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発達障害と遺伝 —最近の知見—

杉 江 陽 子*1. *2, 杉 江 秀 夫*2

はじめに

　発達障害とは，遺伝要因も含めて発達期，特に早期に多くの因子が作用し脳の機能障害が起こり，それによって発達が損なわれた状態をいう．精神発達遅滞，言語発達障害，学習能力の特異的発達障害，運動機能発達障害，広汎性発達障害など非常に広範囲にわたっているが，ここでは自閉性障害(以下自閉症)を例に説明し，精神遅滞，言語発達障害，学習障害について簡単に述べた．注意欠陥/多動障害(精神医学的には行動障害に分類されるが)についても簡単に触れた．

遺伝について

　一般に遺伝病とは，一つの遺伝子に異常(疾患原因遺伝子)があるために病気になってしまう単一遺伝子疾患をいうが，生活習慣病や癌などのように複数の遺伝子に変化(疾患感受性遺伝子)がありその上に環境因子が関わって発病する多因子疾患がある(図1)．今回の発達障害はいずれもその多くが多因子疾患と考えられている．多因子疾患では一つの遺伝子の変化だけで発病することなく，疾患感受性遺伝子の中で変化をもった遺伝子の数が多いほど発病の危険が増すと考えられている．病気が家系内に多く
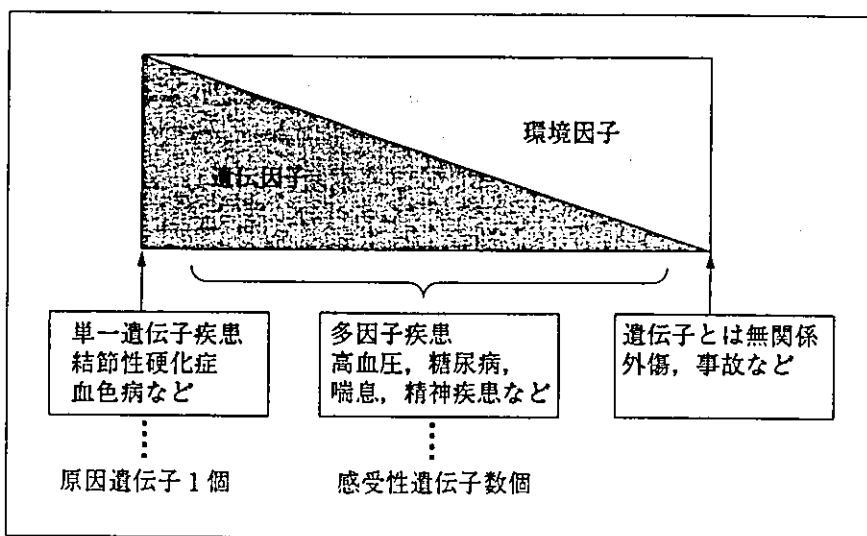

図1　病気にかかわる遺伝因子と環境因子

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みられ，双子研究で一卵性双生児の一致率が二卵性一致率より高く，兄弟の罹患率が一般の罹患率より高いなどの特徴があり，これらが地域を越えて共通して認められると多因子疾患が疑われる．これら多因子疾患の感受性遺伝子をみつけるためにどのようにして研究が進められているかを簡単に紹介する．なお，文中，図中にでてくる略語は表1を参照とする．

表1 略語

VNTR	variable number of tandem repeat
SNP	single nucleotide polymorphism
AUTS1	a susceptibility gene locus for autism on chromosome 15q (7q?)
AUTS2	a susceptibility gene locus for autism on chromosome 7q
AUTS3	a susceptibility gene locus for autism on chromosome 3q
GABA	γ aminobutyric acid A
5-HTT	serotonin transporter
MKRN3/ZNF127	makorin ring finger protein 3
SNRPN	small nuclear ribonucleoprotein polypeptide N
UBE3A	ubiqutin protein ligase E3A
GABRB3	γ-aminobutyric acid A receptor β3
GABRA5	γ-aminobutyric acid A receptor α5
GABRG3	γ-aminobutyric acid A receptor γ3
OCA2	oculo cutaneous albinism
HERC2	hect domain and RLD2
APBA2	amyroid β(A4)precursor protein-binding family A member 2
PTPN9	protein-tyrosine phosphatase nonreceptor-type 9
HOXA1	homeo box A1
RELN	reelin
IMMP2L	inner mitochondrial membrane peptidase 2-like
WNT2	wingless-type MMTV integration site family member 2
FOXP2	forkhead box P2
SPCH1	speech and language disorder 1
5-HT2AR	serotonin 2A receptor
MECP2	methyl CpG-binding protein
AGTR2	angiotensin II receptor
ARHGEF6	rho guanine nucleotide exchange factor 6
ARX	aristaless-related homeobox gene
FACL4	fatty acid-CoA ligase 4
FMR2	fragile-X mental retardation 2
GDI1	guanine dissociation inhibitor 1
IL1RAP1	interleukin-1 receptor accessory proteinlike 1
OPHN1	oligophrenin 1
PAK3	p21-activated kinase
TM4SF2	transmembrane 3 superfamily member 2
VCX-A	variable charged
DYX2	dyslexia specific 2
DYX3	dyslexia specific 3
DAT1	dopamine transpoter
DRD4	dopaine receptor D4

遺伝性疾患の研究の方法

　大きく分けて2つの方法がある．候補遺伝子法とゲノムスクリーン法である．

　候補遺伝子法は従来から行われている方法で，ある仮説のもとに可能性のありそうな遺伝子を選びその遺伝子について直接調べていく方法である．ある仮説とはその疾患の生物学的特徴や染色体異常合併例，単一遺伝子疾患に合併する例などこれまでの多くの報告を参考にして選択される．

　最近の遺伝研究には目覚しいものがあり，ヒトの染色体の地図が完成され，これで得られた情報をもとに研究方法が大規模かつ網羅的になってきている．ヒトには22組の常染色体と1組の性染色体があるが，ゲノムスクリーン法とはまず全部の染色体を見渡して，染色体のどの辺りにその病気を起こす遺伝子がありそうか検討をつける解析方法である．遺伝子は4つの塩基(A，G，T，C)が連なってできているが，その配列のなかで個人差のある部分があり遺伝子多型とよばれている．遺伝子多型には挿入／欠失型，VNTR，マイクロサテライト，SNPがあり，これらが遺伝子上にVNTRで5000個以上，SNPで数百万個ありとても良い指標(マーカー)とされている．マーカーの中からある間隔ごとに250から500個を選んでしらみつぶしに調べ，病気と正常のヒトで比較して病気のヒトである多型に偏って多いマーカーが見つかると，その近くにその病気の感受性遺伝子がある可能性が高いと判断される(連鎖不均衡)．あるいは罹患同胞対を多数のペアでマーカーを調べ，兄弟で一致率の高いマーカーを探す方法などもある．これらの方法は大量の遺伝子を高速で処理する必要があり，それに対処できる専門の施設で行われる．

広汎性発達障害

　自閉症の特徴的な事柄を表2にまとめた．自閉症が遺伝的疾患であるとされる根拠の一つは双子研究と家族研究の結果からである．一卵性双生児の一致率は70%(36〜90%)と高く，二卵性双生児の一致率は10%(0〜23.5%)である．ここで一卵性双生児でもけっして100%でないということは，遺伝だけではない，つまり環境因子などが関与する可能性のあることを示唆していると思われる．兄弟の自閉症発病危険率は2〜6%とされ，一般における発病率0.02〜0.1%より高値であり，一般人口の有病率の約100倍に当たる．関与している遺伝子はおそらく3〜15個位なのではないかと考えられている．

　自閉症の中には染色体異常を伴うものがある．自閉症の染色体異常の頻度は約5〜12%とされ，14と20番を除きほとんどすべての染色体について報告があり，最も頻度が高いのは15番染色体である．脆弱X症候群の6.9〜

表2　自閉症の特徴

定義	DSM-IV, ICD-10
	3歳以前発症
三主徴	対人関係の障害
	コミュニケーションの障害
	行動，興味の限定，常同的
遺伝学的特徴	
頻度	2-5人/10000人〜1人/1000人
男女比	3〜4：1
双子一致率	一卵性36〜90%
	二卵性0〜23.5%
兄弟発症率	2〜6%
生物学的特徴	
精神遅滞の合併	約75%
てんかんの合併	20〜33%
頭が大きい	80%が平均以上
	24%が2SD以上
血中セロトニン値	26〜66%の症例で増加
薬物	SSRI, SDI有効例あり

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
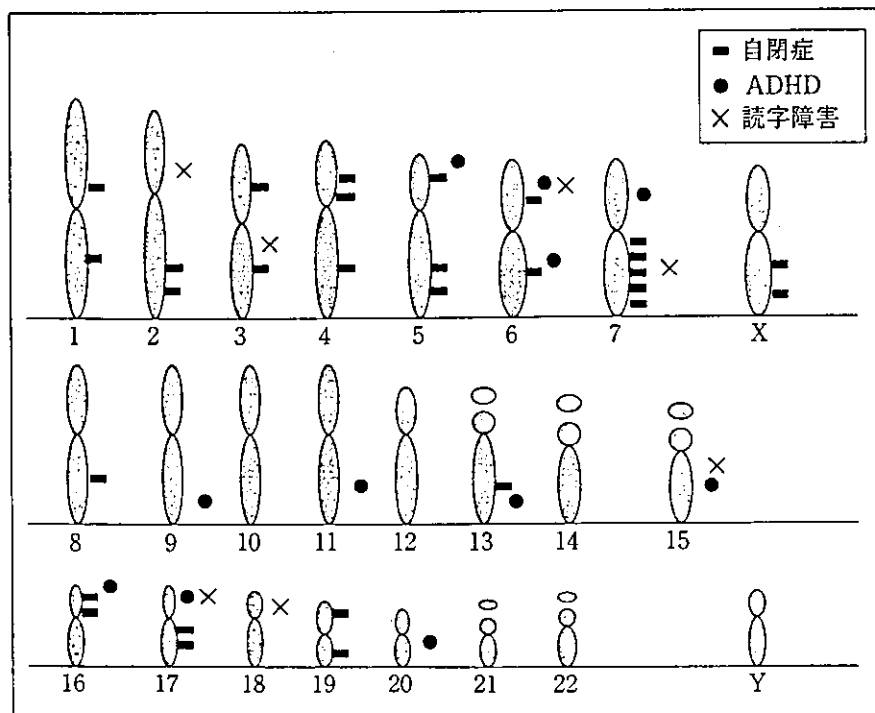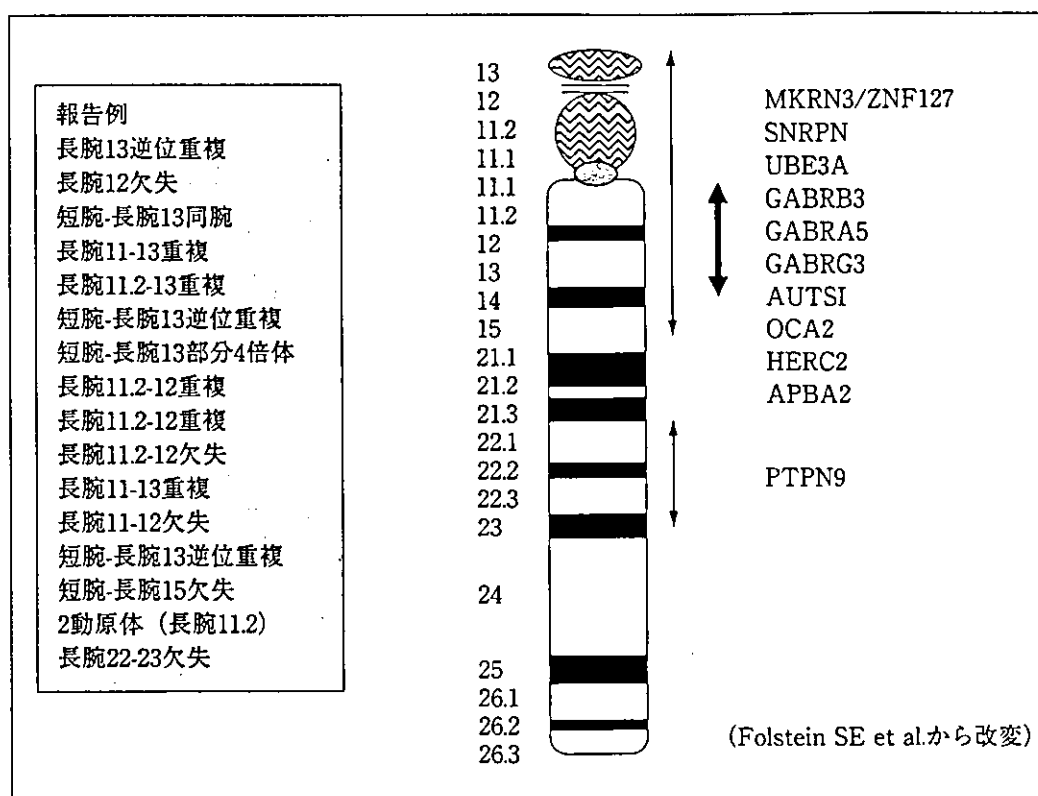

図2　ゲノムスクリーン　候補マーカー部位

報告例
長腕13逆位重複
長腕12欠失
短腕-長腕13同腕
長腕11-13重複
長腕11.2-13重複
短腕-長腕13逆位重複
短腕-長腕13部分4倍体
長腕11.2-12重複
長腕11.2-12重複
長腕11.2-12欠失
長腕11-13重複
長腕11-12欠失
短腕-長腕13逆位重複
短腕-長腕15欠失
2動原体（長腕11.2）
長腕22-23欠失

13
12
11.2
11.1
11.1
11.2
12
13
14
15
21.1
21.2
21.3
22.1
22.2
22.3
23

24

25
26.1
26.2
26.3

MKRN3/ZNF127
SNRPN
UBE3A
GABRB3
GABRA5
GABRG3
AUTSI
OCA2
HERC2
APBA2

PTPN9

（Folstein SE et al.から改変）

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
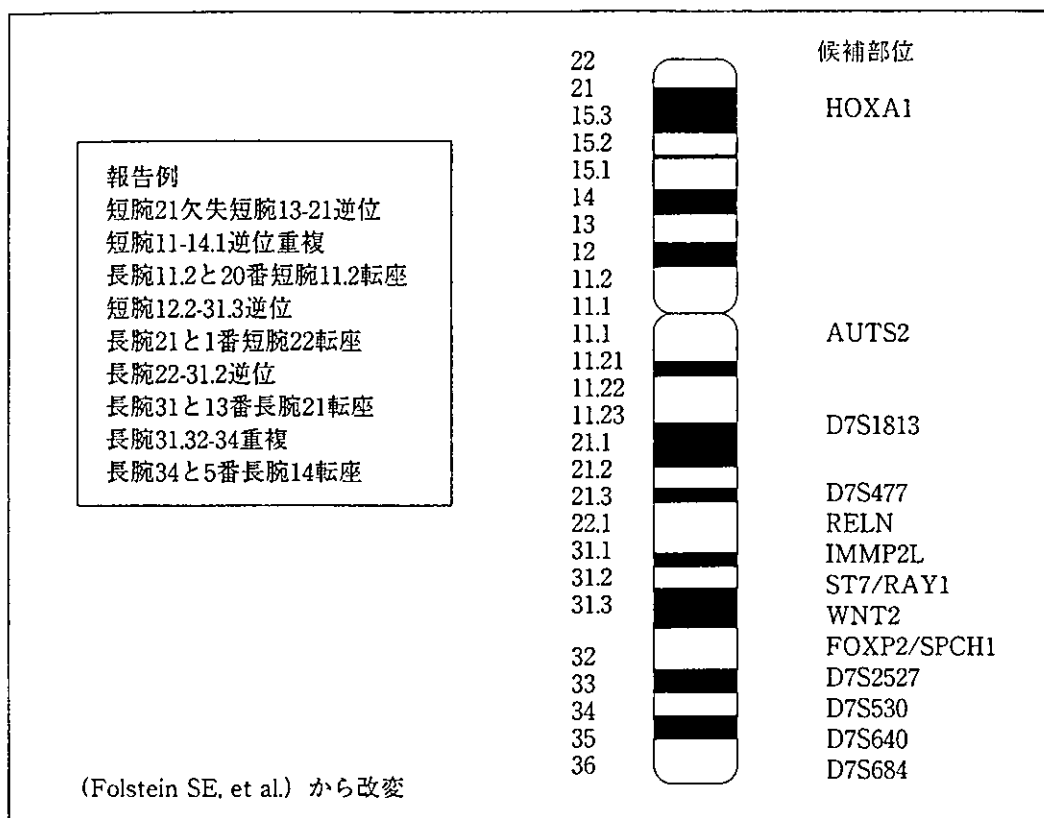
報告例	候補部位

報告例
短腕21欠失短腕13-21逆位
短腕11-14.1逆位重複
長腕11.2と20番短腕11.2転座
短腕12.2-31.3逆位
長腕21と1番短腕22転座
長腕22-31.2逆位
長腕31と13番長腕21転座
長腕31.32-34重複
長腕34と5番長腕14転座

22
21
15.3　　　HOXA1
15.2
15.1
14
13
12
11.2
11.1
11.1　　　AUTS2
11.21
11.22
11.23　　　D7S1813
21.1
21.2
21.3　　　D7S477
22.1　　　RELN
31.1　　　IMMP2L
31.2　　　ST7/RAY1
31.3　　　WNT2
　　　　　FOXP2/SPCH1
32　　　　D7S2527
33　　　　D7S530
34　　　　D7S640
35
36　　　　D7S684

(Folstein SE, et al.) から改変

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

遺伝因子　　　　　　　　　　　環境因子

自閉症遺伝子群
3～15個

染色体異常　　　　　　　　　出生前

単一遺伝子疾患　　　　相互作用　　　周生期～
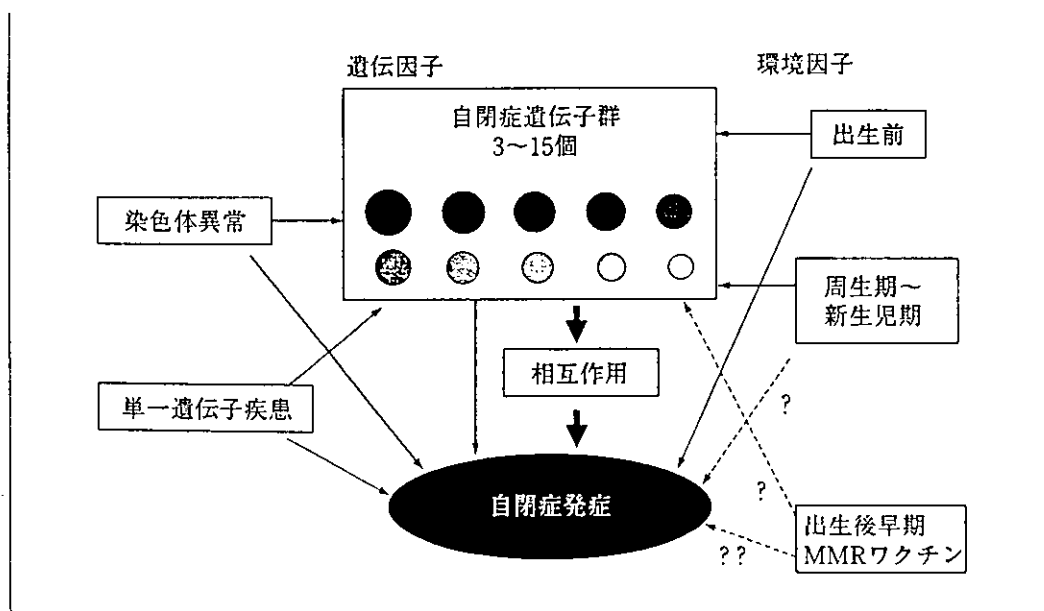　　　　　　　　　　　　　　　　　新生児期

自閉症発症

出生後早期
MMRワクチン

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．

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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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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

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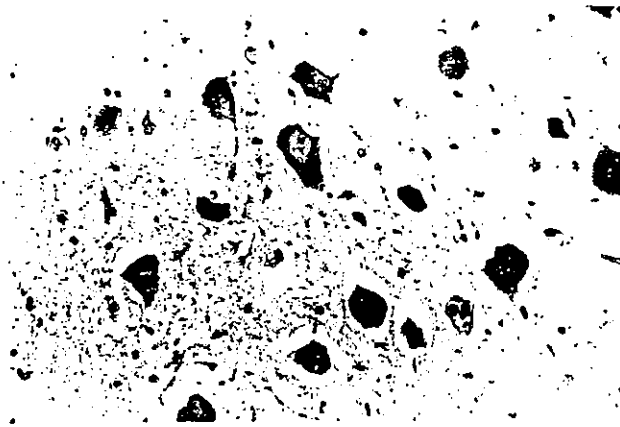
Fig. 1 Medulla oblongata of Case 1. Periodic acid Schiff (PAS)-positive granular deposits are seen in the cytoplasm of neuronal cells (PAS, ×400).
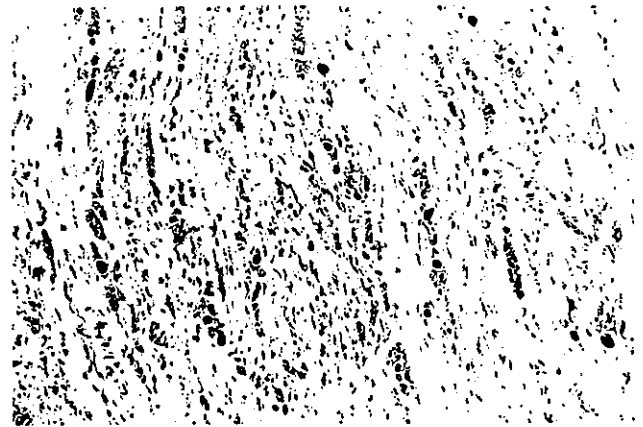

Fig. 2 Iliopsoas muscle of Case 2. Various sizes and shapes of Periodic acid Schiff (PAS)-positive substances are observed in the myocytes (PAS, ×200).

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,EI	M,EI	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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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 (GA$\underline{T}$ to GA$\underline{C}$) on exon 9 and Ala703 (GC$\underline{T}$ to GC$\underline{C}$ 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.
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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 mm³). 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-
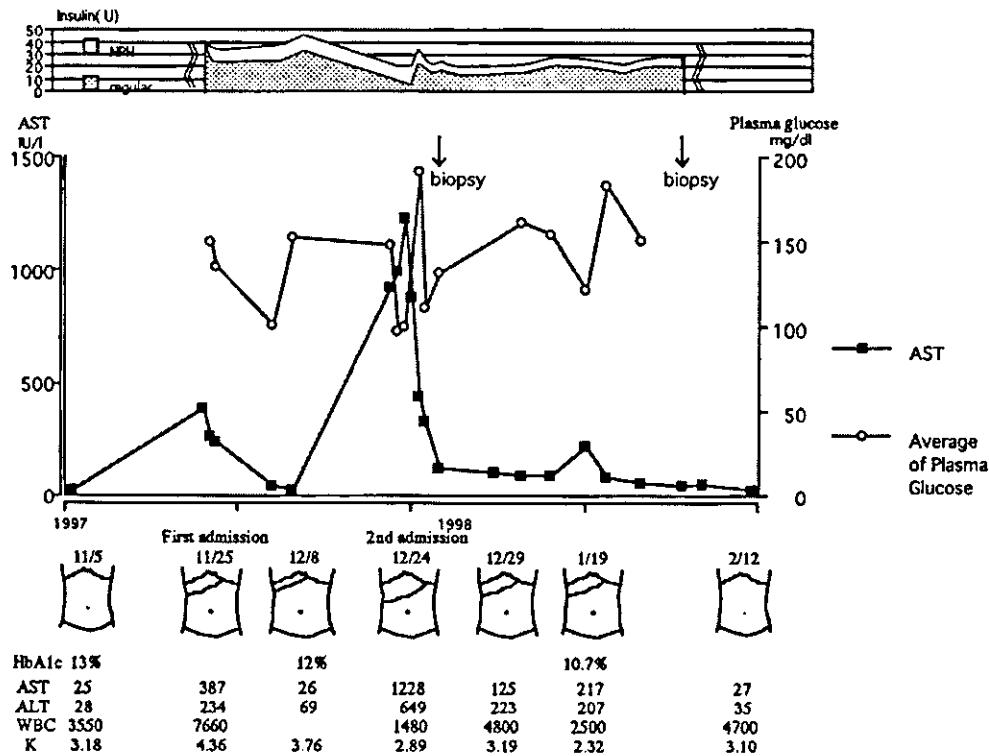
Fig. 1. Clinical course of hepatomegaly and liver function related to plasma glucose, serum K and WBC.

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′,

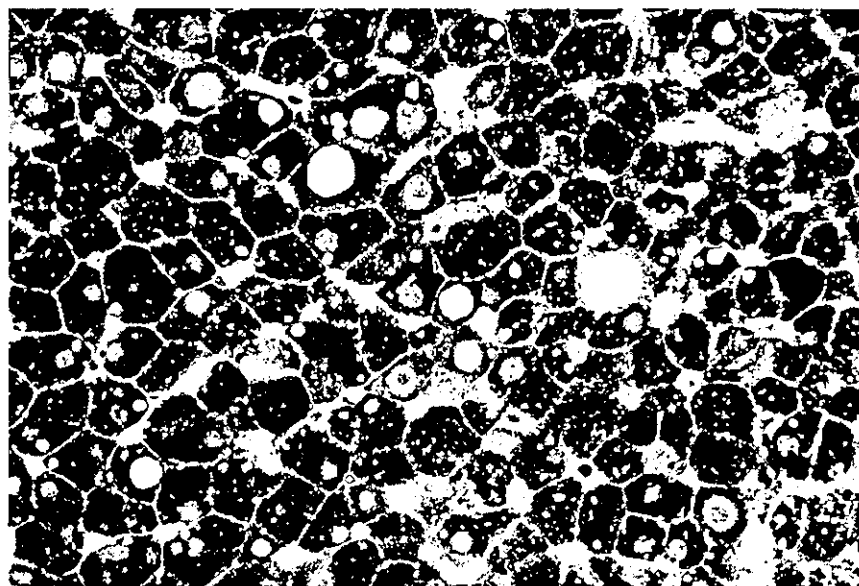Exon 17 + 282 > 5′-GGAAGCCCTCTGAGGTC-ACATACC-3′.

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 acces-

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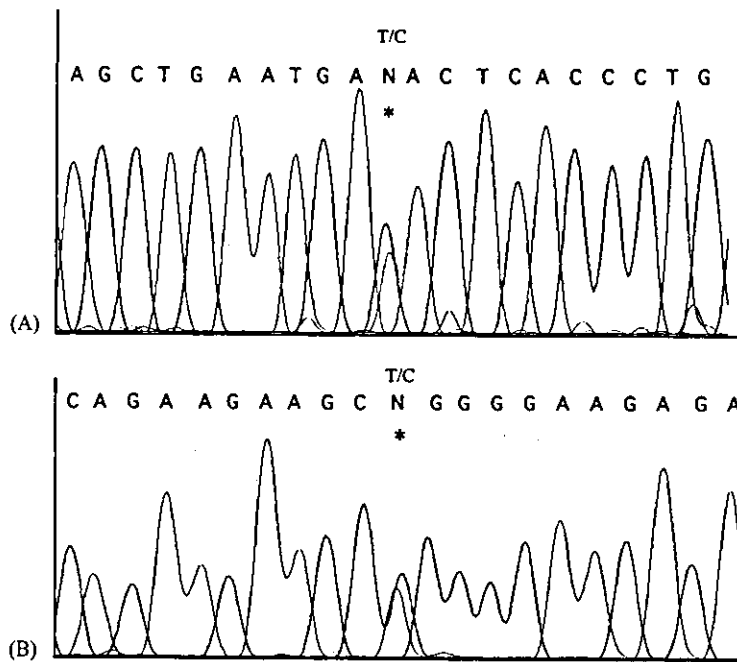
Fig. 3. The electropherograms of *PYGL* gene in the patient. (A) Asp339 (GA**T**/GA**C**) on exon 9. (B) Ala703 (GC**T**/GC**C**) on exon 17.

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

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