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Lipoamide Dehydrogenase Deficiency Due to a Novel Mutation in the Interface Domain

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An infant with a neurodegenerative disorder accompanied by lactic acidemia is described. In muscle homogenate, the activity of lipoamide dehydrogenase (LAD), the third catalytic subunit of pyruvate dehydrogenase complex (PDHc), α-ketoglutarate dehydrogenase complex (KGDHc), and branched-chain keto acid dehydrogenase complex was reduced to 15% of the control. The activity of PDHc was undetectable and the activity of KGDHc was 2% of the control mean. The immunoreactive LAD protein was reduced to about 10% of the control. Direct sequencing of LAD cDNA revealed only one mutation, substituting Asp for Val at position 479 of the precursor form. The mutation resides within the interface domain and likely perturbs stable dimerization. The phenotypic heterogeneity in LAD deficiency is not directly correlated with the residual LAD activity but rather with its impact on the multienzymatic complex activity.

Lipoamide dehydrogenase (LAD) (EC 1.6.4.3) is the third catalytic subunit of pyruvate dehydrogenase complex (PDHc). It promotes the transfer of hydrogen atoms from reduced lipoyl groups to NAD via FAD. LAD is also active in α-ketoglutarate dehydrogenase complex (KGDHc) and in branched-chain keto-acid dehydrogenase complex.

The clinical presentation of LAD deficiency is variable. Patients may present in infancy with severe psychomotor retardation and chronic lactic acidemia or later in life with recurrent episodes of liver failure or myoglobinuria (1–5).

Molecular analysis was reported for 3 patients with the infantile neurodegenerative phenotype. Six mutations were identified, P488L, K72E, R495G, Y35X, E375K and an in-frame deletion of Gly136 (residue numbers in the precursor form) (6, 7, 8). The pathogenicity of the K72E mutation was recently questioned because the K72E enzyme restored full activity to a LAD-deficient yeast strain (9). The common mutation in patients with recurrent episodes of liver failure is G229C; compound heterozygosity for G229C and Y35X mutation is associated with episodic liver disease and moderate neurological involvement (10, 11). A new mutation in the LAD gene is reported in a patient with the severe neurodegenerative phenotype.

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CASE REPORT

The patient was a 9 months old girl of Moslem origin. Her parents denied consanguinity and their 3 other children were healthy. She was born at 36 weeks of gestation following an uneventful pregnancy; birth weight was 2,300 gram and head circumference was 33 cm. On the 3rd day of life she became apathetic and refused to feed. Pallor and tachypnea were noted and the peripheral perfusion was poor. Laboratory investigation revealed hypoglycemia (31 mg%) and severe metabolic acidosis (pH 7.12, pCO2 17 mmHg); plasma lactate was 9 mM (N < 1.5 mM). Plasma carnitine level was normal (45 μM) but the free fraction was relatively low (25 μM). Generalized aminoaciduria was found in the urine and organic acid analysis revealed massive excretion of lactate 1425 mol/mol creatinine (control <0.2 mol/mol creatinine). Branched-chain keto acids and α-ketoglutarate levels were within the normal range. Sodium bicarbonate was repeatedly administered and after muscle biopsy, performed on the 5th day, dichloroacetate, carnitine, thiamin, coenzyme Q and riboflavin were added.

The clinical course was characterized by recurrent episodes of metabolic acidosis, usually triggered by intercurrent infections. At 9 months she was microcephalic, unable to roll over or sit, blind and deaf. Muscle tone was low, postural reflexes were absent and deep tendons reflexes were brisk. Repeated echocardiography revealed mild hypertrophic cardiomyopathy.

METHODS

The activities of the mitochondrial respiratory chain complexes and Mg^{2+} ATPase were determined in mitochondrial enriched frac-

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TABLE 1

Enzymatic Activities in Muscle Homogenate (nmol/min/mg Protein)

Genotype	D479V/D479V	G229C/G229C	G229C/G229C	Control
Pyruvate dehydrogenase complex	Undetectable	0.78	0.9	7.40 ± 1.8
Lipoamide dehydrogenase	18	8	9	123 ± 40
α-Ketoglutarate dehydrogenase complex	0.059	0.43	0.69	3.52 ± 1.22

tion of quadriceps muscle (12–16). PDHc, pyruvate decarboxylase, LAD, and KGDHc activities were determined in muscle homogenate (17–20). In order to study the molecular-biochemical relationship, the activity of LAD, PDHc and KGDHc was also determined in muscle homogenate of 2 patients with LAD deficiency due to homozygosity for the G229C mutation. LAD activity in lymphocytes of the parents was determined as described (21). All assays were performed in triplicates. Immunoblot analysis of LAD and PDHc subunits in muscle was performed as previously described (11).

Total RNA was isolated from fibroblasts of the patient, lymphocytes of her parents and from amniotic cells of the fifth pregnancy. The amniotic cells were obtained by amniocentesis on the 15th gestational week. First strand cDNA was generated using MMLV-reverse transcriptase (Promega, Madison, WI, USA) with the antisense oligonucleotide primer LDP2 (nt 1618 to 1599). This and all other primers were derived from the consensus sequence and nucleotides and amino acids were numbered from the first ATG (22, 23). A 1,662-bp cDNA fragment which contained the entire coding region was PCR-amplified from the reverse transcribed products using the oligonucleotide primers LDP1 (nt −44 to −25) and LDP2. Three additional primers, LDP22 (nt 755 to nt 736) and LDP7 (nt 704 to nt 723) were used for sequence analysis. Direct sequencing was performed on an automatic sequencer (ABI Prism 377, Perkin-Elmer) using Dye Terminator Cycle Sequencing Core Kit (Perkin-Elmer) according to the manufacturer's instructions.

RESULTS

In the mitochondrial enriched fraction the activity of all mitochondrial respiratory chain enzymes was within the normal range. In the muscle homogenate, the activity of PDHc was undetectable, the activity of pyruvate decarboxylase was normal (0.22 nmol/min/mg protein, control 0.24 ± 0.11 nmol/min/mg protein) and the activity of LAD was reduced to 15% of the control. The activity of KGDHc was reduced to less than 2% of the control (Table 1).

In muscle homogenate of the 2 G229C homozygotes, analyzed under the same conditions, the activity of LAD was about 7% of the control, the activity of PDHc was 11 and 12% of the control and the activity of KGDHc was 12 and 19% of the control mean (Table 1).

Immunoblot analysis revealed a reduced amount of LAD protein, 13% in mitochondria and 7.6% in homogenate as compared to control. The 2 subunits of pyruvate decarboxylase (E1α and E1β), the lipoamide transacetylase subunit (E2) and protein X were detected in normal amount and size.

In the lymphocytes of the mother and the father the activity of LAD was 39 and 36 nmol/min/mg protein, respectively (control 80.7 ± 23.6 nmol/min/mg protein). The LAD/citrate synthase ratio was 45% and 55% of the control mean.

Direct sequencing of the entire coding region disclosed an Asp-to-Val substitution at codon 479, (GAT → GTT), designated D479V. Analysis of the cDNA of the parents and amniotic cells revealed heterozygosity for the mutation in all 3 samples.

DISCUSSION

A new patient with severe neurodegenerative disease due to LAD deficiency is presented. Mutation analysis revealed homozygosity for the D479V mutation, which was the only alteration identified in the entire coding region of the LAD cDNA, suggesting that the D479V is the disease-causing mutation. The mutation was also present in a heterozygous form in the cDNA of the parents.

Multiple copies of dimers of LAD are part of the α-keto acid dehydrogenase complexes. Within the complexes, LAD receives 2 electrons from the dihydrolipoyl moiety of the second catalytic subunit, E2. The mutated residue in our patient, Asp479, is located in α-helix XII, which is part of the interface domain of the LAD dimer. In *Azotobacter vinelandii* the Asp442 residue (which is homologous to the human Asp479) is only 4.34Å away from the Phe436 molecule (which is homologous to the human Tyr473) of the opposite monomer (3LAD, Protein Data Bank, Brookhaven National Laboratory).

The α-helix XII and its vicinity creates a large region of negative electrochemical potential over the LAD protein, attributed to 3 highly conserved residues, Glu472, Glu478 and Asp479 (Table 2) (26). In the KGDHc of *Saccharomyces cervisiae*, this negatively charged region is proposed to bind a positively charged sequence in the E2 subunit (23). Similar interaction in mammalian KGDHc is unlikely because in the bovine complex, LAD interacts directly with E1 (24, 25, 27). Nonetheless, the deleterious biochemical phenotype in our patient is indicative of the importance of the Asp479 residue for both PDHc and KGDHc activity. The conformational change imposed by the substitution of the negatively charged aspartate by uncharged valine is

TABLE 2

The α-Helix XII of the LAD Gene and Its Vicinity in the Different Species

Azotobacter vinelandii	M	**E**	F	G	T	S	*A*	***E***	***D***	*L*	*G*	M	M	V	F	A	H	P	A	L	S	E	A	L	H
Pseudomonas fluorescens	M	**E**	F	G	T	S	*A*	***E***	***D***	*L*	*G*	M	M	V	F	S	H	P	T	L	S	E	A	L	H
Bacillus stearothermophilus	I	**E**	A	G	M	T	*A*	***E***	***D***	*I*	*A*	L	T	I	H	A	H	P	T	L	S	E	I	A	M
Pig	L	**E**	Y	G	A	S	*C*	***E***	***D***	*I*	*A*	R	V	C	H	A	H	P	T	L	S	E	A	F	R
Human	L	**E**	Y	G	A	S	*C*	***E***	***D***	*I*	*A*	R	V	C	H	A	H	P	T	L	S	E	A	F	R
Yeast	L	**E**	Y	G	A	S	*A*	***E***	***D***	*V*	*A*	R	V	C	H	A	H	P	T	L	S	E	A	F	K

Note. The highly conserved, negatively charged amino acids are indicated by bold letters. Residues forming the α-helix XII are indicated by italic letters (26). The mutated residues in patients with infantile neurodegenerative phenotype are underlined.

likely interfering with LAD dimerization, leading to its accelerated degradation. It is of note that the urinary α-ketoglutaric acid excretion was within the normal range in our patient *suggesting that in vivo* stability of KGDHc may be higher than expected from the determination of the enzymatic activity. Similar consideration could explain the normal plasma levels of branched chain amino acids.

A defect in LAD is invariably expected to give rise to a serious clinical phenotype because LAD is an essential component in 3 major metabolic pathways: the conversion of pyruvate to acetyl-CoA, the Krebs cycle and the branched chain amino acid degradation. Still, clinical heterogeneity is striking; the severe neurodegenerative phenotype in our patient is in sharp contrast to the lack of neurological involvement in patients homozygous for the G229C mutation (11). This variability is not directly related to residual LAD activity which is higher in the D479V homozygote than in the G229C homozygote (15% vs 7% of the control mean, respectively). Rather, the impact of the mutation should be regarded in the context of the global activity of the enzymatic complex; despite low LAD activity, homozygotes for the G229C mutation still maintain over 10% residual activity of the PDHc and KGDHc, whereas the homozygote for the D479V mutation has less than 2% residual activity of these complexes.

Six mutations, P488L, K72E, R495G, Y35X, E375K and an in-frame deletion of Gly136 were previously reported in 3 patients with infantile neurodegenerative disease (6, 7, 8). Excluding the K72E mutation because of doubtful pathogenicity (9) and including the D479V mutation of our patient, it appears that 3 of 4 missense mutations, which are associated with the severe clinical phenotype, reside within a short fragment in the interface domain (Table 2).

We conclude that the D479V mutation is associated with a severe neurodegenerative phenotype. The mutation interferes with protein stability possibly by perturbing dimerization. The phenotypic heterogeneity in LAD deficiency is not directly correlated with the residual LAD activity but rather with its impact on the multienzymatic complex activity.

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