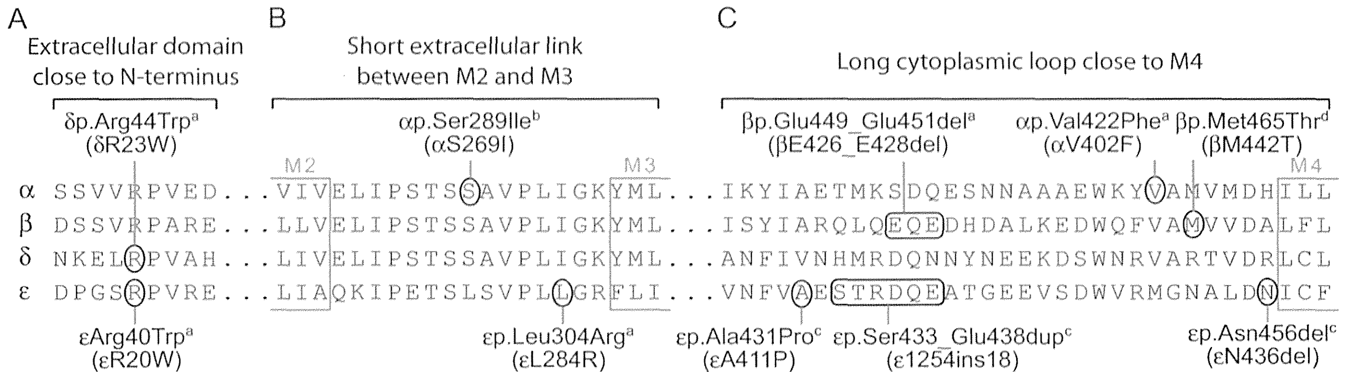

Fig. 5. Positions of the currently identified δp.Arg44Trp, εp.Leu304Arg and βp.Met465Thr, as well as previously reported CMS mutations. Mutations in the extracellular domain close the N-terminal end (A), the short extracellular link between the M2 and M3 transmembrane domains (B), and the long cytoplasmic loop close to the M4 transmembrane domain (C) are indicated. [a]δp.Arg44Trp (current report), εp.Arg40Trp [35], βp.Glu449_Glu451del [36], and αp.Val422Phe [37] cause AChR deficiency (AChR def.). [b]αp.Ser289Ile [38] causes SCCMS. [c]εp.Ala431Pro [24], ε p.Ser433_Glu438dup [23], and εp.Asn456del [34] cause FCCMS. [d]βp.Met465Thr is a currently analyzed polymorphism that shortens channel opening events. Mutations in parentheses are legacy annotations used in the original reports.

mutation in this region, εp.Asn456del (εN436del), destabilizes the diliganded receptor [34]. The C-terminal region of the long cytoplasmic loop of the ε subunit is thus likely to be crucial for stabilizing the open channel. In contrast to the three FCCMS mutations in the C-terminal end, however, βp.Met465Thr mildly shortens channel opening events and has no effect on the fidelity of channel gating, which may represent subunit-specificity and/or position-specificity of the amino acid substitutions.

Excluding δp.Arg44Trp that was previously reported in a healthy subject of unknown ethnicity [17], five of the six mutations in the AChR subunit genes in the current study and the five previously identified *COLQ* mutations [8] are unique to Japanese people. This is in contrast to some CMS mutations that are observed in unrelated families in Western and Middle Eastern countries. Especially, founder effects are implicated in two mutations: p.Asn88Lys in *RAPSN* [9–11] and c.1124_1127dupTGCC in *DOK7* [12]. CMS mutations are all recessively inherited except for those causing SCCMS. As heterozygous carriers of recessive CMS mutations exhibit no clinical phenotypes even by detailed electrophysiological studies, an asymptomatic carrier of a recessive CMS mutation has no disadvantage in transmitting the mutant allele to offspring. Lack of founder effects between the Japanese patients and patients of other nationalities thus suggest that most but not all CMS mutations arose *de novo* in a recent human history or in each family.

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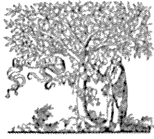
Journal of the Neurological Sciences 340 (2014) 94–98



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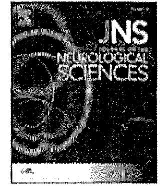

Congenital fiber type disproportion myopathy caused by *LMNA* mutations

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ABSTRACT

A boy, who had shown muscle weakness and hypotonia from early childhood and fiber type disproportion (FTD) with no dystrophic changes on muscle biopsy, was initially diagnosed as having congenital fiber type disproportion (CFTD). Subsequently, he developed cardiac conduction blocks. We reconsidered the diagnosis as possible LMNA-myopathy and found a heterozygous mutation in the *LMNA* gene. This encouraged us to search for *LMNA* mutations on 80 patients who met the diagnostic criteria of CFTD with unknown cause. Two patients including the above index case had heterozygous in-frame deletion mutations of c.367_369delAAG and c.99_101delGGA in *LMNA*, respectively. Four of 23 muscular dystrophy patients with *LMNA* mutation also showed fiber type disproportion (FTD). Importantly, all FTD associated with LMNA-myopathy were caused by hypertrophy of type 2 fibers as compared with age-matched controls, whereas CFTD with mutations in *ACTA1* or *TPM3* showed selective type 1 fiber atrophy but no type 2 fiber hypertrophy. Although FTD is not a constant pathological feature of LMNA-myopathy, we should consider the possibility of LMNA-myopathy whenever a diagnosis of CFTD is made and take steps to prevent cardiac insufficiency.

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1. Introduction

Mutations in the gene encoding nuclear envelope proteins of A-type lamins (*LMNA*) cause several disorders referred to as laminopathies, which include skeletal and cardiac muscle disorders, lipodystrophy, peripheral neuropathy, and premature aging syndromes. Laminopathies predominantly affecting skeletal muscles (LMNA-myopathy) are clinically classified into three different phenotypes; Emery-Dreifuss muscular dystrophy (AD-, AR-EDMD), limb girdle muscular dystrophy type 1B (LGMD1B), and LMNA-related congenital muscular dystrophy (L-CMD). EDMD has distinctive clinical features including early joint contractures, humero-peroneal muscle weakness and dilated cardiomyopathy with conduction defects. LGMD1B is characterized by proximal muscle involvement and cardiomyopathy with conduction defects, but joint contracture is not prominent. L-CMD is an early onset form showing severe weakness of respiratory and neck muscles from infancy. Serum CK levels in LMNA-myopathy are normal to moderately elevated (2–20 times the upper limit of the normal range). Cardiac involvement, such as conduction blocks, dilated cardiomyopathy and sudden death, usually appears after the second decade of life. To minimize the risk of sudden cardiac death, early diagnosis and appropriate cardiac defibrillator implantation is recommended [1–3].

Pathologically, LMNA-myopathy is usually characterized by nonspecific dystrophic changes with variation in fiber size, mild necrotic and regenerating processes, and an increased number of muscle fibers with internalized nuclei. Both type 1 and type 2 fibers are affected. Nuclear abnormalities are common [4]. Interestingly, marked mononuclear cellular infiltrations mimicking inflammatory myopathy can be seen in some patients with the infantile onset form of LMNA-myopathy [5].

We recently experienced a patient with a *LMNA* mutation whose initial diagnosis was congenital fiber type disproportion (CFTD). This patient had shown muscle weakness, hypotonia, and unstable gait from early childhood with no dystrophic changes, but prominent fiber type disproportion (FTD) on his muscle biopsy performed at 4years of age. At his age of 16 years, he was pointed out to have atrial-ventricular conduction block and incomplete right bundle branch block. We thus reconsidered a possible diagnosis of LMNA-myopathy and identified a mutation in the *LMNA* gene.

CFTD is one of the congenital myopathies pathologically defined by smaller type 1 fibers, by at least 12%, than type 2 fibers without structural abnormalities such as nemaline bodies, cores, and central nuclei.

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Clinically, CFTD patients show generalized muscle hypotonia and weakness from infancy, multiple joint contractures, scoliosis, long thin face, and high arched palate. Approximately 30% of individuals with CFTD have mild-to-severe respiratory involvement. Cardiac involvement is seen in less than 10% of affected individuals [6,7]. Six causative genes for CFTD have been identified: *ACTA1* [8], *TPM3* [9], *RYR1* [10], *TPM2* [11], *MYH7* [12] and *SEPN1* [13] encoding α-skeletal actin, α-tropomyosin slow, ryanodine receptor type 1, β-tropomyosin, slow β-myosin heavy chain and selenoprotein N1, respectively.

In this study, we genetically screened CFTD patients for mutations in *LMNA*. We also re-evaluated clinical and pathological findings in patients previously diagnosed as having LMNA-myopathy to ascertain whether these patients have features similar to those of CFTD.

2. Materials and methods

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent. This work was approved by the Ethics Committee of the National Center of Neurology and Psychiatry (NCNP).

2.1. Patients

We examined 80 unrelated muscle biopsies from the NCNP muscle repository. All specimens were from patients who had been diagnosed as having CFTD based on pathological findings as well as clinical features. All cases satisfied the pathological criteria for CFTD; mean type 1 fiber diameter is at least 12% smaller than the mean type 2 fiber diameter, with no structural abnormalities such as nemaline bodies, cores, and increased number of fibers with internal nuclei. In addition, we re-evaluated muscle pathology findings from 23 unrelated patients who had previously been diagnosed as having LMNA-myopathy. We chose genetically confirmed CFTD patients including 7 with *ACTA1* mutation and 2 with *TPM3* mutation for comparison of clinicopathological features. Clinically, all of the patients including in this study had muscle weakness and/or hypotonia from the preschool years (onset age; <6 years).

2.2. Mutation analysis

Genomic DNA was extracted from peripheral lymphocytes or frozen muscle specimens using standard techniques. For mutation screening of *LMNA*, *ACTA1* and *TPM3*, all exons and their flanking intronic regions were amplified by PCR and directly sequenced using an ABI PRISM 3100 automated sequencer (PE Applied Biosystems, Foster City, CA). Primer sequences are available on request.

2.3. Histochemical analysis of biopsied muscles

Biopsied skeletal muscles were frozen with isopentane cooled in liquid nitrogen. Serial frozen sections, 10 μm in thickness, were stained employing histochemical methods including hematoxylin and eosin (H&E), modified Gomori-trichrome (mGT), NADH-tetrazolium reductase (NADH-TR), and ATPases (pH 10.6, pH 4.6 and pH 4.3). For each muscle specimen, the mean fiber diameter was calculated by obtaining the shortest anteroposterior diameters of 100 type 1 and type 2 (A + B) fibers each using ATPase stains. Fiber size disproportion (FSD) was computed as; difference between type 2 fiber diameter (mean) and type 1 fiber diameter (mean) divided by type 2 fiber diameter (mean) × 100%. To obtain muscle fiber size information for age-matched controls, a total of 18 muscle specimens with minimal pathological changes from each age were examined.

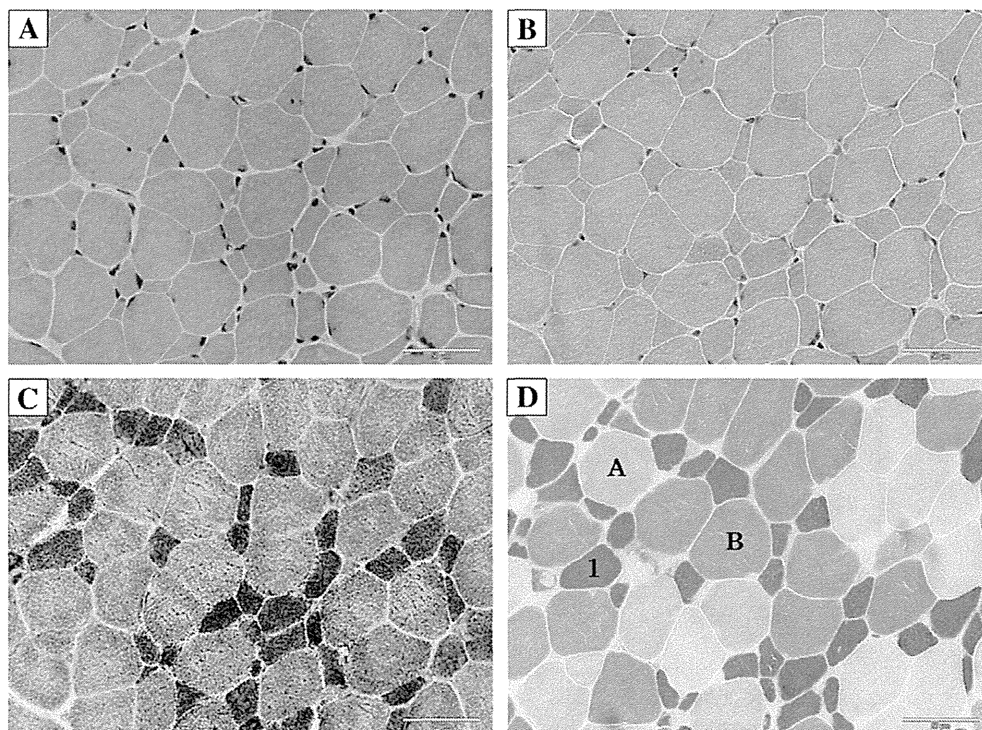

Fig. 1. Muscle biopsy from Patient 1 taken at age 4 years. (A) H&E stain shows marked variation in fiber size with neither fiber necrosis nor regeneration. (B) No nemaline bodies or cytoplasmic inclusions are revealed by mGT stain. On NADH-TR, intermyofibrillar networks are well organized. (D) On ATPase (pH 4.6), type 2A (A) and 2B (B) fibers are larger than type 1 (1) fibers. Bar = 50 μm.

Table 1
Histological features of LMNA-myopathy patients with FTD, CFTD patients with *ACTA1* and *TPM3* mutations.

Patient No.	Muscle Biopsied	Age at Biopsy	Type 1			Type 2A			Type 2B			Type 2C	%FSD	Mutation
			%	Mean Diameter (μM)	SD	%	Mean Diameter (μM)	SD	%	Mean Diameter (μM)	SD			
LMNA mutation														
1	Biceps	4y	52	16.5	5.0	30	39.1	5.3	18	37.1	7.5	0	57	c.367_369delAAG (p.K123del)
2	Quadriceps	2y	48	20.8	3.7	33	24.1	4.2	19	23.8	4.8	0	13	c.99_101delGGA (p.E33del)
3	NA	2y	38	28.6	7.7	50	36.3	4.4	7	31.3	10.5	5	15	c.1583C>A (p.T528K)
4	Biceps	4y	32	22.1	5.9	52	31.2	5.2	15	28.2	5.3	1	25	c.1357C>T (p.R453W)
5	Biceps	4y	56	21.6	5.6	32	40.0	5.8	10	34.0	8.4	2	42	c.1357C>T (p.R453W)
6	Biceps	5y	60	27.5	7.4	28	33.2	7.9	8	29.8	6.3	4	15	c.907T>C (p.S303P)
ACTA1 mutation														
1	Biceps	4y	73	14.5	3.7	26	17.8	3.7	1	–	–	0	18	c.16G>A (p.E6K)
2	Quadriceps	0y6m	60	11.9	3.1	10	18.0	2.8	20	18.8	2.8	10	35	c.143G>T (p.G48C)
3	Quadriceps	0y7m	60	6.8	1.6	29	11.5	2.1	3	–	–	8	44	c.143G>T (p.G48C)
4	NA	0y1m	52	5.6	1.5	28	14.4	2.0	12	10	2.8	8	57	c.668 T>C (p.L223P)
5	Biceps	10y	70	11.9	2.3	27	17.2	3.2	2	–	–	1	31	c.682G>C (p.E228Q)
6	Biceps	0y9m	62	10.5	2.8	23	17.2	2.8	10	18.8	2.8	5	42	c.981T>A (p.M326K)
7	Biceps	0y10m	72	12.0	1.8	22	19.5	3.5	3	–	–	3	36	c.1000C>T (p.P332S)
TPM3 mutation														
1	Biceps	0y5m	56	9.0	2.4	44	24.4	3.2	0	–	–	0	63	c.502C>T (p.R168C)
2	Biceps	0y6m	58	9.7	2.0	20	17.9	2.5	16	17.1	2.4	6	45	c.502C>T (p.R168C)

SD = standard deviation; NA = data not available; dash = not applicable.

2.4. Electron microscopic observation

Muscle specimens were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer. After shaking with a mixture of 4% osmium tetroxide, 1.5% lanthanum nitrate, and 0.2 M s-collidine for 2–3 h, samples were embedded in epoxy resin. Semi-thin sections (1 μm-thickness) were stained with toluidine blue. Ultrathin sections, 50 nm in thickness, were stained with uranyl acetate and lead citrate, and then examined

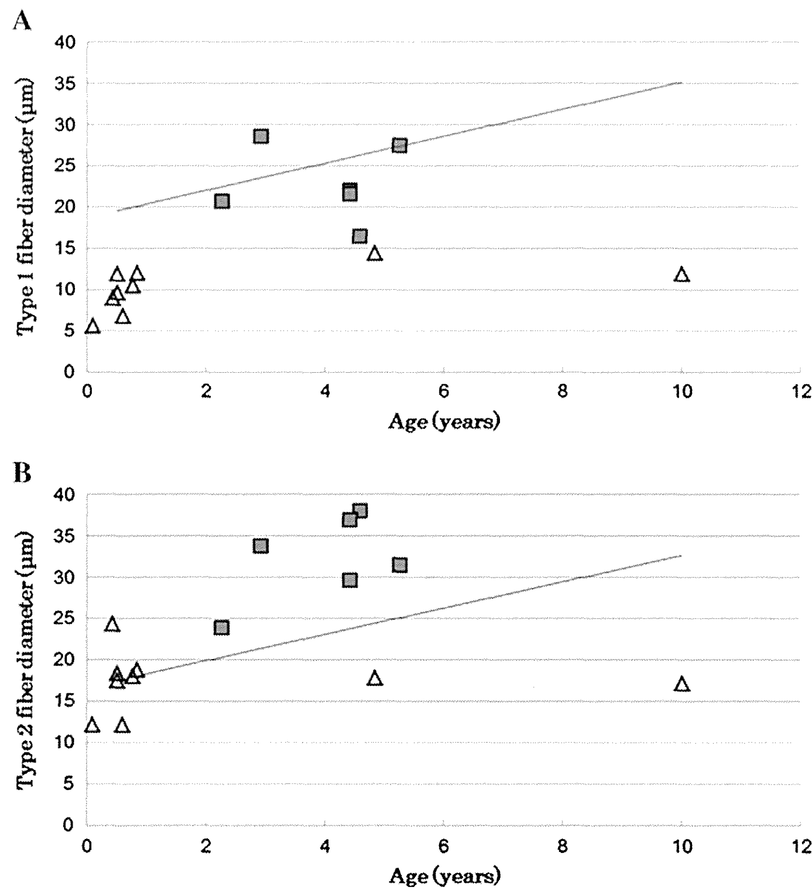

Fig. 2. Composition of mean muscle fiber diameter in each patient. (A) Mean diameters of type 1 fibers, (B) Mean diameters of type 2 fibers. Filled squares represent LMNA-myopathy with FTD, open triangles show CFTD with *ACTA1* or *TPM3* mutations, and the solid line indicates the mean fiber diameter of age-matched controls for children at various ages taken from biopsies classified as normal. CFTD with *ACTA1* and *TPM3* mutations show type 1 fiber atrophy whereas LMNA-myopathy with FTD shows type 2 fiber hypertrophy.

Table 2
Clinical and pathological summary of LMNA-myopathy patients with FTD.

Patient No	Sex	Age at Diagnosis (yr)	Age at biopsy (yr)	Pathological diagnose	Age at walk (mo)	Hypotonia	High arched palate	Respiratory involvement	Cardiac symptoms	Other presenting signs/Symptoms (age/yr)	CK (IU/L)	FSD (%)
1	M	16	4	CFTD	12	Yes	No	No	AV-b, ICRBBB (16 yr)	Joint contractures (4)	330	57
2	M	4	2	CFTD	14	Yes	No	No	No	Joint contractures (2) Dropped head (4) Rigid spine (4)	367	13
3	M	10	2	MD	15	Yes	No	No	No	Joint contractures (2) Rigid spine (8)	1098	15
4	F	4	4	MD	12	Yes	No	No	No	No	1408	25
5	F	13	4	MD	14	No	No	No	No	Lordosis (4) Joint contracture (6) Rigid spine (10)	1985	42
6	F	5	5	MD	18	No	No	No	No	No	303	15

MD; muscular dystrophy, AV-b; atrioventricular block, IRBBB; incomplete right bundle-branch block, PAF; paroxysmal atrial fibrillation.
Patients 1 and 2 were initially diagnosed as having CFTD. Patients 3 to 7 were genetically confirmed to have LMNA-myopathy with FTD. None of the patients had a high arched palate and/ or respiratory involvement. Serum creatine kinase (CK) was mildly elevated in all patients.

under a tecnai spirit transmission electron microscope (FEI, Japan) at 120 kV.

2.5. Statistical analysis

All data are presented as means ± SD. Comparisons among groups were made using Student's t test and analysis of variance (ANOVA). A difference was considered to be statistically significant at a p value less than 0.05.

3. Results

3.1. Mutation analysis

Among the 80 unrelated patients who were diagnosed as having CFTD based on clinical and pathological findings, a heterozygous *LMNA* mutation was identified in two; a previously reported c.367_369delAAG (p.Lys123del) in Patient 1 and a novel c.99_101delGGA (p.Glu33del) in Patient 2 [14]. *ACTA1* mutations found in the 7 CFTD patients were c.16G>A (p.Glu6Lys), c.142G>T (p.Gly48Cys), c.668T>C (p.Leu223Pro), c.682G>C (p.Glu228Gln), c.980T>A (p.Met327Lys), and c.1000C>T (p.Pro334Ser). Two CFTD patients had the same heterozygous c.502C>T (p.Arg168Cys) mutation in *TPM3*. The novel mutations of *LMNA* c.99_101delGGA (p.Glu33del) and *ACTA1* c.980T>A (p.Met327Lys), were not found in either 100 Japanese control chromosomes or the dbSNP and 1000 Genomes databases.

3.2. Histological findings

Histologically, type 1 fiber predominance (more than 55% of type 1 fibers) and type 2B fiber deficiency (less than 5% of type 2B fibers) were observed in 61% and 28%, respectively, of our 80 CFTD cohort. These results are consistent with those of a previous report [7].

Two patients with *LMNA* mutations showed a marked difference in the sizes of type 1 and type 2 fibers, resulting in FSD of 57% and 13%, respectively (Fig. 1). Neither type 1 fiber predominance nor type 2B fiber deficiency was seen (Table 1).

Re-evaluation of genetically confirmed LMNA-myopathy revealed that 4 of 23 patients (17%) had fiber type disproportion (FTD). Their FSD was ranged from 15 to 42%. All 4 patients with FTD also showed some necrotic and/or regenerating fibers in their muscle biopsy and had a diagnosed of muscular dystrophy. These 4 patients with FTD had 3 different mutations. Two mutations of c.1583C>A (p.Thr528Lys) and c.1357C>T (p.Arg453Trp) have already been reported [15,16], whereas the c.907 T>C (p.Ser303Pro) mutation was not reported previously. These mutations were distributed in both central rod and tail domains, but not in the head domain (Table 1).

To clarify whether LMNA-myopathy patients with FTD have specific pathological findings different from those affecting CFTD muscles with known gene mutations, we carefully re-evaluated the muscle pathologies of the 6 LMNA-myopathy patients with FTD, 7 CFTD patients with *ACTA1* mutations, and 2 CFTD patients with *TPM3* mutations. FSD in LMNA-myopathy with FTD, and in CFTD with *ACTA1* and *TPM3* mutations were calculated to be 27.8 ± 17.9% (mean ± SD), 37.7 ± 12.1%, and 54.1 ± 13.1%, respectively. No significant differences were seen in FSD among the 3 groups. We also compared fiber sizes among LMNA-myopathy with FTD, CFTD with *ACTA1* or *TPM3* mutations and age-matched controls. Surprisingly, CFTD with *ACTA1* and *TPM3* mutations showed type 1 fiber atrophy, whereas LMNA-myopathy with FTD showed type 2 fiber hypertrophy with lack of type 1 fiber atrophy (Fig. 2).

In this study, type 1 fiber predominance was seen in 86% of CFTD patients with *ACTA1* mutations and in 100% of those with *TPM3* mutations, but in only 33% of LMNA-myopathy patients with FTD. The percentage of type 1 fibers in LMNA-myopathy was calculated to be 44.6 ± 12.8 (mean ± SD), which was significantly lower than that in CFTD with *ACTA1* mutations (64.1 ± 7.1%) and that with *TPM3* mutations (57.0 ± 1.4%) ($p < 0.05$). Type 2B fiber deficiency was not seen in LMNA-myopathy with FTD (Tables 1, 3), whereas 4 of 7 (57%) patients with *ACTA1* mutations and one (50%) with *TPM3* mutation showed type 2B fiber deficiency.

On electron microscopic (EM) observations, nuclear changes are important pathological findings in skeletal muscles of LMNA-myopathy [4]. We examined the nuclear changes in Patients 2, 4 and 5 on EM, and found a few myonuclei showing abnormal shapes and chromatin disorganization (Fig. 3). Smaller nuclei arranged in a row, giving the appearance of a 'nuclear chain', were also seen (data not shown). However, nuclear abnormalities in patients who had LMNA-myopathy with

Table 3
Comparison of clinical and pathological information between LMNA-myopathy with FTD and CFTD with *ACTA1* and *TPM3* mutations.

Gene mutation	*LMNA*	*ACTA1*	*TPM3*
Number of patients	6	7	2
Onset	Infantile	at birth	< 2 months
Hypotonia	67% (4/6)	100% (7/7)	100% (2/2)
High arched palate	0% (0/6)	57% (4/7)	50% (1/2)
Respiratory involvement	0% (0/6)	57% (4/7)	0% (0/2)
Joint contracture	67% (4/6)	14% (1/7)	0% (0/2)
CK level (IU/L)	963 ± 662	53 ± 15	42 ± 16
Type 1 fiber predominance	33% (2/6)	86% (6/7)	100% (2/2)
Type 2B fiber deficiency	0% (0/6)	57% (4/7)	50% (1/2)

Type 1 fiber predominance and absence of type 2B fibers were common in CFTD caused by *ACTA1* or *TMP3* mutations. Type 2B fiber deficiency was not seen in LMNA-myopathy with FTD. Serum creatine kinase (CK) levels were significantly higher in LMNA-myopathy than in CFTD with *ACTA1* and *TPM3* mutations ($p < 0.05$).

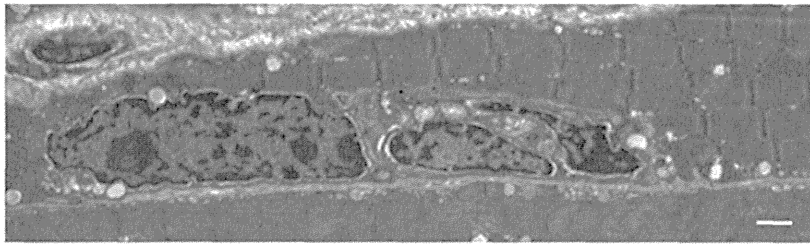

Fig. 3. Myonuclear shape changes in patient 2. Nuclear contours are irregular with a serpentine appearance. Bar = 1 μm.

FTD were milder and less frequent than previously reported for AD-EDMD and LGMD1B muscles [4].

3.3. Clinical findings

Table 2 summarizes the characteristics of the 6 LMNA-myopathy patients with FTD. Patients 1 and 2 were initially diagnosed as having CFTD, and the 4 remaining patients (patients 3 to 6) showed FTD together with dystrophic changes on muscle pathology. All patients had normal antenatal courses and uneventful births. All patients had started walking without delay, but showed a waddling gait and muscle weakness and/or hypotonia from the preschool years. None had a high arched palate or respiratory dysfunction. Four of the 6 (67%) patients had contractures of the ankles and/or elbows which had not been present at birth but appeared with age. Serum creatine kinase (CK) was mildly elevated in all patients.

Sixteen of the 78 (21%) CFTD patients with unknown cause had high CK levels (>200 IU/l), and four of these 16 showed a high arched palate and respiratory involvement.

4. Discussion

FTD can be seen in a single muscle biopsy from patients with several diseases including congenital myotonic dystrophy and centronuclear myopathy [17–20]. Here we identified 2 LMNA-myopathy among patients diagnosed as CFTD. We also found FTD in 17% of muscular dystrophy patients with LMNA mutations. These results suggest that FTD may not be rare in LMNA-myopathy. None of these patients had either a high arched palate or respiratory insufficiency, and serum CK levels were mildly elevated. Pathologically, FTD in LMNA-myopathy is associated with type 2 fiber hypertrophy with lack of type 1 fiber atrophy, whereas type 1 fiber atrophy is seen in CFTD with *ACTA1* or *TPM3* mutations. Unlike CFTD due to *ACTA1* or *TPM3* mutations, type 1 fiber predominance and type 2B fiber deficiency are absent in LMNA-myopathy. These results suggest that *LMNA* analysis should be performed in CFTD patients who has the clinical features such as no high arched palate, no respiratory insufficiency and high CKemia, and has pathological features such as type 2 fiber hypertrophy and lack of type 1 fiber atrophy, type 1 fiber predominance, and type 2B fiber deficiency.

LMNA-myopathy is categorized as muscular dystrophy, and mild necrotic and regenerating processes are usually seen. However, no dystrophic features can be seen as reported herein. Higher CK levels raise the possibility of LMNA-myopathy being dystrophic in nature. On the other hand, in our series, 16 of the 78 (21%) CFTD patients with unknown cause had high CKemia. This result suggests a difficulty in making a differential diagnosis between congenital myopathy and muscular dystrophy in some cases.

Clinically, respiratory insufficiency is common, reportedly being seen in 30% of CFTD patients [7], and in 73% of L-CMD patients [4]. However, 2 CFTD patients with *LMNA* mutations in this study showed no respiratory involvement. Furthermore, in CFTD associated with *LMNA* mutations, FTD is the only pathological abnormality, while prominent dystrophic and/or inflammatory changes are seen in L-CMD. There results suggest that CFTD is the milder form of early onset LMNA-myopathy.

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Review

Metabolomics for Biomarker Discovery in Gastroenterological Cancer

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Abstract: The study of the omics cascade, which involves comprehensive investigations based on genomics, transcriptomics, proteomics, metabolomics, *etc.*, has developed rapidly and now plays an important role in life science research. Among such analyses, metabolome analysis, in which the concentrations of low molecular weight metabolites are comprehensively analyzed, has rapidly developed along with improvements in analytical technology, and hence, has been applied to a variety of research fields including the clinical, cell biology, and plant/food science fields. The metabolome represents the endpoint of the omics cascade and is also the closest point in the cascade to the phenotype. Moreover, it is affected by variations in not only the expression but also the enzymatic activity of several proteins. Therefore, metabolome analysis can be a useful approach for finding effective diagnostic markers and examining unknown pathological conditions. The number of studies involving metabolome analysis has recently been increasing year-on-year. Here, we describe the findings of studies that used metabolome analysis to attempt to discover biomarker candidates for gastroenterological cancer and discuss metabolome analysis-based disease diagnosis.

Keywords: metabolomics; biomarker; serum; gastroenterological cancer; mass spectrometry

1. Introduction to Metabolomics

1.1. Omics

The study of the omics cascade, which involves comprehensive investigations based on genomics, transcriptomics, proteomics, metabolomics, *etc.*, has developed rapidly and now plays an important role in life science research. Genomics makes it possible to find gene mutations and gene polymorphisms, and there is an abundance of reports about genomics-based studies. Transcriptomics is an approach in which biological samples are analyzed to obtain information about the concentrations of a large number of mRNA transcripts in a simultaneous manner. The latter information includes gene expression data related to functional genomics. Recently, the comprehensive analysis of microRNA molecules has attracted increasing attention in the life science field, especially the medical research field. In proteomics, information about protein expression levels and functions, such as about abnormal protein expression, protein phosphorylation, and protein interactions, is evaluated, and a great number of academic papers about such research have been published. Recently, metabolomics or metabolome analysis, which involves the comprehensive analysis of low molecular weight metabolites, has rapidly developed along with improvements in analytical technology leading to its use in a variety of research fields including clinical, cell biology, and plant/food science studies [1–4].

1.2. Characteristics of Metabolomics

Metabolome analysis is used to evaluate the characteristics and interactions of low molecular weight metabolites under a specific set of conditions, for example, at a particular developmental stage, in certain environmental conditions, or after specific genetic modifications. The metabolome mainly represents the endpoint of the omics cascade and is also the closest point in the cascade to the phenotype. Changes in metabolite levels can also be induced by exogenous factors, such as environmental and dietary factors, while genomic information is not basically affected by such factors. Moreover, in addition to expression variations, the metabolome is also affected by the enzymatic activities of various proteins. Therefore, metabolite profiles are considered to be a summary of the other upstream omics profiles, and metabolome analysis might be able to detect subtle changes in metabolic pathways and deviations from homeostasis before phenotypic changes occur [5,6]. Taken together, the metabolite profile of a cell is more likely to represent the cell's status than its DNA, RNA, or protein profile. On the basis of these facts, metabolomics has started to be used in medical research. In such studies, metabolomics has mainly been utilized to discover biomarker candidates for cancer. For example, a search of the papers included in the PubMed database [7] using the keywords "metabolomics", "cancer", and "biomarker" found a total of 377 papers, and the number has been increasing year-on-year. In addition, metabolomics can also be used to examine unknown pathological conditions. Here, we describe the findings of studies that have used metabolome analysis to attempt to

discover biomarker candidates for gastroenterological cancer and discuss metabolome analysis-based disease diagnosis.

2. Metabolism in Cancer

D. Hanahan and R.A. Weinberg suggested that the following characteristics are hallmarks of cancer: sustained proliferation, evasion from growth suppressors, active invasion and metastasis, the enabling of replicative immortality, the induction of angiogenesis, resistance to cell death, the deregulation of cellular energetics, avoidance of immune destruction, tumor-promoting inflammation, and genome instability and mutation [8,9]. Among these characteristics, the deregulation of cellular energetics seems to be particularly related to metabolomics research. Actually, in the study by Hirayama *et al.*, metabolome analysis using capillary electrophoresis-mass spectrometry (CE-MS) demonstrated that colon and gastric tumors produce energy by glycolysis rather than oxidative phosphorylation via the tricarboxylic acid cycle, even in the presence of an adequate oxygen supply, which is known as "the Warburg effect" [10]. The accumulation of significantly higher levels of most amino acids, which are indirectly used as energy sources, in tumor tissue compared with non-tumor tissue has also been reported [10]. In addition, cancer cells obtain energy via glutaminolysis as well as glycolysis [11]. The relationship between "active and metastasis" and metabolites has started to be elucidated. Sarcosine, which is an N-methyl derivative of the amino acid glycine, was identified as a potentially important metabolic intermediary for prostate cancer cell invasion and aggressivity [12]. It was also proposed that glycine metabolism may represent a metabolic vulnerability in rapidly proliferating cancer cells [13]. Thus, metabolites themselves seem to affect cancer cells somehow, and these findings suggest that the pathogenesis of cancer leads to alterations in metabolite levels in the body. If such metabolite alterations influence the metabolite levels in biological fluids such as serum/plasma, urine, and saliva, it may be possible to use the metabolite concentrations of such fluids as biomarkers of cancer.

3. Biomarker Discovery in Gastroenterological Cancer Using Metabolomics

3.1. Biomarker Discovery and Gastroenterological Cancer

Gastroenterological cancer is a group of cancers including esophageal, gastric, colorectal, hepatic, and pancreatic cancer. Oral cancer may be also included in gastroenterological cancer. Gastroenterological cancer remains relatively asymptomatic until it reaches the progressive state, at which point it exhibits poor prognosis. Therefore, methods that facilitate the detection of gastroenterological cancer at an earlier stage are desired, because early stage cancer patients are highly likely to make a complete recovery from such conditions. Regarding gastroenterological cancer, biomarkers that make it possible to accurately predict prognosis, therapeutic efficacy, and adverse effects are also required. Recently, studies on metabolomics-based biomarker discoveries have been widely reported (Table 1). In addition, there are some articles in which the metabolite alterations in tumor tissues were evaluated using tissue metabolome analysis and the results leading to elucidation of pathogenesis of gastroenterological cancer were shown (Table 1). The pathogenesis of gastroenterological cancer is considered to be closely associated with lifestyle factors as well as genome mutations associated with

oncogenes and tumor suppressor genes. Therefore, metabolomics is likely to be a suitable method for biomarker discovery [14], as described in the "Introduction to Metabolomics".

3.2. Metabolomics-Based Biomarker Discovery

Metabolomics-based biomarker discovery studies for gastroenterological cancer have been widely performed by liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), CE-MS, nuclear magnetic resonance (NMR) spectroscopy or Fourier transform-infrared (FT-IR) spectroscopy (Table 1). MS-based techniques exhibit relatively high selectivity and sensitivity during such analyses, and therefore, they have often been used for metabolite profiling. In metabolite profiling, analyses have been performed focusing on the metabolites related to the specific pathways, e.g., amino acids, organic acids, carbohydrates, and lipids [15], and then precise identification of the metabolites and correction for analytical inaccuracies are needed [16]. On the contrary, metabolic fingerprinting is the method for sample classification, and the target samples' spectral patterns based on their biological state and/or origin are available [17]. NMR and FT-IR spectroscopy display relatively low selectivity so they are often used in metabolite fingerprinting aimed at evaluating the differences among biological samples, although there have been some studies in which metabolite profiling was performed using NMR and FT-IR. Therefore, metabolite profiling and metabolite fingerprinting are applied to metabolomics-based biomarker discovery.

3.3. Biomarker Discovery in Gastroenterological Cancer by Metabolomics

Previous studies about metabolome analysis in patients with gastroenterological cancer have analyzed the metabolites present in serum/plasma, saliva, urine, feces, and/or tissue samples, and there is a particular abundance of reports about the metabolites found in serum/plasma samples. Recent reports about metabolome analysis in patients with gastroenterological cancer are listed in Table 1. Metabolome analysis-based attempts to elucidate biomarker candidates for gastroenterological cancer have been carried out using a variety of techniques including GC-MS, LC-MS, CE-MS, NMR, and Fourier transform ion cyclotron resonance (FTICR)-MS. Each approach has different characteristics, and it is impossible to measure all metabolites including hydrophobic and hydrophilic molecules using a single technique. Therefore, in some biomarker discovery studies, a variety of instruments were used, which allowed the researchers to evaluate the potential of a wide range of metabolites as novel biomarkers. However, there were some inconsistencies between the results obtained by different research groups. For example, in the report by Chen *et al.* the urine level of isoleucine was higher in colorectal cancer patients compared with healthy controls [18]. On the contrary, Qiu *et al.* demonstrated that colorectal cancer patients had lower urinary levels of isoleucine [19]. In addition, differences between the presence and absence of significant alterations in metabolite concentrations have also been observed. These discrepancies might have been due to the differences in the methods used to collect the biological samples, and these issues are discussed in Section 4. Thus, although there is an abundance of reports about the use of metabolome analysis to discover biomarker candidates for gastroenterological cancer, no firm conclusions have yet been reached.

Table 1. A list of recent reports in which patients with gastroenterological cancer were subjected to metabolome analysis.

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Oral cancer	Saliva	Lactate; *n*-Eicosanoate	Valine; GABA; Phenylalanine	UPLC-Q-TOF/MS	[20]
Research aim: To discover salivary metabolite biomarkers and to explore salivary metabolomics as a disease diagnostic tool					
Oral cancer	Saliva	Cadaverine; 2-Aminobutyrate; Alanine; Piperidine; Taurine; Piperideine; Pipecolate; Pyrroline hydroxycarboxylate; Betaine; Leucine + Isoleucine; Phenylalanine; Tyrosine; Histidine; Valine; Tryptophan; β-Alanine; Glutamate; Threonine; Serine; Glutamine; Choline; Carnitine	None	CE-TOF-MS	[21]
Research aim: To predict oral cancer susceptibility via saliva-based diagnostics based on metabolomics technology					
Oral cancer	Urine	Alanine; Valine; Serine; Tyrosine; Cystine	6-Hydroxynicotinate; Hippurate	GC-QMS	[22]
Research aim: To establish a diagnostic tool for early stage oral squamous cell carcinoma and its differentiation from other oral conditions by the urinary metabolite profiling approach					
Oral cancer	Serum	Glycerate; Serine; Laurate; *N*-Acetyl-L-aspartate; Asparagine; Ornithine; Heptadecanate	None	GC-QMS	[23]
Research aim: To find metabolite biomarker candidates for detection of early stage oral squamous cell carcinoma					
Esophageal cancer	Mucosal tissue	L-Valine; Naphthalene; 1-Butanamine; Pyrimidine; Aminoquinoline; L-Tyrosine; Isoleucine; Purine; Serine; Phosphate; myo-Inositol; Arabinofuranoside; L-Asparagine; Tetradecanoate; L-Alanine; Hexadecanoate	L-Altrose; D-Galactofuranoside; Arabinose; Bisethane	GC-QMS	[24]
Research aim: To find tissue metabolomic biomarkers that are identifiable and diagnostically useful for esophageal cancer					

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Esophageal cancer	Mucosal tissue	*N*-acetylaspartate; Glutamate; Valine; Leucine + Isoleucine; Tyrosine; Methionine; Phenylalanine; GABA; Phenylacetylglutamine; Glutamic acid γ-H; Unsaturated lipids; Short-chain fatty acids; Phosphocholine; Glycoproteins; Acetone; Malonate; Acetoacetate; Acetate; Trimethylamine; Formate; Uracil; Adenine in ATP/ADP and NAD/NADH; Acetyl hydrazine; Hippurate	Creatine; Glycine; Glutamine; 4-Hydroxyphenylpyruvate; Creatinine; Taurine; Aspartate; myo-Inositol; Cholesterol; Choline; Glucose; Ethanol; α-Ketoglutarate oxime; AMP; NAD	NMR	[25]
Research aim: To find the potential tissue metabolite biomarkers for clinical diagnosis for different stages of human esophageal cancer and new insights for the mechanism research					
Esophageal cancer	Tissue	Choline; Alanine; Glutamate	Creatinine; myo-Inositol; Taurine	NMR	[26]
Research aim: To establish the biochemical profiles of adjacent non-involved tissue and malignant esophageal tumor and to determine the metabolomic changes of tumors with different tumor differentiation for finding metabolomic indicators sensitive to tumor differentiation					
Esophageal cancer	Urine	Urea; Acetate; Pantothenate; 3-Hydroxyisovaleate; Acetone; Formate; 2-Hydroxyisobutyrate; Creatinine; Ethanolamine; 2-Aminobutyrate; Leucine; Succinate; Glutamine; Glucose; Glycine; Tryptophan; Trimethylamine-*N*-oxide; Valine; Lactate; Tyrosine	Dimethylamine; Alanine; Citrate	NMR	[27]
Research aim: To find urinary metabolite signatures that can clearly distinguish both Barrett's esophagus and esophageal cancer from controls					
Esophageal cancer	Serum	Uridine	1-Methyladenosine; N^{2},N^{2}-Dimethylguaosine; N^{2}-Methylguanosine; Cytidine	LC-QqQ/MS	[28]
Research aim: To investigate whether nucleosides can potentially serve as useful biomarkers to identify esophageal adenocarcinoma					
Esophageal cancer	Serum	Lactate; β-Hydroxybutyrate; Lysine; Glutamine; Citrate	Valine; Leucine + Isoleucine; Methionine; Tyrosine; Tryptophan; Myristate; Linoleate	LC-Q-TOF/MS NMR	[29]

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Research aim: To identify the metabolite based biomarkers associated with the early stages of esophageal adenocarcinoma with the goal of improving prognostication					
Esophageal cancer	Serum	β-Hydroxybutyrate; Acetoacetate; Creatine; Creatinine; Lactate; Glutamate; Glutamine; Histine	LDL/VLDL; Unsaturated lipids; Acetate; α-Glucose; β-Glucose; Tyrosine	NMR	[30]
Research aim: To characterize the systemic metabolic disturbances underlying esophageal cancer and to identify possible early biomarkers for clinical prognosis					
Esophageal cancer	Serum	Lactate; Glycolate; Malonate; Fumarate; L-Serine; L-Aspartate; L-Glutamine	Pyruvate	GC-QMS	[31]
Research aim: To investigate the differences in serum metabolite profiles using a metabolomic approach and to search for sensitive and specific metabolomic biomarker candidates					
Esophageal cancer	Plasma	Phosphatidylinositol; Lithocholyltaurine; Phosphatidiate; L-Urobilinogen; 9'-Carboxy-γ-tocotrienol; PC; PE; Sphinganine 1-phosphate; Phosphatidylserine(16:0/14:0); LPC(22:2); Ganglioside GM2(d18:1/24:1(15Z)); Lithocholate 3-*O*-glucuronide; 12-Oxo-20-dihydroxy-leukotriene B4	Desmosine; Isodesmosine; 5-β-Cyprinol sulfate	UPLC-TOF/MS	[32]
Research aim: To search for valuable markers including circulating endogenous metabolites associated with the risk of esophageal cancer					
Gastric cancer	Tissue	2-Aminobutyrate; 3-Aminoisobutanoate; Valine; 2-Hydroxy-4-methyl-pentanoate; Isoleucine; Proline; Uracil; Threonine; Thymine; Dihydrouracil; Aspartate; Pyroglutamate; GABA; Cysteine; Glutamate; Dodecanoate; Asparagine; Putrescine; Cadaverine; Ascorbate; Gluconate; Xanthine; *N*-Acetyl glucosamine; Kynurenine; Inosine	Hydroxyacetate; 3,4-Dihydroxy-2(3H)-furanone; Nicotinamide; Glycerol phosphate; Tetradecanoate; Palmitelaidate; Palmitate; Linoleate; Stearate; Arachidonate; L-Palmitoyl-glycerol; Sucrose; Cholesterol	GC-TOF/MS	[33]

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Research aim: To reveal the major metabolic alterations essential for the development of gastric cardia cancer and to discover a biomarker signature of gastric cardia cancer					
Gastric cancer	Urine	Arginine; Leucine; Valine; Isoleucine; Lactate	Methionine; Serine; Aspartate; Histidine; Succinate; Citrate; Malate	CE-MS	[34]
Research aim: To search for potential tumor markers of gastric cancer in patients' urine samples					
Gastric cancer	Serum	3-Hydroxypropionate; 3-Hydroxyisobutyrate	Pyruvate; Octanoate; Phosphate	GC-QMS	[31]
Research aim: To investigate the differences in serum metabolite profiles using a metabolomic approach and to search for sensitive and specific metabolomic biomarker candidates					
Gastric cancer	Serum	*L*-Valine; Sarcosine; Hexadecanenitrile	*L*-Glutamine; Hexanedioate; 9,12-Octadecadienoate; 9-Octadecenoate; trans-13-Octadecenoate; Nonahexacontanoate; Cholesta-3,5-diene; Cholesterol/Pentafluoropropionate; Cholesterol; Cholest-5-en-3-ol; Fumarate; 2-*O*-Mesyl arabinose; Benzeneacetonitrile; 2-Amino-4-hydroxy-pteridinone; 1,2,4-Benzenetricarboxylate	GC-QMS	[35]
Research aim: To explore the underlying metabolic mechanisms of gastric cancer and to identify biomarkers associated with morbidity					
Colorectal cancer	Mucosal tissue	Lactate; Phosphate; L-Glycine; 2-Hydroxy-3-methylvalerate; L-Proline; L-Phenylalanine; Palmitate; Margarate; Oleate; Stearate; Uridine; 11,14-Eicosadienoate; 11-Eicosenoate; 1-*O*-Heptadecylglycerol; 1-Monooleoylglycerol; Propyl octadecanoate; Cholesterol	Fumarate; Malate; D-Mannose; D-Galactose; D-Glucose; 1-Hexadecanol; Arachidonate	NMR GC-QMS	[36]
Research aim: To reveal that global metabolic profiling of colon mucosae can define metabolic signatures for not only discriminating malignant from normal mucosae but also distinguishing the anatomical and clinicopathological characteristics of colorectal cancer					

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Colorectal cancer	Tissue	Glycine; L-Proline; L-Phenylalanine; L-Alanine; L-Leucine; L-Valine; L-Serine; L-Threonine; L-Isoleucine; Picolinate; L-Methionine; L-Aspartate; β-Alanine; Aminomalonate; 1-Methylhydantoin; Palmitate; Margarate; Oleate; Stearate; 11-Eicosenoate; Myristate; Pentadecanoate; Linolenate; Lignocerate; Phosphate; L-Arabinose; Lactate; Maleate; Pantothenate; Glycerol; 1-Monooleoylglycerol; Uracil; Uridine; Cholesterol	Arachidonate; D-Mannose; D-Galactose; D-Glucose; Fumarate; Malate; Oxalate; Succinate; Ribitol; Squalene	GC×GC-TOF/MS	[37]
Research aim: To investigate whether the metabotype associated with colorectal cancer is distinct from that of normal tissue and whether various biochemical processes are altered by pathogenesis of colorectal cancer					
Colorectal cancer	Urine	Lactate; Arginine; Leucine; Isoleucine; Valine	Histidine; Methionine; Aspartate; Serine; Succinate; Citrate; Malate	CE-IT/MS	[18]
Research aim: To investigate the metabolic profile of urine metabolites and to elucidate their clinical significance in patients with colorectal cancer including possibility as the biomarker candidates for early detection.					
Colorectal cancer	Urine	5-Hydroxytryptophan; 5-Hydroxyindoleacetate; Tryptophan; Glutamate; Pyroglutamate; *N*-Acetyl-aspartate; *p*-Cresol; 2-Hydroxyhippurate; Phenylacetate; Phenylacetylglutamine; *p*-Hydroxyphenylacetate	Succinate; Isocitrate; Citrate; 3-Methylhistidine; Histidine	GC-QMS	[19]
Research aim: To demonstrate the potentials of this noninvasive urinary metabolomic strategy as a complementary diagnostic tool for colorectal cancer					
Colorectal cancer	Serum	None	FAs ($C_{28}H_{46}O_4$, $C_{28}H_{48}O_4$, $C_{28}H_{50}O_4$)	FTICR-MS LC-Q-TOF/MS NMR QqQ-MS	[38]
Research aim: To discover putative metabolomic markers associated with colorectal cancer					

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Colorectal cancer	Serum	Pyruvate; α-Hydroxybutyrate; Phosphate; Isoleucine; β-Alanine; meso-Erythritol; Aspartate; Pyroglutamate; Glutamate; *p*-Hydroxybenzoate; Arabinose; Asparagine; Xylitol; Ornithine; Citrulline; Glucuronate; Glucosamine; Palmitoleate; Inositol; Kynurenine; Cystamine; Cystine; Lactitol	Nonanoate; Creatinine; Ribulose; *O*-Phosphoethanolamine	GC-QMS	[39]
Research aim: To establish new screening methods for early diagnosis of colorectal cancer via metabolomics					
Colorectal cancer	Serum	Lactate; Glycolate; L-Alanine; 3-Hydroxypropionate; L-Proline; L-Methionine; Thioglycolate; L-Glutamate; L-Asparagine; L-Glutamine; Glucuronic lactone	None	GC-QMS	[31]
Research aim: To investigate the differences in serum metabolite profiles using a metabolomic approach and to search for sensitive and specific metabolomic biomarker candidates					
Colorectal cancer	Serum	LPC(16:0); LPC(18:2); LPC(18:1); LPC(18:0); LPC(20:4); LPC(22:6); PC(34:1); LPA(16:0); LPA(18:0); LPC(16:0)	Palmitic amide; Oleamide; Hexadecanedioate; Octadecanoate; Eicosatrienoate; Myristate	DI-FTICR-MS	[40]
Research aim: To discriminate colorectal cancer patients from controls by metabolomic biomarkers and to reveal the stage-related biomarkers for colorectal cancer and the changing trends of four lipid species in the colorectal cancer progression					
Hepatic cancer	Tissue	Arachidyl carnitine; Tetradecanal; Oleamide	β-Sitosterol; L-Phenylalanine; LPC(18:2); Glycerophosphocholine; LPE(18:3); Chenodeoxycholate glycine conjugate; LPC(22:6); Quinaldate; LPE(18:0); LPC(18:0); LPC(20:4)	LC-LTQ-Orbitrap-MS	[41]
Research aim: To select characteristic endogenous metabolites in hepatitis B virus-related hepatocellular carcinoma patients and to identify their molecular mechanism and potential clinical value					

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Hepatic cancer	Urine	Octanedioate; Glycine; Tyrosine; Threonine; Butanedioate	Heptanedioate; Ethanedioate; Xylitol; Urea; Phosphate; Propanoate; Pyrimidine; Butanoate; Trihydroxypentanoate; Hypoxanthine; Arabinofuranose; Hydroxyproline dipeptide; Xylonate	GC-QMS	[42]
Research aim: To investigate the urinary metabolic difference between hepatocellular carcinoma patients and normal subjects and to find biomarkers for hepatocellular carcinoma					
Hepatic cancer	Serum	Cortisol; GCA; GCDCA; C16:1-CN; FAs (C16:1, C16:0, C18:2, C18:1, C18:0, C20:5, C20:4, C20:2, C22:6, C22:5)	Tryptophan; LPC(14:0); LPC(20:3); LPC(20:5); C10-CN; C10:1-CN; C8-CN; C6-CN	LC-Q-TOF/MS	[43]
Research aim: To study the related metabolic deregulations in hepatocellular carcinoma and chronic liver diseases and to discover the differential metabolites for distinguishing the different liver diseases					
Hepatic cancer	Plasma	LPC(24:0); Glycodeoxycholate; Deoxycholate 3-sulfate	LPC(14:0); LPC(16:0); LPC(18:0); LPC(18:1); LPC(18:2); LPC(18:3); LPC(20:4); FA(24:0); FA(24:1); LPC(20:2); LPC(20:3); LPC(20:5)	UPLC-QqQ/MS GC-QMS	[44]
Research aim: To evaluate the molecular changes in the plasma of hepatocellular carcinoma patients and to provide new insights into the pathobiology of the diseases					
Hepatic cancer	Feces	LPC(18:0); LPC(16:0)	Chenodeoxycholate dimeride; Urobilin; Urobilinogen; 7-Ketolithocholate	UPLC-Q-TOF/MS	[45]
Research aim: To find fecal metabolite biomarkers for distinguishing liver cirrhosis and hepatocellular carcinoma patients from healthy controls					
Pancreatic cancer	Saliva	Cadaverine; 2-Aminobutyrate; Alanine; Putrescine; Methylimidazole acetate; Trimethylamine; Piperidine; Leucine + Isoleucine; Phenylalanine; Tyrosine; Histidine; Proline; Lysine; Glycine; Ornithine; Burimamide; Ethanolamine; GABA; Aspartate; Valine; Tryptophan; β-Alanine; Glutamate; Threonine; Serine; Glutamine; Hypoxanthine; Choline; Carnitine	Taurine; Glycerophosphocholine	CE-TOF-MS	[21]

Table 1. *Cont.*

Disease	Specimen	Upregulated Metabolites	Downregulated Metabolites	Analytical Method	Ref.
Research aim: To reveal the comprehensive salivary metabolic profiles of pancreatic cancer patients and healthy controls and to identify cancer-specific biomarkers with high discriminative ability					
Pancreatic cancer	Tissue	Taurine	Succinate; Malate; Uridine; Glutathione; UDP-*N*-Acetyl-D-glucosamine; NAD; UMP; AMP	UPLC-TOF/MS	[46]
Research aim: To investigate the differences in the metabolite profiles of normal and pancreas tumor tissue with a goal of developing prognostic biomarkers					
Pancreatic cancer	Serum	Lactate; Thiodiglycolate; 7-Hydroxyoctanoate; Asparagine; Aconitate; Homogentisate; *N*-Acetyl-tyrosine	Glycine; Urea, Octanoate; Glycerate; Decanoate; Laurate; Myristate; Palmitate; Urate; Margarate; Stearate	GC-QMS	[47]
Research aim: To evaluate the differences in the metabolomes between pancreatic cancer patients and healthy volunteers and to aid the discovery of novel biomarkers					
Pancreatic cancer	Serum	Arabinose; Ribulose	Valine; 2-Aminoethanol; *n*-Caprylate; Threonine; Nonanoate; Methionine; Creatinine; Asparagine; Glutamine; *O*-Phosphoethanolamine; Glycyl-Glycine; 1,5-Anhydro-D-glucitol; Lysine; Histidine; Tyrosine; Urate	GC-QMS	[48]
Research aim: To construct a diagnostic model for pancreatic cancer using serum metabolomics and to confirm its diagnostic performance					
Pancreatic cancer	Plasma	Arachidonate; Erythritol; Cholesterol; *N*-Methylalanine; Lysine; Deoxycholylglycine; Cholylglycine; LPC(16:0); Tauroursodeoxycholate; Taurocholate; LPC(18:2); PE(26:0); PC(34:2)	Glutamine; Hydrocinnamate; Phenylalanine; Tryptamine; Inosine	GC-TOF/MS LC-IT/MS LC-LTQ-Orbitrap-MS	[49]
Research aim: To seek novel metabolic biomarkers of pancreatic cancer					

In this review, we searched for the articles, in which the evaluations of differences between cancer and control were performed by metabolomics using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis-mass spectrometry (CE-MS), Fourier transform ion cyclotron resonance (FTICR-MS) and nuclear magnetic resonance (NMR), via PubMed database and so on, and their articles were shown in Table 1. In Table 1, the instruments used for metabolomics were described to specify the analytical method, and the aim of each article was also stated. In Table 1, upregulated or downregulated metabolites in serum/plasma, saliva, feces or urine of the cancer patients compared with healthy controls were listed. Regarding tissues, upregulated or

downregulated metabolites in tumor tissues compared with non-tumor (normal) tissues in cancer patients were shown. Table 1 shows the list of metabolites that were demonstrated to be significantly changed between cancer and control in each article. In some articles, many metabolites with the significant alterations between cancer and control were exerted. Regarding these articles, only metabolites that were determined as biomarker candidates based on each criterion was listed in Table 1.

Abbreviations: GABA, γ-Aminobutyrate; LPC, Lysophosphatidylcholine; PC, Phosphatidylcholine; LPA, Lysophosphatidate; LPE, Lysophosphatidylethanolamine; PE, Phosphatidylethanolamine; FA, Fatty acids; GCDCA, Glycochenodeoxycholate; GCA, Glycocholate; UDP, Uridine diphosphate; NAD, Nicotinamide adenine dinucleotide; UMP, Uridine monophosphate; AMP, Adenosine monophosphate; ATP, Adenosine triphosphate; CE-IT/MS, Capillary electrophoresis-ion-trap/mass spectrometry; CE-MS, Capillary electrophoresis-mass spectrometry; CE-TOF-MS, Capillary electrophoresis-time-of-flight mass spectrometry; DI-FTICR-MS, Direct infusion-Fourier transform ion cyclotron resonance-mass spectrometry; FTICR-MS, Fourier transform ion cyclotron resonance-mass spectrometry; GC×GC-TOF/MS, Two-dimensional gas chromatography-time-of-flight mass spectrometry; GC-QMS, Gas chromatography-quadrupole mass spectrometry; GC-TOF/MS, Gas chromatography-time-of-flight mass spectrometry; LC-IT/MS, Liquid chromatography-ion-trap/mass spectrometry; LC-LTQ-Orbitrap-MS, Liquid chromatography-linear ion trap quadrupole-Orbitrap-mass spectrometry; LC-QqQ/MS, Liquid chromatography-triple quadrupole/mass spectrometry; LC-Q-TOF/MS, Liquid chromatography-quadrupole-time-of-flight/mass spectrometry; NMR, nuclear magnetic resonance; QqQ-MS, Triple quadrupole-mass spectrometry; UPLC-QqQ/MS, Ultra performance liquid chromatography-triple quadrupole/mass spectrometry; UPLC-Q-TOF/MS, Ultra performance liquid chromatography-quadrupole-time-of-flight/mass spectrometry; UPLC-TOF/MS, Ultra performance liquid chromatography-time-of-flight/mass spectrometry.