転帰・後遺症

転帰	１．治癒　２．軽快　３．不変　４．増悪 ５．死亡　６．転院　７．不明　８．その他（＿＿＿＿＿＿＿＿）
現在の日常生活動作	食事；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助 更衣；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助 移動；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助 排泄；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助 整容；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助 入浴；　１．自立　２．準備のみ　３．観察　４．部分介助　５．全介助

患者会活動報告原稿

ｇｌｕｔ１異常症患者会です。
本会は平成 20 年 6 月に、顧問医師である大阪大学・金沢大学・浜松医科大学連合小児発達学研究科　　下野九理子先生、国立精神・神経センター病院　小児神経科　青天目　信先生にご協力いただき発足いたしました。

会員数は全国で２０家族程度と小さな会ではありますが、患者とその家族の精神的サポートと、患者がよりよい生活ができるような社会をめざして活動しています。
定例会は月に 1 度名古屋市内で程度行い、活動の計画・準備などをしています。
また、年 3 回の会報誌の発行や、会員・医師向けに病気や食事療法の情報提供もしています。

患者とその家族のサポートとしては、希少病故に病気に詳しい医師と出会えず、不安感を持っている患者家族に専門医をセカンドオピニオンとして紹介したり、患者やその家族の交流のため年１回の交流会と 2 年に 1 回の総会を開催しています。

これまでには患者会総会・交流会を各 2 回中部・関西地区で開催いたしました。
交流会では、初めて関西、中部、関東の患者とその家族が対面しました。
総会では、勉強会を開催し、顧問医師に病気についての「メカニズム」「症状」「治療法」などの講演をしていただきました。講演を聴き終えた会員の中には、初めて自分の子供の病気のことを詳しく知ったという方も多く、あらためて患者会の存在意義を感じました。

社会への周知活動としては、協力企業の社内誌への投稿、日本てんかん協会の会報誌「波」への投稿、第 52 回先天代謝異常学会　患者会フォーラム参加・患者会ブースの開設、第 53 回同学会　懇親会・患者会交流会参加などが、いままでに行った主な活動です。特に日本てんかん協会会報誌への掲載については、Ｇｌｕｔ－１異常症がてんかんと診断され、発見が遅れることもあるということから、単に病気を知ってもらうだけではなく、病気の早期発見へのきっかけになるよう願い、実現したものです。
また、滋賀県立小児保健医療センター院長　藤井達哉先生の編集のもと、本年発売された「ケトン食の基礎から実践まで」にもケトン食を実践している患者・家族の立場から執筆協力をさせていただきました。

その他には食事療法に必要な特殊ミルク　ケトンフォーミュラーが今後も安定供給されるよう、厚生労働省に登録ミルク認定の要望書を提出しました。

以上が現在までの主な活動です。

グルコーストランスポーター1欠損症症候群の実態と診断治療指針に関する研究班
第2回班会議

glut1異常症患者会活動報告

平成23年12月18日（日）

glut1異常症患者会　海上美佳

活動報告

平成20年

6月　glut1異常症患者会発足

平成21年

4月 3日（金）名古屋市内にて会食
4日（土）愛・地球博公園内児童館にて交流会
5日（日）名古屋市内にて交流会

平成21年

8月 8日（土）　第1回glut1異常症患者会総会
・勉強会（名古屋ウィルあいち）

9月　てんかん協会「波の会」会報へ投稿

平成22年

4月17日（土）第2回患者会交流会開催
（京都市障害者スポーツセンター）

8月15日（土）フェニルケトン尿症親の会会合に参加

10月　患者会リーフレット作成

10月21日（木）～23日（土）
第52回日本先天代謝異常学会
「希少疾患のQOL向上のために」
患者会ブース開設・患者会フォーラムへの参加
（大阪国際会議場）

平成23年

3月　「ケトン食の基礎から実践まで～ケトン食に関わるすべての方へ」(診断と治療社出版)

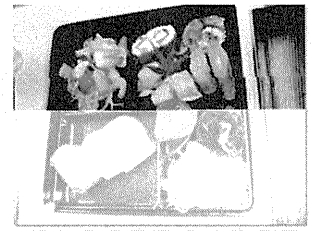
患者会として執筆協力しました！

5月　厚生労働省に患者会より要望書を提出

「ケトンフォーミュラ」を
登録特殊ミルクに認定してもらうための要望

平成23年

8月6日(土)　第2回glut1異常症患者会総会
(京都市障害者スポーツセンター)



食堂の方が作ってくれた低糖質弁当
(低糖質パンは会員今井さん作)

平成23年

11月24日(金)～26日(日)

第53回日本先天代謝異常学会

～先天代謝異常症その高みをめざして風を感じよう～

第10回アジア先天代謝異常シンポジウム

医療関係者の懇親会と患者会の交流会に参加

(ホテルニューオータニ幕張)

1. グルコーストランスポーター1欠損症症候群(GLUT1DS)とは

概説

・グルコース輸送蛋白1(Glucose transporter type 1 ; GLUT1)欠損症は、脳のエネルギー代謝基質である glucose が中枢神経系に取り込まれないことにより生じる代謝性脳症で、1991 年に De Vivo らにより初めて報告された。

疫学

現在までに全世界で 200 例程度の報告があります。今回の国内調査では 57 例の本症候群が発見されておりますので国内だけでも未診断例を含めまだ相当数いるのではないかと考えられる。

病因

本症は常染色体優性の遺伝性疾患であり、90%に SLC2A1(GLUT1)遺伝子 (1p35-31.3)におけるヘテロ接合性の変異を認め*1、大多数は de novo である。

臨床症状・診断

・生下時には異常を認めない。てんかん発作は乳児早期に発症し、オプソクローヌスに疑似した異常眼球運動発作や無呼吸発作が先行することがある。

・発作型は全般性強直間代、ミオクロニー、非定型欠神、定型欠神、脱力、部分発作とさまざまであるが、てんかん発作のない症例も報告されている。

・筋緊張低下を認める。小脳失調、痙性麻痺、ジストニアなどの複合的な運動障害が遅発性に出現する。構語障害は全例に認め、失調性である。

・認知障害は、学習障害の程度から重度精神遅滞までさまざまである。社会性があり、親しみやすい。重症例で後天性小頭症が合併する。

・運動失調、精神錯乱、嗜眠・傾眠、不全片麻痺、全身麻痺、睡眠障害、頭痛、嘔吐を発作性に認めることがある。最近、発作性労作誘発性ジスキネジアにおいてSLC2A1遺伝子のヘテロ接合性変異が同定されたが、てんかん発症は遅く、髄液糖低値も有意でなく、GLUT1 欠損症の典型例とは異なっている。

・本症に認める症状は、空腹、運動により増悪し、特に早朝空腹時に強く、食後に改善する。年齢とともに改善し、思春期を経て安定してくる*2。

臨床検査所見

・髄液検査*3 では, 低血糖の不在下に髄液糖は 40 mg/dl 以下*4、髄液糖／血糖比は 0.45 以下(平均 0.35)、髄液乳酸値は正常～低下を呈する。

・頭部CT・MRI では大脳萎縮,髄鞘化遅延など非特異的所見を呈する。

・発作間欠期脳波では背景脳波の徐波化を認める。てんかん波はないことが多いが、初期に焦点性棘波を、成長とともに 2.5-4 Hz の全般性棘徐波を認める。脳波異常は食事*5 やグルコース静

注で改善する(図 1)。
・遺伝子検査にて確定診断されるが、遺伝子異常がない場合は赤血球 3-O- methyl-D-glucose 取り込み試験や赤血球膜における GLUT1 免疫反応定量を行う。

治療
・抗てんかん剤に対しては治療抵抗性である。
・グルコースに代わりケトンをエネルギー源として供給するケトン食療法(3:1～4:1)が早期診断のもとに開始されるべきである。著者らが使用している Atkins 式ダイエット変法*6 は、従来のケトン食に比べ調理が容易で、カロリー、蛋白制限がないため空腹感がなく、長期継続しやすい利点がある。本症では、尿のケトスティックス検査で 2～3+程度維持できれば有効である。
・GLUT1 を抑制する薬剤(phenobarbital、chloral hydrate、theophylline)や飲食物(alcohol、caffeine)を避けるべきである。

診断基準

1. **空腹時、食前での神経学的症状(複雑運動障害*、てんかん発作**)の憎悪とその食後での改善**
2. **髄液検査所見で髄液糖／血糖比＜0.45*****
3. **SLC2A1(GLUT1)遺伝子 (1p35-31.3)におけるヘテロ接合性の変異******
4. **赤血球 3-O- methyl-D-glucose 取り込み試験の低下*******

*痙性麻痺、小脳失調、ジストニアなどの運動障害
**欠神発作, ミオクロニー発作, 部分発作や失立発作
***早朝空腹時に血糖採血後に髄液検査施行(髄液検査のストレスで血糖が上昇する場合があるので)
****90%で陽性
*****T295M 変異では取込み低下を示さない。

確定診断

①1±2 と 3 あるいは 4 を満たす
②診断が強く疑われる
a) 1+2 **のみ**
b) 1 **と参考所見のひとつを満たす場合、髄液検査へ**
c) 2 **と参考所見を満たす場合**

参考所見

臨床経過

1. 乳児期発症:チアノーゼ発作, 発作性異常眼球運動、ミオクロニーなどの発作性症状と筋緊張低下、発達遅滞が主体
2. 幼児期以降発症:発達遅滞、筋緊張低下、固定性の複雑運動障害の合併、治療抵抗性のてんかん発作(欠神発作, ミオクロニー発作, 部分発作や失立発作)の出現。また発作性の精神錯乱、嗜眠・傾眠、不全片麻痺や

交代性片麻痺、全身麻痺、睡眠障害、頭痛、嘔吐を認めることがある。発作性労作誘発性ジスキネジア症状を呈する例も存在。重症例では後天性小頭症の報告がある。認知障害は、学習障害の程度から重度精神遅滞までさまざまである。社会性があり、親しみやすい。

検査所見

頭部 CT・MRI：非特異的所見

頭部 PET 所見：大脳皮質の全体的な取り込み低下と基底核・視床の特異な取り込みの亢進

発作間欠期脳波：食事前後で背景脳波、てんかん発射の憎悪と改善、幼児期以降に全般性 2.5-4Hz 棘徐波複合が出現する場合がある。

治療

1. 診断が確定次第ケトン食の導入

長期維持の観点から修正アトキンス食、古典的ケトン食 3:1 が推奨される。早朝尿のケトン体の 3+以上が目標

2. ケトン食治療でてんかん発作が抑制できない場合抗てんかん薬治療

- **ただし GLUT1 を抑制する PB, DZP, chloralhydrate 使用は避ける。また日常生活では theophylline、飲食物(alcohol, green tea, caffeine)などの注意**

グルコーストランスポーター1欠損症症候群（GLUT1DS）の診断治療ガイドライン

グルコーストランスポーター１欠損症症候群（GLUT1DS）の診断治療ガイドライン作成にあたって

GLUT1-DSは、脳のエネルギー代謝基質であるグルコースが中枢神経系に取り込まれないことにより生じる代謝性脳症で、慢性の脳低血糖状態が持続することによりてんかん発作や神経・精神的退行が進行していくが、ケトン食治療により治療可能な疾患と考えられています。本症候群は、1991年に米国のDeVivo博士が初めて報告した先天性代謝疾患であり、当初は難治性てんかんの原因疾患のひとつと考えられてきました。その後、欧州においても報告が認められるようになり日本においても2006年頃より患者の報告が相次いでおりました。今回、厚生労働省　難治性疾患克服研究事業のひとつ「グルコーストランスポーター１欠損症症候群の実態と診断治療指針に関する研究」斑において全国調査を行い国内で57例の確定あるいは疑い例が診療されておりました。そのほとんどの患者は様々なケトン食療法が試みられておりました。本症は、治療抵抗性のけいれん発作や嘔吐発作、失調、発作性不随意運動が認められ、抗てんかん薬などでは症状の改善が乏しいのですが、ケトン食治療はその多くの症状について劇的な改善をもたらします。逆に診断・治療が遅れれば慢性の脳内低血糖状態のため中枢神経障害が不可逆的に進行していくものと考えられます。そのためGLUT1DSでは早期診断が非常に重要となります。このガイドラインはまだ不完全と思われますが、内容につきまして皆様の忌憚のないご意見をいただければ幸いです。また疑わしい症例の診断や治療につきまして当研究班にご相談いただければ幸いです。

グルコーストランスポーター１欠損症症候群の実態と診断治療指針に関する研究

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分担研究者
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高橋悟　旭川医科大学小児科　講師
夏目淳　名古屋大学小児科　準教授
柳原恵子　大阪府立母子保健総合医療センター小児神経科，同免疫研究所兼任臨床研究員
下野九理子　大阪大学・金沢大学・浜松医科大学連合小児発達学研究科　助教
藤井達哉　滋賀県立小児保健医療センター病院長

ｇｌｕｔ１異常症患者会

グルコーストランスポーター1欠損症症候群（GLUT1DS）の診断と治療

GLUT1DS を疑う

乳児期：発作性異常眼球運動を伴う意識減損、チアノーゼを伴う発作、無呼吸発作、ミオクロニー発作性
重症例では筋緊張低下、発達遅滞を伴う
幼児期：空腹時に目立つけいれん発作や意識消失発作、運動性失調で食後に改善

↓

GLUT1DS の診断

髄液検査で髄液糖/血糖比＜0.45
↓
SLC2A1（GLUT1）遺伝子検査
赤血球 3-O- methyl-D-glucose 取り込み試験の低下

↓

ケトン食治療開始

入院してケトン食治療導入
　修正 Atkins 食が第一候補
その他
　MCT2：1 ケトン食
　古典的 3：1 ケトン食

↓

ケトン食の維持と必要であれば一般的治療の追加

てんかん発作が抑制困難：ケトーシスの維持（尿中ケトン 2+ 以上）、それでも困難であれば発作型に応じ抗てんかん薬の併用（PB や DZP を避ける）
痙性麻痺：リハビリ
失調：TRH やセレジスト

疾患概要

【疾患名】	グルコーストランスポーター1欠損症症候群(GLUT1DS)
【患者数】	国内で少なくとも57例の存在を確認
【概要】	GLUT1DSは、脳のエネルギー代謝基質であるグルコースが中枢神経系に取り込まれないことにより生じる代謝性脳症で、慢性の脳低血糖状態が持続することによりてんかん発作や神経・精神的退行が進行していくものと考えられている。
【原因の解明】	常染色体優性の遺伝性疾患であり、90%に*SLC2A1*(GLUT1)遺伝子(1p35-31.3)におけるヘテロ接合性の変異を認め、大多数はde novoである。
【主な症状】	乳児期早期より発作性異常眼球運動、無呼吸発作やミオクロニーを含むけいれん発作で発症し、その後、様々な型のてんかん発作、発達遅滞、筋緊張低下、痙性麻痺、小脳失調、ジストニアなどの神経学的異常を呈する。症状は、空腹時、食前に悪化し、食後に改善する特徴がある。髄液糖低値(髄液糖/血糖＜0.45)が特徴である。
【主な合併症】	様々な程度の小頭症、低身長、精神遅滞、発作性不随意運動や周期性嘔吐症を合併する。
【主な治療法】	本症はケトン食療法により、神経細胞のエネルギー供給物質をグルコースからケトン体に代用させることができるため、永続的な改善が期待できる。ケトン食療法が第一選択となるが、長期継続が可能なカロリー制限、蛋白質摂取制限や水分制限の少ない修正アトキンス食等が望ましい。
【研究班】	GLUT1DSの実態と診断治療指針に関する研究班

留意事項
研究が採択された場合については、当該資料についてはホームページ等で公開する予定です。

Ⅲ. 研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
伊藤康	先天代謝異常症：Glut1 欠損症。		今日の神経疾患治療指針 第2版。	医学書院	東京	2011	未定
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Ⅳ. 研究成果の刊行物・別刷

Journal of Human Genetics (2011) 56, 846–851

www.nature.com/jhg

ORIGINAL ARTICLE

SLC2A1 gene analysis of Japanese patients with glucose transporter 1 deficiency syndrome

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Glucose transporter 1 deficiency syndrome (Glut1-DS) is a congenital metabolic disorder characterized by refractory seizures with early infantile onset, developmental delay, movement disorders and acquired microcephaly. Glut1-DS is caused by heterozygous abnormalities of the *SLC2A1* (*Glut1*) gene, whose product acts to transport glucose into the brain across the blood-brain barrier. We analyzed the *SLC2A1* gene in 12 Japanese Glut1-DS patients who were diagnosed by characteristic clinical symptoms and hypoglycorrhachia as follows: all patients had infantile-onset seizures and mild to severe developmental delay, and ataxia was detected in 11 patients. For the 12 patients, we identified seven different mutations (three missense, one nonsense, two frameshift and one splice-site) in exons and exon–intron boundaries of the *SLC2A1* gene by direct sequencing, of which six were novel mutations. Of the remaining five patients who had no point mutations and underwent investigation by multiplex ligation-dependent probe amplification, a complex abnormality with deletion and duplication was identified in one patient: this is the first case of such recombination of the *SLC2A1* gene. Changes in regulatory sequences in the promoter region or genes other than *SLC2A1* might be responsible for onset of Glut1-DS in the other four patients (33%) without *SLC2A1* mutation.
Journal of Human Genetics (2011) **56**, 846–851; doi:10.1038/jhg.2011.115; published online 20 October 2011

Keywords: glucose transporter 1 deficiency syndrome; mutation; *SLC2A1*

INTRODUCTION

Glucose transporter 1 deficiency syndrome (Glut1-DS: OMIM no. 606777) was first described by De Vivo *et al.*[1] in 1991 as a disease with impaired glucose transport across the blood-brain barrier and characterized by hypoglycorrhachia, seizures and developmental delay. Validation of their observation was confirmed by the discovery that Glut1-DS was caused by genetic abnormalities of the *SLC2A1* (*Glut-1*) gene (OMIM *138140) encoding Glut1, by which glucose transport across the blood-brain barrier is mediated. The *SLC2A1* gene is located on the short arm of chromosome 1 (1p34.2)[2] and is about 35 kb in length, containing 10 exons.[3] Almost 60 mutations including missense, nonsense, frameshift and splice-site mutations of the *SLC2A1* gene have been reported to date in Glut1-DS patients.[4] In addition, chromosomal abnormalities, such as microdeletions including the *SLC2A1* gene, have also been recently reported.[5,6]

Here, we report the clinicogenetic characteristics of 12 Japanese patients with Glut1-DS. Initial mutation analysis was performed by direct sequencing, and multiplex ligation-dependent probe amplification (MLPA) was subsequently performed in the remaining patients in whom no point mutation was identified. This is the first report documenting clinicogenetic features in a series of Japanese patients with Glut1-DS.

MATERIALS AND METHODS

Patients

In all 13 Glut1-DS patients, who were diagnosed at Osaka University Hospital or whose blood sample had been sent for genetic analysis from other institutions from 2004 to 2011, were subjected to the study. Diagnosis was made according to clinical and laboratory features: infantile-onset seizures, developmental delay, EEG abnormalities aggravated by fasting and improved by food intake, low CSF/blood glucose ratio (<0.5) after 4–5 h fasting despite low to normal CSF lactate concentration and normal levels of CSF cells and protein. 3-*O*-Methyl-D-Glucose (3-OMG) uptake by erythrocytes was measured according to previously reported methods.[7] This study was exempted from full review and approved by the Osaka University Graduate School of Medicine Institutional Review Board. Written informed consent was obtained from the parents of all the children who were recruited to this study.

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Received 14 June 2011; revised and accepted 15 September 2011; published online 20 October 2011

Mutational analysis of *SLC2A1*

Genomic DNA was isolated from heparinized blood using QuickGene DNA whole blood Kit S and Nucleic Acid Isolation System QuickGene-810 (FUJIFILM, Tokyo, Japan). PCR primer sets were designed to cover the exons and exon–intron junctions of the *SLC2A1* gene. The primers used are shown in Table 1.[8] PCR was conducted in 20 µl volume containing 50 ng genomic DNA, 0.5 µM each primer, 0.2 mM each dNTP and 1 U Taq DNA polymerase (Takara Taq, Takara Bio Inc. Otsu Shiga, Japan). The PCR reaction used 35 cycles of amplification with denaturation at 95 °C for 4 min, annealing for 30 s at 64 °C for 1 min, extension at 72 °C for 2 min, and followed by a final extension at 72 °C for 10 min. The amplified products were purified with Takara SUPREC-PCR (Takara Bio Inc.). The *SLC2A1* gene was directly sequenced using 20 ng of purified DNA with BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, USA). The pathogenetic nature of novel mutations was confirmed by their absence in 100 control subjects.

MLPA analysis

The principle for MLPA, which is a technique for measuring allele dosage by the hybridization of the complementary probes, has been described previously.[9] The SALSA SLC2A1 region test reagent set (P138 probe mix; MRC-Holland, Amsterdam, The Netherlands) contains one probe for each exon of the *SLC2A1* gene and 12 reference probes (www.mrc-holland.com). All runs included DNA from unrelated controls for calibration. MLPA was entrusted to FALCO biosystems (Kyoto, Japan).

RESULTS

Clinical characteristics

The clinical signs and laboratory data for the study patients are listed in Table 2. There were three boys and nine girls ages 6–15 years. All patients showed early-onset (2–11 months of age) and refractory seizures. Seizure type was diverse and included generalized tonic-clonic, atonic, absence and complex partial seizures. A total of 8 patients had microcephaly below −2 s.d. All of them had developmental delay to various degrees of severity. All except one had truncal ataxia and eight showed dystonia.

Laboratory examination revealed hypoglycorrhachia (CSF glucose 26–38 mg dl^{-1} and CSF/blood glucose ratio: 0.28–0.48) in all patients. 3-OMG uptake by erythrocytes was also decreased (to 47–56% of controls) in eight patients, but one patient (case 5) was within the normal range of erythrocyte 3-OMG uptake. The clinical features of two informative cases are detailed later in this section.

Table 1 Primer set for the direct sequencing of the *SLC2A1* gene

Exon	*Forward primer*	*Reverse primer*
1	5′-AACAGCGAGCGTGCCGGTCGCTAGT-3′	5′-TAAGGCGGGCAGGAGTCTGCGCCTT-3′
2	5′-CTCCCAGACACGCCTATAACAGT-3′	5′-GGCTGGTGTCCATAAGCCAACG-3′
3–4	5′-GCTTGCTCACCCAGGCTGCAT-3′	5′-GTGCCAGGCAGGTAGATCCT-3′
5–8	5′-AAAGGGGGTCAGGGCAGAGGCGCTCA-3′	5′-GCATCCCTCACTCTCCAGAACCT-3′
9–10	5′-AACTTTTCCCCCTCTCCGTCATC-3′	5′-TGTGCTCCTGAGAGATCCTTA-3′

SLC2A1 mutations on direct sequencing

A summary of the results of the *SLC2A1* gene analysis is shown in Table 3. Direct sequencing of the *SLC2A1* gene revealed mutations in seven patients (cases 2, 5, 6, 7, 9, 10 and 11). Among them, three missense mutations (cases 5, 6 and 9) were noted. The mutation found in case 9 was the same as previously reported by Wang *et al.*[9] A nonsense mutation was found in case 11 (c.1272 T>A, p.Y424X). Cases 7 and 11 showed frameshift mutations (case 7: c.707_708delAC, p.D236GfsX4 and case 11: c.431_432delTG, p.V144GfsX2). A splice-site mutation was found in case 2 (c.679+1G>A). Collectively, direct sequencing of the gene revealed seven mutated alleles among 12 Glut1-DS patients, of which six were novel mutations. The novel missense mutation found in case 2, 5, 6, 7 and 11 was not found in 100 normal control subjects. The location for each mutation on the *SCL2A1* gene is indicated in Figure 1. No specific localization of mutations was found.

Quantitative analysis by MLPA

Further analysis using the MLPA method was performed in five patients in whom direct sequencing failed to disclose any mutation. In one patient (case 12), relative copy number was decreased from exon 1–8 and increased from exon 9–10. Although her father did not have any abnormalities, her mother had an increased relative copy

Table 2 Clinical characteristics of Glut1-DS patients

			Biochemical data			*Clinical signs*					
Patient no.	*Gender*	*Age at study (y)*	*CSF/blood glucose (mg dl^{-1})*	*CSF/blood glucose ratio*	*Erythrocyte 3-OMG uptake (% normal)*	*Seizure onset (mo)*	*Seizure type*	*Micro cephaly*[a]	*Mental impairment*[b]	*Ataxia*[c]	*Dystonia*[d]
1	F	7	27/73	0.36	NA	2	GTC, CPS, Ato	+	+++	+++	+
2	F	12	32/89	0.39	56	2	GTC, CPS	+	+++	++	+
3	M	15	35/90	0.39	48	2	CPS, Abs	±	++	++	+
4	F	12	26/83	0.31	52	3	GTC, CPS, Abs, Ato	+	+++	+++	+
5	M	14	38/78	0.48	102	3	GTC,GT	+	+++	+++	–
6	F	6	28/85	0.32	Decreased	6	GTC, Abs, CPS	–	++	++	+
7	F	6	28/90	0.32	NA	6	CPS	–	+	+	+
8	F	9	33/82	0.4	Decreased	6	Abs, Ato, CPS	+	+	+	–
9	F	10	36/87	0.41	56	8	GTC, Abs	–	+	–	–
10	M	14	29/82	0.35	47	9	GTC, CPS	+	++	++	+
11	F	4	26/90	0.28	NA	9	GT,Abs	–	++	++	–
12	F	15	30/84	0.38	51	11	CPS, Ato	+	+++	++	+

Abbreviations: Abs, absence seizure; Ato, atonic seizure; CPS, complex partial seizure; CSF, cerebrospinal fluid; GT, generalized tonic seizure; GTC, generalized tonic-clonic seizure; mo, month; NA, not assessed; 3-OMG, 3-*O*-methyl-D-glucose; y, year.
[a]Grade: –, ⩽1 s.d. (standard deviation); ± ⩽1.5 s.d.; +, ⩽2 s.d.
[b]Grade: –, none (DQ (developmental quotient) 70–100); +, mild (DQ 50–70); ++, moderate (DQ 35–50); +++, severe (DQ 20–35).
[c]Grade: –, none; +, mild; ++, moderate; +++, severe.
[d]Grade: –, absent; +, present.

Table 3 Results of *SLC2A1* gene analysis in Glut1-DS patients

Patient no.	*Mutation*	*Location*	*Nucleotide change*	*Type of mutation*	*Family member analysis*
1	Not found				
2	c.679+1G>A	Intron 5	c.679+1G>A	Splice-site	
3	Not found				
4	Not found				
5	p.M344T	Exon 8	c.1031T>C	Missense	Father (−) Mother (+) Brother (+)
6	p.E41K	Exon 3	c.121G>A	Missense	
7	p.D236GfsX4	Exon 6	c.707_708delAC	Frameshift	
8	Not found				
9	p.G130S[a]	Exon 4	c.388G>A	Missense	Father (−) Mother (−) Brother (−)
10	p.Y424X	Exon 9	c.1272T>A	Nonsense	
11	p.V144GfsX2	Exon 4	c.431_432delTG	Frameshift	
12	del Ex1–8, dup Ex9–10	Exon 1–10		Deletion Duplication	Father (−) Mother (dup Ex9–10)

[a]Mutation previously reported in other patients.[9]

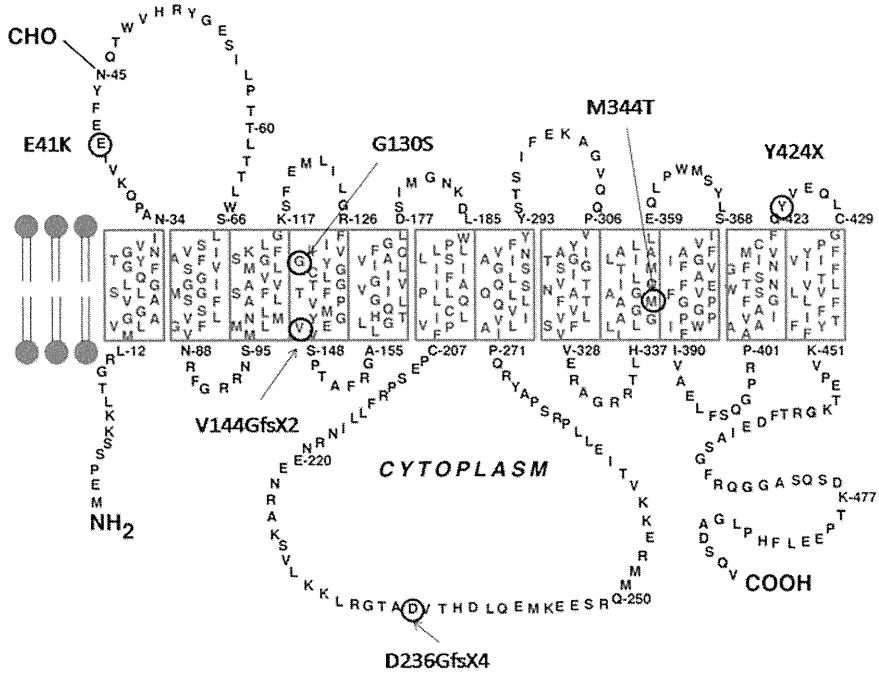

Figure 1 Location of six novel mutations (three missense, two frameshift and one nonsense) from Glut1-DS patients in the Glut1 protein transmembrane configuration. Mutations are scattered without a specific hotspot.

number between exon 9–10. This suggests that her mother originally had duplication in exon 9–10 as a polymorphism and that further chromosomal recombination in front of the duplication occurred, leading to the onset of Glut1-DS in the proband (Figure 2).

Case 5 presentation

This 14-year-old male was the first child born to non-consanguineous parents. He was born at 37 gestational weeks (birth weight 2410 g) without asphyxia by normal delivery. He developed tonic seizures at 3 months of age and his seizures were frequent and refractory to 10 anti-epileptic drugs including adrenocorticotropic hormone. His cognitive impairment was severe and he has never learned a single word. He manifested hypotonia, increased tendon reflex and ataxia but no dystonic posture. At the age of 9 years, he was suspected to be suffering from Glut1-DS because of low CSF glucose concentration (Table 1), although glucose uptake by erythrocytes was not decreased. Direct sequencing analysis revealed a novel missense mutation (c.1031T>C, p.M344T).

A younger brother of the proband was also born by normal delivery. At 4 years of age, he developed monthly tonic-clonic seizures while in a fasting state before breakfast. He showed neither ataxia nor dystonia on fasting. His CSF glucose was slightly low (CSF/blood glucose; 41/71 mg dl^{-1}). Although he did not show any motor disability, his cognitive function was mildly impaired. He has the same mutation as his brother.

When screened, the mother and a brother were also found to have the relevant mutation of the *SLC2A1* gene but not their father (Figure 3). The mother's history revealed one episode of seizures at 6 years of age. The two brothers of the mother had been diagnosed with schizophrenia and autism, respectively, but we were unable to gain

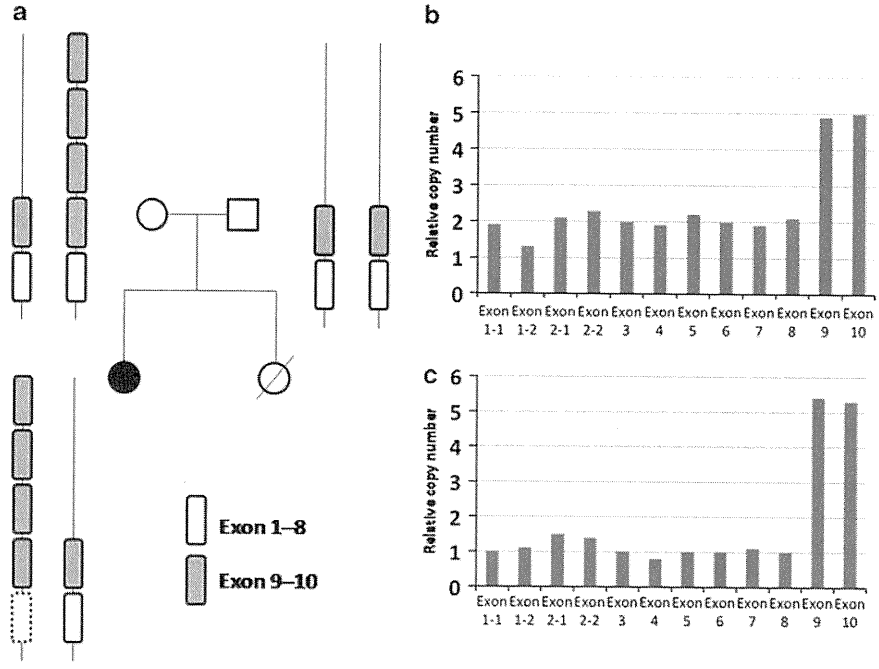

Figure 2 Family pedigree (**a**) and MLPA results in case 12. Although a relative copy number of around two was observed for normal controls, the mother of the proband had a copy number of about five in exon 9–10 (**b**). In case 12, there is a copy number of around one from exon 1–8 and about five from exon 9–10 (**c**). This means that case 12 has *de novo* microdeletion in exon 1–8 and duplication in exon 9–10. The latter is not pathogenetic as it is shared with her asymptomatic mother.

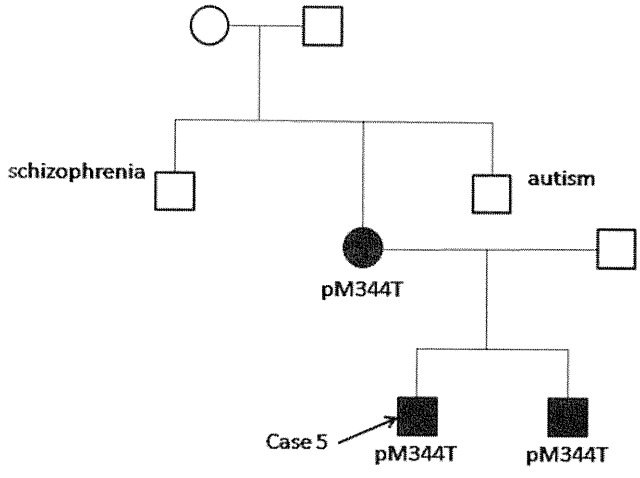

Figure 3 Family pedigree of case 5. The proband, his brother and mother had same mutation. His father did not have the mutation. The mother's older brother was diagnosed with schizophrenia in his fourth decade of life and her younger brother was diagnosed with autism and was institutionalized in a shelter for intellectually disabled people.

consent for their *SLC2A1* gene analysis. The pathogenetic nature of the mutation was confirmed by its absence in his father and one hundred non-consanguineous normal control subjects.

Case 12 presentation

This 15-year-old female was the first-born child of healthy non-consanguineous parents. She was born after full-term pregnancy by vaginal delivery with no complication. Her birth weight was 2836 g. She developed truncal ataxia and hypotonia at 6 months of age. At 11 months of age, she developed hemiconvulsions and atonic seizures just before breakfast. Phenobarbital was started and her seizures were well controlled. When phenobarbital was withdrawn at 5 years of age, atonic seizures recurred on fasting. Although she started to speak words at 15 months of age, her intellectual delay became severe thereafter. At 8 years of age, she was diagnosed with Glut-1 DS on the basis of clinical features as well as hypoglycorrhachia and decreased 3-OMG uptake by erythrocytes (Table 1).

At the age of 12 years old, she was admitted to our hospital to start a ketogenic diet. She had microcephaly (head circumference 50.8 cm) but no other facial dysmorphia. Although she could walk without support, she easily fell to the ground and was unable to walk for a long distance because of spastic gait and ataxia. She could have a simple conversation, her intelligence quotient was low at 32 and she had dysarthria. MPLA analysis revealed duplication in exon 9–10, deletion in exon 1–8 and duplication in exon 9–10. Her mother possessed the same duplication in exon 9–10 without any neurological symptoms.

DISCUSSION

Classic Glut1-DS is characterized by early-onset epilepsy, developmental delay and acquired microcephaly, which is an aspect of encephalopathy. Klepper *et al.*[10] proposed diagnostic criteria for Glut1-DS as follows: seizures, developmental delay, complex movement disorder and fasting EEG changes improving postprandially. Original laboratory criteria for Glut1-DS consisted of hypoglycorrhachia ($<40\,mg\,dl^{-1}$), low CSF/blood glucose ratio (<0.4) and reduced erythrocyte glucose uptake (uptake cutoff point at 60% uptake).[6] After the discovery of *SLC2A1* as a gene responsible for Glut1-DS, many mutations have been recognized that show autosomal dominant inheritance. However, as more cases with documented gene abnormalities of the *SLC2A1* gene have been reported, atypical cases have come into light such as a case of movement disorder without

epilepsy[11] and another with autosomal recessive inheritance.[12] Furthermore, it was recognized that a wide clinical syndromes existed resulting from different *SLC2A1* gene mutations. For example, Glut1-DS was reported to occur in >10% of patients with early-onset absence epilepsy and some of them expressed mild manifestations.[13] Furthermore, some patients with *SLC2A1* mutations have been reported to have milder hypoglycorrhacia (40–52 mg dl^{-1}) and relatively high CSF/blood glucose ratio (0.45–0.57) as compared with original Glut1-DS criteria. However, no Glut1-DS patients showed normal CSF glucose concentration.[14] On the other hand, Glut1-DS cases have been reported which had normal erythrocyte glucose uptake.[15] Therefore, in order not to overlook milder or atypical cases of Glut1-DS, usage of a broader criteria including mild hypoglycorrhacia or normal erythrocyte glucose uptake may be useful. These varied clinical and laboratory findings indicate the increasing importance of gene testing in the diagnosis of the patients suspected of having Glut1-DS. We report *SLC2A1* gene analysis in 12 Japanese patients who were diagnosed on the basis of clinical features including early-onset epilepsy and hypoglycorrhachia. This is the first report of a large number of Japanese patients with Glut1-DS being subject to genetic analysis.

We analyzed genomic abnormalities for SLC2A1 including the case with typical clinical pictures and hypoglycorrhacia regardless of erythrocyte glucose uptake and found eight abnormalities including seven mutations, of which six were novel. The locations of each mutation in the transmembrane configuration of the Glut1 protein are shown in Figure 1. Although several hot spots for recurrent mutations have been previously reported, as many as 60 mutations have been reported scattered in transmembrane, intracellular and extracellular domains.[16]

We made sure that they didn't match the reported polymorphism in the database (Japanese Single-Nucleotide Polymorphisms),[17] and also confirmed that the mutations were not present in one hundred healthy controls. All mutations caused the change of amino acid in SLC2A1. The nonsense and frameshift mutations are highly likely to induce the reduced function of Glut 1. In case of the missense mutation, two patients out of three (cases 6 and 9) with missense mutations showed evidence of abnormal Glut 1 function supported by reduced 3-OMG uptake. The pathogenicity of the missense mutation in the remaining one patient (case 5) was unfortunately unsupported by reduced 3-OMG uptake. Recently Glut1-DS patients with T295M mutation have been reported to have normal 3-OMG uptake in erythrocytes. However, in functional studies, Glut1 conformational change asymmetrically affects the efflux of glucose from cells as compared with its influx.[15] Thus, influx measured by erythrocyte glucose uptake can be normal.[18] Although we did not measure either glucose efflux or conformational change, we can speculate that this kind of mechanism could be associated with case 5.

In addition, we revealed the first case with a complex rearrangement of deletion and duplication using the novel MLPA method (case 12). In this patient, deletion of exon 1–8 was the pathophysiological recombination, because her mother with same duplication did not have any symptoms and Glut1-DS is a congenital metabolic disorder induced by haploinsufficiency of the *SLC2A1* gene.[12]

With respect to the genotype–phenotype correlation, we could not find a definite relationship between clinical severity indicated by seizure onset and/or CSF glucose concentration, CSF/blood glucose ratio and 3-OMG uptake. For example, case 5 had relatively mild hypoglycorrhachia, he had the same *SLC2A1* mutation as his brother and mother, which indicates they were familial cases with an autosomal dominant trait. The proband case 5 manifested symptoms of 'encephalopathy' with refractory seizures and severe developmental delay. Despite his brother having the same CSF glucose concentration as that of the proband, he had considerably milder symptoms. The description of mutation-positive family members of case 5 agrees with previous reports documenting highly variable clinical symptoms in a Glut1-DS family, ranging from no clinical manifestation at all to the severe classic phenotype.[13]

Regarding seizure type, the predominant seizure types were generalized tonic-clonic seizure and complex partial seizures followed by absence and atonic seizures. Glut1-DS would appear to be a syndrome with variable generalized epilepsy. It is possible that most of our cases harbored classic and relatively severe phenotypes, which probably limited the ability to detect any correlation between genotype and phenotype.

Finally, no *SLC2A1* mutation gene was found in five or our 12 patients (33.3%) despite the presence of hypoglycorrhachia and typical clinical symptoms of Glut1-DS. Leen *et al.*[16] reported that 59% of 132 Glut1-DS patients were negative on genetic analysis for *SLC2A1* mutation. They suspected that regulatory sequences of the *SLC2A1* gene such as promoter sequences and/or sequences deep within introns may have been underdiagnosed. In our series, there were no clinical differences between mutation-positive and -negative cases. Thus, mutation in regulatory sequences and/or other modulating genes could well affect Glut1 activity. Furthermore, loss of activity of hexokinase I, which involve glucose transport in the endothelial cells, may contribute to pathophysiology in these cases.[19] In conclusion, as it is now possible to improve the prognosis of Glut1-DS with a ketogenic diet, it is important to make a definite diagnosis as early as possible. As there is a wide range of clinical manifestations and false-negative patients when using the original criteria for Glut1-DS diagnosis, it is important to identify genetic abnormalities not only by the direct sequencing but also by MLPA. Further studies are needed for the detection of potentially secondary or modifier genes.

ACKNOWLEDGEMENTS

This work was supported by Eisai (project no. J109001037) and UCB Japan (project no. J099001110).

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