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特集 脳・神経系のMRI

Ⅱ．疾　患

神経皮膚症候群

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要旨

神経皮膚症候群のうち結節性硬化症と神経線維腫症1型は，小児神経学領域においてしばしば遭遇する代表的疾患である．これらの疾患は特徴的・特異的な画像所見を呈し，またそれぞれ年齢により異なるMRI画像所見を呈する．

本稿では，それぞれについて症例を呈示し，その一般的疾患概念とMRI画像について述べた．

Key Words
結節性硬化症
神経線維腫症
神経皮膚症候群

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はじめに

神経皮膚症候群は，外胚葉・中胚葉の発生異常に基づく皮膚および神経組織に発生異常をきたす疾患群の総称である．神経系・皮膚のみならず多臓器にわたり発生異常所見を示し，またしばしば発生異常組織からの腫瘍を伴う．現在多くの神経皮膚症候群が報告されているが，本稿では，代表的疾患である結節性硬化症と神経線維腫症1型について症例を呈示し，その画像所見について述べたい．

結節性硬化症

1．症例呈示

症例1　神経疾患について家族歴のない女児．出生前，周産期に特記すべき点なし．定頸が不良であることを4カ月健診で指摘されていたが様子をみていた．6カ月からシリーズを形成するtonic spasmsを認めたため受診，覚醒時脳波にてヒプスアリスミアを認めた．身体所見では体幹，臀部に白斑が散見された．腎エコー，心エコーを施行したが正常であった．MRI（図1）は1歳10カ月時のSE（spin echo）法T1, T2強調画像とガドリニウムによる造影画像である．多数の皮質結節がT2強調画像で高信号を示し，T1強調画像では白質と等信号ないし低信号を示

している．ガドリニウムによる造影効果は認められない．脳室上衣下結節は両側モンロー孔近傍，側脳室体部に認められ，T2強調画像で大脳白質よりやや高信号ないし等信号，T1強調画像で低信号ないし等信号を示している．モンロー孔近傍の結節はガドリニウムによる造影効果を認める．

症例2　2歳9カ月の女児で主訴は発達遅滞と半身のけいれんであり，身体所見で症例1と同様体幹を中心に白斑を認めた．腎エコー，心

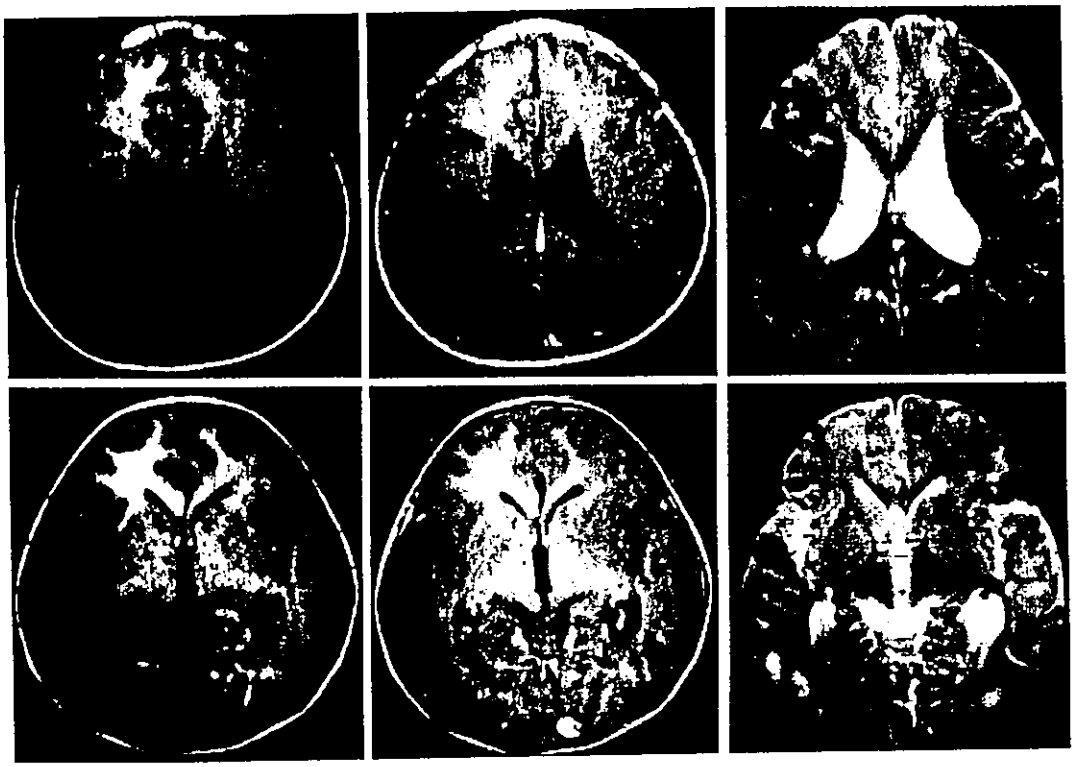

図1　1歳10カ月女児，結節性硬化症
左：T1強調画像，中：T1強調画像ガドリニウム造影像，右：T2強調画像

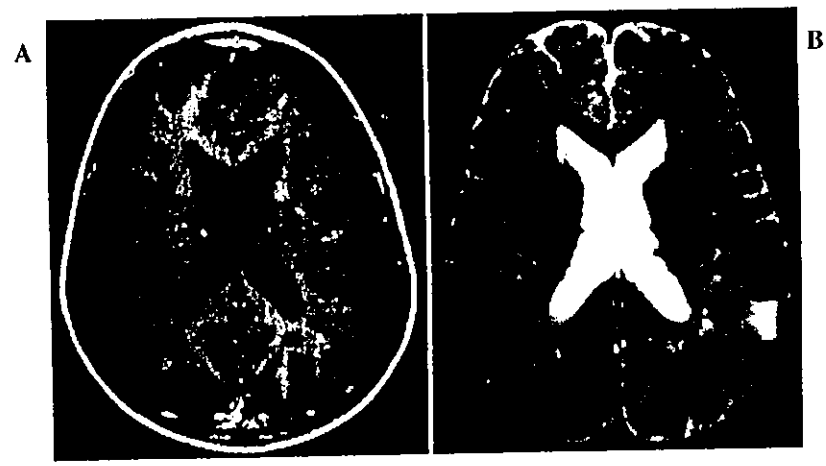

図2　2歳9カ月女児，結節性硬化症
A：ガドリニウム造影T1強調画像，B：T2強調画像

エコー所見は正常であった．MRI（図2）はガドリニウム造影T1強調画像，T2強調画像である．T1強調画像では造影効果を認めない低信号を認める皮質結節が認められる．この皮質結節はT1強調像で低信号，T2強調画像で高信号であり，そこから脳室へ刷毛で履いたような高信号域を認めている．脳室上衣下結節はガドリニウムによる造影効果を軽度有する小結節として側脳室体部壁に多数認められている．

2．概念およびMRI所見

結節性硬化症はBourneville-Pringle病ともよばれる，きわめて高い浸透率（95%）を呈する常染色体優勢遺伝を示し，きわめて多彩な表現型を示す神経皮膚症候群の代表的疾患である．孤発例が70%であるが，孤発例であることは両親が結節性硬化症でないことを診察とともに，可能であれば放射線学的に確認する必要がある．また両親が結節性硬化症でなくても1～2%の率で次子が罹患する可能性がある．旧来てんかん，知能障害，血管線維腫が三主徴とされるが，これをすべて同時に満たすものは全体の3割にすぎない．Barkovichの教科書に記載されている診断基準を表1に示す[1]．責任遺伝子は今のところ*TSC1*と*TSC2*の二つの遺伝子が判明しており，前者は第9番染色体長腕（9q34）に局在しhamartinをコードし，後者は第16番染色体短腕（16p13.3）に局在しtuberinをコードしている．

MRI上の小児期中枢神経所見は主に上衣下結節，皮質結節，白質病変の三つでさらに加齢とともに巨大星細胞腫が加わる．上衣下結節は脳室から不規則に突出した小結節としてみられ，髄鞘形成が未熟な乳児期では上衣下結節はT1強調像，T2強調像でそれぞれ相対的な高信号，低信号を示す．このため未熟児では上衣下出血と見誤ることがあり注意を要する．年齢とともに髄鞘化が進むと白質と同信号となる．比較的大きな上衣下結節の場合石灰化の程度によりT2強調像では低信号を示す．ガドリニウムによる造影効果については，まったく変化のないものから顕著に造影効果を認めるものまでさまざまであるが，その程度が臨床像に関連することはない．皮質結節は病理肉眼的に平滑白色調でやや肥大した脳回として認められる．組織学的には奇異な巨大神経細胞，神経なのかグリア細胞なのか判定しがたい不確定巨大細胞の出現とグ

表1　結節性硬化症の診断基準（文献1）より引用）

Ⅰ．一次所見
1. 顔面血管線維腫*
2. 爪下線維腫*
3. 皮質結節（組織学的）
4. 上衣下結節ないし巨大星細胞腫（組織学的）
5. 脳室壁にそった石灰化多発性上衣下結節（放射線学的）
6. 多発性網膜星細胞腫*

Ⅱ．二次所見
1. 一親等の近親者に結節性硬化症
2. 心横紋筋肉腫（組織学的ないし放射線学的）
3. 網膜の過誤腫ないし脱色素斑*
4. 皮質結節（放射線学的）
5. 非石灰化上衣下結節（放射線学的）
6. 隆起皮様皮膚斑［shagreen patch］*
7. 前額線維隆起斑*
8. 肺リンパ管筋腫症（組織学的）
9. 腎血管筋脂肪腫（組織学的ないし放射線学的）
10. 腎嚢胞（組織学的）

Ⅲ．三次所見
1. 色素脱失斑*
2. "紙ふぶき様"皮膚病変*
3. 腎嚢胞（放射線学的）
4. 歯エナメル陥凹
5. 過誤腫性直腸ポリープ（組織学的）
6. 骨嚢胞（放射線学的）
7. 肺リンパ管筋腫症（放射線学的）
8. 大脳白質"遊走経路"ないし異所性灰白質（放射線学的）
9. 歯肉線維腫*
10. 他臓器過誤腫（組織学的）
11. 点頭てんかん

確定診断：Ⅰが1個以上，Ⅱが2個以上，あるいはⅡが1個以上かつⅢが2個以上
推定診断：Ⅱが1個かつⅢが1個，あるいはⅢが3個
疑い診断：Ⅱが1個，あるいはⅢが2個

*：臨床的に明らかであれば組織学的証明は必要なし

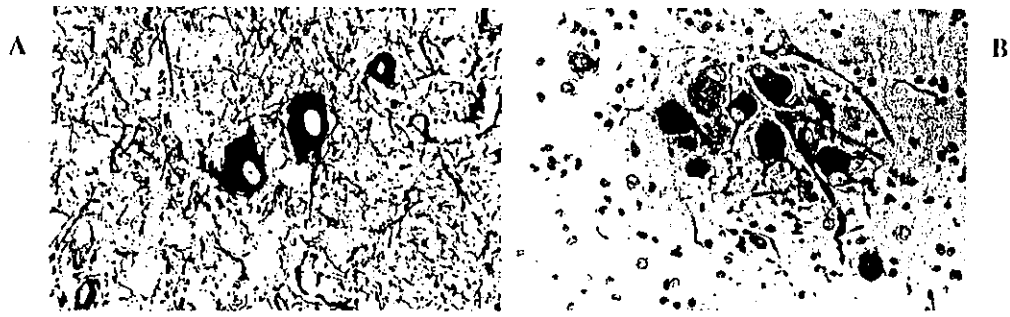

図3　結節性硬化症皮質結節
巨大神経細胞（A：ニューロフィラメント染色），不確定巨大細胞（B：ネスチン染色）

リオーシスと，髄鞘化の形成不良ないし破壊が認められる（図3）[2]．大脳のみに存在することが多いが1割程度の患者には小脳にもみられる．MRIはそれぞれの年齢により所見が異なる．すなわち，新生児期ではT1, T2強調像で白質の信号強度に比較してそれぞれ高信号，低信号を示す．この異常信号は皮質結節部から脳室へむかう部分にも波及する場合がある．乳児期後期となるとT1, T2強調画像でそれぞれ低信号，高信号を示すようになる．これらの病変はFLAIR法によるT2強調像でより明確に示しうる[3]．MRI画像では皮質結節領域の皮質は結節部との境界が明瞭であるが，組織学的には当該部位の皮質にも異常巨大異形成細胞などの出現する皮質結節病変が存在する．皮質結節内側と白質の境界はMRI画像，組織所見同様不明瞭である．石灰化を示す皮質結節はT1強調像で高信号を示す場合があり，また石灰化に加えて変性を示す皮質結節はガドリニウムによる造影効果を示す場合がある．解像度の高いT2強調像においては，皮質結節から上衣下結節にかけての白質に線状に伸びる高信号を示す場合あり，これは同部位に異形性巨大細胞が束状に集族して認められるからであるという説がある．巨大星細胞腫は加齢とともにモンロー孔付近に生ずる良性腫瘍（WHO grade Ⅰ）でしばしばモンロー孔を閉鎖して水頭症の原因になりうる．MRI画像上，信号強度の違いや造影効果有無によって上衣下結節と区別をすることは不可能であるが，直径が12 mmを超えたものや増大傾向を示す場合を巨大星細胞腫と考える．

神経線維腫症1型

1．症例呈示

症例3　学習障害を主訴に来院した7歳女児である．家族歴に特記すべき点はない．身体所見では体幹中心に径20 mmまでのカフェ・オ・レ斑を多数認め，腋窩には褐色の雀卵斑を認めた．眼科的には虹彩に異常を認めず，視覚検査も正常であった．図4に示すMRIはSE法およびFLAIR法によるT2強調画像であるが両側淡蒼球と視床枕に高信号域を認めた[4]．

症例4　軽度の精神遅滞を有する14歳女児で体幹に多数のカフェ・オ・レ斑を認めた．眼科を受診し虹彩結節を指摘された．図5では両側淡蒼球のT2延長像に加えて左脳梁膨大にFLAIR法T2強調像でより明瞭となり，ガドリニウムによる造影効果を有する病変を認めた[4]．

2．概念およびMRI所見

神経線維腫症1型は別名von Recklinghausen病とよばれ，神経皮膚症候群のなかでももっとも多く，発生頻度は1/4,000人とされる．第17番染色体長腕（17q11.2）に存在するneurofibrominをコードする癌抑制遺伝子の異常によりもたらされる，常染色体優勢遺伝を示す疾患で

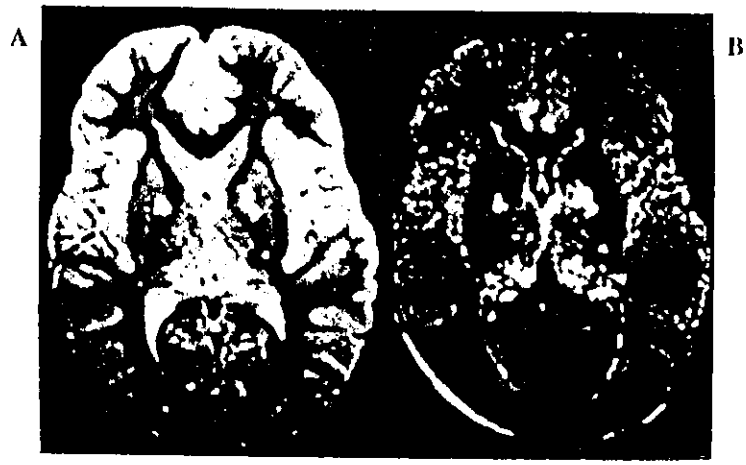

図4　7歳女児，神経線維腫症1型
A：SE法T2強調画像，B：FLAIR法T2強調画像

図5　14歳女児，神経線維腫症1型
A：SE法T1強調画像，C：ガドリニウム造影SE法T1強調画像，B：SE法T2強調画像，D：FLAIR法T2強調画像

表2　神経線維腫症1型の診断基準

1. 最大径5 mm以上（思春期以降では15 mm以上）のカフェ・オ・レ斑が6個以上存在する
2. 2個以上の神経線維腫（型を問わず）があるか1個以上の叢状神経線維腫（plexiform neurofibroma）が存在する
3. 腋窩あるは鼠径部に雀卵斑様色素斑が存在する
4. 視神経膠腫が存在する
5. 2個以上のLisch nodule（虹彩結節：有色の虹彩過誤腫）が存在する
6. 蝶形骨異形成あるいは長幹骨骨皮質の非薄化などの明らかな骨病変が存在する
7. 第一親等に神経線維腫1型が存在する

ある．カフェ・オ・レ斑は乳児期にすでに認められ，数・大きさともに思春期まで増大する．皮膚神経線維腫は思春期ころに出現し，生涯増えつづける．虹彩結節は虹彩に認められる過誤腫であり罹患成人のほとんどにみられる．その他，脊椎側彎症，皮膚神経線維腫症の合併した長幹骨過成長，血管異形成，精神遅滞，学習障害を認める．また，症例の約15%に神経膠腫を視神経から始まり，視交叉，視索，外側膝状体，そして視放線というように視覚路に沿って成長するように認めるが，そのほとんどはWHO grade Ⅰの比較的良性なpilocytic astrocytomaである[11]．腫瘍が視神経にとどまるものは，無症状である場合も多く比較的予後はよいが，腫

瘍が視索を巻き込んで成長していく場合は，必ずしも予後は良好でない．腫瘍が視床下部を巻き込むかたちで成長すれば，思春期早発症となる．MRI画像はT1強調像で3 mm以下のスライス幅で水平断と冠状断で，視覚路を観察する．この場合気をつけるべき点は視神経周囲のくも膜腔拡大による見かけの視神経肥大を，腫瘍自体による視神経の肥大と見誤らないようにすることである．視神経膠腫は自然に消退する場合もあり過度の治療は避けるべきである．視神経以外にも脳幹とくに延髄に神経膠腫を認めるるが，一般的にみられる脳幹部神経膠腫よりも予後は良好であり，視神経膠腫と同様自然に退縮する場合もある．このほか小脳・大脳に星状細胞腫を認める場合がある．小児期における神経線維腫1型のMRI画像上もっとも特徴的な所見として，T2強調画像で周囲への圧迫像や浮腫などの変化を伴わない高信号領域を大脳，小脳，脳幹部に認める．これは症例の75%，視神経膠腫を合併する症例においては95%以上に認められるが，起源が今のところ確定していないため，UBO（unidentified bright object）とよばれることがある．このT2延長像はミエリンの空砲化によるものであったとする唯一の病理報告がある[6]．興味深いことに通常T1強調像では現れにくく，通常3歳以降から出現し10～12歳ころまで増加し，そして成人では認められなくなる．部位としては，橋，延髄，小脳白質，内包，淡蒼球などが多いが，海馬，視床枕，脳梁膨大部にも認められ，とくにFLAIR法による観察が優れている[4]．神経線維腫症1型の学童は学習障害が多いとされるが，これらのUBOの出現時期や，その出現部位を考察すると興味深いものがある．たとえば視床枕は視覚路に外側膝状体を介さない結合をしているが，一説によれば視覚刺激を重要であるものと無視してよいものに分けるための視空間認知に関連する機能を有するという．その障害は可逆的であるにせよ視覚認知障害をもたらし，結果として学習障害の原因となりうる可能性がある．また，脳梁膨大部は視覚野皮質と対側の角回を連絡する経路が通るが，その障害は読書障害をもたらす可能性がある[4]．

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III. けいれん・意識障害を起こす疾患の診療のポイント

15. 代謝障害の診療のポイント

山内秀雄*
Hideo Yamanouchi

Key words：けいれん，低血糖，低カルシウム血症

低血糖症

【症状・診察所見のポイント】

低血糖症の症状として自律神経症状（発汗，心悸亢進，顔面蒼白，振戦，嘔吐）とそれにひき続く中枢神経症状（頭痛，めまい，視力障害，構音障害，失語，健忘，失見当識，失調，片麻痺，知覚障害，意識障害）とが認められる。診察所見としては，低身長（下垂体機能不全），巨舌・臍ヘルニア（Beckwith-Biedemann 症候群），色素沈着（Addison 病など），肝腫大の有無（糖原病など）をチェックする。また，発症年齢，発症時間や食事との関係（ガラクトース血症はミルク摂取後，果糖不耐症は果糖摂取後）などを聴取する。

【診断のための検査のポイント】

低血糖そのものの定義は成熟児では 30 mg/dl，未熟児は 20 mg/dl，生後 72 時間以降は 40 mg/dl 以下とされている。低血糖時に鑑別診断の目的で行う検査項目として，血中インスリン（通常は低下しているが，膵 β 細胞過形成・腺腫，Beckwith-Wiedemann 症候群，ロイシン過敏症などでは高インスリン血症となる），アラニンと乳酸（ともに glucose-6-phospatase 欠損症，fructose-1,6-diphosphatase 欠損症では高値を示す），成長ホルモン（下垂体機能不全），コルチゾール（副腎不全），カルニチン値（カルニチンサイクルやミトコンドリア β 酸化異常による非ケトン性低血糖症）の測定を行う。また尿中ケトン体の有無（非ケトン性低血糖症，高インスリン血症はケトン陰性である）をチェックする。

【鑑別診断のポイント】

新生児期に限って出現するものには，① 基質不足や酵素活性未熟によるもの（低出生体重児，重症呼吸障害，妊娠中毒母体の児），② 高インスリン血症（糖尿病母体の児，胎児赤芽球症，多血症など），③ 母体の薬剤投与（トルブタマイド，スルフォニールウレア，プロプラノロール）などがある。

新生児期以降における低血糖症の鑑別診断で重要なものは，① 高インスリン血症（島細胞腫，β 細胞腺腫・過形成，ロイシン過敏症），ホルモン欠損症），② ホルモン欠損症（先天性下垂体機能低下症，GH・ACTH 欠損症，Addison 病，グルカゴン欠損症，エピネフリン欠損症），③ アミノ酸代謝異常（メープルシロップ尿症など），④ 糖原病（glucose-6-phosphatase 欠損症（GSD I），amylo-1,6-glucosidase 欠損症（GSD IV），肝 phosphorylase 欠損症（GSD VI），glycogen synthetase 欠損症），⑤ 非ケトン性低血糖（カルニチン欠損症，CPT 欠損症，acyl-CoA dehydrogenase 欠損症），⑥ その他，酵素欠損症（fructose-1,6-bisphosphatase 欠損症，phosphoenolpyruvate carboxykinase 欠損症，ガラクトース血症，果糖不耐症）などがある。

低血糖時に施行した検査結果を参考にして絶食試験，グルカゴン負荷試験を追加施行し鑑別診断を行う。ロイシン負荷試験は高インスリン血症の診断に用いるが重度の低血糖を起こす可能性があ

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り，注意を要する。ところで幼児期に好発する最も頻度の多い低血糖症として“ケトン性低血糖”があるが，低血糖時アラニンは低値で逆にアラニン投与で血糖が上昇することから，糖新生基質不足によるものと推察されている。

【治療のポイント】

持続する低血糖傾向は脳の不可逆的な障害をもたらす可能性があり，いずれの年齢も血糖値40 mg/dl以下は絶対的な治療対象である。新生児低血糖の場合，20%グルコースを2 ml/kgをゆっくり静注，その後4〜8 mg/kg/分の速度で血糖を50 mg/dl以上に維持する。これで目標血糖値を維持できなければハイドロコルチゾン5 mg/kgを静注しその後8時間ごとに2 mg/kgを静注する。また，グルカゴン0.05〜0.1 mg/kg静注し0.025〜0.05 mg/kg/時で持続静注してもよい。詳細は文献2)をご参照いただきたいが，糖原病ではたとえばG6P欠損症の場合，1歳まではグルコースとして最低8〜9 mg/kg/分の量を与え，糖原病用ミルク昼間用を年齢に合わせて8分割して与える。1歳以上ならグルコースとしての摂取量は5〜7 mg/kg/分とし，離乳食（炭水化物：脂肪：蛋白質を70：15：15とする）の分だけミルクを減らす。2歳以下ではミルク，それ以上ではコーンスターチを眠前・4時間後に2〜3 mg/kg与える。

低カルシウム血症

【症状・診察所見のポイント】

新生児期には副甲状腺ホルモン，ビタミンD，カルシトニンによるカルシウム調節機構が未熟であり，とくに生後24〜48時間では血清カルシウムは低値を示しやすい。新生児・乳児期にみられる重要な症状の一つにけいれんがあるが，これは通常意識障害を伴わない短いものでfocalあるいはmultifocal clonic seizureである。筆者は以前単純部分てんかんと安易に誤診しまったことがあったので注意されたい。年長児ないし成人にみられるような助産婦手位はかえってみられない場合が多く，また新生児期においては通常テタニーの際にみられるChvostek徴候は一般新生児にも非特異的にみられるため，低カルシウム血症の徴候として認識されえない可能性がある。新生児では易刺激性，筋けいれん，振戦などの神経系興奮状態を示す症状を呈するほか，無呼吸・チアノーゼ（喉頭攣縮による可能性あり），哺乳力低下，嘔吐，腹部膨満などの非特異的症状をきたす。まれであるがQT延長を伴う徐脈，低血圧，うっ血性心不全などを認めることがある。診察では特に遺伝性疾患に関する家族歴，生活・食習慣（筆者らは乳児期に室内のみで育児，豆乳のみを与えていたくる病患者を経験したことがある），易感染性の有無（CATCH 22に伴う細胞性免疫不全），頸部手術放射線照射の有無の聴取を行った後，皮下石灰化の有無，低身長，第4・5指短縮，円形顔貌の有無（Albright徴候），幅広い鼻根と狭い鼻翼幅，開放性鼻声，先天性心疾患示唆する心雑音の有無（CATCH 22）を確認する。口腔外科的に歯の異常を確認し，眼科的には白内障の有無を確認する。

【診断・鑑別診断のポイント】

血清総カルシウムが7.5 mg/dl以下，イオン化カルシウム濃度2.5 mg/dl以下であれば低カルシウム血症と診断する。腎不全を除外すると，副甲状腺ホルモン（PTH）ないしビタミンDに関連するものがほとんどである。血清マグネシウムを測定して低値でないことを確認した後（Mg低値であれば低Mg血症に伴うPTH分泌不全ないし抵抗性），血清リン値をみて低ければビタミンDに関連し，血清リン値が高値を示せば腎不全ないし副甲状腺ホルモンに関連している。高リン血症でさらにintact PTH低値を示せば特発性副甲状腺機能低下症，intact PTH高値であればEllisworth-Howard試験を行い尿中リンの排泄をみて，不変であれば偽性副甲状腺機能低下症，増加していれば偽性特発性副甲状腺機能低下症である。放射線学的には頭部CTを施行し，基底核石灰化の有無を確認し，単純X線像でくる病の変化（長幹骨骨幹端の骨端線の拡大，カッピング・フレイングなど）を確認する。

【治療のポイント】

急激な神経症状を認めた場合，グルコン酸カル

シウムの静注を考慮する。8.5%カルチコールを1～2 ml/kg を 20 分以上かけて one shot で静注する。速度は 1 ml/分をこえないようにする。持続点滴投与をする場合は不整脈，静脈炎，皮下石灰化をきたしやすいことを充分説明したうえで，乳児には 3～4 ml/kg/日，年長児には 1～2 ml/kg/日を投与する。慢性期には尿中 Ca/Cr が 0.3 以下となるようにビタミン D_3 製剤を併用して血清カルシウム値を調節する。

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ORIGINAL PAPER

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Neonatal intrahepatic cholestasis caused by citrin deficiency: severe hepatic dysfunction in an infant requiring liver transplantation

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Abstract Adult-onset type 2 citrullinaemia (CTLN2) is caused by a deficiency of the citrin protein encoded by the *SLC25A13* gene. Citrin, an aspartate glutamate carrier in mitochondria, is an essential component of the malate-aspartate NADH shuttle. Recently, citrin deficiency has been reported to manifest as neonatal intrahepatic cholestasis. We report here five cases with neonatal intrahepatic cholestasis caused by citrin deficiency. Genetic diagnosis revealed compound heterozygotes of 851del4/IVS11+1G→A in two patients, IVS11+1G→A/E601X, and IVS11+1G→A/unknown in each one patient and homozygote for S225X in one patient. All cases revealed high levels of alpha-fetoprotein, which are not observed in CTLN2 patients. The condition was self-limiting and spontaneously disappeared after 5–7 months of age in four patients. However, one patient developed hepatic dysfunction from the age of 6 months and required a living-related liver transplantation at the age of 10 months. The patient showed complete recovery after transplantation, and now at the age of 3 years, shows normal growth and mental development. *Conclusion:* we report the first case of neonatal intrahepatic cholestasis caused by citrin deficiency with severe hepatic dysfunction requiring a living-related liver transplantation. Patients with this disorder should be followed up carefully, even during infancy.

Keywords Argininosuccinate synthetase · Cholestasis · Citrin · Citrullinaemia · Liver transplantation

Abbreviations *AGC* aspartate glutamate carrier · *ASS* argininosuccinate synthetase · *CTLN1* classical citrullinaemia · *CTLN2* adult-onset type 2 citrullinaemia · *NICCD* neonatal intrahepatic cholestasis caused by citrin deficiency

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Introduction

Citrullinaemia is classified into classical citrullinaemia (CTLN1, OMIM 215700) and adult-onset type 2 citrullinaemia (CTLN2, OMIM 603471). CTLN1 is an autosomal recessive disease caused by argininosuccinate synthetase (ASS) deficiency on chromosome 9q34 [3]. CTLN1 is characterised by neonatal/infantile-onset of severe hyperammonaemia, irritability, lethargy, poor feeding, and tachypnoea. On the other hand, CTLN2 is characterised by late onset (11 to 79 years), frequent attacks of hyperammonaemia, mental derangement, sudden attacks of unconsciousness, and ultimately death within a few years of onset [9, 15]. The CTLN2 locus was identified to chromosome 7q21.3, and the causative gene, *SLC25A13*, has been determined [8]. The *SLC25A13* gene encodes calcium-binding mitochondrial protein, designated citrin. Citrin, an aspartate glutamate carrier (AGC) [13], plays an important role in the malate-aspartate NADH shuttle, urea synthesis, and

gluconeogenesis [10]. Impairment of citrin could lead to failure in supply of aspartate from mitochondria to the cytoplasm for synthesis of argininosuccinate, and cause high citrulline and ammonia levels (Fig. 1). Recently, neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) has been reported with *SLC25A13* mutations as a new disease entity (OMIM 605814) [12, 16, 18, 21]. Nine different mutations in *SLC25A13* have been detected in CTLN2 and NICCD patients [8, 21, 22].

In previous reports [12, 16, 18], patients with NICCD showed recovery within several months. However, we experienced a patient who underwent living-related liver transplantation at the age of 10 months because of deteriorating liver function. We report here clinical and biochemical features of five NICCD cases including the first infant case with liver transplantation.

Case reports

Case 1

The girl, weighing 3,114 g at birth, was born at 39 weeks of gestation. She was the second live-born child of unrelated parents with no family history. Screening for galactosaemia, homocystinuria, phenylketonuria, and maple syrup urine disease was negative. She received milk-based formulas and breast milk. At 1 month of age, failure to thrive was noted with a body weight of 3,400 g. At 2 months, she presented with jaundice and laboratory tests showed a total bilirubin of 7.7 mg/dl, direct bilirubin of 3.8 mg/dl, aspartate aminotransferase of 162 IU/l, and alanine aminotransferase of 79 IU/l. These tests improved gradually, but worsened again at 6 months of age. She was admitted to Osaka City General Hospital at 7 months. Her weight was 6,610 g. She had light yellow-coloured stools, splenomegaly (3.0 cm below the costal margin), and hypoglycaemia (21 mg/dl) without hyperinsulinaemia (0.0 μU/ml). Biochemical data on admission are shown in Table 1. We suspected tyrosinaemia type 1. Urine analysis showed a significant rise of *p*-hydroxyphenylacetate and *p*-hydroxyphenylpyruvate, but no succinylacetone. The fumarylacetoacetate hydrolase activity in skin fibroblasts was within the normal range. There was no past history of hepatitis A, B and C, cytomegalovirus, herpes virus, or Epstein-Barr virus infection. Biliary tract diseases and cystic fibrosis, glycogen storage disease and α_1-antitrypsin deficiency (185 mg/dl) were excluded. Doppler ultrasound studies did not show abnormalities of the portal vein or a portosystemic shunt. She showed no developmental delay or neurological abnormalities.

Medium-chain triglycerides and a low phenylalanine-tyrosine formula with supplementation of fat-soluble vitamins were administered. However, hepatic dysfunction with hypercitrullinaemia (239 μmol/l) and hypoglycaemia progressed in spite of intensive treatment. She manifested poor feeding and activity. We considered that she had progressive hepatic failure. She underwent living-related liver transplantation at 10 months of age. The histopathological findings of liver specimens included diffuse fatty changes of hepatocytes, cholestasis in lobules with proliferation of bile ducts, portal-to-portal bridging fibrosis, and pseudolobule. At 3 years of age, she was diagnosed with NICCD by genetic analysis, and currently has a normal plasma amino acid pattern, hepatic function, and normal development. Urea cycle enzymes were measured in a postoperative native liver specimen, which was stored for 2.5 years at −80°C (Table 2).

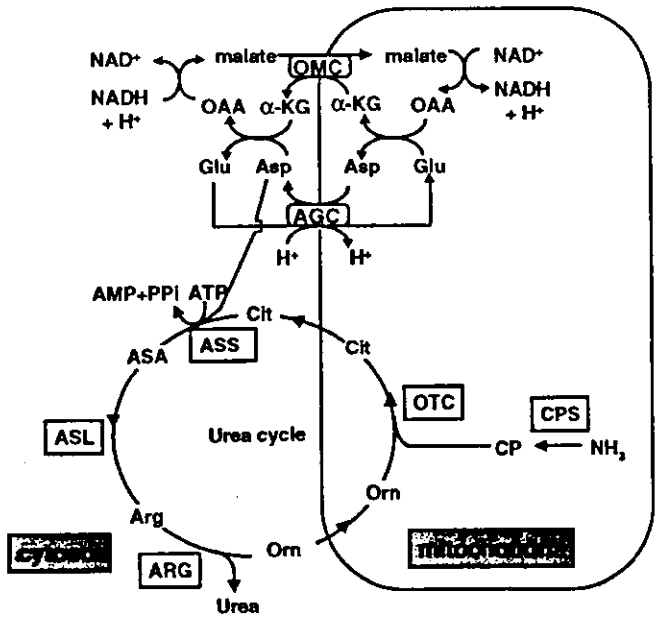

Fig. 1. Metabolic map of the urea cycle and malate-aspartate NADH shuttle. (*AGC* aspartate glutamate carrier; *ARG* arginase; *ASA* argininosuccinic acid, *ASL* argininosuccinate lyase; *CP* carbamylphosphate; *CPS* carbamylphosphate synthetase;*α-KT* α-ketoglutarate; *OAA* oxaloacetic acid; *OMC* α-ketoglutarate/malate carrier; *OTC* ornithine transcarbamylase)

Cases 2–5

These four unrelated patients had healthy parents. Newborn mass screening showed Cases 3 and 4 were positive for phenylalanine (242 and 157 μmol/l, respectively), while Case 5 had high levels of methionine (268 μmol/l) and galactose (1.1 mmol/l). Cases 2–5 had white-cream coloured stools but no hepatosplenomegaly. Cases 2–4 showed mild failure to thrive and Case 5 had hypoglycaemia (31 mg/dl). The laboratory findings are listed in Table 1. Known causes of neonatal cholestasis were eliminated in these patients, including infectious hepatitis, metabolic disease, and biliary tract disease. None showed developmental delay or neurological abnormalities. Without specific treatment other than feeding with medium-chain triglycerides or lactose-free formulas with supplementation of fat-soluble vitamins, biochemical abnormalities in Cases 2–5 improved by the age of 5–7 months.

Control subjects

Seven control newborn subjects were found to have high levels of galactose (0.22–1.4 mmol/l) on newborn mass screening. They did not have the enzyme deficiency for galactosaemia, and surveys of nine different mutations in *SLC25A13* were negative. The cause of mild intrahepatic cholestasis (total bile acid: >40 μmol/l) could not be specified. Biochemical data in control subjects returned to near-normal levels within 2 months.

Methods

The nine known mutations were diagnosed as follows [8, 21,22]: (1) the different length of amplified DNA (851del4 and 1638ins23), (2) the restriction fragments length polymorphisms after DNA amplification with polymerase chain reaction (IVS11+1G→A by Sau3AI digestion, S225X by *Alu* I, IVS13+1G→A by *Pst* I, 1800ins1 by *Tru*1I, R605X by *Bsh*1236 I, E601X by *EcoR* I, and E601K by *EcoR* I), (3) the multiple DNA diagnosis method by using GeneScan/SNaPshot analysis. Informed consent for genetic analysis was obtained from all parents.

Table 1. Biochemical data in five cases of NICCD. (*ALP* alkaline phosphatase, *PSTI* pancreatic secretory trypsin inhibitor)

	Case 1	Case 2	Case 3	Case 4	Case 5	Control subjects	Reference range
Age (months)	7	1	1	1	1	1	
Sex	F	M	M	M	F	M 3, F 4	
Gestational Age (weeks)	39	40	36	38	39	39 ± 1	37–41
Birth weight (g)	3144	2970	2058	2416	1930	3108 ± 39	2164–3928
Total protein (g/dl)	6.0	4.4	4.3	5.6	5.1	5.5 ± 0.4	4.6–7.4
Total bilirubin (mg/dl)	5.9	4.6	6.6	6.3	8.2	2.5 ± 1.7	0.2–1.0
Direct bilirubin (mg/dl)	2.9	3.1	3.4	4.3	2.5	0.7 ± 0.4	0.0–0.4
AST (IU/l)	191	50	64	142	47	45 ± 10	15–55
ALT (IU/l)	78	32	23	67	20	39 ± 14	5–45
γ-GTP (IU/l)	292	78	347	323	219	47 ± 23	5–32
ALP (IU/l)	1774	2444	2849	3889	1530	1005 ± 265	145–420
Total bile acid (μmol/l)	88	265	259	319	220	48 ± 30	5–25
Prothrombin time (%)	29	96	Not determined	63	37	Not determined	75–100
Hepaplastin test (%)	22	Not determined	45	Not determined	32	Not determined	70–130
PSTI (ng/ml)	Not determined	33	107	42	Not determined	32 ± 6	34 ± 12
Ammonia (μg/dl)	67	196	102	166	97	Not determined	18–74
Alpha-fetoprotein (ng/ml)	207000	136000	379000	309000	152570	6400 ± 10460	260–6400[a], 2–55[b]
Threonine (μmol/l)	294	547	962	730	532	179 ± 44	102 ± 20
Citrulline (μmol/l)	87	397	484	611	218	34 ± 8	28 ± 41
Methionine (μmol/l)	246	56	196	705	597	52 ± 19	23 ± 8
Tyrosine (μmol/l)	182	87	139	275	298	125 ± 34	71 ± 23
Arginine (μmol/l)	118	105	206	196	240	107 ± 59	85 ± 13
Phenylalanine (μmol/l)	56	35	41	37	41	70 ± 21	61 ± 14
Fischer ratio	0.76	1.2	1.4	0.36	0.78	1.7 ± 0.4	2.3 ± 0.6
Threonine/serine ratio	2.1	3.8	3.9	3.9	1.9	1.1 ± 0.2	0.8 ± 0.9
Galactose (mmol/l)	Not determined	3.1	5.1	2.9	0.2	0.2 ± 0.2	< 0.06
*SLC25A13*mutation[c]	I/II	II/VIII	I/II	IV/IV	I/-	Negative	

[a]Normal values at 1 month
[b]Normal values at 7 months
[c]*SLC25A13* mutations I, II, IV, and VIII were 851del4, IVS11 + 1G→A, S225X, and E601X, respectively

Results and discussion

We examined our subjects for nine mutations of *SLC25A13*, which had been previously observed in alleles of 92% of patients with early- and late-onset citrin deficiency [21]. These mutations were not present in the control subjects tested. It was therefore presumed that mild intrahepatic cholestasis in our control subjects was not due to citrin deficiency. On the other hand, mutations in *SLC25A13* were detected in both alleles of Cases 1–4 and in a single allele of Case 5, and accordingly were diagnosed as NICCD. The characteristic clinical features (Table 1) of these five paediatric patients are: (1) white coloured or yellow-white coloured stools, (2) poor body weight gain until 1 month after birth, (3) high levels of direct bilirubin, total bile acid, alkaline phosphatase, and γ-glutamyl transpeptidase, (4) high levels of citrulline, tyrosine, methionine, high threonine/serine ratio, low branched-chain amino acids/aromatic amino acid ratio (Fischer ratio), as previously described in CTLN2 patients [14], (5) low levels of vitamin K-dependent coagulation factor, (6) mild hyperammonaemia, and (7) high levels of alpha-fetoprotein, which are characteristic in NICCD since it has not been observed in CTLN2 patients [7,9]. Alpha-fetoprotein in our NICCD patients may have increased due to premature hepatocytes and/or hepatic damage and regeneration. Hepatocyte growth factor was also high (1.57 ng/ml) in Case 1. Pancreatic secretory trypsin inhibitor levels are high in CTLN2 patients [7,9], however, our infant patients (Cases 2 and 4) showed normal levels except for Case 3. Hypoglycaemia was seen in Cases 1 and 5, and was caused by the disturbance of gluconeogenesis. The AGC functions to provide substrates for gluconeogenesis as a part of the pathway for conversion of amino acids to glucose [10].

To date, 22 liver transplantations in CTLN2 adult patients have been performed [1, 5, 6, 7, 9, 17]. Case 1 was the first NICCD case requiring liver transplanta-

tion. The difference between Case 1 and the other four patients is clear if viewed through the progressive changes in cholestasis indices. In Cases 2–5, direct bilirubin, alkaline phosphatase, total bile acid, and alpha-fetoprotein improved with time and the values had nearly normalised by 5–7 months after birth (Fig. 2). In contrast, while cholestasis in Case 1 tended to improve up to 6 months after birth, similar to Cases 2–5, it worsened later, necessitating liver transplantation. The alpha-fetoprotein in Case 1 was also high until liver transplantation was performed. The genotype of Case1 is a compound heterozygote of 851del4 and IVS11+1-G→A (Table 1). These two mutations are prevalent, accounting for 33% and 40%, respectively, in Japanese patients with citrin deficiency [21]. Their compound heterozygotes account for 20%. At present, ten patients with the same genotype including our Cases 1 and 3 have developed NICCD [21]. However, two of them, Case1 and a case with NICCD (Hirayama et al., personal communication) required liver transplantation in early infancy, and the condition of the remaining eight patients improved spontaneously [12, 16,18]. Therefore, the relapse of hepatic dysfunction in Case 1 from 6 months after birth is not due to the genotype. At the time of liver transplantation, Case 1 had no infections of hepatitis A, B and C, cytomegalovirus, herpes virus or Epstein-Barr virus, and also had no particular events such as bacterial infection. We could not identify the triggers of relapse after 6 month of age in Case 1. Furthermore, the histological findings at the liver transplantation in Case 1 could not specify the cause of hepatic dysfunction. Adult patients with CTLN2 also do not show pathognomonic histopathological features, but rather were reported to vary from no pathological findings or fatty change to severe pathological lesions such as cirrhosis and chronic hepatitis [1, 5, 6, 9, 17, 20].

The primary cause of CTLN2 is citrin deficiency and low hepatic ASS activity is a secondary effect [22]. The prominent characteristics of CTLN2 are still a quantitative decrease of ASS protein in the liver (13.1 ± 13.3% of control, $n=99$; from 0.5% to 79%) [7, 9,22]. On the other hand, three cytosolic enzymes of ASS, argininosuccinate lyase, and arginase in a NICCD patient were within the normal range in the liver biopsy specimens after normalisation of all clinical and biochemical data [16]. The ASS and arginase activities in the resected

Table 2. Activities of urea cycle enzymes in the liver of Case 1. (*ASL* argininosuccinate lyase, *CPS* carbamoylphosphate synthetase, *OTC* ornithine transcarbamylase)

Enzymes	Case 1		Controls[a]
	(U/mg protein)	(%)	
CPS	0.015	56	0.027 ± 0.013 ($n=28$)
OTC	0.36	48	0.75 ± 0.29 ($n=23$)
ASS	0.005	22	0.023 ± 0.013 ($n=30$)
ASL	0.045	82	0.055 ± 0.021 ($n=32$)
Arginase	3.0	20	14.9 ± 3.3 ($n=36$)

[a]The controls are given as mean ± SD with the number of control samples in parenthesis. The ages of the controls are 3 days to 6 years

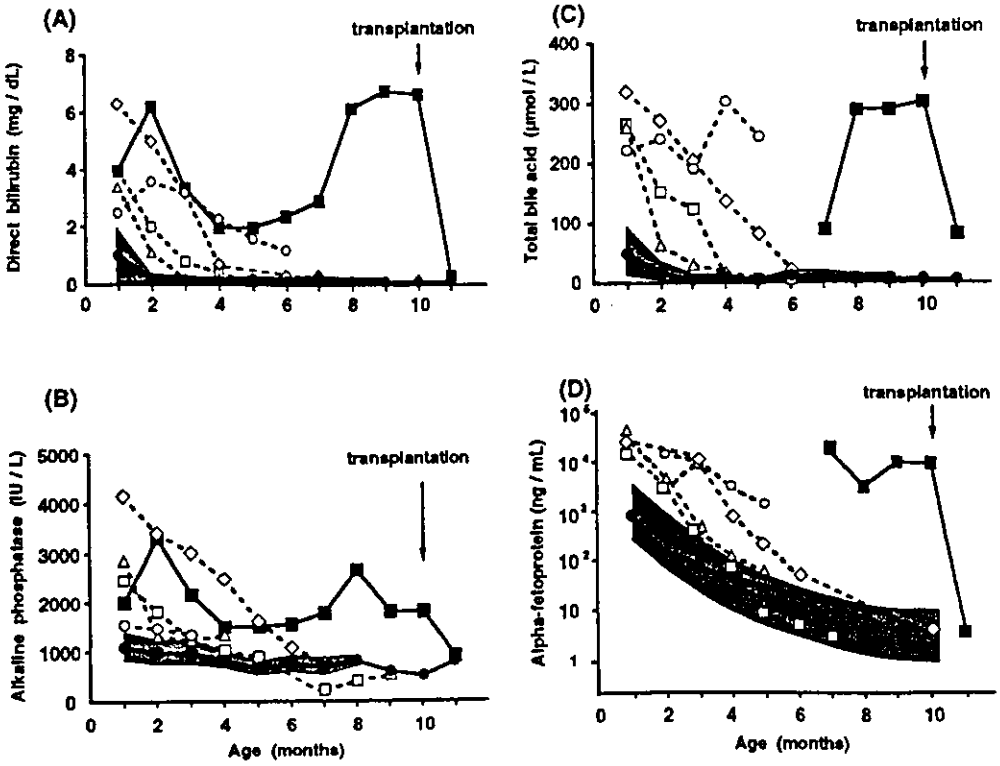

Fig. 2. Serial changes in direct bilirubin (A), alkaline phosphatase (B), total bile acid (C), and alpha-fetoprotein (D) in five patients with NICCD. Case 1 (*solid squares*), Case 2 (*open squares*), Case 3 (*open triangles*), Case 4 (*open diamonds*), Case 5 (*open circles*), average data of seven control subjects (*solid circles*). The *shaded* area indicates variation (± SD) of the data values of control subjects (A), (B) and (C) or reference range of alpha-fetoprotein (D) [19]

native liver specimen of Case 1 were reduced to 22% and 20% of control, respectively, as shown in Table 2. Several studies have reported decreases of ASS accompanied with carbamoyl phosphate synthetase, argininosuccinate lyase, and/or arginase in patients with CTLN2 [2, 4, 5, 6, 17]. Deterioration of liver tissue results in reduction of activities of all five enzymes of the urea cycle, for example, 36% to 45% in liver cirrhosis [11]. Therefore, the reduced ASS activity in liver specimens of Case 1 is primarily caused by citrin deficiency. In other words, we suspect that deterioration of liver function in Case 1 is primarily caused by citrin deficiency.

Citrin deficiency resulting from mutation of *SLC25A13* is associated with the development of hypercitrullinaemia, followed by intrahepatic cholestasis in infancy. The conditions in most NICCD patients are often self-limiting and spontaneously disappear because of maturation of hepatocytes and/or some adaptations or compensations of other mitochondrial carriers. After 10 or more years, compensatory failure is likely to occur with resultant relapse of the disease in adulthood. However, one of our cases had very severe phenotype of NICCD that required liver transplantation at the age of 10 months. We suspect that some patients with hypertyrosinaemia of an unknown cause may result from NICCD. This severe phenotype of NICCD may not be that rare therefore patients with NICCD should be followed up carefully, even during infancy.

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Short communication

Alterations and diversity in the cytoplasmic tail of the fusion protein of subacute sclerosing panencephalitis virus strains isolated in Osaka, Japan☆

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Abstract

We determined the nucleotide sequence of the fusion (F) gene of three strains (Osaka-1, -2, and -3) of nonproductive variants of measles virus (MV). These viral strains were isolated in Osaka, Japan, from brain tissues of patients with subacute sclerosing panencephalitis (SSPE). Phylogenetic analysis revealed a close relationship among the three strains of SSPE virus. The cytoplasmic tail of the F protein, predicted from sequence analysis of the gene, is altered in all three SSPE strains when compared to the MV field strains. However, the extent and mode of alteration are different in each strain. The F protein of the Osaka-1 strain has six nonconservative amino acid substitutions and a 29-residue elongation of its cytoplasmic tail. The F protein of the Osaka-3 strain has two nonconservative substitutions and a 5-residue truncation of its C-terminus. Although the termination codon is not altered in the F protein of the Osaka-2 strain, five or six amino acids are changed in the cytoplasmic tail of the F protein of the two sibling viruses of this strain. The significance of the altered cytoplasmic domain of the SSPE viruses in the SSPE pathogenesis is discussed.

Keywords: Measles virus; Subacute sclerosing panencephalitis virus; Fusion gene; Mutation

The fusion (F) gene of measles virus (MV) (2373-nucleotides long for the Edmonston strain) encodes an inactive F precursor glycoprotein (F_0, 550-amino acids long). This spike protein is transported to the Golgi apparatus where cellular proteases cleave the F_0 into disulfide-linked subunits, F_1 and F_2. In general, the F gene has been

☆ The nucleotide sequence data reported in this paper for nucleotide sequences with accession numbers AF179430–AF179441 have been deposited in the DDBJ, EMBL, and GenBank™ databases.

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thought to be well conserved among the paramyxoviruses (Barrett et al., 1987; Buckland et al., 1987), particularly among MVs (Rota et al., 1992). In combination with the hemagglutinin (H) protein, another glycoprotein spike of MV, the F protein is responsible for fusion of the viral envelope with the host cell membrane and for syncytium formation in the virus-infected cells. In contrast to MV isolated from acute measles, genetic information is limited on the F protein of subacute sclerosing panencephalitis (SSPE) virus. This nonproductive MV variant with defective matrix (M) protein is isolated from brains of patients with SSPE, a fatal degenerative disease of children caused by its persistent infection of the central nervous system. The molecular mechanisms of cell-to-cell spread of SSPE virus in the brain are still unclear, although a recent report (Lawrence et al., 2000) has shown that a microfusion not mediated by CD46 might be associated with in vitro transmission of MV from neuron to neuron.

The envelope genes (F, H, and M) of MV might have evolved in the transition from acute to persistent infection in infected brains. In such specific environments, some specified mutations might have selectively survived. For example, alterations of the cytoplasmic tail of the F protein have been reported in MV persistent infection (for a review, see Griffin and Bellini, 1996). However, most sequence data were obtained from directly cloned genes from SSPE brain specimens, and might not represent the replicating viral genome. Several (the MF, Biken, Niigata-1, Yamagata-1, and IP-3-Ca strains) of a few replicable isolates of SSPE virus from diseased human brains have been sequenced (Cattaneo et al., 1987, 1988a,b, 1989; Ayata et al., 1989, 1991; Komase et al., 1990; Yoshikawa et al., 1990; Wong et al., 1991). Therefore, genetic data from more isolates are needed to clarify whether the persistence and neuropathogenicity of the virus are associated with particular mutations.

We have reported three strains (Osaka-1, -2, and -3) of nonproductive SSPE virus isolated in Osaka, Japan (Ogura et al., 1997), and have characterized the defective M gene from the Osaka-1 and -2 strains (Ayata et al., 1998a; Seto et al., 1999) and compared it with those from local contemporary isolates from patients with acute measles (Ayata et al., 1998b). In this paper, we further determined the complete nucleotide sequences of the F gene of these strains of SSPE virus in order to analyze their common mutations associated with viral persistence in the brain. The several sibling viruses isolated from different portions of the same patient's brain by cocultivating with the different cell types were compared with each other in order to investigate viral spreading in the brain.

MV strains and sibling viruses of SSPE strains sequenced for this study were previously described (Ogura et al., 1997; Ayata et al., 1998b). All of the strains, including those referred to in this paper, are listed in Table 1.

Total RNA preparation from the virus-infected cells passaged in the least possible times was subjected to reverse transcription primed with a random primer (nonadeoxynucleotide mixture, Takara Shuzo, Otsu, Japan) according to the method described previously (Ayata et al., 1998b). The sequence including the open reading frame for the F protein was amplified by polymerase chain reaction (PCR) with a set of primers, MVF-m5 (5′-AATGTCCATCATGGGTCTCAAGGT-3′) and MVF-g6 (5′-CATTGTGGATGATCTTG-CACCCTA-3′) prepared on the basis of the published sequence for the Edmonston strain of MV. To amplify the F gene of the Osaka-1 strain, MVF-m6 (5′-AACGTCCATCATGGGTCTCAA-GAT-3′) was substituted for the MVF-m5 forward primer. Amplification was performed with 30 cycles of three steps (98 °C for 10 s, 55 °C for 30 s, and 72 °C for 1 min), and Pyrobest DNA polymerase was used as recommended by the manufacturer (Takara Shuzo). Amplified products were purified according to the method described previously (Ayata et al., 1998b). A portion of the F gene was sequenced directly with a Thermosequenase II dye terminator cycle sequencing kit (Amersham Phamacia Biotech, Piscataway, NJ) and a model 373S or Prism 310 sequencer (Applied Biosystems, Foster City, CA). Also, the PCR products covering the open reading frame for the F protein were cloned into a pBluescript II KS plasmid and several clones were sequenced.

The sequences for the 5′ noncoding and 3′ noncoding region of the F gene were determined by the PCR products encompassing the M–F and F–H junctions, respectively. The primer pairs used were MVM-m7 (5′-CAGCCAGCAGCCGACGGCAA-3′) and MVF-g5 (5′-GACCGGTTCAGAGTGTAGCTTCA-3′); and MVF-m4 (5′-ATCCTGATTGCAGTGTGTCT-3′) and MVH-g3 (5′-CCACTCTTCAAAATCATCGG-3′) for PCR encompassing the M–F and F–H junctions, respectively. These primers were constructed based on the published sequences for several MVs and SSPE viruses. Amplification was performed with 40 cycles of two steps (98 °C for 20 s and 68 °C for 5 min), and an LA-PCR kit was used as previously described (Ayata et al., 1998b). A portion of the PCR products was sequenced directly as described above. Additional nine primers were constructed for sequencing F genes for both orientations, on the basis of the sequences conserved among several strains of MVs and SSPE viruses. These primers were MVF-m1 (5′-GGAATCCCAKAATCAAGACTCATC-3′), MVF-m2 (5′-CTACTAATCAGGCAATTGAG-3′), MVF-m3 (5′-CAACCCAAGGGTACCTTATC-3′), MVF-g2 (5′-TTGATGACGAAGRGGAGACTTGTG-3′), MVF-g3 (5′-TGCCCGGTAGTCGAGGTGAA-3′), MVF-g4 (5′-GAGTTATCCGGGCCTTTATT-3′), and MVF-g7 (5′-TGTGGTGGATTGATCTTTCG-3′).

An unrooted phylogenetic tree drawn by a CLUSTAL W analysis, based on the sequence of the F protein coding region of the Edmonston strain (1653 nucleotides), showed all sibling viruses of the three SSPE strains, and the Nagahata and the Masusako strains of MV in the same cluster in agreement with the results shown by our previous M gene analysis (Ayata et al., 1998b) and also by the phylogenetic analysis based on the sequence of the 3′end of the N gene of selected sibling SSPE viruses (Osaka-1 Fr/V, Osaka-2 Fr/V, and Osaka-3 Bs/V) (data not shown).

Our sequence result for the F gene of the Nagahata strain was different by three nucleotides

Table 1
Measles and SSPE virus strains compared in this study

Virus	Strain	Sibling virus	Portion of brain for isolation	Cell line for isolation	Location, year of isolation[a]
Measles	Edmonston[b]			Human kidney	Boston, 1954
	Toyoshima			Vero	Osaka, 1959
	Nagahata[c]			Vero	Osaka, 1971
	Masusako			Vero	Osaka, 1983
SSPE	Osaka-1				Osaka, 1993 (1969)
		Fr/V	Frontal lobe	Vero	
		Fr/H	Frontal lobe	TIG-1[d]	
		Oc/V	Occipital lobe	Vero	
	Osaka-2				Osaka, 1994 (1984)
		Fr/V	Frontal lobe	Vero	
		Fr/B	Frontal lobe	B95a	
	Osaka-3				Osaka, 1995 (1971)
		Bs/V	Brain stem	Vero	
		Bs/B	Brain stem	B95a	
		Oc/V	Occipital lobe	Vero	

[a] Year of primary measles infection is shown in parentheses.
[b] Richardson et al. (1986).
[c] The F gene sequence of the Nagahata strain was first reported by Watanabe et al. (1995), but it differed by three nucleotides from our sequence.
[d] Diploid cells derived from human embryonic lung cells.

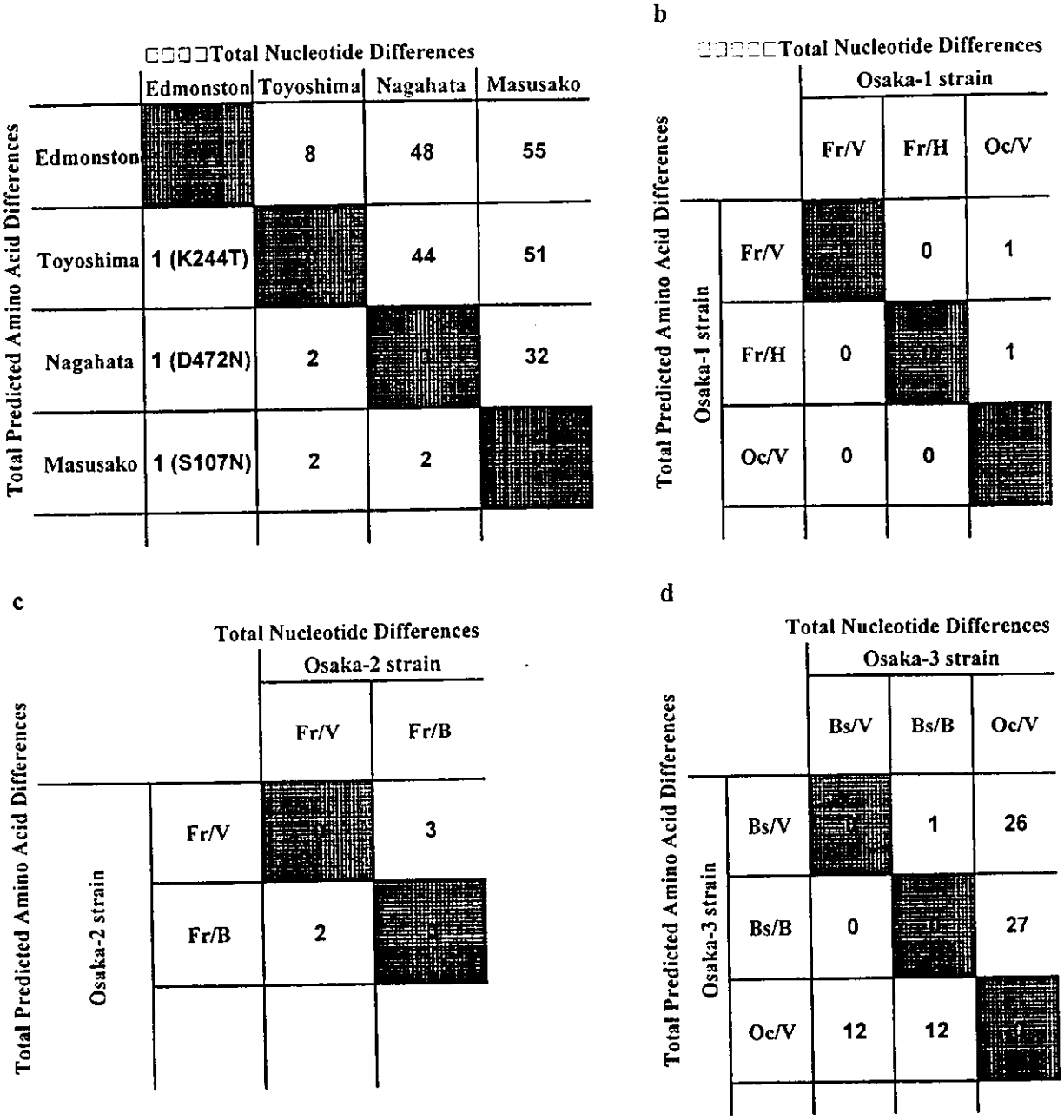

Fig. 1. Comparison of total nucleotide differences and total predicted amino acid differences among MV strains (a), among sibling viruses of SSPE virus Osaka-1 (b), -2 (c), or -3 (d) strains. Position of amino acid replacement is shown in parentheses.

from that previously reported by Watanabe et al. (1995). In their sequencing data, an A to G substitution at nucleotide number 1158 resulted in an additional amino acid substitution, a glutamine to arginine change, at residue 192 (numbered from the second AUG codon according to Cathomen et al., 1995). Total nucleotide differences and total amino acid differences among the MV strains

were shown in Fig. 1a. The Masusako strain isolated in 1983 was different from the Nagahata strain isolated in 1971 by 32 nucleotides and from the Toyoshima strain isolated in 1959 by 51 nucleotides. Despite the numerous nucleotide substitutions, only two nonconservative changes were found in the deduced amino acid sequence, showing that the F protein of the three MV strains isolated in Osaka was highly conserved. When the F protein sequences of the three MV strains were compared with that of the Edmonston strain, only a single amino acid change was noted in each strain. There were no insertions or deletions in the F gene sequence of these strains. In addition, it was confirmed that the Nagahata and the Masusako strains, which were isolated at roughly the same time and the same place as the primary measles infection in our SSPE patients, were the most adequate strains for use as a reference in comparison with the F gene of the Osaka-1, -2, and -3 strains (Table 2). This result was consistent with that from our previous M gene analysis (Ayata et al., 1998a).

When whole F gene sequences were compared, a single G insertion was observed in the 5′ noncoding region of the Osaka-1 and Osaka-3 strains (data not shown). For detailed analysis, we compared numbers of nucleotide differences of the F genes and amino acid differences of the predicted F proteins among the eight sibling viruses of the three SSPE virus strains and the two selected MV strains (the Nagahata and the Masusako) (Table 2), and among the sibling viruses of each SSPE strains (Fig. 1b, c, and d). The F gene sequence of the Osaka-1 Fr/V sibling virus of the Osaka-1 strain was different from that of the Nagahata strain by 45 nucleotides (Table 2). The sequence of the Osaka-1 Fr/H virus was identical to that of the Osaka-1 Fr/V virus, and only one nucleotide difference without amino acid replacement was found in the sequence of the Osaka-1 Oc/V virus (Fig. 1b). The F gene sequence of the Osaka-2 Fr/V sibling virus was different from that of the Nagahata strain by 51 nucleotides. The Osaka-2 strain was more closely related to the Masusako strain; the nucleotide differences of the F gene between the Osaka-2 Fr/V and the Masusako strain decreased to 27 (Table 2). There were three nucleotide differences in the sequence between the Osaka-2 Fr/V and the Osaka-2 Fr/B viruses, and two of the three differences were nonconservative changes (Fig. 1c). The F gene sequence of the Osaka-3 Bs/V sibling virus was different from that of the Nagahata strain by 46 nucleotides (Table 2). The F gene sequence of the Osaka-3 Bs/B virus was identical to that of the Osaka-3 Bs/V virus excepting a single nucleotide, which difference did not result in an amino acid change (Fig. 1d). A total of 26 nucleotide differences, 12 of which were nonconservative changes, were noted between the Osaka-3 Bs/V and the Osaka-3 Oc/V sibling viruses (Fig. 1d), whereas the F gene sequence was very similar among the sibling viruses of the Osaka-1 or -2 strains and among the Osaka-3 Bs/V and Bs/B sibling viruses (Fig. 1b, c, and d).

Table 2
Comparison of nucleotide differences between SSPE strains and MV field strains

Strain	Sibling virus	Nagahata	Masusako
Osaka-1	Fr/V	45[a] (13)[b]	57[a] (13)
	Fr/H	45[a] (13)	57[a] (13)
	Oc/V	46[a] (13)	58[a] (13)
Osaka-2	Fr/V	51 (12)	27 (12)
	Fr/B	48 (10)	24 (10)
Osaka-3	Bs/V	46[a] (14)	56[a] (14)
	Bs/B	45[a] (14)	55[a] (14)
	Oc/V	48[a] (14)	59[a] (14)

[a] A single nucleotide insertion is included in the 5′-noncoding region.

[b] Amino acid differences in the region of amino acid residues 1–550 are shown in parentheses.

In all three SSPE strains no amino acid substitution was found at the glycosylation sites (amino acid residues 29, 61, and 67), at the subtilisin-related protease cleavage site (amino acid residues 108–112), at the disulfide sites (amino acid residues 68 and 195), or at the palmitoylation sites (amino acid residues 503, 515, and 521) (data not shown). In addition, the hydrophobic region located at amino acid residues 113–136 was conserved (data not shown). This conservation may be natural, given that these sites are essential for viral multiplication, especially through fusion ac-