25% に自閉症がみられることもよく知られている．染色体異常部位にある遺伝子の異常が自閉症と関係していると推測され候補遺伝子として研究が進められている(後述)．ゲノムスクリーンの報告は 1998 年より始まりこれまでに約 10 の報告がある．22 の常染色体のうち 17 の染色体上に候補部位が認められている．それらの報告のうち可能性の高いとされる候補部位について図 2 に示したが，研究結果は必ずしも一致していない．その理由は，診断基準の差，解析方法の差，マーカーの差，サンプル数の差があげられる．その中で最も注目されているのは，第 7 染色体長腕，そして 15 番長腕，3 番長腕である．自閉症遺伝子の可能性のある遺伝子として AUTS 1(15 q，7 q?)，AUTS 2(7 q)，AUTS 3(3 q)が想定されている．

候補遺伝子の研究はここ 5 年間急速に増えさらに今後も増えていくと思われる．候補遺伝子の選択には以下のような事柄を参考にしている．①染色体異常を有する自閉症例で異常のある染色体の切断部位や重複部位にある遺伝子，②症候性自閉症でみつかった異常遺伝子，③自閉症の生物学的特徴，④その遺伝子欠損動物の症状，などである．①の方法の例として図 3 に 15 番染色体における自閉症の染色体異常と候補遺伝子の例をあげた．自閉症でみられた 15 番染色体の異常は逆位，重複，欠失などあるが，どれも長腕の 11-13 に集中しており，この部位にある遺伝子について調べられている．特に神経伝達物質の GABA 関連の受容体遺伝子である GABRB 3 では，1998 年にあるマーカーで連鎖不均衡がみられたとの報告があり，1999 年にはこれを否定するあるいは判断できないとの報告が続き，また 2000 年にははじめの報告の部位は関係なかったが，それより少し離れた部位で連鎖不均衡がみられたなどと研究者により結果が異なりまだ結論が出ていない状態である．③の方法の例として，自閉症ではセロトニンの血中値の高い症例があり，セロトニン再取り込み阻害剤の効く症例がみられることから，5-HTT を候補遺伝子とする研究がなされた．1997 年にはプロモーター部位で 44 塩基

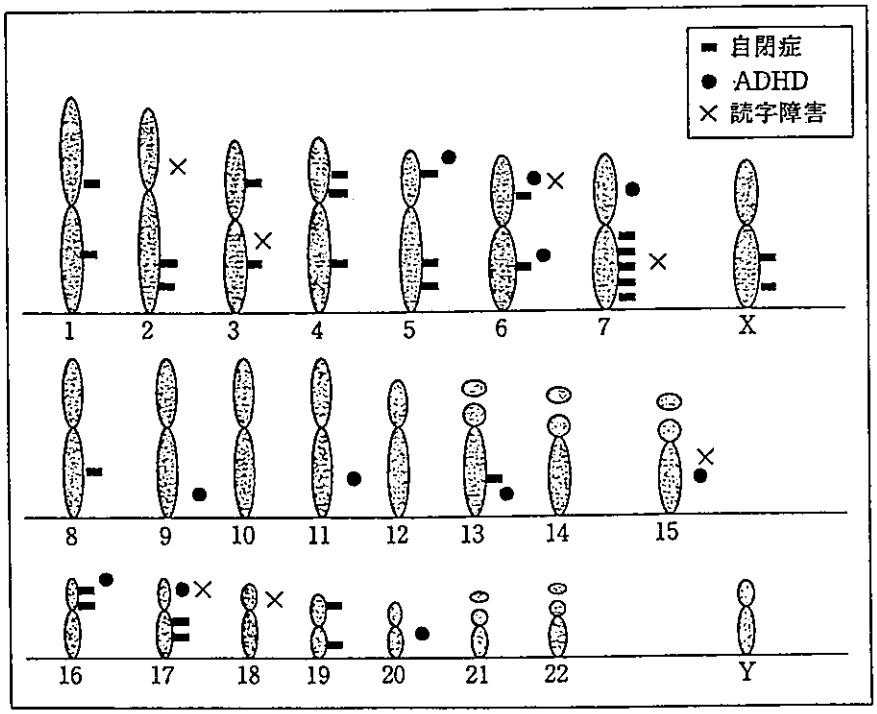


図 2　ゲノムスクリーン　候補マーカー部位

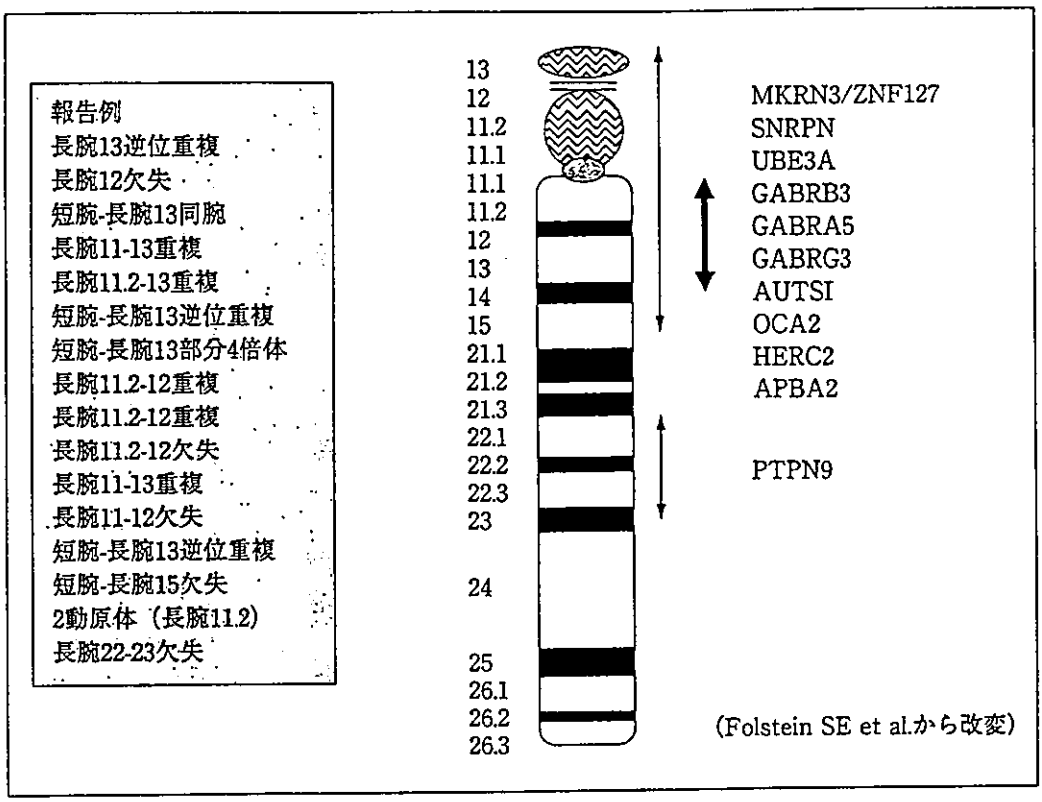


**図3 第15番染色体における自閉症の染色体異常と候補部位**

分長さが短い方(s)が自閉症と関係あり，とする報告と逆に長い方(l)が関係するという報告が出され，2000年にはどちらも関係しないとの報告がだされ3つに分かれている．この多型は日本人と欧米人で人種差があり，lとsの比が正常欧米人では6対4に対して正常日本人では1対7で長いヒト割合が少ない．われわれが調べたのでは日本人の自閉症は正常と差はなかった．ゲノムスクリーンの結果が報告されたあとからは，候補マーカーの近くにある遺伝子の中で脳発達に機能をもつ遺伝子が候補として選ばれるようになった．図4にゲノムスクリーンで最も報告の多かった7番染色体における自閉症の染色体異常と，候補マーカーあるいは候補遺伝子をあげた．染色体異常部位は15番とは異なり全体にわたっているが，ゲノムスクリーンでは長腕に集中している．この中のWNT2という遺伝子は脳を含む多くの器官の発達に影響を与え，この欠損マウスは社会性行動に問題がみられることから候補遺伝子として研究された．自閉症ではWNT2遺伝子の翻訳領域で正常ではみられないある変異が2例と別の変異が1例にみられ，非翻訳領域の多型の1つに連鎖不均衡がみられたことから，このWNT2の変異が自閉症の罹患性を増加させる可能性があるとしている．自閉症と関係するとされる環境因子は母親の甲状腺機能低下，先天性甲状腺機能低下，母親のサリドマイド内服，母親の飲酒，先天性サイトメガロ感染症，先天性風疹症候群が知られている．サリドマイドでは受精後20～24日(ちょうど神経管が造られ，中枢神経が複数の菱形部に分かれる時期)の内服の場合にみられているが，これはサリドマイドが直接この時期の脳に作用し発病に関わっているのか，あるいはこのころ発現している感受性遺伝子の一つに作用することにより発病に影響しているのかわかっていない．図5に自閉症の遺伝についての考え方をまとめてみた．自閉

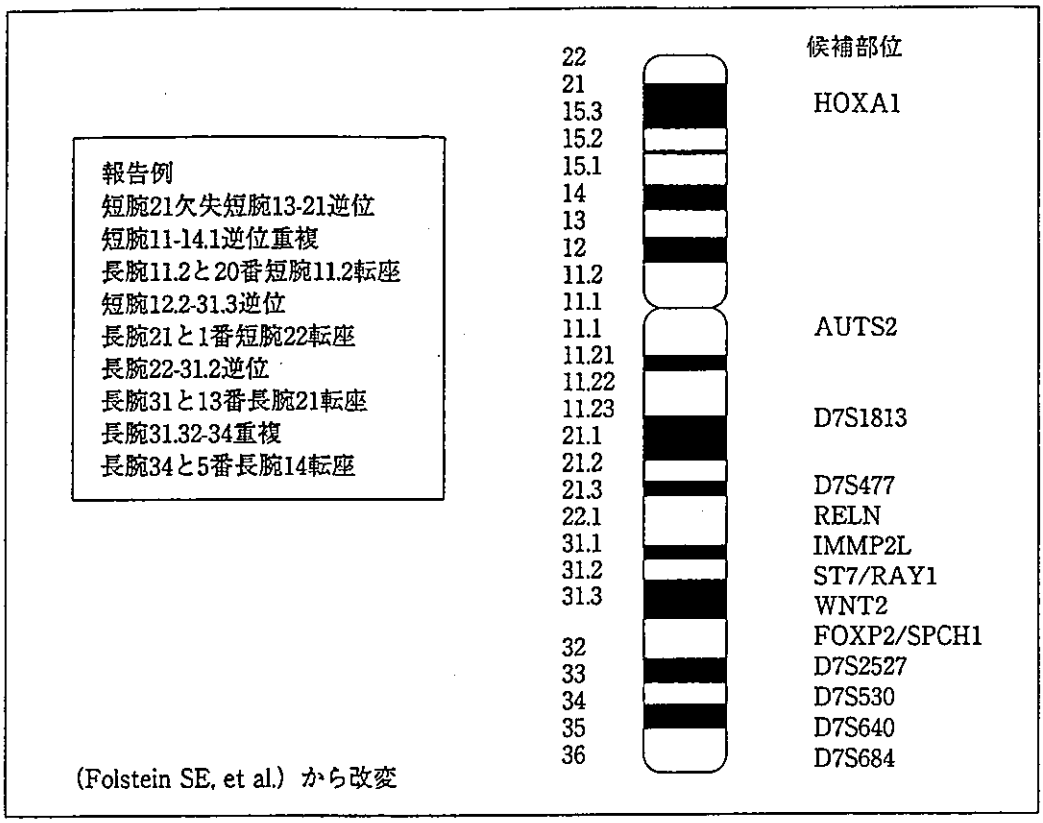


**図4　第7番染色体における自閉症の染色体異常と候補部位**

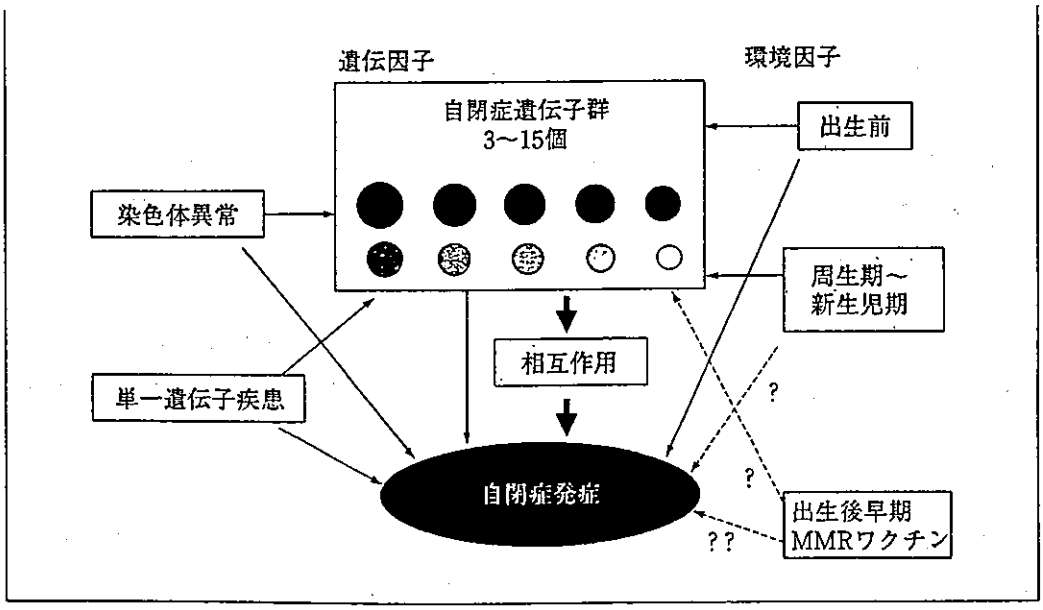


**図5　自閉症発症の関与因子**

症感受性遺伝子としては3〜15個が想定され，それぞれ自閉症の発病に影響を与える程度に強弱があるであろう．なかには自閉症にみられた染色体異常の部位に含まれている遺伝子があるかもしれないし，自閉症を合併する単一遺伝子疾患と関連する遺伝子があるかもしれない．また環境因子が直接発症に影響するのか遺伝子に働いてそこから影響するのかわかっていない．

われわれは5-HT 2 AR の多型 102 T/T では他の2つの多型(T/C, C/C)に比べて新生児期早期に軽度でも異常があると自閉症の発病が増加する印象をもっているが推測段階である．おそらくこれら感受性遺伝子が相互作用していると考えられているが，どのような相互作用のもとに発病するのかもわかっていない．以上のように自閉症の遺伝研究は候補遺伝子の絞り込みが盛んに行われ確実に進んでいるのは間違いないが，現状はまだ非常に混沌とした状態にある．

## レット症候群

広汎性発達障害の一項目に分類されており，女児に特有で乳幼児期より退行がみられ，小頭症，歩行障害，対人関係障害，常同運動の手もみが特徴である．この責任遺伝子は1999年にMECP 2 という遺伝子であることがつきとめられた．MECP 2 は染色体 Xq 28 にあり，これはメチル化されたDNAに特異的に結合する蛋白をコードする遺伝子で成熟した神経系の正常機能に必要な蛋白質とされている．ただしこの疾患ほとんどが弧発例であり，突然変異でMECP 2 に異常が起こり発病するが，家族歴をもちいわゆる遺伝するという形はまれである．

## 精神遅滞

精神遅滞の頻度は1～3%とされている．精神遅滞のみが主症状となる場合(非症候性)と染色体異常や，さまざまな症候群の症状となっている場合(症候性)があり，原因が判明しているものは20～25%とされている．精神遅滞の遺伝は自閉症とは異なり，その原因がさらに広く異質性が強いためまとめて述べるのは困難である．しかしX染色体連鎖性精神遅滞(XLMR)とよばれて注目されている一群がある．精神遅滞は男子が20～30%多いのは一部はXLMRによると考えられている．非症候性XLMRの原因遺伝子は今のところ11個(AGTR 2, ARHGEF 6, ARX, FACL 4, FMR 2, GDI 1, IL 1 RAPL 1, OPHN 1, PAK 3, TM 4 SF 2, VCX-A)みつかっているが，これらはいずれも発達期の脳神経細胞の形成，成長，そして成長後も神経シナプスの機能に深く関わっている遺伝子とされている．

## 言語発達障害

言語発達障害の頻度は3～6%とされる．家族性の会話言語障害の一家系として三代にわたる大きな家系(KE)についてはその原因遺伝子が 7 q 31 にある FOXP 2 であることがつきとめられた．そのきっかけとなったのはその家系とはまったく無関係だが同じ症状の言語会話障害をもつ別の一人のヒトに染色体異常があり，その異常の転座断端にある遺伝子を候補としてKE家系のメンバーを調べていき，症状のあるメンバーに認められ，症状のないメンバーに認められない遺伝子異常をみつけ出し，さらにこの異常が他の正常のヒトではみられないことを確認している．この遺伝子は胎児発生に重要な作用をもつとされている．

## LD(学習障害)

LDは4～14%と高頻度(ただし日本では1～2%)にみられ，読字障害，算数障害，書字表出障害に大きく分けられている．読字障害はLDの80%にみられるが，遺伝的研究は主にこの読字障害についてなされている．家族研究では読字障害の兄弟の40%，親の27～49%，子の23～65%が読字障害と診断されている．連鎖研究では染色体 2 p, 3, 6 p, 7 q, 15, 17 p, 18 p (図2)が候補にあげられ，特に 6 p は注目されている．候補遺伝子は SPCH 1, FOXP 2, DYX 2, DYX 3 があげられている．

## AD/HD(注意欠陥/多動障害)

AD/HDの頻度は3～10%と多く，学校生活，

社会生活上多くの課題をもっている．AD/HDの家族研究では一卵性一致率55～92%，兄弟一致率25～35%，父親との一致率15～30%，母親との一致率15～20%とされている．ゲノムスクリーンでは5p，6p，6q，7p，13q，16p1，17p11，20q(図2)が候補部位として報告されている．候補遺伝子としては神経伝達物質に関心が強く，ドーパミン転送拮抗薬であるメチルフェニデートの有効例の多いことからドーパミン関連遺伝子が注目されている．なかでもDAT1は1995年関係ありと報告されてから，毎年数件ずつの報告があるが，関係あるなしが約半々の状態である．DRD4の第3エクソンの48塩基の繰り返し回数の多型で7回が関係ありとする報告があり，その後20を超える報告があるが結果はこれも関係あるなしが半々に分かれている．その他のドーパミン受容体や他の神経伝達物質についての報告も多々あるがいまのところ決定的なものはみつかっていない．

## おわりに

以上発達障害に関する遺伝について現在まで判明していることの一部について述べた．異なる発達障害においてゲノムスクリーン法の候補部位が共通している部位があり，(自閉症とAD/HDで5p13，16p13，17p13，自閉症と読字障害で7q31，AD/HDと読字障害で6p21，6q12)，これらの発達障害が共有する症状と関連があるのかどうか，今後症状の絞込みとさらに細かいマーカー(SNP)の検索へと研究はすすめられていくと思われる．

## 参考文献

1) Folstein SE and Rosen-Sheidley B. Genetics of autism：Complex aetiology for a heterogeneous disorder. Nature Reviews Genetics 2001；2：943-955.
2) 中村祐輔．先端のゲノム医学を知る．羊土社．2001.

*Pediatrics International* (2004) **46**, 474–477

Patient Report

# Congenital form of glycogen storage disease type IV: A case report and a review of the literature

KEINCHI MARUYAMA,[2] TOMOKO SUZUKI,[2] TAKENOBU KOIZUMI,[2] HIDEO SUGIE,[1] TOKIKO FUKUDA,[1] MASATAKA ITO[1] AND JUNKO HIRATO[3]

[1]*Department of Pediatric Neurology, Hamamatsu City Medical Center for Developmental Medicine, Hamamatsu,* [2]*Department of Neonatology, Gunma Children's Medical Center, Gunma, and* [3]*First Department of Pathology, Gunma University School of Medicine, Gunma, Japan*

**Key words** glycogen storage disease type IV, hydrops fetalis, respiratory distress.

Glycogen storage disease (GSD) type IV is a very rare autosomal recessive disorder, which is caused by deficiency of alpha-1,4-glucan:alpha-1,4-glucan 6-glycosyl transferase, also known as the branching enzyme. The most common clinical feature is hepatosplenomegaly in the infantile period, and progressive liver cirrhosis results in death by 5 years of age. The disease, however, has been revealed to include three other clinical subtypes: congenital subtype, which is characterized by severe neonatal hypotonia or fetal death; childhood subtype, which starts between 2 and 7 years of age with cardiomyopathy; and adult subtype, in which adult onset myopathy is predominant.[1] Among the four clinical subtypes, the congenital one is the most severe variant of the disease and only a few cases have been reported.[1–9] Here we report additional cases, in whom the clinical and pathological findings were consistent with the congenital form of GSD type IV and the diagnosis was made enzymologically.

## Case reports

### *Case 1*

A boy was delivered to a 36-year-old G1P1 mother by cesarean section at 37 weeks and 4 days' gestation because she had a history of cesarean section. His birthweight was 2616 g and the Apgar scores were 2 and 3 at 1 and 5 min after birth. The family history was unremarkable. Since about 2 weeks before the delivery, the mother had had reduced fetal movement and polyhydramnios. Shortly after birth he was endotracheally intubated. When he was admitted to Gunma Children's Medical Center, Gunma, Japan, at 1 h after birth, severe hypotonia was seen, and X-ray examination revealed cardiomegaly. The serum aspartate aminotransferase (ASAT) was 120 IU/L; alanine aminotransferase (ALAT) was 25 IU/L; lactic acid dehydrogenase (LDH) was 1617 IU/L; alkaline phosphatase (ALP) was 782 IU/L; and creatine phosphokinase (CPK) was 2154 IU/L. Blood gas analysis revealed pH, 7.39; $PaO_2$, 421.1 mmHg; $PaCO_2$, 22.3 mmHg; and base excess, −11.0 mmol/L. Although he was treated with mechanical ventilation and inotropic drugs, he had had bradycardia since 7 h after birth and he died at 14 h after birth. Pathological findings showed that the hepatocytes, cardiomyocytes and skeletal muscle cells contained slightly basophilic masses which were periodic acid-Schiff (PAS)-positive and diastase-resistant. In addition, the heart included cardiomyocytes with vacuoles. In the vacuoles, there were PAS-positive granules, which were also observed in the hepatocytes, hepatic histiocytes and nerve cells of the brain stem nuclei, thalamus and cerebellum (Fig. 1). As accompanying lesions, he had multiple thrombosis in the lungs and hemorrhagic lesions in the subdural and subarachnoidal space, lungs, gastrointestinal tract, heart, kidneys and testes, and these findings suggested that he had disseminated intravascular coagulation. Biochemical analysis of branching enzyme with a frozen and preserved autopsy specimen of the liver revealed that activity of the enzyme was 0.1 Pi mg/min per mg (normal controls, 14.7 and 11.6 Pi mg/min per mg).

### *Case 2*

Two years after the birth of Case 1, his younger sister was born by cesarean section at 34 weeks and 6 days' gestation, weighing 2544 g. The Apgar scores were 2 and 4 at 1 and 5 min after birth. The mother had had polyhydramnios since 29 weeks' gestation, and ultrasonography revealed fetal

Correspondence: Kenichi Maruyama, Department of Neonatology, Gunma Children's Medical Center, 779 Shimohakoda, Hokkitsu, Gunma 377-8577, Japan. Email: maruken@gcmc.pref.gunma.jp

Received 22 July 2003; revised 17 November 2003; accepted 22 December 2003.

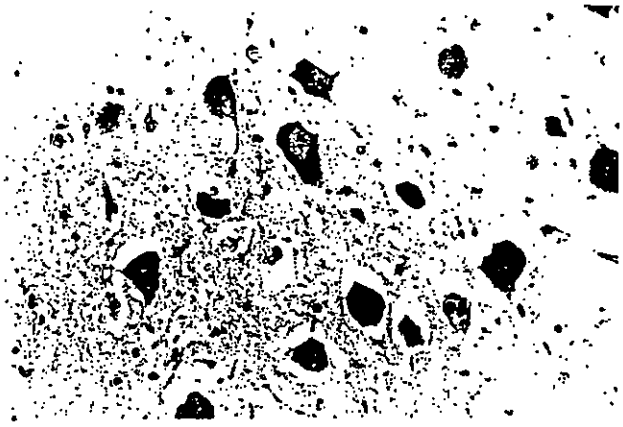

Fig. 1 Medulla oblongata of Case 1. Periodic acid Schiff (PAS)-positive granular deposits are seen in the cytoplasm of neuronal cells (PAS, ×400).

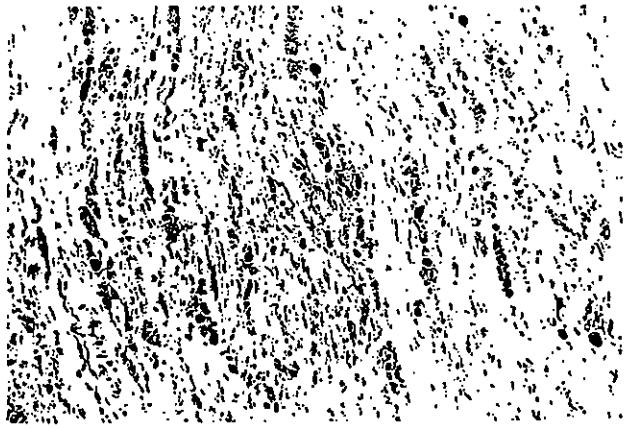

Fig. 2 Iliopsoas muscle of Case 2. Various sizes and shapes of Periodic acid Schiff (PAS)-positive substances are observed in the myocytes (PAS, ×200).

hydrothorax at 34 weeks' gestation. At birth, the patient had subcutaneous edema, hypotonia, poor spontaneous respiration and response to very painful stimuli. An X-ray showed decreased aeration in both lungs and right-sided hydrothorax. The serum ASAT was 70 IU/L; ALAT, 19 IU/L; LDH, 1298 IU/L; ALP, 600 IU/L; and CPK, 1474 IU/L. Mechanical ventilation and continuous chest drainage were commenced. Cells in the pleural fluid consisted of mononuclear cells. Because the effusion was reduced, enteral feeding started at 3 days of age. At 7 days of age, chylous effusion appeared in the right thoracic cavity again, and an X-ray showed both-sided pleural effusion at 10 days of age. She needed chest drainage until 25 days of age. Although her patent ductus arteriosus became symptomatic at 36 days of age, it was successfully treated with indomethacin. Joint contracture of the extremities had been apparent since the second week of life. Since Case 1 was diagnosed as having GSD type IV, we analyzed her red blood cells yielded at 38 days of age, and very low activity of the branching enzyme was seen (the patient, 0.05 μmole Pi/min per gHb; and controls, 0.9–1.8 μmole Pi/min per gHb). At 82 days of age, serum C-reactive protein turned to positive, and blood culture at 87 days of age revealed *S. epidermidis*. She died at 89 days of age. Pathological examination revealed PAS-positive deposits in the heart, liver, skeletal muscle, and central nervous system, including the brain, brain stem and cerebellum (Fig. 2). Additionally, fibrously thickened endocardium, and hypertrophied cardiomyocytes in the middle layer of the myocardium were found. Myofibers of the skeletal muscles were degenerated and reduced. She also had findings suggesting infection, such as hyperplasia of the granulocytes in the bone marrow. Branching enzyme activity in the quadriceps muscle, liver and brain, which were harvested at autopsy, was all 0 nmole Pi/min per mg (control muscles, 55.9–80.2 nmole Pi/min/mg; control livers, 107.2–150.3 nmole Pi/min per mg; and control brains, 1.2–3.1 nmole Pi/min per mg).

## Discussion

The congenital form of GSD type IV develops with severe symptoms during the fetal or neonatal period. In a literature search, we could find 12 cases of the disease[1–9] and the characteristics of the cases are shown in Table 1. Four previous cases had polyhydramnios during the fetal period.[1,5,6] Diminished fetal movement was documented in two cases.[6,9] Hydrops fetalis was seen in four cases, of which three had artificial termination of pregnancy.[8,9] Two of the reported cases died *in utero*.[1] Both our cases had polyhydramnios. Case 2 also presented hydrops fetalis, which had been suspected due to fetal cardiac failure caused by the metabolic disease before birth, but the cause of the fetal abnormality was postnatally elucidated to be due to chylothorax.

In the live born cases, the main clinical features are respiratory or circulatory distress and hypotonia.[1–3,6–8] Four of the cases had arthrogryposis[1–3,7,8] and three patients had hypoplastic lungs,[1,5,6] which could cause respiratory distress. These two abnormalities were thought to be secondary to the neuromuscular disorder. Four of the six patients whose outcome was described, died within 1 year after birth.[1,4,6,8] Case 1, who had hypotonia and severe neonatal asphyxia, died at 14 h after birth. In Case 2, mechanical ventilation was needed for life because of severe respiratory insufficiency due to severe hypotonia. She also had joint contracture, and her autopsy showed cardiac wall thickening, which was seen in one previously reported case.[6]

Although the prenatal or postnatal findings, such as hydrops fetalis, respiratory distress and hypotonia, were common to

**Table 1** Cases of congenital form of glycogen storage disease type IV

| | Schochet[2] (1970) Zellweger[3] (1972) | Shin[4] (1988) | Benirschke[5] (1990) | van Noort[1] (1993) | van Noort[1] (1993) | van Noort[1] (1993) | Tang[6] (1994) | Di Rocco[7] (1998) | Alegria[8] (1999) | Cox[9] (1999) | Cox[9] (1999) | Cox[9] (1999) | Case 1 | Case 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | Female | | | Female | Female | Male | Male | Male | Female | Female | | | Male | Female |
| Gestational age (week) | | | | 36 | 38 | 36 | 34 | | 34 | | | | 37 | 34 |
| Birth weight (g) | 2610 | | | 2200 | 2416 | 1730 | 2319 | | | | | | 2616 | 2544 |
| Prenatal findings | | | | | | | | | | | | | | |
| Polyhydramnios | | | + | | + | + | + | | | | | | + | + |
| Hydrops fetalis | | | | | | | | | + | + | + | + | | + |
| Diminishing fetal movement | | | | | | | + | | | + | | | + | |
| Intrauterine growth retardation | | | | + | + | + | | | | | | | | |
| Clinical/pathological findings | | | | | | | | | | | | | | |
| Respiratory/circulatory distress† | | | | | + | | + | | + | | | | + | + |
| Hypotonia | + | | | | | | + | + | + | | | | + | + |
| Hypoplastic lungs | | | + | + | + | + | + | | | + | | | | |
| Cardiac wall thickening | | | | | | | + | | | | | | | + |
| Arthrogryposis | + | | | | + | | | + | + | + | | | | + |
| PAS-positive deposit | L,M,H,C | | L,M,H | L,M,H,C | L,M,H,C | L,M,H,C | L,M,H,C | L,M | L,M,H,C | M | M,E,I | M,E,I | L,M,H,C | L,M,H,C |
| Low branching enzyme activity | L,W,F | R | | | | | F,M | F | F | | | F | L | L,R,M,C |
| Branching enzyme gene mutation | | | | | | | + | | | | | | | |
| Outcome | Died | Died | | IUFD | Died | IUFD | Died | Alive | Died | ATP | ATP | ATP | Died | Died |
| Age | 28 months | 0 day | | | 0 day | | 4 weeks | 1 year | 3 days | | | | 14 hours | 89 days |

†Cases with intensive therapy such as mechanical ventilation or circulatory support. L, liver; M, muscle; H, heart; C, central nervous system; E, epidermis; I, intestine; W, white blood cell; F, fibroblast; R, red blood cell; IUFD, intrauterine fetal death; ATP, artificial termination of pregnancy.

several cases, they were not specific to the disease. Therefore pathological or enzymological examination is necessary for a diagnosis. Characteristic pathological findings of GSD type IV are deposition of basophilic substances in the organs involved. These substances are PAS-positive, but they are not digested by diastase. All of the reported cases in whom pathological findings were documented had these deposits in muscle.[1–3,5–9] Abnormality in the central nervous system, liver or heart was proven in more than half of the pathologically examined cases.[1–3,5–8] In our two cases, PAS-positive deposits were found in the muscle, central nervous system, liver and heart.

Measurement of the branching enzyme activity was performed in six reported cases: fibroblasts were used for the measurement in three cases; fibroblasts, liver and white blood cells in one; fibroblasts and muscle in one; and red blood cells in one.[2–4,6–9] In Case 1, we harvested a liver specimen on postmortem examination and proved lowered branching enzyme activity. Case 2 had low activity of the enzyme in the red blood cells, liver, muscle and brain.

The etiology of heterogeneity of clinical features in GSD type IV is unclear. Van Noort *et al.* attempted to explain it by pathological and enzymological findings.[1] Difference in the distribution of the organs involved due to the clinical subtypes was found pathologically. But the difference could not be proven enzymologically because in most cases limited organs were used for measurement of the branching enzyme activity. In Case 2, we could examine the main organs involved and found correspondence between pathological and enzymological findings. Another approach to the etiology is genetical analysis. According to a report on the genetical analysis of the branching enzyme in GSD type IV, 3 and 2 point mutations were detected in two patients with the classical form and one with the non-progressive hepatic form, respectively, and a patient with the congenital form had a large defect representing the loss of one full exon.[10] In our cases, the parents did not want to have genetic counseling, and we could not obtain parental consent to the genetical analysis. In order to elucidate the mechanism causing the clinical variation in GSD type IV, the relationship between pathological, enzymological and genetical findings should be investigated in further cases.

## References


1 van Noort G, Straks W, Van Diggelen OP, Hennekam RCM. Congenital variant of glycogenosis type IV. *Pediatr. Pathol.* 1993; **13**: 685–98.

2 Schochet SS Jr, McCormick WF, Zellweger H. Type IV glycogenosis (amylopectinosis). Light and electron microscopic observations. *Arch. Pathol.* 1970; **90**: 354–63.

3 Zellweger H, Mueller S, Ionasescu V, Schochet SS, McCormick WF. Glycogenosis type IV: A new cause of infantile hypotonia. *J. Pediatr.* 1972; **80**: 842–4.

4 Shin YS, Steigueber H, Klemm P, Endres W, Schwab O, Wolff G. Branching enzyme in erythrocytes. Detection of type IV glycogenosis homozygotes and heterozygotes. *J. Inher. Metab. Dis.* 1988; **11**: 252–4.

5 Benirschke K, Kaufmann P. Fetal storage disease. In: *Pathology of the Human Placenta*, 2nd edn. Springer-Verlag, New York, 1990; 460–3.

6 Tang TT, Segura AD, Chen YT *et al.* Neonatal hypotonia and cardiomyopathy secondary to type IV glycogenosis. *Acta Neuropathol.* 1994; **87**: 531–6.

7 Di Rocco M, Doria-Lamba L, Marino C *et al.* Atypical congenital form of glycogen storage disease (GSD) type IV. *J. Inher. Metab. Dis.* 1998; **21** (Suppl. 2): 95.

8 Alegria A, Martins E, Dias M, Cunha A, Cardoso ML, Maire I. Gycogen storage disease type IV presenting as hydrops fetalis. *J. Inher. Metab. Dis.* 1999; **22**: 330–2.

9 Cox PM, Brueton LA, Murphy KW *et al.* Early-onset fetal hydrops and muscle degeneration in siblings due to a novel variant of type IV glycogenosis. *Am. J. Med. Genet.* 1999; **86**: 187–93.

10 Bao Y, Kishnani P, Wu JY, Chen YT. Hepatic and neuromuscular forms of glycogen storage disease type IV caused by mutations in the same glycogen-branching enzyme gene. *J. Clin. Invest.* 1996; **97**: 941–8.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Diabetes Research and Clinical Practice 65 (2004) 175–182
DIABETES RESEARCH AND CLINICAL PRACTICE
www.elsevier.com/locate/diabres

Case report

# Intermittent and recurrent hepatomegaly due to glycogen storage in a patient with type 1 diabetes: Genetic analysis of the liver glycogen phosphorylase gene (*PYGL*)

Masako Tomihira[a], Eiji Kawasaki[b], Hiromu Nakajima[c], Yutaka Imamura[d], Yuichi Sato[a], Michio Sata[e], Masayoshi Kage[f], Hideo Sugie[g], Kiyohide Nunoi[a,*]

[a] *Division of Endocrinology and Metabolism, St. Mary's Hospital, 422 Tsubukuhonmachi, Kurume, Fukuoka, Japan*
[b] *Unit of Metabolism/Diabetes and Clinical Nutrition, Nagasaki University School of Medicine, Nagasaki, Japan*
[c] *Department of Clinical Laboratory, Osaka Medical Center for Cancer and Cardiovascular Diseases (OMCC), Osaka, Japan*
[d] *Division of Hematology, St. Mary's Hospital, 422 Tsubukuhonmachi, Kurume, Fukuoka, Japan*
[e] *Second Department of Internal Medicine, Kurume University of School of Medicine, Fukuoka, Japan*
[f] *First Department of Pathology, Kurume University of School of Medicine, Fukuoka, Japan*
[g] *Department of Pediatric Neurology, Hamamatsu City Medical Center for Developmental Medicine, Shizuoka, Japan*

Received in revised form 12 February 2003; accepted 12 December 2003

**Abstract**

We report a 19-year-old woman who had a history of type 1 diabetes with recurrent glycogen accumulation in the liver. During her infantile period she presented with no hepatomegaly nor growth retardation. On admission she was diagnosed with diabetic ketoacidosis (DKA). She also had hepatomegaly and elevated transaminase levels, but these abnormalities had resolved after administration of insulin. However, 4 weeks after DKA marked hepatomegaly and elevated transaminases were reappeared with simultaneous hypoglycemia which suggested an impaired glycogenolysis in the extraordinary conditions. We supposed the partial deficiency of liver glycogen phosphorylase activity in this patient and analyzed the liver glycogen phosphorylase gene (*PYGL*). Deduced amino acid sequence of the PYGL in this patient was completely identical to that reported by Burwinkel et al. (Y15233), however, the nucleotide sequence of PYGL cDNA was heterozygous for substitutions at positions Asp339 (GAT to GAC) on exon 9 and Ala703 (GCT to GCC on exon 17, respectively. These SNPs were also screened in 51 Japanese normal subjects by PCR-based direct sequencing or PCR–RFLP method. The same genotype observed in this patient was detected in 2 of 51(3.9%) normal subjects. These results suggest that the structure of PYGL coding sequence in this patient is unlikely to account for her excessive liver glycogen accumulation. Further studies including genetic analysis on the promoter region of the gene are necessary to clarify the etiology of susceptibility to excessive liver glycogen storage in patients with type 1 diabetes.


*Keywords:* Type 1 diabetes; Mauriac syndrome; Liver glycogen storage; PYGL; Polymorphisms

* Corresponding author. Tel.: +81-942-35-3322; fax: +81-942-34-3575.
*E-mail address:* nu@st-mary-med.or.jp (K. Nunoi).

0168-8227/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.diabres.2003.12.004

## 1. Introduction

Marked hepatomegaly due to liver glycogen storage and liver dysfunction in the patients with poorly controlled type 1 diabetes mellitus was reported by Mauriac in 1930 and referred to as Mauriac syndrome [1]. The classical features of Mauriac syndrome were hepatomegaly, cushingoid facies, dwarfism, delayed sexual maturation, and hyperlipidemia. Glycogen-induced hepatomagaly in type 1 diabetes mellitus was well recognized and reported [2–9]. Hepatic glycogen metabolism is regulated by complicated mechanisms and glycogen accumulation is promoted by hyperglycemia and insulin effect [10–12]. It is supposed that in such cases excessive glycogen storage is caused by extraordinary hyperglycemia and following acute therapeutic insulin effect. Hepatomegaly occurred characteristically in the cases with diabetic ketosis or ketoacidosis, unstable blood glucose levels from hyperglycemia to hypoglycemia and regressed when stable glucose levels were maintained. The frequency of excessive liver glycogen storage in patients with type 1 diabetes mellitus is not so often or relatively rare. Chatila et al. reviewed the clinical and pathological features of 11 cases with hepatocellular glycogenesis confirmed by liver biopsy at Yale University for the period from 1954 to 1995 [13]. In Japan we reviewed seven cases with hepatic glycogen storage after diabetic ketoacidosis confirmed by liver biopsy for the period from 1975 to 1998 [14].

On the other hand, several basic deficiencies of enzymes for glycogen metabolism cause glycogen storage in the liver. Deficiency of liver glycogen phosphorylase typically causes glycogen storage disease type VI (Hers disease) [15]. The clinical features of this disease are hepatomegaly in infantile period, early fasting hypoglycemia, elevated transaminase, hyperlipidemia and ketosis [16]. It takes a benign course. Hepatomegaly and growth retardation usually improve with age and disappear. With regard to the clinical features, glycogen-induced hepatomagaly in type 1 diabetes and hepatic phosphorylase deficiency have several similarities.

We experienced a case of type 1 diabetes with excessive glycogen accumulation in the liver and simultaneous hypoglycemia which suggested impaired glycogenolysis in the extraordinary conditions. In addition, based on the several similarities with clinical features of glycogen storage disease type VI, we supposed that partial inhibition of liver glycogen phosphorylase occurred in our case. Not all the patients with type 1 diabetes with or without ketoacidosis exhibit abnormal glycogen accumulation during insulinization. Therefore, it should be clarified whether any functional abnormality in the liver glycogen phosphorylase accounts for such a phenomenon. The present study aimed to clarify the relation between the genetic abnormalities of *PYGL* gene and the tendency to glycogen accumulation during the clinical course of extraordinary hyperglycemia and rapidly increasing insulin action such as release of glucose toxicity in a patient with type 1 diabetes.

## 2. Case report

A 19-year-old Japanese woman, who had a history of type 1 diabetes since 15 years of age, was admitted to our hospital due to diabetic ketoacidosis (DKA). During her infantile period she presented with no hepatomegaly nor growth retardation and her development had been normal. The parents and siblings were healthy. On admission her height was 162 cm with a weight of 45 kg. She had drowsy consciousness level, hyperglycemia (492 mg/dl) and acidemia (pH 6.980). She was confirmed as having type 1 diabetes based on a low level of fasting serum C-peptide (0.1 ng/ml) and positive for GAD autoantibodies (11.4 U/ml). She also had hepatomegaly extending 5 cm below the right costal margin and transaminase levels were elevated (AST 387 U/l, ALT 234 U/l), but these abnormalities had resolved after administration of insulin, i.e. continuous venous infusion of regular insulin (total 41 U/30 hours) during DKA and subcutaneous insulin injection four times a day (regular 30 U and NPH 10 U) after recovery from DKA (Fig. 1). However, four weeks after DKA marked hepatomegaly extending 10 cm below the right costal margin and elevated transaminases (AST 1228 U/I, ALT 649 U/l) reappeared with hypoglycemia (FPG 47 mg/dl), hypokalemia (2.89 mEq/l) and leucocytopenia (WBC/1480 $mm^3$). The values of plasma glucagon, adrenalin, noradrenalin and cortisol were within normal ranges. Serological examinations of viral hepatitis and autoimmune hepatitis were negative and the examination of bone marrow aspira-

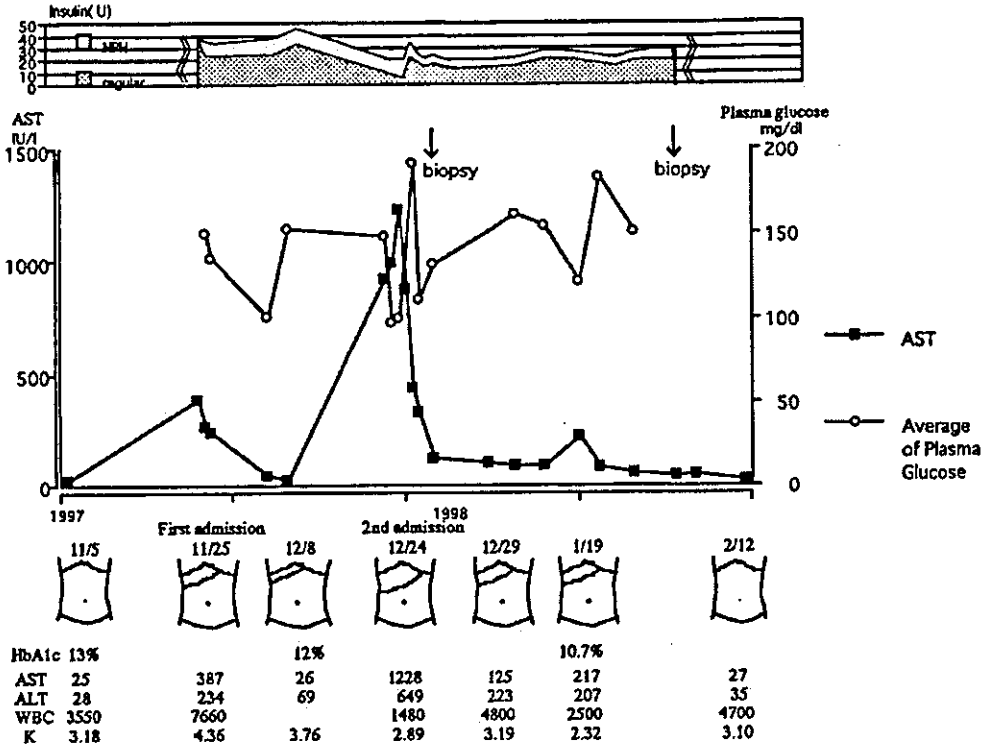


Fig. 1. Clinical course of hepatomegaly and liver function related to plasma glucose, serum K and WBC.

tion showed no malignant findings. The liver biopsy showed extensive glycogen deposition and mild steatosis by both light microscopy and electron microscopy. The evidence of glycogen deposition in the cytoplasm of hepatocytes was obtained in a PAS stain and diastase digestion (Fig. 2). Her hepatomegaly disappeared in accordance with the maintenance of stable blood glucose levels 10 weeks after DKA. However, she presented with recurrent hepatomegaly 3 cm below the right costal margin 5 months later during the repeated poor diabetic control periods.

## 3. Materials and methods

### 3.1. PYGL gene analysis

To analyze the coding region of *PYGL* gene, total RNA was prepared from the patient's peripheral blood lymphocytes. The first-strand cDNA was synthesized and PYGL cDNA was amplified by reverse-transcription PCR method. The sequence-specific primers used were taken from the sequence for human PYGL as previously reported (GenBank accession number M14636) [19]. The amplified cDNA fragments were subcloned into the pCR2.1 vector. Multiple clones for each PCR fragment were sequenced by ABI Prism 310 DNA Sequencer.

Genomic DNA were obtained from peripheral blood lymphocytes from the patient and 51 independent Japanese healthy subjects. Exons 9 and 17 of the *PYGL* gene were amplified by PCR using genomic DNA and sequence-specific primers for each exon.

Exon 9 − 95 < 5′-GTGGGCATATCAGTGCTTTC-TCCAG-3′,
Exon 9 + 151 > 5′-AGTCTTTCAACTGCAGCAT-TCTGG-3′,
Exon 17 + 52 < 5′CTCGGGGACAGGCAATATG-AAGTT-3′,
Exon 17 + 282 > 5′-GGAAGCCCTCTGAGGTC-ACATACC-3′.

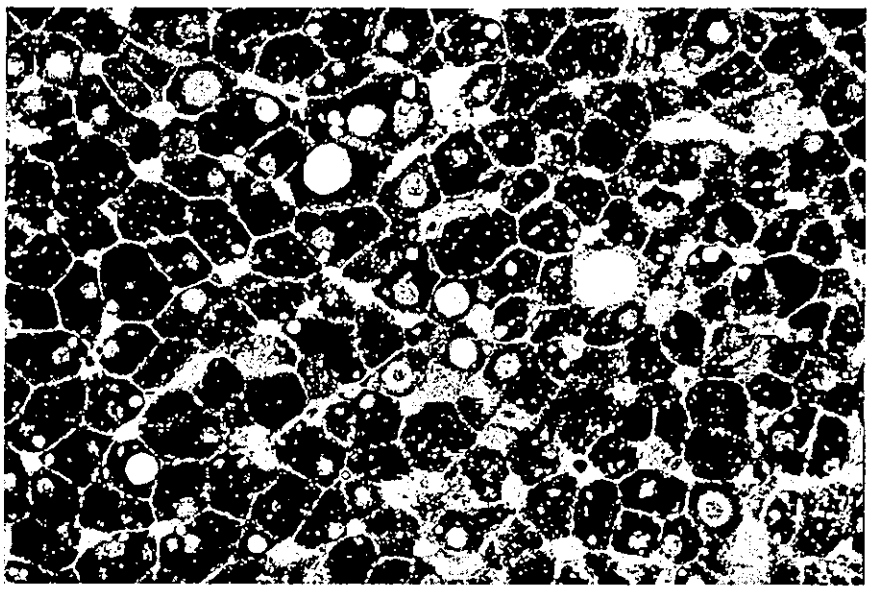

Fig. 2. Histological findings of the liver. The evidence of glycogen deposition in cytoplasm of hepatocytes with a PAS stain and diastase digestion. (PAS stain, ×200).

The nucleotide sequences were analyzed by direct sequencing and were confirmed by cloning the PCR product into pGEM-T vector.

### 3.2. Enzyme assay

Total phosphorylase activity in the presence of AMP was determined by the method of DiMauro et al. [17]. Phosphorylase b kinase in erythrocytes was measured in the presence of exogenous phosphorylase b as described by Lederer et al. [18].

### 3.3. Statistical analysis

Allele or haplotype frequencies were calculated on control subjects by direct counting. The estimate haplotype frequencies (EH) program was used to determine maximum-likelihood estimates of disequilibrium ($D_{ij}$) between two SNPs, where $D_{ij} = h_{ij} - p_i q_j$ and $p_i$ and $q_j$ are the frequencies for allele $i$ at locus 1 and for allele $j$ at locus 2, respectively [20].

## 4. Results

The PYGL cDNA sequence of the patient obtained was compared with that published (GenBank accession number Y15233) [21]. The deduced amino acid sequence of the PYGL in this patient was completely identical to that reported by Burwinkel et al. However, the nucleotide sequence of PYGL cDNA was heterozygous for substitutions at two positions, Asp339 (GAT to GAC) on exon 9 and Ala703 (GCT to GCC) on exon 17, respectively, (Fig. 3A and B). These nucleotide substitutions were confirmed by direct sequencing of genomic DNA or PCR-RFLP method. We have also screened 51 unrelated Japanese normal subjects. In all 102 chromosomes screened, 37 (36.3%) and 65 (63.7%) were GAT and GAC at codon 339, respectively, 94 (92.2%) and 8 (7.8%) were GCT and GCC at codon 703 (Table 1). Genotypic frequencies of these two SNPs were statistically consistent in Hardy–Weinberg equilibrium. As shown in Table 2, the genotype observed in this patient, GAT/GAC at codon 339 and GCT/GCC at codon 703, was detected in 2 of 51 (3.9%) normal subjects. To examine the linkage disequilibrium between these SNPs, haplotype frequencies were estimated by maximum likelihood methods based on the EH program [20]. The linkage disequilibrium found all of the four possible haplotypes defined by these SNPs. The most common haplotype is codon 339 GAC/codon 703 GCT which accounts for about 60% of the possible haplotypes. The

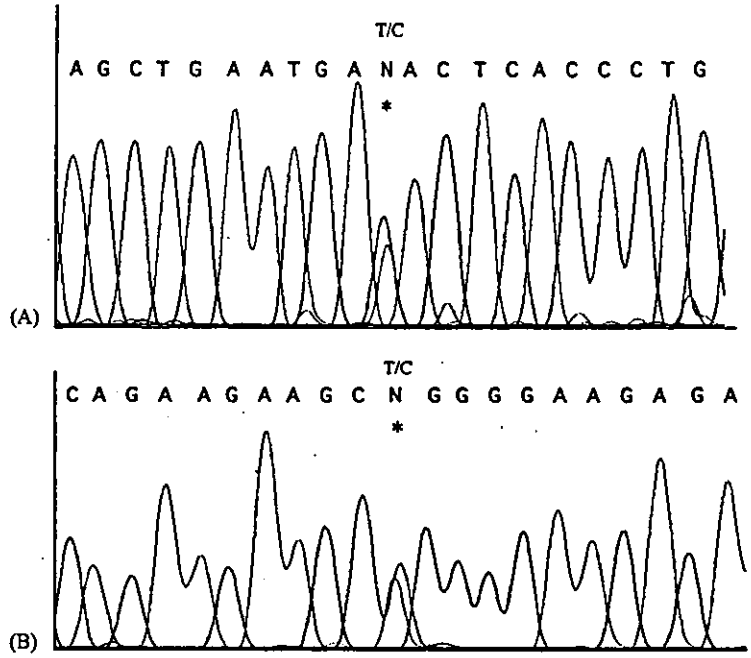


Fig. 3. The electropherograms of *PYGL* gene in the patient. (A) Asp339 (GA<u>T</u>/GA<u>C</u>) on exon 9. (B) Ala703 (GC<u>T</u>/GC<u>C</u>) on exon 17.

haplotypes comprising codon 703 GC<u>C</u> were relatively rare among them (Table 3).

We could not obtain the liver glycogen phosphorylase activity in liver biopsy specimens, however, the leucocyte phosphorylase activity and erythrocyte phosphorylase kinase activity were measured. The total leucocyte phospholylase activity in this

Table 1
Allele and genotype frequencies of *PYGL* gene polymorphisms in healthy controls

| | Polymorphism | | Frequency ($n = 51$) |
|---|---|---|---|
| Allele | Codon 339 | GA<u>T</u> | 37/102 (36.3) |
| | | GA<u>C</u> | 65/102 (63.7) |
| | Codon 703 | GC<u>T</u> | 94/102 (92.2) |
| | | GC<u>C</u> | 8/102 (7.8) |
| Genotype | Codon 339 | GA<u>T</u>/GA<u>T</u> | 8/51 (15.7) |
| | | GA<u>C</u>/GA<u>T</u> | 21/51 (41.2) |
| | | GA<u>C</u>/GA<u>C</u> | 22/51 (43.1) |
| | Codon 703 | GC<u>T</u>/GC<u>T</u> | 44/51 (86.3) |
| | | GC<u>C</u>/GC<u>T</u> | 6/51 (11.8) |
| | | GC<u>C</u>/GC<u>C</u> | 1/51 (1.9) |

Data are *n* (%).

Table 2
Pairwise genotype distribution of *PYGL* gene SNPs in 51 healthy controls

| Codon 339 genotype | Codon 703 genotype | | |
|---|---|---|---|
| | GC<u>T</u>/GC<u>T</u> | GC<u>T</u>/GC<u>C</u> | GC<u>C</u>/GC<u>C</u> |
| GA<u>T</u>/GA<u>T</u> | 7 (0.14) | 1 (0.02) | 0 (0) |
| GA<u>T</u>/GA<u>C</u> | 18 (0.35) | 2 (0.04) | 1 (0.02) |
| GA<u>C</u>/GA<u>C</u> | 19 (0.37) | 3 (0.06) | 0 (0) |

Percentage in parentheses refers to observed haplotype frequencies.

Table 3
Estimated haplotype frequencies of the SNPs in the *PYGL* gene in 51 healthy controls

| Haplotype | | Estimated HF |
|---|---|---|
| Codon 339 | Codon 703 | |
| GA<u>T</u> | GC<u>T</u> | 0.333 |
| GA<u>T</u> | GC<u>C</u> | 0.030 |
| GA<u>C</u> | GC<u>T</u> | 0.589 |
| GA<u>C</u> | GC<u>C</u> | 0.049 |

Data are *n* (%). The haplotype frequencies were estimated based on the EH program. HF, haplotype fiequency.

patient was 18.4 nmol/min/mg, which was not decreased compared to that in normal control subjects (19.3 ± 3.9 nmol/min/mg, mean ± SD; $n$ = 4). Phosphorylase kinase activity in erythrocytes was 47.9 nmol/min/gHb and within normal range (53.3 ± 7.9 nmol/min/gHb, mean ± SD; $n = 4$).

## 5. Discussion

Glycogen induced hepatomegaly in type 1 diabetes and glycogen storage disease type VI have some similar clinical features such as liver dysfunction, fasting hypoglycemia and ketosis. The differences between them are the age-at-onset of disease; i.e. youth period versus infantile period, and the background of disease; i.e. the state of type 1 diabetes versus the hereditary enzyme deficiency. Glycogen-induced hepatomagaly in type 1 diabetes mellitus had been reported and the features common to all reported cases were the state of type 1 diabetes and the lability of the diabetic control. Vallance-Owen et al. pointed out the normal or increased amounts of liver glycogen in patients dying in diabetic coma without insulin therapy and that such high blood glucose levels alone could cause the liver glycogen accumulation on a basis of circulating insulin [6]. In addition, they found that the patients dying in diabetic coma, having received insulin, showed a uniformly increased deposition in the liver over the controls. In our patient, on the first admission due to diabetic ketoacidosis she already had hepatomegaly. On the second admission during adequate insulinization marked hepatomegaly reappeared 4 weeks after recovering from DKA with the improvement of insulin sensitivity due to the release of glucose toxicity. Manderson et al. reported two cases with liver glycogen accumulation in unstable diabetes and analyzed the glycogen contents and phosphorylase activity in the liver [9]. The liver glycogen content was high and the phosphorylase activity appeared to be lower than normal but did not approach the levels associated with glycogen storage disorder. At that time the other enzymes related to liver glycogen storage, such as glucose-6-phosphatase, acid maltase and amylo-1,6-glucosidase were assayed and these values were normal. Therefore they suggested that the elevated liver glycogen levels seen in some patients with brittle diabetes mellitus are not the result of enzyme deficiency but are secondary to wide fluctuations in blood sugar and frequent doses of soluble insulin. However, the doses of regular insulin (8–35 U per day) administrated in our patient were not so high. In addition, she presented with recurrent mild hepatomegaly thereafter during the repeated poor diabetic control periods. Therefore, it is suggested that her liver glycogen phophorylase systems were latently impaired and inhibited transiently in the extraordinary conditions.

Glycogen storage disease type VI (Hers disease) is caused by the deficiency of liver glycogen phosphorylase and suspected as an autosomal recessive inheritance. The mutations of *PYGL* gene in glycogen storage disease type VI were identified and reported previously [21,22]. Burwinkel reported the mutations of PYGL of three patients with glycogen storage disease type VI and these are two splicing-site mutations and two missense mutations [21]. Chang determined that Mennonite glycogen storage disease type VI was caused by a single base pair change in a splice donor site of intron 13 in the *PYGL* gene, which showed heterogeneous PYGL mRNA lacking all or part of exon 13 in affected persons [22]. Liver glycogen phosphorylase is regulated by multiple allosteric effectors and hormonal controls, therefore it is relatively difficult to assess the enzyme activities in various conditions [9,21,23]. In addition, phosphrylase kinase deficiency also causes deficiency of glycogen phosporylase in liver glycogen metabolism [24,25]. Therefore it is suggested that genetic analysis of related enzymes contribute to the diagnosis of liver glycogen storage disease type VI and the detection of the susceptibility to such liver glycogen storage in extraordinary conditions.

The sequences of human PYGL mRNA have been reported previously [19,21,22]. The nucleotide sequence of PYGL cDNA in this patient was identical to that reported by Burwinkel et al. (GenBank accession number Y15233), except for the two heterozygous positions, Asp339 (GA<u>T</u>/GA<u>C</u>) and Ala703 (GC<u>T</u>/GC<u>C</u>). This is the first report on the SNP at codon 703 (GC<u>T</u>/GC<u>C</u>). These nucleotide substitutions were also found in Japanese healthy controls, indicating that they are SNPs of PYGL. Burwinkel et al. found polymorphisms in the PYGL coding sequence at Asp50 (GA<u>C</u>/GA<u>T</u>), Asp339 (GA<u>T</u>/GA<u>C</u>), Thr671 (AC<u>C</u>/AC<u>T</u>) and Val221/Ile221 (<u>G</u>TC/<u>A</u>TC) [21]. Considering the clinical course of our patient

that marked hepatomegaly and elevated transaminases reappeared with the tendency of hypoglycemia and hypokalemia 4 weeks after DKA, it is suggested that liver glycogen phosphorylase activity was transiently inhibited. At this point, we could not obtain the value of her liver glycogen phosphorylase activity. However, the sequence analysis of PYGL c DNA in this patient suggests that there are no structural defects in her amino acid sequence. Later the leucocyte phosphorylase activity and erythrocyte phosphorylase kinase activity were measured and these values were within normal range. The enzyme activities of these blood cells seemed to parallel that of liver and be useful in the diagnosis of glycogen storage disease type VI [26].

Single nucleotide polymorphisms are the most common type of genetic variations. Most SNPs are neutral but a proportion of SNPs contribute to disease susceptibility and resistance. In this study we screened 51 unrelated Japanese normal subjects at the heterozygous sites on exons 9 and 17 of PYGL by direct sequencing or PCR–RFLP. The same genotype observed in this patient was detected in 2 of 51 (3.9%) normal subjects. The association of these SNPs and liver glycogen accumulation in type 1 diabetes is unknown and further genetic analysis including the promoter region is necessary to reveal the susceptibility to this phenomenon in our patient.

We were also interested in the transient appearance of marked leucocytopenia accompanied by the peak of hepatomegaly. The mechanism of such leucocytopenia is unknown and analysis of glycogen contents and enzyme assays on glycogen metabolism in WBC at this point may reveal the mechanism.

## Acknowledgements

The authors thank Emeritus Professor Masayuki Hirata of Tokyo Women's Medical University for his advice and Akemi Tyuman for assistance with gene analysis.

## References

[1] P. Mauriac, Gros ventre, hepatomegalie, troubles de la croissnce chez les enfants diabetiques traites plucieurs annes par l'insuline, Gaz. Hebd. Med. Bordeaux. 26 (1930) 402–410.

[2] F. Mandell, W. Berenberg, The Mauriac syndrome, Am. J. Dis. Child. 127 (1974) 900–902.

[3] H.S. Traisman, Mauriac's syndrome. A complication of poorly managed diabetes, Clin. Pediatr. 3 (9) (1964) 520–522.

[4] E.W. Brian, A.J. Schecher, E.L. Persons, Unusual glycogen storage in a case of diabetes Mellitus, Arch. Intern. Med. 59 (1937) 685–690.

[5] A. Marble, P. White, I.K. Nogan, R.M. Smith, Enlargement of the liver in diabetic children. I. Its incidence, etiology and nature, Arch. Intern. Med. 62 (1938) 740–750.

[6] J. Vallance-Owen, Liver glycogen in diabetes Mellitus, J. Clin. Pathol. 5 (1952) 42–53.

[7] J.I. Goodman, Hepatomegaly and diabetes mellitus, Ann. Intern. Med. 39 (1953) 1077–1087.

[8] R.W. Evance, T.R. Littler, H.S. Pemberton, Glycogen storage in the liver in diabetes mellitus, J. Clin. Path. 8 (1955) 110–113.

[9] W.G. Manderson, M.T. McKiddie, D.J. Manners, J.R. Stark, Liver glycogen accumulation in unstable diabetes, Diabetes 17 (1968) 13–16.

[10] G.I. Shulman, R.A. DeFronzo, L. Rosseti, Differencial effect of hyperglycemia and hyperinsullinemia on pathways of hepatic glycogen repletion, Am. J. Physiol. 260 (1991) 731–735.

[11] H.K. Ortmeyer, N.L. Bodkin, B.C. Hansen, Insulin regulate liver glycogen synthase and phosphorylase activity reciprocally in ruthus monkeys, Am. J. Physiol. 272 (1997) 133–138.

[12] K.F. Peterson, D. Laurent, D.L. Rothma, G.W. Cline, G.I. Shulman, Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans, J. Clin. Invest. 101 (1998) 1203–1209.

[13] R. Chatila, B. West, Hepatomegaly and abnormal liver tests due to glycogenesis in adults with diabetes, Medicine (Baltimore) 75 (6) (1996) 327–333.

[14] M. Tomihira, Y. Uchizono, Y. Sato, et al., Recurrenrt hepatomegaly due to glycogen storage after diabetic ketoasidosis in a case of type 1 diabetes mellitus, J. Jpn. Diabetes Soc. 43 (10) (2000) 879–885. In Japanese.

[15] Y.T. Chen, A. Burchell, Glycogen storage disease, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Basis of Inherited Disease, vol 1, McGraw-Hill, New York, NY, 1995, pp. 935–966.

[16] H. Hers, Etudes emzymatiques sur fragments he'patiques, Revue. Int. Hepatol. 9 (1959) 35–55.

[17] S. DiMauro, G.B. Hartwing, A. Hays, et al., Debrancher deficiency, neuromuscular disorder in 5 adults, Ann. Neurol. 5 (1979) 422–436.

[18] B. Lederer, G. van de Werve, T. de Barsy, H.G. Hers, The autosomal form of phosphorylase kinase deficiency in man reduced activity of the muscle enzyme, Biochem. Biophys. Res. Commun. 92 (1980) 169–174.

[19] C.B. Newgard, K. Nakano, P.K. Hwang, R.J. Fletterick, Sequence analysis of the c DNA encoding human liver glycogen phosphrylase reveals tissue-specific codon usage, Proc. Natl. Acd. Sci. U.S.A. 83 (1986) 8132–8136.

[20] J.D. Terwilliger, J. Ott, Linkage Disequilibrium Between Alleles at Marker Loci, Johns Hopkins University Press, Baltomore, 1994.
[21] B. Burwinkel, H.D. Bakker, E. Herschkoviz, S.W. Moses, Y.S. Shin, M.W. Killian, Mutation in liver glycogen phosphorylase gene (*PYGL*) underlying Glycogenosis type IV (Hers disease), Am. J. Hum. Genet. 62 (1998) 785–791.
[22] S. Chang, M.J. Rosenberg, H. Morton, C.A. Francomano, L.G. Biesecker, Identification of a mutation in liver glycogen phosphorylase in glycogen storage disease type IV, Hum. Mol. Genet. 7 (5) (1998) 865–870.
[23] J.W. Hudson, G.B. Golding, M.M. Crerar, Evolution of allosteric control in glycogen phosphorylase, J. Mol. Biol. 234 (1993) 700–721.
[24] B. Lederer, van F. Hoof, van den G. Berghe, H.G. Hers, Glycogen phosphorylase and its converter enzymes in haemolysates of normal human subjects and of patirnts with type VI glycogen storage dosease, Biochem. J. 147 (1975) 23–35.
[25] I. Marie, C. Baussan, N. Moatti, M. Mathieu, A. Lemonnier, Biochemical diagnosis of hepatic glycogen storage diseases: 20 years French experience, Clin. Biochem. 24 (1991) 169–178.
[26] N. Dahan, C. Baussan, N. Moatti, A. Lemonnier, Use of platelets, mononuclear and polymorphonuclear cells in the diagnosis of glycogen storage disease type IV, J. Inher. Metab. Dis. 11 (1988) 253–260.

ELSEVIER

Brain & Development 26 (2004) 57–60

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case report

# Pyruvate dehydrogenase E1α subunit deficiency in a female patient: evidence of antenatal origin of brain damage and possible etiology of infantile spasms

Naoko Wada[a], Toyojiro Matsuishi[a], Michiko Nonaka[b], Etsuo Naito[c], Makoto Yoshino[a,*]

[a]*Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830-0011, Japan*
[b]*Neonatal Intensive Care Unit, St. Mary's Hospital, Kurume, Japan*
[c]*Department of Pediatrics, School of Medicine, University of Tokushima, Tokushima, Japan*

Received 21 May 2002; received in revised form 30 January 2003; accepted 18 March 2003

**Abstract**

Enlargement of the lateral ventricles and atrophy of the brain were documented ultrasonographically in utero at as early as 28th week of gestation in a female patient with lactic acidosis due to deficiency of the pyruvate dehydrogenase E1α subunit, demonstrating that the changes characteristic of this disease can occur antenatally. The mechanism of infantile spasms in this disease may be linked to mosaicism of the brain cells involving the normal enzyme and the mutant enzyme.


*Keywords:* Pyruvate dehydrogenase; E1α deficiency; Antenatal brain damage; Infantile spasms

## 1. Introduction

Pyruvate dehydrogenase complex (PDHC) is an enzyme complex consisting of five enzymes. The first enzyme of the complex, pyruvate dehydrogenase (E1), is a tetramer consisting of two α-subunits and two β-subunits. A deficiency of α-subunit (E1α) is the most common cause of congenital PDHC deficiency with lactic acidemia [1]. The α subunit gene locus is assigned to Xp22.1 [2]; and accordingly, this gene is subjected to random inactivation. E1α deficiency is often associated with various types of brain damage and neurological symptoms [3], and, notably, a significant difference has been shown in symptoms between female and male patients; and infantile spasms have been encountered almost exclusively in female patients. The antenatal origin of such brain damage has been suspected [4–6], but no supportive evidence has yet been found. Mutations in the gene encoding the α-subunit have been documented in patients with E1α deficiency. We herein report a female patient with E1α deficiency who developed infantile spasms, and in whom anomalous development of the brain was antenatally demonstrated.

## 2. Case report

This female patient was born after 40 weeks of uncomplicated pregnancy to healthy nonconsanguineous parents. The birth weight was 2020 g. A routine ultrasonographic study performed at the 28th week of gestation showed enlargement of the lateral ventricles of the fetus. A similar finding was noted at the 32nd week of gestation (Fig. 1A). Due to early detection of this enlargement, the baby was transferred to a neonatal intensive care unit immediately after birth. Laboratory findings at this time indicated metabolic acidosis and hyperammonemia (151 μmol/l). Auditory-evoked brainstem response was negative. Elevated concentrations of lactic acid (5.8 mmol/l) and pyruvic acid (0.6 mmol/l) in blood were first found at the age of 70 days. The karyotype of the patient was 46, XX. At the age of 6 months, she developed tonic seizures with series formation, which was diagnosed as infantile spasms. An electroencephalographic tracing taken at this time showed modified hypsarrythmia. Because the

* Corresponding author. Tel.: +81-942-31-7565; fax: +81-942-38-1792.
*E-mail address:* yoshino@med.kurume-u.ac.jp (M. Yoshino).

0387-7604/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0387-7604(03)00072-X

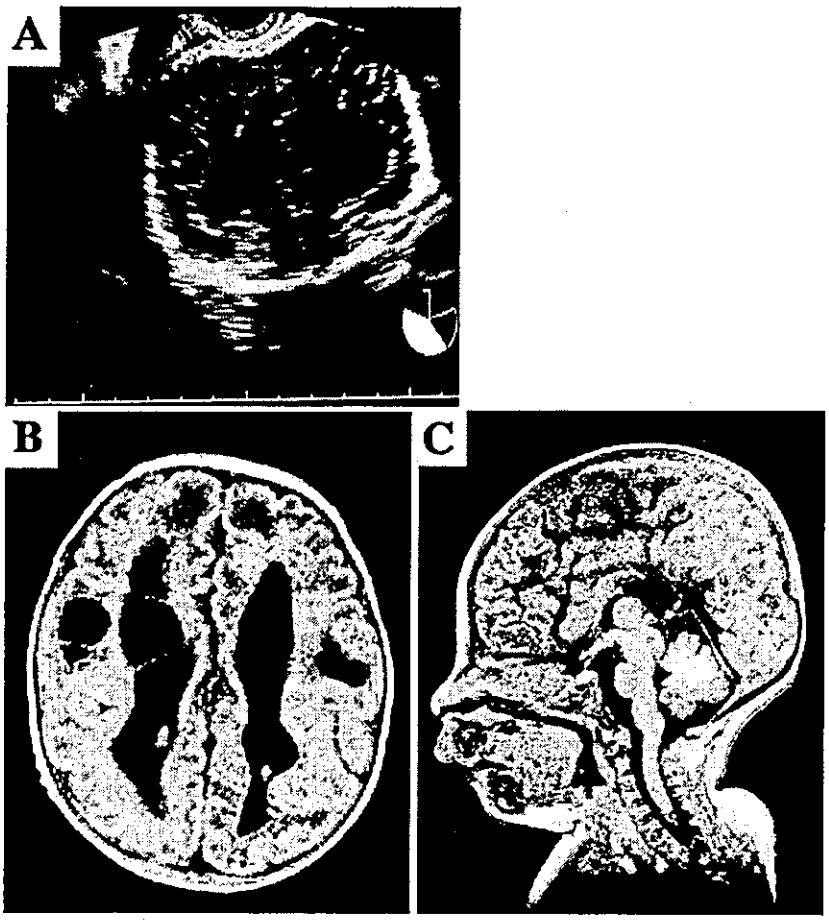


Fig. 1. Enlargement of the lateral ventricles of the fetus was reconfirmed by ultrasonography at the 32nd week of gestation (A). T1-weighted magnetic resonance imaging of the brain recorded at 1 month of age revealed atrophic change of the cerebrum with multiple cystic changes in the brain substance, formation of the intraventricular septum, enlargement of the posterior horns of the lateral ventricles (B), atrophy of the brainstem, and partial agenesis of the corpus callosum (C).

seizure was refractory to vitamin B6, the patient was hospitalized at the age of 6 months for evaluation and management of the seizure. Physical examination on admission revealed a female infant with microcephaly, facial dysmorphism; frontal bossing, laterally upslanting palpebral fissures, depressed nasal bridge, short upturned nose and shark mouth. She had achieved virtually no psychomotor development and had moderate generalized hypotonia with trunkal predominance. The laboratory tests revealed elevated blood concentrations of lactic acid (5.5 mmol/l) and pyruvic acid (0.6 mmol/l), and elevated alanine (1.2 mmol/l) and proline (0.44 mmol/l) levels in serum. Presumptive diagnosis of PDHC E1α deficiency was made at this time. The seizure was ameliorated by the administration of sodium valproate and clonazepam, and the patient was subsequently discharged.

Thiamine hydrochloride at a dose of 10 mg/kg/day was started at the age of 8 months. The patient was readmitted at the age of 11 months, when elevated concentrations of lactic acid (6.2 mmol/l) and pyruvic acid (0.6 mmol/l) in cerebrospinal fluid were found, for the further evaluation. At the age of 13 months, the patient was placed on a high-fat formula. At 16 months of age, after diagnosis had been confirmed by enzyme measurements, administration of sodium dichloroacetate (DCA) was started and maintained at a dose of 50 mg/kg/day for the first 3 months, then tapered to 25 mg/kg/day later (DCA administration had been withheld per parental request until the diagnosis had been confirmed by enzyme analysis). The concentrations of lactic acid and pyruvic acid in blood decreased, with marked fluctuations, shortly after the introduction of DCA and have remained at levels approximately half the pretreatment values. The pretreatment concentrations of lactic acid and pyruvic acid in cerebrospinal fluid were close to those in the blood, and the former levels decreased to approximately two-thirds the pretreatment value 7 days after the initiation of DCA. Nevertheless, no changes in clinical signs have occurred since the introduction of the therapy.

The cranial computerized tomographic (CT) scan on the first day of life showed multiple leukomalacia and enlargement of the lateral ventricles with irregularity of the ventricular walls (figure not shown). Cranial magnetic resonance imaging, first performed at the age of 1 month, revealed, in addition to essentially the same findings as those shown in the previous CT scan, atrophic change of the cerebrum with multiple cystic changes in the brain substance, formations of the intraventricular septum, enlargement of posterior horns of the lateral ventricles (Fig. 1B), atrophy of the brainstem and partial agenesis of corpus callosum (Fig. 1C). The enlargement of the ventricles and atrophic changes of the cerebrum became more marked on the follow-up imagings performed at the ages of 6, 12, and 14 months (figures not shown), irrespective of treatment.

The overall activity (unit, nmol/min/mg protein) of DCA-activated PDHC in cultured skin fibroblasts, measured as described elsewhere [7], was moderately decreased; 0.75 (control range, 2.38 ± 0.60) in the presence of 0.4 mmol/l thiamine pyrophosphate, and 0.53 (control range, 2.31 ± 0.62) in the presence of $1 \times 10^{-4}$ mmol/l thiamine pyrophosphate. The DCA-activated activity (unit, nmol/h/mg protein) of E1 was 0.69 (control range, 10.7 ± 4.0). Mutational analysis of the E1α gene of the present patient, performed as previously described [7], revealed a deletion of one of the seven base-pair (AGTAAGA) segments of the tandem repeat (nt positions 927–940) in exon 10, creating a termination codon downstream of the deleted segment (nt positions 974–976 in the wild-type sequence).

## 3. Discussion

It has been postulated that in female patients with E1α deficiency, structural anomalies and degenerative changes in the brain would occur antenatally [4–6]. Observations in our case first demonstrate this postulation, and then confirm that significant retardation of fetal brain development occurs as early as the end of the second trimester in this disease. Additional damage to the brain would be superimposed between that time and delivery because development of the fetal brain becomes more dependent on PDH E1α activity after the mid-organogenesis stage [8]. These observations also suggest that postnatal intervention alone is of limited therapeutic effect. This disease should be included as a differential diagnosis when dilatation of the lateral ventricles is found antenatally.

Scrutiny of clinical records of patients with E1α deficiency [3] revealed that male patients who survived the neonatal period and early infancy generally have a milder phenotype, including mental retardation, ataxia and mild carbohydrate-sensitive lactic acidemia, than female patients, who usually present with seizures, severe developmental delay and structural abnormality of the central nervous system. This observation seems inconsistent with a general rule that in most X-linked dominant diseases, hemizygous male patients usually have a more severe phenotype than heterozygous female patients. A possible explanation for this apparent discrepancy may be as follows. Males who carry a mutant E1α allele that leads to severe deficiency of the enzyme are selected antenatally, and consequently male patients who harbor a mutant E1α allele that results in a less severe enzyme deficiency survive the neonatal period and present as male patients with a mild phenotype. In females, on the other hand, possession of a mutant allele leading to severe enzyme deficiency could still be consistent with a live birth, depending on a pattern of X-inactivation; such individuals would then present a more severe phenotype than male patients who have the mild phenotype.

The pathogenesis of infantile spasms is variable. Neuropathological findings reported in infantile spasms include gross developmental malformations, such as agenesis of the corpus callosum and multifocal dysplastic lesions [9]. The latter is considered to have particular relevance to the mechanism of infantile spasms [10,11]. These cerebral lesions have been observed almost exclusively in female patients with E1α deficiency [3,4], with the exception of one male patient [3]. The vast majority of patients presenting with infantile spasms have been female [12,13]. The presence of such multiple lesions may be linked in part to the pathogenesis of infantile spasms in this disease.

The mosaicism of brain cells may have an implication also in terms of the therapeutic effect of DCA. DCA may not be meaningful in improving pyruvate oxidation in the mutant cells, although it may be effective in reducing the lactic acid released from mutant cells through activation of the enzyme in normal cells. Thus, DCA may have limited therapeutic effect on brain metabolism in female patients.

The seven-base-pair deletion in exon 10 has been reported in multiple patients [14,15]. Clinical presentation among those patients, including the present one, was variable. Though both the nature of mutation and the pattern of the lyonization may affect the phenotype, the former seems to have a more significant influence, as this particular mutation would result in a severe impairment of cellular function in half of the cells of the central nervous system; this is because the mutation is expected to express null activity of the enzyme, given that the wild-type allele and the mutant allele are equally inactivated.

## Acknowledgements

This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan (No. 12670796). We thank Dr M. Ito (Department of Pediatrics, School of Medicine, University of Tokushima) for the determinations of DCA concentrations; and Drs F. Koga (Neonatal Intensive Care Unit, St. Mary's Hospital), and S. Yoshizawa and S. Nakagawa