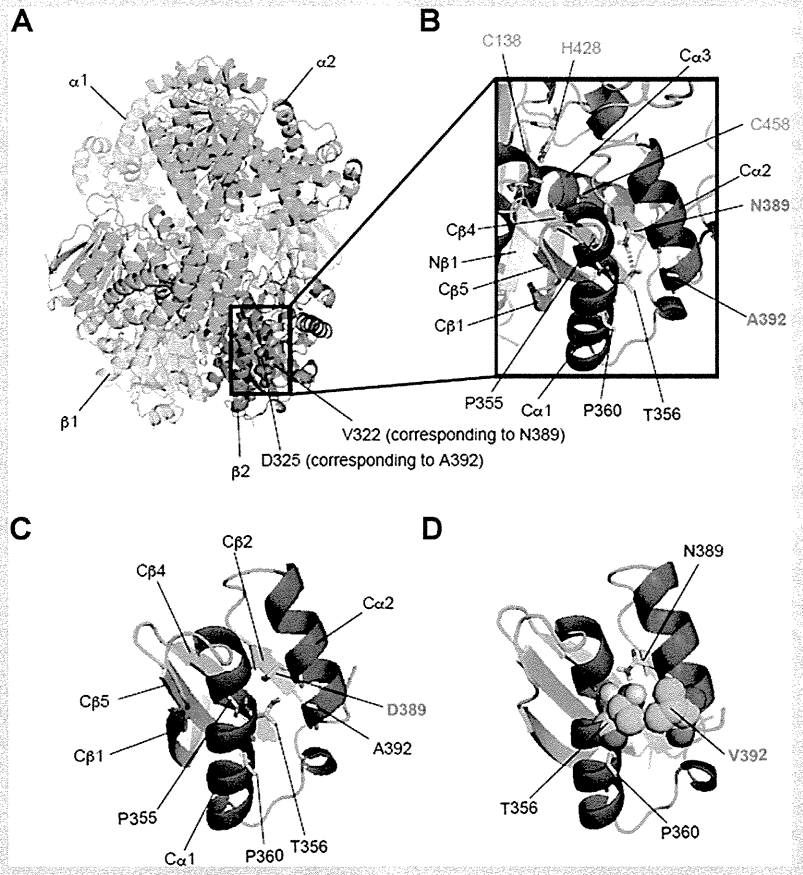

FIG. 3. Structure and homology modeling of MTP. A: α2β2 tetrameric structure of MTP. Molecules colored in cyan and green are the α-subunits [α1 and α2, respectively] of MTP from *Pseudomonas fragi*, whereas those colored in yellow and orange are the β-subunits [β1 and β2, respectively] [Ishikawa et al., 2004]. V322 and D325 are depicted as sphere models. These residues correspond to N389 and A392 of human MTP. B: Enlarged view of the homology model of one of the β-subunits of human MTP. α-helices and β-strands are colored in red and yellow, respectively. Hydrogen, carbon, oxygen, nitrogen, and sulfur atoms are colored in gray, cyan, red, blue, and yellow, respectively. Secondary structure nomenclature [Nβ1, Cβ1, Cβ4, Cβ5, Cα1, Cα2, and Cα3] defined in the yeast thiolase structure [Mathieu et al., 1997] is also indicated. Helices corresponding to Lα1 [171–175], Lα2, and Lα3 of yeast thiolase [V171-L260] have been removed for clarity. A hydrogen bond between the amino group of N389 and the hydroxyl group of T356 is depicted as a dotted line [pink]. C: View around the N389D mutation in the homology model of the β-subunit of human MTP. D: View around the A392V mutation. T356 and V392 are depicted as sphere models.

reported the case of a female patient with MTP deficiency and hypoparathyroidism. Hypoparathyroidism became apparent when she was admitted to the hospital because of fasting-induced rhabdomyolysis at 15 months of age. She had severe hypotonia, respiratory failure, and peripheral polyneuropathy without renal failure. Her serum iPTH concentration was low (4 pg/ml) with severe hypocalcemia (0.95 mmol/L), and the enzyme activities of MTP in the fibroblasts were all reduced: LCKT activity was absent, and LCHAD and LCEH activities were 30% and 52% of the control mean, respectively. These results indicate that she had MTP deficiency with a neuromyopathic phenotype, caused possibly by an *HADHB* mutation encoding LCKT. Labarthe et al. [2006] also reported a female case with hypoparathyroidism and MTP deficiency caused by a *HADHB* mutation. Hypoparathyroidism (iPTH <5 pg/ml) and severe hypocalcemia (1.2 mmol/L) became evident when she was 4 months old. A homozygous mutation (c.1165A>G, [p.N389D]) in *HADHB* was identified. Her serum iPTH concentration reached normal levels after vitamin D therapy, although she developed peripheral polyneuropathy with decreased nerve conduction velocity. LCKT activity was not reported, but numerous episodes of fasting-induced rhabdomyolysis suggested that she had a defective β-subunit of MTP. Indeed, we demonstrated that the N389D β-subunit does not associate with the wild-type α-subunit (Fig. 2C). Taken together, the sibling patients presented here and the two previously reported cases have similar clinical features of infantile onset hypoparathyroidism, peripheral polyneuropathy, and rhabdomyolysis.

A dysfunction in mitochondrial energy metabolism and/or toxicity of accumulated long-chain fatty acids in the parathyroid glands may have contributed to the pathogenesis of hypoparathyroidism in the patients with MTP deficiency [Saudubray et al., 1999]. In fact, hypoparathyroidism has also been reported in other disorders of mitochondrial fatty acid oxidation, including LCHAD deficiency [Tyni et al., 1997] and MCAD deficiency [Baruteau et al., 2009]. However, this hypothesis cannot explain why hypoparathyroidism has not been reported in patients with the most common inborn mitochondrial fatty acid β-oxidation disorders of carnitine palmitoyl transferase II and very-long-chain acyl-CoA dehydrogenase deficiencies or the severe form of MTP deficiency (a lethal phenotype). Thus, the mechanisms underlying the pathogenesis of the disease presented here remains to be elucidated. Tyni et al. [1997] reported the autopsy findings of a patient with hypoparathyroidism and LCHAD deficiency where the parathyroid glands were severely hypoplastic. These findings suggest that mutations of the proteins associated with β-oxidation cause hypoparathyroidism by congenital malformations of parathyroid glands.

Our studies demonstrate decreased expression of the mutant β-subunits (N389D and A392V) and a failure of those subunits to associate with the wild-type α-subunit (Fig. 2). Moreover, N389D and A392V are located close to the active site and are likely have an immediate impact on the structure and function of the catalytic core of human MTP (Fig. 3C,D). A recent study demonstrated that the β-subunit of MTP interacts and colocalizes with the estrogen receptor α or β in the mitochondria and suggested an important role of the β-subunit in estrogen-mediated lipid metabolism [Zhou et al., 2012a,b]. From this perspective, mutant N389D and A392V β-subunits may cause mitochondria dysfunction, including MTP deficiency due to a failure to associate with the α-subunit of MTP and other proteins that result in dysfunction of parathyroid glands. Further case studies are required to determine whether the specific mutations located in the proximity of the active site of the β-subunit are associated with hypoparathyroidism and MTP deficiency.

Early diagnosis and treatment are important for patients with the neuromyopathic phenotype of MTP deficiency, since peripheral polyneuropathy is progressive [Spiekerkoetter et al., 2004; Yamaguchi et al., 2012]. Our study has demonstrated that MTP deficiency should be considered when patients have hypoparathyroidism as the initial presenting feature in infancy.

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【長期予後と成人後の医学的問題】

先天代謝異常症

深尾敏幸

キーワード●先天代謝異常症，予後，成人期，治療

はじめに

先天代謝異常症は，一般的にまれな遺伝性疾患で，代謝系における特定の酵素等の欠損・欠乏により代謝に異常が生じるために起こる疾患である．アミノ酸代謝異常症，尿素サイクル異常症，有機酸代謝異常症，脂肪酸代謝異常症，糖代謝異常症，金属代謝異常症などのように分類されたり，障害される細胞内小器官を中心にミトコンドリア病，ライソゾーム病，ペルオキシソーム病などのように分類されたりする．日本先天代謝異常学会では，先天代謝異常症を身近に感じてもらい，日本全体の診療レベルを向上するために10年前から日本先天代謝異常学会セミナーを開催している．最近は毎年300～400名が参加しており，先天代謝異常症診療の裾野は確実に広がってきている．

I 先天代謝異常症の治療と予後

平成26年度にはタンデムマス法による新生児マス・スクリーニングが全国に広がり，対象疾患が6疾患から，アミノ酸代謝異常症，脂肪酸代謝異常症，有機酸代謝異常症のいくつかが追加されて19疾患へと増加した．追加された疾患は，多くが新生児期～乳幼児期に低血糖，代謝性アシドーシス，高アンモニア血症などの重篤な発作を来す可能性がある疾患である．初回発作で死亡したり後遺症を残す可能性が高い疾患であり，早期診断，治療開始により，予後の改善が期待されている．

この15年で先天代謝異常症に対する治療成績が大きく向上した．①海外では使われていたが日本では未承認という薬剤が，いくつか国内での使用が可能となったこと，②酵素製剤などの新規治療薬剤の開発が進んだこと，③骨髄移植，肝移植などの移植治療が取り入れられたこと，④血液浄化法など急性期治療技術が向上したことなどである．それに伴い，長期生命予後は向上している．代謝異常は生涯にわたって存在し，小児期にのみ治療すればよいという疾患は少なく，成人後の対応が問題化している．

以下に代表的な先天代謝異常症とその予後，成人期の問題点について記載したい．

1. アミノ酸代謝異常症

●フェニルケトン尿症

アミノ酸代謝異常症の代表的疾患であるフェニルケトン尿症（phenylketonuria；PKU）は，無治療では重度の精神発達遅滞を来す．新生児マス・スクリーニングにより新生児期に診断されることで，無症状のうちからフェニルアラニン（Phe）除去ミルクを併用したPhe制限食療法を行うことにより知的予後は改善した．食事制限は特に小児期に重要であるが，生涯続けることが望ましい[1-4]．成人期においても高Phe血

Long-term prognosis and medical problems in adulthood for patients with inborn errors of metabolism
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症は，抑うつなどの精神症状，MRIで白質病変を来すとされており[2,3]，また酸化ストレスの亢進を介して全身的な影響を及ぼすことが示唆されている[4]からである．また女性については，妊娠時に胎児が高Phe環境にさらされることで生じる知的障害，先天異常（マターナルPKU）を防ぐため，食事療法の継続が必要で，一旦緩和してしまうと，妊娠前に厳しく制限し直すことが必要となる．

しかし本邦において，2004年の調査では，1977～1981年に出生した23～27歳の患者の60%，およびそれ以降に出生した18～22歳の患者の30%はPhe制限食を中止していた[1]．高校生までは食事療法がうまくいっていた症例でも，大学への進学や就職により親元を離れたり，忙しくて外来受診が難しくなり，食事療法が中断もしくは大幅に緩和されてしまうことが経験される．20歳を超えると小児慢性特定疾患による公費補助がなくなり，治療用ミルク，低タンパク食品の購入など経済的な負担が大きいことも中断に大きく影響していると考えられる［成人期のフェニルケトン尿症（PKU）．特殊ミルク情報2011；47：6-39］．

2. 尿素サイクル異常症

● オルニチントランスカルバミラーゼ欠損症

尿素サイクル異常症は，一般に高アンモニア血症を来す予後の悪い疾患群であり，その代表がオルニチントランスカルバミラーゼ（OTC）欠損症である．本症はX連鎖遺伝をとり，多くは男児が発症するが，X染色体不活化の偏りで女児にも発症することが比較的高い疾患である．

2009年のアンケート調査[5]によれば，新生児発症（重症型）のOTC欠損症の男児においては，新生児期に20%が亡くなっているが，そこで救命された症例は20歳までほぼ生存している．新生児期以降発症のOTC欠損症男児例においても，20歳における生存率は1998年の報告が45%であったのに対し，2009年の調査では89.4%と改善し，新生児期以降発症の女児例においても29.8%から83.8%と改善している[5]．この生命予後の改善は，薬物投与，血液浄化による急性期治療，タンパク制限などの治療が確立し，また肝移植が導入されて救命できる症例が増えてきたためと考えられる．

しかし生命予後は改善されているが，神経学的予後は発作時のピークのアンモニア値が高い場合，厳しい現実がある．360μmol/L以上であった症例の15%は死亡し，51%が知的な発達遅滞を残し，15%は発達遅滞がないものの画像，脳波に異常をもっている．わずか8%が正常発達し，画像や脳波に異常がないというのが現状である[5]．このように，成人期を迎えた多くの患者がハンディキャップをもち，さらに治療継続が必要な状態と言える．

3. 有機酸代謝異常症

● ビタミンB_{12}不応性メチルマロン酸血症

有機酸代謝異常症の代表的な疾患の1つがビタミンB_{12}不応性メチルマロン酸血症（methylmalonic acidemia；MMA）である．詳細は省略するが，2009年に行われた日本におけるアンケート調査[6]では，ビタミンB_{12}不応性MMAは69.8%が20歳で生存しており，生命予後という点では非常に良くなっている．しかし，成人例を含めた69%が重篤な発作の後遺症と考えられる精神発達遅滞を来しており，また腎障害も35.7%に合併している．

最近はコントロール不良例では肝移植が行われることがあり，臨床的に食事制限が緩和され，アシドーシス発作が著しく少なくなりQOLに改善がみられている．しかし多くの肝移植症例は，それ以前の発作後遺症により精神発達遅滞を合併しているのが現状である[6]．新生児マス・スクリーニングの対象疾患となったので，今後は予後の改善が期待される．

4. ライソゾーム病

ライソゾーム病の中からは，小児期に典型的症状を来すムコ多糖症II型と，小児期よりは成人期に典型的症状を来すファブリー病の2つ

を取り上げる．共に，現在は酵素補充療法という治療法があるX連鎖遺伝のライソゾーム病である．

(1) ムコ多糖症II型

ムコ多糖症II型はα-イズロネート2-スルファターゼの欠損症で，グリコサミノグリカン(GAG)の蓄積を特徴とする．発症年齢，疾患の重症度，進行速度には著しい差がある．通常，幼児期に診断されることが多い．従来，重症型の患者では中枢神経系症状，進行性の気道疾患，心疾患により20歳以前に死亡した[7]．軽症型の患者では正常な知能を維持したままで成人初期まで生存する場合が多いが，関節拘縮，難聴の進行，気道狭窄による呼吸障害，心臓弁膜症の進行などで生活が制限される．早期に診断し，早期に酵素補充療法，骨髄移植を行うことがQOLの改善には重要と考えられ，今後，酵素補充療法などにより成人期に治療継続が必要な症例が増えてくると思われる．

小児期発症のライソゾーム病では，成人期の治療は多くの場合，小児期から診ている医師がそのまま引き続き行って，他診療科との連携をとっている場合が圧倒的に多いと考えられる．

(2) ファブリー病

ファブリー病は，α-ガラクトシダーゼA酵素の活性が低下することにより，心臓，腎臓，血管，皮膚，角膜，神経など全身の臓器にスフィンゴ糖脂質の蓄積が生じ，多臓器障害を起こす疾患である．典型的ファブリー病の男性患者では，幼少時より四肢末端の痛みの発作，発汗障害があり，この時期の診断のきっかけになるが，一般には成人期に入って皮膚の被角血管腫，角膜の混濁，尿タンパクから腎不全という腎臓機能障害，心臓肥大などの心臓機能障害や一過性脳虚血発作，多発性小梗塞などの脳血管障害も出現する[8]．女性がかなり発症するX連鎖遺伝をとるため，女性保因者のフォローも重要である[8]．

まれな疾患と考えられてきたが，日本において比較的頻度の高い疾患であることが明らかになってきており[9]，成人例として見つかった患者は循環器内科，腎臓内科，神経内科などで診療されている例も多い．酵素補充療法は，男児の場合臓器障害の進行していない小児期からすべきと考えられ，小児期に診断された患者は小児科において開始することになる．成人患者においても小児科の先天代謝異常症を専門にしている医師が酵素補充療法を行い，他の循環器内科，腎臓内科，神経内科との連携で診ているところもある．

II 先天代謝異常症における成人期の問題

上記に示したファブリー病以外の疾患の長期予後，成人期の問題は共通している部分が多い．治療管理が向上し，生命予後が改善し，多くの先天代謝異常症患者において，成人期の診療が重要となってきている．生命予後は良くなってはいるが，精神運動発達にハンディキャップをもって成人期を迎えている患者が多い．ハンディキャップをもった患者の場合は，引き続き家族による診療へのかかわりが重要であることは言うまでもないが，知的問題がない思春期以降の患者に対し，家族依存の治療体制から本人中心への治療への移行が必要となる．

だれが成人期の先天代謝異常症患者を中心に診て行くのか？　おそらく日本の現状では，小児期に診てきた小児科の医師がそのまま継続するパターンが多いと考えられる．しかし当然であるが，小児科医は加齢に伴う成人期の合併疾患の診療が苦手であり，小児科のみでは対応できない問題が加齢と共に大きくなってくる．成人期に主要な症状を呈するファブリー病のような疾患以外でも，通常は小児期に発症する先天代謝異常症が成人後に発症することもあり[10]，成人の診療にあたる医師が先天代謝異常症についても知識をもつことは重要である．

ヨーロッパの先天代謝異常学会では2010年に先天代謝異常症の成人に関心のある臨床医が

集まり，ヨーロッパ先天代謝異常学会成人代謝異常臨床医グループを形成し，2011年の年次集会から成人の先天代謝に関するワークショップ等を開催して非常に関心が高まってきている．内科，神経科，遺伝科，内分泌科，成人代謝異常クリニックなどの肩書きの医師でグループを形成しているが，まだ日本からの参加はない．将来的にはヨーロッパ同様に，内科，総合診療科の中で成人期の先天代謝異常症患者の診療を行う担当医ができてくるのも1つの方向性ではないかと考える．しかし小児科の中でも先天代謝異常症を専門にする医師が少ない中で，すぐに実現する状況にはない．先天代謝異常学会に内科関係の医師がもっと参加しやすい環境づくりをすることが重要と考えられる．

おわりに

最後に，先天代謝異常症の多くは小児慢性特定疾患であり，20歳未満までの医療費公費負担がなされている．今後，特定疾患に一部移行すると期待されるが，移行しなかった疾患においては，成人期の医療費の負担が大きな問題となる点を指摘したい．

成人期を迎えた先天代謝異常症患者が，患者の状況に応じた適切な診療を受けられる仕組みを作り上げていくことが，日本先天代謝異常学会としての1つの今後の使命である．

謝辞：現在，厚生労働科学研究費補助金難治性疾患克服研究事業「新しい先天代謝異常症スクリーニング時代に適応した治療ガイドラインの作成および生涯にわたる診療体制の確立に向けた調査研究」(研究代表者：遠藤文夫)において，先天性代謝異常症の成人期における問題点についての検討がなされており，熊本大学大学院生命科学研究部小児科学分野・遠藤文夫教授にご校閲いただきました．ここに深謝いたします．

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ORIGINAL INVESTIGATION

A hemizygous *GYG2* mutation and Leigh syndrome: a possible link?

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Abstract Leigh syndrome (LS) is an early-onset progressive neurodegenerative disorder characterized by unique, bilateral neuropathological findings in brainstem, basal ganglia, cerebellum and spinal cord. LS is genetically heterogeneous, with the majority of the causative genes affecting mitochondrial malfunction, and many cases still remain unsolved. Here, we report male sibs affected with LS showing ketonemia, but no marked elevation of lactate and pyruvate. To identify their genetic cause, we performed whole exome sequencing. Candidate variants were narrowed down based on autosomal recessive and X-linked recessive models. Only one hemizygous missense mutation (c.665G>C, p.W222S) in glycogenin-2 (*GYG2*) (isoform a: NM_001079855) in both affected sibs and a heterozygous change in their mother were identified, being consistent with the X-linked recessive trait. *GYG2* encodes glycogenin-2 (GYG2) protein, which plays an important role in the initiation of glycogen synthesis. Based on the structural modeling, the mutation can destabilize the structure and result in protein malfunctioning. Furthermore, in vitro experiments showed mutant GYG2 was unable to undergo the self-glucosylation, which is observed in wild-type GYG2. This is the first report of *GYG2* mutation in human, implying a possible link between GYG2 abnormality and LS.

Introduction

Glycogen is a large branched polysaccharide containing linear chains of glucose residues. Glycogen deposits in skeletal muscle and liver serve as shorter-term energy storage in mammals, while fat provides long-term storage. Glycogen biosynthesis begins with self-glucosylation of glycogenins by covalent binding of UDP-glucose to tyrosine residues of the glycogenins and the subsequent extension of approximately ten glucose residues (Pitcher et al. 1988; Smythe et al. 1988). Glycogen particles are formed by the continued addition of UDP-glucose to the growing

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glycogen chain by glycogen synthase, and introduction of branches every 10–14 residues by the glycogen branching enzyme (Krisman and Barengo 1975; Larner 1953). To date, two glycogenin paralogues have been identified in human, glycogenin-1 (GYG1) and glycogenin-2 (GYG2). These proteins have been shown to form homodimers, heterodimers and larger oligomers (Gibbons et al. 2002). GYG1 (muscle form) is expressed predominantly in muscle while GYG2 (liver form) is expressed mainly in liver, heart and pancreas (Barbetti et al. 1996; Mu et al. 1997). Biallelic GYG1 abnormality is known to cause muscle weakness and cardiac arrhythmia in humans through GYG1 autoglucosylation failure (Moslemi et al. 2010). However, human disease due to GYG2 abnormality has never been reported.

Leigh syndrome (LS; MIM #256000) was first described as a subacute necrotizing encephalomyelopathy by Dr. Denis Leigh in 1951 (Leigh 1951). LS is a progressive neurodegenerative disorder with an estimated incidence of 1:40,000 live births (Rahman et al. 1996). Onset is usually in early childhood (typically before age 2) (Naess et al. 2009; Ostergaard et al. 2007). Clinical manifestations of LS are observed in the central nervous system (CNS) (developmental delay, hypotonia, ataxia, convulsion, nystagmus, respiratory failure and dysphagia), peripheral nervous system (polyneuropathy and myopathy) and extraneural organs (deafness, diabetes, cardiomyopathy, kidney malfunction and others) (Finsterer 2008). The neurological features depend on the affected regions and degree of severity. The presence of bilateral, symmetrical, focal hyperintense T2-weighted MRI signals in basal ganglia (mainly putamen), thalamus, substantia nigra, substantia ruber, brainstem, cerebellum, cerebral white matter or spinal cord is diagnostic of LS (Farina et al. 2002; Medina et al. 1990). Neuropathological studies revealed that these lesions reflect neuronal necrosis, gliosis and vascular proliferation (Brown and Squier 1996; Leigh 1951). In the majority of LS cases, lactate, pyruvate or the lactate/pyruvate ratio is increased in blood and cerebrospinal fluid (Finsterer 2008). To the best of our knowledge, 37 nuclear genes are known to be mutated in LS, in addition to some mitochondrial genes (Antonicka et al. 2010; Debray et al. 2011; Finsterer 2008; Lopez et al. 2006; Martin et al. 2005; Quinonez et al. 2013). Thus, inheritance patterns of LS include mitochondrial, autosomal recessive and X-linked recessive modes (Benke et al. 1982; van Erven et al. 1987).

We encountered a Japanese family with affected brothers showing atypical LS without marked elevation of lactic or pyruvic acid and unknown etiology. A unique genetic variant was identified by whole exome sequencing (WES), which may be associated with atypical LS phenotype in this family.

Materials and methods

Subjects

Peripheral blood samples of affected brothers diagnosed with LS and their parents were collected after obtaining written informed consent. DNA was extracted from peripheral blood leukocytes using QuickGene-610L (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. Lymphoblastoid cell lines derived from all family members were established. The Institutional Review Boards of Yokohama City University School of Medicine approved this study.

Causative gene identification

Whole exome sequencing was performed in two affected individuals (II-2 and II-3 in Fig. 1a) as described in the Supplementary methods. All candidate variants based on autosomal and X-linked recessive models were checked by Sanger sequencing in the parents and affected siblings. PCR products amplified with genomic DNA as a template were sequenced on an ABI3500xl autosequencer (Applied Biosystems, Foster City, CA) and analyzed using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI). As the pedigree tree might also indicate mitochondrial inheritance of this disease and LS is known to be caused by mitochondrial genome mutations, we screened the entire mitochondrial genome by the algorithm reported previously (Picardi and Pesole 2012), using exome data (detailed in Supplementary methods).

Structure modeling

To evaluate the effect of the GYG2 missense mutation (c.665G>C, p.W222S in isoform a: NM_001079855) on its function at the molecular structural level, the mutated molecular structure was constructed, and the free energy change caused by the mutation was calculated using the FoldX software (version 3.0) (Guerois et al. 2002; Khan and Vihinen 2010). As crystal structure of human GYG2 is unavailable, that of human GYG1 (Protein Data Bank code; 3T7O) was used as a structural model. The mutation was introduced into one subunit of the GYG1 homodimer. The ligands included in the crystal structure of GYG1 were ignored in the calculation, because the FoldX energy function could not deal with the ligands. The calculation was repeated three times, and the resultant data were presented as an average value with standard deviations.

Preparation for mammalian expression vectors

Human glycogenin-2 isoform a cDNA clone (IMAGE Clone ID: 100008747) integrated in pENTR221 was purchased from Kazusa DNA Research Institute (Chiba, Japan). The

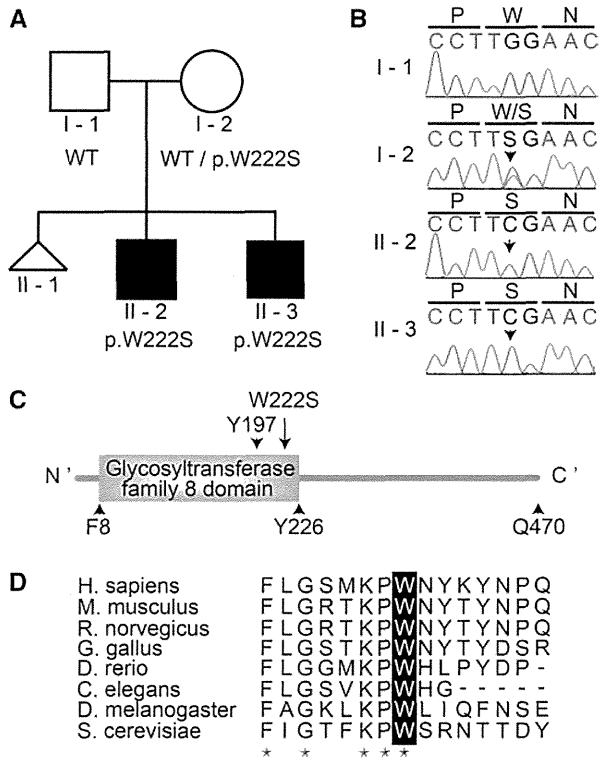

Fig. 1 Mutation Analysis of *GYG2*. **a** Pedigree of the family with a unique type of LS and a *GYG2* mutation (c.665G>C, p.W222S). *Square*, *circle* and *triangle* denote male, female and spontaneous abortion, respectively. *White* and *black symbols* indicate unaffected and affected individuals, respectively, while the affection status of the spontaneous abortion is unknown. **b** Electropherograms of a *GYG2* mutation. **c** The functional domain of human GYG2 (isoform a). The substitution of p.W222S is located within the glycosyltransferase family 8 domain (*yellow square*). **d** The evolutionary conservation of the W222 in GYG2. *Red stars* indicate identical amino acids from *S. cerevisiae* to *H. sapiens*. Sequences were aligned using CLUSTALW (http://www.genome.jp/tools/clustalw/)

missense mutation (c.665G>C, p.W222S) was introduced by Site-directed mutagenesis using the QuikChange II XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). Wild-type and mutant C' V5/6xHis tagged GYG2 constructs were created using pcDNA-DEST40 (Invitrogen, Carlsbad, CA) by LR recombination in Gateway system (Invitrogen). To create the untagged construct, the last codon was altered to a stop codon by mutagenesis.

Self-glucosylation analysis

Glucosyltransferase activity of GYG2 was measured as previously described (Lomako et al. 1988), with slight modifications. In brief, COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, Schnelldorf, Germany) containing 10 % heat-inactivated fetal bovine serum (FBS) (Gibco-BRL, Grand Island, NY), 2 mM L-glutamine (Sigma-Aldrich) and 1 % penicillin–streptomycin (Sigma-Aldrich). As previously described (Mu and Roach 1998), the ~80 % confluent COS-1 cells (~1 × 10^7) were transiently transfected by X-treamGENE9 DNA transfection reagent (Roche Applied Science, Foster City, CA) with 5 μg of either a wild-type Human GYG2 (isoform a) expressing plasmid or the same plasmid into which the W222S encoding mutation had been introduced. After 24 h, the cells were collected and lysed in 300 μl of buffer consisting of 50 mM HEPES, 0.5 % Triton X-100, 1 × EDTA-free protease Inhibitor Cocktail tablets (Roche Applied Science), 1 × phosphatase inhibitor cocktail (Nacalai Tesque Inc., Kyoto, Japan) and 0.5 mM β-mercaptoethanol (Mu et al. 1997). After centrifugation at 14,000 rpm for 15 min, 10 μl of the soluble fractions were mixed with 10 μl of 2 × reaction buffer containing 100 mM HEPES (pH7.5), 10 mM MgCl, 4 mM dithiothreitol (DTT) and 40 μM UDP-[^{14}C]-glucose (250 mCi/mmol; PerkinElmer, Waltham, MA) (Cao et al. 1993). After incubation at 30 °C for 30 min, the reaction was stopped by addition of 20 μl of 2 × Laemmli sample buffer (Sigma-Aldrich) (Viskupic et al. 1992). 15 μl of each sample was subjected to SDS-polyacrylamide gel electrophoresis. After treatment with Gel drying solution (Bio-Rad Laboratories, Hercules, CA) for 30 min, gels were dried. Dried gels were then exposed on X-ray film for 2 weeks to detect the incorporation of UDP-[^{14}C]-glucose into GYG2. In addition, the ^{14}C-signal intensities were evaluated using an imaging analyzer, BAS2500 (Fujifilm). Three independent experiments were performed.

Western blot analysis

For the detection of GYG2 protein, rabbit polyclonal anti-GYG2 antibodies (1:500 dilution; Abcam Inc., Cat.#HPA005495, Cambridge, MA) and horse-radish peroxidase (HRP)-conjugated anti-rabbit IgG (1:10,000 dilution; Jackson ImmunoResearch, Cat.#111-035-003, West Grove, PA) were used. Immunoblot chemiluminescence was performed using SuperSignal West Dura as substrate (Thermo Fisher Scientific, Waltham, MA). The chemiluminescence signal images were captured by FluorChem 8900 (Alpha Innotech, San Leandro, CA). Signal intensities were measured by AlphaEase FC (Alpha Innotech). Three independent experiments were performed.

Results

Clinical finding

Patient II-2 (Fig. 1a; Table 1) is a 26-year-old male born to non-consanguineous parents. His mother previously had a

Table 1 Clinical features of the presenting patients affected with LS

	II-2	II-3
Sex	M	M
Age (years)	26	19
Common clinical phenotype		
Psychomotor retardation	+	+
Failure to thrive	+	+
Swallowing difficulties	–	–
Spasticity	+	+
Rigidity	+	+
Pathological reflexes	+	+
Ataxia	+	+
Athetoid movements	+	+
Convulsions	+	+
Ophthalmoplegia	+	+
Strabismus	+	+
Gastrointestinal problems	+	+
Renal agenesis	NA	+
Pes equinovarus	+	+
Uncommon clinical phenotype		
Increase of ketone body	+	+

NA not assessed

spontaneous abortion. He was born at 39 weeks gestation without asphyxia after an uneventful pregnancy. His body weight was 3,680 g (+1.6 SD), his height was 50.0 cm (−0.5 SD), and his head circumference (HC) was 34.0 cm (−0.5 SD). His early developmental milestones were normal with head control and reach to toys at 4 months, roll at 6 months and grasp with two fingers at 7 months. At 10 months, he was referred to our hospital because of an inability to sit. His body weight was 9,120 g (±0.0 SD), his height was 76.0 cm (+1.3 SD), and his HC was 48.0 cm (+1.4 SD). He could smile and swallow well. Bilateral strabismus was noted. No minor anomalies were noticed. Muscle tone was normal. Deep tendon reflexes were normal with negative Babinski sign. He showed athetoid movements of trunk and extremities. He showed pes equinovarus at traction response. Levels of lactate and pyruvate were normal with 12.2 and 0.89 mg/dl (L/P ratio = 13.7), respectively. Other laboratory examinations, including blood gas, blood sugar, ammonia, AST, ALT, BUN, Creatine, TSH, T3, T4, amino acids, and urine organic acid analyses were all normal. Electroencephalogram (EEG) showed no abnormalities. He was suspected to have dyskinetic cerebral palsy and referred to the division of rehabilitation. He could crawl at the age of 2. At 6 years, he experienced a loss of consciousness followed by generalized tonic–clonic convulsion with fever and was admitted to another hospital. He was diagnosed with bilateral infarction of the basal ganglia. Although EEG showed no abnormalities, clonazepam was started with the suspicion of symptomatic epilepsy. At the age of 9, he was referred to us again. His weight was 19.1 kg (−4.5 SD), his height was 115.0 cm (−2.8 SD). He lost the ability to speak several words and switched handedness from right to left. He also showed other signs of regression: including spasticity with elevated deep tendon reflexes and positive Babinski sign. In addition, he suffered bilateral hip joint dislocations and the foot deformity became worse. Contractures were noted in all extremities. Brain magnetic resonance imaging (MRI) revealed a bilateral necrotic lesion of the globus pallidus (Fig. 2a, b). EEG and motor conduction velocities were normal. Laboratory examinations, including lactate and pyruvate, were all normal. At the age of 12, he was admitted with acute bronchitis, at that time he showed an increase of blood ketone bodies: acetoacetic acid, 720 μmol/l; 3OHBA, 974 μmol/l and urine ketone (+++). Blood levels of ammonia (18 μmol/l), sugar (125 mg/dl) and lactate/pyruvate (5.1/0.29 mg/dl) were all within normal range. The values of blood ketone bodies returned to normal level with the cease of fever. Deficiencies of 3-ketothiolase and succinyl-CoA:3-oxoacid CoA transferase were ruled out by enzyme analysis using fibroblasts. His clinical symptoms and repeated MRI show the non-progressive course of his disease. Currently he is unable to sit or speak any words. Despite the addition of carbamazepine and lamotrigine, he still exhibits generalized tonic–clonic convulsion a few times a year. He also takes medicine for hypertonicity including dantrolene sodium, diazepam, baclofen and levodopa.

Patient II-3 (Fig. 1a; Table 1), the younger brother of II-2, was born uneventfully. He was born at 37 week's gestation without asphyxia after an uneventful pregnancy. His body weight was 3,668 g (+1.5 SD), his height was 50.0 cm (+0.5 SD), and his HC was 36.0 cm (−0.5 SD). He suffered from bacterial meningitis of unknown origin at 1 month of age. He became unconscious followed by convulsion and gastroenteritis at 1 year and 11 months. Brain MRI showed marked swelling of the basal ganglia (Fig. 2c, d). He was diagnosed with bilateral infarction of the basal ganglia. After this event, he became left handed. When he was 2 years old, surgery was performed to correct bilateral inner strabismus. He was referred to our hospital at the age of 4 for evaluation. His body weight was 11.0 kg (−2.2 SD), his height was 92.5 cm (−1.2 SD), and his HC was 49.5 cm (−1.3 SD). He could respond with a smile to his mother's voice. Motor milestones were delayed with no head control. No minor anomalies were noticed. Muscle tone was hypotonic. Deep tendon reflexes were exaggerated with positive Babinski sign and ankle clonus. He showed pes equinovarus. He showed a significant increase of blood acetoacetic acid of 1,270 μmol/l and 3-OHBA of 3,270 μmol/l. Levels of blood lactate and pyruvate were normal (6.2 and 0.48 mg/dl, respectively, L/P ratio = 12.9).

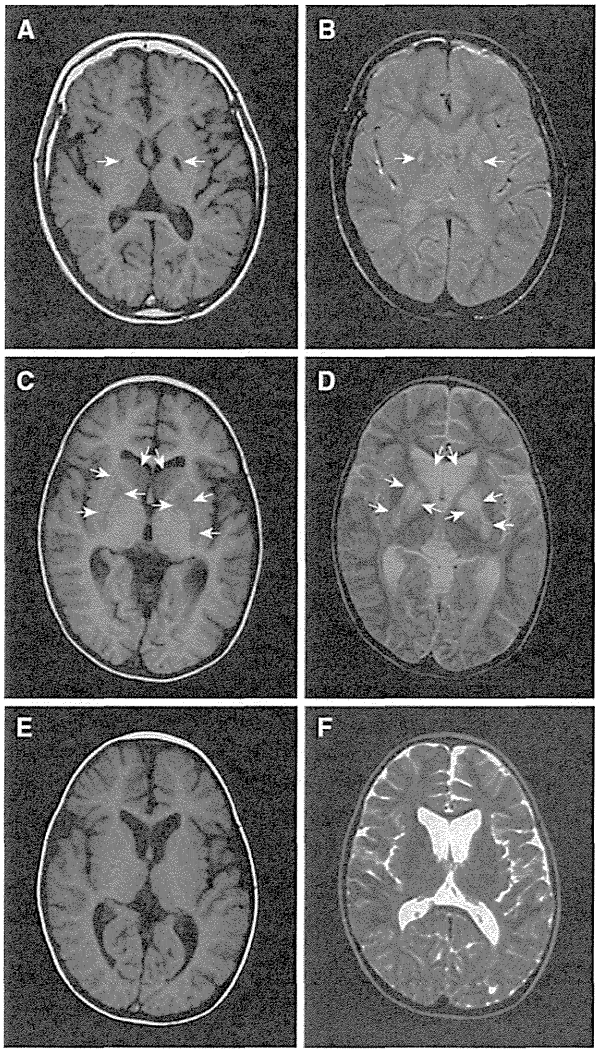

Fig. 2 Brain MRI of affected patients with a *GYG2* mutation. **a**, **b** (Patient II-2): T1 (**a**) and T2 (**b**) weighted brain magnetic resonance imaging (MRI) show necrotic lesion of bilateral globus pallidus (*arrows*). T2 elongation is observed at deep white matter at 1 year. **c–f** (Patient II-3): MRI at 1 year and 11 months shows swellings of caudate nuclei, globus pallidus, and putamen with the decreased T1 intensity (**c**) and increased T2 signals (**d**). *Arrows* indicate swollen lesions in basal ganglia. At 4 years (**e**, **f**), swelling of basal ganglia disappeared with continued mild high intensity in T2 weighted image (**f**)

Lactate and pyruvate levels of cerebrospinal fluid were slightly elevated with 11.3 and 1.11 mg/dl, respectively. Other laboratory examinations, including blood gas, blood sugar, ammonia, AST, ALT, BUN, Creatine, TSH, T3, T4, amino acids, and lysosomal enzymes were all normal. Urine organic acid analyses showed an increase of acetoacetic acid, 3-OHBA, and 3-OH-isovaleric acid. EEG showed no paroxysmal discharges. Muscle biopsy showed no specific abnormalities and no ragged red fibers. Staining for cytochrome c oxidase was normal (data not shown). Brain MRI disclosed T2 elongation in the basal ganglia and cerebral deep white matter (Fig. 2e, f). At the age of 5, he showed lethargy with fever. At 6 years, he again showed lethargy. Biochemical analysis disclosed a significant increase of blood ketone bodies: acetoacetic acid, 1,337 μmol/l; 3-OHBA, 4,845 μmol/l and urine ketone (+++). Blood levels of ammonia (28 μmol/l), sugar (78 mg/dl), lactate (5.1 mg/dl) and pyruvate (0.43 mg/dl) were all within normal range. Blood gas analysis revealed metabolic ketoacidosis with an increase of anion gap; 22.4 mEq/l (normal range 12 ± 2). His consciousness and biochemical measurements returned to normal within a few days with intravenous fluid infusion. Similar ketoacidosis attacks were repeatedly observed and agenesis of the left kidney and neurogenic bladder were recognized at the age of 8. He started intermittent urinary catheterization, and suffered from repeated urinary tract infections, resulted in chronic renal failure. Repeated brain MRI shows the progression of cerebral and cerebellar atrophy. He is now 19 years old and shows no gain of motor or intellectual abilities from the age of 4. He takes dantrolene sodium and diazepam for hypertonicity, and spherical charcoal, allopurinol for renal failure.

Identification of a *GYG2* variant by exome sequencing

A total of 2,433,011,483 bps (II-2) and 7,926,169,749 bps (II-3) were mapped to RefSeq coding DNA sequence (CDS). 83.3 and 96.0 % of CDS were covered by ten reads and more. We used only NGS data of II-3 for selecting candidate variants as the lower-quality NGS data of II-2 may lead to erroneous conclusion. Based on the hypothesis that this syndrome is inherited in an autosomal recessive or an X-linked recessive fashion, we focused on homozygous or compound heterozygous variants on autosomes and hemizygous variants on the X chromosome. While nine variants in four candidate genes were selected by in silico flow, only one hemizygous missense mutation in *GYG2* gene agreed with the familial segregation pattern (autosomal recessive or X-linked recessive) (Table S1, S2). The c.665G>C (p.W222S) in *GYG2* (isoform a: NM_001079855) was hemizygous in affected sibs and heterozygous in their mother, consistent with the X-linked recessive model, and was confirmed by Sanger sequence (Fig. 1b). The variant was absent in our in-house Japanese exome data (n = 418), the 1,000 Genomes database and ESP6500. Furthermore, no pathological variants in mtDNA were detected by exome sequence (Supplementary Results, Figure S1). In addition, a total of 21 LS patients (12 males and 9 females) were screened, but no pathological changes were found in *GYG2*.

GYG2 encodes GYG2 proteins with at least five isoforms: isoform a (NM_001079855), isoform b (NM_003918),

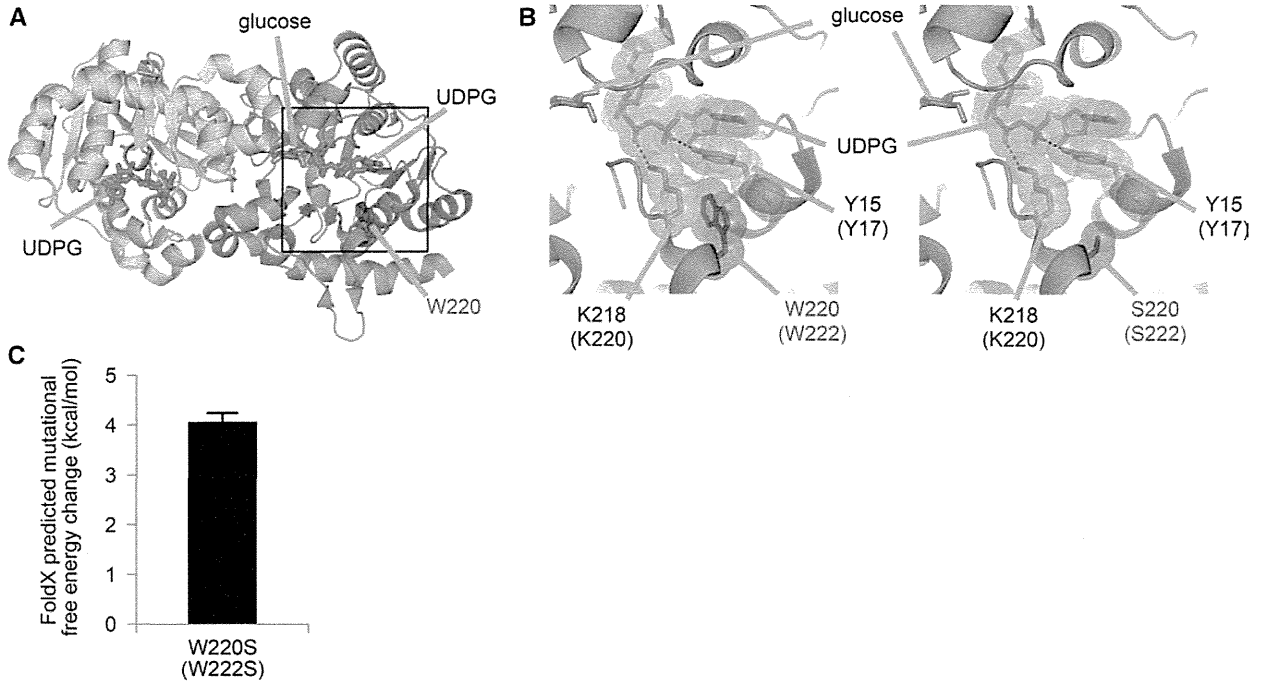

Fig. 3 Molecular structural consideration of the W222S mutation of GYG2. **a** Crystal structure of human GYG1 (Protein Data Bank code; 3T7O) (Chaikuad et al. 2011). Each monomer is colored *yellow* and *cyan*. α-helices, β-sheet and loops are drawn as *ribbons*, *arrows* and *threads*, respectively. The side chain of W222, glucose and UDP-glucose (UDPG) are shown as *sticks* in *red*, *orange* and *green*, respectively. Amino acid numbering shown is for human GYG1 with that for human GYG2 in *parenthesis*. The *squared area* corresponds to the close-up views in (**b**). **b** Detailed views of structures of the wild-type (*left*) and mutated GYG2 (p.W222S) (*right*). Amino acid residues at positions of 15, 218 and 220 and UDPG are shown as sticks with van der Waals representation and annotations. Hydrogen bonds are depicted as *dotted lines*. **c** Calculated free energy change upon the p.W222S mutation of GYG2 using FoldX software. All the molecular structures were drawn using PyMOL (www.pymol.org)

isoform c (NM_001184702), isoform d (NM_001184703), and isoform e (NM_001184704). At least two GYG2 isoforms (isoform a and b) are expressed preferentially in liver, heart and pancreas (Mu et al. 1997), while the detailed expression and function of other isoforms are undetermined. GYG2 has a glycosyltransferase family 8 domain and initiates glucose addition on its Tyrosine residue (Y197 in isoform a) via *O*-glycosylation (self-glucosylation) and can also attach an additional 7–10 residues of UDP-glucose to itself (Bollen et al. 1998; Lomako et al. 2004; Zhai et al. 2001). The W222 within the glycosyltransferase family 8 domain is evolutionarily highly conserved from *S. cerevisiae* to *H. sapiens* (Fig. 1c, d). In addition, all isoforms contain this residue. Thus, it is thought that this mutation may impair its biological function.

Structural consideration of the p.W222S mutation in human GYG2

The amino acid residue W222 of GYG2 (isoform a) was mapped to the crystal structure of human GYG1 (Chaikuad et al. 2011), since no experimental structure of GYG2 was available. W222 is involved in a hydrophobic core near the UDP-glucose (UDPG) binding site along with Y17 and K220 (Fig. 3a, b). The side chains of Y17 and K220 are hydrogen-bonded to UDPG, and the former also makes van der Waals contacts with the uridine ring of UDPG in a stacking mode. Therefore, the formation of the hydrophobic core appears to be a prerequisite for UDPG binding. To estimate the impact of the W222S mutation on the protein stability, we modeled the mutant structure and calculated the free energy change upon the mutation using the FoldX software. As a result, the mutation was predicted to destabilize the protein structure with about 4 kcal/mol increase in free energy (Fig. 3c). This suggests that the W222S mutation would impair UDPG binding (Fig. 3b).

Self-glucosylation analysis

To see the functional effects of the *GYG2* mutation in vitro, glucosyltransferase activity monitoring by self-glucosylation was measured using wild-type (WT) and W222S mutant (Mut) GYG2 (isoform a) transiently

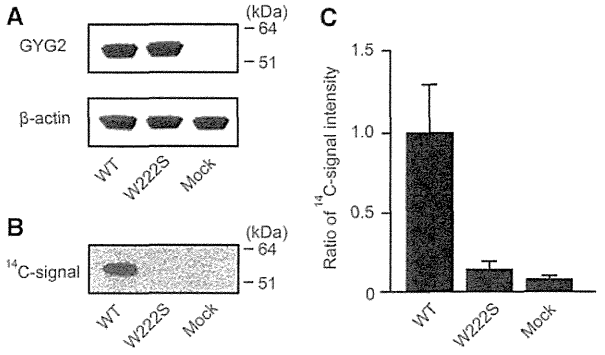

Fig. 4 Enzyme activity of GYG2. **a** Western blot analysis of recombinant GYG2. Wild-type (WT) and mutant (p.W222S) GYG2 was detected at the expected size (52 kDa). β-actin (42 kDa) was used as an internal control. **b** Autoradiography images presenting ^{14}C glucosylation toward GYG2. The signal was detected in WT, but undetected in mutant, with similar levels to Mock. **c** Graphic presentation of autoglucosylation of GYG2. The activity detected in Mock might be due to the endogenous glycogenin. *Error bars* represent the standard error of the mean

overexpressed in COS-1 cells. By immunoblotting, the expected 52 kDa bands of recombinant WT and Mut GYG2 were detected with similar expression levels (Fig. 4a). While WT GYG2 showed reasonable glucosyltransferase activity, Mut GYG2 almost completely lost the enzyme activity and was similar to the Mock level (Fig. 4b, c).

Expression analysis of GYG1 and GYG2

To observe tissue distribution of the human *GYG1* and *GYG2*, expression analysis was performed using multiple tissue cDNA panels. *GYG1* was expressed preferentially in skeletal muscle and heart from fetus to adult stages as previous reports (Barbetti et al. 1996). *GYG2* is dominantly expressed in liver from fetus through adult stages and moderately expressed in brain, heart, pancreas and kidney (Supplementary Results, Figure S2). To be marked, *GYG1* is not expressed in liver and brain where *GYG2* is highly expressed.

Discussion

In this study, we analyzed unique brothers affected with LS who were born to non-consanguineous healthy parents after uneventful pregnancies. Patient II-2 and II-3 developed LS accompanied by delayed developmental milestones at 10 months and 13 months of age, respectively. Their age of onset, clinical features and brain imaging were compatible with the diagnosis of LS. Interestingly, CNS abnormalities were observed (developmental delay, convulsion, athetoid movements, nystagmus, hypotonia, spasticity, increased deep tendon reflex and abnormal reflection), but involvement of peripheral nerve and extraneural organs was obscure. Based on the facts including (1) male (X-linked recessive), (2) normal lactate/pyruvate, (3) ketonemia/ketonuria, and (4) CNS predominant symptoms, the hemizygous *GYG2* mutation was highlighted a primary culprit.

In this study, we first identified a human *GYG2* mutation in affected brothers with LS with ketonemia/ketonuria but normal blood lactate/pyruvate. We can hypothesize a pathomechanism of the *GYG2* impairment in this family based on the canonical pathway of glycogen metabolism (Fig. 5). As glycogen storage in liver might be decreased because of the GYG2 malfunction, glucose is easily depleted. To keep appropriate blood glucose concentrations, the metabolism would be shifted toward gluconeogenesis and beta-oxidation to create glucose and energy sources like Acetyl-CoA (Garber et al. 1974; Laffel 1999; Randle et al. 1964). Excess beta-oxidation would result in overproduction of ketone bodies, consistent with the observation of ketonemia and ketonuria. However, pyruvate and lactate could be normally metabolized in gluconeogenesis and/or TCA cycle and would not accumulate in the body as seen in the majority of LS patients. Interestingly, both patients showed normal blood glucose level while showing LS manifestations which might be due to tissue energy depletion. In GYG2-deficient patients, the CNS was dominantly affected, while the effect of this abnormal metabolism was thought to extend to the entire body. This predominance could be explained by high glucose consumption as the primary energy source in brain (Amaral 2012; Magistretti and Pellerin 1999) and glycogen depletion in brain tissue level, while the blood sugar level was maintained by the other compensatory mechanism. This is similar to the muscle specific phenotypes (muscle weakness and arrhythmia) observed in patients with deficiencies of "muscle form" GYG1 in the absence of hypoglycemia (Moslemi et al. 2010). Remarkably, glycogen was less in the muscle tissue of GYG1 depleted patient (Moslemi et al. 2010). These evidences might indicate that it is not always linked between glucose level in the peripheral blood and glycogen/energy supply in tissue level while we could not show the loss of glycogen in liver or brain tissues because the materials were not available. In addition, deficiencies in two paralogous enzymes, GYG1 and GYG2, result in different human diseases suggesting they are unable to compensate each other in specific organs.

The *GYG2* mutation is probably causative for LS in this family. However, it is possible that the mutation is just coincidence because we just showed genetic evidences (due

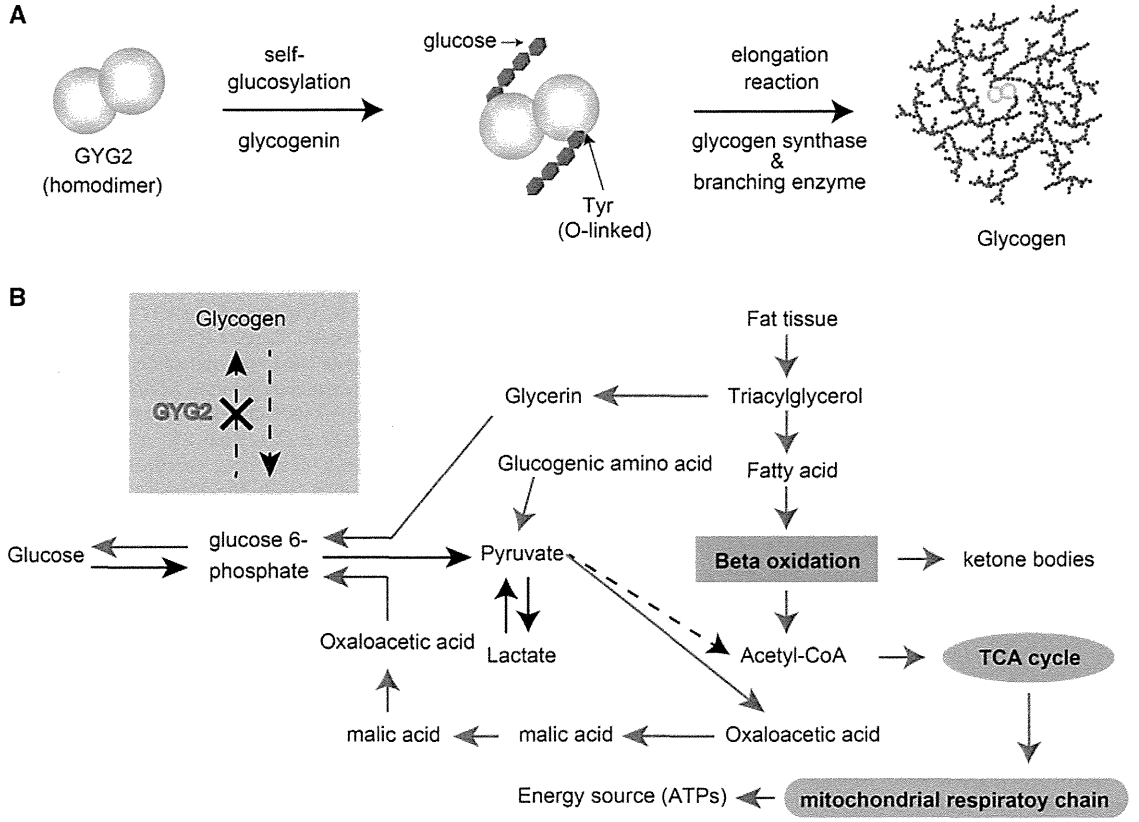

Fig. 5 Biochemical metabolisms in glycogen storage and glycolysis pathways. **a** Schematic presentation of glycogen biosynthesis. GYG2 has a catalytic capability for *O*-linked self-glucosylation at Tyrosine (Y197 in isoform a) and adds approximately 10 glucose molecules. By the subsequent elongating reactions by glycogen synthase and branching enzyme, giant molecule "glycogen" is formed. **b** Modeled biochemical pathway in GYG2 impairment. As the GYG2 impairment results in the absence of glycogen storage, glycogen is easy to be depleted and gluconeogenesis is induced from fat tissues and glucogenic amino acids. The reactions in mitochondria are shown in *yellow shadow*. While increased acetyl-CoA inhibits the pyruvate dehydrogenase complex which irreversibly converts pyruvate to acetyl-CoA (as shown as *dotted line*), it accelerates gluconeogenesis through pyruvate–oxaloacetic acid–malic acid–oxaloacetic acid. Triacylglycerol was metabolized into glycerin and fatty acid. Fatty acid was used for beta-oxidation and ketone production. The *arrows* indicate the directions of normal metabolism. *Red arrows* indicate the predicted predominant pathways in GYG2-deficient patients

to its rarity and familial co-segregation) and GYG2 loss of function by in vitro study without showing any sufficient data on how the *GYG2* mutation causes LS.

In conclusion, we describe the first human variant of *GYG2* which may be associated with the atypical LS phenotype in this family. Further studies are absolutely needed to conclude whether *GYG2* abnormality leads to atypical LS observed in this family.

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Conflict of interest The authors declare that they have no conflict of interest.

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Brachial plexus MRI showed increased signal intensity with contrast enhancement of the right long thoracic nerve, suggesting inflammation (Fig. 1). The patient was given oral prednisolone, 1 mg/kg daily for 21 days, with dose tapering over the next month. Three months later, her symptoms were stable, but electrophysiological follow-up showed a slight reduction of fibrillation potentials in right serratus anterior muscle.

Scapular winging is caused by imbalanced action of scapular muscles. The clinical examination based on the muscle group affected (trapezius, rhomboids, serratus anterior) is still the first guide for interpretation both of "pure" and "complicated" phenotypes.[1] Isolated scapular winging due to serratus anterior weakness is usually caused by traumatic or postsurgical long thoracic nerve injury, or it can occur as a manifestation of neuralgic amyotrophy, also known as Parsonage-Turner syndrome (PTS). PTS is a clinical syndrome characterized by attacks of extreme pain at onset, patchy weakness in the upper extremities, and atrophy of affected muscles.[2]

The available evidence suggests that PTS has a complex pathophysiology that includes an underlying predisposition, susceptibility to dysfunction of some peripheral nervous system structures, and an autoimmune trigger.[3] Cases of PTS are described after an immune event, such as pregnancy, childbirth, vaccination, or infection.[2]

Our patient did not have a history of previous surgery or trauma involving the thoracic area. Other possible causes were excluded by the laboratory evaluation. Moreover, she did not experience acute pain at onset, but only subsequent musculoskeletal pain due to compensation for the deficit. Since childbirth, an immunological trigger, occurred 3 days before symptom onset and approximately 4% of patients with PTS do not experience pain,[2] we made a diagnosis of post-partum PTS.

Currently, neuralgic amyotrophy cannot be diagnosed confidently by a single test, and often the presence of extreme pain of the upper limb together with patchy paresis helps in the diagnosis. In PTS without pain at onset, neuroimaging studies can be very useful, because they can exclude other causes such as intervertebral disc disease, tumors, or entrapment neuropathies. Furthermore, the evidence of contrast enhancement on MRI suggests a possible inflammatory etiology, thus supporting early administration of corticosteroids even in absence of pain, to block further evolution of the clinical picture and to possibly speed up functional nerve recovery.

In conclusion, contrast-enhanced MRI can provide crucial help in identifying different phenotypes of PTS and can guide therapy.

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A PRIMIGRAVIDA WITH VERY-LONG-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY

We report a woman who had the adult-onset form of very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency[1,2] and developed acute postpartum heart failure due to cardiomyopathy.

A 34-year-old primigravida visited our clinic due to nocturnal dyspnea. She had suffered from exercise-induced muscle pain since she was 5 years of age. Her food intake was insufficient due to hyperemesis gravidarum, and she developed severe generalized myalgia at 14 weeks' gestation. Her serum creatine kinase was 21,530 IU/L, and myoglobinuria was detected. From 15–30 weeks' gestation, her appetite recovered, and frequency of myalgia decreased. She delivered a baby by Caesarean section at 37 weeks without any complications.

She was transferred to our hospital on postpartum day 10 for treatment of heart failure. Serum glucose, creatine kinase, lactate, pyruvate, ammonia, and cardiac troponin T were within the normal ranges, but brain natriuretic peptide was increased to 3194.5 pg/ml. The left ventricular ejection fraction on echocardiography was 30%. Furosemide and carperitide were administered intravenously, and oral losartan and carvedilol were started on day 6 after admission. Sixty days after admission, echocardiography revealed no pericardial effusion and normal movement of the basal portion of the septum without any medications. There was no muscle weakness during or after pregnancy.

A muscle biopsy showed only mild fiber size variation without pathological findings. We measured acyl-CoA dehydrogenase activities using frozen muscle biopsy specimens.[3] Palmitoyl-CoA activity was reduced

Table 1. Results of β-oxidation enzyme analysis.

	Patient	Controls
Carnitine (free)	3.7	12.9 ± 3.7
Carnitine (total)	5.8	15.7 ± 2.8
Carnitine palmytoyl transferase II	0.9	1.6 ± 0.4
Acyl-CoA-dehydrogenase (substrate: C16:0)	4.1	24.5 ± 6.1
Acyl-CoA-dehydrogenase (substrate: C8:0)	6.1	8.1 ± 2.5
Acyl-CoA-dehydrogenase (substrate: C4:0)	15.7	16.8 ± 3.9
C16:0/C8:0	0.7	3.8 ± 1.4
C16:0/C4:0	0.3	1.8 ± 0.2
C8:0/C4:0	0.4	0.5 ± 0.1

compared with normal controls, but octanoyl-CoA activity was within the normal range (Table 1).

We sequenced the acyl-CoA dehydrogenase (very-long-chain) (*ACADVL*) gene in both the patient and controls.[4,5] DNA was extracted from muscle biopsy specimens using conventional methods. Sequence analysis of *ACADVL* revealed a novel homozygous c.272C>T mutation (p.P91L).

Patients with missense mutations or single amino acid deletions in the adult-onset form of VLCAD deficiency show energy deficiency and exercise-induced rhabdomyolysis or myoglobinuria.[6,7] During early pregnancy, our patient was malnourished due to hyperemesis gravidarum and suffered severe myalgia and rhabdomyolysis. She also developed acute heart failure at 2 weeks postpartum. She lacked the capacity for proper fatty acid oxidation and was in a catabolic state. In addition to a poor nutritional state, delivery may have served as a trigger for acute energy failure.

The nature of heart failure in our patient is quite different from that of rhabdomyolysis. Because Ca^{2+} homeostasis is altered in the VLCAD$^{-/-}$ mouse heart, abnormal intracellular Ca^{2+} handling can cause arrhythmias and lethal cardiac failure.[8] The accumulation of long-chain acylcarnitines in the heart has been shown to lead to cardiomyopathy and arrhythmia due to calcium uncoupling disorders.[9] Although cardiomyopathy has been reported in the childhood form of severe VLCAD deficiency,[10] 13% of patients with adult-onset VLCAD deficiency also develop cardiomyopathy.[6,11] Unlike the childhood form, patients with the adult-onset form due to missense mutations or single amino acid deletions have slight residual VLCAD activity and can avoid severe heart failure when treated with medium-chain triglycerides as the major source of dietary fat.[12,13] VLCAD deficiency should be included in the differential diagnosis of acute postpartum cardiomyopathy.

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慢性疾患をもって成人に至る子どもや青年に提供される医療環境—現状と課題

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キーワード●トランジション，連携，自己管理能力，社会的支援体制

はじめに

小児期発症の慢性疾患を抱えたまま成人になる患者が増加している．かつては治療が困難であった疾患が，医学の進歩で克服されてきたからであるが，この問題がクローズアップされてきたのは実数が増えたからというよりも，その中で行き場を失った患者が増えてきたためと考えることもできる．昔から小児期発症の慢性疾患を抱えて成人する患者は存在していた．筆者の大先輩の医師たちは，そういった患者が何歳になっても診療を続けていた．免疫疾患，神経疾患，内分泌疾患，先天代謝異常など，分野に関係なくである．かつて，その先輩医師たちに「何歳まで小児科で診るのですか」と尋ねたとき，「何歳までででも診るしかないだろう」と一喝されたのを覚えている．

ところが，今は洋の東西を問わず，「トランジション」問題が盛んに議論されている．長年診療を受けていた小児科の名医の退職をきっかけに，患者が今後どうすればいいのか悩んでしまう事例が多発している．そのいちばんの理由は，われわれが大先輩たちほどの全人的医療レベルを兼ね備えられなくなったからであると断言できる．それほど医療は良い意味でも悪い意味でも専門分化され，高度化している．総合診療医や家庭医に注目が集まっているのも自明の理である．

では小児科医は総合内科医に紹介状を書いてお願いすればいいのかというと，そうではない．今，大先輩ほどの全人的医療能力を持たないわれわれは何をすればいいのか．現状を分析し，今後の課題を探りたいと思う．

Ⅰ トランジションとは

小児期発症の慢性疾患を抱えた成人期の患者を小児科医が診療し続ける場合の問題点として，成人期特有の疾患（高血圧，がんなど）への対応が小児科医には困難であることがまず挙げられる．また小児科に身を置くことで，患者自身が自立し，自己管理能力を身に付けることが阻まれるとしたら，それは大きな問題である．

これらの問題に対して，日本小児科学会「移行期の患者に関するワーキンググループ」は提言を発表した[1]．そこでは，「移行期と呼ぶのは小児期医療から成人期医療へと移り変わりが行われる（と期待される）段階であって，何歳から何歳までという年齢より，患者ごとに機能的に定義される段階を指している」とされている．

また，2002年に出された米国の小児科学会，家庭医療学会，内科学会・内科専門医学会の合同声明は，トランジションとは，「a family-centered, continuous, comprehensive, coordinated, compassionate, and culturally compe-

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