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Phospholipid Abnormalities in Children With Barth Syndrome

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OBJECTIVES We sought to identify characteristic lipid abnormalities in patients with Barth syndrome (BTHS) and to correlate the lipid profile to phenotype and genotype.

BACKGROUND Barth syndrome typically includes cardiomyopathy, skeletal myopathy, neutropenia, growth retardation, and 3-methylglutaconic aciduria, and it is commonly associated with mutations in the tafazzin (*TAZ*) gene, whose products are homologous to phospholipid acyltransferases. However, clinical features of BTHS have also been found in patients with normal *TAZ* gene.

METHODS We analyzed molecular species of phospholipids in left and right ventricle, skeletal muscle, platelets, lymphoblasts, and fibroblasts from 19 children with BTHS (positive *TAZ* mutation), 6 children with BTHS-like syndromes (wild-type *TAZ*), 4 children with isolated cardiomyopathy (wild-type *TAZ*), and various controls.

RESULTS Cardiolipin, the specific lipid found only in mitochondria, was decreased in all tissues from BTHS patients, whereas concentrations of other phospholipids were normal. The molecular composition of cardiolipin was altered in all tissues from BTHS patients. The molecular compositions of phosphatidylcholine and phosphatidylethanolamine were altered in the heart. Cardiolipin abnormalities were only found in children with true BTHS, not in children with BTHS-like disease or with isolated cardiomyopathy. The degree of cardiolipin deficiency was tissue-specific but did not correlate with severity or specific phenotypic expression of BTHS.

CONCLUSIONS Abnormal cardiolipin is a specific diagnostic marker of cardiomyopathies caused by *TAZ* mutations. These mutations lead to alterations in the fatty acid composition of several phospholipids, supporting the idea that *TAZ* encodes a human acyltransferase. (J Am Coll Cardiol 2003;42:1994–9) © 2003 by the American College of Cardiology Foundation

Barth syndrome (BTHS) (MIM 302060) is a rare cause of congestive heart failure in infants. In its typical presentation, it is a multisystem disorder that includes cardiomyopathy, cyclic neutropenia, growth retardation, proximal and distal muscle weakness, and elevated excretion of 3-methylglutaconic acid (1–4). Barth syndrome is an inherited disease linked to the tafazzin gene (*TAZ*, G4.5) on the X chromosome (5–8). Sequence homology of the *TAZ* gene with genes coding for phospholipid acyltransferases triggered speculations that BTHS may be caused by a defect in phospholipid metabolism (9). This idea was supported by two observations, namely reduced transfer of linoleic acid into cardiolipin of BTHS fibroblasts (10), and absence of tetralinoleoyl-cardiolipin in platelets (11,12), fibroblasts (13), and muscle tissue (11) from BTHS patients. These abnormalities are consistent with an acyltransferase deficiency in BTHS, and they raise the question whether the disease involves other phospholipids besides cardiolipin.

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Clinical manifestations of BTHS are extremely variable. Each symptom may range from mild to severe, generating vastly different clinical patterns (3,4). Furthermore, cardiac involvement can present as dilated cardiomyopathy (DCM), left ventricular noncompaction, or endocardial fibroelastosis (7,8,14,15). Growth retardation, neutropenia, and muscle weakness are inconsistently present and may vary in severity. This makes it difficult to diagnose BTHS on clinical findings alone and to distinguish BTHS from closely related diseases, especially other infantile cardiomyopathies. In fact, some cases of familial cardiomyopathy with all associated symptoms of BTHS had normal *TAZ* gene. In one such case, the BTHS-like disease could be linked to a mutation of mitochondrial DNA (16), but in other cases the genetic cause has not yet been identified. The question arises then as to whether the cardiolipin abnormality described in BTHS is also present in BTHS-like disease with wild-type *TAZ*. Is cardiolipin deficiency strictly the consequence of *TAZ* mutations or is it associated with symptoms of BTHS? If so, does the severity of the symptoms correlate with the magnitude of cardiolipin deficiency?

METHODS

Patients. The protocols used for harvesting human specimens were approved by the institutional review committees of New York University Medical Center (platelets), New York Presbyterian Hospital (heart, skeletal muscle, fibro-

Abbreviations and Acronyms

BTHS = Barth syndrome
DCM = dilated cardiomyopathy
HPLC = high-performance liquid chromatography
ICM = ischemic cardiomyopathy
LV = left ventricle
RV = right ventricle
TAZ = tafazzin

blasts), Hospital for Special Surgery (platelets, skeletal muscle), Texas Children's Hospital (heart), Johns Hopkins University (lymphoblasts), and the Academic Medical Center Amsterdam (fibroblasts). Patients, and/or their guardians, gave written, informed consent in all cases. The study included children with BTHS, BTHS-like disease, and isolated cardiomyopathy (Table 1). Children with BTHS had mutations in the *TAZ* gene and met either one of the following clinical criteria: 1) cardiomyopathy plus at least one noncardiac symptom of BTHS, or 2) no cardiomyopathy but at least three noncardiac symptoms of BTHS. Noncardiac symptoms included neutropenia, growth retardation, proximal and distal skeletal muscle weakness, and 3-methylglutaconic aciduria (>15 mg 3-methylglutaconic acid/g creatinine). Children with BTHS-like disease met the same clinical criteria as did children with BTHS, but they did not have *TAZ* mutations. We also studied three healthy heterozygous females (42 to 46 years) who carried a *TAZ* mutation. Several controls were used in this study. Cardiac tissue was obtained from six adults with brain death (22 to 51 years), five adults with ischemic cardiomyopathy (ICM) (59 to 67 years), and nine adults with DCM (37 to 66 years). Skeletal muscle biopsies were obtained from five children with Prader-Willi syndrome (2 to 15 years) and from nine pediatric (1 to 18 years) and four adult (28 to 47 years) patients in whom diagnostic biopsies had shown minimal nonspecific changes. Platelets were obtained from five normal children (1 to 15 years) and eight normal adults (23 to 56 years). All patients were male, unless otherwise specified.

Table 1. Study Patients

Patient	Specimen (Age)*	*TAZ* Mutation		CM	NP	GR	SW	MA
		Location	Effect					
BTHS								
1	RV/LV (0.5)	Exon 4	Missense	LVNC	+	+	−	+
2	LB (1)	Exon 8	Missense	DCM	+	+	+	+
3	FB (1)	Intron 2	Frameshift	DCM	+	+	+	+
4	SM (2), PT (14)	Exon 6	Frameshift	DCM	+	+	−	+
5	LB (3) PT (8)	Intron 1	Splice site	DCM	+	+	+	+
6	FB (3)	Exon 4	Missense	DCM	+	+	+	+
7	FB (4), PT (14)	Exon 2	Frameshift	DCM	+	−	+	+
8	LB (4)	Intron 1	Splice site	DCM	+	+	+	+
9	PT (5)	Exon 2	Stopcodon	DCM	+	+	+	+
10	FB (5)	Exon 4	Stopcodon	DCM	+	+	+	+
11	SM (6), PT (12)	Exon 1	Frameshift	DCM	+	+	+	+
12	PT (6)	Exon 8	Missense	DCM	+	+	−	+
13	PT (7)	Exon 2	Frameshift	DCM	+	+	+	ND
14	PT (8)	Intron 9	Splice site	None	−	+	+	+
15	LB (9), PT (18)	Exon 2	Frameshift	DCM	−	−	+	+
16	PT (10)	Exon 4	Stopcodon	None	+	+	+	+
17	RV/LV (12)	Exon 2	Splice site	DCM	+	+	−	−
18	PT (15)	Exon 2	Splice site	EFE	+	+	+	+
19	PT (15)	Exon 10	Missense	DCM	−	−	−	+
BTHS-like disease								
20	SM (0.3)	None	None	DCM	+	−	−	ND
21	PT (2)	None	None	LVNC	+	+	+	+
22	PT (8)	None	None	DCM	+	+	+	+
23	PT (9)	None	None	DCM	+	+	+	+
24	PT (14)	None	None	None	+	+	+	+
25	RV/LV (15)	None	None	DCM	+	−	−	ND
Isolated cardiomyopathy								
26	SM (0.1)	None	None	LVNC	−	−	−	−
27	LV (11)	None	None	DCM	−	−	−	−
28	LV (16)	None	None	DCM	−	−	−	−
29	LV (19)	None	None	DCM	−	−	−	−

All patients were male, except for Patient 22. *The age (years) at the time of tissue harvest is given in parentheses.

BTHS = Barth syndrome; CM = cardiomyopathy; EFE = endocardial fibroelastosis; FB = fibroblasts; GR = growth retardation; LB = lymphoblasts; LVNC = left ventricular noncompaction; MA = 3-methylglutaconic aciduria; ND = not determined; NP = neutropenia; PT = platelets; SM = skeletal muscle; SW = skeletal muscle weakness.

Table 2. Phospholipid Composition in BTHS Patients With *TAZ* Mutation

Specimen	Patient Group	Phospholipid Composition (mol% Phosphorous)				
		PC	PE	CL	PI + PS	Other
Heart ventricle	Control	46.6 ± 0.8	24.4 ± 3.1	11.5 ± 0.2	12.7 ± 0.1	4.6 ± 2.4
	BTHS	46.7 ± 1.7	28.5 ± 2.6	5.5 ± 0.5*	12.6 ± 0.6	6.7 ± 1.4
Platelets	Control	55.0 ± 0.6	25.0 ± 0.6	0.6 ± 0.1	17.6 ± 0.3	1.8 ± 0.3
	BTHS	56.0 ± 1.0	26.0 ± 0.5	0.1 ± 0.1*	16.1 ± 0.4	1.9 ± 0.3
Lymphoblasts	Control	44.6 ± 0.5	27.3 ± 2.3	5.4 ± 0.8	14.4 ± 1.6	8.2 ± 1.0
	BTHS	43.0 ± 1.2	30.9 ± 1.5	2.8 ± 0.2*	16.7 ± 1.1	6.6 ± 1.3

Four samples were studied in each group. *Significant difference between BTHS and control ($p < 0.01$).
BTHS = Barth syndrome; CL = cardiolipin; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PS = phosphatidylserine.

Specimens. Cardiac tissue was excised from whole hearts explanted for transplantation. Paired samples from the left ventricle (LV) and right ventricle (RV) were obtained in most cases. Skeletal muscle biopsies were removed under surgical conditions. All tissues were frozen in liquid nitrogen immediately after harvest. Platelets were isolated from anticoagulated blood by differential centrifugation. Lymphoblasts and fibroblasts were grown in standard cell culture media. All samples were stored at −80°C before analysis.

Measurements. Samples were homogenized in aqueous buffer, and the protein concentration was determined (17). Lipids were extracted into chloroform/methanol (18) and separated by two-dimensional thin-layer chromatography (19). Phospholipids were quantified by the determination of phosphate after ashing (20). Microanalysis of cardiolipin was described in detail elsewhere (21). In brief, cardiolipin was methylated with diazomethane and then derivatized with naphthylacetic anhydride in the presence of an internal standard (oleoyl-tristearoyl-cardiolipin). The derivative was purified by solid-phase extraction and analyzed by high-performance liquid chromatography (HPLC) with fluorescence detector. The concentration of individual cardiolipins relative to the internal standard was derived from the chromatogram by computer-assisted manual peak integration. Molecular species of phosphatidylcholine and phosphatidylethanolamine were analyzed as 1,2-diacyl-3-dinitrobenzoylglycerol derivatives as described by Takamura et al. (22) with some modifications (23). The two phospholipids were purified by thin-layer chromatography, treated with phospholipase C, and derivatized with 3,5-dinitrobenzoyl chloride. Molecular species of phosphatidylcholine and phosphatidylethanolamine were resolved by reversed-phase HPLC and identified by fatty acid analysis. Species composition was calculated from peak integrals of the chromatogram.

Statistics. Data are given as mean ± SEM. Study groups were compared by the Student *t* test. Both RV and LV were compared by paired *t* test. Differences were considered to be statistically significant for p values below 0.05.

RESULTS

Heart muscle. We compared cardiac tissue from children with BTHS to cardiac tissue from children with isolated DCM, as well as adults with DCM, adults with ICM, and adult organ donors. The non-BTHS groups had similar lipid composition, and therefore they were considered nor-

Table 3. Cardiolipin in Heart

Patient Group	N	Specimen	Cardiolipin Content (nmol/mg Protein)	Cardiolipin Composition (mol%)			
				L_4	L_3O	L_2O_2	Other
Adults							
Control	6	LV	12.0 ± 1.3	79.5 ± 2.1	12.1 ± 1.6	4.4 ± 0.7	4.0 ± 0.9
		RV	10.3 ± 1.2*	78.0 ± 1.3	13.3 ± 0.8	4.3 ± 0.5	4.4 ± 1.3
ICM	5	LV	8.6 ± 1.3	73.2 ± 3.0	17.0 ± 1.9	5.4 ± 1.1	4.3 ± 0.5
		RV	13.5 ± 2.5	79.8 ± 1.4	11.4 ± 0.8	3.5 ± 0.3	5.3 ± 0.9
DCM	9	LV	10.5 ± 0.9	76.4 ± 1.6	14.8 ± 0.8	5.4 ± 0.5	3.3 ± 0.6
		RV	12.8 ± 1.4	79.0 ± 1.5	13.2 ± 0.9	4.6 ± 0.6	3.1 ± 0.5
CHF	14	LV	9.8 ± 0.8	75.3 ± 1.5	15.6 ± 0.9	5.4 ± 0.5	3.7 ± 1.6
		RV	13.1 ± 1.2*	79.3 ± 1.0*	12.6 ± 0.7*	4.2 ± 1.5*	3.9 ± 1.9
Children							
BTHS (*TAZ* mutation)	2	LV	10.4 ± 3.7	3.5 ± 2.5†‡	6.7 ± 2.5†	0.1 ± 0.1†	89.8 ± 5.1†‡
		RV	6.9 ± 2.9	4.5 ± 3.5†	13.5 ± 9.3	14.8 ± 14.7	67.1 ± 27.7†
BTHS-like disease (normal *TAZ*)	1	LV	32.4	33.9	4.6	5.2	56.3
		RV	9.4	73.7	9.9	0.1	16.3
DCM (normal *TAZ*)	3	LV	15.2 ± 3.2	80.9 ± 3.9	12.0 ± 2.7	3.5 ± 0.9	3.5 ± 0.4

Patients were male except for one patient in the adult control group and one patient in the adult dilated cardiomyopathy (DCM) group. The congestive heart failure (CHF) group contained adult patients with ischemic cardiomyopathy (ICM) and DCM. *Significant difference between left ventricle and right ventricle (paired *t* test, $p < 0.05$). †Significant difference between Barth syndrome (BTHS) and adult DCM ($p < 0.001$). ‡Significant difference between BTHS and child DCM ($p < 0.001$).
L_4 = tetralinoleoyl-cardiolipin; L_3O = trilinoleoyl-oleoyl-cardiolipin; L_2O_2 = dilinoleoyl-dioleoyl-cardiolipin; *TAZ* = tafazzin.

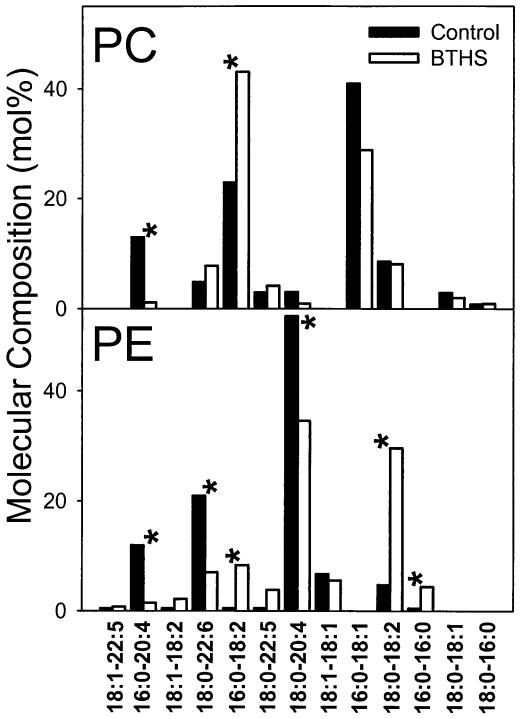

Figure 1. Molecular composition of cardiac phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from controls and Barth syndrome (BTHS) patients with tafazzin (*TAZ*) mutation. Data are means of four measurements (two left ventricle samples and two right ventricle samples). **Asterisks** indicate a statistically significant difference between control and BTHS ($p < 0.05$). Diacylglycerol species are listed in the sequence in which they elute from the chromatography column. 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 20:4 = arachidonic acid; 22:5 = docosapentaenoic acid; 22:6 = docosahexaenoic acid.

mal controls (data not shown). Cardiac tissue from BTHS patients contained less cardiolipin than the controls, but all other phospholipids were present in normal proportions (Table 2). The molecular composition of cardiolipin was also abnormal in BTHS. The most striking feature was an almost complete absence of the predominant molecular species, tetralinoleoyl-cardiolipin. In contrast, normal levels of tetralinoleoyl-cardiolipin were found in children without *TAZ* mutations, who had either isolated cardiomyopathy or BTHS-like disease (Table 3). The molecular compositions of phosphatidylcholine and phosphatidylethanolamine were also altered in BTHS, with severe deficiency of the 1-palmitoyl-2-arachidonoyl-glycerol species in both phospholipids (Fig. 1). Likewise, a deficiency of arachidonic acid was noted in phospholipid-bound fatty acids from skeletal muscle of BTHS patients (data not shown).

Cardiolipin levels from different anatomical sites were compared. In adult organ donors (control cardiac tissue), the LV contained 17% more cardiolipin than did the RV and about twice as much cardiolipin than the atrium (atrial cardiolipin: 5.8 ± 0.7 nmol/mg protein, n = 6). However, in adult patients with congestive heart failure the RV contained 34% more cardiolipin and 4% more tetralinoleoyl-cardiolipin than the LV. In BTHS, no consistent difference existed between RV and LV (Table 3).

Skeletal muscle. Muscle biopsies from children with BTHS showed extremely low content of cardiolipin, primarily due to deficiency of tetralinoleoyl-cardiolipin. In contrast, both cardiolipin concentration and cardiolipin composition were normal in children with other cardiac and skeletal muscle diseases, including BTHS-like disease (no *TAZ* mutation), isolated left ventricular noncompaction, and Prader-Willi syndrome (Table 4).

Platelets. In children with BTHS, platelets contained very little cardiolipin but normal amounts of other phospholipids (Table 2). Cardiolipin deficiency was due to loss of all three major molecular species (i.e., tetralinoleoyl-cardiolipin, trilinoleoyl-oleoyl-cardiolipin, and dilinoleoyl-dioleoyl-cardiolipin). In children with BTHS-like disease (no *TAZ* mutation), both cardiolipin total concentration and composition were normal. Heterozygous carriers of *TAZ* mutations also had normal cardiolipin (Table 5). In contrast to heart and skeletal muscle, platelets from BTHS patients did not show alterations in the fatty acid composition of total phospholipids (data not shown).

Lymphoblasts and fibroblasts. Cardiolipin was reduced by about half in lymphoblasts from BTHS patients, whereas all other phospholipids were normal (Table 2). In fibroblasts, cardiolipin was reduced by about 75% (0.29 ± 0.05 nmol/mg protein in BTHS vs. 1.22 ± 0.10 nmol/mg protein in controls, n = 4, $p < 0.001$). Although normal fibroblasts contained very little tetralinoleoyl-cardiolipin

Table 4. Cardiolipin in Skeletal Muscle

Patient Group	N	Cardiolipin Content (nmol/mg Protein)	Cardiolipin Composition (mol%)			
			L_4	L_3O	L_2O_2	Other
Adults						
Control	4	1.9 ± 0.3	78.6 ± 2.7	15.8 ± 0.9	4.1 ± 1.1	1.5 ± 0.7
Children						
Control	9	2.2 ± 0.3	78.9 ± 2.1	14.7 ± 1.1	3.2 ± 1.1	3.2 ± 0.6
Prader-Willi syndrome	5	1.8 ± 0.1	77.4 ± 0.6	15.8 ± 0.7	5.0 ± 0.2	1.9 ± 0.4
BTHS (*TAZ* mutation)	2	0.4 ± 0.1*	17.7 ± 2.9*	17.9 ± 11.3	13.5 ± 1.9*	50.9 ± 10.2*
BTHS-like disease (normal *TAZ*)	1	2.1	78.9	13.3	5.1	2.8
Left ventricular noncompaction (normal *TAZ*)	1	2.9	80.7	11.4	7.8	0.1

Patients were male except for two patients in the adult control group, four patients in the pediatric control group, and two others in the Prader-Willi group. *Significant difference between BTHS and child control ($p < 0.02$).

Abbreviations as in Table 3.

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Table 5. Cardiolipin in Platelets

Patient Group	N	Cardiolipin Content (nmol/10^9 Cells)	Cardiolipin Composition (mol%)			
			L_4	L_3O	L_2O_2	Other
Adults						
Control	8	3.2 ± 0.3	42.0 ± 2.1	36.6 ± 1.3	12.4 ± 1.7	9.0 ± 1.4
BTHS carrier	3	2.5 ± 0.1	46.0 ± 0.9	34.7 ± 2.2	11.8 ± 2.0	7.4 ± 1.0
Children						
Control	5	2.9 ± 0.4	42.4 ± 4.5	34.9 ± 1.6	14.7 ± 3.5	7.0 ± 1.1
BTHS (*TAZ* mutation)	12	0.3 ± 0.1*	0.8 ± 0.6*	1.4 ± 0.9*	1.3 ± 1.0*	97.0 ± 2.2*
BTHS-like disease (normal *TAZ*)	4	2.7 ± 0.4	45.5 ± 2.5	35.9 ± 1.2	10.7 ± 1.6	8.0 ± 0.7

Patients were male except for all BTHS carriers, three patients in the adult control group, and one patient in the BTHS-like group with normal *TAZ*. *Significant difference between BTHS and child control ($p < 0.001$).
Abbreviations as in Table 3.

and normal lymphoblasts contained virtually none, the cardiolipin composition of both cell types changed dramatically in BTHS (data not shown), suggesting that many cardiolipin species may be affected by the disease.

DISCUSSION

In patients with BTHS, we found abnormal molecular compositions of several phospholipids, including cardiolipin, phosphatidylcholine, and phosphatidylethanolamine. The data indicate a maldistribution of fatty acids (i.e., fatty acids lost their preference for specific phospholipids). For instance, the characteristic predominance of linoleic acid in cardiolipin was missing in BTHS. Conversely, the content of linoleoyl was increased in phosphatidylcholine and phosphatidylethanolamine. The notion of misdirected fatty acids was consistent with the presumed acyltransferase defect in BTHS. The most striking consequence of this defect was the absence of tetralinoleoyl-cardiolipin, a major molecular species in several control tissues (24). However, the molecular composition of cardiolipin was altered even in lymphoblasts that did not contain any tetralinoleoyl-cardiolipin, suggesting a general impairment of fatty acid trafficking from and to cardiolipin. In phosphatidylcholine and phosphatidylethanolamine, deficiency of palmitoyl-arachidonoyl-glycerol species was the most prominent abnormality. It was not found in every tissue, but may have functional implications for eicosanoid-dependent signal transduction in BTHS hearts.

Barth syndrome varies in its clinical presentation and it is usually, but not invariably, associated with mutations in the *TAZ* gene. Thus, the question arises how phospholipid abnormalities relate to the various phenotypes and genotypes. Of 25 children who met clinical criteria of BTHS, we found abnormal cardiolipin only in the 19 patients with *TAZ* mutations. In contrast, neither cardiac or noncardiac features nor the overall severity of the disease was specifically linked to cardiolipin deficiency. The absence of phenotypic correlation makes it unlikely that the pathophysiology of BTHS can be explained solely on the basis of cardiolipin abnormalities. Instead, there must be additional factors that modify disease expression.

The association between tetralinoleoyl-cardiolipin deficiency and *TAZ* mutations suggests that *TAZ* is involved in the transfer of linoleoyl groups to cardiolipin. The *TAZ* activity is absent in most BTHS patients because the mutation results in loss of the entire protein. However, in five of our patients (Patients 1, 2, 6, 12, and 19), we found missense mutations that cause substitutions of single amino acids in exons 4, 8, or 10, indicating that these exons are crucial for acyltransferase activity.

The *TAZ* mutations can result in a broad spectrum of cardiac phenotypes, including DCM, left ventricular noncompaction, and endocardial fibroelastosis (7,8,14,15,25). Although BTHS with DCM is probably the most characteristic presentation of *TAZ* mutations, it is clinically indistinguishable from BTHS-like disorders without *TAZ* mutations. Thus, on purely clinical grounds, it is impossible to differentiate true BTHS from related cardiomyopathies. We show that cardiolipin is a valuable diagnostic tool to identify those cardiomyopathies that are caused by *TAZ* mutations. Diagnostic cardiolipin analysis in platelets has become even more attractive since the availability of a rapid mass-spectrometric technique (12). We propose that cardiolipin analysis should be included in the evaluation of all children with cardiomyopathy in whom X-linked inheritance is suspected.

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