All patients were treated by methylprednisolone pulse therapy. Intravenous immunoglobulin (IVIG) was administered to 4 patients in the severe group and 1 patient in the mild group (Table 1).

Neurological sequelae included epilepsy or severe MI in addition to regression in the severe group. The neurological status of the 4 patients with underlying disease in the severe group had been stable before encephalopathy. However, their status evidently worsened after

encephalopathy. Mild MR was observed in all patients in the mild group; however, 2 patients with underlying disease had mild MR before encephalopathy (Table 1).

3.2. Serum markers

Seven out of the 10 patients studied showed maximum values of AST and ALT within 10 days of admission, and all patients showed maximum values of LDH and CK within 10 days. These markers returned to normal levels approximately 1 month later (Table 2). The maximum LDH values observed in the severe group were higher than those in the mild group, but that difference was only marginally significant ($p = 0.055$). The platelet counts reached the minimum value between the first and fourth day of hospitalization and returned to normal levels shortly thereafter (Fig. 1). The

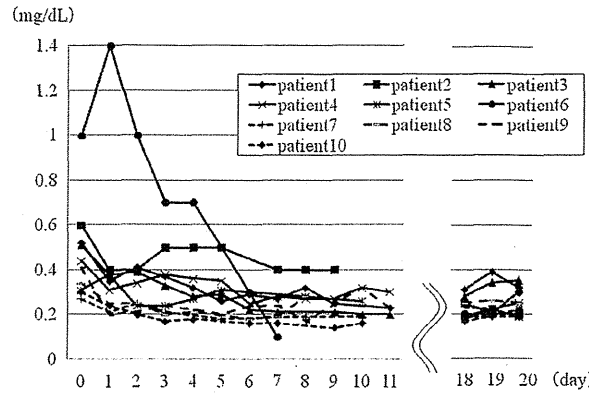

Fig. 2. Change in serum Cr levels, Cr: creatinine. The solid line represents the severe group (patients 1–6), and the dotted line represents the mild group (patients 7–10).

Table 2
Comparison of serum markers between the severe and mild groups.

		Severe group ($n = 6$)		Mild group ($n = 4$)		p Value
		Median (range)		Median (range)		
Maximal AST	(IU/L)	600	(220–1341)	372	(114–719)	0.2
Maximal ALT	(IU/L)	253	(132–1350)	162	(49–373)	0.2864
Maximal CK	(IU/L)	1247	(572–4723)	281	(141–524)	0.1356
Maximal LDH	(IU/L)	1204	(1018–5211)	875	(655–1057)	0.055
Maximal Cr	(mg/dL)	0.515	(0.44–0.6)	0.32	(0.285–0.375)	0.019*
Cr in the convalescent phase	(mg/dL)	0.24	(0.19–0.3)	0.21	(0.18–0.24)	0.3359
Maximal BS	(mg/dL)	219	(176–252)	311	(118–411)	0.3938
Minimal Plt	($\times 10^3/\mu L$)	9.4	(9.1–10)	20	(17.5–22.5)	0.0325*

AST: aspartate aminotransferase, ALT: alanine aminotransferase, CK: creatine kinase, LDH: lactate dehydrogenase, Cr: creatinine, BS: blood sugar, Plt: platelet.
Statistical analysis was performed using the Mann–Whitney U test.
* $p < 0.05$.

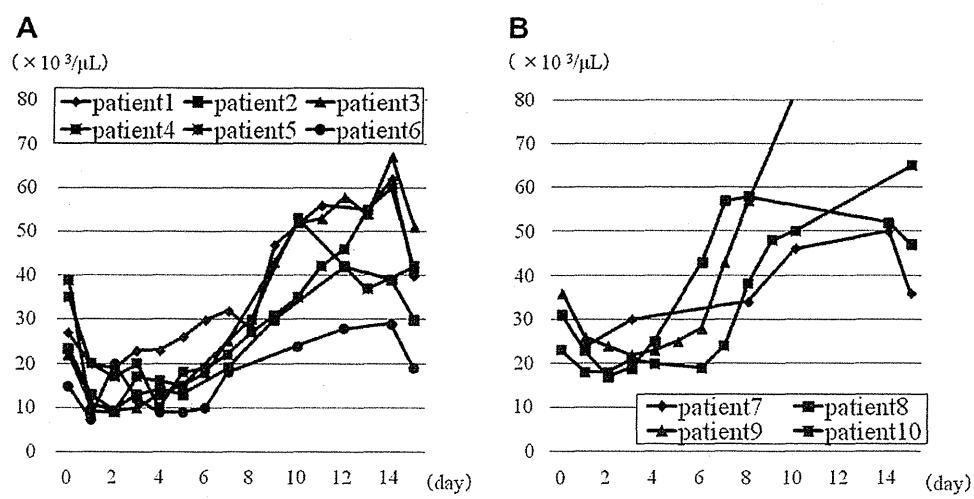

Fig. 1. Change in platelet counts. (A) Severe group, (B) mild group.

Table 3
Maximum Cr values and urine volume in the acute phase and Cr values in the convalescent phase.

Patient	Maximal Cr levels in the acute phase (mg/dL)	Cr levels, 97.5% (mg/dL)	Urine volume on day 0 (mL/kg/h)	Cr levels in the convalescent phase (mg/dL)
1	0.52	0.48	4.66	0.31
2	0.6	0.45	1.47	0.3
3	0.51	0.4	2.35	0.28
4	0.44	0.37	1.18	0.18
5	0.38	0.32	Not measured	0.19
6	1.4	0.31	4.52	0.2
7	0.27	0.4	Not measured	0.19
8	0.34	0.37	6.5	0.23
9	0.41	0.37	3.94	0.25
10	0.3	0.32	3.67	0.17

Cr: creatinine.
Cr 97.5%: 97.5 percentile value in age-matched Japanese children.

minimum platelet count was significantly lower in the severe group than in the mild group ($p < 0.05$). The maximum Cr value was observed on the day of onset or the following day in all patients (Fig. 2, Table 3); however, that maximum value was significantly higher in the severe group than in the mild group ($p < 0.05$). During the first week of illness, the Cr value was higher in the severe group than in the mild group, and no significant difference was observed between the 2 groups on the 20th day ($p = 0.336$). No significant differences were observed in any of the other markers between the 2 groups (Table 2).

3.3. Brain MRI and MRS

The median period of time elapsed between hospital admission and confirmation of the presence of BTA was 7 days. BTA was observed in different regions, with 2 patients in the mild group showing global involvement with spared pericentral areas. MRI performed during the chronic phase showed severe brain atrophy in all patients in the severe group, which was absent before encephalopathy, and mild to intermediate atrophy in all patients in the mild group (Table 4). None of the patients presented any thalamic lesions, diffuse brain edema, or hernia compressing the brain stem throughout the clinical course.

MRS was performed for 9 patients during the BTA period. A lactate peak was observed in 5 out of the 6 patients included in the severe group and 1 out of the 3 patients in the mild group (Fig. 3). In the mild group, the only patient presenting a lactate peak also showed intermediate cerebral atrophy during the remote period, while the 2 patients without a lactate peak showed only mild atrophy. The NAA/Cr ratio was 0.88–1.34 (median, 0.97) in the severe group and 1.13–1.19 (median, 1.17) in the mild group. No significant differences were observed between the 2 groups ($p = 0.178$; Table 4).

4. Discussion

Hayakawa proposed the integration of the 3 most common acute encephalopathies in Japan (namely, acute encephalopathy with febrile convulsive status epilepticus or AEFCES, AESD, and acute encephalopathy with biphasic clinical course) into one type of encephalopathy because of the overlap in clinical features and similarities in pathomechanisms. However, he reported that only 36% patients experienced all the symptoms in the triad: BTA, status epilepticus at illness onset, and biphasic clinical course [4]. Hayashi defined acute encephalopathy with reduced diffusion (AED), which encompasses a spectrum that includes not only typical AESD but also atypical AESD with a monophasic

Table 4
Findings of brain MRI and MRS.

Patient	Brain MRI (acute phase)			Brain MRI (chronic phase)
	Position of BTA	MRS:lactate peak	MRS:NAA/Cr	
1	Whole brain	(+)	0.88	Severe atrophy
2	Whole brain	(+)	0.98	Severe atrophy
3	Whole brain	(+)	0.97	Severe atrophy
4	Bioccipital lobe predominant	(+)	0.48	Severe atrophy
5	Right hemisphere	(−)	1.34	Severe atrophy of right hemisphere
6	Bifrontal lobe predominant	(+)	No data	Severe atrophy
7	Right hemisphere	(−)	1.19	Mild atrophy of right hemisphere
8	Whole brain (central sparing)	Not done	Not done	Moderate atrophy
9	Bioccipital lobe predominant	(−)	1.17	Mild atrophy
10	Whole brain (central sparing)	(+)	1.13	Moderate atrophy

BTA: bright tree appearance, MRI: magnetic resonance imaging, MRS: MR spectroscopy.
MRS was performed by a point-resolved spectroscopy sequence (PRESS).
Echo time (TE)/repetition time (TR) = 1800/136.
NAA: N-acetyl aspartic acid.
Cr: creatine + phosphocreatine.

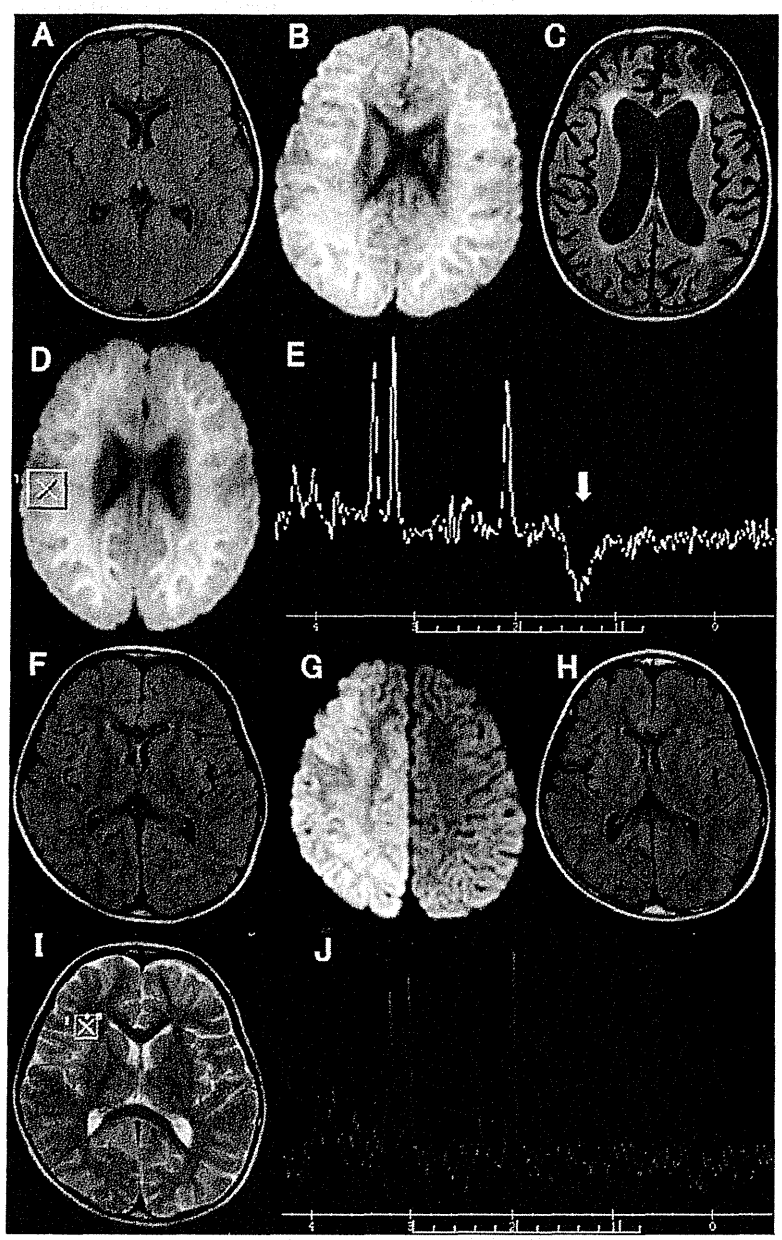

Fig. 3. Findings of brain MRI and MRS. Two typical cases are shown. Patient 3 (severe group). (A) FLAIR (day 1): No abnormal lesion is observed. (B) Diffusion-weighted imaging (DWI; day 6): Abnormal high intensities are observed in the whole subcortical white matter. (C) FLAIR (3 years later): Diffuse cerebral atrophy is observed. (D) Region of interest (ROI) on MRS. (E) MRS (point-resolved spectroscopy sequence (PRESS): repetition time (TR)/echo time (TE), 1800/136) shows a lactate peak. The arrow indicates the lactate peak. Patient 7 (mild group). (F) FLAIR (day 1): No abnormal lesion is observed. (G) DWI (day 6): Abnormal high intensities are observed in the right hemispheric cortical and subcortical white matter. (H) FLAIR (1 year later): Cerebral atrophy was observed in the right hemisphere. (I) ROI of MRS. (J) MRS (PRESS: TE/TR, 1800/136) shows no lactate peak. MRI: magnetic resonance imaging MRS: MR spectroscopy FLAIR: fluid-attenuated inversion recovery.

clinical course, or more severe subtypes [5]. In our study, only 3 patients showed a biphasic clinical course. However, it is worth pointing out that the other patients were treated with antiepileptic agents immediately after the onset of status epilepticus. We diagnosed 8 patients with AED, but we could not categorize 2 patients who presented with altered consciousness but no seizures at onset. In the present study, regardless of the clinical symptoms, all patients presented with BTA and some similitudes in laboratory data, including maximum LDH and CK values within 10 days, minimum platelet counts within the 1st–4th days, and maximum Cr values on the day of onset or following day. Therefore, we could assume a similar pathophysiology for all patients.

We also considered patients with BTA from the 3rd to 9th days as cases of acute encephalopathy with BTA. Overexpression of glutamate, abnormal increase in intracellular calcium ion levels, and subsequent neuronal cell death can be suggested as the possible pathomechanisms behind this encephalopathy. Takanashi reported an association between the appearance of a glutamine/glutamate complex peak on MRS during the BTA period and delayed neuronal cell death [6]. In our study, cases that showed a lactate peak displayed severe brain atrophy and subsequent severe neurological sequelae. To the best of our knowledge, this is the first study reporting the appearance of a lactate peak during the BTA period. A lactate peak on MRS generally reflects an elevation in anaerobic glycolysis or a disorder in aerobic glycolysis; therefore, it appears during the acute phase of an ischemic brain injury [9]; hypoxia [10]; a disorder of the tricarboxylic acid cycle, such as a mitochondrial disease [11]; and a state of imbalance between energy supply and demand, such as brain tumor [12]. We believe that energy failure may have led to neuronal necrosis and severe brain atrophy during the remote period in patients presenting the lactate peak.

In neural cells, NAA is synthesized from acetyl CoA and aspartate through L-aspartate N-acetyltransferase. The Cr peak observed on MRS comprised both Cr and phosphocreatine. The Cr peak is known to be stable; therefore, it is frequently used as the standard for other chemicals [13,14]. During the acute phase of brain infarction, the lactate peak appears immediately after onset; however, the Cr peak does not change within the first 24 h after onset. Therefore, it is believed that the NAA/Cr ratio reflects the metabolic rate of the neural cells and axons, while a decrease in NAA/Cr reflects the decrease or dysfunction in neural cells. Aaen et al. reported a decrease in NAA/Cr and the appearance of lactate peaks in patients with severe brain traumatic injury [15]. In the present study, no significant difference was observed between the mild and severe groups, although we considered that the NAA/Cr tended to be lower in the severe group. Nevertheless, we cannot underestimate the effect that the low sample size could have in these results.

Hayashi reported that AST, LDH, and CK levels reached a maximum value within 10 days from the onset of the illness and that they were significantly higher in the severe group [5]. Ishii reported an abrupt increase in neuron-specific enolase in all patients during the 2nd period (mean, 85.4 ± 40.6 ng/μL), and these high values were maintained for a month before they gradually decreased [16]. Ishikawa proposed that the presence of a high initial serum Cr value could be a useful marker to differentially diagnose prolonged febrile convulsions and AEFCES. On the other hand, they could not identify the reason for the increase of Cr levels, and no renal failure was observed [17]. The changes in serum markers detected in this study were similar to those reported by the abovementioned previous studies. Serum Cr levels showed the highest values either on the day of onset or the following day, and platelet counts showed the lowest values at some point between the 1st and 4th days. Within 10 days after the onset of the illness, 7 of the 10 patients presented their highest values of AST and ALT, and all patients displayed their highest values of LDH and CK. However, the reason behind the correlation between poor prognosis and increased LDH levels and a significant decrease in platelet counts remains unclear.

Five out of the 6 patients in the severe group had serum Cr levels higher than the 97.5 percentile of age-matched controls in their initial measurements; the remaining patient also had a value markedly higher than the 97.5 percentile of age-matched controls by the following day [18]. Their values were higher than the values during the convalescent period around the 20th day. The primary symptom of acute kidney injury is oliguria or anuria. The increase in serum Cr levels reflects the decrease in Cr clearance. Waikar reported that in patients with a >50% decrease in Cr clearance, a 50% increase in serum Cr levels is reached in 12–48 h [19]. Eight out of the 10 patients received urinary catheters, and the urine volume was accurately measured to ensure that the urine volume remained constant. Therefore, we could conclude that these patients did not have acute kidney injury during the acute phase. The increase in serum Cr levels may thus reflect a neurological dysfunction, given that these increased levels also correlate with the incidence of neurological sequelae.

Cr is the last product of creatine metabolism. Creatine is produced primarily in the liver, kidney, and pancreas and is stored in the skeletal muscle as phosphocreatine. Phosphocreatine plays an important role for buffering energy and is essential for the ATP-generating reaction:

$$\text{ADP} + \text{phosphocreatine} \leftrightarrow \text{ATP} + \text{creatine}$$

This energy-buffering mechanism also exists in the brain [20]. The concentration of creatine in the brain is approximately 200-fold higher than that in the serum [21], with the brain presenting the second highest concentration of creatine after the skeletal muscle. Creatine in the brain is directly supplied from the circulatory system through the blood–brain barrier and through production within astrocytes [20]. It is stored in the brain as phosphocreatine and converted to creatine by the brain isozyme for creatine kinase. Creatine is ultimately converted to Cr and released into the blood vessels through the blood–cerebrospinal fluid barrier [22].

Two possible mechanisms may explain the higher Cr value detected in the severe group. The more severe destruction of the blood–cerebrospinal fluid barrier in

the severe group may lead to the flood of Cr. Overproduction and leakage of Cr, synthesized by the phosphocreatine–creatine reaction facilitated by the energy demand in acute encephalopathy, can also explain the higher Cr values. Kubota reported a significant decrease in serum ATP levels during the acute phase of acute encephalopathy compared with the levels detected during the convalescent phase and ascribed it to mitochondrial dysfunction [23]. Although energy expenditure increases tremendously during the acute phase of acute encephalopathy, the production and supply of ATP cannot satisfy the demand, leading to ATP shortage. Although this hypothesis needs to be verified, all patients in the severe group showed significantly higher serum Cr values on the day of onset or the following day. This information may help in differentiating severe from mild cases, even in the early phase of the disease.

A disorder of cytokine secretion is not the main pathomechanism in acute encephalopathy with BTA [24]. In fact, it has been suggested that methyl prednisolone pulse therapy or IVIG therapy are not effective for this encephalopathy. Alternative promising therapies include cerebral hypothermia, edaravone, cyclosporine, high-dose antithrombin, and hemodialysis. The efficacy of cerebral hypothermia has been reported in patients with febrile convulsive status epilepticus [25], neonatal hypoxic ischemic encephalopathy [26], and encephalopathy after resuscitation in adults [27]. The mechanism of cerebral protection in cerebral hypothermia is assumed to be the inhibition of glutamate release [28]. Therefore, it is believed that cerebral hypothermia can be effective against acute encephalopathy with BTA, which leads to delayed neuronal cell death through the over-release of glutamate. However, the therapeutic time window to use cerebral hypothermia is reportedly only a few hours after the onset of the illness [29]. Cerebral hypothermia performed earlier with the detection of increase in serum Cr levels on the day of onset or the following day, rather than with the detection of BTA from the 3rd to 7th days, may lead to a better prognosis. In conclusion, serum Cr levels on the day of onset or the following day, minimum platelet count during the first 4 days, and a lactate peak in the BTA lesion on MRS were significant predictors of poor prognosis. Among these, the initial serum Cr level is expected to be an excellent predictor of prognosis in the acute phase.

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