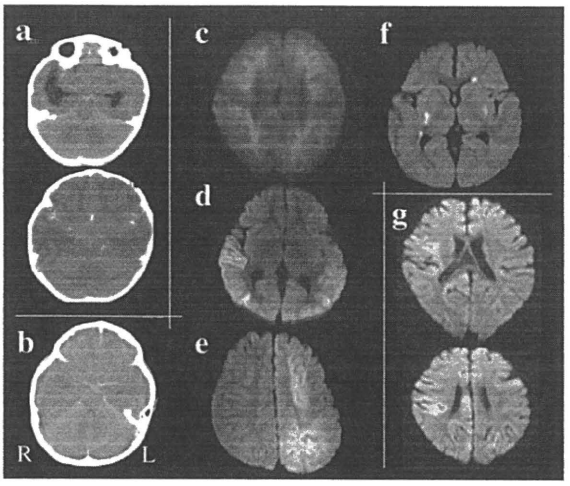

Fig. 1. (a) Brain CTs on 2nd and 21st day of hospitalization in Case 1 showing disappearance of cistern between brainstem and cerebellum (upper image) and severe brain edema (lower image). (b) Brain CT on admission showing severe brain edema in Case 2. (c–g) Brain MRIs showing abnormal high intensities in diffusion-weighted images in Cases 6,7,12,11 and 10, respectively.

3.4. Patient profile for the study of the blood ATP level

Twenty-five patients were included in this study. The diagnoses of the 25 patients were as follows: 10 patients with acute encephalopathy (mean age: 3 years and 11 months, age range: 7 months–10 years and 8 months, one IAE, one *Salmonella*-associated, one HHV-6-associated, three unknown virus-associated, one methylmalonic aciduria, one hepatic encephalopathy, one hemolytic uremic syndrome, and one septic encephalopathy (Case 2 in Table 1)), nine febrile seizure status (mean age: 1 year and 5 months, age range: 4 months–4 years 9 months), and six mitochondrial disease (mean age: 9 years and 8 months, age range: 2–25 years, two partial cytochrome c oxidase deficiency, three Leigh syndrome, and one chronic progressive external ophthalmoplegia). All 10 patients with acute encephalopathy were analyzed regarding the blood ATP levels in the acute phase (within 24 h of disease onset), and five of the 10 patients were also analyzed in the convalescent phase. Among the 15 patients who were analyzed for CPT II polymorphism, only Cases 2 and 12 were included in this study.

4. Methods

4.1. Analysis of CPT II polymorphism

Genomic DNA from whole blood was purified as previously described [15]. PCR of five exons of the CPT II gene was carried out with intron-based primers in genomic DNA. For haplotype analysis, the CPT II exon four region was cloned into the pCR® 2.1 vector (Invitrogen). The sequences of the PCR products and cloned CPT II gene were analyzed employing the ABI DyeDeoxy Terminator Cycle Sequencing Kit with an ABI-PRISM 3100 Genetic Analyzer (PE-Applied Biosystems). Each PCR product was sequenced at least twice independently.

4.2. Preparation of patients' lymphoblasts and culture

Blood samples (2 mL) were obtained from patients by venipuncture into a sterile EDTA blood collection tube. Lymphocytes were separated from peripheral blood, diluted (1:1, v/v) with sterile saline, by centrifugation ($800\times g$, 20 min) over 2 mL of Lymphoprep (Nycomed). The lymphocyte layer was recovered and washed twice with PBS by centrifugation at $250\times g$ for 10 min each, and then maintained in PRMI-1640 (GIBCO) supplemented with 12.5% FCS. Cells were incubated with 5% CO_2 at 37 °C for 7 days. Lymphoblastic cell lines were established by infecting peripheral blood lymphocytes with the Epstein Barr virus. Cells were grown in suspension in an SC flask (Greiner 658190) in an upright position, in 10 ml of PRMI-1640 medium that contained 12.5% FCS, maintained at 37 °C. Fluid was routinely changed every 2 days by removing the medium above the settled cells and replacing it with an equal volume of fresh medium.

4.3. Analysis of CPT II activity

CPT II activities of patients' lymphoblasts were analyzed as previously described [14]. To prepare whole cell extracts, cells were harvested and washed twice with PBS (−) at $250\times g$ for 10 min and then lysed with 0.5 mL of ice-cold lysis buffer (5 mM Tris–HCl buffer, pH 7.4, containing 1% Tween-20 and 0.5 M KCl), then centrifuged at $1{,}47{,}600\times g$ for 1 h at 4 °C. To analyze the heat stability of CPT II, cell lysates were pre-incubated at 30, 37 and 41 °C for 0–120 min. Protein concentrations in the cell lysates were measured using the BCAc Protein Assay Kit (Thermo SCIENTIFIC).

4.4. Measurement of blood ATP levels

ATP concentrations in whole blood lysate were measured by an ENLITEN® ATP assay system bioluminescence detection kit (Promega) according to the instructions provided by the manufacturer and the values were expressed as ATP levels in whole blood.

5. Results

5.1. CPT II polymorphism in the patients

As shown in Table 1, among the 15 patients studied, seven had a thermolabile F352C CPT II variant (1 F352C only and six [F352C + V368I]), four V368I only,

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two [V368I + M647 V], and two no polymorphisms. In 12 patients with acute encephalopathy (Cases 1–12), six (Cases 1–3 and 5–7) had a thermolabile F352C CPT II variant (1 F352C only and five [F352C + V368I]), and five (Cases 8–12) had the V368I CPT II variant (4 V368I only and one [V368I + M647 V]) and one (Case 4) showed no CPT II variant. Two patients with acute encephalopathy who died (Cases 1 and 2) had a thermolabile F352C CPT II variant (1 F352C only and the other [F352C + V368I]). In three patients with febrile delirium associated with influenza infection (cases 13–15), only case 13 (brief febrile seizure and unusually long febrile delirium) had the [F352C + V368I] CPT II variant. No other reported CPT II mutations or polymorphisms were detected.

There was no significant difference in the age at onset (41.0 ± 23.3 vs. 24.3 ± 12.7 months of age, $p = 0.18$), duration of high fever (52.0 ± 35.3 vs. 63.0 ± 44.9 h, $p = 0.28$), and duration of seizures (40.5 ± 40.1 vs. 56.7 ± 23.4 h, $p = 0.12$) between the six patients with acute encephalopathy with a thermolabile F352C CPT II variant (Cases 1–3,5–7) and six patients with acute encephalopathy without this thermolabile variant (Cases 4,8–12) (Mann–Whitney U-test).

5.2. Lymphocyte CPT II activity in the patients

As shown in Fig. 2(b), CPT II activity using peripheral lymphocytes of a patient with a thermolabile F352C CPT II variant was significantly reduced to about 50% during incubation for 120 min at 41 °C as compared to those at 30 and 37 °C. All patients with a thermolabile F352C CPT II variant showed a significant reduction of CPT II activity at 41 °C.

Fig. 2(a) shows CPT II activity in a patient with the V368I CPT II variant without reduction even at 41 °C.

5.3. Blood ATP levels in patients with acute encephalopathy

As shown in Fig. 3, ATP levels in the extracts of whole blood in the acute phase of encephalopathy during high fever were significantly low (0.58 ± 0.16 mM, $n = 10$) compared with those in the convalescent phase (1.08 ± 0.27 mM, $n = 5$) and with those of patients with febrile seizure status (1.01 ± 0.36 mM, $n = 9$). The blood ATP levels in the acute phase of encephalopathy revealed no significant difference when compared to those of patients with mitochondrial disease exhibiting several symptoms (0.79 ± 0.39 mM, $n = 6$).

6. Discussion

Although the precise pathomechanisms of acute encephalopathy have yet to be clarified, it is postulated that some genetically-determined factors might be involved, because some types of acute encephalopathy are more frequent in Japanese than in Caucasians. Chen et al. [12] demonstrated that the thermolabile phenotype of CPT II variations such as the F352C CPT II variant or complex [F352C + V368I] CPT II variant might be a principal genetic background of IAE in Japanese. On the basis of the analysis of fatty acid oxidation and cellular ATP production in COS-7 cells transfected with wild-type and variant *CPT2* cDNAs at 37 and 41 °C, Yao et al. [14] suggested that the compound *CPT2* variants with thermolabile phenotypes are the main cause of multiple-organ failure, particularly in high ATP-consuming organs as well as endothelial cells and play a major role in the etiology of IAE.

In the 12 patients with acute encephalopathy studied, six patients (Cases 1–3 and 5–7) had thermolabile F352C CPT II variants (F352C CPT II variant alone in one case and complex [F352C + V368I] CPT II variants in five cases), which were reported to be frequently noted in severe IAE patients [12,14]. Of the six patients, two patients (Case 1, IAE and Case 2, *Hemophilus influenzae*-associated septic encephalopathy) died despite intensive care. Case 2, who died of fatal septic encephalopathy [16], showed a high acylcarnitine ratio ((C16 + C18:1)/C2:0.203) on admission. This value corresponded to the ratio (>0.09) of the high-risk group of patients with IAE showing a fatal outcome, thus reflecting the disorder of mitochondrial β-oxidation. [12]. The remaining six patients (Cases 4 and 8–12) with acute encephalopathy without a thermolabile F352C CPT II variant followed a relatively mild clinical course (Table 1). Out of the six patients, five had a V368I CPT II variant.

As shown in Fig. 2, the CPT II activities of lymphocyte in patients with the F352C CPT II variant showed thermal instability, that is, a marked activity reduction at 41 °C, while those in patients with the V368I CPT II variant did not. There was no significant difference in the age at onset, duration of high fever, and duration of seizures between the six patients with the F352C CPT II variant (Cases 1–3 and 5–7) and six patients without this variant (Cases 4 and 8–12). Therefore, taken together, it seems likely that a thermolabile F352C CPT II variant might be related to the severity of disease, that is, the rapidity of progression of brain edema. In Caucasians, two polymorphisms of CPT II, p.V368I and p.M647 V, occur with a frequency of 0.5 and 0.25, respectively, exhibiting a Hardy–Weinberg equilibrium. A third polymorphism, p.F352C, occurs with a frequency of 0.21 exclusively in the Japanese population [17]. Therefore, this thermolabile F352C CPT II variant might be one of the predisposing factors to trigger the pathomechanism of acute encephalopathy in Japanese.

The CPT system regulates the entry of long-chain fatty acids into the mitochondrial matrix for β-oxidation. Fatty acid oxidation is an important source of acetyl-CoA for maintaining the tricarboxylic acid cycle.

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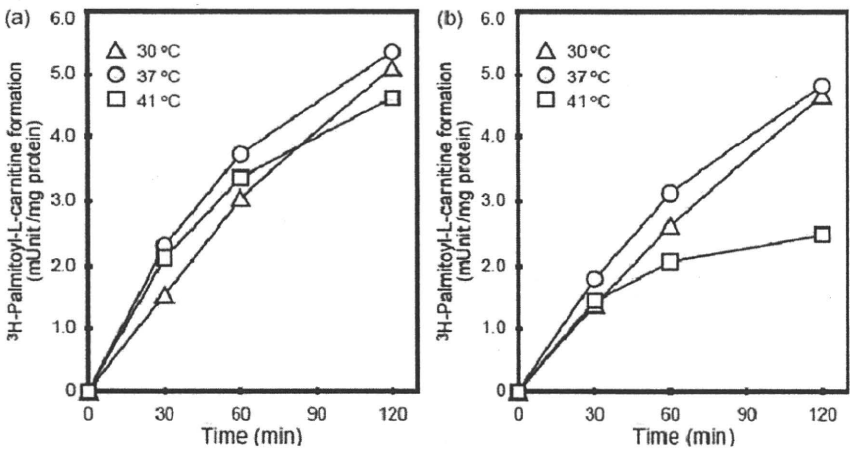

Fig. 2. (a) Lymphocyte CPT II activity in case 12 (influenza-associated encephalopathy) with V368I CPT II variant at 30, 37 and 41 °C. No definite reduction of CPT II activity was observed at 41 °C. (b) Lymphocyte CPT II activity in Case 1 (influenza-associated encephalopathy) with a thermolabile F352C CPT II variant at 30, 37 and 41 °C. At 41 °C, the CPT II activity decreased to about 50% of that at 37 °C after 2-h-incubation.

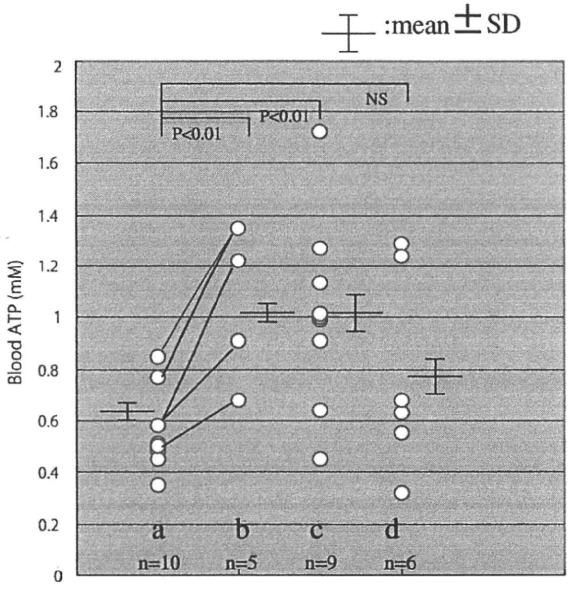

Fig. 3. ATP levels in whole blood in patients with acute encephalopathy (acute (a) and convalescent phase (b)), febrile seizure status (c) and mitochondrial disease (d). In five patients with acute encephalopathy, blood ATP level recovered at convalescent phase.

The CPT II is ubiquitously expressed in all tissues that require fatty acid oxidation as an energy-producing pathway [18]. CPT II deficiency is a disorder of long-chain fatty acid oxidation. It is classified into three clinical types based on the age at onset and disease severity: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. It is clear that our patients' clinical manifestations did not correspond to any of these three types. The thermolabile instability of the F352C CPT II variant in our cases explains the situation whereby impaired energy metabolism could occur during high fever due to a secondary CPT II deficiency in spite of the absence of symptomatic manifestations of CPT II disorder in daily life at a normal temperature [12,14].

Olpin et al. [19] reported based on mutation analysis that when CPT II activities are above 20% of controls, fatty acid oxidation in fibroblasts is usually within the normal range (>70% of controls). However, under heat stress, fasting, acidosis, and seizures, moderately lowered CPT II activity due to the thermolabile F352C CPT II variant may accelerate the disease process of acute encephalopathy.

Blood ATP levels in the acute phase of encephalopathy during high fever were significantly lower than those in the convalescent phase and also with those of patients with febrile seizure status. This suggests that mitochondrial energetic failure may be more severe in patients with acute encephalopathy, and the pathological process of acute encephalopathy should differ from the febrile seizure status. The low levels of ATP in the acute phase of encephalopathy were normalized in the convalescent phase in line with clinical recovery. Interestingly, blood ATP levels in the acute phase of encephalopathy corresponded to those of mitochondrial disease with several symptoms. Yao et al. [14] showed that COS-7 cells transfected with thermolabile [F352C + V368I] CPT II variants exhibited significantly decreased fatty acid oxidation and subsequent intracellular ATP reduction at 41 °C. The decreased ATP levels seemed to reflect systemic mitochondrial dysfunction including the blood brain barrier (BBB) at the acute phase of encephalopathy in our cases. The ATP demand per body weight is so high in infants that a thermolabile CPT II variant induced-ATP reduction might lead to a greater susceptibility to the pathophysiology of encephalopathy in children than in adults.

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The brain capillary endothelium is characterized by a greater density of mitochondria than that of peripheral capillaries [20]. This greater mitochondrial density is required to maintain the significant active transport mechanisms, electrochemical gradients, autoregulatory adjustments, and regulation of tight junctional complexes. As such, the requirement of a constant ATP supply may make the BBB particularly susceptible to acute hypoxic insult [21]. From a similar perspective, BBB breakdown may occur at an initial stage of encephalopathy under the condition of ATP reduction, thus leading to subsequent brain edema due to complex cascade of hypercytokinemia, excitotoxicity, and oxidative stress. Although there is one hypothesis that cytokine storm due to virus–glial cell interaction might cause endothelial cell damage (BBB breakdown) leading to brain edema and neuronal injury [11], we consider that endothelial cell damage might induce in turn cytokine production resulting in neuronal damage in patients with thermolabile F352C CPT II variant irrespective of encephalopathy type .

In three patients with febrile delirium associated with influenza virus infection (Cases 13–15), Case 13 with a thermolabile F352C CPT II variant developed a short seizure and an intermittent confused state with visual hallucinations and agitation lasting 6 h. Cases 14 and 15 without F352C CPT II variant showed short-term consciousness alteration and abnormal behavior without seizures. All patients' brain MRIs were normal, and they fully recovered. Although more extensive study is needed, the grade of febrile delirium associated with influenza virus was more severe in a case with a thermolabile F352C CPT II variant when compared with that in cases without F352C CPT II variant.

Given that a thermolabile CPT II variant might be one of the predisposing factors for acute encephalopathy, we should revise the therapeutic strategy from the acute phase. Considering the rapid progression of encephalopathy and associated low CPT II activity during high fever, immediate hypothermia, sufficient glucose infusion, and L-carnitine supplementation should be adopted as treatment options. We speculate that the immediate hypothermia led to the recovery of the lowered CPT II activity and, thus, mitochondrial energy failure became minimal in many tissues including the brain capillary endothelium, leading to less severe damage to the central nervous system.

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小児の発達症候学

ヒトの随意運動の発達

久保田雅也

随意運動の諸相

- 随意運動とはその名のごとくわれわれが意図して（随意的に）身体を動かす（あるいは止める）ことである.
- しかしわれわれの身体を駆動した意図はどこから生まれ何によって制御されているのか，そもそもその随意性はどこまで意識できるのか，意識できない部分はどういう神経学的基盤を有するのか，「心」と「身体」の関係はどこまで記述できるのかなど，答えの不明な問いは際限なく出てくる.
- ここでは随意運動の諸相を Tourette 症候群，9 か月乳児，表情筋の収縮に情動性と随意性で解離のみられた症例から神経学的基盤を考察してみる.

Tourette 症候群（TS）にみる随意運動の発達

WBC：World Baseball Classic

- マリナーズのイチローが，「2009 年の WBC 最後の打席が最も雑念の多かった打席であった」と述懐していた．卓越した打撃技術にとっても雑念[*1]は大きな影響を与えるということであろう.
- それでもイチローはヒットを打ち，英雄となった.
- イチローが獲得していた打撃の内部モデルは，雑念に影響されないほど身体がおぼえ込んでいたということかもしれない.
- この「身体がおぼえ込む」「身体が勝手に反応する」ということは随意運動が複雑になればなるほど必要になってくる.
- 熟練の過程は意識した運動から無意識の運動への変化，自動化の過程である.
- 「意識して意のままに」動かすことを「随意性」とすると「勝手に反応した身体」は「随意性」をはずれる.
- この「随意性」とは何であろうか.「随意性」と「不随意性」は「意識」と「無意識」と同様，お互いを排除する概念なのであろうか.
- Tourette 症候群（TS）の主たる徴候としてのチックは，瞬き，顔しかめ，肩をすくめる，咳払い，発声などさまざまであるが，これらは一定時間止めようと思えば止めることが可能なので，随意性があるといえる.
- しかし無理に止めるとその後増加し，止め続けることは困難となって随意性の及ばない状況となるが，ミオクローヌスなどのようなまったくの不随意運動とも異なる[*2].
- 多くの TS の患者ではチックの出現前に「前兆としての衝動やもやもやした不快感[*3]」が現れる.
- TS の本来の不随意性はこの不快な前兆にあり，チック自体の随意性は大きく損なわれてはいないとする説もある.

*1
内部からの己の技術に対するゆらぎや外部からの期待やゆさぶり.

*2
随意性とも不随意性とも確定しにくい運動を準随意運動（quasi-volitional movement）と呼ぶこともある.

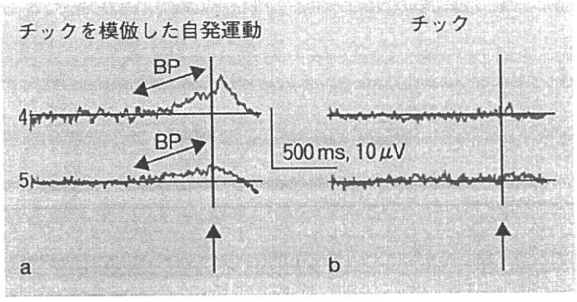
*3
premonitory urge, premonitory sensation.

●花粉症の患者でかゆい眼をこすり続けたり，瞬きを繰り返した後，花粉症が治癒しても瞬きがチックとして持続することがある．何らかの感覚運動ゲーティングの異常を示唆する．

●また強迫性障害が基盤にあり，不合理とわかっていてもしっくりくるまでやり続けるという"just-right" phenomena の側面もある．

●TS の患者で上肢のチックとそれを模倣した自己ペースでの随意運動の運動誘発電位（MRCP）をみる（❶）と，チックでは運動準備電位が出現していない[1)*4, *5]．

❶ 2 人の Tourette 症候群でのチック（上肢屈曲，b）およびそれを模倣した場合の運動誘発電位（a）



†：筋収縮開始．

（Karp BI, et al. 1996[1)] より引用，一部改変）

●われわれが自己ペースで随意的に身体を動かす 1 秒以上前から両側補足運動野および運動前野は活性化され，意識下で運動準備が始まる．

●自己ペースでの随意運動とはいえ「随意性」の中には意識下での過程も含まれることになる．

●チックでその運動準備にかかわる過程が記録できない場合があるということは，その「随意性」が自己ペースでの随意運動とは異なるということである．

●この「勝手に動く」チックと先のイチローの「身体が勝手に反応する」打撃技術とはどこが異なるのであろうか．

●「習慣化」しているという点では共通であるが，その獲得過程を学習という面からみると強化学習の過程に違いがあるのかもしれない．

●またチック自体は高度の集中を要する行為*6，歌唱やスポーツの最中には通常起こりにくい．

●彦坂は，チックは本来は報酬がないはずの行動に基底核のレベルで報酬としての信号が入り，それが強化されて繰り返されることにより起こるのではないかと述べている[2)]．

●なぜ不合理とわかっていても儀式のように同じ行為・行動を繰り返すのであろうか，また何を打ち消したいのか，その繰返し行為そのものが目的なのか，または単に習慣化しただけなのか．

●ヒトの正常な（生理的な）行為・行動と心理・情動機制の仕組みの中にその本態はすべてあると思われるが．

●チックの頻度が抗精神薬*7で減少することから，TS ではドパミン神経伝達亢進が示唆される．

●野村[3)] は TS の主病態を黒質と腹側被蓋野のドパミン神経の線条体への投射の低活性が発達過程に生じ*8，基底核間接路ではドパミン伝達過剰状態が生じ，皮質-基底核-視床-皮質回路*9 が機能変容を起こす点に求めている．

●TS の病態には不快な前兆とチック発現*10，その習慣化*11，併発症発現*12，一部でのチック固定化など，随意運動を修飾する多様な系の発達期の異常が基盤にある．

BP：bereitschaftspotential（運動準備電位）

MRCP：movement related cortical potentials

＊4
運動準備電位がチックにも出現した例も同論文に報告されている．

＊5
この運動準備電位は自己ペースでの筋収縮に先立つ緩やかな陰性電位から成り立ち，成人で 1 秒以上前から出現し，その早期成分は両側補足運動野および運動前野，後期成分は反対側運動野および運動前野が起源だとされる．

＊6
goal-directed behavior.

＊7
ハロペリドールやリスペリドンなどドパミン D_2 受容体阻害薬．

＊8
受容体過感受性が基盤にあり，発達過程に出現する経年齢性減衰変化の加速された状態．

＊9
運動系では補足運動野，感覚運動野，非運動系では眼窩前頭皮質，前帯状回が重要．

＊10
感覚運動ゲーティング，ドパミン伝達過剰．

＊11
変容した強化学習．

＊12
非運動系回路の変容．

- われわれの随意運動のなかには本来的に，意識した制御の及ばない要素があるということをTSの病態は指し示してくれている．

乳児における随意運動と他者の運動の観察

- 乳児における随意運動は1歳までに劇的に変化するが，その「随意性」と「反射性」の解析は容易ではない．
- Southgateら[4]は9か月乳児での到達運動の際の脳波の周波数変化，とくにαリズムの減衰に着目して解析を行った．
- ❷aに示すように乳児の手の到達運動開始前からαリズムの減衰が始まっている．
- また他者の手の到達運動を観察したときにもα領域の減衰が認められる(❷b)[*13]．
- 9か月乳児での自身の到達運動や他者の到達運動の観察の際のαリズムの減衰は年長児・成人における同様の条件でのμリズムの減衰・消失に等価であると推定できる．
- μリズムは感覚運動皮質に起源を有し，ミラーニューロンシステム[*14]（下前頭回，上側頭溝，下頭頂小葉）から制御を受ける．
- 減衰したαリズムの起源は不明だが，μリズムと同様に考えることも可能である．
- また手の到達運動開始前からαリズムが減衰したことは，その運動準備性の側面も推定させる．
- つまり乳児の物に対する到達運動は，少ない運動パターンの中で随意的[*15]に行われたとみなしてよい．
- それを視覚情報として受け取った場合には，すでに獲得された運動レパートリーの運動表象[*16]へ自動的に変換されるということも表している．

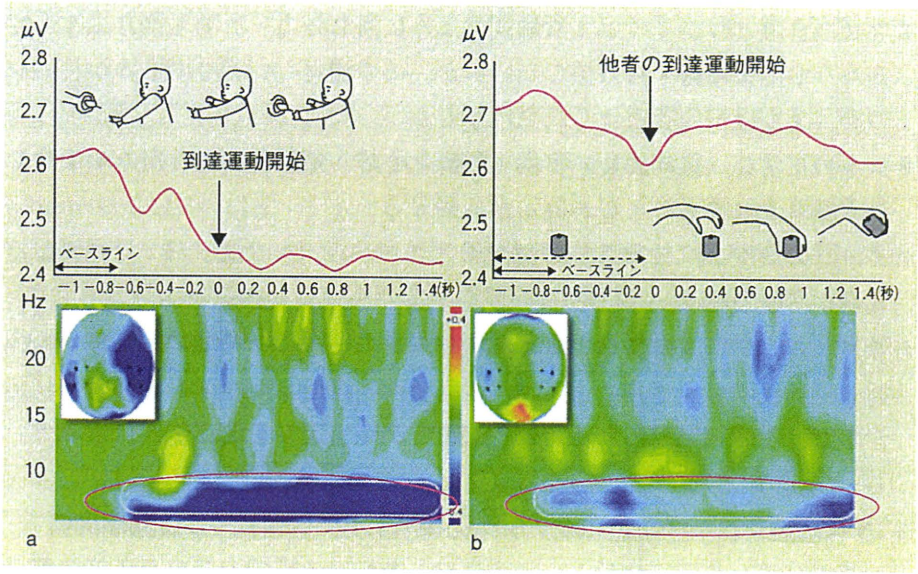
*13
これは何度か課題を繰り返すうちに認められたもので，行為の予測や期待とも関連する．
これらの減衰は6か月の乳児では認められていない．
年長児・成人においても他者の運動の観察のみでμリズムの減衰・消失が起こる．

*14
リゾラッティはミラーニューロンシステムの第一の役割を「他者の行為の意味の理解」とし「行為の共有空間を生み出す」もので，認知的側面とは異なるとしている[5]．
また見る側があたかも自分が行っているかのように観察できるかどうかは観察者本人の運動レパートリーによって決まり，観察した行為を運動の言語でコードし，その行為を再現可能にするという，模倣における根本的な役割を果たしているとした[5]．

*15
目標志向性に，goal-directed.

*16
motor representation.

❷ 9か月乳児の手の到達運動および他者の到達運動観察時のα波の減衰



a：手の到達運動，b：他者の到達運動観察．

(Southgate V, et al. 2009[4]より引用，一部改変)

- 随意性という場合，熟練するとどこまで意識されているかを問えないのは乳児でも同じかもしれない．

表情筋における随意性の解離

- 笑いはほぼヒトに特有の表情であり，その中枢性支配は複雑である．
- 時に脳血管障害において症例の❸に示すように笑えるにもかかわらず，「イー」という発声を命じると顔片側下半分の収縮ができない解離した状態に遭遇する．
- これは顔面の随意的収縮と笑いでは機能している中枢神経系の回路が異なるために起こる．
- サルでは，皮質から脳幹顔面神経核へは5つの回路がある．これによると顔面上半分は補足運動野（M2），吻側帯状運動皮質（M3）から両側支配を受け，下半分は対側一次運動皮質（M1），腹側外側運動前野（LPMCv），尾側帯状運動皮質（M4）から片側支配を受ける（❺）*17．
- M2，M3，M4は前大脳動脈の支配を受け，M1，LPMCvは中大脳動脈の支配を受けるため，障害された部位により臨床症状に差異が生まれる．
- ヒトでもほぼ相同と考えられ，M1，LPMCvは対側顔面下半分を強力に支配するためこの部分の皮質や皮質下白質が障害されると随意運動（たとえば「イーと発声する」）での当該部位の収縮が障害される*18．
- ところが笑ったときの表情はM1，LPMCv以外の関与（とくにM4）が大きいため，たとえM1，LPMCvが障害されても他が障害を免れていれば❸左写真のように保たれる．
- 2つの帯状運動皮質（M3，M4）は辺縁系からの強力な入力があり，笑いという情動と不可分な行為は単純な随意運動とは別経路でなされているの

*17
Morecraftら[6]の研究による．

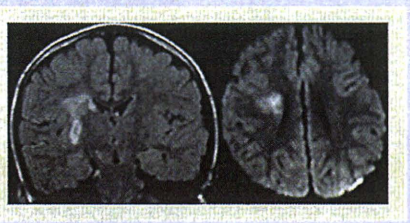
*18
❸の右写真での左鼻唇溝消失．

症例

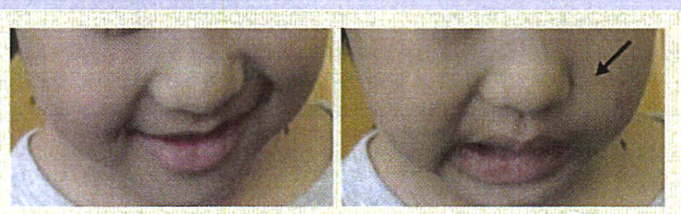
7歳，男児（❸）．

診断：原因不明の脳梗塞．転倒後に発症．左上肢の巧緻性低下，左下肢の軽度痙性と顔面筋収縮に笑いと随意運動で解離を認めた．MRI上右深部白質，右島，右内包後脚，右大脳脚外側に信号異常あり（❹）．

❹ 症例の頭部MRI

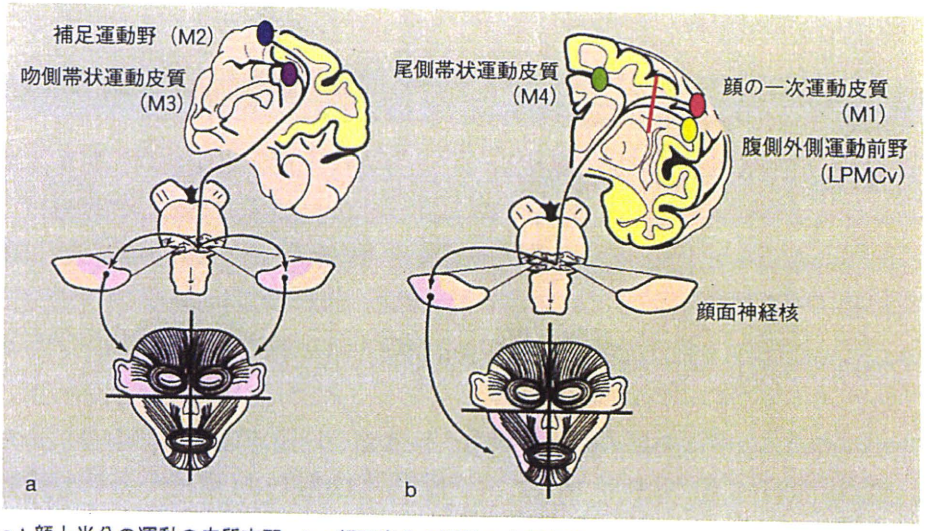


❸ 表情筋の随意性の解離



笑ったときには顔面筋は対称に収縮するが，「イー」という発声では左表情筋は収縮せず非対称となる（矢印）．

❺ 顔面筋の中枢からの支配様式



a：顔上半分の運動の皮質支配，b：顔下半分の運動の皮質支配．
症例1，2における病変（—）．

（Morecraft RJ, et al. 2001[6] より引用，一部改変）

であろう*19．

- Iwase ら[7] は PET を用いた研究で，コミックをみて笑ったときの笑いの程度は両側補足運動野と左被殻の活性が優位に相関したが，一次運動皮質の活性とは相関しなかったと報告した．
- これに対し単純な顔の随意運動では一次運動皮質と補足運動野の活性化を認めている．
- こういう運動の情動性支配は表情筋だけなのであろうか．
- Morecraft らの図[6] では表情筋と同様に MI 以外に M2，M3，M4 に体部位局在性をもって刺激により上下肢を収縮させるニューロンがある．
- 通常の随意運動では M1 の支配が絶大で前面に出ることはなく，情動刺激による運動では表情の影に隠れて目立たないが上下肢の情動性運動はあるのではないだろうか*13．
- 表情筋を中心とした運動の情動性支配は，四肢の目標志向的な goal-directed 随意運動とは異なる発生と発達を示す．

*19
臨床的には M1，LPMCv が保たれて M3，M4 が障害された場合，逆に単純な顔の随意運動は可能だが笑えないという状態になる．

PET：positron emission tomography

*13
いわゆる「火事場のばか力」も，情動性出力も含めて皮質を総動員した結果かもしれない．

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神経生理学的検査からみる発達

MEGを用いた小児の発達の解析

久保田雅也

MEG：magnetoencephalogram（脳磁図）

良性Rolandてんかんとは

- 良性Rolandてんかん*1（BRE）は❶の特徴を有する小児期特有のてんかん症候群である．
- この症候群は年齢依存性に脳波異常や発作の発現，消失がみられ，とくに感覚運動皮質の発達上の変容が基盤にあると考えられる．
- この特異な症候群の脳波変化と発達特性の解析はてんかんと発達を考察する際に重要な知見を提供する．
- 「良性」と冠されることから無治療経過観察も可能な場合があるが，その定義は以下のような臨床脳波学的定義であり，不均一な症候群と考えられる．

*1
中心・側頭部に棘波焦点を有する良性小児てんかん．

BRE：benign rolandic epilepsy

睡眠とRDの関係

- 終夜脳波の観察によると，Roland発射（RD，❷）は覚醒時では認めないこともあるが，non-REM睡眠で増加，REM睡眠で激減する（❸）．
- とくにREM期から浅睡眠（睡眠第Ⅰ，Ⅱ段階）へ向かう時期で急増し，深睡眠（睡眠第Ⅲ～Ⅳ段階）からREM期に向かう時期に急減する．
- REM期は脳幹コリン作動系の活性化とセロトニン作動系，ノルアドレナリン作動系の抑制により形成される．
- これに対しnon-REM期には脳幹コリン作動系は抑制される．
- RDの出現頻度と脳幹コリン作動系の活性は逆相関するといえる．

RD：rolandic discharges

REM：rapid eye movement

RDの電流源

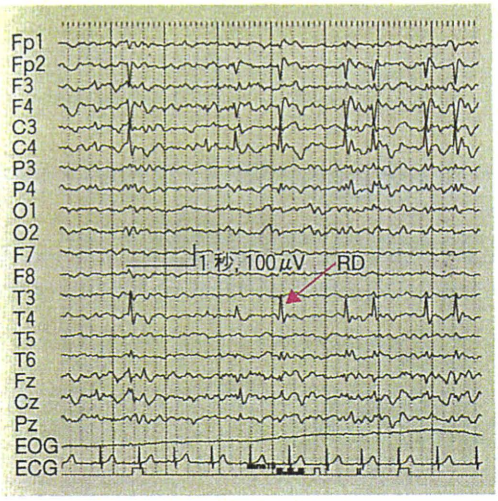
- MEGによる検索では，典型的BREのRD電流源は中心溝周囲の口腔顔面の一次感覚運動皮質にある[2]（❹）．
- この局在はSylvius発作の起始部と考えられる*2．

*2
このことは口唇刺激による誘発磁場とRDの頂点の局在解析から初めて確かめられた[3]．

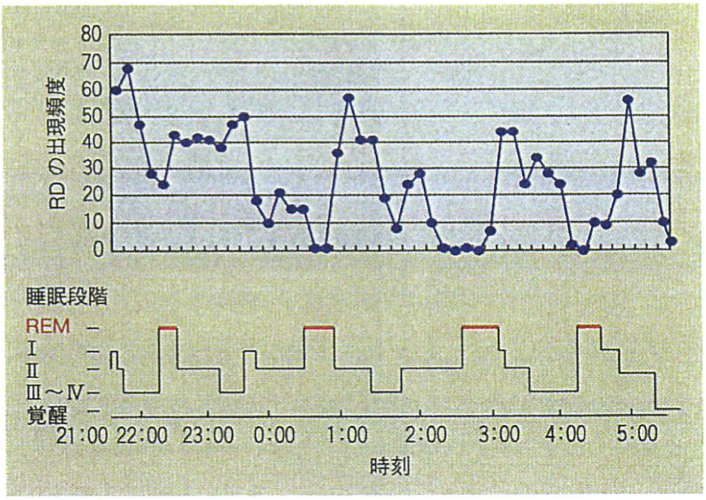
❶ 良性Rolandてんかんの特徴

発症年齢	3～12歳（平均7歳）．
典型的発作型	寝がけや起きがけの片側口腔顔面の運動発作，唾液分泌過剰，発語停止（これらをSylvius発作と呼ぶ）などの単純部分発作，上肢の運動発作，および二次性全般化．
脳波	中心側頭部のRD（❷）．右中心側頭部に高振幅陰性鋭波を認めるが，時に小さい陽性棘波が先行する．また前頭部には高振幅陰性鋭波に対応した陽性鋭波を認める．頭頂部にまで及ぶことや律動化や全般化することもある．
経過	発作コントロールは良好で，10歳前後での発作の消失が先行し，その後RDは遅くとも14～15歳までには消失する．急性期に空間認知の異常，注意集中困難，学習障害などを認めることがある．
病理	中枢神経系に器質的異常を認めず．
原因	不明であるが，患者の同胞の脳波検査の解析からRD自体が常染色体優性遺伝で伝わり，その中のごく一部（10%以下）が発作を発現することが示唆され，遺伝的要因が強いと考えられる[1]．男児優位であることも遺伝的要因が単純ではないことを示唆している．

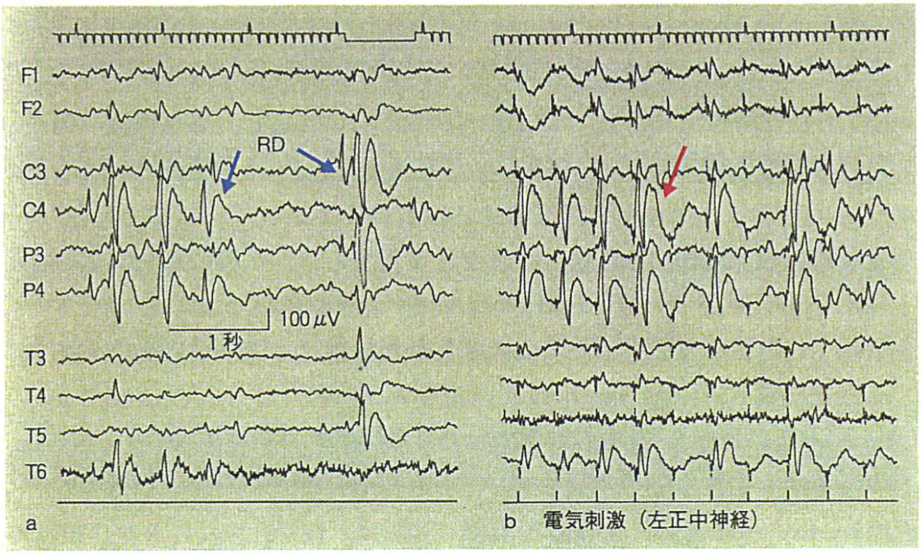
❷ BRE 7 歳男児の睡眠脳波



❸ 睡眠段階と RD の出現頻度

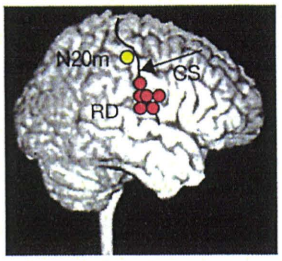


❺ 左正中神経の電気刺激で C4，P4 に出現した RD（a）と類似した波形（b. →）



a：通常の脳波での RD（→），b：左正中神経刺激での RD 類似波形（C4，P4）

❹ N20m と RD の位置



N20m（●）は中心溝（CS）後方の一次感覚皮質の場所を示し，RD の電流源はその下方，口腔顔面の一次感覚運動皮質に存在する（●）．

CS：central sulcus

＊3
巨大 SEP
体性感覚誘発電位（SEP）は正中神経の電気刺激で頭皮上脳波を加算平均して得られる電位．同様にして得られる磁場を体性感覚誘発磁場（SEF）という．皮質の過興奮性により反応が巨大化したものを巨大（giant）SEP（SEF）という．正中神経刺激での巨大 SEP は手の感覚処理過程にかかわる皮質の過興奮性を示唆する．

SEP：somatosensory evoked potentials

SEF：somatosensory evoked fields

＊4
すでに 1970 年代から De Marco が TES と SESE の解析を行い，先駆的な業績をあげている[5]．

TES：tactile evoked spikes

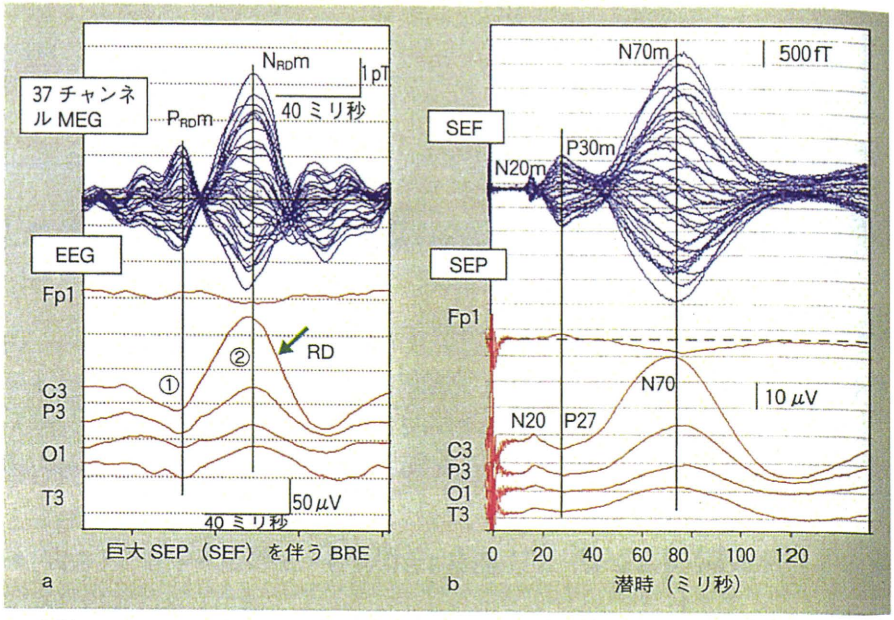
SESE：somatosensory evoked spikes epilepsy

- 症例によっては，手の一次感覚運動皮質や二次感覚皮質に局在が広く分布して認められる．

巨大誘発電位を有する症例

- ときに BRE の患者で正中神経の電気刺激で反対側頭頂部に RD と類似の波形（巨大 SEP[*3]）を認める[4]（❺ b）が，形態および頂点間潜時からこれらは同一の起源をもつと考えられる．
- この巨大誘発電位の起源や変化を探ることで RD の起源や発達特性に接近することができる[*4]．
- これらの患者で RD および正中神経刺激による反応を，脳波脳磁図同時記録したものが❻である．
- 自発脳波および MEG における RD と体性感覚誘発電位および磁場（SEP，SEF[*3]）は形態，頂点間潜時ともに対応していることがわかる．

❻ RD および体性感覚誘発反応（正中神経）の脳波脳磁図同時記録



a：左側 RD の MEG と EEG，b：右正中神経刺激による SEF と SEP.

EEG：electroencephalogram（脳波）

＊5
P30m
m：magnetic

＊6
Lafora 病，ガラクトシアリドーシス，セロイドリポフスチン病など．

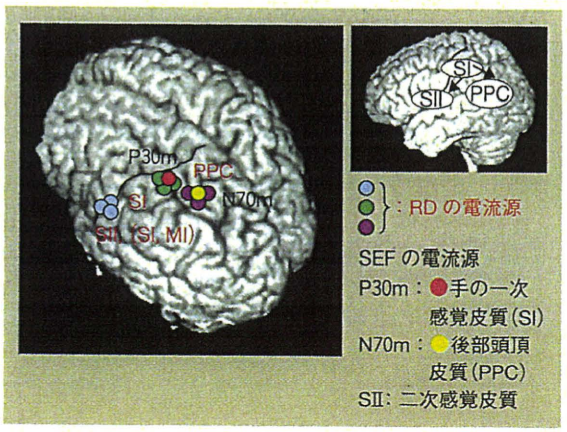
PPC：posterior parietal cortex

＊7
一部の症例では N70m 電流源は二次感覚皮質（SII）に局在した．

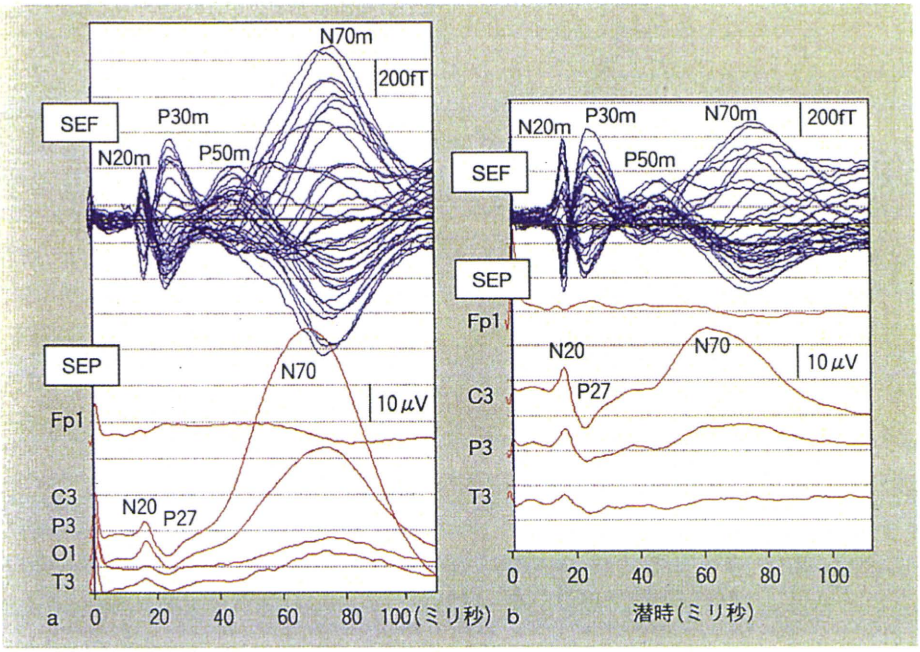
＊8
ascending sequential maturation.

- RD の初期陽性波（❻①）は P30（P30m[*5]）に対応し，陰性鋭波（❻②）は巨大中潜時成分 N70（N70m）に対応する．
- 皮質反射性ミオクローヌスを有する進行性ミオクローヌスてんかん[*6] の患者では巨大 SEP が出現するが，巨大化する成分は SEP における初期（P25-N33）成分であり，BRE で巨大化する中潜時成分とは異なる．
- それぞれの頂点の電流源を求めてみると❼のようになり，P30m は手の一次感覚皮質（SI），N70m は後部頭頂皮質（PPC，おそらく Brodmann の area 5, 7）に局在し，RD の初期陽性波と陰性鋭波の電流源は P30m，N70m の電流源に重畳した．
- RD と正中神経電気刺激による SEP，SEF は，波形，空間的分布，頂点の時間的関係において対応することがわかる．
- 正中神経刺激による巨大 SEP の有無で BRE を 2 群に分けると，巨大 SEP を有する群は P30m，N70m の電流源強度が有意に大きいものであった．
- これに対し，初期皮質反応である N20m は電流源強度に有意差はなかった．
- このことから，RD の発生の要因は視床から手の一次感覚皮質（SI）へ到達以降の感覚情報処理過程（P30m-N70m の巨大化），とくに中潜時成分の巨大化にあると思われる[*7]．
- 高次な機能ほど成熟に時間を要する[*8] とすると，SII，PPC における反応の巨大化は感覚処理過程における SII，PPC の成熟遅延と思われる．
- 遅れた成熟がくるころに，けいれん源性は終息する．
- ❽に示すように，発作のあった 9 歳時に比較す

❼ 巨大 SEP を有する BRE の RD 電流源と SEF 電流源



❽ 巨大 SEP を有する BRE の 9 歳(a)および 11 歳(b)の SEP(SEF)右正中神経刺激



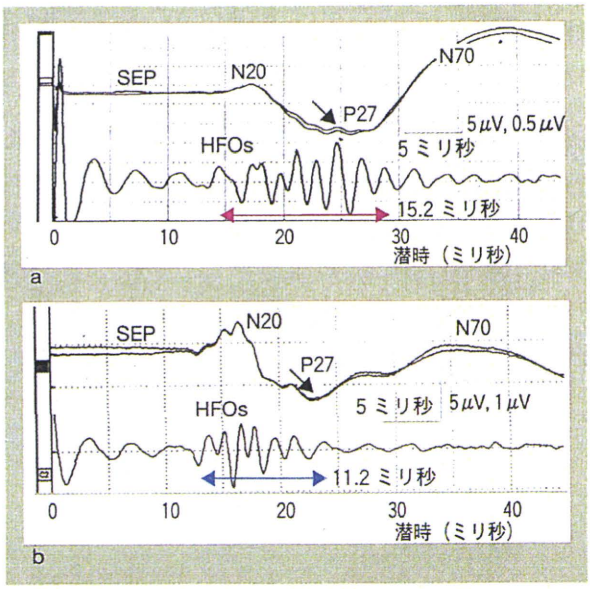
ると，発作消失後の 11 歳時での N70（N70m）は振幅，電流源強度がともに低下した*9.

- BRE の少なくとも一部は，高次の感覚処理過程に関連する皮質の過興奮性が発作および RD 出現に関与する.
- RD を有する者のうち 9 割以上は臨床的に発作を起こさないが，その理由は不明である.

SEP における高周波振動（HFO）

- 通常の正中神経刺激による SEP の記録に 400～800 Hz のフィルター処理を行うと，初期成分 N20，P27（N20m，P30m にほぼ等価）に重畳した約 600 Hz の高周波成分がとらえられる.
- 一般に BRE と他の小児期発症てんかん症候群で比較すると，BRE のほうが 600 Hz HFO の持続が長いことが知られている[6].
- 先述の巨大 SEP（巨大 N70）を有する BRE では，それをもたない BRE に比してさらに振幅が大きく潜時の長い 600 Hz HFO を認める（❾）.
- 後者では N20 にほぼ重畳するが，前者では P27 にまで及ぶ.
- 同様の 600 Hz HFO*10 持続の増加は Parkinson 病や一部の進行性ミオクローヌスてんかんで認められる.
- この高周波の生理学的意義については諸説ある

❾ 通常の正中神経刺激 SEP とフィルター処理（400～800 Hz）して得られた高周波振動（HFOs）



a：巨大 SEP（N70）を有する BRE 8 歳女児.
b：巨大 SEP（N70）をもたない BRE 8 歳男児.

*9
図示していないが脳波上の RD の振幅，出現頻度も 11 歳で減少した.

HFO：high frequency oscillation

*10
600 Hz HFO は前半（N20 に重畳）と後半（P27 に重畳）で生理学的意義が異なるが，前半は基底核機能とも関連し，後半はとくに GABA 作動系の burst を反映する.

GABA：γ-aminobutyric acid（γ-アミノ酪酸）

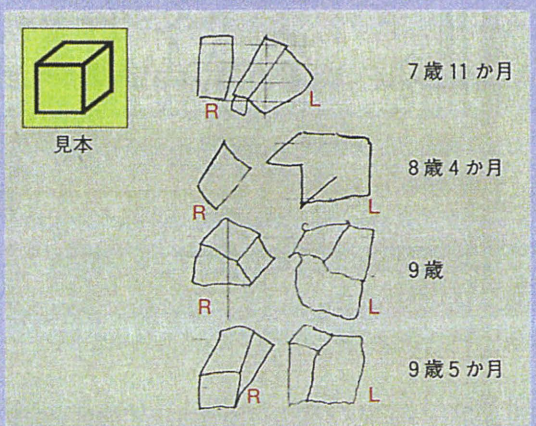
が，橋本は体性感覚 3b 野の 4 層に存在する GABA 作動性抑制性介在ニューロンの活動を反映していると推定している[7]．

- GABA 作動性抑制性介在ニューロンは覚醒とともにアセチルコリンやセロトニンによって賦活され，下流のグルタミン酸作動性錐体細胞の抑制を増強する．
- この 600 Hz HFO は覚醒時に出現するが，non-REM 睡眠中は消失する[8]．REM 期にはその振幅は減衰するが，覚醒時同様認める[8]．
- 興味深いのは ❸ に示したように RD は REM 期や覚醒時にほぼ消失するが non-REM 期に増加し，600 Hz 高周波振動とは相反した振舞いをみせることである．
- 巨大 N70 に関しても睡眠中の振幅は増大する．
- 以上から，正常において睡眠段階を規定するコリン作動系やセロトニン作動系の GABA 系を介した錐体細胞のフィードフォワード抑制や脱抑制に未熟性があり，600 Hz 高周波振動の持続延長と RD の相反性の基盤となっている可能性がある．
- 今後 RD と睡眠，高周波振動，高次機能のさらに詳細な検討が必要であろう．

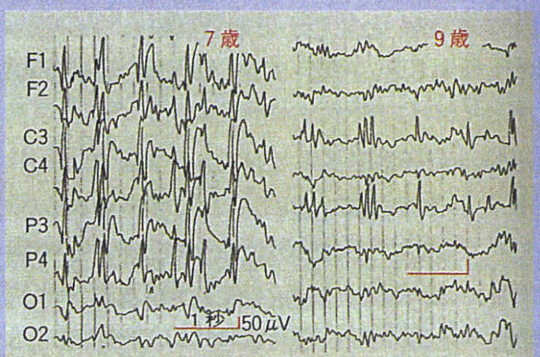
症例 1　BRE

11 歳，男児．右利き．6 歳で典型的 Sylvius 発作が始まり，月に 2 回以上となったためバルプロ酸（VPA）の服用を開始．7 歳での WISC-R の結果：V-IQ 101，P-IQ 112，F-IQ 107 であったが，block design や object assembly は低得点であった．立体描写は ❿ のように 7～8 歳では拙劣であり，臨床的にはごく軽度の注意集中困難や多動を認めたが，とくに治療は要さず．⓫ に示すように脳波の改善とともに立体描写は改善し（❿），ADHD 様の行動も消失した．左右の半球から出現していた RD が左のみに減少した時期と臨床症状の改善が符合する．右半球の RD と空間認知の異常を示唆する報告がある[9]が，本例も右頭頂葉の機能不全が立体描写に象徴される構成機能を阻害していた可能性がある．

❿ 症例 1 における立体描写の推移



⓫ 症例 1 の脳波の変化



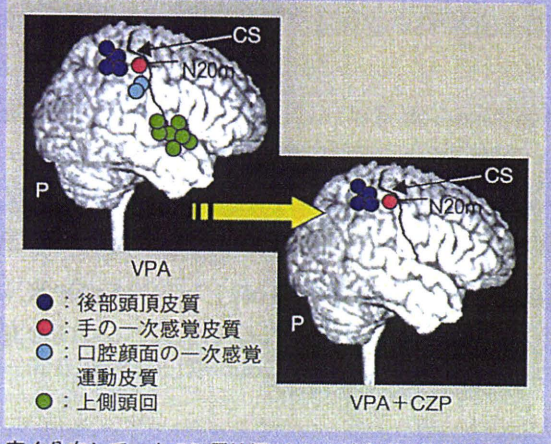
7 歳時（a）両側から律動的に出ていた RD が 9 歳（b）になり左半球に限局した．

VPA：valproic acid
WISC-R：Wechsler intelligence scale for children-revised
ADHD：attention deficit hyperactivity disorder（注意欠陥・多動性障害）

症例 2　口舌失行を伴う Roland てんかん

10 歳，男児[10]．3 歳で典型的 Sylvius 発作が始まり，週 2 回以上となったため，カルバマゼピンの服用を開始．無効であったためバルプロ酸（VPA）に変更．発作頻度は 2 か月に 1 回に減少．その後発作はほぼ消失したが，9 歳時の神経学的所見としては口舌失行，四肢協調運動障害，流涎，不明瞭な発音を認めた．MEG による RD 電流源の解析では後部頭頂皮質，手の一次感覚皮質，口腔顔面の一次感覚運動皮質，上側頭回に広く分布する電流源を認めた（⑫）．また，ひらがなを読む事象関連磁場の解析では，右口腔顔面の一次感覚運動皮質に持続した活性を認めた（⑬）．頻発する RD と臨床症状の関連を考慮し，RD の減少をねらってクロナゼパム（CZP）を加えたところ，流涎の消失，発音の改善，口舌失行の改善を認めた．CZP 投与後の RD 電流源は後部頭頂皮質のみとなり（⑫），RD 頻度も減少した．本例は多彩な高次機能の問題を示す非典型的な Roland てんかんだが，発作以外の症状にも RD 自体が関連したと考えられ，抗てんかん薬の使用が奏効した．RD 電流源の分布と強度により Roland てんかんの症状は多彩になると考えられる．CZP 投与により RD の減少をみたことは RD の出現に GABA 作動系を介した制御が関与していることを示唆する．

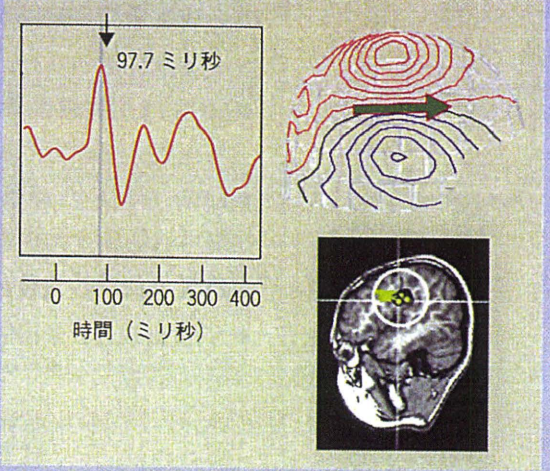
⑫ 症例 2 の CZP 追加前後の MEG 所見



広く分布していた RD 電流源が CZP 追加後，後部頭頂皮質のみに限局した．

CZP：clonazepam

⑬ ひらがなを読む事象関連磁場



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Determinants of outcomes following acute child encephalopathy and encephalitis: pivotal effect of early and delayed cooling

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ABSTRACT

Background Acute encephalopathy/encephalitis is one of the most important causatives of mortality and neurological deficit during childhood. The aim of this retrospective observational study was to investigate clinical variables and therapeutic options associated with the outcome of children with acute encephalopathy/encephalitis.

Methods Relationships between the clinical information at admission and the neurological outcome evaluated using Pediatric Cerebral Performance Category Scale (PCPC) at 12 months after admission were assessed in 43 patients who were treated at 10 Japanese paediatric intensive care units.

Results Sixteen patients were cared for at normothermia, whereas mild hypothermia was applied to 27 children. In univariate analysis, ages ≤18 months, marked elevation in serum lactate dehydrogenase (LD) and aspartate transaminase, diagnosis of either acute necrotising encephalopathy or haemorrhagic shock and encephalopathy syndrome and longer hypothermic periods were associated with increased risks of death or severe neurological deficit, whereas hypothermia showed pivotal effects: the outcome of children cooled after 12 h of diagnosis was statistically invariant with normothermic children, but was significantly worse compared with children cooled ≤12 h. In multivariate analysis, younger ages and elevated serum LD were associated with adverse outcomes, whereas early initiation of cooling was related to favourable outcomes. For normothermic children, PCPC scores were dependent on the computed tomographic findings suggestive of cerebral oedema, serum LD levels and Glasgow Coma Scale at admission. For hypothermic children, PCPC scores depended on longer delays in cooling initiation.

Conclusion Without therapeutic hypothermia, the outcome of children was determined by variables suggestive of the severity of encephalopathy/encephalitis at admission. Hypothermia may have pivotal impacts on the outcome of children according to the timing of cooling initiation following acute encephalopathy/encephalitis.

What is already known on this topic

- Encephalopathy/encephalitis affects more than 1000 Japanese children per annum, with approximately a half of these children resulting in mortality or permanent neurological deficits.
- Hypothermia is neuroprotective following perinatal asphyxia and adult cardiac arrest; however, no randomised controlled trials have been performed for child encephalopathy/encephalitis.

What this study adds

- Therapeutic hypothermia for children with acute encephalopathy/encephalitis may alter the outcome of patients according to the delay in cooling initiation.
- Delayed cooling after 12 h of initial neurological signs of encephalopathy/encephalitis is likely to be deleterious, whereas there remains a possibility that early cooling is neuroprotective.
- Randomised controlled studies of early hypothermia in acute encephalopathy/encephalitis are urgently required.

Acute encephalopathy/encephalitis affects more than 1000 children per year in Japan.1 Of various causatives, flu infection has recently been recognised as one of the commonest triggers in Asian countries.[1 2] According to a recent report on 148 cases of flu-related encephalopathy/encephalitis in childhood, 32% resulted in mortality, whereas 28% led to permanent neurological deficit.[2] There currently is no established therapeutic intervention which ameliorates the outcome of acute child encephalopathy/encephalitis. Therapeutic hypothermia has been demonstrated to be neuroprotective for encephalopathy following perinatal asphyxia[3–5] and adult cardiac arrest.[6 7] Although the neuroprotective effect of therapeutic hypothermia for other pathological conditions has not been demonstrated in the clinical setting,[8–10] several guidelines have already approved careful use of hypothermia for acute cerebral injury in childhood.[11] Consequently, despite the lack of randomised controlled trials which evaluated the protective effect of hypothermia for child encephalopathy/encephalitis, therapeutic

hypothermia for this clinical condition has already become popular among Japanese paediatric intensivists.[12]

We performed a retrospective observational study in children with acute encephalopathy/encephalitis to investigate the dependence of outcomes on clinical backgrounds and therapeutic options. We hypothesised that (1) mild systemic hypothermia initiated shortly after the development of encephalopathy/encephalitis improves the outcome of children without increasing the incidence of adverse events, (2) without hypothermia, the outcome of children would depend on the severity of encephalopathy/encephalitis at admission; however, (3) with therapeutic hypothermia, the outcome would depend on the timing of cooling initiation.

METHODS

This study was performed under the guidance and approval of the local ethical committee.

Study population

Children between 1 month and 14 years old, who developed acute encephalopathy/encephalitis between January 1997 and July 2008 and were cared for at paediatric intensive care units of 10 Japanese tertiary centres, were retrospectively enrolled into the study. Acute encephalopathy/encephalitis was defined as progressive loss or impairment of consciousness with or without other neurological signs such as seizures.[13] Patients with traumatic brain injury, febrile convulsions without prolonged unconsciousness and bacterial meningitis were not included.

Treatment of encephalopathy/encephalitis

For encephalopathic children, six centres routinely provided therapeutic hypothermia throughout the study period; one centre introduced hypothermia only after January 2003; another centre provided cooling only when the routine cranial CT at admission showed significant cerebral oedema; two centres never provided hypothermia. Following the diagnosis of acute encephalopathy/encephalitis, all centres which provided therapeutic hypothermia induced systemic cooling to 33.5–35°C within 48 h of the primary neurological manifestation. Mattresses, through which temperature-adjustable water circulated, were applied over the ventral and/or dorsal trunk of children; hypothermia was initially maintained for 48–72 h according to the protocol of each centre. During cooling, patients were given continuous infusion of thiopental (up to 3 mg/kg/h) or midazolam (up to 1 mg/kg/h) unless contraindicated; in addition, intravenous vecronium or pancronium (up to 0.1 mg/kg/h) was considered when shivering was uncontrollable. At all centres, patients were rewarmed 0.5–1°C per day; rewarming was postponed or slowed down with either the deterioration of neurological findings or signs suggestive of the recurrence of brain oedema with critically increased intracranial pressure. The core temperature was monitored using either rectal or urinary bladder temperature probes; invasive and non-invasive brain temperature monitoring was additionally performed in some children using ventricular, nasopharyngeal or forehead thermo-flux probes. Cranial CT was obtained at timings of admission except for three hypothermic and one normothermic children, of whom MRI was available shortly after admission. CT scans were repeated when the increase in intracranial pressure was suspected; experienced radiologists assessed signs suggestive of cerebral oedema, such as sulcal effacement, size reduction of lateral ventricles and basal cisterns and the loss of grey/white matter differentiation, to assign scores of 0 (up to mild oedema), 1 (moderate oedema) and 2 (severe oedema) (see online supplementary figure 1 for representative CT appearances).

Data collection

Clinical variables were collected for each patient including the age; body weight; sex; type of encephalopathy/encephalitis; preceding infection; Glasgow Coma Scale (GCS) at admission; timing of cooling initiation (hours after the initial neurological manifestation); potential serum markers of tissue damage such as aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LD) at admission; CT scores at admission; additional treatments (intravenous steroid and/or immunoglobulin) and outcome. Preceding infection by influenza virus, adenovirus, respiratory syncytial virus and rotavirus was confirmed, with at least one positive result of viral culture, antigen test, reverse transcription PCR or significantly raised titres in paired serum samples. Types of encephalopathy/encephalitis were defined according to the definition proposed by Mizuguchi and colleagues[13]; for further analyses, acute necrotising encephalopathy, haemorrhagic shock and encephalopathy syndrome and Reye's syndrome were grouped and discriminated from other types of encephalopathy/encephalitis such as acute encephalopathy with refractory seizures and acute disseminated encephalo-myelitis because of their relationship with unfavourable outcome. Adverse events such as hypotension (defined as systolic blood pressures below average values −2 SD for the patients' age in accordance with the Task Force on Blood Pressure Control in Children),[14] pneumonia, thrombocytopenia (<150 000/μl), coagulation disorders (either prothrombin time >12 s, international normalised ratio of prothrombin time >1.2 s or activated partial thromboplastin time >45 s), arrhythmias (excluding brady cardia) and hypokalaemia (<3.5 mEq/l) were also recorded.

Outcome assessment

The neurological performance at 12 months from the development of encephalopathy/encephalitis was assessed by the Pediatric Cerebral Performance Category Scale (PCPC).[15 16] Additional standard outcome scales, that is, the Glasgow Outcome Scale (GOS) and the Pediatric Overall Performance Category Scale (POPC), were also obtained to enable comparison with other studies. For PCPC and POPC, scores 1–6 represent normal performance, mild disability, moderate disability, severe disability, coma or vegetative state and death, respectively, whereas for GOS, scores 1–5 represent death, persistent vegetative state, severe disability, moderate disability and good recovery, respectively.

Statistical analysis

Clinical variables and therapeutic options associated with adverse acute events (hypotension, pneumonia, thrombocytopenia, coagulation disorders, arrhythmias and hypokalaemia) and unfavourable outcomes (PCPC >3) were examined using the univariate logistic regression analysis: independent variables were dichotomised either at the 75th percentile (AST, ALT and LD) or medically relevant breakpoints (tables 1 and 2). Independent variables with p values less than 0.05 were further tested using multivariate analysis with backward stepwise elimination of non-significant variables.

Eventually, paradoxical dependences of the outcome on early and late hypothermia were observed: to further investigate the types of relationships between clinical factors and

Kawano G, Iwata O, Iwata S, *et al. Arch Dis Child* (2010). doi:10.1136/adc.2009.180554

Table 1 Baseline characteristics, treatments, adverse events and outcome

	Hypothermia (n=27)	Normothermia (n=16)
Baseline characteristics		
Age (month)	45.0 (25.7–64.4)	29.6 (18.8–40.3)
Body weight (kg)	15.3 (11.3–19.3)	12.9 (10.8–15.0)
Sex (female)	14	13
GCS at admission	4.2 (3.2–5.2)	5.6 (3.2–8.1)
Initial CT findings*		
Moderate oedema	5	4
Severe oedema	3	2
Other lesions	3	4
Preceding infection		
Influenza virus	13	4
Adenovirus	0	1
Rotavirus	1	0
Human herpesvirus 6/7	2	1
Others or no preceding infection	11	10
Type of encephalopathy/encephalitis		
Acute necrotising encephalopathy	3	5
Haemorrhagic shock and encephalopathy syndrome	4	1
Acute encephalopathy with refractory seizures	11	5
Others	9	5
Serum markers for tissue damage		
<6 h of admission		
AST (IU/l)	1915 (−534 to 4364)	150 (53–247)
ALT (IU/l)	154 (69–240)	100 (−4 to 209)
LD (IU/l)	2105 (462–3749)	552 (451–652)
48–72 h after admission		
AST (IU/l)	1039 (22–2057)	3003 (874–5132)
ALT (IU/l)	550 (21–1079)	1453 (473–2435)
LD (IU/l)	2725 (910–4539)	3499 (1471–5526)
Treatments		
Steroid	24	10
Immunoglobulin	14	4
Timing of cooling initiation (h)	16.7 (10.3–23.0)	–
Cooling duration (h)	99.7 (78.1–121.3)	–
Adverse events		
Hypotension	11	7
Pneumonia	9	2
Thrombocytopenia (<150 000)	12	9
Coagulation disorder	9	7
Arrhythmias	0	0
Hypokalaemia (<3.5 mEq)	16	10
Outcome		
Mortality	2	4
Death or severe disability	11	7
PCPC	2.9 (2.3–3.6)	3.3 (2.2–4.3)
POPC	3.0 (2.3–3.7)	3.3 (2.3–4.3)
GOS	3.6 (3.0–4.1)	3.3 (2.5–4.2)

ALT, alanine transaminase; AST, aspartate transaminase; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; LD, lactate dehydrogenase; PCPC, Pediatric Cerebral Performance Category Scale; POPC, Pediatric Overall Performance Category Scale.
Data are shown as number of patients or mean (95% CI).
*Excluding three hypothermic and one normothermic children of whom CT was not obtained at admission.

outcomes; PCPC scores were compared with scale or rank-ordinal clinical variables (GCS and CT scores at admission and variables which were included within the final logistic regression model) using Spearman's rank correlation coefficient for subgroups of normothermic and hypothermic patients, respectively. Intergroup comparisons between normothermic and hypothermic children were also performed: the incidence of adverse events and initial CT scores were compared using either the χ^2 test or Fisher's exact test, whereas scores for GCS and PCPC were compared using the analysis of variance on ranks.

RESULTS

Of 43 children, preceding viral infection was identified in 27 participants, 17 of which were flu. Acute necrotising encephalopathy, haemorrhagic shock and encephalopathy syndrome and acute encephalopathy with refractory seizures were observed in 8, 5 and 16 children, respectively (table 1); however, none developed Reye's syndrome or acute disseminated encephalo-myelitis. No child had hypoxic–ischaemic events before admission; however, one child with preceding flu infection experienced cardiac arrest at admission (hypothermia group). Sixteen children (9–71 months, range) were cared for at normothermia, whereas 27 children (5–213 months) were cooled at 16.7±16.1 h (mean±SD) following the initial neurological signs of encephalopathy/encephalitis. All children were initially cooled to 34–35°C; three children required moderate hypothermia <34°C for the control of severe brain oedema. Seventeen children required intravenous vecronium or pancronium because of shivering resistant to thiopental and/or midazolam. For these children, subsequent duration of cooling ≤35°C was 99.7±37.9 h including the rewarming period.

Intergroup analysis for normothermic and hypothermic patients

There was no difference in the background clinical variables, serum markers of tissue damage, CT findings, incidence of acute adverse events and PCPC scores between normothermic and hypothermic children.

Determinants of outcomes within the overall study population

In the univariate analysis, the incidence of acute adverse events was unrelated with clinical variables and therapeutic options; unfavourable outcomes at 12 months were associated with longer hypothermic periods (p=0.030), serum AST and LD higher than the 75th percentiles (p=0.020 and 0.006, respectively), younger ages ≤18 months (p=0.020) and diagnosis of either acute necrotising encephalopathy or haemorrhagic shock and encephalopathy syndrome (p=0.021), whereas early initiation of hypothermia ≤12 h was related to a reduced risk of adverse outcomes (p=0.004) (table 2). Because of a significant intercorrelation observed between AST and LD (r^2=0.97), only LD but not AST was considered in the multivariate analysis as a representative serum marker of tissue damage. Multivariate analysis identified younger ages (p=0.038) and elevated serum LD levels (p=0.021) as independent variables associated with adverse outcomes, whereas early initiation of cooling was associated with a reduced risk of adverse outcomes (p=0.017).

Intragroup analysis for normothermic and hypothermic patients

In normothermic children, PCPC was dependent on CT scores, GCS and LD levels at admission (p=0.040, 0.004 and 0.002, respectively: figure 1A and online supplementary figures 2 and 3), whereas in hypothermic children, PCPC depended on the timing of cooling initiation (p=0.001: figure 1A–B, see online supplementary figures 4 and 5 for comparisons with other outcome scales).

DISCUSSION

Our results suggested that the age and temperature control are both important factors associated with the outcome of acute encephalopathy/encephalitis in childhood. Younger ages ≤18 months and marked elevation of serum LD greater than the 75th percentile were associated with severe disability or death

Table 2 Determinants of adverse outcomes following acute child encephalopathy/encephalitis

	Outcome							
	Good (PCPC ≤3)		Poor (3 <PCPC)		OR (95% CI)			
	n=25	%	n=18	%	Average	Lower	Upper	p Value
Univariate analysis								
Baseline characteristics								
Age (≤18 months)	5	20.0	10	55.6	5.00	1.30	19.30	0.020
Sex (female)	17	68.0	9	50.0	0.47	0.14	1.64	0.237
Moderate to severe oedema on initial CT*								
	9	40.9	6	35.3	0.79	0.21	2.92	0.721
AST† (190 IU/<)	3	12.5	8	47.1	6.22	1.33	29.01	0.020
ALT† (91 IU/<)	4	16.7	6	35.3	2.73	0.63	11.79	0.179
LD† (835 IU/<)	2	8.3	9	50.0	11.00	1.98	61.26	0.006
Types and severity of encephalopathy/encephalitis								
GCS (≤4)	16	64.0	15	83.3	2.81	0.64	12.41	0.172
Types of encephalopathy/encephalitis associated with adverse outcomes‡								
	4	16.0	9	50.0	5.25	1.28	21.57	0.021
Therapeutic options								
Steroid	19	76.0	15	83.3	1.58	0.34	7.38	0.562
Globulin	11	44.0	7	38.9	0.81	0.24	2.78	0.738
Hypothermia	16	64.0	11	61.1	0.88	0.25	3.09	0.847
Timing of cooling initiation								
Early hypothermia (≤12 h)	14	56.0	3	16.7	0.05	0.01	0.39	0.004
Late hypothermia (12 h <)	2	8.0	8	44.4	1	Reference		
Normothermia	9	36.0	7	38.9	0.19	0.03	1.22	0.112
Target temperature								
Normothermia	9	36.0	7	38.9	1	Reference		
Mild hypothermia (34–35°C)	14	56.0	10	55.6	0.92	0.26	3.30	0.896
Moderate hypothermia (33–34°C)	2	8.0	1	5.6	0.64	0.05	8.62	0.739
Cooling duration (≤35°C)								
None (normothermia)	9	36.0	7	38.9	2.18	0.53	9.02	0.283
≤96 h	14	58.3	5	29.4	1	Reference		
96 h <	1	4.2	5	29.4	14.00	1.30	150.89	0.030
Multivariate analysis								
Age (≤18 months)					7.70	1.12	52.89	0.038
Timing of cooling initiation: early hypothermia (≤12 h)					0.09	0.01	0.65	0.017
LD† (835 IU/l <)					13.66	1.48	126.10	0.021

ALT, alanine transaminase; AST, aspartate transaminase; GCS, Glasgow Coma Scale; LD, lactate dehydrogenase; PCPC, Pediatric Cerebral Performance Category Scale.

*Excluding three hypothermic and one normothermic children of whom CT was not obtained.

†AST, ALT and LD values were dichotomised at the 75th percentile.

‡Including acute necrotising encephalopathy, haemorrhagic shock and encephalopathy syndrome and Reye's syndrome (see the method section and the reference 13 for detail).

after 12 months. In contrast, therapeutic hypothermia might have pivotal effects on the outcome: the incidence of unfavourable outcome for children cooled after 12 h of diagnosis was invariant with normothermic children but was significantly higher compared with children cooled within 12 h. Delayed cooling after 12 h of acute events may be deleterious, whereas early cooling is likely to be neuroprotective. Further prospective studies with larger populations are required to delineate a group of patients who may have the benefit of cooling after acute child encephalopathy/encephalitis.

Limitation of the study

This study was a retrospective observational study based on a limited number of patients from 10 tertiary centres. Because of the variation in the patients' background and the interinstitutional differences in the therapeutic strategy, our study does not have the power to demonstrate the neuroprotective effect of therapeutic hypothermia with the direct comparison between two therapy modes. The therapy mode was determined by the policy of each centre where the children received treatments. Hence, although we did not observe any significant interinstitutional difference in unfavourable outcomes, it is possible that the outcome of children was affected by the quality of intensive care provided at the specific centre and the temperature control. Further, one institution provided hypothermia only for children with evidence of severe brain oedema, which may have led to the underestimation of the benefits of therapeutic hypothermia.

Determinants of outcomes following acute encephalopathy/encephalitis

Univariate analyses in the overall study population identified the eventual cooling duration, age of patients, serum AST and LD at admission, types of encephalopathy/encephalitis and timing of cooling initiation as significant independent variables. It may be counterintuitive that longer cooling periods led to worse outcomes; however, children with more severe cerebral oedema might eventually require slower rewarming compared with peers, presumably leading to adverse outcomes despite special care. Acute necrotising encephalopathy and haemorrhagic shock and encephalopathy syndrome, which had been linked with adverse outcomes in previous studies,[13] were consistently associated with an increased incidence of unfavourable outcomes in our study population. However, these variables were not included within the final multivariate logistic model. In contrast, the age of patients, serum LD and cooling at different timings were all identified as significant determinants of the outcome in the final multivariate model.