表2 浜松市知的障害児保育者研修会（平成9年度から開始）

初級コース（20名）	上級コース（15名）
発達障害の基礎講義と実習	初級コース研修修了者対象
講義（3日間）	講義，グループワーク（2日間）
(1) 発達障害児の概念	(1) 統合保育の現場から
(2) 精神遅滞	(2) 言葉の発達について
(3) ダウン症	(3) 作業療法士から見た保育へのヒント
(4) 集団保育での知的発達障害児への関わり方	実習（2日間）
(5) 自閉症	知的障害児通園施設
(6) AD/HD（注意欠陥/多動性障害）	
(7) LD（学習障害）	
(8) 発達障害児の家族支援	
実習（1日）	
知的障害児通園施設での実習	

参加スタッフおよび関連機関	
センター	浜松市
医師，臨床心理士，保健師，保育士	障害福祉課，保育課，浜松市教育委員会
言語聴覚士，作業療法士	

表3 発達教育研修会（平成14年度から開始）

浜松市教育委員会新規学校選択研修のひとつ（定員15名）6回のシリーズ研修	
(1) 教育委員会指定校での軽度発達障害児（センターにかかっている）の授業参観	
研究協議：担任，研修参加者，教育委員会，センタースタッフを交えた討議	
(2) 夏季（センターにおいて）	
軽度発達障害の基礎講義	
事例検討	
発達障害児への訓練の見学	
参加スタッフ：	
浜松市発達医療総合センター	浜松市教育委員会学校教育課
医師，臨床心理士，言語聴覚師，	
作業療法士，保健師，MSW	

している．

(b) 発達教育研修会（定員15名）（表3）

センターと浜松市教育委員会とで平成13年秋準備会を発足し，小中学校教職員の研修制度について検討のうえ，平成14年度から浜松市教育委員会新規学校選択研修のひとつとして発足した．6回のシリーズ研修で以下の2つの構成からなる．①浜松市教育委員会指定校（毎年新たに指定される）における軽度発達障害児（センター受診中の児）の授業参観：授業参観ではあらかじめ配布された机の配置図に，対象児の机にマークがしてあり，研修を受講中の教員は同級生に対象児が特定児であることに気づかれないように配慮して，授業での児の状態を見守った．またその際の担任の対応などについても記録して後の協議の資料とした．その後研究協議として担任，研修参加者，教育委員会，センタースタッフ（小児神経科医，臨床心理士，保

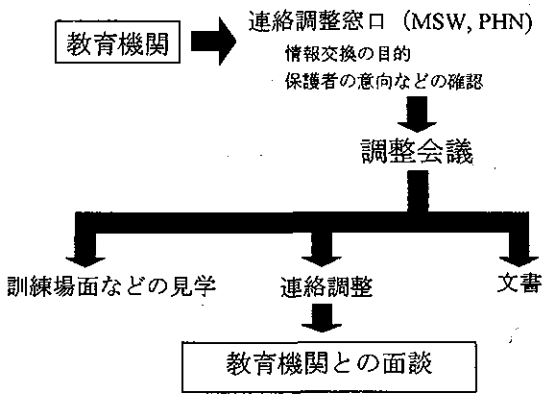

図3 医療現場と教育現場のスタッフ同士の面談

健師）による児への対応・処遇に関する討論を行った．②夏季休暇を利用した発達障害の基礎講義，事例検討，軽度発達障害児への訓練参観．参加教職員は学校長推薦などを得て，出張扱いで公務として参加した．

4．教育現場担当者との面談システムについて（図3）

図3に面談への流れを示す．はじめに述べたように発達障害児を包括的にサポートするには，児が社会生活を送っている現場のスタッフとの連携が不可欠である．この考え方から平成10年から現場教師とセンタースタッフによる児への対応に関する面談事業を開始した．ソーシャルワーカー，保健師が面談窓口（現在は総合相談室）を開設し，面談日程の設定，保護者への説明および同意などを取り付けた．また医療情報を開示する際にはその範囲についてあらかじめ保護者の希望を聞き（例えば発達テスト結果の開示の制限，診断名の開示の制限など），例え教育現場のスタッフから診断名などの具体的な説明を求められても，保護者の希望の範囲での面談を行った．面談時間は原則1時間で，児の特徴について専門的な説明および教育現場でどのようなサポート，対応が必要かの議論を中心に情報交換を行った．面談児の診断ではAD/HD，精神遅滞，自閉性障害が最も多く，知的に正常な範囲に属する軽度発達障害の児が多かった（図4）．面談に来院した教育機関としては小学校および幼稚園が多かった（図5）．

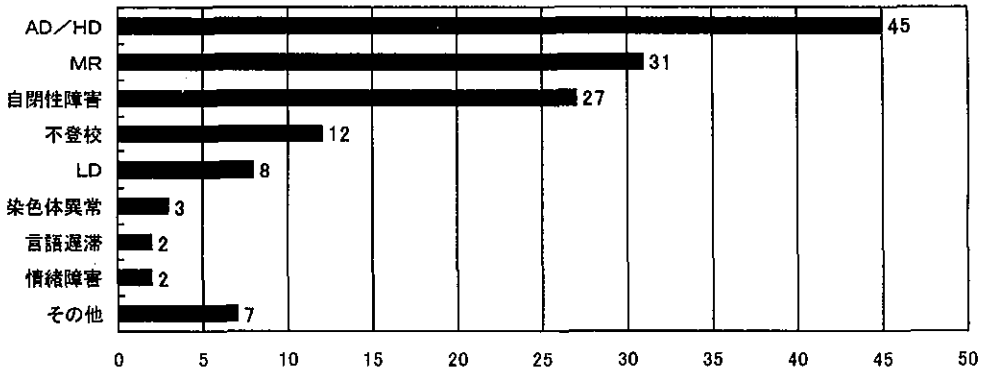

図4 平成15年度における，面談児の診断

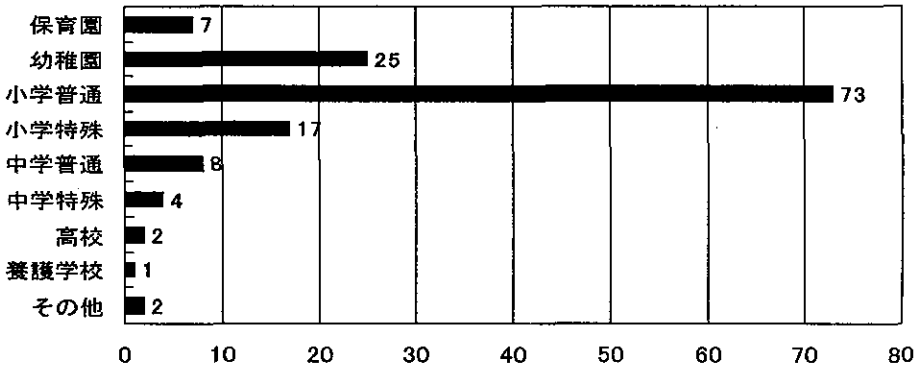

図5 平成15年度における面談に来院した教育機関の内訳

5．面談についての評価の分析について

当センターに受診する患児について教育機関と面談を行う上で，面談が教育機関での指導に役立っているのか，教育機関との連絡調整窓口（現在総合相談室）の機能について十分に理解が得られているかなどについて把握する事を目的としてアンケート調査を行った．対象は当センター小児神経科に受診中の患児のうち，平成12年4月～平成13年3月に面談を行った教育機関の保育士，教師等（以下教職員とする）である．面談後1ケ月を目安に，アンケートを91の教育機関へ送付した．なお本調査にあたっては保護者の同意を得て行った．82機関，95人の教職員から回答を得た（回収率84.5%）．窓口が面談の手順を整えるシステムについては，「センターのスタッフと学校の間に入ってきめ細かく対応してくれた」「窓口が決まっていることで連絡がスムーズに行える」「医師に直接連絡することはためらわれるが，担当者がいると密に連絡できる」などがあった．患児の個人医療情報の取扱について窓口から説明をうけた上で，「確認書」を作成する意義については「当然のこと」，「権利擁護の点で重要なこと」などがあった．面談で説明した内容については「説明が具体的で丁寧だった」「対応方法を具体的に教えてもらえた」があった．ただし「子どもの状態の説明に専門用語が使われたため難解だった」などの感想もあった．面談の効果について「対応方法が明確になった」，「子どもの様子を理解できた」，「今後の指導に自信が持てた」などがあった．今後も当センターと積極的に連携を取ろうと思うかについて「協力して関わるほうが効果がある」，「多方面のアプローチが必要」などがあつた．また少数に「保護者とセンターの見解にずれがなく，保護者を通して連携することで十分」という回答もあった．当センターで窓口を設け「確認書」作成の手続きを取る目的は，患児のプライバシー保護が目的の一つであるが，それについては「当然のこと」と認識している教職員が圧倒的であり，倫理的なことを理解して面談を行うという考え方に対して好意的であった．しかし自由記述の中には「保護者の了解がなくても診断名など教えてもらいたい」，「クラス選択の資料として知能検査の結果を数値で教えて欲しい」などがあり，今後も十分な配慮が必要と考えられる．当センターとの面談については，88.4%の者が分かりやすかったと評価している．教職員は問題行動への具体的な対応方法を切実に求めており，説明も難解な専門用語を交えず具体性のあるものを必要としていた．また面談で得た情報は教育機関内で有効に活用されている傾向にあり，子どもの状態の改善につながらなくとも，教職員が指導に自信が持てることや，教職員間で共通理解が得られることが重要であることが，アンケートの自由記述などから明らかになった．面談を実施した教職員の94.1%が今後も積極的に連携を行いたいと回答していることから，継続的な助言を求めていると言える．医療現場での方法論を教育現場にそのまま当てはめることは困難であるが，教育機関の体制や問題行動に対する考え方を，我々医療機関が十分に理解した上で，教育機関で実行可能なことを医療と教育の両面から模索し，解決策を見出す連携が重要であることがわかった[7]．

6．今後の課題

センターにおける医療・教育連携のシステムつくりの経緯と現状について述べた．この成果は平成15年2月8日に浜松市で厚生労働科学研究発表会として報告した[6]．約380名の出席者とともに浜松市の医療教育連携について議論がされた．今後はこの成果を元に医療の領域，教育の領域がさらに理解を深め合い議論してゆくことで軽度発達障害児の適切な教育環境への適応支援が行われることを期待したい．さらに近年は「虐待」の問題も複雑に関係している事例もあり，今後さらに幅広い連携が求められてゆくことが想定される．特別支援教育の施行に伴い連携の重要性が増すことが予想され，現場からの連携の構築のみならず医療・教育・福祉・保健にさらに担当行政の意識改革も重要である[8,9,10]．

謝　辞

本研究の一部は厚生労働科学研究（平成12—14年度）「知的障害児の医学的診断のあり方と療育・教育連携に関する研究」，厚生労働科学研究（平成16年度）「知的障害児者の機能退行の要因分析と予防体系開発に関する研究」，精神神経疾患研究委託費（14公—2）の援助を受けた．またこの研究に関しては浜松市発達医療総合センター，療育センター附属診療所，療育課，総合相談室，および浜松市保健福祉部障害福祉課，浜松市教育委員会学校教育課のスタッフの多大な理解，努力と協力によることを付記する．

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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*)

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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

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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-

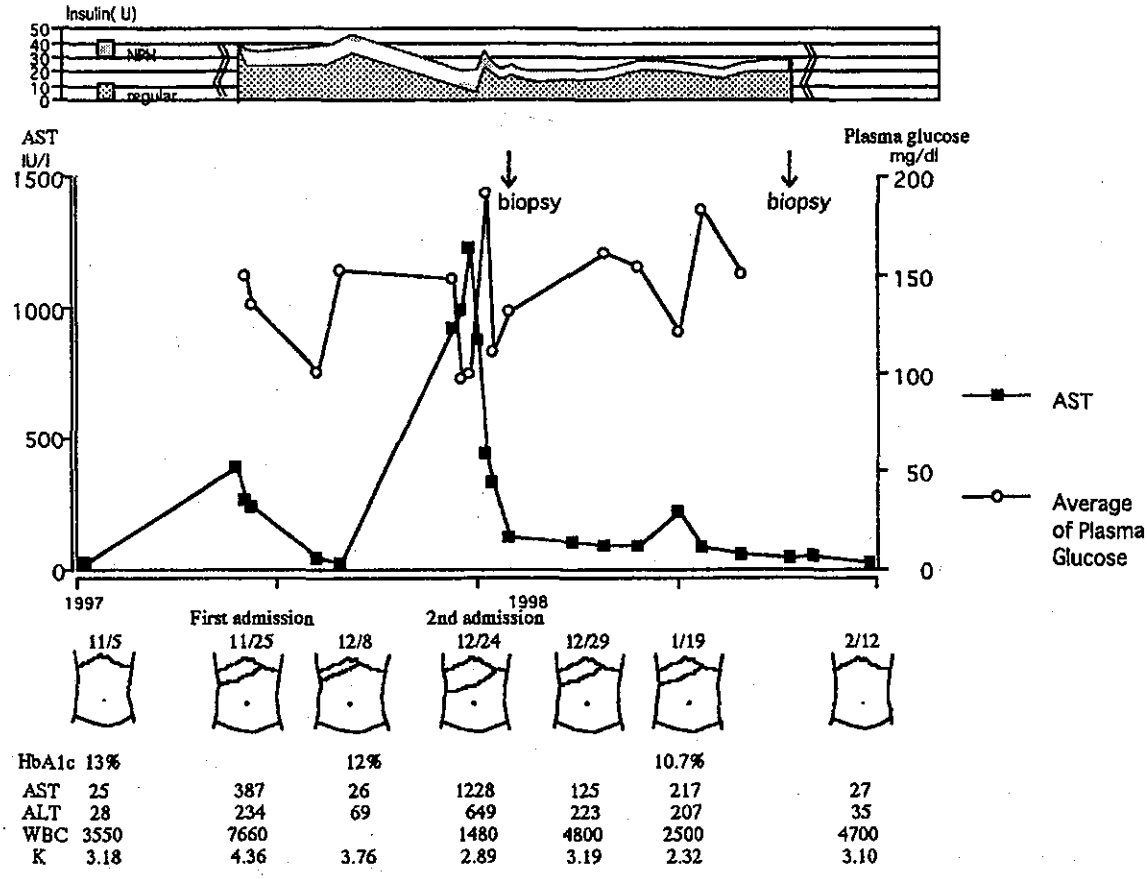

Fig. 1. Clinical course of hepatomegaly and liver function related to plasma glucose, serum K and WBC.

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′.

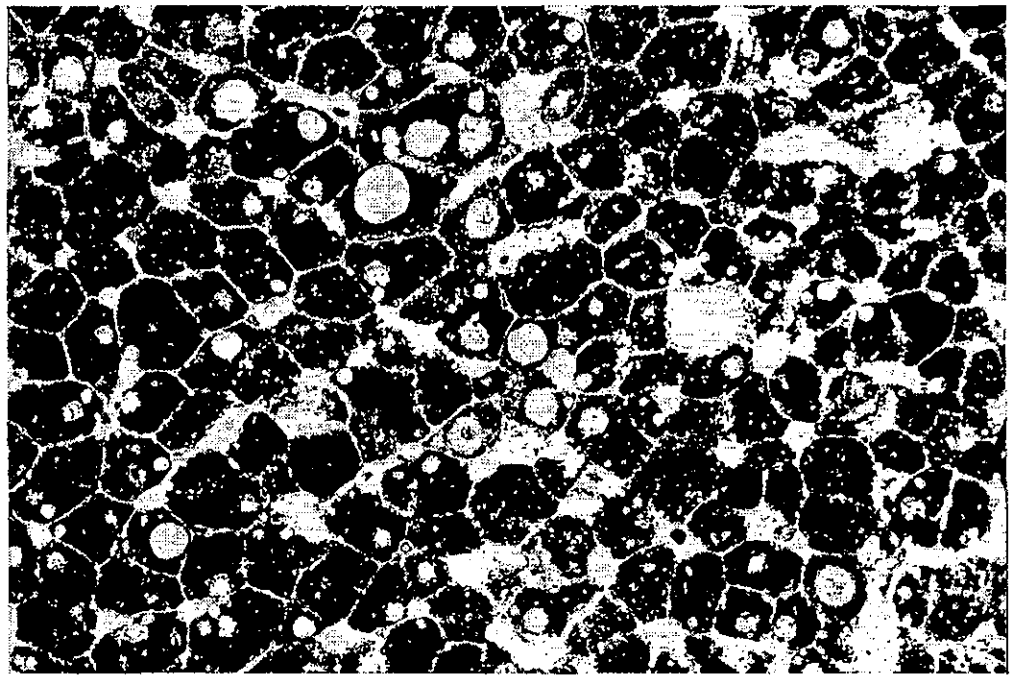

Fig. 2. Histological findings of the liver. The evidence of glycogen deposition in cytoplasm of hepatocytes with a PAS stain and diastase digestion. (PAS stain, ×200).

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 (GA<u>T</u> to GA<u>C</u>) on exon 9 and Ala703 (GC<u>T</u> to GC<u>C</u>) 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 GA<u>T</u> and GA<u>C</u> at codon 339, respectively, 94 (92.2%) and 8 (7.8%) were GC<u>T</u> and GC<u>C</u> 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, GA<u>T</u>/GA<u>C</u> at codon 339 and GC<u>T</u>/GC<u>C</u> 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 GA<u>C</u>/codon 703 GC<u>T</u> which accounts for about 60% of the possible haplotypes. The

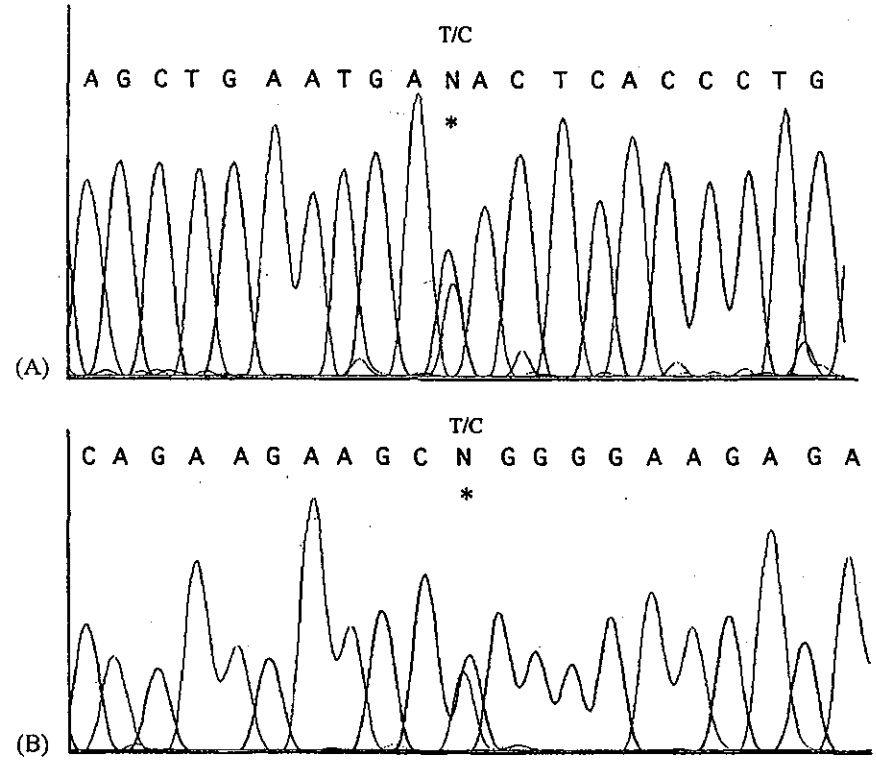

Fig. 3. The electropherograms of *PYGL* gene in the patient. (A) Asp339 (GAT/GAC) on exon 9. (B) Ala703 (GCT/GCC) on exon 17.

haplotypes comprising codon 703 GCC 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	GAT	37/102 (36.3)
		GAC	65/102 (63.7)
	Codon 703	GCT	94/102 (92.2)
		GCC	8/102 (7.8)
Genotype	Codon 339	GAT/GAT	8/51 (15.7)
		GAC/GAT	21/51 (41.2)
		GAC/GAC	22/51 (43.1)
	Codon 703	GCT/GCT	44/51 (86.3)
		GCC/GCT	6/51 (11.8)
		GCC/GCC	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		
	GCT/GCT	GCT/GCC	GCC/GCC
GAT/GAT	7 (0.14)	1 (0.02)	0 (0)
GAT/GAC	18 (0.35)	2 (0.04)	1 (0.02)
GAC/GAC	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	
GAT	GCT	0.333
GAT	GCC	0.030
GAC	GCT	0.589
GAC	GCC	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.

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

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Patient Report

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

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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

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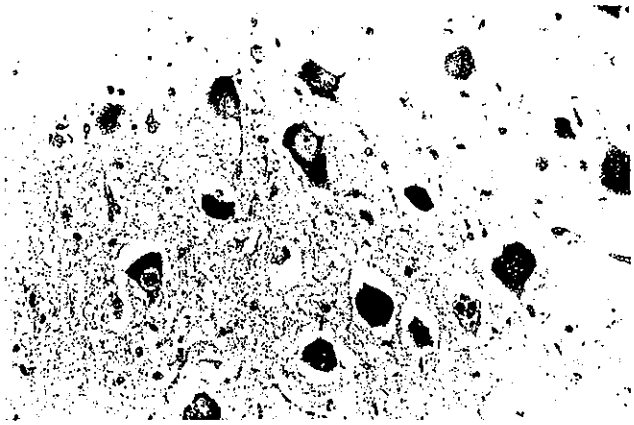

Fig. 1 Medulla oblongata of Case 1. Periodic acid Schiff (PAS)-positive granular deposits are seen in the cytoplasm of neuronal cells (PAS, ×400).

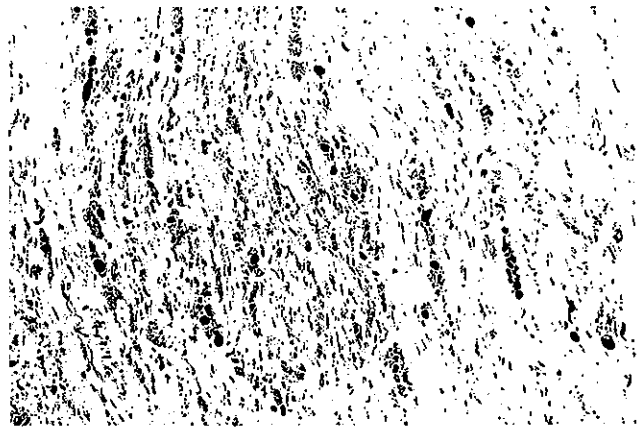

Fig. 2 Iliopsoas muscle of Case 2. Various sizes and shapes of Periodic acid Schiff (PAS)-positive substances are observed in the myocytes (PAS, ×200).

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.

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＜症例報告＞

インフルエンザ予防接種による oculo-respiratory syndrome の1例

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Ⅰ はじめに

インフルエンザウイルスの予防接種は，その効果への評価が改善するに伴い，接種率が向上してきている。群馬県における知的障害者の更生施設においてもワクチン接種が実施されてきているが，2000～2001年には約60%の利用者が接種を受け副反応の発生率は1.1%であった。われわれの重度知的障害者の更生施設では，2000年よりインフルエンザの発生予防および軽症化のためにほぼ全員の利用者に予防接種が実施されているが，本報告では2002～2003年の患者発生の状況および副反応と思われる興味ある症例の経験を報告する。

Ⅱ 方法と結果

常法によるインフルエンザ予防接種を受けた利用者数は，2001～2002年と2002～2003年の2シーズンにはそれぞれ491名，487名とほとんど同一（95.5%）であったが，患者発生寮数は4寮から1寮となり大きく改善された。患者発生数も21名から7名へと減少した。寮の中で感染者が発生した場合で，更に発熱者がでた場合には後鼻腔ウイルス抗原を調べ迅速に対処した。また，利用者を支援する職員の予防接種者数は不十分ではあるが，接種率の上昇（50/373＝13.3%，147/373＝49.4%）が得られた。このことは職員の感染予防意識の向上を反映しており，この点も患者減少に効果を示したと考えられる。

Ⅲ 症例提示

【症例1】 男，53歳

既往歴：2002年10月より高血圧症に対する服薬以外特記するものはない。

現病歴：生来健康であったが，2002年12月10日13時30分ごろ，インフルエンザHAワクチン（LotNo.HA031A）を0.5mL，左上腕伸側皮下に接種した。夕方になって右目に幕がかかり，像がぼけるようになり，20時ごろより痒みが生じるようになった。11日朝，眼科医を受診し，アレルギー性潰瘍性結膜炎の診断のもとに抗アレルギー薬（フサコール®点眼）が処方された。午後より咽喉から前胸部の異常感，全身倦怠感，発熱，意欲の減退感が生じた。12日昼ごろより

痒みが生じ，夕方のアルコール摂取により増強したので，13日，本診療所外来を受診した。

来院時現症：眼脂，眼瞼・眼球結膜の発赤，眼瞼周囲の発赤，顔面の浮腫状腫脹，全身に大小不同の深-浅紅色の多形紅斑，予防接種部位の発赤腫脹（10×10cm），左上腕-前腕の浮腫性腫脹，左脇窩リンパ節腫脹を認めた。口腔内および心肺，腹部に異常所見を認めなかった。脳神経症状には異常がなかった。

検査所見：白血球数5400（分画B0, E3, N55, L36, M5.3），CRP<0.3, ALT36, AST40, LDH353, CK129, 抗influenza抗体 H_1N_1<10, H_3N_2<10

臨床経過と予後：以上の所見よりインフルエンザ予防接種による副反応の診断のもとに，アタラックス®-P，グリチロン®の経口投与ならびに前記の点眼を行い，呼吸，眼症状は発症36時間には完治したが皮膚症状は徐々に改善し，5日目ごろには後遺症はなく治癒した。

Ⅳ 考　　案

これまでに報告されたインフルエンザ予防接種による副反応は，末梢神経障害をはじめとして多種にわたる。カナダのNational Advisory Committee on Immunization(NACI) の報告では2000～2001年に実施されたインフルエンザワクチンによって，2450名の副反応患者が発生し，眼または呼吸器症状は1735名と多発した。3社で作成されたワクチンはFluviral S/Fを用い，1200万本であったが，両眼と呼吸器症状および顔面浮腫状腫脹を主症状とするoculo-respiratory syndrome（以下ORSと略す）の患者は960名発生し，その96%の925名が接種されたワクチンは380万本を作成した1社製であった。カナダ政府は以上の症状を呈する副反応を新しい特異な疾患として認定し，予防接種に際して注意を喚起し，更に，賛同を得た発症患者への再接種を翌年に実施して，再発率が低いことを報告[1)~3)]した。1995～1996年のイタリアでは類似の粒子のワクチンによって，接種した65歳以上の24.7%の人に副反応が発生した報告[4)]があった。カナダでのコール酸Naを用いたトリバレントワクチンは，電顕ではaggregated unsplit virionが認められたが，添加物以外の混合物はなく，抗原は不活化されており病因は不明であった[1)]。カナダのORSの主要症状は予防接種後2～24時間以内に発症し48時間以内に改善する両側の眼球発赤，種々の呼吸器症状，顔面浮腫を呈し，発疹，掻痒，発熱を伴い40～59歳の女性に多かったという。

わが国ではニューカレドニア/20/99/H_1N_1，パナマ/2007/99/H_3N_2，山東/7/97株が使用されているが，製品の調整は各社独自である。本例は男性であるが，臨床経過はORSと一致し，本邦第1例と思われる。全身の発疹を含む全身症状を呈し，経過も遷延したのでより症状が重かったと思われた。抗アレルギー薬の早期投与が有効であったが，今後は本疾患の発生も考えられるので早期発見と治療が望まれる。

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〈シンポジウム 7—3〉医原性神経疾患と生物化学神経毒による神経障害

Reye 症候群と小児の薬剤性脳症

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（臨床神経，43：873—876, 2003）
Key words：Reye 症候群，アスピリン，RNA ウイルス脳炎，コレステロールエステル，ACAT

はじめに

Reye 症候群は，Reye ら，Johnson ら，Wood (1963) が報告した急性ミトコンドリア障害で急性脳症，選択的肝臓障害，内臓への脂肪酸浸潤をしめす疾患である[1~3]．Table 1 に示すように，先行するウイルス感染症，とくに上気道や消化器感染症や水痘に罹患後数日して脳浮腫による脳圧が亢進し，不幸な転機を取る．森嶋らによると，本疾患と現在注目を集めているインフルエンザ脳症とはことなる疾患である[4]と考えられている．欧米においては，発熱をともなうウイルス感染症のときにアセチルサルチル酸（アスピリン）を服用することによって発症すると考えられ，アスピリンの服用を発熱時に禁忌とする事によってその発症数が激減した[5]．わが国では，従来からアスピリンの使用が少ないこともあって，本症との関連は明らかではないが，同様にアスピリン使用の制限により減少している[6]．また，本症の類似疾患として Reye 様疾患が報告され，わが国における種々に薬物の服用によるミトコンドリアの脂肪酸代謝障害や先天性脂肪酸代謝異常症によって発症することが示されている．本疾患は，ウイルス感染の結果，宿主細胞の核酸におきる変化や，たんぱくの合成などを通して細胞の脂肪酸代謝障害をおこし，この代謝系に影響を持つ薬物の服用や先天性代謝障害によって異常が増強されることが主たる病態と考えられている．本シンポジウムではこれまでに報告された研究を総括すると共に感染によって発現した中枢神経系の ACAT による細胞膜のコレステロール回転亢進が果たす病因的役割に言及する．

わが国における Reye 症候群患者の推移

欧米では，Reye 症候群（以下 RS と略す）の病態解明の研究が進行していたが，Table 1 に示すように，先行するウイルス感染症，季節性，種々のサイトカインの関与，肝臓の病態などが明らかになった．解熱薬としてのアスピリンの使用頻度と本疾患発生との相関が証明された結果，アスピリンの使用制限が提案され実践された結果，RS 症例の発生頻度が激減したとの報告が出されている[7]．わが国における本症の疫学調査は，厚生省の班研究の一つとして，1984 年山下文雄によっ

Table 1　Reye 症候群の概念

- Reye et al (1963)
- Antecedent URI, bowel infect or varicella
- Viral RNA diverts host ER
- Endotoxin like activities
- Cytokine cascade (TNF etc)
- NH_3, FFA elevation
- Hepatic FA infiltration
- Mitochondrial damage

Table 2　Reye 症候群の分類

	急性脳浮腫	肝機能異常	肝生検異常	特徴的病理所見
確定的	●	●	●	●
疑似	●	●	●	×
臨床的	●	●	×	×

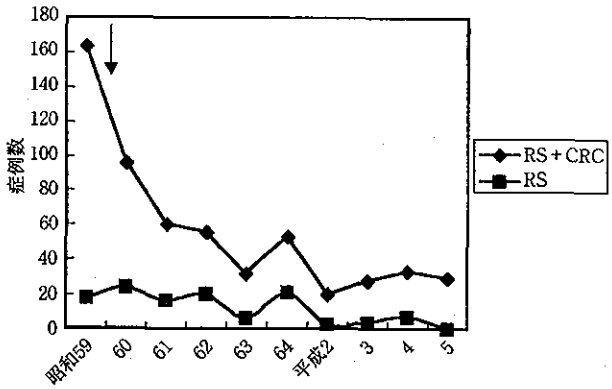

Fig. 1　RS+CRS 推定発生数の年次推移（北川．平成 8 年）
↓は厚生省によるアスピリン市販の自粛指導

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（受付日：2003 年 5 月 17 日）

Table 3　臨床像の違い

	Reye 症候群	Flu 脳症
Flu 型	B	A
年齢	学童	乳幼児
先行感染（発症病日）	4—7	0—1
特徴的嘔吐	+	−
意識障害	++	++
薬の使用	アスピリン	解熱薬
先行感染逸脱酵素	++	++
NH3	↑	→
血糖	↓	↑
MRI 異常	++	− or +→
死亡率（%）	50	30

Table 4　Reye 様症候群を起こす代謝異常症

Acyl CoA dehydrogenase deficiency
MCAD, VLCAD, MADD, 3-OH ADD
Glutaric aciduria
type Ⅱ, 3-OH 3-methyl
Carnitine palmitoyl transferase deficiency
Amino acid metabolic diseases
Fructose-1,6-diphosphatase deficiency
OCT- or CPS-deficiency

Table 5　Reye 症候群発症が疑われる薬物

解熱薬（ASA, DFN, AAP）
気管支拡張薬（テオドール）
抗菌薬
抗ヒ薬（ベリアクチン，タベジール）
脳代謝促進薬（HPA）
抗てんかん薬（VPA, DZP, ECG）
制吐薬（ナウゼリン）
止痢薬

Table 6　膜脂質の代謝カスケード

PC, PE, PS
プロスタグランジン形成
PI
DAG, フォスフォイノシトール
Ca イオン
中性プロテアーゼ活性化
SM
セラミド
スフィンゴシン

て開始され，堀　誠から北川照男へとバトンタッチされ1996年に終了された[6]．Table 2に示すように，RSには確定的(臨床経過および病期中の肝生検組織に特徴的病変陽性)，擬似(臨床経過および病期中の肝生検異常陽性)，臨床的(臨床経過

Table 7　Fatty acids composition of cholesterol esters in the rat brain with measles encephalitis

	1		2	Serum
	L	R	L + R	
14:0	6.1	2.0	10.3	tr
16:0	45.3	50.1	45.1	9.2
16:1	3.8	15.0	12.1	2.8
18:0	25.0	9.9	10.2	tr
18:1	21.4	17.9	17.6	8.0
18:2	tr	tr	tr	20.9
20:4	tr	tr	tr	56.8

Values are expressed as percentages of total fatty acids. L and R, left and right hemispheres; tr, trace. Experiments 1 and 2 indicate separate experiments with measles virus.

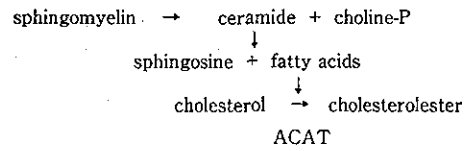

Fig. 2　ACAT の関与の模式図

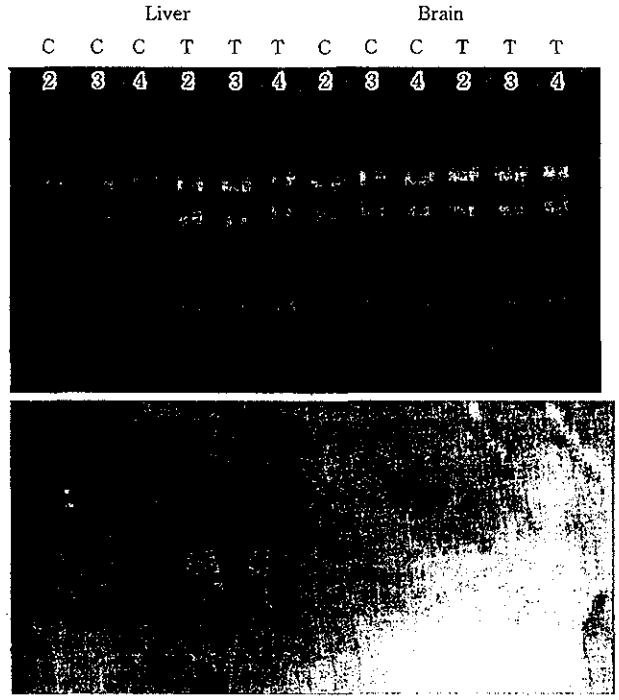

Fig. 3　Northern blotting of ACAT-1 in measles virus-infected rat brain
上図はETBr染色，下図はNothern blotting.
C：コントロール，T：感染
Total RNA preparations were prepared from infected rat brain by ISOGEN. Fourteen mg of total RNA was fractionated by electrophoresis with 1% agarose gel containing formaldehyde, and then was transferred to nylon membrane. Control rats were treated with culture medium for measles virus.