Mitochondrial Medicine 2014: Pittsburgh, PA June 4-7, 2014.

ガバペンチンが有効であった GNAO1 変異をもつヒョレアアテトーシスの一例
高木真理子1, 佐藤睦美1, 安西里恵1, 奥田美津子 1, 露崎悠 1, 高野亨子 1,2, 井合瑞江 1, 中村和幸3,4, 才津浩智3, 小坂仁1,5, 山下純正 1
1 神奈川県立こども医療センター神経内科, 2 信州大学医学部遺伝医学予防医学講座, 3 横浜市立大学医学部遺伝学講座, 4 山形大学小児科, 5 自治医科大学小児科学講座
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重度精神遅滞, 難治性てんかんの臨床像を示し, PIGO 遺伝子変異が同定された 1 例
安西里恵1, 佐藤睦美1, 高木真理子1, 奥田美津子 1, 露崎悠 1, 高野亨子 1,4, 井合瑞江 1, 中村和幸2,3, 才津浩智2, 小坂仁1,5, 山下純正 1
1 神奈川県立こども医療センター神経内科, 2 横浜市立大学医学部遺伝学, 3 山形大学医学部小児科, 4 信州大学医学部遺伝医学, 5 自治医科大学医学部小児科
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Whole exome sequencing reveals molecular basis of childhood cerebellar atrophy
Hitoshi Osaka1,2, Yu Tsuyusaka1, Mizue Iai2, Sumimasa Yamashita2, Nobuyuki Shimozawa3, Yoshikatsu Eto4, Hirotomo Saitsu5
1Department of Pediatrics, Jichi Medical School, 2Division of Neurology, Kanagawa Childrens Medical Center, 3Division of Genomics Research, Life Science Research Center, Gifu University, 4Advanced Clinical Research Center & Asian LSD Center, Institute of Neurological disorders, 5Department of Human Genetics, Graduate School of Medicine, Yokohama City University
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Effect of CYP2C19 polymorphisms on stiripentol administration in cases of Dravet syndrome
Takeshi Kouga1,2, Mariko Takagi3, Rie Anzai3, Mutsumi Sato3, Mitsuko Okuda3, Kyoko Takano3, Mizue Iai3, Sumimasa Yamashita3, Hitoshi Osaka2,3
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Mutational and functional analysis of Glucose transporter 1 deficiency syndrome.
Sachie Nakamura[1)], *Hitoshi Osaka*[1)], *Shinichi Muramatsu*[2)], *Shiho Aoki*[1)], *Eriko F. Jimbo*[1)], *Takanori Yamagata*[1)]
Department of Pediatrics[1)], Department of Neurology[2)], Jichi Medical University
2014.10.18-22 第 64 回アメリカ人類遺伝学会 (サンディエゴ)

ミトコンドリア DNA m.3243A>T 変異を認めた mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes の 1 例
池田尚広 1), 山崎雅世 1), 鈴木峻 1), 門田行史 1), 小坂仁 1), 杉江秀夫 1), 新保裕子 2), 山形崇倫 1)
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Infantile Neuroaxonal Dystrophy 様の脳 MRI 所見を示した SLC9A6 変異を有する一例

山本亜矢子 1,2, 和田敬仁 2,3, 新保裕子 2, 松本直通 4, 小坂仁 2,5

1 大和市立病院小児科, 2 神奈川県立こども医療センター神経内科, 3 京都大学大学院医学研究科医療倫理学/遺伝医療学, 4 横浜市立大学大学院医学研究科遺伝学, 5 自治医科大学小児科学講座 56 回日本小児神経学会 2014.5.28-2014.5.30. 浜松

治療可能な小脳失調 : Cerebral Folate Transport Deficiency の同胞例

露崎悠 1, 井合瑞江 1, 安西里恵 1, 佐藤睦美 1, 高木真理子 1, 奥田美津子 1, 高野亨子 1,3, 小坂仁 1,2, 山下純正 1, 才津浩智 4

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当院における副腎白質ジストロフィー6 例の臨床的検討

宮内彰彦 1, 門田行史 1, 池田尚広 1, 川原勇太 1, 長嶋雅子 1, 小坂仁 1, 杉江秀夫 1, 森本哲 1, 渡辺浩史 3, 下泉秀夫 3, 下澤伸行 2, 山形崇倫 1

1 自治医科大学小児科, 2 岐阜大学生命科学総合研究支援センターゲノム研究分野, 3 国際医療福祉リハビリテーションセンターなす療育園 56 回日本小児神経学会 2014.5.28-2014.5.30. 浜松

非造影灌流画像, ASL で最も鋭敏にとらえた MELAS の脳卒中様発作の一例

奥田美津子 1, 佐藤睦美 1, 安西里恵 1, 高木真理子 1, 露崎悠 1, 高野亨子 1,2, 井合瑞江 1, 小坂仁 1,3, 山下純正 1

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頸部動脈解離による脳梗塞

佐藤睦美 1, 高木真理子 1, 安西里恵 1, 奥田美津子 1, 露崎悠 1, 高野亨子 1,2, 小坂仁 1,3, 井合瑞江 1, 山下純正 1

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くも膜下出血を合併した Reversible cerebral vasoconstriction syndrome の女児例

吉原尚子 1,2, 和田敬仁 1,3, 高木真理子 1, 佐藤睦美 1, 安西里恵 1, 奥田美津子 1, 露崎悠 1, 小坂仁 1,4, 高野亨子 1,5, 井合瑞江 1, 山下純正 1

1 神奈川県立こども医療センター神経内科, 2 日本赤十字社医療センター小児科, 3 京都大学医学部医療倫理学遺伝医療学講座, 4 自治医科大学小児科学講座, 5 信州大学医学部付属病院遺伝医学講座

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早期ステロイドパルス療法によるけいれん重積型急性脳症発症予防効果の検討

池田尚広, 山形崇倫, 谷口祐子, 宮内彰彦, 石井朋之, 長嶋雅子, 門田行史, 小坂仁, 杉江秀夫自治医科大学小児科 56 回日本小児神経学会 2014.5.28-2014.5.30. 浜松

日内変動を伴うジストニアを認める自閉症スペクトラム障害の男児例

宮内彰彦[1)]、門田行史[1)]、長嶋雅子[1)]、杉江秀夫[1)]、小黒範子[2)]、小坂仁[1)]、山形崇倫[1)]

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2014.9.20　第61回日本小児神経学会関東地方会（筑波）

H. 知的所有権の取得状況

1. エリスロポエチン発現増強剤。国際出願　国際公開番号：WO2014/080640A1
2. 生体試料中のアミンの測定方法およびその方法を用いる患者のスクリーニング方法
   特願2011-019561　特許第5662182号

厚生労働科学研究費補助金（難治性疾患政策研究事業）
分担研究報告書

脳クレアチニン欠乏症候群の診断基準作成および疫学調査に対する研究

分担研究者　後藤知英
地方独立行政法人神奈川県立病院機構 神奈川県立こども医療センター　神経内科　部長

研究要旨：脳クレアチン欠乏症候群の診断には脳 MRI 検査機器による脳 magnetic resonance spectroscopy（MRS）で異常所見（クレアチンピークの減衰）を検出することが重要である。神奈川県立こども医療センターでは年間559件の新規紹介があり、このうち発達遅滞・自閉症・てんかんのいずれかを主訴に受診したものは352件であった（2013年度実績。2014年度については集計中)。これらの症例に対して、ほぼ全例で脳MRSを含めた頭部MRI検査を実施した。その結果、2014年度は1症例においてクレアチン輸送体欠損症が強く疑われる所見が得られ、生化学的・遺伝学的検査を実施して診断の確定を進めている。352件のうち約半数が男児であるとした場合、前述の有病率から当院で遭遇すると期待されるクレアチン輸送体欠損症の症例数は0.5～6.2人となる。したがって、2014年度の1年間に1症例が検出されたことは予測値の範囲内にあったことになる。来年度も引き続きMRS検査による患者スクリーニングを進めていく予定である。

A．研究目的

脳クレアチン欠乏症候群はクレアチン産生にかかわる酵素（グアニジノ酢酸メチル基転移酵素、アルギニン・グリシンアミジノ基転移酵素）あるいは細胞内への輸送体（クレアチン輸送体）の機能異常によって、脳内のクレアチンの欠乏を生じる先天性代謝疾患である。臨床的には精神遅滞、言語発達遅滞、てんかんなどを引き起こすことが知られている。特にクレアチン輸送体の異常によるもの（SLC6A8 遺伝子欠損症）は遺伝性精神遅滞のうち脆弱X症候群に次ぎ頻度が高い疾患とされ、精神遅滞を有する男性の 0.3～3.5%、アメリカでは 42,000 人、世界では100 万人と推定されている。

脳クレアチン欠乏症候群は発達遅滞やてんかんといった非特異的な臨床像を呈するため、診断には脳 MRI 検査機器による脳 magnetic resonance spectroscopy（MRS）で異常所見を検出することが重要である（クレアチンピークの減衰)。我が国では MRI 検査機器は広く普及しており発達遅延やてんかんの診断の上でルーチンの検査となっている。しかし、脳MRSは検査手技あるいは検査時間の制約のため実施される症例は限られている。このことから、未診断となっている脳クレアチン欠乏症候群症例が、我が国にも多数存在する可能性がある。

本研究においては、患者を集積し診断基準を作成するとともに、本邦における有病率を推定することが目的である.

B. 研究方法

神奈川県立こども医療センターにおける年間559件の新規紹介症例のうち、発達遅滞・自閉症・てんかんのいずれかを主訴に受診したもの352件（2013年度実績。2014年度については集計中であるが、例年同数程度の受診がある）のほぼ全例対して、脳MRSを含めた頭部MRI検査を実施した。

（本研究は、当センターの倫理委員会で承認されている.）

C. 研究結果

2014年度は352症例のうち、1症例においてクレアチン輸送体欠損症が強く疑われる所見が得られ、生化学的・遺伝学的検査を実施して診断の確定を進めている。

D. 考察

脳クレアチン欠乏症候群は発達遅延の原因として潜在的に多い疾患と推測されている。352件のうち約半数が男児であるとした場合、前述の有病率から当院で遭遇すると期待されるクレアチン輸送体欠損症の症例数は0.5～6.2人となる。したがって、2014年度の1年間に1症例が検出されたことは予測値の範囲内にあったことになる。来年度も引き続きMRS検査による患者スクリーニングを進めていく予定である。

E．結論

脳クレアチン欠乏症候群は、発達遅滞、自閉症、てんかんの鑑別診断として重要であるが、まだ臨床現場での認知度が低いことが予想される．欧米での有病率を考慮すると、日本国内の大多数の症例は診断されていないと考えられる．

来年度は診断基準作成にむけて、既に診断されている症例の臨床情報を集積するとともに、臨床現場への周知を進めていく予定である．

G．研究発表　（本研究に関連するものに限る）

1．論文発表　なし
2.　学会発表　なし

H. 知的所有権の取得状況
なし

厚生労働科学研究費補助金（難治性疾患政策研究事業）
分担研究報告書

脳クレアチン欠乏症候群の分子遺伝学的診断に関する研究

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研究要旨

治療可能な代謝疾患のひとつに脳内クレアチンが欠乏するクレアチン代謝異常症があげられる。クレアチン代謝異常症は、重度の精神遅滞を主要症状とし、てんかん、自閉症、重度の言語障害などの様々な臨床症状を有する。その先天的な原因として、クレアチン生合成に関わるグアニジノ酢酸メチルトランスフェラーゼ(GAMT)、アルギニン・グリシンアミジノトランスフェラーゼ(AGAT)、およびクレアチントランスポーター(SLC6A8)の遺伝子の欠損が知られている。臨床症状を有する患者の脳を磁気共鳴スペクトル装置(MRS)で調べて脳内クレアチン値の低下を確認することにより診断されるが、原因遺伝子を特定する手掛かりを得るには、尿や血清中のクレアチン関連化合物（特にグアニジノ酢酸／クレアチニン比、クレアチン／クレアチニン比）を測定する必要がある。本研究では、クレアチン代謝異常症が疑われる症例において、まず尿中のクレアチン関連化合物をHPLC法で測定し、次いで血液からRNA 、ゲノムDNAを抽出して遺伝子解析を行う。

A. 研究目的

クレアチン代謝異常症は、重度の精神遅滞を主要症状とし、てんかん、自閉症、重度の言語障害などの様々な臨床症状を有する疾患である。海外におけるクレアチン代謝異常症の報告が多いが、日本での報告は当院で解析した数例にとどまる。その先天的な原因として、クレアチン生合成に関わるグアニジノ酢酸メチルトランスフェラーゼ(GAMT)、アルギニン・グリシンアミジノトランスフェラーゼ(AGAT)、およびクレアチントランスポーター(SLC6A8)の遺伝子の欠損が知られている[1-3]。臨床症状を有する患者の脳を磁気共鳴スペクトル装置(MRS)で調べて脳内クレアチン値の低下を確認することにより診断されるが、原因遺伝子を特定する手掛かりを得るには、尿や血清中のクレアチン関連化合物（特にグアニジノ酢酸／クレアチニン比、クレアチン／クレアチニン比）を測定する必要がある。

2011年に当研究室において、高速液体クロマトグラフィー(HPLC)-UV 検出器でポリマー系の陽イオンクロマトグラフィー用カラムを用いた生体試料中のクレアチン化合物の測定方法及びその方法を用いるクレアチン代謝異常症の患者のスクリーニング方法を開発し[4]、本邦の初症例を含むクレアチン代謝異常症 6 家系（SLC6A8 欠損症:5 家系、GAMT 欠損:1 家

系）の診断を行った[5-7]。生体試料分析において、SLC6A8 欠損症は尿中のクレアチン/クレアチニン比が上昇、GAMT 欠損症は尿、血漿、髄液中のグアニジノ酢酸濃度上昇を特徴とする。

尿のスクリーニング、脳内 MRS、遺伝子検査から早期診断、治療応用を目指す。

B. 研究方法

1. 尿中のクレアチニン関連化合物の測定

採取した尿は、直ちに凍結し、－80℃で保存する。

HPLC 測定には、表面にカルボキシル基の付いたポリマー系ゲルが充填された弱酸性陽イオン交換カラム(Shodex YS-50)を用い、薄いリン酸またはギ酸の水溶液を溶離液として UV 210nm で検出する。まず、グアニジノ酢酸、クレアチン、クレアチニンの標準溶液(1～10 μM)を測定し、各成分について、濃度を X-軸、ピーク高さを Y-軸にとって検量線を作成する。

患者尿検体の測定は、凍結尿 500 μl に等量のアセトニトリルを添加後、氷上に静置し、遠心分離により蛋白を除去後の上清を 10～100 倍希釈し、評価する。

2. 遺伝子検査

末梢血液（EDTA2Na）5ml（3ml をゲノム DNA 抽出、2ml を RNA 抽出）に使用する。cDNA 合成には Primescript RT reagent kit (Takara)を用いる。

SLC6A8, GAMT, AGAT 各遺伝子の全コーディング領域をカバーするプライマーを独自に設計、プライマー内側特に 3’側に SNP が入らないことを確認する。

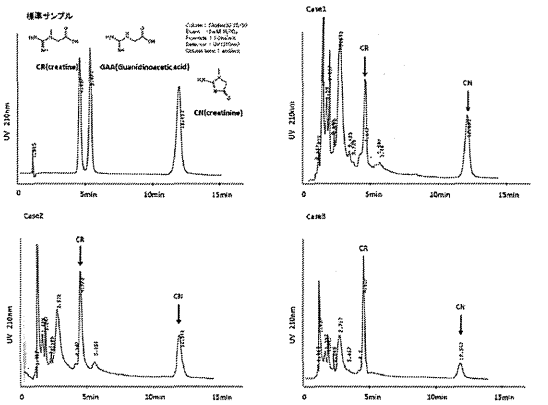
C. 研究結果

臨床学的クレアチン代謝異常症が疑われた 3 症例(男児 2 名、女児 1 名)について尿中のクレアチン化合物を HPLC 法で測定した(表 1、図 1)。1 例(Case3)の男児に関して、尿中クレアチン／クレアチニン比が同年齢の正常上限値の約 3 倍上昇を認めた。2 例(Case1, 2)に関しては尿中クレアチン／クレアチニン、グアニジノ酢酸／クレアチニン比は正常範囲内であった。

表 1. 尿中クレアチン／クレアチニン比

Patient (year)	gender	クレアチン／クレアチニン比
Case1 (2y5m)	male	1.10 (ref. 0.2-2.03)
Case2 (3y2m)	female	1.81 (ref. 0.2-2.03)
Case3 (2y)	male	6.45 (ref. 0.2-2.03)

図 1. クロマトグラム



D. 考察

本研究期間にクレアチン代謝異常症が疑われた 3 患者の尿中のクレアチン化合

物を測定したところ、1例(Case3)においてクレアチン／クレアチニン比の上昇を認めた。MRS検査では脳内クレアチン値の低下が認められたことにより、クレアチントランスポーター(SLC6A8)欠損症が疑われた。
遺伝子検査により確定診断を行う。

E. 結論

尿中のクレアチン関連化合物のHPLC測定は、クレアチン代謝疾患の診断に有用である。

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G. 研究発表

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藤進、鈴木良介、三宅茂太、新保裕子 第10回 craniosynostosis 研究会, 名古屋, 2014年7月

H. 知的財産権の出願・登録状況

１．特許取得

特許第5662182号

発明の名称： 生体試料中のアミン測定方法およびその方法を用いる患者のスクリーニング方法

特許権者： 地方独立行政法人神奈川県立病院機構

発明者:: 和田敬仁、新保裕子、小坂仁

出願番号： 特願2011-019561

出願日： 平成23年 2月1日

登録日： 平成26年12月12日

Ⅲ．研究成果の刊行に関する一覧表

書籍

著者署名	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

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kato H, Miyake F, Shimbo H, Ohya M, Sugawara H, Aida N, Anzai R, Takagi M, Okuda M, Takano K, Wada T, Iai M, Yamashita S, Osaka H.	Urine screening for patients with developmental disabilities detected a patient with creatine transporter deficiency due to a novel missense mutation in SLC6A8.	Brain Dev	36	630-633	2014
Akiyama T, Osaka H, Shimbo H, Nakajiri T, Kobayashi K, Oka M, Endoh F, Yoshinaga H.	A Japanese adult case of guanidinoacetate methyltransferase deficiency.	JIMD	12	665-669	2014
van de Kamp JM, Errami A, Howidi M, Anselm I, Winter S, Phalin-Roque J, Osaka H, van Dooren SJ, Mancini GM, Steinberg SJ, Salomons GS.	Genotype-phenotype correlation of contiguous gene deletions of SLC6A8, BCAP31 and ABCD1.	Clin Genet	87	141-147	2015
野崎 章仁、熊田知浩、柴田実、藤井達哉、和田敬仁、小坂仁.	尿中クレアチン/クレアチニン比と家族歴より診断に至ったクレアチントランスポーター欠損症の1家系　本邦3家系目.	脳と発達	47	49-52	2015

Ⅳ. 研究成果の刊行物・別刷

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Brain & Development 36 (2014) 630–633

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Case report

Urine screening for patients with developmental disabilities detected a patient with creatine transporter deficiency due to a novel missense mutation in *SLC6A8*

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Abstract

Creatine transporter deficiency (CTD) is an example of X-linked intellectual disability syndromes, caused by mutations in *SLC6A8* on Xq28. Although this is the second most frequent genetic cause of intellectual disabilities in Europe or America after Fragile X syndrome, information on the morbidity of this disease is limited in Japan. Using the HPLC screening method we have established recently, we examined samples of urine of 105 patients (73 males and 32 females) with developmental disabilities at our medical center. And we have found a family with three ID boys with a novel missense mutation in *SLC6A8*. This is the second report of a Japanese family case of CTD. A systematic diagnostic system of this syndrome should be established in Japan to enable us to estimate its frequency and treatment.

Keywords: Intellectual disability; X-linked; Mental retardation; Creatine deficiency syndrome; Urine screening

1. Introduction

Cerebral creatine deficiency syndromes (CCDSs) are a group of inborn errors of creatine metabolism, including two autosomal recessive disorders that impair the biosynthesis of creatine, arginine:glycineamidinotransferase deficiency (MIM 602360) and guanidinoacetate-methyltransferase deficiency (MIM 601240), and the X-linked disorder, creatine transporter deficiency (CTD; MIM 300036). The common clinical features of CCDSs are intellectual disability, delayed language, autistic behavior and epilepsy [1].

CTD is caused by mutations in *SLC6A8* on Xq28, and reported to constitute 1% of males with ID of unknown etiology [2] or 2.1% of male with nonsyndromic X-linked intellectual disability (XLID) [3]. Although this is the second most frequent genetic cause of intellectual disabilities in Europe or America after Fragile X syndrome, only one case report of a Japanese patient with a deletion of the *SLC6A8* gene has been published by Osaka [4], and we have little information on the frequency of this disease in Japan.

To diagnose the three types of CCDs, we have established a new simple HPLC screening method to determine the concentrations of guanidinoacetic acid (GAA), creatine (CR), and creatinine (CN) in urine [5]. Here we report the second case of Japanese familial case of CTD with a novel missense mutation of the *SLC6A8* gene.

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2. Case report

The proband is a six-year-old boy, who was referred to our medical center because of his psychomotor retardation and delayed speech at age 4 years 11 months. He was born after an uneventful and full-term pregnancy. He sat until 12 months and walked at 22 months. He had no overt dysmorphism. He had febrile seizures three times. He could speak only a few words. His language comprehension was much better than expression, and he could obey several simple orders. His cognitive function seems to be that of a 3–4 years old. His neurological examination showed no abnormal findings, including pyramidal, extrapyramidal, or cerebellar signs. At 6 years and 4 months old, his height was 112.7 cm (−0.6 S.D.), and weight 18.8 kg (−0.7 S.D.), and head circumference 47.8 cm (−2.2S.D.), suggesting relative acquired microcephalus. He had no apparent autistic features.

The clinical pictures of the patient, his two younger brothers and mother are summarized. (Fig. 1 and Table 1).

We detected an abnormal pattern in the patient's urine by the HPLC method, showing CR/CN ratio was extremely high and GA within normal range, during the examination of the urine samples of 105 patients (73 males and 32 females) with developmental disabilities at our medical center.

These results prompted us to suspect a diagnosis of CTD. The urines of his two brothers and their mother also showed abnormal pattern. (Fig. 2).

^{1}H-MRS, examined using 3.0T MR system at the left basal ganglia of the index case showed a marked reduction of brain creatine peak. Brain T2-weighted and FLAIR MRI showed a high signal at the left trigone, and hypoplasia of the corpus callosum.

Genetic analysis of genomic DNA and cDNA from the patient showed a novel missense mutation in the exon 12 of the *SLC6A8* gene [c.1681G>C; p.Gly561Arg]. The two brothers had the same mutation. This glycine is a highly conserved amino acid among the creatine transporters of several species (chimpanzee, cattle, dog, mouse, rat and zebrafish). This amino acid substitution was not registered in the SNP database, and we did not find this substitution in 50 controls. Therefore this mutation is likely to be a pathogenic mutation for this family. Their mother has the same mutation heterogeneously.

3. Discussion

This is the second report of a Japanese familial case of CTD with a novel missense mutation of the *SLC6A8* gene, diagnosed by our new screening method of urinary GAA concentration and CR/CN ratio for individuals with developmental disabilities.

Several reviews have been published to characterize the clinical, laboratory, molecular, and imaging profiles of CTD, and the patients can presented with various symptoms, including growth disorder, hypotension, pyramidal/extrapyramidal findings, and attention deficit

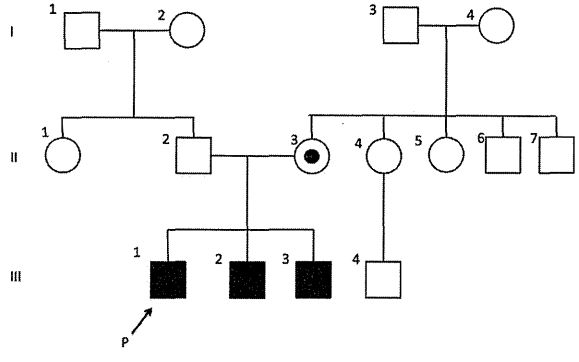

Fig. 1. Pedigree chart of the family.

Table 1
Clinical features of the family.

	III-1; 6 Yr	III-2; 4 Yr	III-3; 2 Yr	II-3; 29 Yr
Gestational age	38.6 w	38.5 w	40.6 w	40 w
Birth Wt/HC	2776 g/32.5 cm	2632 g/–	2882 g/–	2960 g/NA
Present Wt/Ht/HC (S.D.)	−0.6/−0.7/−2.2	−0.4/−1.7/NA	−2.2/−3.4/−4.2	−2.1/−2.2/NA
Walking	24 months	22 months	24 months	18 months
Language development	Words only	5 words only	One words only	16 months
Meaningful words	Perception > Expression 24-months	Perception > Expression 20-months	Perception > Expression 21-months	
Seizure (Onset)	Febrile Sz (5 Yr)	Febrile Sz (2 Yr 10 Mo)	Febrile Sz Status (11 Mo) Epilepsy (1.5 Yr)	Epilepsy (14 Yr)
EEG	No Sz discharge	Not done	No Sz discharge	P/O-dominant diffuse Sp&W
Brain MRI/CT	Hypoplasia of corpus callosum	Normal (CT only)	Delayed myelination, hypoplasia of corpus callosum, small pituitary grand	Normal
Characteristics	Obey a simple order, understand his situation, good memory of sight information	Obey a simple order	Restless, hyperactive	Poor at putting in order, easy to be deceived
Creatine/Creatinine (mg/dl)	55.2/10.9	175.7/27.5	110.3/16.5	294.0/131.6
Ratio	5.07	6.39	6.7	2.23

Yr, year(s); Wt, weight; HC, head circumference; w, week(s); Ht, height; NA, not assessed; EEG, electroencephalogram; Sz, seizure; Mo, month(s); P/O, Parietal and occipital cortex; Sp&W, spike and waves; MRI, magnetic resonance imaging; CT, computed tomography.

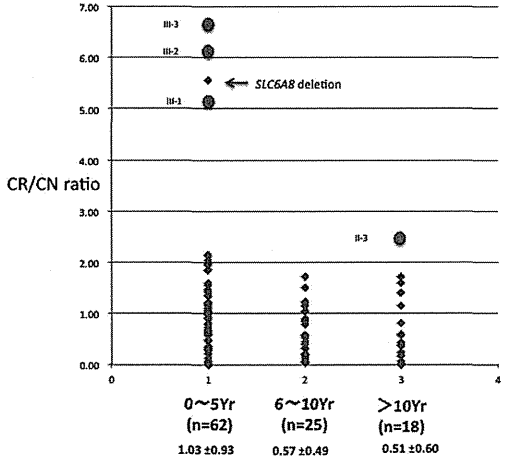

Fig. 2. The distribution of the ratio of creatine (CR)/creatinine (CN) by age of normal controls and the family. "SLA6A8 deletion" is the patient reported by Osaka [4]. (Yr, years; *n*, number).

hyperactivity disorder (ADHD) have been reported [1]. Our patients of the family presented with a broad spectrum of clinical phenotypes and relatively mild intellectual disability without autistic features or extrapyramidal abnormal movement, and they had expressive language disorder rather than receptive language disorder. Their relatively mild phenotype may suggest that their creatine transporter has some residual activity, although we have not confirmed it yet. Our result indicates that short stature and acquired relative microcephaly may be a good indication to suspect CTD as a diagnosis for patients with ID.

Up to now, no treatments had been established for CTD, although oral supplementation of creatine monohydrate is known to be effective for GAMT and AGAT deficiency. Recently it was reported that the creatine analog, cyclocreatine, improved the cognitive abilities of brain-specific *Slc6a8* knockout mouse, and this might be a therapeutic agent in the near future [6].

Female carriers of X-linked CTD can commonly manifest, although usually less severely than affected males, as the mother of our index case presents. There is no consistent skewing of X-inactivation in peripheral tissues, indicating that there is no selection against CTD [7]. We emphasize the utility of our screening method for patients of CCDSs because of its cost performance, although the screening method may not be suitable for detecting female patients with CTD because of the high rate of false positive results [8].

The family was diagnosed during the examination of the urine samples of 105 patients with developmental disabilities at our medical center by our screening HPLC method. Considering that CTD is a frequent cause of intellectual disabilities in Europe or America, many patients in Japan should remain to be diagnosed. We have to develop systematic strategies for diagnosis, treatment and prevention of CCDSs as early as possible, because CCDSs are potentially treatable disorders [9].

Acknowledgements

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JIMD Reports
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CASE REPORT

A Japanese Adult Case of Guanidinoacetate Methyltransferase Deficiency

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Received: 12 April 2013 / Revised: 12 May 2013 / Accepted: 26 May 2013

Abstract Guanidinoacetate methyltransferase (GAMT) deficiency is a rare disorder of creatine synthesis resulting in cerebral creatine depletion. We present a 38-year-old patient, the first Japanese case of GAMT deficiency. Developmental delay started after a few months of age with a marked delay in language, which resulted in severe intellectual deficit. She showed hyperactivity and trichotillomania from childhood. Epileptic seizures appeared at 18 months and she had multiple types of seizures including epileptic spasms, brief tonic seizures, atypical absences, complex partial seizures with secondary generalization, and "drop" seizures. They have been refractory to multiple antiepileptic drugs. Although there have been no involuntary movements, magnetic resonance imaging revealed T2 hyperintense lesions in bilateral globus pallidi. Motor regression started around 30 years of age and the patient is now able to walk for only short periods. Very low serum creatinine levels measured by enzymatic method raised a suspicion of GAMT deficiency, which was confirmed by proton magnetic resonance spectroscopy and urinary guanidinoacetate assay. *GAMT* gene analysis revealed that the patient is a compound heterozygote of c.578A>G, p.Gln193Arg and splice site mutation, c.391G>C, p.Gly131Arg, neither of which have been reported in the literature. We also identified two aberrant splice products from the patient's cDNA analysis. The patient was recently started on supplementation of high-dose creatine and ornithine, the effects of which are currently under evaluation. Although rare, patients with developmental delay, epilepsy, behavioral problems, and movement disorders should be vigorously screened for GAMT deficiency, as it is a treatable disorder.

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Competing interests: None declared

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Introduction

Guanidinoacetate methyltransferase (GAMT; OMIM 601240) deficiency is a rare autosomal recessive disorder of creatine synthesis resulting in cerebral creatine depletion (Stöckler et al. 1994, 1996b). Guanidinoacetate (GAA) accumulates in body fluids. Symptoms of GAMT deficiency usually emerge after a few months of life, such as intellectual disability, speech delay, autistic behaviors, epileptic seizures, and involuntary movements (Mercimek-Mahmutoglu et al. 2006). Making a diagnosis of GAMT deficiency is challenging; nonetheless, early diagnosis is crucial because this disorder is treatable (Stöckler et al. 1996a). Only approximately 80 cases have been reported to date, mostly from Europe and the Middle East. Here we report on the first Japanese patient with GAMT deficiency with two novel gene mutations.

Case Report

The patient, a 38-year-old female with intractable epilepsy and severe mental retardation, was born at full term with a birth weight of 3,260 g. There were no pre- or perinatal complications. She is the third of four children of Japanese non-consanguineous healthy parents. The first child, a boy, started having epileptic seizures after 1 year of age with unknown cause and died at 28 years of age at an institution for the mentally handicapped. The other two children have been healthy.

Although the patient showed a social smile by 3 months and head control by 4 months of age, her development has been delayed since then. She sat alone at 14 months, walked alone at around 20 months, and became able to take the stairs one step at a time with support around 5 years of age. She has spoken no meaningful words and gained little language comprehension. Her medical chart at 7 years of age described her as speechless, unable to follow verbal commands, but able to run and walk up the stairs one step at a time without support. She showed no involuntary movements. She was hyperactive and had trichotillomania. Neuropsychological assessment at 7 years 7 months by analytic test for development in infancy and childhood (Enjoji and Yanai 1961) demonstrated her developmental quotient was 14. Around 30 years of age, she was unable to walk for a long time but was able to take the stairs with support. At 32 years of age, she was no longer able to run. Currently, at 38 years of age, the patient has severe intellectual deficit with no speech or language comprehension. She still has trichotillomania. Her transport is mostly by wheelchair, although she is able to walk slowly for short periods. Her muscle tone is normal and there are no involuntary movements.

The onset of epilepsy was at around 18 months of age, characterized by epileptic spasms and brief tonic seizures. At 2 years of age, atypical absences appeared. Despite therapy with multiple antiepileptic drugs, the patient continued to have these seizures until 15 years of age, when her seizures were suppressed by valproic acid and clonazepam. When they recurred at 20 years of age, her seizures were characterized by consciousness impairment with head and body version to left followed by generalized tonic-clonic convulsions lasting up to 1 minute, suggesting complex partial seizures with secondary generalization. At around 23 years, brief "drop" seizures occurring in clusters started. She has continued to have these seizures since then, although she has been tried on multiple antiepileptic drugs including phenobarbital, valproic acid, clonazepam, phenytoin, clobazam, topiramate, lamotrigine, and levetiracetam.

Electroencephalograms (EEGs) at 2–12 years of age showed a slow background activity, generalized 1.5–2.5 Hz slow spike-wave bursts and some multifocal spikes, consistent with Lennox-Gastaut syndrome. EEGs after adolescence showed multifocal spike-waves with anterior head predominance and intermittent generalized slow spike-waves. The most recent EEG at 38 years of age demonstrated background slowing and no spikes during wakefulness but intermittent focal polyspikes and polyspike-waves over bilateral anterior and left posterior head regions during sleep.

Laboratory blood tests demonstrated low levels of serum creatinine (5–7 μmol/L by enzymatic method; normal range 40–71 μmol/L). Subsequent tests using enzymatic methods demonstrated serum creatine levels were below detection limit (normal range 23–92 μmol/L). Proton magnetic resonance spectroscopy (^{1}H-MRS) demonstrated absent creatine peak (Fig. 1a). Brain magnetic resonance imaging (MRI) demonstrated T2 high-intensity lesions in globus pallidi (Fig. 1b). Analysis of urinary creatine metabolites by weak-acid ion chromatography (Wada et al. 2012) demonstrated elevated GAA (548.53, 782.52 mmol/mol creatinine; normal 3–78 mmol/mol creatine (Almeida et al. 2004)) and creatine below detection limit. These findings suggested GAMT deficiency.

Genomic DNA analysis of the *GAMT* gene (Suppl. Table 1) showed a compound heterozygosity for two novel point mutations, an exonic splicing mutation c.391G>C located at the last nucleotide of exon 3 and a missense mutation c.578A>G, p.Gln193Arg in exon 6 (Fig. 2a). Analysis of cDNA revealed two aberrantly spliced transcription products at the allele of splicing mutation (Fig. 2b, c). One transcript had the complete exon 3 (64-bp) deletion by exon skipping and the other transcript was aberrantly spliced at exon 2 involving intron 2 insertion (44-bp) followed by exon 3 skipping, resulting in a 20-bp deletion. Both transcripts are expected to result in frame shift and premature termination of p.Val110Glyfs*30 and p.Ile111Profs*73, respectively. A novel A to G transition on exon 6 (c.578A>G) results in the replacement of arginine by glutamine at position 193 (p.Gln193Arg). This missense variation was not found in 100 control alleles. Glutamine193 is highly conserved in evolution (Fig. 2d), suggesting this mutation represents a pathogenic mutation.

This patient was recently started on supplementation of high-dose creatine and ornithine, and its effects are currently under evaluation.

Discussion

We reported on the first Japanese case of an adult patient with GAMT deficiency. Cases have been reported mostly from Europe and the Middle East (Mercimek-Mahmutoglu et al. 2006).

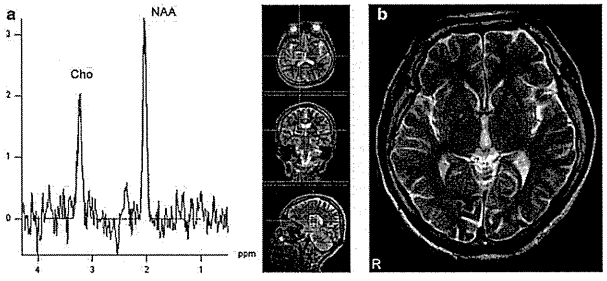

Fig. 1 MR spectroscopy and MRI from the patient with GAMT deficiency. (**a**) ^{1}H-MRS at the right basal ganglia demonstrates absence of creatine peak. (**b**) T2-weighted brain MRI shows high-intensity lesions in bilateral globus pallidi. *Cho* choline; *NAA* N-acetylaspartate

Compared with cases in the literature, our patient showed similar MRI findings and clinical course, with severe intellectual deficit, intractable epilepsy, behavioral problems, but she lacked involuntary movements. Although no definite progression of symptoms was seen during adolescence and young adulthood, motor regression slowly started around 30 years of age. This suggests GAMT deficiency can be slowly progressive if untreated.

Onset of symptoms in GAMT deficiency is from a few months to young childhood (Longo et al. 2011). Intellectual disability is seen in all cases and is severe (IQ < 35) in the majority, especially with profound speech disturbance (Mercimek-Mahmutoglu et al. 2006). Epilepsy is the second most frequent symptom, intractable in most cases, and partially responsive to antiepileptic drugs in two thirds (Leuzzi et al. 2013). Various types of seizures, such as generalized tonic-clonic seizures, absences, myoclonic seizures, myoclonic-astatic seizures, and partial seizures with secondary generalization, have been reported (Leuzzi et al. 2013). Involuntary movements, behavioral problems, and abnormal MRI signals in globus pallidi are seen in some cases. Adult cases that help to understand the natural history of GAMT deficiency are scarce (Schulze et al. 2003; Caldeira Araújo et al. 2005). Progression of neurological deficits, such as paraparesis, hypertonia, and rigidity, has been reported in some cases (Caldeira Araújo et al. 2005).

GAMT gene analysis revealed a compound heterozygosity of two novel mutations: c.391G>C splice donor site of exon 3 and c.578A>G, p.Gln193Arg in exon 6. The former led to two abnormal transcripts lacking exon 3, resulting in a premature stop codon. Reverse transcription polymerase chain reaction detected a higher expression level of the allele with the c.578A>G mutation, which implies the degradation of mRNA from the allele with the splice site mutation by nonsense-mediated mRNA decay (Fig. 2b). Gln193Arg substitution by the latter mutation is presumed to destabilize the tertiary structure of GAMT (Komoto et al. 2002) by increasing the bulkiness and changing the neutral to a positively charged residue, as Gln193 is situated in the middle of α-helix and protrudes into this enzyme.

Making a diagnosis of GAMT deficiency is challenging, because of its nonspecific symptoms and limited access or capacity of ^{1}H-MRS. GAA assay may not be readily available. While not as specific as GAA, measurement of creatinine is helpful, as creatinine can be low in GAMT deficiency (Verhoeven et al. 2000). It should be warned that creatinine may also be low in patients with decreased muscle volume. Another caveat is that creatinine measurement by Jaffé method is not as sensitive in detecting GAMT deficiency as the enzymatic method or high-performance liquid chromatography (Verhoeven et al. 2000). Our patient showed low levels of serum creatinine as determined by enzymatic method, which directed us to the diagnosis of GAMT deficiency. The assay of creatine and creatinine is also important to detect creatine transporter 1 deficiency, another type of cerebral creatine deficiency, as the urinary creatine/creatinine ratio is elevated in this disorder (Salomons et al. 2003; Verhoeven et al. 2005). GAA is a more sensitive marker than creatine and creatinine in GAMT deficiency and arginine: glycine amidinotransferase deficiency, the other type of cerebral creatine deficiency (Verhoeven et al. 2005). Therefore, blood and urine tests of creatinine, creatine and GAA should be a part of the workup for developmental delay and/or epilepsy with unknown cause, if creatine and GAA measurements are available.

Early diagnosis is crucial to achieve a favorable outcome in GAMT deficiency. Ideally, treatment should be initiated as early as possible before the creatine pool supplied from maternal body during gestation becomes

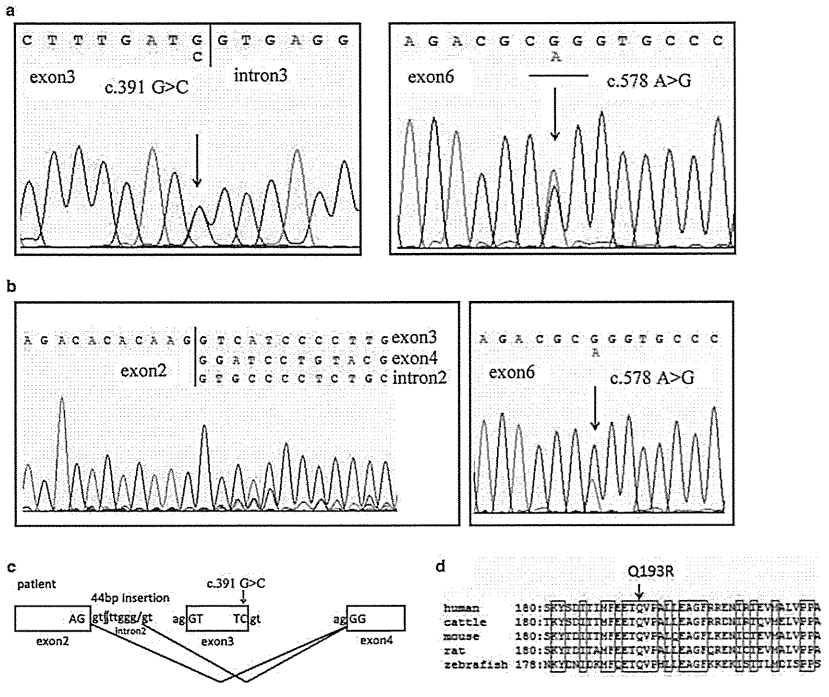

Fig. 2 Genetic analysis of the mutation in *GAMT*. (**a**) Chromatogram of genomic DNA analysis in a patient shows the heterozygote of c.391G>C (*left*) and c.578A>G (*right*). (**b**) cDNA analysis in the patient shows two aberrantly spliced transcription products (*left*) and c.578A>G (*right*). (**c**) c.391G>C mutation causes two aberrant splicing products: one with complete exon 3 (64-bp) skipping and the other involving intron 2 insertion (44-bp) followed by exon 3 skipping. (**d**) Aligned GAMT amino acid sequence of the patient with several other animals, revealing Gln193 is highly conserved among species

depleted and clinical symptoms appear. Presymptomatic treatment has been shown to be successful in achieving normal development (Schulze et al. 2006; El-Gharbawy et al. 2013). Even when diagnosed later, creatine supplementation with reduction of GAA by arginine restriction and ornithine supplementation can alleviate symptoms and prevent further progression of the disease (Schulze et al. 2001). GAMT deficiency is a good candidate for neonatal mass screening. Elevated GAA levels in neonatal blood (Schulze et al. 2006; El-Gharbawy et al. 2013) and amniotic fluid (Cheillan et al. 2006) have been reported, and validity of these tests needs to be elucidated.

In conclusion, we presented a 38-year-old patient, the first Japanese case of GAMT deficiency with two novel gene mutations. We should always include this disorder on the list of differential diagnoses when seeing patients with neurological symptoms such as intellectual disability, epilepsy, behavioral problems, and involuntary movements, since GAMT deficiency is a treatable disorder.

Take-Home Message

A 38-year-old patient, the first Japanese case of guanidinoacetate methyltransferase deficiency with two novel gene

mutations (splice site mutation and missense mutation) was reported.

Compliance with Ethics Guidelines

Contributions of Individual Authors

Tomoyuki Akiyama, Hitoshi Osaka, Hiroko Shimbo, and Tomoshi Nakajiri: Drafting/revising the manuscript for content, analysis, and interpretation of data

Katsuhiro Kobayashi, Makio Oka, Fumika Endoh, and Harumi Yoshinaga: Drafting/revising the manuscript for content

Guarantor for the Article

Tomoyuki Akiyama

Details of Funding

None

Details of Ethics Approval

This study was approved by the ethics board at Kanagawa Children's Medical Center.

Conflict of Interest

Tomoyuki Akiyama, Hitoshi Osaka, Hiroko Shimbo, Tomoshi Nakajiri, Katsuhiro Kobayashi, Makio Oka, Fumika Endoh, and Harumi Yoshinaga declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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Short Report

Genotype–phenotype correlation of contiguous gene deletions of *SLC6A8, BCAP31* and *ABCD1*

van de Kamp J.M., Errami A., Howidi M., Anselm I., Winter S., Phalin-Roque J., Osaka H., van Dooren S.J.M., Mancini G.M., Steinberg S.J., Salomons G.S. Genotype–phenotype correlation of contiguous gene deletions of *SLC6A8, BCAP31* and *ABCD1*. Clin Genet 2015: 87: 141–147. © John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, 2014

The *BCAP31* gene is located between *SLC6A8*, associated with X-linked creatine transporter deficiency, and *ABCD1*, associated with X-linked adrenoleukodystrophy. Recently, loss-of-function mutations in *BCAP31* were reported in association with severe developmental delay, deafness and dystonia. We characterized the break points in eight patients with deletions of *SLC6A8, BCAP31* and/or *ABCD1* and studied the genotype–phenotype correlations. The phenotype in patients with contiguous gene deletions involving *BCAP31* overlaps with the phenotype of isolated *BCAP31* deficiency. Only deletions involving both *BCAP31* and *ABCD1* were associated with hepatic cholestasis and death before 1 year, which might be explained by a synergistic effect. Remarkably, a patient with an isolated deletion at the 3′-end of *SLC6A8* had a similar severe phenotype as seen in *BCAP31* deficiency but without deafness. This might be caused by the disturbance of a regulatory element between *SLC6A8* and *BCAP31*.

Conflict of interest

The authors have no conflict of interest.

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Key words: clinical genetics – creatine transporter deficiency – deletion – intellectual disability – liver disease – metabolic disorders – neurology – X-linked adrenoleukodystrophy

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Loci for the genes *SLC6A8* and *ABCD1* are within a 55-kb span of Xq28. Loss-of-function mutations in *SLC6A8* are associated with X-linked creatine transporter deficiency (CRTR-D), which is characterized by severely reduced brain creatine on ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) and an increased creatine/creatinine ratio in urine. Males present with intellectual disability, severe speech delay, behavioral problems and seizures. The creatine uptake defect can be confirmed in cultured fibroblasts (1).

Loss-of-function mutations in *ABCD1* are associated with X-linked adrenoleukodystrophy (X-ALD), which is characterized by reduced β-oxidation of very long chain fatty acids (VLCFAs), demyelination of white matter and adrenal cortex atrophy. Elevated plasma VLCFA is present at birth. The phenotypic expression of *ABCD1* mutations varies widely. The most severe form, childhood cerebral X-ALD, has an onset usually after 3 years of age; it is characterized by neurological deterioration, often starting with behavioral problems and learning deficits, and later progresses to total disability and death (2).

BCAP31 is located between *SLC6A8* and *ABCD1*. It is in a head-to-head orientation with *ABCD1* and a tail-to-tail orientation with *SLC6A8*. In 2002, Corzo et al. (3) reported three male newborns with large *ABCD1* deletions that extended into *BCAP31* (*DXS1357E*). They had profound hypotonia, developmental delay, hepatic cholestasis and death prior to their first birthday. This severe neonatal presentation has never been observed in isolated *ABCD1* defects. The extent of the contiguous gene deletions was not determined in all the three boys, but the patient with the smallest deletion was characterized and showed that the critical region included the 5′ coding exons of *BCAP31* and *ABCD1*. The syndrome was named 'contiguous *ABCD1 DXS1357E* deletion syndrome' (CADDS). A fourth CADDS patient with a similar phenotype has been reported; he had a large deletion spanning seven genes: *BCAP31*, *ABCD1*, *PLXNB3*, *SRPK3*, *IDH3G*, *SSR4* and *PDZD4* (4).

Large deletions involving *SLC6A8* were reported in three boys with a more severe presentation than in classic CRTR-D; they had pronounced hypotonia and developmental delay, severe failure to thrive and dystonia or choreathethoid movements (5, 6). In one patient, the deletion extended into *BCAP31* (6). However, the deletion size was not determined in the other two patients (5).

These studies suggest that the clinical phenotypes associated with *ABCD1* or *SLC6A8* deficiencies were exacerbated by concomitant knockout of *BCAP31*. Just recently, isolated loss-of-function mutations in *BCAP31* were reported in association with a severe phenotype combining deafness, dystonia and cerebral hypomyelination (DDCH, MIM 300475) (7). Conclusions regarding the contribution of the separate genes in contiguous gene deletions involving *SLC6A8*, *BCAP31* and/or *ABCD1* were hampered by the fact that the deletion size was not determined in all the seven reported patients (3, 5). We characterized the break points in five patients and provide an update of the patients who were alive at the time of the previous report (5, 6). In addition, we describe two new patients with a CADDS and one patient with an isolated partial *SLC6A8* deletion. We discuss the genotype–phenotype correlations in all the 10 patients.

Materials and methods

Materials and patients

DNA was isolated from blood or cultured fibroblasts of eight patients with suspected large gene deletions of *SLC6A8* and/or *ABCD1*. Three patients were suspected of *SLC6A8* deletions and five patients of *ABCD1* deletions, based on clinical and biochemical features and the absence of polymerase chain reaction (PCR) products of the involved gene. Case reports of two patients with *SLC6A8* deletions (5, 8) and three patients with CADDS (3) were previously reported. In addition, the genotype and phenotype of two previously reported contiguous gene deletion patients (4, 6) were reviewed (patients 9 and 10).

Break point analysis

Multiplex ligation-dependent probe amplification (MLPA) using the P049 kit with probes for several exons of *SLC6A8*, *BCAP31*, *ABCD1* and neighboring genes was performed to confirm the deletions and to estimate their size. To narrow down the regions of the break point, PCRs of about 200 bp in intervals of ~5–10 kb were designed flanking the deleted MLPA probes. Finally, long-range PCR over the break point was performed followed by DNA sequencing to reveal the exact break points. All primers were designed with a high specificity for the X-chromosome, as a paralogous gene region occurs on chromosome 16.

RNA analysis of *BCAP31*

RNA was isolated from the available fibroblasts of patients 2–6 and 9. Subsequently, cDNA was synthesized using oligodT. In order to study whether the deletions resulted in truncated transcripts, we amplified specific regions of the *BCAP31* transcript (i.e. exons 1–8, 1–4 and 5–8) using specific reverse transcription polymerase chain reaction (RT-PCR) primers.

Results

Break point analysis

The break points were sequenced by long-range PCR in seven patients (Appendix S1, Supporting information). Although long-range PCR was unsuccessful in eighth patient (patient 6), MLPA and locus-specific PCR analyses narrowed down the break point sites to between exons 5 and 8 in *BCAP31* and between exons 7 and 8 in *ABCD1*. In total, of the 10 patients reported here, 2 had isolated partial *SLC6A8* deletions and 8

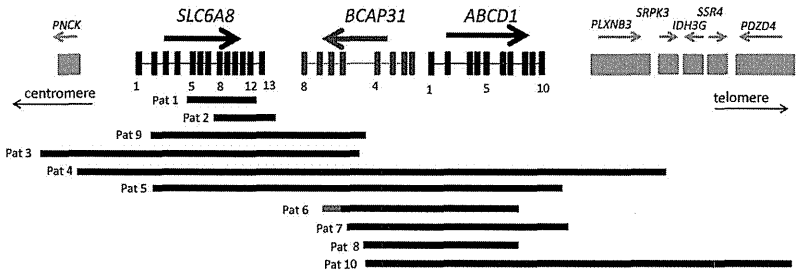

Fig. 1. Location and size of the deletions on Xq28. Deletions are depicted with black bars. The exact break point in patient 6 is unknown and the uncertainty of the involvement of *BCAP31* exons 6 and 7 in the deletion is depicted by a gray bar.

had a contiguous gene deletion involving *BCAP31* and *SLC6A8* and/or *ABCD1* (Fig. 1; Table 1).

Genotype–phenotype correlation

The clinical features of the patients are summarized in Table 1. The patients with contiguous gene deletions involving *BCAP31* ($n=8$) shared many features: profound developmental delay, severe failure to thrive, sensorineural hearing loss and childhood death. Seizures occurred in some. All the patients with deletions involving both *BCAP31* and *ABCD1* ($n=6$) developed cholestatic liver disease and died in the first year of life. It is not documented whether the cause of death was related to liver failure in all cases. By contrast, patients with deletions of *SLC6A8* and *BCAP31* but not *ABCD1* ($n=2$) did not develop cholestatic liver disease, survived until at least 6 years and developed severe dystonia and choreoathetosis after 3 years. Patient 2 with an isolated deletion of exons 8–13 of *SLC6A8* also had a severe presentation with death at 8 years, but without sensorineural hearing loss. By contrast, patient 1 with an isolated deletion of exons 5–12 of *SLC6A8* had a phenotype consistent with classic CRTR-D.

RNA analysis of *BCAP31*

RT-PCR confirmed the absence of *BCAP31* transcripts in patients 4–6. A truncated transcript of exons 1–4 was present in patients 3 and 9. In patient 2, a full-length *BCAP31* transcript was detected (Appendix S1, Supporting information). Because patient 2 had a severe phenotype that suggested BCAP31 deficiency, the open reading frame and splice sites of *BCAP31* gDNA were additionally sequenced; no pathogenic mutation was identified.

Discussion

The phenotype of patients with contiguous gene deletions involving *BCAP31* was more severe, overall, than the isolated defects of *SLC6A8* (causing CRTR-D) or *ABCD1* (causing X-ALD); this suggests an important role for *BCAP31* in patients harboring these contiguous gene deletions. *BCAP31* encodes B-cell-receptor-associated protein 31 (BAP31), an integral membrane protein that is localized in the endoplasmic reticulum (ER) membrane (9). It is a protein-sorting factor that controls the fates (egress, retention, survival and degradation) of newly synthesized integral membrane proteins (10). However, BAP31 is also involved in apoptosis, participating in ER–mitochondrial apoptosis signaling. The mitochondrial fission protein Fission 1 (Fis1) interacts with BAP31 at the ER, forming a platform for recruitment and activation of procaspase-8 during Fas-mediated apoptosis (11). BAP31 is cleaved by caspase-8, generating p20 that remains integrated in the membrane (9, 12). p20 induces apoptosis (9) by causing a rapid transfer of ER calcium into the mitochondria, which leads to mitochondrial recruitment of dynamin-like protein 1 (Dlp1) and mitochondrial fission (12). By contrast, full-length BAP31 inhibits Fas-mediated apoptosis (13). BAP31 also associates with the components of the cytoskeleton actomyosin complex, suggesting that BAP31 may play a role in the structural organization of the cytoplasm (14).

Recently, loss-of-function mutations in *BCAP31* were found in seven individuals from three families presenting with severe motor and intellectual disability, dystonia, sensorineural deafness, hypomyelination, failure to thrive and early death. Fibroblasts of affected individuals showed altered ER morphology and disorganized Golgi; however, contrary to expectation, there was not an excessive accumulation of unfolded proteins or exacerbated cell death (7). The profound developmental delay, sensorineural hearing loss, failure to thrive and childhood death in the patients with contiguous gene deletions involving *BCAP31* are very similar to the isolated *BCAP31* defects and confirm the association of loss of *BCAP31* with this phenotype.

Neonatal hepatic cholestasis leading to liver failure and death in the first year was restricted to the patients with deletions involving both *ABCD1* and *BCAP31* and

Table 1. Clinical features. Patient order is based on the location of the deletion (from centromeric to telomeric)

Patient	1	2	9	3	4	5	6	7	8	10	CRTR-D	BCAP31	X-ALD
Deletion size Involved genes	2.1 kb *SLC6A8*	4.9 kb *SLC6A8*	19 kb *SLC6A8, BCAP31*	40 kb *PNCK, SLC6A8, BCAP31*	110 kb *PNCK, SLC6A8, BCAP31, ABCD1, PLXNB3, SRPK3*	64 kb *SLC6A8, BCAP31, ABCD1*	34–42 kb *BCAP31, ABCD1*	50 kb *BCAP31, ABCD1*	31 kb *BCAP31, ABCD1*	90 kb *BCAP31, ABCD1, PLXNB3, SRPK3, IDH3G, SSR4, PDZD4*	Isolated defect *SLC6A8*	Isolated defect *BCAP31*	Isolated defect *ABCD1*
Age	40 years	Died 8 years, septic shock	9 years	Died 8 years, unknown cause	Died <5 months	Died 4 months, LF, HF	Died 8 months	Died 11 months, LF, GI bleeding	Died 4 months, RF, GI bleeding	Died 6 months, pneumonia, sepsis	Normal life expectancy	Death, 7 months –24 years[a]	Average death at 9.4 years
Development	Walking at 2 years, speaks single words	Smiles, eye contact, no milestones attained	Profound delay, no head control	Some eye contact, no milestones attained	?	Profound delay	Delayed, smiles, alert and active at 4 mo, sedated at 7 months	Profound delay	Profound delay	No milestones attained	Mild-severe delay, walking at mean age of 2 years	No milestones attained or only head control[a]	Early development normal, onset neurological deterioration usually >3 years
Motor symptoms	–	Profound axial hypotonia, hypertonic limbs, quadriplegia	Hypertonic	Profound neonatal hypotonia	Hypertonic	Profound neonatal hypotonia	Hypotonia	Profound neonatal hypotonia	Profound neonatal hypotonia	Hypotonia	Mild hypotonia	Pyramidal signs, quadriplegia	Neurological deterioration >5 years
Extrapyramidal	–	Dystonia from 3 months; severe choreoathetosis from 4–5 years	Severe dystonia and athetosis from 4 months	Severe choreoathetosis from 3 years	?	–	–	Frequent episodes of opisthotonus, bruxism	–	–	Mild choreoathetosis or dystonia in some patients	Severe dystonia	–
Seizures (onset)	2 years	–	4 years status	4 years	?	2 months	–	–	2 months	–	–	+/–	+/–
FTT (height and weight)	–	–3 to –4 SD	–3 to –4 SD	–3 SD	?	++, IUGR	–3 to –4 SD	++	++	–6 SD, IUGR	+/–	–2 to –8 SD, IUGR	–
Head circumference	0 SD	–3 SD	?	–2.5 SD	?	?	–3 SD	?	?	–10 SD	Normal	–2 to –5 SD	Normal
Hepatic	–	–	Mildly elevated liver transaminases	Transient elevated liver transaminases	Cholestasis	Cholestasis	Cholestasis	Cholestasis	Cholestasis	Cholestasis	–	Transient elevated liver transaminases	–
Congenital SNHL	–[b]	–[c]	+	+	+	?	?	+	+	+	–	+	–
Ophthalmological	–	Strabismus	Does not peruse objects	Pigmentary retinopathy at 3 years	?	?	–	Cataract	?	Blind	Strabismus	Strabismus, optic atrophy	–
Dysmorphic	Mild	–	–	+[d]	?	–	Mild[d]	–	–	+[d]	Mild atypical	+/–	–