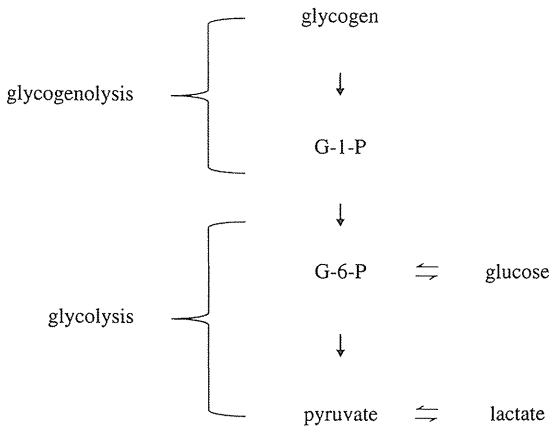

図1 グリコーゲン分解と解糖系

G-1-P: Glucose-1-phosphate, G-6-P: glucose-6-phosphate

モルのグルコースから38個のATPを生み出す重要なエネルギー産生系を担っていて，筋収縮のエネルギー源として利用される．

従って，ATP産生が目的の筋肉における解糖で異常があれば，解糖段階でできる2個のATP以外にもさらにその下流のミトコンドリアへの基質供給という点でも障害が波及するため，筋収縮に対応するATP供給不全のため筋症状として労作時筋痛，筋硬直，横紋筋融解症などが出てくる．肝臓では解糖の障害はグルコース産生の障害となり，低血糖，あるいはグリコーゲン蓄積による肝腫大という症状となる．しかし症状は一臓器にとどまらず多様性を示す場合もある．これは解糖の酵素系には臓器特異性があることが原因である．

Ⅱ グリコーゲン代謝異常症の今までの報告の特徴と経緯

グリコーゲン代謝異常症の報告は，大きく3つの時期に分類できると思われる．Ⅰ期（1929～1965年）はvon Gierke病（Ⅰ型）の報告がされ，初期の古典的な肝腫大を伴う「糖原病」の概念が提唱された時期である．また別にこの時期には筋グリコーゲン代謝異常症としてMcArdle病，垂井病が筋エネルギー枯渇型として報告され，それぞれギリシャ数字で病型の順番がつけられていった．Ⅱ期（1965～1995年）は新たに嫌気解糖の酵素異常が発見され，いわゆるdistal glycolysis（図1）に関与する酵素系の障害が次々に報告された時期である．distal glycolysisの障害ではグリコーゲンの蓄積が必ずしも著明でないことも注目された．Ⅲ期（1996年～）従来から報告されていた病型での新たな表現型の報告や，二次性のグリコーゲン代謝異常症，さらに新規酵素異常症の発見が報告されてきた時期である．同時にこの時期には酵素補充療法，ビタミンB6療法などの治療が登場してきた．

グリコーゲン代謝異常症は最初の報告から既に90年近く経過し，ある程度解決した疾患と考えられがちであるが，近年は新たな知見，病態の解明，治療など今後もさらに発展が期待される分野である[2]．

表1 各臓器における解糖の目的

臓器	役割
肝臓	グルコース供給，グリコーゲン貯蔵
筋肉	筋収縮のためのATP供給
脳	神経細胞へのATP供給（short term?）

ATP: adenosine triphosphate

Ⅲ 新たなグリコーゲン代謝異常症とMcArdle病の治療

従来のグリコーゲン代謝異常症とは異なる症状を示す症例については，鑑別診断をしてゆく過程でぜひ知っておく必要がある．今回紹介するのは①0型の筋型，もう一つは②Ⅳ型の神経筋型である．両者ともグリコーゲン合成系の酵素異常である（図2）

1. 0b型[3]

11歳の女児．幼児期から反復する運動時あるいは運動後の意識消失があり，軽度の筋力低下も見られていた．有機酸，脂肪酸異常症などの代謝検査のワークアップは正常で，前腕運動負荷試験で乳酸の上昇は認めなかった．筋組織化学ではPAS染色で染色性がほとんどないという特異な組織化学所見であった（図3）．グリコーゲン合成系の異常が示唆され，筋組織のグリコーゲン含量は0.03%wet weight（0.94±0.55）とグリコーゲンの枯渇が明らかで，グリコーゲン合成酵素は0.9 mol/min/mg（42.0±11.2）であり，グリコーゲン合成酵素欠損症（0b型）と診断した．遺伝子検査では*GYS1*遺伝子にc.1230-2A>G，c.1810-2A>G，の遺伝子変異が認められた．この症例も含め，欧米での報告例も多くが突然死をきたしており予後不良である．この病型は症例報告が少なく，本症の症候，病態，治療法などは確立されていないが，不良な予後なため，早急に解明が待たれる．

2. Ⅳ型[4]

生後0日の男児．生直後から呼吸障害，重度の筋緊張低下が認められ，人工呼吸器管理が行われたが，生後14日で死亡した．剖検時の病理検査でPAS陽性，ジアスターゼで消化できない物質の蓄積が心，筋，肝，脳に認められ，polyglucosanと考えられ，グリコーゲン分枝酵素欠損症（Ⅳ型）が疑われた．生化学分析ではグリコーゲン分枝酵素は筋，肝，脳ともにほとんど活性が無く，グリコーゲン分枝酵素欠損症（Ⅳ型）の神経筋型（致死性）と診断した．

上記2症例から学ぶものは，グリコーゲン代謝異常症は，従来考えられていたより臨床症状に広がりがあり，かつ予後不良であることである．鑑別をする上でこのようなグリコーゲン代謝異常症の存在を知っておく必要があり，正確な診断をしておくことで，遺伝相談などに有用である．

3. McArdle病（Ⅴ型）とビタミンB6療法

筆者は神経研究委託費（荒畑班，清水班）でMcArdle病のビタミンB6療法を6例の本症患者で行い，有意に血清CKが

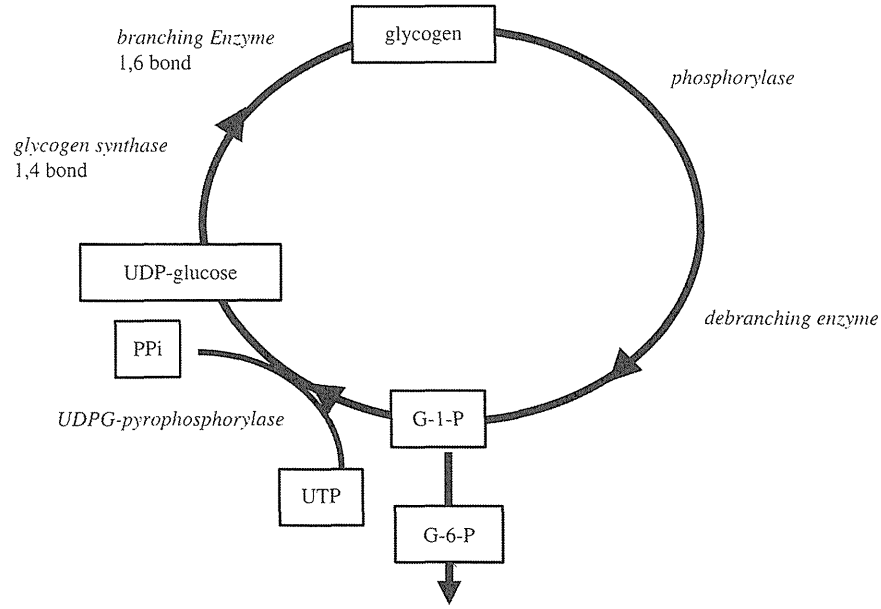

図2 グリコーゲンの合成と分解系

UTP: Uridine triphosphate

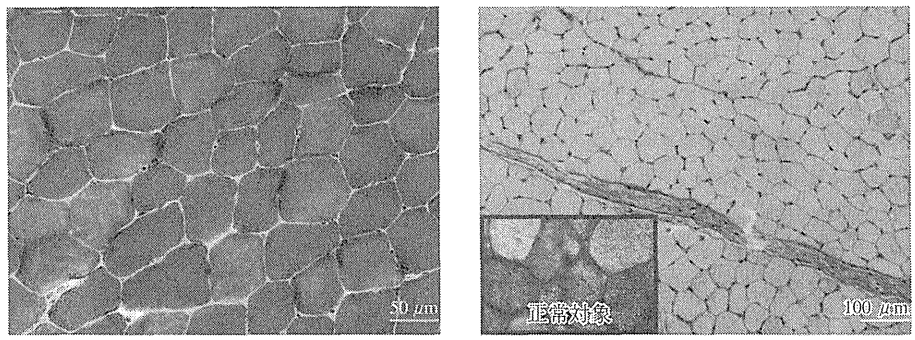

図3 筋組織化学検査

A：Gomori-Trichrome 染色．形態の異常は認めないがやや筋細胞膜下のミトコンドリアの集積を認める．
B：PAS 染色．正常対照に比較して PAS 染色性が著しく低下している（グリコーゲンの枯渇）．
（国立精神神経医療研究センター西野一三部長による）

低下することを始めて報告した．一方イギリスでは Quinlivan らが McArdle 病の症例に対してビタミン B6 投与は有意な効果が無いことを報告していたが[5]，最近本邦からビタミン B6 の投与で効果がある症例が報告された[6]．筋ホスホリラーゼ蛋白はビタミン B6 と結合して存在しているため，ホスホリラーゼが欠損している McArdle 病では，ビタミン B6 の二次的欠乏が起こっていると推測される．イギリスと我が国の報告で効果に差がある点については，以下のことが考えられる．

欧米の遺伝子変異で頻度が多いのは p. Arg50 で酵素活性がほぼ 0 であるのに反し，日本人では好発遺伝子変異が p. Phe 710 del であり[7]，日本人 McArdle 病患者ではある程度の残存酵素活性があることから，ビタミン B6 補充によりホスホリラーゼ酵素活性の賦活ができるのではないかと考えられている．我々は McArdle 病の治療基準を表 2 のようにまとめた．日本人 McArdle 病では我々の経験ではある程度の残存酵素活性が認められるので，ビタミン B6 療法は試みるべき治療法であると考えられる．また Andersen らが報告したように，運動前の糖分の服用は運動機能の改善に役立つので併用することで本症患者の日常生活動作（ADL）の改善が期待できる[8]．一方 McArdle 病は運動と関連した筋症状が主体と考えられて

表2 McArdle 病治療基準

1. ビタミン B6 は筋細胞の障害を軽減する可能性が示唆されるので，将来的な筋細胞の障害蓄積を緩和することを期待して一日 25～50 mg を服用する．ただし血中濃度の上昇には個人差があるため，PAL で 50～100 ng/ml となるように服用量を調節する．
2. Cornstarch などの糖分は，運動する前に 1～2 g/kg 摂取することで効果が期待できる．
3. 急激な運動は避け，準備運動を穏やかに行った後に，運動を開始すること．
4. ビタミン B6 の長期服用については定期的な副作用チェックを行うこと．

表 3　1994 年～2014 年における糖原病診断支援症例数

		累積診断確定症例数	今年度診断確定数
Type 0	Glycogen synthase	1	0
Type Ⅰa, b	Von Giercke	43	2
Type Ⅱ	Pompe	68	1
Type Ⅲ	Cori	49	0
Type Ⅳ	Andersen	12	0
Type Ⅴ	McArdle	53	1
Type Ⅵ	Hers	17	0
Type Ⅶ	Tarui	14	0
Type Ⅷ	Phosphorylase kinase	160	10
Type Ⅸ	Phosphoglycerate kinase	7	0
Type Ⅹ	Phosphoglycerate mutase	0	0
Type Ⅺ	Kanno	2	0
Type Ⅻ	Aldolase-A	1	0
Type ⅩⅢ	beta-enolase	0	0
Type ⅩⅣ	Phosphoglucomutase	3	0
GLUT2	Fanconi-Bickel	1	0
GLUT1	De Vivo	1	0
		432	14

いるが，高齢になると，筋力低下（進行性）が認められる．長期にビタミン B6 で治療し筋障害を抑制しておくことが，長期予後としては改善が期待されるかもしれない．

Ⅳ　脳におけるグリコーゲンの役割についての考察

脳のグリコーゲン含量は肝臓，骨格筋に比較すると極めて少量であり，その生理学的な存在意義は十分に解明されていない．筆者が丁度研修医だったころ，進行性に脳障害を呈した女児例の剖検脳において著明なグリコーゲンの蓄積が認められ，cerebral glycogenosis として報告された症例があり，現在までおぼろげながらグリコーゲンと脳代謝について気になっていた[9]．脳における解糖機構はおそらく一過性の低酸素，虚血などの嫌気状態での緊急時において，脳代謝の補助的エネルギー供給分担機構として，neuroprotection に寄与していると思われる．例えば新生児仮死のような嫌気状態を考えると，短期間の脳エネルギーレスキューの一端を担っているのではないかと推定される．嫌気解糖の基質である脳内のグリコーゲンは，ほぼアストロサイトに局在しており[10]，またそれはグリコーゲン分解の key enzyme である脳型ホスホリラーゼ（brain form，*PYGB*）の局在とほぼ一致している[11]．これらを踏まえて筆者は一つの仮説を考えてみた．

つまり新生児仮死児の予後をみると，同程度の仮死状態に暴露されながら，正常発達から重篤な脳障害を残す一連の障害 spectrum を形成している[12]．この説明として急性期での嫌気解糖に何らかの個体差があるためではないかという仮説である．

新生児低酸素状況の持続は脳血流の低下も伴い，低酸素性虚血性脳（hypoxic-ischemic encephalopathy；HIE）として，一次神経細胞壊死及び遅発性神経細胞壊死を経て最終的な神経細胞死に至る．遅発性神経細胞壊死は一次神経細胞壊死がトリガーとなって招来すると考えられている．新生児仮死では低酸素状態のため好気性のエネルギー産生が障害されるため，嫌気性解糖によって生成される ATP，乳酸あるいは脂肪酸代謝によって生成されるケトン体などが補助的に脳エネルギー代謝を担っている．すなわち緊急時の最初のエネルギー供給は短時間ではあるものの，嫌気解糖に依存しているはずである．

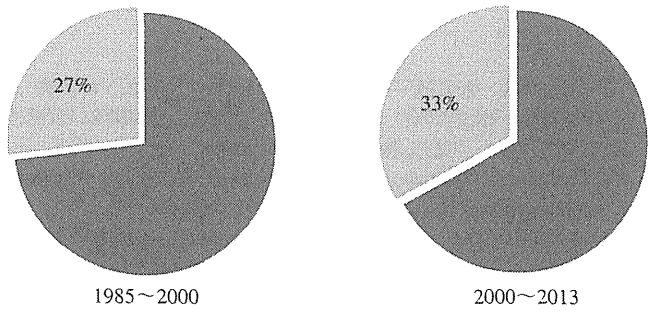

図 4　当科における代謝性ミオパチー診断症例の内訳
■：診断不明，▒：診断例

我々はホスホリラーゼの脳型 isozyme の機能変化の存在の有無に注目して検討を行っている．初期段階での結果であるが，一定の違いが認められているため今後症例を重ねてゆく予定である．この病態が明らかになれば脳型ホスホリラーゼ活性誘導目的にビタミン B6 投与による治療法の開発の可能性も考えられ，周生期の早期脳保護および予後改善，早期介入などに寄与できるとのではないかと考えている．

Ⅴ　診断の問題点

我々は主に糖原病の酵素診断を 30 年継続してきた（表 3）．しかし約 1/3 の症例で確定診断ができたのみで，大部分は原因不明である（図 4）．現在の検索法の限界であり，生体での複雑な代謝病態を in vitro で単純に解明するという安易なものではないと考えられる．今後次世代シーケンサーなどを利用した新たな手法を用いての診断へのブレークスルーが期待される[12]．また最近は PGM1-CDG の報告のように，糖鎖の問題も糖原病にはかかわっていることが明らかにされ[13,14]，グリコーゲン代謝異常は古くからある疾患でありながら新たな展開を迎えている．

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著者の利益相反：本論文発表内容に関連して開示すべき事項なし．

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Application of multiplex ligation-dependent probe amplification, and identification of a heterozygous Alu-associated deletion and a uniparental disomy of chromosome 1 in two patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency

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Abstract. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL) deficiency is an autosomal recessive disorder affecting the leucine catabolic pathway and ketone body synthesis, and is clinically characterized by metabolic crises with hypoketotic hypoglycemia, metabolic acidosis and hyperammonemia. In the present study, we initially used PCR with genomic followed by direct sequencing to investigate the molecular genetic basis of HMGCL deficiency in two patients clinically diagnosed with the condition. Although we identified a mutation in each patient, the inheritance patterns of these mutations were not consistent with disease causation. Therefore, we investigated *HMGCL* using multiplex ligation-dependent probe amplification (MLPA) to determine the copy numbers of all exons. A heterozygous deletion that included exons 2-4 was identified in one of the patients. MLPA revealed that the other patient had two copies for all *HMGCL* exons. Paternal uniparental isodisomy of chromosome 1 was confirmed in this patient by microarray analysis. These findings indicate that MLPA is useful for the identification of genomic aberrations and mutations other than small-scale nucleotide alterations. To the best of our knowledge, this is the first study describing HMGCL deficiency caused by uniparental disomy.

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Abbreviations: HMGCL, 3-hydroxy-3-methylglutaryl-CoA lyase; MLPA, multiplex ligation-dependent probe amplification; CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism; LOH, loss of heterozygosity

Key words: multiplex ligation-dependent probe amplification, mitochondrial 3-hydroxy-3-methylglutaryl CoA lyase, Alu element, uniparental disomy, microarray

Introduction

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL; EC 4.1.3.4) deficiency is an autosomal recessive disorder that affects leucine catabolism and ketogenesis. The *HMGCL* gene, located on chromosome 1p36.1, contains nine exons and spans approximately 25 kb (1). In the majority of HMGCL-deficient patients, the first hypoglycemic crisis occurs before the age of one, while one-third of all cases may have neonatal onset. In acute episodes, laboratory data have shown patients with non- or hypoketotic hypoglycemia with high levels of free fatty acids and severe metabolic acidosis with liver dysfunction and hyperammonemia (2). In Japan, HMGCL deficiency is one of the inborn errors of metabolism screened for in newborns by tandem mass spectrometry. Six Japanese HMGCL-deficient patients, including those previously reported (3) were re-evaluated (2). Among them, three had neonatal onset. Follow-up data showed that two patients experienced hypoglycemic crises even after ten years of age. Developmental delay and epilepsy were noted in two and three patients, respectively (2).

We recently encountered two Japanese HMGCL-deficient patients, whose inheritance patterns of single nucleotide muta-

tions were not consistent with transmission within their families. *HMGCL* has 23 Alu elements in introns. Recombination between Alu elements results in genomic deletions associated with a number of human genetic disorders (4). Hence, we hypothesized that these patients may have an intragenic deletion by non-equal homologous recombination between Alu elements (5-7). Large homozygous deletions can be suspected by the absence of the deleted exons detected by PCR amplification. However, the detection of heterozygous deletions is difficult using routine PCR amplification of genomic DNA and direct sequencing. Multiplex ligation-dependent probe amplification (MLPA) has been proven to be an efficient and reliable technique for the copy number analysis of each exon in a gene (5,8-10). In the present study, we applied MLPA for the analysis of copy numbers in exons of *HMGCL* and confirmed mutations in the two patients with HMGCL deficiency.

Patients and methods

Patients. Patient 1 was of the female gender, born to non-consanguineous parents, who presented with hypoglycemia at the age of 2 days. She also experienced hypoketotic hypoglycemic crises at the age of 6, 8 and 13 months. She was diagnosed as having HMGCL deficiency at the age of 13 months by urine organic acid analysis, which detected 3-hydroxymethylglutarate, 3-methylglutaconate and 3-hydroxy-3-methylglutarate. The patient is currenlty 13 years old. She has epilepsy and developmental delay.

Patient 2 was of the male gender, born to non-consanguineous parents, who presented with vomiting and unconsciousness at the age of 3 months. He was diagnosed as having HMGCL deficiency at the age of 3 months by urine organic acid analysis and blood acylcarnitine analysis. He has experienced ten or more hypoketotic hypoglycemic crises, the last of which was at the age of 4 years. He is currently 8 years old and has achieved normal development. A case report for this patient has been previously published in Japanese (11).

Mutation analysis at the genomic DNA level. The present study was approved by the Ethics Committee of the Graduate School of Medicine, Gifu University, Gifu, Japan. Genomic DNA was purified from peripheral blood samples using Sepa Gene kits (Sanko Junyaku Co., Ltd., Tokyo, Japan). Mutation screening was performed at the genomic level by PCR and direct sequencing, using a set of primer pairs that amplify fragments, including exons and their intron boundaries. The primer sequences are presented in Table I.

Establishment of MLPA for the analysis of HMGCL. The MLPA reaction is an efficient and reliable technique for the analysis of exon copy numbers. We designed a pair of MLPA probes for each *HMGCL* exon, using the human MLPA probe design program (H-MAPD), as previously described (12). The MLPA probe sets for the *HMGCL* exon are listed in Table II. MLPA reactions were performed according to the manufacturer's instructions (MRC-Holland BV, Amsterdam, The Netherlands) using 100 ng of genomic DNA, the EK1 MLPA reagent kit and the P200-A1 Human DNA reference kit, which includes reference probes and MLPA control fragments (MRC-Holland BV). The PCR products were separated

Table I. Amplification primer for *HMGCL* exons.

Exon	Foward primer	Sequence	Reverse primer	Sequence	Product size (bp)
1	HL1s	5'-GTGGAGCCAGCTTCGGAAGT-3'	HL1as	5'-GGGAGGGTCCAGGACTCCAACG-3'	324
2	HL2s	5'-ATGAATTCGGTCTCCCTGGGAATTG-3'	HL2as	5'-TAACTTGTGCAGAGGAATCACATC-3'	275
3	HL3s	5'-ATGAATTCTGCATTTTGAGGCTGTTT-3'	HL3as	5'-TTTGCTGCAACACAGTGCTATG-3'	325
4	HL4s	5'-ATGAATTCCTGCTCTTGGTGATGACT-3'	HL4as	5'-GATCACAGAGCAGTGAGTGGCA-3'	314
5	HL5s	5'-GAACCCAGGAGGTGGAGGTTGCA-3'	HL5a	5'-ATAAGCTTGAACGGTACAGAGGAAAGGA-3'	329
6	HL6s	5'-CTGGCACTGAATTGTACCAT-3'	HL6as	5'-GGGTGAATGAATGAAGTCAGGA-3'	336
7	HL7s	5'-AACTGAGTGCGTCATACCCAGA-3'	HL7as	5'-CAGAGCTGTACACTTCACATCTG-3'	473
8	HL8s	5'-ATGAATTCGGCAACAGACGATTGGG-3'	HL8as	5'-GAGCCACTGCGCCTGGCTAACC-3'	366
9	HL9s	5'-CCTGGTGTTGAGGGCATACC-3'	HL9as	5'-TGCCAGGAGAGACCTCTGTGTA-3'	300

Table II. MLPA probes for the *HMGCL* gene.

Exon	Product length (base)	Primer name	Length	Probe sequence
1	104	MLPA-HMGCLEX1L	50	GGGTTCCCTAAGGGTTGGA[5016]*TGGACTGCCGCGGGGGATTCTGGGCCAAGAT*
		MLPA-HMGCLEX1R	54	[5047]*GGCAGCAATGAGGAAGGCGCTTCCGCGGCGA*TCTAGATTGGATCTTGCTGGCAC
2	108	MLPA-HMGCLEX2L	52	GGGTTCCCTAAGGGTTGGA[9872]*CACCTCATCTATGGGCACTTTACCAAAGCGGGT*
		MLPA-HMGCLEX2R	56	[9905]*GAAAATTGTGGAAGTTGGTCCCCGAGATGGACT*TCTAGATTGGATCTTGCTGGCAC
3	112	MLPA-HMGCLEX3L	54	GGGTTCCCTAAGGGTTGGA[12925]*GAAGCAGGACTCTCTGTTATAGAAACCACCAGCTT*
		MLPA-HMGCLEX3R	58	[12960]*TGTGTCTCCTAAGTGGGTTCCCCAGGTGAGCCCTA*TCTAGATTGGATCTTGCTGGCAC
4	116	MLPA-HMGCLEX4L	56	GGGTTCCCTAAGGGTTGGA[13730]*TTCCTGGCATCAACTACCCAGTCCTGACCCCAAATTT*
		MLPA-HMGCLEX4R	60	[13767]*GAAAGGCTTCGAGGCAGCGGTAAGAGGATAGCTTGTTT*CTAGATTGGATCTTGCTGGCAC
5	120	MLPA-HMGCLEX5L	58	GGGTTCCCTAAGGGTTGGA[16193]*TTGTTCCATAGAGGAGAGTTTTCAGAGGTTTGACGCAAT*
		MLPA-HMGCLEX5R	62	[16232]*CCTGAAGGCAGCGCAGTCAGCCAATATTTCTGTGCGGGG*TCTAGATTGGATCTTGCTGGCAC
6	124	MLPA-HMGCLEX6L	60	GGGTTCCCTAAGGGTTGGA[19636]*TCATTCCTCCCCTGTCTTCCCACAGGTACGTCTCCTGTGCT*
		MLPA-HMGCLEX6R	64	[19677]*CTTGGCTGCCCTTATGAAGGGAAGATCTCCCCAGCTAAAGT*TCTAGATTGGATCTTGCTGGCAC
7	128	MLPA-HMGCLEX7L	62	GGGTTCCCTAAGGGTTGGA[22152]*TACTCAATGGGCTGCTACGAGATCTCCCTGGGGGACACCATTG*
		MLPA-HMGCLEX7R	66	[22195]*GTGTGGGCACCCCAGGGATCATGAAAGACATGCTATCTGCTGT*TCTAGATTGGATCTTGCTGGCAC
8	132	MLPA-HMGCLEX8L	64	GGGTTCCCTAAGGGTTGGA[25930]*TCTAGATGGGAGTGAGTGTCGTGGACTCTTCTGTGGCAGGACTTG*
		MLPA-HMGCLEX8R	68	[25975]*GAGGCTGTCCCTACGCACAGGGGGCATCAGGAAACTTGGCCACAG*TCTAGATTGGATCTTGCTGGCAC
9	136	MLPA-HMGCLEX9L	66	GGGTTCCCTAAGGGTTGGA[27975]*CTCAGGCTACCTGTAAACTCTGAGCCCCTTGCCCACCTGAAGCCCTG*
		MLPA-HMGCLEX9R	70	[28022]*GGGATGATGTGGAAATAGGGGCACACACAGATGATTCATGGATGGGG*TCTAGATTGGATCTTGCTGGCAC

Left primer sequence (5'→3'), GGGTTCCCTAAGGGTTGGA; right primer sequence (5'→3'), TCTAGATTGGATCTTGCTGGCAC. Hybridization sequences are shown in italics. MPLA, multiplex ligation-dependent probe amplification.

by capillary electrophoresis on an ABI 3130xl genetic analyzer (Applied Biosystems, Warrington, UK). GeneMapper v4.0 software (Applied Biosystems) was used to analyze the separated products and to retrieve peak intensities corresponding to each probe in the different samples. Integrated peak areas were exported to an Excel 2003 spreadsheet. Data generated from a combination of the *HMGCL* synthetic probe mix and the P200-A1 probe mix were intra-normalized by dividing the peak area of the amplification product of each probe by the total area of only the reference probes in P200-A1. Secondly, normalization was achieved by dividing this intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference samples.

Deletion breakpoint characterization. The region surrounding the deletion from intron 1 to intron 4 in patient 1 was amplified using three primer pairs as follows: fragment A: sense primer (In1s1, 5'-ACGAACGGTGGTAAAGAGGCAACAG-3') located at position g.6421-6445 in intron 1 and antisense primer (Ex5as, 5'-TTGGCTGACTGCGCTGCCTTCAGGA-3') located at position g.16255-16231 in exon 5; fragment B: sense primer (In1s3, 5'-GTGATGATTCCAGGAGGTCAGA GGA-3') located at position g.8701-8725 in intron 1 and antisense primer Ex5as; and fragment C: sense primer In1s3 and antisense primer (In4as1: 5'-GAGAGGCATAGGACAGA TTCTCC-3') located at position g.15110-15088 in intron 4 (GenBank accession no. NG_013061).

PCR was carried out for 40 cycles at 94°C for 1 min, 64°C for 2 min, 72°C for 2 min followed by a 5-min extension at 72°C using Takara r-Taq (Takara Shuzo Co., Ltd., Shiga, Japan) and a Takara PCR thermal cycler. After subcloning into the pGEM-T Easy vector (Promega, Madison, WI, USA), the fragments were sequenced.

Comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) microarray analysis. Whole genomic copy number analysis was performed for patient 2 using an Agilent SurePrint G3 Hmn CGH + SNP 180K Microarray kit (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. Genomic DNA extracted from peripheral blood was used as a template. Data were extracted using Feature Extraction version 9 (Agilent Technologies) and the results were visualized using Agilent Genomic Workbench version 6.5 (Agilent Technologies).

Results

Mutation analysis. Mutations were screened at the genomic level using PCR amplification followed by direct sequencing. Patient 1 had a heterozygous c. 31C>T (p.R11*) mutation in exon 1, and her mother did not have this mutation (Fig. 1). No mutation in the maternal allele was identified. The DNA of the father was not available. Patient 2 had a homozygous c.242G>A (p.W81*) mutation in exon 3. Genomic analyses of his patients revealed that the father was heterozygous for the p.W81* mutation; however, the mother did not have the p.W81* mutation (Fig. 2). Hence, we hypothesized the following: i) the maternal allele may have a large deletion not including exon 1 in patient 1; and ii) the maternal allele in patient 2 may have a deletion including exon 3.

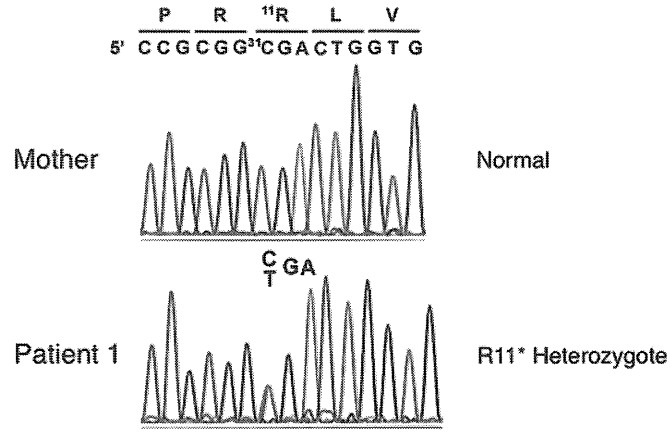

Figure 1. Sequence analysis of patient 1 and her mother. The c.31C>T (p. R11*) mutation site is shown.

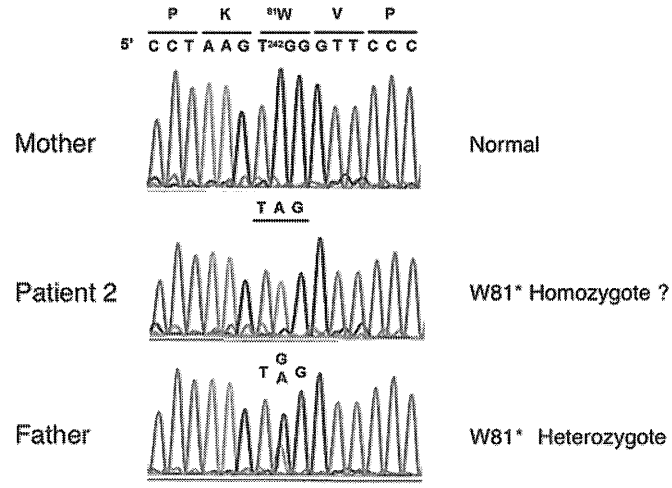

Figure 2. Sequence analysis of patient 2 and his parents. The c.242G>A (p.W81*) mutation site is shown.

MLPA analysis of HMGCL. We successfully performed MLPA for *HMGCL*. Similar patterns of amplification were obtained in three controls, enabling copy number to be evaluated in the patient samples (Fig. 3). In patient 1 and his mother, only one copy of exons 2-4 was present, whereas two copies of all other exons were present, suggesting a large deletion including exons 2-4 in the maternal allele (Fig. 3). Patient 2 and his parents all had two copies of all *HMGCL* exons (Fig. 3). This means that both copies of exon 3 in patient 2 were from the father. We, therefore, suspected that patient 2 has a paternal uniparental disomy of the *HMGCL* region.

Determination of breakpoints in patient 1. Long-range PCR amplification using DNA from patient 1 yielded fragments that included an approximate 4-kb deletion (Fig. 4A). A large deletion from g.9326 to g.13806 (4481 bp; NG_013061) was identified. We confirmed that one breakpoint was within an Alu element in intron 1 and the other was in a non-Alu element in intron 4 (Fig. 4B).

CGH and SNP arrays in patient 2. To confirm the copy number and SNP haplotype of the *HMGCL* region, microarray analysis

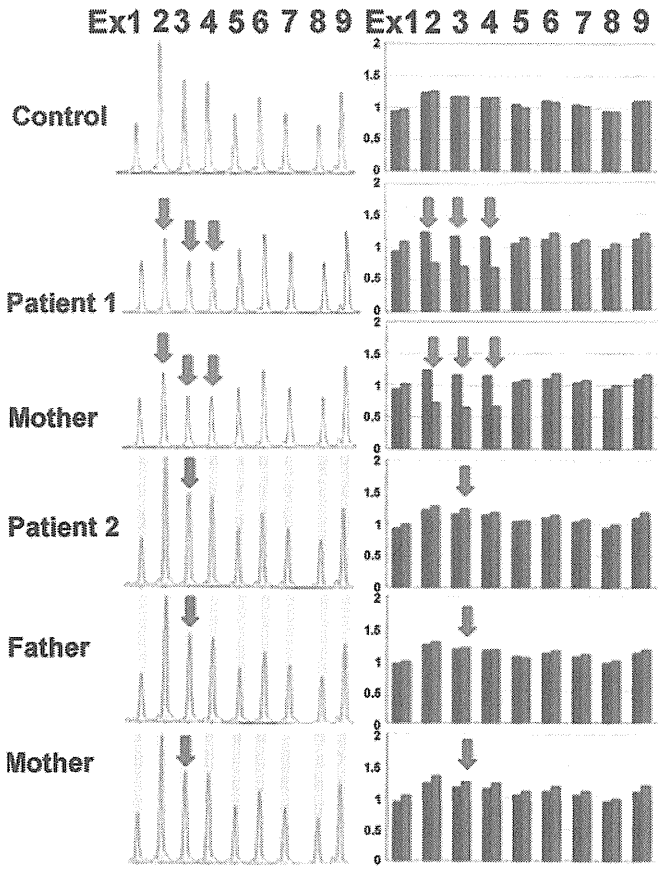

Figure 3. Multiplex ligation-dependent probe amplification (MLPA) of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase (*HMGCL*) gene. The MLPA profiles of each *HMGCL* exon are shown. The peaks derived from the P200-A1 reference probes followed by the peak of exon 9 are not shown in this figure. The histogram shows the calculated exonic dosage normalized as described in the Materials and methods. Blue and red columns indicate the dosage in the subject and in one control, respectively. Red arrows indicate that the bands had a significantly decreased dosage compared with the control, indicating that only one copy of exons 2-4 was present in patient 1 and her mother. Patient 2 and his parents had two copies of each exon including exon 3 (green arrows).

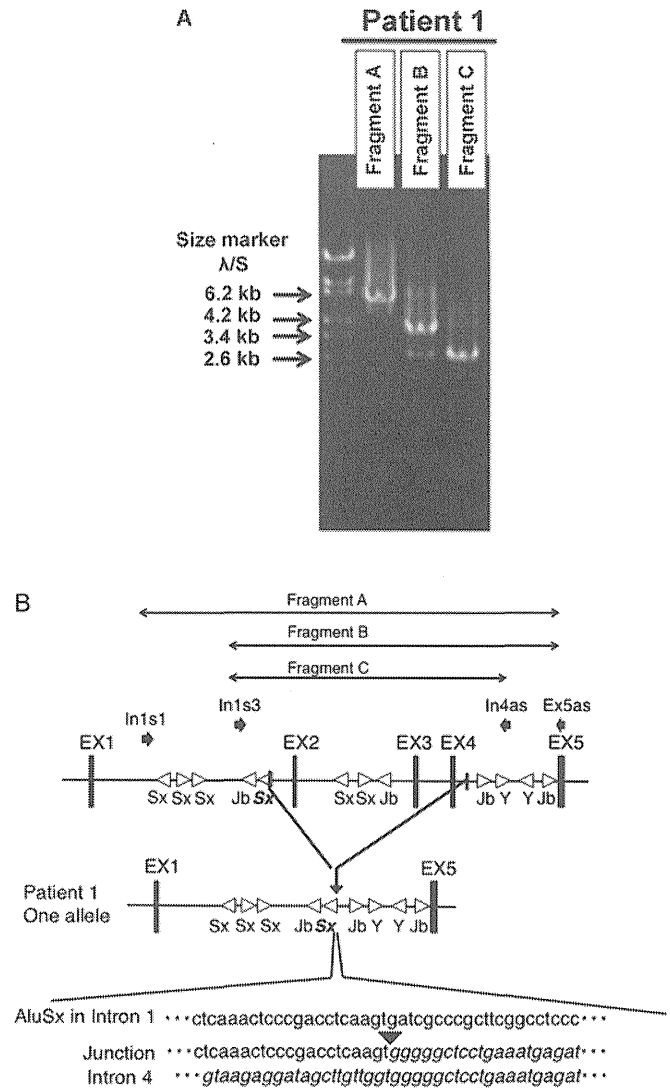

Figure 4. Characterization of a deletion that includes exons 3 and 4. (A) Long-range PCR. Fragments A, B and C were electrophoresed following amplification using genomic DNA and primer pairs of In1s1 and Ex5as, In1s3 and Ex5as, and In1s3 and In4as, respectively. The position of primers and their orientation are indicated in panel B. (B) Schematic representation of the rearranged mitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase (*HMGCL*) gene in patient 1. Alu elements in introns 2-4 are indicated by arrowheads. Patient 1 had breakpoints within an Alu-Sx element in intron 1 and in a non-Alu sequence in intron 4, as indicated by the inverted triangle.

using CGH + SNP array was performed for patient 2. In the CGH array, no copy number aberration was detected in the whole of chromosome 1 including the *HMGCL* region, which confirmed the homozygous status of the *HMGCL* mutation (Fig. 5).

Furthermore, a loss of heterozygosity (LOH) for almost all of chromosome 1, where *HMGCL* is located, was revealed. This indicated paternal uniparental disomy of chromosome 1. In conclusion, a homozygous mutation in *HMGCL* was determined to be caused by non-Mendelian inheritance due to paternal uniparental isodisomy of the whole chromosome 1, including the 1p36.1 region.

Discussion

In the present study, we investigated the molecular genetic basis of two Japanese HMGCL-deficient patients using standard Sanger sequencing. Although we identified a mutation in each patient, inheritance patterns were not consistent in their families. Human *HMGCL* is an Alu element-rich gene, having 23 Alu elements within 23 kb. We first hypothesized that a heterozygous intragene deletion caused by non-equal homologous recombination between Alu elements was a possible cause of *HMGCL* mutation in these patients. Therefore, we used the MLPA method to detect the copy numbers of each exon in *HMGCL*.

Patient 1 had a heterozygous p.R11* mutation from the father, but a mutation from the mother was not identified by conventional sequence analysis. MLPA revealed that patient 1 and her mother had only one copy of exons 2-4, which was consistent with our hypothesis. However, this large deletion that included exons 2-4 was not caused by non-equal Alu-mediated homologous recombination. One breakpoint was within an Alu element in intron 1 and the other was in a non-Alu element in intron 4. There were no homologous sequences around the breakpoint. The genesis of this deletion is unknown.

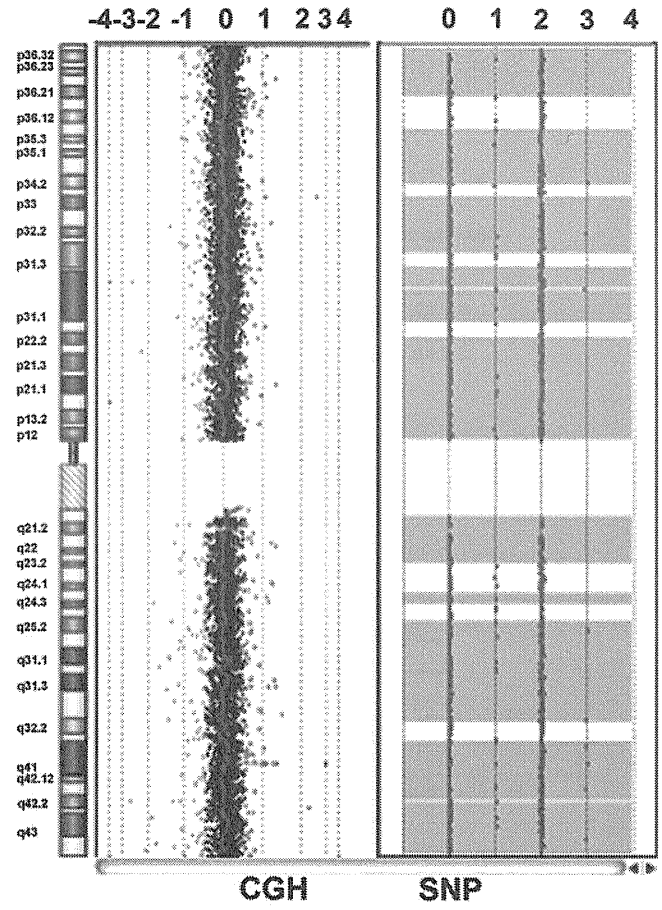

Figure 5. Results of chromosomal microarray testing using the comparative genomic hybridization and single nucleotide polymorphism (CGH + SNP) platform. The results of genomic copy number (left) and loss of heterozygosity (LOH) status (right) are shown for chromosome 1 of patient 2 according to the chromosome view of the Agilent Genomic Workbench. No genomic copy number aberration was detected in chromosome 1. Regions showing LOH status, judged by computer analysis, are depicted in purple; other non-LOH regions are considered to be false due to SNP artifacts. Patient 2 was, therefore, regarded to have LOH across the entire chromosome 1.

Patient 2 had an apparent homozygous p.W81* mutation and his father was heterozygous for this mutation; however, his mother did not have this mutation. MLPA revealed that patient 2 had two copies of each *HMGCL* exon. Hence, our new hypothesis was that LOH of the *HMGCL* region may be the molecular basis for HMGCL deficiency in this patient. We successfully identified a paternal uniparental isodisomy of almost the entire chromosome 1 by microarray analysis using a CGH + SNP array. To the best of our knowledge, this is the first case of HMGCL deficiency shown to be caused by uniparental disomy.

There are two types of uniparental disomy, heterodisomy and isodisomy. In heterodisomy, a pair of non-identical chromosomes is inherited from one parent and in isodisomy a single chromosome from one parent is duplicated. Isodisomy is potentially dangerous as it may lead to the duplication of lethal recessive genes, while heterodisomy is essentially benign. Uniparental disomy in humans is mainly caused by meiotic non-disjunction events followed by i) gamete complementation; ii) trisomy rescue; iii) monosomy duplication; and iv) somatic crossing over (13). Our present results from *HMGCL* gene analysis, MLPA and CGH + SNP arrays indicate that patient 2 had a monosomic duplication of paternal chromosome 1. Partial and whole uniparental disomy of chromosome 1 has been reported in autism, fumarase deficiency, Stargardt disease, Pelizaeus-Merzbacher-like disease, leptin receptor deficiency, rhizomelic chondrodysplasia punctata type 2 and CD45-deficient severe combined immunodeficiency (14-20). This study demonstrates the advantage of using MLPA method for the analysis and identification of such heterozygous gene alterations. The identification of such mutations may facilitate mutation analysis in newly diagnosed patients.

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特集　慢性疾患をもつ子どもの成人へのtransition

【長期予後と成人後の医学的問題】

先天代謝異常症

深尾敏幸

キーワード●先天代謝異常症，予後，成人期，治療

はじめに

先天代謝異常症は，一般的にまれな遺伝性疾患で，代謝系における特定の酵素等の欠損・欠乏により代謝に異常が生じるために起こる疾患である．アミノ酸代謝異常症，尿素サイクル異常症，有機酸代謝異常症，脂肪酸代謝異常症，糖代謝異常症，金属代謝異常症などのように分類されたり，障害される細胞内小器官を中心にミトコンドリア病，ライソゾーム病，ペルオキシソーム病などのように分類されたりする．日本先天代謝異常学会では，先天代謝異常症を身近に感じてもらい，日本全体の診療レベルを向上するために10年前から日本先天代謝異常学会セミナーを開催している．最近は毎年300～400名が参加しており，先天代謝異常症診療の裾野は確実に広がってきている．

I 先天代謝異常症の治療と予後

平成26年度にはタンデムマス法による新生児マス・スクリーニングが全国に広がり，対象疾患が6疾患から，アミノ酸代謝異常症，脂肪酸代謝異常症，有機酸代謝異常症のいくつかが追加されて19疾患へと増加した．追加された疾患は，多くが新生児期～乳幼児期に低血糖，代謝性アシドーシス，高アンモニア血症などの重篤な発作を来す可能性がある疾患である．初回発作で死亡したり後遺症を残す可能性が高い疾患であり，早期診断，治療開始により，予後の改善が期待されている．

この15年で先天代謝異常症に対する治療成績が大きく向上した．①海外では使われていたが日本では未承認という薬剤が，いくつか国内での使用が可能となったこと，②酵素製剤などの新規治療薬剤の開発が進んだこと，③骨髄移植，肝移植などの移植治療が取り入れられたこと，④血液浄化法など急性期治療技術が向上したことなどである．それに伴い，長期生命予後は向上している．代謝異常は生涯にわたって存在し，小児期にのみ治療すればよいという疾患は少なく，成人後の対応が問題化している．

以下に代表的な先天代謝異常症とその予後，成人期の問題点について記載したい．

1. アミノ酸代謝異常症

●フェニルケトン尿症

アミノ酸代謝異常症の代表的疾患であるフェニルケトン尿症（phenylketonuria；PKU）は，無治療では重度の精神発達遅滞を来す．新生児マス・スクリーニングにより新生児期に診断されることで，無症状のうちからフェニルアラニン（Phe）除去ミルクを併用したPhe制限食療法を行うことにより知的予後は改善した．食事制限は特に小児期に重要であるが，生涯続けることが望ましい[1~4]．成人期においても高Phe血

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症は，抑うつなどの精神症状，MRIで白質病変を来すとされており[2,3]，また酸化ストレスの亢進を介して全身的な影響を及ぼすことが示唆されている[4]からである．また女性については，妊娠時に胎児が高Phe環境にさらされることで生じる知的障害，先天異常(マターナルPKU)を防ぐため，食事療法の継続が必要で，一旦緩和してしまうと，妊娠前に厳しく制限し直すことが必要となる．

しかし本邦において，2004年の調査では，1977～1981年に出生した23～27歳の患者の60%，およびそれ以降に出生した18～22歳の患者の30%はPhe制限食を中止していた[1]．高校生までは食事療法がうまくいっていた症例でも，大学への進学や就職により親元を離れたり，忙しくて外来受診が難しくなり，食事療法が中断もしくは大幅に緩和されてしまうことが経験される．20歳を超えると小児慢性特定疾患による公費補助がなくなり，治療用ミルク，低タンパク食品の購入など経済的な負担が大きいことも中断に大きく影響していると考えられる[成人期のフェニルケトン尿症(PKU)．特殊ミルク情報2011；47：6–39]．

2. 尿素サイクル異常症

● オルニチントランスカルバミラーゼ欠損症

尿素サイクル異常症は，一般に高アンモニア血症を来す予後の悪い疾患群であり，その代表がオルニチントランスカルバミラーゼ(OTC)欠損症である．本症はX連鎖遺伝をとり，多くは男児が発症するが，X染色体不活化の偏りで女児にも発症することが比較的高い疾患である．

2009年のアンケート調査[5]によれば，新生児発症（重症型）のOTC欠損症の男児においては，新生児期に20%が亡くなっているが，そこで救命された症例は20歳までほぼ生存している．新生児期以降発症のOTC欠損症男児例においても，20歳における生存率は1998年の報告が45%であったのに対し，2009年の調査では89.4%と改善し，新生児期以降発症の女児例においても29.8%から83.8%と改善している[5]．この生命予後の改善は，薬物投与，血液浄化による急性期治療，タンパク制限などの治療が確立し，また肝移植が導入されて救命できる症例が増えてきたためと考えられる．

しかし生命予後は改善されているが，神経学的予後は発作時のピークのアンモニア値が高い場合，厳しい現実がある．360μmol/L以上であった症例の15%は死亡し，51%が知的な発達遅滞を残し，15%は発達遅滞がないものの画像，脳波に異常をもっている．わずか8%が正常発達し，画像や脳波に異常がないというのが現状である[5]．このように，成人期を迎えた多くの患者がハンディキャップをもち，さらに治療継続が必要な状態と言える．

3. 有機酸代謝異常症

● ビタミンB_{12}不応性メチルマロン酸血症

有機酸代謝異常症の代表的な疾患の1つがビタミンB_{12}不応性メチルマロン酸血症(methylmalonic acidemia；MMA)である．詳細は省略するが，2009年に行われた日本におけるアンケート調査[6]では，ビタミンB_{12}不応性MMAは69.8%が20歳で生存しており，生命予後という点では非常に良くなっている．しかし，成人例を含めた69%が重篤な発作の後遺症と考えられる精神発達遅滞を来しており，また腎障害も35.7%に合併している．

最近はコントロール不良例では肝移植が行われることがあり，臨床的に食事制限が緩和され，アシドーシス発作が著しく少なくなりQOLに改善がみられている．しかし多くの肝移植症例は，それ以前の発作後遺症により精神発達遅滞を合併しているのが現状である[6]．新生児マス・スクリーニングの対象疾患となったので，今後は予後の改善が期待される．

4. ライソゾーム病

ライソゾーム病の中からは，小児期に典型的症状を来すムコ多糖症II型と，小児期よりは成人期に典型的症状を来すファブリー病の2つ

を取り上げる．共に，現在は酵素補充療法という治療法があるX連鎖遺伝のライソゾーム病である．

(1) ムコ多糖症II型

ムコ多糖症II型はα-イズロネート2-スルファターゼの欠損症で，グリコサミノグリカン(GAG)の蓄積を特徴とする．発症年齢，疾患の重症度，進行速度には著しい差がある．通常，幼児期に診断されることが多い．従来，重症型の患者では中枢神経系症状，進行性の気道疾患，心疾患により20歳以前に死亡した[7]．軽症型の患者では正常な知能を維持したままで成人初期まで生存する場合が多いが，関節拘縮，難聴の進行，気道狭窄による呼吸障害，心臓弁膜症の進行などで生活が制限される．早期に診断し，早期に酵素補充療法，骨髄移植を行うことがQOLの改善には重要と考えられ，今後，酵素補充療法などにより成人期に治療継続が必要な症例が増えてくると思われる．

小児期発症のライソゾーム病では，成人期の治療は多くの場合，小児期から診ている医師がそのまま引き続き行って，他診療科との連携をとっている場合が圧倒的に多いと考えられる．

(2) ファブリー病

ファブリー病は，α-ガラクトシダーゼA酵素の活性が低下することにより，心臓，腎臓，血管，皮膚，角膜，神経など全身の臓器にスフィンゴ糖脂質の蓄積が生じ，多臓器障害を起こす疾患である．典型的ファブリー病の男性患者では，幼少時より四肢末端の痛みの発作，発汗障害があり，この時期の診断のきっかけになるが，一般には成人期に入って皮膚の被角血管腫，角膜の混濁，尿タンパクから腎不全という腎臓機能障害，心臓肥大などの心臓機能障害や一過性脳虚血発作，多発性小梗塞などの脳血管障害も出現する[8]．女性がかなり発症するX連鎖遺伝をとるため，女性保因者のフォローも重要である[8]．

まれな疾患と考えられてきたが，日本において比較的頻度の高い疾患であることが明らかになってきており[9]，成人例として見つかった患者は循環器内科，腎臓内科，神経内科などで診療されている例も多い．酵素補充療法は，男児の場合臓器障害の進行していない小児期からすべきと考えられ，小児期に診断された患者は小児科において開始することになる．成人患者においても小児科の先天代謝異常症を専門にしている医師が酵素補充療法を行い，他の循環器内科，腎臓内科，神経内科との連携で診ているところもある．

II 先天代謝異常症における成人期の問題

上記に示したファブリー病以外の疾患の長期予後，成人期の問題は共通している部分が多い．治療管理が向上し，生命予後が改善し，多くの先天代謝異常症患者において，成人期の診療が重要となってきている．生命予後は良くなってはいるが，精神運動発達にハンディキャップをもって成人期を迎えている患者が多い．ハンディキャップをもった患者の場合は，引き続き家族による診療へのかかわりが重要であることは言うまでもないが，知的問題がない思春期以降の患者に対し，家族依存の治療体制から本人中心への治療への移行が必要となる．

だれが成人期の先天代謝異常症患者を中心に診て行くのか？　おそらく日本の現状では，小児期に診てきた小児科の医師がそのまま継続するパターンが多いと考えられる．しかし当然であるが，小児科医は加齢に伴う成人期の合併疾患の診療が苦手であり，小児科のみでは対応できない問題が加齢と共に大きくなってくる．成人期に主要な症状を呈するファブリー病のような疾患以外でも，通常は小児期に発症する先天代謝異常症が成人後に発症することもあり[10]，成人の診療にあたる医師が先天代謝異常症についても知識をもつことは重要である．

ヨーロッパの先天代謝異常学会では2010年に先天代謝異常症の成人に関心のある臨床医が

集まり，ヨーロッパ先天代謝異常学会成人代謝異常臨床医グループを形成し，2011年の年次集会から成人の先天代謝に関するワークショップ等を開催して非常に関心が高まってきている．内科，神経科，遺伝科，内分泌科，成人代謝異常クリニックなどの肩書きの医師でグループを形成しているが，まだ日本からの参加はない．将来的にはヨーロッパ同様に，内科，総合診療科の中で成人期の先天代謝異常症患者の診療を行う担当医ができてくるのも1つの方向性ではないかと考える．しかし小児科の中でも先天代謝異常症を専門にする医師が少ない中で，すぐに実現する状況にはない．先天代謝異常学会に内科関係の医師がもっと参加しやすい環境づくりをすることが重要と考えられる．

おわりに

最後に，先天代謝異常症の多くは小児慢性特定疾患であり，20歳未満までの医療費公費負担がなされている．今後，特定疾患に一部移行すると期待されるが，移行しなかった疾患においては，成人期の医療費の負担が大きな問題となる点を指摘したい．

成人期を迎えた先天代謝異常症患者が，患者の状況に応じた適切な診療を受けられる仕組みを作り上げていくことが，日本先天代謝異常学会としての1つの今後の使命である．

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The role of lipoxygenases in pathophysiology; new insights and future perspectives

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ABSTRACT

Lipoxygenases (LOXs) are dioxygenases that catalyze the formation of corresponding hydroperoxides from polyunsaturated fatty acids such as linoleic acid and arachidonic acid. LOX enzymes are expressed in immune, epithelial, and tumor cells that display a variety of physiological functions, including inflammation, skin disorder, and tumorigenesis. In the humans and mice, six LOX isoforms have been known. 15-LOX, a prototypical enzyme originally found in reticulocytes shares the similarity of amino acid sequence as well as the biochemical property to plant LOX enzymes. 15-LOX-2, which is expressed in epithelial cells and leukocytes, has different substrate specificity in the humans and mice, therefore, the role of them in mammals has not been established. 12-LOX is an isoform expressed in epithelial cells and myeloid cells including platelets. Many mutations in this isoform are found in epithelial cancers, suggesting a potential link between 12-LOX and tumorigenesis. 12R-LOX can be found in the epithelial cells of the skin. Defects in this gene result in ichthyosis, a cutaneous disorder characterized by pathophysiologically dried skin due to abnormal loss of water from its epithelial cell layer. Similarly, eLOX-3, which is also expressed in the skin epithelial cells acting downstream 12R-LOX, is another causative factor for ichthyosis. 5-LOX is a distinct isoform playing an important role in asthma and inflammation. This isoform causes the constriction of bronchioles in response to cysteinyl leukotrienes such as LTC_4, thus leading to asthma. It also induces neutrophilic inflammation by its recruitment in response to LTB_4. Importantly, 5-LOX activity is strictly regulated by 5-LOX activating protein (FLAP) though the distribution of 5-LOX in the nucleus. Currently, pharmacological drugs targeting FLAP are actively developing. This review summarized these functions of LOX enzymes under pathophysiological conditions in mammals.

Contents

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1. Introduction

Lipoxygenases (LOXs) catalyze the oxygenation of polyunsaturated fatty acids such as arachidonic acid and linoleic acid [1,2]. The oxygenated lipids initiate subsequent biological reactions, activate cellular signaling mechanisms through specific cell surface receptors, or are further metabolized into potent lipid mediators. LOX can be found not only in mammals, but also in plants. Historically, biochemical characterizations have been performed mainly on soybean LOX isoforms. While the overall structure of mammalian LOX enzymes seems to be similar, each isoform has unique properties, such as substrate specificity (Table 1, reviewed in [3]). In most cases, the structure depends on the shape of the substrate cavity and the coordination of histidine residues or alternatives to a non-heme iron atom at the catalytic center [4,5]. Importantly, LOX enzymes require a lag period for the activation of enzymes from an inactive ferrous form to an active ferric form by either molecular oxygen or lipid hydroperoxides. Enzymatic activity is also regulated by the N-terminal β-barrel region of polypeptides, where this region has a similar amino acid sequence to the C2-like domain; thus, Ca^{2+}-mediated activation via interaction with the plasma membrane has been proposed. Earlier studies have shown that LOX enzymatic activity can be inhibited by phenolic antioxidants such as nordihydroguaiaretic acid and caffeic acid, suggesting a beneficial role of dietary polyphenol intake [6]. Alternatively, synthesized drugs for LOX are relatively limited thus far. The 5-LOX inhibitor zileuton has been accepted and used successfully for the control of asthma. Currently, inhibitors for 5-LOX activating protein are actively developed by many pharmaceutical companies [7]. These inhibitors essentially modulate the transportation of 5-LOX from the nucleus to the cytoplasm, leading to suppressive 5-hydroperoxyeicosatetraenoic acid (5-HPETE) production. This mode of action of 5-LOX inhibitor is unique, and there are no similar regulatory mechanisms and drugs for other LOX isoforms.

From a genetic point of view, the alignment of LOX isoform nucleotides encoded by arachidonate lipoxygenase (*ALOX* in humans and *Alox* in mice) genes has revealed that *ALOX5* and other *ALOX* genes have separate origins. The other *ALOX* genes seem to have originated from fewer genes, as human *ALOX* genes are found in a cluster in chromosome 17p13.1 and murine *Alox* genes are found in chromosome 11 as active enzymes [8]. The expression levels of *ALOX* genes are partially controlled by cytokines, such as *ALOX15*, whose expression increases in response to Th2 cytokines. *ALOX* enzymatic activity is also regulated by tissue distribution and cell type. *ALOX12B*, *ALOXE3*, and *ALOX15B* are expressed mainly in the skin and other epithelial cells, whereas *ALOX15*, *ALOX12*, and *ALOX5* are expressed in hematopoietic/immune cells. They are involved in atherosclerosis, neuronal disorder, immune modulation, skin diseases, and maintenance of the epithelium. The roles of human enzymes (Table 2) seem to be slightly different from what is expected from phenotypes of knockout mice (Table 3), which shows that these oxygenated lipids are uniquely and finely regulated in humans and mice.

2. 15-Lipoxygenase (15-LOX)

15-LOX is a prototypical enzyme catalyzing oxygenation of polyunsaturated fatty acids. Among various mammalian species, rabbit reticulocyte LOX has been characterized from earlier studies and often used as standard for biochemical assays. When the

Table 1
Properties of LOX enzymes.

Proteins	15-LOX	15-LOX-2	12-LOX	12R-LOX	eLOX-3	5-LOX	FLAP
Human							
Gene	*ALOX15*	*ALOX15B*	*ALOX12*	*ALOX12B*	*ALOXE3*	*ALOX5*	*ALOX5AP*
Products[a]	15S-HPETE	15S-HPETE	12S-HPETE	12R-HPETE	Epoxyalcohols	5S-HPETE	NA
Expression	Leukocytes	Epithelium, leukocytes	Myeloids, skin, epithelium	Skin, epithelium	Skin, epithelium	Leukocytes	Leukocytes
Mouse							
Gene	*Alox15*	*Alox15b*	*Alox12*	*Alox12b*	*Aloxe3*	*Alox5*	*Alox5ap*
Products[a]	12S-, 15S-HPETE	8R-HPETE, epoxyalcohols	15S-, 12S-HPETE	12R-HPETE	Epoxyalcohols	5S-HPETE	NA
Expression	Leukocytes	Skin, epithelium, leukocytes	Platelet, skin, epithelium	Skin, epithelium	Skin, epithelium	Leukocytes, epithelium	Leukocytes

NA, not available. [a]Arachidonic acid as a substrate except eLOX-3 where 12R-HPETE as a substrate.

Table 2
Human diseases that potentially links to lipoxygenase genes.

Genes	Atherosclerosis/heart disease	Immune response	Neurological disorder	Cancer	Skin disease	Others
ALOX15	A near null mutant (T560M) in coronary artery disease [140]			Rectal cancer [100] Colon cancer [141] Adenoma recurrence [142] Breast cancer [39] Prostate cancer [12]		Bone mineral density↓[56,143–144]
ALOX15B	↑ in carotid plaque with thrombosis [43] ↑ in carotid lesion [32] Variants in coronary artery disease [145]			Esophageal cancer [35] Adrenocortical tumor [36] Breast cancer [39] Epithelial tumors [37] Prostate cancer cells [38] Head and neck carcinoma [40] ↑ in TAMs isolated from renal cell carcinoma [42]		
ALOX12	Variants in subclinical atherosclerosis [146]	Variants in Toxoplasmosis [147]	Bipolar disorder [49] Schizophrenia [50]	Methylation in AML[61] Esophageal squamous cell carcinoma [64] Rectal cancer [100] Adenoma recurrence [142] Colorectal cancer [148] E261R mutation Breast cancer [48] Colon cancer [47] Colorectal cancer [65] Colorectal adenoma [149]		Bone mineral density↓[54–57,150] Fat mass↑[53]
ALOX12B					ARCI [81–82, 94,151] NCIE [84–86]	
ALOXE3					ARCI [82, 94,151] NCIE [84,86]	
ALOX5	Variants in atherosclerosis [152] ↑ in atherosclerosis [153] Variants in subclinical atherosclerosis [146]	Variants in asthma [97,154,155] Variants in AHR [98,99]		Inverse correlation Rectal cancer [100] Ovarian cancer [101] Colon cancer [47]		
ALOX5AP	Variants in subclinical atherosclerosis [146]	Variants in asthma [126,156]		Rectal cancer [100] Adenoma [100]		

AHR, airway hyperresponsiveness; AML, acute myeloid lymphoma; ARCI, autosomal recessive congenital ichthyosis; NCIE, nonbullous congenital ichthyosiform erythroderma; TAM, tumor-associated macrophages.

potential link between atherosclerosis and its inhibition with antioxidants was explored, 15-LOX has been hypothesized to initiate and/or promote atherosclerosis through low density lipoprotein (LDL) oxidation. This is based on the "oxidative LDL theory" that oxidation of lipids induces atherosclerosis and its inhibition by antioxidants prevents atherogenesis. To initiate oxidation in vivo, there must be some initiators for lipid peroxidation. One potential candidate includes carbon-centered free radicals, which react with molecular oxygen to give rise to peroxyl radicals. Since these radicals trigger oxidation of lipids continuously, therefore, generation of such free radical-generating initiators must be tightly regulated in vivo. Lipid hydroperoxides generated from polyunsaturated fatty acids by LOX enzyme can induce this reaction since these oxidation products further decompose into other free radicals in the presence of metal ions. 15-LOX can be found in macrophages and other immune cells as well as epithelial cells. Human and mouse enzymes are known to be induced by Th2 cytokines such as IL-4 and IL-13 via STAT6-dependent manner [9,10].

2.1. *ALOX15*

It is widely accepted that cyclooxygenase (COX) inhibitor nonsteroidal anti-inflammatory drugs (NSAIDs) induce colon cancer in humans [11]. One suggested reason is that the balance between COX and LOX determines tumorigenesis critically. Under low COX activity, arachidonic acid released from cell membranes in response to external stimuli is preferentially metabolized by LOX

Table 3
Phenotypes of LOX-deficient mice.

Genes	Atherosclerosis	Immune response	Neurological disorder	Others
Alox15	↓ in ApoE KO [19–21] ↓ in LDLR KO [22,23] VSMC response↓[24]	LTC4↑[26] Arthritis↑[157] *Schistosoma mansoni* infection→[158] Th1↓[159] IL-12 ↓, TNF-α → [160] Acute lung injury↓[161] Phagocytosis↑[162]	Peripheral diabetic neuropathy↓[163] Diabetic autonomic neuropathy→[163]	Osteoclast development↑[28] Insulin resistance↓[164] Angiogenesis↓[165] Myeloid differentiation↓[27] Erythrocyte development→[26] Angiogenesis↓[166] ER stress↓[167] Hypertension↓[168] Inflammatory neovascularization↓[169] Nonalcoholic fatty liver disease↓[170] Diabetes associated pp38 and pErk↓[171] Obesity↓[29] Ischemic cardioprotection↓[25] Airway epithelial injury in asthma↓[172]
Alox15b				Epidermal permeability barrier↓ [44]
Alox12		Platelet sensitivity↑[67]		Carcinoma (B6/129)↓[68] Papilloma (SENCAR)↓[68] Basal transepidermal water loss↑[69]
Alox12b				Skin barrier↓[87,95] Ichthyosiform↑[88]
Aloxe3				Skin barrier↓[95]
Alox5	↓ in LDLR KO [114]	PAF-induced lethal shock↓[102,103] OVA-induced asthma↓[108] *Schistosoma mansoni* infection↓[158] Early female mortality (MRL-lpr/lpr)↓[109] Toxoplasma gondii elimination↓[107] Tumor-infiltrating macrophages↑[121] OVA/alum-induced Th2↓[159] Peritonitis↓[105] LTB4↓[173] Acute pancreatits↓[104] *Borrelia burgdorferi* elimination↓[106] *Histoplasma capsulatum* elimination↓[174]	Anxiety-like behavior (C57BL/6)↑[117] Synaptic dysfunction↓[119] Anxiety-like behavior (B6/129)↓[175]	Inflammatory neovascularization↓[169] Endotoxin-induced Hypoxic pulmonary vasoconstriction↓[176] $Apc^{\Delta 468}$-induced intestinal polyposis↓[121–123].
Alox5ap	↓ in COX-2 KO [115]	PAF-induced shock↓[127] Zymozan-induced peritonitis↓[127] Collagen-induced arthritis↓[129] Cerebral inflammation↓[128]	Improved Alzheimer's disease-like phenotype [133] Anxiety-like behavior↑[134]	

ApoE, apolipoprotein E; ARCI, autosomal recessive congenital ichthyosis; KO, knockout; LDLR, low-density lipoprotein receptor.

enzymes. There is evidence that a 15-LOX metabolite 13S-HPODE (13S-hydroperoxyoctadecaenoic acid) generated from linoleic acid induces apoptosis in colon cancer cells; thus, defective expression of *ALOX15* in colon cancers could promote tumorigenesis [11]. *ALOX15* expression itself is controlled, at least in part, by the epigenetic process, as an alteration of methylation in the *ALOX15* promoter has been observed in prostate cancer patients [12]. Furthermore, the expression of 15-LOX in epithelial cancer cells is tightly regulated by additional mechanisms. As mentioned, STAT6 is a critical regulator of *ALOX15* expression regulated by its phosphorylation and acetylation, as well as histone modification [13,14]. Recent studies have also shown the *ALOX15* expression can be modulated by the chromatin-dependent STAT6-independent mechanism [15,16]. Biochemically, the produced 13 S-HPETE interacts with PPAR-δ, followed by the induction of apoptosis prior to carcinogenic conditions [17]. The importance of 15-LOX-derived metabolites has also been defined by its aberrant failure in conditional transgenic mice, expressing it in the mouse prostate, inducing prostatic intraepithelial neoplasia once the apoptotic function is dysregulated [18].

2.2. *Alox15*

Atherosclerosis is an inflammatory disease characterized by an accumulation of lipid-loaded macrophages in blood vessels. Evidence has suggested a close link between *Alox15* expression and atherosclerosis in the mouse, characterized mostly on the atherosclerosis-prone genetic background of mice lacking the *ApoE* and/or LDL receptor [19–23]. In both cases, the initiation and/or development of atherosclerosis depends on 15-LOX enzymatic

activity. Recent studies have suggested that atherosclerosis is involved, at least in part, in sterile inflammation characterized by augmented IL-1β and the activation of caspases. The accumulation of lipids in blood vessels causes a failure of vascular function; therefore, disruption of *Alox15* shows impaired migration of vascular smooth muscle cells [24]. In addition, ischemia-induced cardioprotection plays an important role in maintaining proper heart function. A previous study showed that *Alox15* deficiency caused its impairment after reperfusion-mediated preconditioning by PKC activation in the heart [25]. These results suggest that this enzyme might also contribute to the pathogenesis of cardiovascular disease.

Lipid hydroperoxides, the primary reaction products of *Alox15* from polyunsaturated fatty acids, readily induce oxidative stress through their decomposition to free radicals. Thus, *Alox15*-deficient mice might produce less oxidative stress under physiological conditions. However, *Alox15*-deficient mice have shown an increase in oxidative stress markers such as isoprostane 8-epi-prostaglandin F2a in a zymosan-induced peritonitis model [26]. These apparently paradoxical results might be explained by the concomitantly enhanced 5-LOX-, but not 15-LOX-, dependent mechanism, which facilitates the accumulation of neutrophils by 5-HPETE and leukotrienes, thereby leading to enhanced oxidative stress at the site of inflammation in *Alox15*-deficient mice.

Accumulating evidence suggests that *Alox15* is critically involved in the regulation of cell differentiation. For example, the development of hematopoietic stem cells into the myeloid lineage is impaired in *Alox15*-deficient mice [27]. Alternatively, the formation of osteoclasts in *Alox15*-deficient mice and mice treated with enzymatic inhibitors has indicated its attenuation [28]. This impaired bone-desorbing osteoclastogenesis is negatively regulated by osteoblast formation, which derives from mesenchymal stem cells. Given that this bone-absorption is impaired in *Alox15*-deficient mice, bone-generating osteoblast formation is likely to be enhanced. In this case, adipocytes, which also stem from mesenchymal stem cells, would be impaired. Consistent with this speculation, *Alox15*-deficient mice have displayed impaired obesity [29], indicating a critical role of *Alox15* in cell differentiation.

3. 15-Lipoxygenase, type B (15-LOX-2)

15-LOX-2 shows higher similarity to 15-LOX, initially discovered in the skin in humans [30]. Human 15-LOX-2 generates 12R-HPETE from arachidonic acid specifically, showing unusual specificity for the regioisomeric lipid mediators in contrast to most isozymes, which produce S-regioisomers. Furthermore, mouse ortholog of 15-LOX-2 generates 8S-HETE and 8S-, 15S-diHPETE from arachidonic acid, thus enzymologically murine *Alox15b* is considered to be similar to human *ALOX12* [31]. Due to this, there are almost no overlapping diseases/phenotypes in the human and mouse in this isoform.

3.1. ALOX15B

Although a link between atherogenesis and *ALOX15* expression has long been hypothesized, it is known that the expression of *ALOX15B* in human carotid plaque macrophages is higher compared to *ALOX15* [32,33]. An in vitro experiment of *ALOX15B* silencing reported an attenuated lipid accumulation in human macrophages, indicating that it is functional for lipid uptake into the cells [34]. Thus, it is suggested that *ALOX15B* plays an important role in the initiation and development of atherosclerosis in humans.

Several studies have shown the downregulation of *ALOX15B* in epithelial tumors, suggesting that *ALOX15B* has an antiproliferative role [35–40]. This reduction of 15-LOX-2 in tumor cells was restored by an inhibitor for COX enzyme, demonstrating that its expression is negatively regulated by prostaglandins, at least in part [35]. PPAR-γ, a nuclear receptor regulated by endogenous LOX products, was upregulated in some epithelial tumors, suggesting that the downregulation of *ALOX15B* is autonomously controlled by PPAR-γ in epithelial cancer cells [37]. In prostate epithelial cells, *ALOX15* expression is positively regulated by transcription factor Sp1, whereas transcription factor Sp3, which is closely related to Sp1, negatively regulates its expression, suggesting that *ALOX15B* expression is critically regulated by multiple regulators [41]. Apart from epithelial cells, a separate study demonstrated increased *ALOX15B* expression in tumor-associated macrophages from renal cell carcinoma, suggesting that *ALOX15B* expression is distinctly regulated in epithelial cancer cells and macrophages [42]. Similar to tumor-associated macrophages, the upregulation of *ALOX15B* in carotid plaque macrophages has also been described [32,43].

3.2. Alox15b

There are a limited number of studies involving *Alox15b*. In chimeric mice transplanted with *Alox15b*-silenced bone marrow cells in mice lacking LDL receptor showed defective atherogenesis, suggesting that 15-LOX-2 is required in the murine atherosclerotic model [34]. As mentioned previously, the enzymatic action of murine 15-LOX-2 preferentially generates 12S-HPETE rather than 15S-HPETE from arachidonic acid, suggesting that mouse *Alox15b* and human *ALOX12* have similar roles in vivo. The skin of *Alox15b*-deficient mice has been shown to display an ichthyosiform appearance, as in *Alox12b*- and *Aloxe3*-deficient mice [44]. This example clearly shows that the skin phenotype found in *Alox15b*-deficient mice seems to require specific oxidation products from polyunsaturated fatty acids. Furthermore, this result suggests that *Alox15b* could be a functional alternative for *Alox12b* and *Aloxe3*.

4. 12-Lipoxygenase (12-LOX)

12-LOX enzymes derived from *ALOX12* in humans and its murine ortholog *Alox12* (also known as platelet-typed 12S-LOX) produce 12S-HPETE from arachidonic acid. These enzymes are expressed in leukocytes in humans and in platelets, megakaryocytes, and skin in mice [45,46]. Genetic studies have suggested that *ALOX12* gene polymorphisms are associated with cancers [47,48], neurological disorders [49,50], hypertension [51,52], fat mass [53], and bone mineral density [54–57]. The mechanism of regulation of 12-LOX expression has been studied in several models. The *ALOX12* promoter region contains at least four binding sites for RUNX1, leading to its suppressive effect in human erythroleukemic cells [58]. *Alox12* expression is also regulated by transcription factor p63 in the skin, which plays a key role in the development and terminal differentiation of the epidermis [59]. Similar to other isozymes, human 12-LOX has a catalytic domain in the C-terminal. Truncation of the N-terminal domain (which needs to be explored in terms of function) decreases its enzymatic activity at approximately 20% without altering substrate specificity [60].

4.1. ALOX12

Since the discovery of the high expression of LOX in platelets, the mechanism of its expression has been studied in detail. A previous study reported that *ALOX12* expression was attenuated in platelets by the haplodeficiency of RUNX1, a hematopoietic transcription factor associated with familial thrombocytopenia, platelet dysfunction, and a predisposition to acute leukemia in patients