図2　LC/MS アミノ酸分析計の仕組み

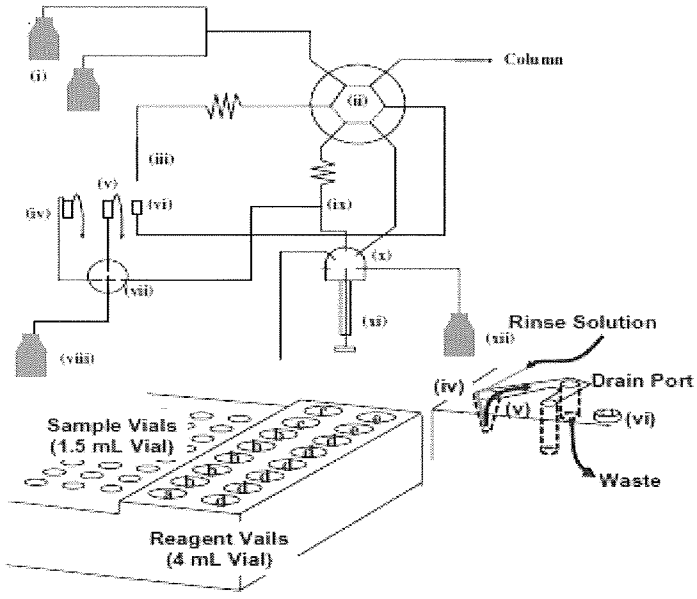

表1　血漿検体を分析した場合の分析精度（CV 値）およびアミノ酸回収率

Table 4. Recovery rates of the amino acids spiked in plasma sample ($N = 5$)

Amino acid		Plasma			
		Concentration [μM]	CV [%]	Recovery rates[%]	
				250 μM addition	25 μM addition
Glycine	Gly	266.3	5%	111%	78%
Alanine	Ala	387.8	2%	107%	78%
Serine	Ser	123.0	3%	108%	93%
Proline	Pro	105.7	8%	97%	100%
Valine	Val	154.9	8%	98%	106%
Threonine	Thr	111.4	4%	99%	77%
Taurine	Tau	40.7	5%	92%	100%
Isoleucine	Ile	45.8	4%	102%	113%
Leucine	Leu	77.6	8%	111%	110%
Aspragine	Asn	44.1	7%	100%	106%
Glutamine	Gln	717.7	5%	81%	57%
Glutamic Acid	Glu	14.9	7%	98%	95%
Methionine	Met	26.0	8%	107%	104%
Histidine	His	65.8	6%	99%	72%
Phenylalanine	Phe	45.8	5%	106%	105%
Arginine	Arg	79.8	3%	100%	105%
Citrulline	Cit	19.5	7%	91%	94%
Tyrosine	Tyr	59.4	4%	105%	112%
Trptophan	Trp	52.0	7%	103%	100%
Ornithine	Orn	40.3	6%	102%	109%
Lysine	Lys	144.1	6%	97%	86%
Cystine	Cys2	12.5	13%	107%	59%

表2　LC/MS法とニンヒドリン法の比較

Table 5. Amino acid concentrations in plasma from 10 healthy volunteers and calculated Fischer index as analysis by LC-MS and ninhydrin (values are given in μM as means ± standard deviation)

Amino acid		LC-MS	Ninhydrin[a]
Glycine	Gly	267.9 ± 40.2	262.3 ± 40.1
Alanine	Ala	422.0 ± 51.1	413.3 ± 50.5
Serine	Ser	123.0 ± 19.5	131.5 ± 19.2
Proline	Pro	174.4 ± 48.0	177.7 ± 44.0
Valine	Val	215.3 ± 43.9	222.4 ± 42.3
Threonine	Thr	135.1 ± 23.5	144.6 ± 30.9
Taurine	Tau	47.8 ± 10.3	47.7 ± 5.7
Isoleucine	Ile	63.8 ± 16.3	63.8 ± 15.3
Leucine	Leu	106.9 ± 26.9	112.4 ± 24.0
Aspragine	Asn	50.6 ± 8.3	56.3 ± 6.4
Glutamine	Gln	579.4 ± 116.6	585.5 ± 112.1
Glutamic acid	Glu	24.3 ± 15.6	33.6 ± 12.1
Methionine	Met	30.1 ± 5.6	30.1 ± 6.5
Histidine	His	77.5 ± 5.6	80.8 ± 8.4
Phenylalanine	Phe	63.3 ± 8.8	63.9 ± 8.8
Arginine	Arg	100.0 ± 12.4	102.5 ± 16.2
Citrulline	Cit	29.3 ± 3.8	32.2 ± 5.2
Tyrosine	Tyr	66.1 ± 8.4	70.2 ± 10.3
Tryptophan	Trp	63.4 ± 9.5	57.5 ± 9.1
Ornithine	Orn	53.3 ± 11.3	56.9 ± 10.9
Lysine	Lys	174.3 ± 30.1	193.2 ± 29.4
Cystine	Cys2	36.5 ± 9.8	50.6 ± 8.3
Fischer index		2.98 ± 0.50	2.95 ± 0.42

[a] Amino acids detected with ninhydrin reagent were determined with post-column derivatization on an automatic amino acid analyzer (L-8800; Hitachi High-Technologies, Tokyo, Japan) after protein precipitation by 5-sulfosulytiric acid.

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
遠藤文夫	尿素サイクル異常症	日本先天代謝異常学会	症例から学ぶ先天代謝異常症	診断と治療社	東京	2009	67-69

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Endo F et.al	Mutant alleles associated with late-onset ornithine transcarbamylase deficiency in male patients have recurrently arisen and have been retained in some population	J Hum Genet.	55(2)	18-22	2010
Endo F et.al	Differences between the amino acid concentrations of umbilical venous and arterial blood.	Arch Dis Child Fetal Neonatal Ed.	94(2)	F155-6	2009
Endo F et.al	Management of undifferentiated sarcoma of the liver including living donor liver transplantation as a backup procedure.	J Pediatr Surg.	44(2)	e33-8	2009
大浦敏博	シトリン欠損症研究の進歩～発症予防・治療法の開発に向けて	日本小児科学会雑誌	113巻11号	1649-53	2009
大浦敏博　他	テトラヒドロビオプテリン（BH_4）反応性フェニルアラニン血症診断のためのBH_4供給について	日本小児科学会雑誌	113巻11号	1758	2009
大浦敏博　他	テトラヒドロビオプテリン（BH_4）反応性高フェニルアラニン血症に対する天然型BH_4製剤塩酸サプロプテリンの適正使用に関する暫定指針	日本小児科学会雑誌	113巻3号	649-653	2009

Ⅳ. 研究成果の刊行物・別刷

F 尿素サイクル異常症 Meet the Expert

1 尿素サイクル異常症の発症形態

アンモニアから尿素を合成するための代謝経路である尿素サイクルあるいはこれに関連したアミノ酸の転送などの異常によって，血液中のアンモニアおよび関連したアミノ酸などの代謝産物が血液中あるいは体液中で上昇し，高アンモニア血症とそのほかの症状が出現する．尿素合成に関与する5種の酵素の欠損がある(表1)．

重症の酵素欠損では尿素サイクルの最初の4種の酵素(CPSI，OTC，ASS，ASL)の欠損あるいは関連した活性化因子の合成に必要な酵素(NAGS)の欠損の場合，生後数日以内にアンモニアと疾患に関連したそのほかの代謝物の蓄積にいたる．出生時は正常であるがアンモニアの上昇とともに急速に脳浮腫とこれに関連した徴候を示す．不活発，食欲不振，過呼吸，呼吸不全，低体温，けいれん，後弓反張，昏睡など多彩な症状がみられる．尿素サイクルの酵素の部分的な欠損によって軽症の症状で発症も多い．軽症患者ではアンモニアの蓄積は新生児期以降のいかなる時期にも発症し，血中アンモニア濃度の上昇あるいは精神発達遅延，失調，四肢麻痺など様々な症状が出現する．アンモニアの蓄積は，発熱，感染，飢餓その他のストレスなどによって誘発される．

2 検査値の異常

a. 血中アンモニア

本症の診断では幅広い臨床症状を念頭においてアンモニアを測定することが必須である．先天代謝異常症が疑われる例では血糖と血液ガスと共にかならずアンモニアを測定する．意識障害，けいれん，肝腫大，精神発達の遅れ，原因不明のトランスアミナーゼの増加例などがあれば必ずアンモニアの測定を試みる．

b. 一般生化学検査

BUNの低値は尿素サイクルの機能低下の可能性を示唆する．AST，ALTの上昇はよくみられる．代謝性アシドーシス，尿酸の高値は有機酸血症の存在を疑わせ，白血球減少，血小板減少はメチルマロン酸血症あるいはプロピオン酸血症の存在を疑わせる．

c. 血中尿中アミノ酸の分析

シトルリン血症，アルギニノコハク酸尿症，アルギニン血症はアミノ酸分析でほぼ診断でき

表1 高アンモニア血症をもたらす先天代謝異常症(アンモニア代謝異常症)

1	尿素サイクルの一次的機能異常	カルバミルリン酸合成酵素欠損症，オルニチントランスカルバミラーゼ欠損症，古典型シトルリン血症(アルギニノコハク酸合成酵素欠損症)，アルギニノコハク酸尿症(アルギニノコハク酸分解酵素欠損症)，アルギニン血症(アルギナーゼ欠損症)
2	尿素サイクルに関連したアミノ酸転送障害	シトリン異常症(成人発症シトルリン血症II型)，リジン尿性蛋白不耐症(リジン尿症)，高オルニチン血症－高アンモニア血症－ホモシトルリン尿症候群(Hyperornithinaemia-hyperammonaemia-homocitrullinuria HHH症候群)
3	尿素サイクルに関連した転送障害	高オルニチン血症を伴う脳脈絡膜脳膜色素変性症 Gyrate atrophy of retina(オルニチンケト酸アミノ基転移酵素欠損症)，1-pyrroline-5-carboxylate synthetase 欠損症
4	その他のアンモニア代謝障害	高インスリン血症高アンモニア血症症候群
5	有機酸代謝障害に伴う高アンモニア血症	メチルマロン酸血症，プロピオン酸血症，全身性カルニチン欠乏症　など

る．また血中アルギニン，シトルリンの低下，オルニチンの上昇，低下も重要な所見である．シトルリンの低値はCPS I欠損症，OTC欠損症，リジン尿症(LPI)の診断に重要である(図1，表2)．

d. 脳MRIあるいはCT検査……

高アンモニア血症による脳浮腫は急性期に出現する．長期間高アンモニア血症の影響を受けると脳萎縮が認められる．

e. 有機酸分析・尿オロト酸……

鑑別診断には尿中有機酸分析あるいは血液アシルカルニチンの分析を行う．尿中オロト酸あるいはウラシルなどの増加は尿素サイクル異常症を疑わせる．ただしCPSI欠損症とNAGS欠損症では尿中にオロト酸は排泄されないので鑑別診断に重要である．尿オロト酸排泄をマーカーとしたOTC欠損症のヘテロ接合体女性の診断ではアロプリノール負荷試験が行われる．

f. 酵素診断・遺伝子診断確定診断……

最終診断には酵素診断あるいは遺伝子診断が必要な場合がある．また出生前診断や発症前診断を目的にする場合にも酵素診断および遺伝子診断が必要になる場合がある．ただし個々の遺伝子診断については状況により可能な場合があるというのが現状である．

3 保存しておくべき試料

保存用の資料として血清，尿は診断に重要である．特に濾紙血液は極めて有用である．濾紙を用いてDNA分析も可能でありタンデムマスでも一部のアミノ酸の解析は可能．線維芽細胞，白血球，肝臓は酵素活性の測定および遺伝子診断に用いることができる．

4 治療と管理

治療の原則は血中アンモニアの低下を図り，脳障害を防止することである．ピークのアンモニアが480 μmol/L以上あるいは初期のアンモニアが300 μmol/Lの場合，知的障害が生じるので血中アンモニアの低下を図る．急性増悪期には血液透析，ろ過透析(一部では腹膜透析)による血症アンモニア濃度の低下を図り，経静脈的なアルギニン塩酸塩水溶液の投与，安息香酸ナトリウム，フェニル酢酸ナトリウム(フェニル酪産ナトリウム)の投与が行われる．最初の24～48時間には蛋白質の摂取を強く制限し，食事中の窒素の含有量を減少させる．また炭水化物によるエネルギーの投与(経静脈的な高濃度グルコース投与)を投与して体蛋白質の分解を防止する．経静脈的な水溶液の投与によって生理機能を安定させる．ただし，水分の過剰な投与は脳浮腫を招きあるいは悪化させる．これらの処置によって神経学的障害をできるだけ軽減することが重要である．安定期(慢性期)には食事による蛋白制限，必須アミノ酸の投与，シトルリン(アルギニン)，安息香酸ナトリウム，フェニル酢酸ナトリウム(フェニル酪酸ナトリウム)の経口投与を行いアンモニア値を安定させるとともに急性増悪の予防を行う．

表2 尿素サイクル異常症におけるアミノ酸の異常

NAGS欠損症	グルタミン，グルタミン酸の上昇，シトルリン低下，アルギニン低下
CPSI欠損症	グルタミン，グルタミン酸の上昇，シトルリン低下，アルギニン低下
OTC欠損症	グルタミン，グルタミン酸の上昇，シトルリン低下，アルギニン低下
シトルリン血症(古典型)	シトルリン増加，アルギニン低下，グルタミン，グルタミン酸増加
アルギニノコハク酸尿症	血中アルギニノコハク酸増加，シトルリン増加，アルギニン低下，グルタミン，グルタミン酸増加，尿中アルギニノコハク酸増加
アルギニン血症	血中アルギニン増加

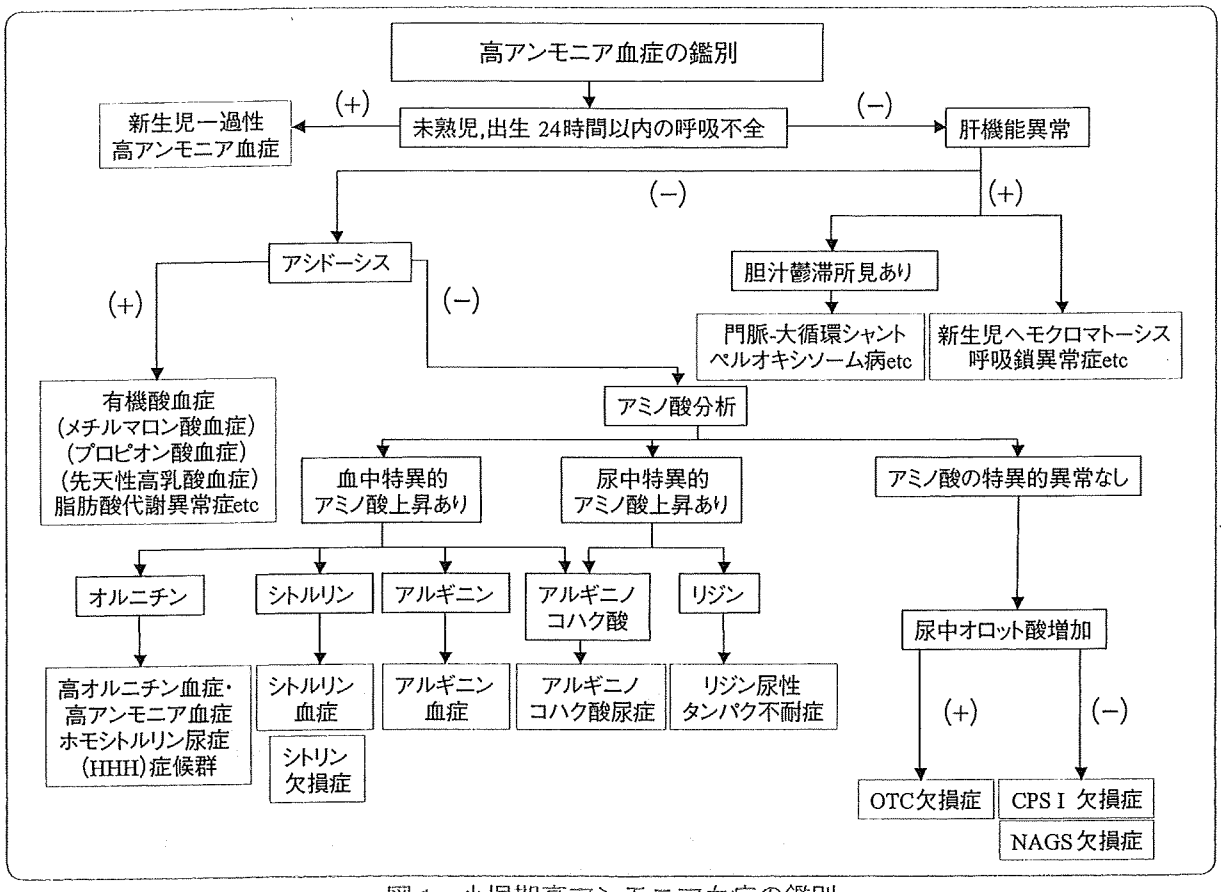

図1　小児期高アンモニア血症の鑑別

新生児期小児期に発症する高アンモニア血症の鑑別診断を示す．尿素サイクル異常症以外の肝臓に関連した疾患，門脈体循環シャント，アミノ酸の転送に関連したアミノ酸代謝異常症，シトリン異常症など鑑別すべき疾患は多い．

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ORIGINAL ARTICLE

Mutant alleles associated with late-onset ornithine transcarbamylase deficiency in male patients have recurrently arisen and have been retained in some populations

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We performed haplotype analysis using nine single nucleotide polymorphisms in the ornithine transcarbamylase gene to explore the ancestral origins of three mutations associated with late-onset phenotype in male patients: p.R40H, p.R277W and p.Y55D. Overall, 8 haplotypes were defined among 14 families carrying p.R40H, 5 families carrying p.R277W and 2 families with p.Y55D mutations. Of nine Japanese families carrying p.R40H, eight exhibited haplotype (HT)1, whereas the other family harbored HT2. Among three Caucasian families, one Spanish and one Australian family bore HT3; one Austrian family had HT4. Two US patients harbored HT2 and HT4. Among families carrying p.R277W, HT5 was found in one Japanese, one Korean and one US family. Two other US families had HT2 and HT6. Two families carrying p.Y55D, both Japanese, shared HT1. These results indicate that the p.R40H mutation has arisen recurrently in all populations studied, although there is evidence for a founder effect in Japan, with most cases probably sharing a common origin, and to a lesser extent in subjects of European ancestry (HT3). It is evident that p.R277W mutation has recurred in discrete populations. The p.Y55D mutation appears to have arisen from a common ancestor, because this transversion (c.163T>G) occurs rarely.
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Keywords: haplotype; late-onset; male; ornithine transcarbamylase deficiency; recurrent mutation; single nucleotide polymorphism

INTRODUCTION

Ornithine transcarbamylase (OTC) deficiency (OMIM no. 311250) is the most common inherited disorder of the urea cycle and is transmitted as an X-linked trait.

The locus of the gene encoding OTC is on the short arm of the X chromosome within band Xp21.1.[1] The gene spans 74 kb with an open reading frame of 1062 nucleotides distributed into 10 exons and 9 introns.[2,3] The phenotypes of females heterozygous for a mutant OTC allele vary from asymptomatic carrier state to overt, even fatal disease, depending first on the nature of the gene mutation, second on X-inactivation pattern and third on other genes and environmental factors. In contrast, in hemizygous male patients, the phenotype is determined by the nature of mutation and other yet unknown factors (other genes/environment). Such male patients most commonly develop symptoms of hyperammonemia in the neonatal period or in early infancy and their disease is often fatal.[4] However, there are some male patients in whom the onset of the disease is delayed until the preschool age period[5] through to adulthood.[6,7] Some affected males within the same families may remain asymptomatic for life.[8] Their condition is now recognized as 'late-onset OTC deficiency in male patients', accounting for ~30% of male patients.[9] Such male patients reproduce at a fitness value of 0.49.[10] Although the majority of mutations at human OTC locus are 'private', being observed in single families only,[11] several mutations have been observed repeatedly in discrete families,[12–14] mainly affecting CpG dinucleotides. Among those, the c.119G>A (p.R40H) and c.829C>T (p.R277W) mutations have been repeatedly reported in multiple ethnicities. In our previous series of Japanese families, the c.119G>A (p.R40H) mutation was encountered in a cluster.[7,10,15] In addition, we identified another novel mutation, c.163T>G (p.Y55D) in two discrete families.[10,16] It is not

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known whether or not these mutations share a common ancestral origin or have arisen recurrently.

Polymorphic sites in the human OTC gene having potential for family tracking were reported previously. These include single nucleotide substitutions, insertions and short tandem repeats.[9,17–22] After completion of the HapMap Project, single nucleotide polymorphisms (SNPs) have become available for haplotype analysis on a given gene.

However, SNP-based haplotype analysis of the human OTC locus has not been reported previously. We aimed to determine the haplotypes of these mutant OTC alleles to explore the origins of these mutations.

MATERIALS AND METHODS

Families

A total of 14 families with the c.119G>A (p.R40H) mutation, 5 families with the c.829C>T (p.R277W) mutation and 2 families with the c.163T>G (p.Y55D) mutation were studied. The 14 families with the p.R40H mutation consisted of 9 families from Japan, 1 family each from Spain, Australia and Austria, and 2 families from the United States. The families with p.R277W included 1 family from Japan, 1 family from Korea and 3 families from US. Two families with p.Y55D were both Japanese. The probands were all male, except the proband in family 11, a 13-year-old symptomatic girl who carried the p.R40H mutation, as did her asymptomatic mother. Families 1–9, 15, 20 and 21 were Japanese; family 16, Korean; and families 10–12, Caucasians.

None of the families in the present series were known to be related to each other. Demographic information, including ethnic background, of the US families (13, 14, 17–19) was not available because of the US Personal Data Protection Act. Haplotype analysis was performed on the proband from each family and their relatives, when specimens were available from them. In family 11, the DNA specimens from parents of the proband were also analyzed to determine the mutation-bearing allele. In family 12, the DNA specimen of the proband was prepared from the liver tissue obtained after it had been inadvertently transplanted to a woman.[23]

Haplotype analysis

A total of 9 tagged SNPs were selected on the Haploview[24] with r^2 of 0.80 and minor-allele frequency of 0.05 (Table 1). The haplotype frequencies of Japanese in Tokyo (JPT), Utah residents with Northern and Western European ancestry from CEPH collection (CEU) and Yoruban in Ibadan, Nigeria (YRI) were available on http://hapmap.ncbi.nlm.nih.gov/index.html.en. The nucleotide combinations in *Pan troglodytes* (chimpanzee) were obtained on http://www.ensembl.org/index.html to estimate the human ancestral alleles. The nucleotide in the polymorphic site 6 (rs5963421 for human) for *P. troglodytes* was represented by that in *Pongo pygmaeus abelii* (orangutan), because this nucleotide was not available for *P. troglodytes*.

The SNPs were determined by the TaqMan probe-based real-time PCR on LightCycler LC-480 (Roche Diagnostics GmbH, Mannheim, Germany). The PCR was carried out in a total volume of 20 μl, containing 20 ng genomic DNA, 10 μl LC-480 Probe Master (Roche) and 1 μl 20× Probe/Primer Mix (Applied Biosystems, LLC, Foster City, CA, USA). The probes used were labeled as either FAM or VIC. The temperature was programmed as follows: a pre-incubation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 60 s and extension at 72 °C for 1 s. At the end of the annealing step, the fluorescence signal was measured. After the PCR reaction, the temperature was decreased to 40 °C as a cooling step.

Ethical considerations

The Ethical Committee of Kurume University approved this project and specimens were obtained in accordance with respective institutional bioethical standards and relevant bioethical regulations or guidelines in each country.

RESULTS

Haplotypes defined in the families and their allelic frequency and heterozygosity

The data on the nine SNPs employed and the haplotypes determined in the families by the use of these SNPs are summarized in Table 1. Six discrete haplotypes (HT's) (1–6) were found among the probands. The parents of the female proband in family 11 carried two additional HT's, 7 and 8.

Table 1 Haplotypes generated by single-nucleotide polymorphism in human ornithine transcarbamylase locus

			Allele frequency heterozygosity			*Haplotype*								
Polymorphic sites	*Tagged SNPs*	*Nucleotide*	*JPN*	*CEU*	*YRI*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	Pan troglodytes
1	rs5917576	G/A	0.91/0.09 0.164	0.50/0.50 0.500	0.83/0.17 0.282	G	G	G	A	G	G	A	G	G
2	rs17274134	G/C	0.53/0.47 0.498	0.08/0.92 0.147	0.19/0.81 0.308	G	C	C	C	C	C	C	C	C
3	rs6417794	G/C	0.77/0.23 0.354	0.79/0.21 0.332	0.88/0.12 0.211	G	C	C	G	G	C	G	G	G
4	rs6609709	G/A	0.11/0.89 0.196	0.23/0.77 0.354	0.30/0.70 0.420	A	A	A	A	G	A	A	A	A
5	rs2235125	G/A	0.38/0.62 0.471	0.63/0.36 0.454	0.22/0.78 0.343	A	G	G	G	A	G	A	A	A
6	rs5963421	A/T	0.82/0.18 0.295	0.60/0.40 0.480	0.92/0.08 0.147	A	A	T	T	A	A	A	A	A
7	rs17274141	C/T	0.46/0.53 0.488	0.13/0.87 0.226	0.08/0.92 0.147	C	T	T	T	T	T	C	C	T[a]
8	rs5963428	A/T	0.05/0.95 0.095	0.24/0.76 0.365	0.43/0.47 0.404	T	T	T	T	A	T	T	T	T
9	rs12557315	C/T	0.82/0.18 0.295	0.80/0.20 0.320	0.98/0.02 0.039	C	T	C	C	C	C	C	C	C

Abbreviations: JPT, Japanese in Tokyo; CEU, Utah residents with Northern and Western European ancestry from CEPH collection; YRI, Yoruban in Ibadan, Nigeria.
[a]Surrogated by the nucleotide in this position in *Pongo pygmaeus abelii* (Orangutan) because it was not available for *P. troglodytes*.
Shaded cells indicate nucleotide bases different from those in *P. Troglodytes*. The segments enclosed by frames indicate those which may have been involved in recombinatory event to form HT4 or generation of HT's 3 and 7 from HT4.

Association of the three mutant alleles and particular haplotypes

The results of the haplotype analysis of the 21 families are summarized in Table 2. Among the nine Japanese probands carrying the p.R40H mutation, those belonging to families 1–8, who resided in an area within a radius of 140 km, all had HT1, whereas family 9, who lived 650 km away from that area, had HT2. One Spanish family and one Australian family had HT3. In this Australian family, the proband (a girl) bore HT3/8; her mother, 3/7; and father, HT8. Previous analysis had shown that mother carried the mutant allele. Therefore, it was determined that the mutation was linked with the HT3 allele. The Austrian family and one US family shared HT4. The proband in the remaining one US family had HT2. Thus, in the 14 families carrying the p.R40H mutation, this mutation was associated with four different HT's, indicating that it had appeared anew at least four times in the Japanese and the Caucasian populations. Similarly, the p.R277W mutation was associated with three different HT's in our five families carrying this mutation. Three families that carried p.R277W, one Japanese, Korean and US family, shared HT5 each. One of the remaining two US families carried HT6 and the other, HT2. The two Japanese families that carried the p.Y55D mutation shared HT1.

Evolutional order of the haplotypes

To estimate the evolutional order of these haplotypes, nucleotides in each polymorphic site were compared with those in *P. troglodytes* (Table 1). HT8 differed only in polymorphic site 7 (T-to-G) from that of *P. troglodytes*. HT1, HT5, HT6 and HT7 exhibited nucleotide changes in two polymorphic sites, whereas in HT2, HT3 and HT4, there were nucleotide changes in three polymorphic sites.

DISCUSSION

Although the majority of mutations at the OTC locus are 'private' in patients with neonatal and infantile presentation,[11] some mutations have been found to recur in unrelated families.[12] Among mutations associated with late-onset OTCD in male patients, the two mutations, p.R40H and p.R277W, have been most frequently reported in multiple different families.[7,12–15,25] The p.Y55D mutation has been found only in two unrelated Japanese families.[10,16] It was not known whether or not the recurring mutations shared a common ancestral origin or had arisen independently. The present study suggests that the p.R40H mutation occurred at least four times, or even five times, if the family 14 is non-Japanese American, in the Japanese and in the Caucasian populations. It appears very likely that those eight Japanese families (1–8) share a common ancestral origin and the mutant allele had been retained in the population in this small area. The other Japanese family (family 9) had a distinct haplotype, however, suggesting this mutation had arisen recurrently. Families 10 and 11, both Caucasian, may also have a common ancestral origin. Although the Australian family has no known Spanish ancestry, the proband's maternal grandfather (not studied) was of North Italian descent and could conceivably share ancestry with the Spanish family. It remains possible that the mutation occurred recurrently, but it is noteworthy that the allele in these families has a low haplotype frequency (0.033) among

Table 2 Haplotypes of mutant alleles carrying the three mutations

Family	*1–6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Mutation	p.R40H								
Haplotype	1	1	1	2	3	3	4	4	2
Reference no.	Harada *et al.*[7a]	Matsuda *et al.*[25]	Present report	Present report	Arranz *et al.*[27]	Pinner *et al.*[28]	Plöchl *et al.*[23]	Tuchman *et al.*,[11] Tuchman *et al.*[12]	
Ethnicity	Japanese	Japanese	Japanese	Japanese	Caucasian	Caucasian	Caucasian	Unknown	Unknown
Residential country	Japan	Japan	Japan	Japan	Spain	Australia	Austria	United States	United States
Allele frequency									
JPT	0.408			0.151	0.031		0.133		0.151
CEU	0.067			0.155	0.033		0.356		0.155
YRI	0.056			0.022	<0.05[b]		0.044		0.022

Family	*15*	*16*	*17*	*18*	*19*	*20*	*21*
Mutation	p.R277W					p.Y55D	
Haplotype	5	5	5	6	2	1	1
Reference no.	Numata *et al.*[10]	Kim *et al.*[29]	McCullough *et al.*[14]			Nishiyori *et al.*[16]	Numata *et al.*[10]
Ethnicity	Japanese	Korean	Unknown	Unknown	Unknown	Japanese	Japanese
Residential country	Japan	Korea	United States	United States	United States	Japan	Japan
Allele frequency							
JPT	0.061			<0.05[b]	0.151	0.408	
CEU	0.175			<0.05[b]	0.155	0.067	
YRI	0.122			0.056	0.022	0.056	

Abbreviations: JPT, Japanese in Tokyo; CEU, Utah residents with Northern and Western European ancestry from CEPH collection; YRI, Yoruban in Ibadan, Nigeria.
[a]Patients 1, 3, 4, 8. 9 and 10 in reference Harada *et al.*[7]
[b]Below minimum-allele frequency.

Caucasians. Families 12 and 13 share a haplotype, but there is insufficient information to say whether this represents the effect of common ancestry or of recurrence on the same haplotype. HT4 is the most common in Caucasians (allele frequency 0.356 in the CEPH Caucasian samples) and there is no information about the ethnicity of family 13.

Among the families with the p.R277W mutation, the Japanese and the Korean families (families 15 and 16) could have stemmed from a common ancestor. Family 18 bore HT6, confirming recurrent origin of this mutation.

The families that harbored the p.Y55D mutation (families 20 and 21) shared HT1. This mutation has been found among the Japanese population alone to date.[10,16] In contrast to the p.R40H and p.R277W mutations, which involve CpG dinucleotides and hence are likely to recur, this transversion (T-to-G) is not common among single base substitutions in X chromosome genes in general[26] and in the OTC gene in particular.[12,14,27] This characteristic of the p.Y55D mutation would again support that the affected families share a common ancestral origin.

The search for polymorphic markers and the determination of their allelic frequency is required for exploring the origin of an allele. As expected, different types of polymorphic markers in the OTC gene or in its vicinity are known and include SNPs in the coding region,[9,17,20] in the 5′ untranslated region[21] and extragenic microsatellite markers.[18,19,22] Four SNPs, A-to-G in codon 46 in exon 2 (E2 46), A-to-T in intron 3 (IVS3-8nt), A-to-G in intron 4 (IVS4-7nt) and A-to-G in codon 270 of exon 8 (E8 270), were found to be informative among a US population,[17] though their informativity varied somewhat in the Iberian population and in the Mozambiquan population.[18] One additional SNP (IVS3-39_insT) was informative in a Spanish population,[20] indicating that informativity of these SNPs varied between populations. The nine tagged SNPs we used in the present study were all highly informative, except rs5963428, which presented 0.095 heterozygosity in JPT (Table 1). These tagged SNPs were all informative in CEU and YRI also, with the exception of rs12557315 in YRI. We thus considered it appropriate to apply these tagged SNPs to define haplotypes in other ethnicities also. There are several advantages of using these tagged SNPs in determining haplotypes. First, these SNPs generally show high heterozygosity frequency, allowing high informativity. Second, the tagged SNPs are located over the entire span of the OTC gene, reducing misdiagnosis due to an extragenic recombination event when diagnosis by linkage analysis is necessary in the absence of an identifiable mutation. Third, it permits easy differentiation between intragenic recombination and mutation of one particular marker single nucleotide (both rare events). Finally, the analytical efficiency of this technique is high as amplification and data acquisition of up to seven specimens can be completed simultaneously within 90 min under the conditions employed.

Both the p.R40H and p.R277W mutations arise in CpG dinucleotides, which represent mutational hot spots, consistent with our finding that they have recurred in different populations. Recurrent point mutations occur evenly among most CpG dinucleotides in the OTC gene.[9,12,14,27] These two mutations arise in arginine codons. It is not surprising that these single nucleotide changes have a higher chance to recur than others, because four of six codons that encode for arginine contain CpG dinucleotides. Indeed, scrutiny of a recent OTC mutation update[9] reveals that 20 discrete mutations have occurred in a total of 15 arginine codons (ratio: 20/15=1.33) in the human OTC gene, whereas this ratio is less than 1.0 in the vast majority of codons encoding for other amino acids.

Haplotype 8 differed by one nucleotide from that in *P. troglodytes*, whereas the other seven HT's exhibited two or three nucleotide differences. This possibly indicates that HT8 is the oldest among the eight HT's identified in the present series. HT's 3 and 4 shared segments consisting of identical nucleotides (polymorphic sites 5–9) and HT's 4 and 7 had jointly another identical segment (polymorphic sites 1–3) (Table 1). It is thus possible that HT3 and HT7 were generated by a recombination of the HT4 allele with another allele, or conversely HT4 was generated by a recombination of HT3 with HT7.

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Management of undifferentiated sarcoma of the liver including living donor liver transplantation as a backup procedure

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Abstract We present the cases of 3 children with huge undifferentiated sarcoma of the liver who were treated with surgical excision including liver transplantation as an option and adjuvant chemotherapy. All 3 patients were males aged 10, 13, and 15 years old. The size of the tumor was 10, 15, and 20 cm in diameter, respectively. The youngest patient is disease free and doing well 43 months after resection. The 13-year-old patient presented with tumor rupture and underwent operation. The primary tumor and the ruptured tissue fragments were removed and he was given postoperative chemotherapy. The patient is disease free and doing well 52 months after surgery. The oldest patient had an unresectable tumor in the hilar region. Preoperative chemotherapy was given but later discontinued owing to severe side effects. He underwent living donor liver transplantation followed by postoperative chemotherapy. The patient had recurrent tumor 24 months after transplantation that was excised at reoperation. He is doing well and is disease free 18 months after the second procedure. Complete removal of the tumor including total hepatectomy and transplantation when indicated and suitable pre- and/or postoperative chemotherapy is an effective treatment for children with undifferentiated sarcoma of the liver.

Undifferentiated sarcomas of the liver (USL) in children are often huge tumors and sometimes unresectable at the time of diagnoses. In the early 1980s, results of treatment for USL were poor, with a 5-year survival rate of 20% to 40% [1-8]. The advent of effective chemotherapy and complete macroscopic removal of the tumor have yielded improved results for children with USL [9-11]. In patients with unresectable tumor, total hepatectomy to remove the tumor completely and liver transplantation are some of the

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treatment options. However, the effects of immunosuppressant therapy and chemotherapy on recurrence of disease are not clear.

In this study, we describe 3 children with huge USL tumor who were treated by surgical removal including liver transplantation and chemotherapy.

1. Patients

1.1. Case 1

A 13-year-old adolescent boy presented to a local hospital because of back pain while playing rugby football. Laboratory data showed mild anemia, and abdominal computed tomography (CT) scan showed a huge low-density area in the right lobe of the liver. Initial laboratory data included hemoglobin level (Hb) of 10.4 g/dL; hematocrit (Hct) of 32.6%; red blood cell count (RBC) of 371×10^4 per microliter; white blood cell count (WBC) of 3200 per microliter; C-reactive protein concentration (CRP) of 1.42 mg/dL; glutamic oxaloacetic transferase (GOT) of 39 IU/L (normal range [NR], 10-34); glutamic pyruvic transferase (GPT) of 40 IU/L (NR, 8-37); and lactate dehydrogenase (LDH) of 322 IU/L. Serum alpha-fetoprotein, carcinoembryonic antigen (CEA), and carbohydrate antigen (CA19-9) levels and hepatitis serology were negative. The initial diagnosis was traumatic hematoma. However, abdominal ultrasonography and enhanced abdominal CT scan showed a solid and cystic multilobular mass that was compatible with USL and he was referred to our hospital (Fig. 1A). As the severity of symptoms increased, rupture of the tumor was suspected and he underwent emergency laparotomy. Tumor rupture was confirmed at operation (Fig. 1B). The entire tumor including portions of the ruptured tissues was removed and postoperative chemotherapy using vincristine (VCR), adriamycin D (ADR), cyclophosphamide, cisplatin (CDDP), and VP16 was administered. The size of the tumor was 20 cm in diameter. Histologic findings showed USL (Fig. 1C). Immunohistochemistry of the tumor revealed vimentin (+), α1-antitrypsin (+), desmin (+), cytokeratin (−), CD34 (−), and S-100 protein (−). The patient has been doing well and is disease free 52 months after resection.

1.2. Case 2

A 10-year-old boy visited a local hospital because of symptoms consistent with gastroenteritis. Abdominal ultrasonography showed a huge homogenous hypoechoic mass in the right lobe of the liver and he was referred to our hospital. Enhanced abdominal CT scan showed a large low attenuation mass occupying the right lobe of the liver (Fig. 2A), and an open biopsy was performed to confirm a tissue diagnosis of tumor to facilitate preoperative chemotherapy. Unfortunately, intraoperative histologic findings from 3 different parts of the tumor showed only connective tissue surrounding the tumor and blood tinged gelatinous clot inside the tumor. Laboratory data included Hb of 10.2 g/dL; Hct of 31.3%; RBC of 375×10^4 per microliter; WBC of 6100 per microliter; CRP of less than 0.1 mg/dL; GOT of 25 IU/L;

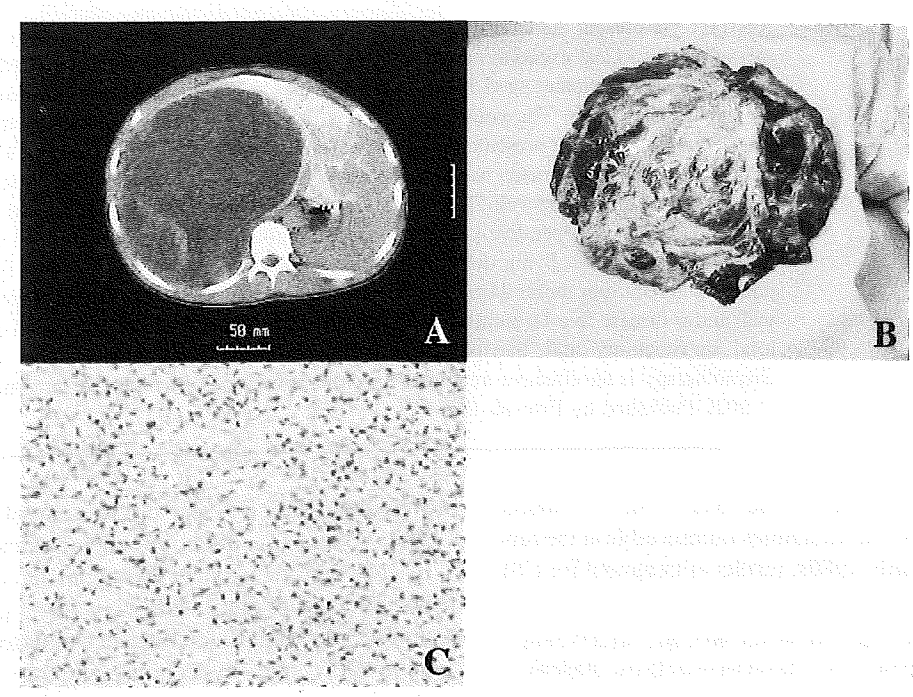

Fig. 1 A, Enhanced abdominal CT scan shows a large low attenuated mass in the right lobe of the liver. B, The tumor was composed of friable necrotic tissue, gelatinous material, and pseudocapsule. C, Diffuse proliferation with immature spindle cells.

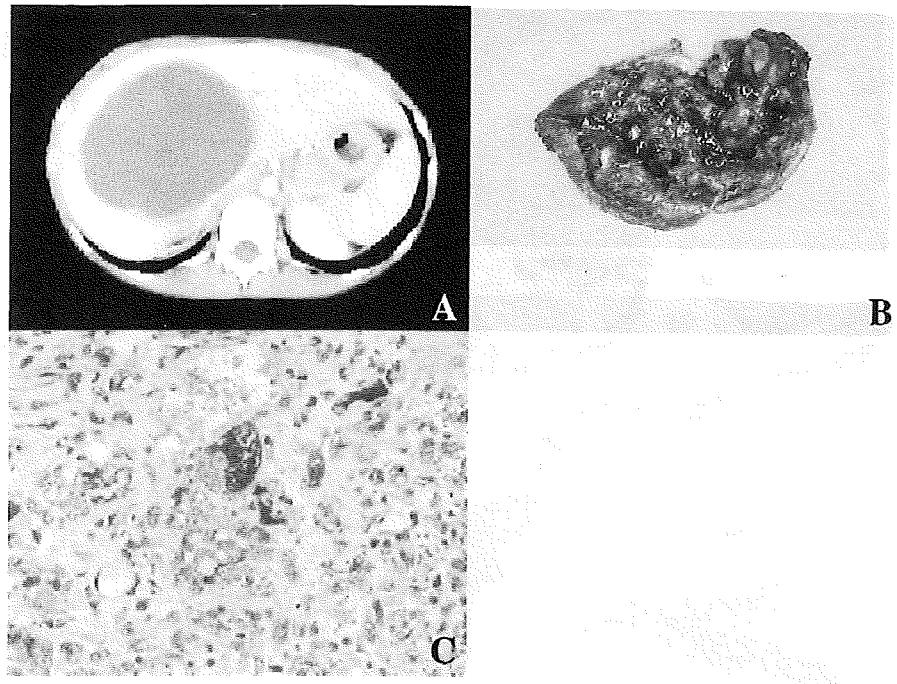

Fig. 2 A, Enhanced abdominal CT scan shows a large low attenuated mass in the right lobe of the liver. B, The tumor was composed of friable necrotic tissue, gelatinous material, and pseudocapsule. C, Diffuse proliferation with immature spindle cells.

GPT of 19 IU/L; and LDH of 437 IU/L. Serum CA19-9 level was 44.3 IU/L (NR, <37). Serum alpha-fetoprotein and CEA as well as hepatitis serology were negative. He was initially diagnosed with a traumatic hematoma. A drainage tube was inserted into the tumor for 1 month, with approximately 10 mL of bloody discharge draining per day, but the size of the mass did not change. We therefore decided to remove the mass surgically. The tumor was removed completely, with right lobectomy of the liver. The tumor was 10 cm in diameter and weighed 380 g (Fig. 2B). Histologically, the tumor consisted of a fibrous pseudocapsule and a compact area of spindle cells, findings compatible with USL (Fig. 2C). Immunohistochemistry of the tumor revealed vimentin (+), α1-antitrypsin (+), desmin (+), cytokeratin (−), CD34 (−), and S-100 protein (−). Postoperative chemotherapy using VCR, adriamycin D, cyclophosphamide, CDDP, and VP16 was administered. The patient has been doing well and is disease free for 36 months after resection.

1.3. Case 3

A 15-year-old adolescent boy visited a local hospital because of jaundice and was diagnosed with a 15-cm tumor at the hilar region of the liver (Fig. 3A). Laboratory data included Hb of 12.0 g/dL; Hct of 37.2%; RBC of 403 × 10^4 per microliter; WBC of 9100 per microliter; CRP of less than 0.1 mg/dL; GOT of 100 IU/L; GPT of 77 IU/L; and LDH of 240 IU/l. Serum CA19-9 level was 353.4 IU/L (NR, <37). Serum alpha-fetoprotein and CEA as well as hepatitis serology were negative. Percutaneous needle biopsy of the tumor was performed and yielded histologic findings compatible with USL. Neoadjuvant chemotherapy including VCR (2 mg/d), actinomycin D (0.5 mg/d), and cyclophosphamide (2.2 g/d) was administered. However, the chemotherapy had to be discontinued owing to severe side effects including bone marrow suppression and sepsis. Surgical removal was considered but the tumor was still considered unresectable (Fig. 3B). An extended right lobectomy of the liver, along with resection of the left main portal vein and placing a jump graft between the portal trunk and the umbilical portion of the portal vein, was considered, but the extent of tumor invasion of the portal vein and hepatic artery was unclear. We therefore prepared for living donor liver transplantation as a backup procedure. During the operation, we exposed all the vessels and bile duct in the hilum of the liver and found that the tumor had invaded the entire length of the portal vein bifurcation and hepatic arteries. Hence, we abandoned an attempt at partial hepatectomy and decided to proceed with total hepatectomy and liver transplantation. The donor was the patient's mother who had an identical blood type. The donor graft was the left lateral segment; the graft weight/recipient body weight ratio was 0.7. In the recipient, the entire liver, including the entire tumor, was removed. The total operative time was 14 hours and 11 minutes, and blood loss was 1605 mL. He recovered uneventfully and was discharged 14 days after transplantation. Histologic findings of the tumor were compatible with USL (Fig. 3C). Immunohistochemistry of the tumor revealed vimentin (+), α1-antitrypsin (+), desmin (−), cytokeratin (−), CD34 (−), and S-100 protein (−). He was readmitted to his local hospital for postoperative chemotherapy consisting of VCR (1 mg/mo), actinomycin D (0.5 mg/mo), and

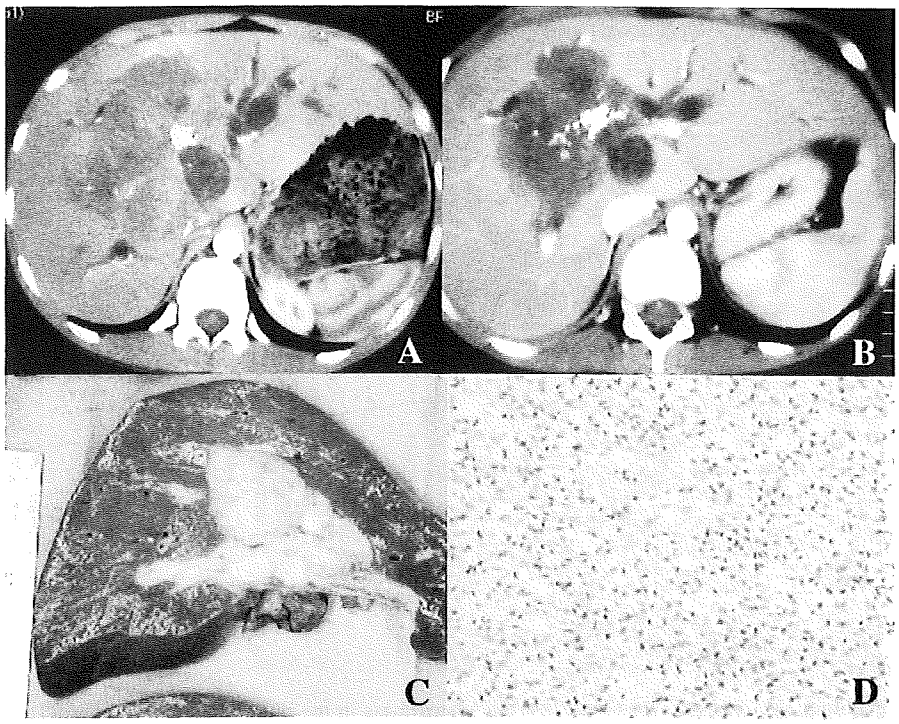

Fig. 3 A, Enhanced abdominal CT scan before chemotherapy shows a large heterogeneous low-density mass occupying the hilar region of the liver. B, Follow-up CT scan after one cycle of chemotherapy shows a marked reduction in the size of the primary tumor; however, it still occupies the hilum of the liver. C, A solitary stromal tumor is seen at the hilar region of the explanted liver. D, Diffuse proliferation with immature spindle cells.

cyclophosphamide (600 mg/mo). The patient had a recurrent tumor at the hilar region of the liver 24 months after transplantation. The recurrent tumor was removed surgically and he was treated with additional postoperative chemotherapy with VCR, adriamycin D, cyclophosphamide, CDDP, and VP16. The patient has done well and is disease free 18 months after the second procedure.

2. Discussion

In the early 1980s, results of treatment for USL were not satisfactory, with the 5-year survival rate ranging from 20% to 40% [1-8]. The advent of effective chemotherapeutic regimens and complete macroscopic removal of the tumor have yielded better results for children with USL [9-11]. Kim et al [9] described 6 children with USL treated by surgical removal and chemotherapy, 5 of whom were alive without recurrence at a mean follow-up of 63 months. Bissogno et al. [10] reported 17 cases of USL, including 2 with ruptured tumor, treated by surgical removal and pre- and/or postoperative chemotherapy. Ten of these children are alive, with follow-up ranging from 2.4 to 20 years, including one of the children with a ruptured tumor.

Undifferentiated sarcoma of the liver most frequently occurs in patients between 5 and 15 years of age. Clinical manifestations include no specific abnormalities on laboratory evaluation and variable degrees of cystic mass noted on abdominal CT and ultrasonography. In our series, only mild anemia and mild elevation of serum transaminases were detected. Radiologic methods, including CT and ultrasonography scans, cannot differentiate USLs from mesenchymal hamartomas. The latter usually present in younger patients, up to 2 years old [12], and they often have elevated serum alpha-fetoprotein levels. The serum alpha-fetoprotein in this series was negative in each case. However, some patients with USL may also present at less than 1 year of age [10]. Regarding the immunohistchemical studies of the tumor, all 3 tumors were positive for vimentin and α1-antitrypsin, 2 were positive for desmin, and all 3 tumors were negative for cytokeratin, CD34, and S-100 protein.

In the second case, treatment was delayed because of misdiagnosis of the tissue biopsy from the capsule of the tumor and the gelatinous coagulum fluid aspirated from the cystic region for cytology. Because USL may be composed of several compartments (ie, cystic and solid regions), needle biopsies from several areas are needed to achieve an accurate diagnosis. Chowdhary et al [13] have also reported misdiagnosis with an initial biopsy in a patient with USL. However, USL should be considered in the differential diagnosis of children with variable degrees of cystic liver mass.

Recently, the outcomes of patients with USL have improved after treatment with multiagent chemotherapy. Although it is difficult to determine which of the drugs are most effective, CDDP and ADR seem to be generally

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キーワード：シトリン，*SLC25A13*，アスパラギン酸・グルタミン酸輸送体（AGC），
シトリン欠損による新生児肝内胆汁うっ滞症（NICCD），
成人発症II型シトルリン血症（CTLN2）

はじめに

乳児期早期に発症する古典型シトルリン血症（citrullinemia type I：CTLN1）は1962年McMurrayらによりはじめて報告された[1]．しかしわが国ではこれとは別に成人の肝脳疾患患者において高シトルリン血症を合併する症例が報告されており，成人発症II型シトルリン血症（adult-onset type II citrullinemia：CTLN2）と呼ばれていた．本稿ではCTLN2の原因遺伝子*SLC25A13*及びその遺伝子産物シトリン（citrin）の発見とシトリン欠損による新生児肝内胆汁うっ滞症（neonatal intrahepatic cholestasis caused by citrin deficiency：NICCD）の疾患概念の確立までの経緯を述べた後，NICCDの臨床像を概説し，さらにその治療法およびCTLN2の発症予防法について最近の知見を述べる．

CTLN2の原因遺伝子の解明

1968年宮腰らは慢性反復性肝脳疾患患者において高シトルリン血症を合併する症例があることを初めて報告した[2]．その後同様の症例が報告されたが，多くは20～50歳代の成人期に突然の行動異常，見当識障害，精神不穏などの意識障害で発症し，検査上は高シトルリン血症，高アンモニア血症が特徴で，数年の経過で死亡していた．また，殆どの症例で豆類を異常に好み，米飯，甘い物を好まないという偏食が見られた．佐伯らは患者肝アルギニノコハク酸合成酵素（Argininosuccinic acid synthetase：ASS）の動力学的性質を解析し，肝臓特異的にASS蛋白の低下があり量的異常を示す肝脳疾患をCTLN2と命名し，全身のASS欠損により乳児期早期に発症するCTLN1と区別した[3]．

その後しばらくはCTLN2の真の原因は不明であったが，1999年Kobayashiらにより原因遺伝子*SLC25A13*が発見され，その遺伝子産物はシトリンと命名された[4]．現在シトリンはミトコンドリア内膜に局在するアスパラギン酸・グルタミン酸輸送体（aspartate-glutamte carrier：AGC）であることが明らかにされている[5]（図1）．AGCはミトコンドリアで生成するアスパラギン酸を細胞質に供給するとともに，リンゴ酸・アスパラギン酸シャトルを構成して細胞質のNADH還元当量をミトコンドリアに輸送する機能を持っている．シトリン（AGC）の機能喪失は尿素・蛋白合成，好気的解糖，糖新生，さらにはエネルギー代謝などに障害を与えるが，その結果シトリン欠損患者では多彩な症状を呈するものと推察されている[6]．

NICCDの発見

1997年筆者らは新生児マススクリーニング（NBS）陽性を契機に受診した患児の中に特異なアミノ酸異常を伴う新生児肝炎例を7例報告した[7]．その臨床的特徴は①NBSにてメチオニン，フェニルアラニンもしくはガラクトース陽性，②高度の胆汁うっ滞，肝障害，③複数のアミノ酸（シトルリン，メチオニン，スレオニンなど）やガラクトースの一過性の上昇，④脂肪肝であった．いずれの症例も乳糖除去粉乳や中鎖脂肪酸（MCT）フォーミュラ，脂溶性ビタミンが投与され，肝機能は1歳までに改善，発育発達は正常であった．当時は新たな疾患群と考え報告したが，その原因は不明のままであった．

CTLN2は通常小児期には無症状であると考えられていたが，前述の7例においてもシトルリン値の一過性上昇があることに着目し，検体の採取可能であった3症例においてKobayashiらの単離した*SLC25A13*遺伝子の解析を行った．その結果3例全例にCTLN2と同じ遺伝子変異が同定され，これらの症例の胆汁うっ滞の原因がシトリン欠損によることが明らかにされた[8]．一方Tazawaらは脂肪肝を伴う新生児肝内胆汁うっ滞症例において*SLC25A13*異常を見出し[9]，TomomasaらもCTLN2を発症した男児が新生児肝炎の既往があることによりシトリン欠損が新生児期に胆汁うっ滞症を発症することを報告した[10]．これらの症例はCTLN2とは異なり，主に肝内胆汁うっ滞症を呈す