

# Molecular Analysis of Abnormal Pyruvate Dehydrogenase in a Patient with Thiamine-Responsive Congenital Lactic Acidemia

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## ABSTRACT

A patient who responded to thiamine therapy with reduction of lactate in the blood and cerebrospinal fluid and clinical improvement was studied. Cultured lymphoblastoid cells of this patient were found to show reduced activities of pyruvate dehydrogenase complex (PDHC) and pyruvate dehydrogenase, decreased affinity of PDHC for thiamine pyrophosphate, and defective activation of PDHC by pyruvate dehydrogenase phosphatase. PDHC deficiency in fibroblasts and biopsied muscle of this patient was also due to the decreased affinity of PDHC for thiamine pyrophosphate. A mutation in the E<sub>1</sub>α subunit containing the thiamine binding site and serine phosphorylation site regulating the activation/inactivation of PDHC was characterized by the polymerase chain reaction and

DNA sequencing. A single A→G transition was identified at position 131, resulting in the substitution of Arg-44 for His-44. This mutation must be a *de novo* mutation because it was not found in either parent's genomic DNA. In this study, we have obtained the first evidence at the molecular level for a mutation of thiamine-responsive PDHC deficiency. (*Pediatr Res* 36: 340-346, 1994)

### Abbreviations

PDHC, pyruvate dehydrogenase complex  
E<sub>1</sub>, pyruvate dehydrogenase  
TPP, thiamine pyrophosphate  
DCA, sodium dichloroacetate  
CSF, cerebrospinal fluid

Congenital lactic acidemia is known to be heterogeneous and to be caused by genetically determined defects in the activities of a variety of enzymes. It is most often caused by a defect in the PDHC, which catalyzes the oxidative decarboxylation of pyruvate (1). The PDHC is a multienzyme system composed of E<sub>1</sub>, dihydrolipoyltransacetylase, and dihydrolipoyldehydrogenase. E<sub>1</sub> is composed of two nonidentical subunits, E<sub>1</sub>α and E<sub>1</sub>β and has a tetrameric structure, α<sub>2</sub>β<sub>2</sub>. One mechanism for regulation of PDHC activity is phosphorylation/dephosphorylation of serine residues on the E<sub>1</sub>α subunit (2). The binding site for TPP, which is an essential cofactor for PDHC, is also thought to be between the E<sub>1</sub>α and E<sub>1</sub>β subunits (3). Therefore, the E<sub>1</sub>α subunit plays an important role in thiamine-dependent decarboxylation of pyruvate.

Many patients with congenital lactic acidemia have been reported to show reduction of blood lactate and clinical improvement in response to thiamine administration (4, 5). This response is thought to occur because the mutant enzyme has decreased affinity for thiamine or because thiamine stabilizes the abnormal enzyme, but it has not been studied in detail. In this article, we described for the first time the biochemical and molecular analysis of thiamine dependency in a patient with congenital lactic acidemia.

## METHODS

**Case report.** The patient was the second child of unrelated parents and was born after 37 wk of gestation after a normal pregnancy and delivery. His birth weight was 1928 g, and he showed hypotonia and poor feeding from birth. The family history was negative for neurologic disease. He was admitted to the hospital for further examination at 2 mo of age. On admission, the blood lactate (10.2 mM) to blood pyruvate (0.79 mM) ratio was 13:1. The concentrations of lactate and pyruvate in the CSF were significantly elevated, being 11.5 and 1.1 mM,

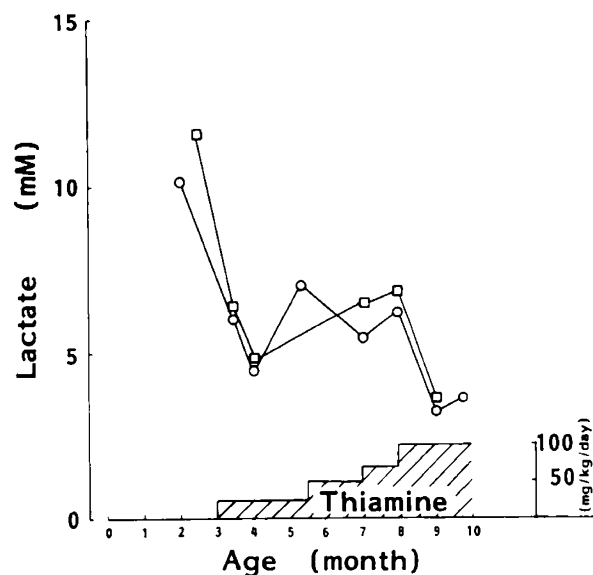
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respectively. Amino acid analysis of plasma showed an elevated alanine level at 1840  $\mu\text{mol/L}$  (normal range, 220–490  $\mu\text{mol/L}$ ). Repeat blood glucose concentrations and other routine blood studies were normal. The findings on EEG and ECG were unremarkable. Muscle biopsy specimens showed no significant morphologic changes. No ragged red fibers were found. Administration of thiamine was started at the age of 3 mo. He responded to thiamine therapy (100 mg/d, 25 mg/kg/d) with reduction in the concentrations of lactate in the blood (5.9 mM) and CSF (6.0 mM) as shown in Figure 1. When the patient was 5 mo of age, the concentrations of lactate (6.9 mM) and pyruvate (0.65 mM) in the blood were elevated again. Increase of doses of thiamine (50 mg/kg/d) led to reduction of blood lactate and pyruvate (5.4 and 0.64 mM, respectively). Head computed tomography scan and magnetic resonance imaging scan when the patient was 8 mo of age showed no significant changes except mild atrophy. The finding on EEG was unremarkable. However, the concentrations of lactate (6.3 mM) and pyruvate (0.75 mM) in the blood increased again. When the patient was 9 mo of age, high doses of thiamine (600 mg/d, 100 mg/kg/d) led to marked reductions of lactate in the blood (3.0 mM) and CSF (3.1 mM), as shown in Figure 1. An alanine concentration was also reduced to 330  $\mu\text{mol/L}$ . After 6 mo of thiamine treatment, the patient showed an improvement of feeding and hypotonia and then gained weight and developed head control. Thiamine therapy of this patient led to the clinical improvement, consistent with the reduction of lactate.

**Radioisotopes and chemicals.** [ $^{14}\text{C}$ ]pyruvate was obtained from NEN Research Products (Boston, MA). [ $^{35}\text{S}$ ]deoxy ATP was purchased from Amersham International (Bucks, UK). DCA was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Other compounds were from Sigma Chemical Co. (St. Louis, MO).



**Figure 1.** Effect of thiamine on the concentration of lactate in blood and CSF of a thiamine-responsive patient. Shown is the change of lactate concentration in blood (○) and CSF (□).

**Cell culture.** Lymphoblastoid cell lines derived from the thiamine-responsive patient, his parents, and 16 disease-free Japanese persons were established after incubation with EBV and cyclosporin A as described by Anderson and Gusella (6). Lymphoblastoid cells were grown in RPMI 1640 medium supplemented with 15% FCS (Gibco Laboratories, Grand Island, NY). Fibroblasts were grown in Eagle's minimal essential medium (Nissui, Tokyo, Japan) with 10% FCS. Confluent cells were harvested and washed twice with PBS.

**Assay of rate of decarboxylation of [ $^{14}\text{C}$ ]pyruvate.** The rate of decarboxylation of [ $^{14}\text{C}$ ]pyruvate was measured by a modification of the method of Naito *et al.* (7). Briefly, lymphoblastoid cells were harvested, washed, and suspended with PBS. The cells were incubated in 0.2 mL of PBS containing 2.5 mmol/L [ $^{14}\text{C}$ ]pyruvate (0.1 mCi/mmol) with or without thiamine-HCl (4 mM) and with or without TPP (1 mM) in closed vessels at 37°C for 1 h.

**Assays of PDHC and other enzymes.** Three kinds of activities of PDHC in cultured lymphoblastoid cells and cultured fibroblasts were determined by a modification of the method of Naito *et al.* (7). For determination of the DCA-activated activity, the harvested cells were incubated for 30 min at 37°C in PBS containing 5 mM DCA to activate PDHC. The incubation was stopped by stop solution (30 mM NaF, 30 mM DCA, 6 mM EDTA), and then ice-cold homogenizing buffer was added to the cell pellet obtained by centrifugation. The suspension was frozen rapidly, and then the PDHC activity was determined as described previously (7). Native activity was measured without preincubation of 5 mM DCA. For assay of total activity, the homogenate was preincubated with a partially purified preparation of a broad specificity protein phosphatase from rabbit liver (8) and 3.7 mM  $\text{MnCl}_2$  for 30 min at 37°C, and then the PDHC activity was measured as described above. The PDHC activities in the presence of various concentrations of TPP were measured after their additions to the assay mixture. The activity of  $\text{E}_1$  in cultured lymphoblastoid cells was determined by the method of Chuang *et al.* (9). The activity of DCA-activated  $\text{E}_1$  was measured after preincubation with 5 mM DCA for 30 min as described above. The  $\text{E}_1$  phosphatase activities in lymphoblastoid cells were determined by the method of Ito *et al.* (10). The pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities in cultured fibroblasts were determined by the method of Atkin *et al.* (11).

**Immunoblot analysis.** PDHC was purified from ox heart, and rabbit antibodies were prepared against PDHC,  $\text{E}_1\alpha$ , and  $\text{E}_1\beta$  proteins (12). Immunoblot analysis was performed with these antibodies as described previously (12).

**Amplification of human  $\text{E}_1\alpha$  and  $\text{E}_1\beta$  mRNA, subcloning, and DNA sequencing.** RNA isolation from lymphoblastoid cells and cDNA synthesis were performed (13). Sets of sense and antisense oligonucleotides were designed so that the PCR products generated covered the entire nor-

mal human cDNA sequence, and each contained an *EcoRI* restriction site for subcloning. The synthetic oligonucleotides for E<sub>1</sub>α were PDHA-A, PDHA-B, PDHA-C, and PDHA-D as described previously (13). The synthetic oligonucleotides for E<sub>1</sub>β were PDHB-5 (5'-GGAATTCGATAGAGGACACGACCAAG-3') and PDHB-3 (5'-ATAAATTTCAACGAATTCATATCAAGTCC-3') selected according to the human E<sub>1</sub>β cDNA sequence (14, 15). Thirty-five cycles of the polymerase chain reaction were performed with a GeneAmp DNA amplification reagent kit (Perkin-Elmer Cetus, Norwalk, CT). One cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Subcloning and DNA sequencing with [<sup>35</sup>S]deoxy ATP were performed as described previously (13).

**Analysis of genomic DNA.** Genomic DNA was isolated from cultured lymphoblastoid cells by the proteinase K method (13). Specific amplification of genomic DNA encompassing the region of a point mutation detected in the mRNA of the E<sub>1</sub>α subunit of this patient was attempted with oligonucleotides PA3F (5'-TGGAGGATAATAAC-TACCTTATTCATTT-3') and PA3R (5'-ACAGTTC-CACCACAAACCTACCAC-3'). These primers were selected according to the human E<sub>1</sub>α genomic DNA sequence (16). The DNA amplified by PCR were digested with *NaeI*.

**Protein determination.** Protein concentrations during purification of ox PDHC and SDS-PAGE were determined by the method of Bradford (17). The protein concentrations of other samples were measured by the method of Lowry *et al.* (18) with BSA as a standard.

**Statistical analysis.** Statistical significance was determined by *t* test.

## RESULTS

**Decarboxylation of [1-<sup>14</sup>C]pyruvate in intact lymphoblastoid cells.** Intact lymphoblastoid cells from normal subjects and the thiamine-responsive patient incubated with [1-<sup>14</sup>C]pyruvate alone showed decarboxylation rates of 78.6 and 15.9 nmol/h/mg of protein, respectively, indicating an 80% decrease in the rate in the thiamine-responsive variant cells. Addition of a high concentration (4 mM) of thiamine-HCl to intact normal and thiamine-responsive cells increased the decarboxylation rates of

80.9 and 35.2 nmol/h/mg of protein, respectively, resulting in the increase of the decarboxylation rates by 2 and 121%, respectively. Addition of a high concentration (1 mM) of TPP to intact normal and thiamine-responsive cells increased the decarboxylation rates of 85.0 and 40.3 nmol/h/mg of protein, respectively, resulting in the increase of the decarboxylation rates by 8 and 153%, respectively.

**Activities of PDHC and other enzymes.** The activities of the PDHC in cultured lymphoblastoid cells from normal subjects and the patient were assayed in three different conditions (native, DCA activated, and total) and at two different concentrations of TPP (0.4 mM and 1 × 10<sup>-4</sup> mM) (Table 1). When assayed in the presence of 0.4 mM TPP, the DCA-activated activity of normal controls was 2-fold of native activity, whereas the DCA-activated activity of the patient was almost the same activity in native state, indicating that PDHC of this patient was not sufficiently activated by only his own endogenous E<sub>1</sub> phosphatase; the total activity, measured after *in vitro* activation with an excess amount of a broad specificity protein phosphatase, was within the normal range, indicating that PDHC in this patient was activated by the large amount of exogenous E<sub>1</sub> phosphatase. Furthermore, the activity of the E<sub>1</sub> phosphatase in this patient was within normal range (the patient had 226.8 and controls had 204.4 ± 48.2 mU PDHC activated/min/mg protein). These results indicated that this patient had a disorder of the mechanism for activation of PDHC except E<sub>1</sub> phosphatase. In the presence of 1 × 10<sup>-4</sup> mM TPP, corresponding to the TPP concentration within normal human blood cells, all three activities of PDHC of this patient were extremely low, although no difference was observed in the PDHC activities of control cells in the presence of 0.4 mM and 1 × 10<sup>-4</sup> mM TPP. Furthermore, the activities of PDHC of his parents were within normal range in the presence of either 0.4 mM or 1 × 10<sup>-4</sup> mM TPP (data not shown). Pyruvate carboxylase and phosphoenolpyruvate carboxylase activities of cultured fibroblasts of this patient were normal (data not shown).

**Affinity of PDHC for TPP.** For further explanation of why thiamine had a therapeutic effect in this patient and the addition of thiamine-HCl or TPP to intact lymphoblastoid cells from this patient increased the rate of decarboxylation of [1-<sup>14</sup>C]pyruvate, we investigated the

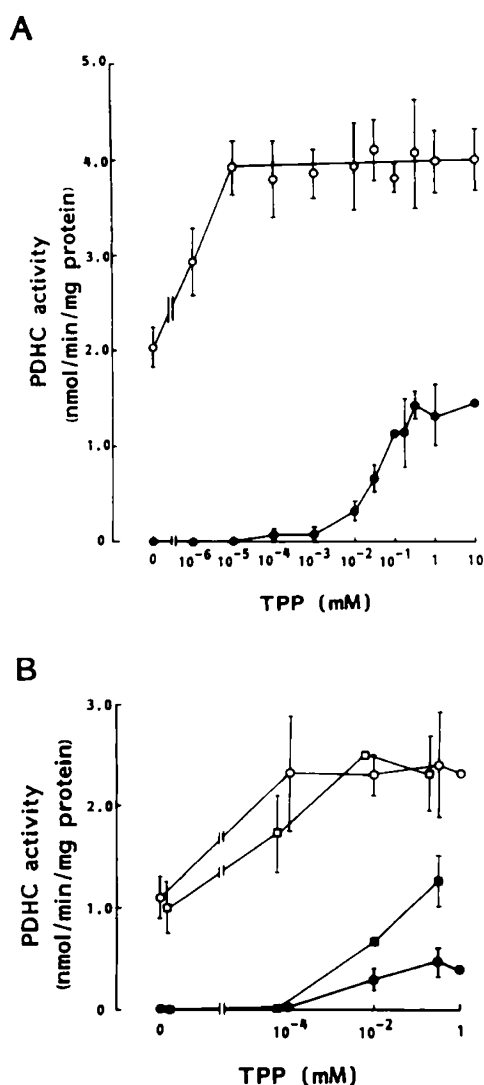
Table 1. Activities of PDHC in lymphoblastoid cells\*

	PDHC (nmol/min/mg protein)		
	Native	DCA activated	Total
With 0.4 mM TPP			
Patient	1.41 ± 0.26 (3)	1.41 ± 0.11† (4)	3.13 ± 0.41 (4)
Controls	2.09 ± 0.62 (12)	4.07 ± 0.68† (16)	4.03 ± 0.89 (13)
With 1 × 10 <sup>-4</sup> mM TPP			
Patient	0.06 ± 0.02† (3)	0.06 ± 0.05† (3)	0.21 ± 0.12† (3)
Controls	1.96 ± 0.29† (3)	3.90 ± 0.34† (4)	4.19 ± 0.29† (4)

\* Values are means ± SD with the number of determinations in parentheses.

† *p* < 0.001 vs controls.

affinities of normal and mutant PDHC for TPP. As shown in Figure 2A, normal cells showed approximately 50% of the full PDHC activity in the absence of TPP, and addition of  $1 \times 10^{-5}$  mM TPP restored the full activity, the PDHC activity being constant at concentrations of more than  $1 \times 10^{-5}$  mM TPP. However, cells from the thiamine-responsive patient showed extremely low PDHC activities with TPP concentrations of 0 to  $1 \times 10^{-3}$  mM and exponential increase of activity with TPP concentration above  $1 \times 10^{-3}$  mM. Addition of a high concentration (0.4 mM) of TPP restored up to 35% of the full PDHC activity of normal cells. These data indicated that the PDHC of this patient showed decreased affinity for TPP.



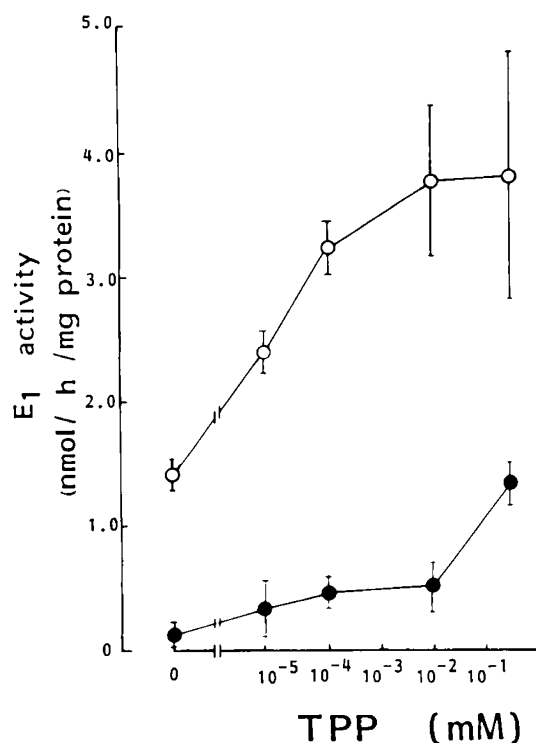
**Figure 2.** Effect of TPP on the activity of DCA-activated PDHC in disrupted lymphoblastoid cells, disrupted fibroblast cells, and biopsied muscle from normal subjects and a thiamine-responsive patient. *A*, Activity of PDHC in disrupted lymphoblastoid cells was measured at various concentrations of TPP. Shown are normal controls (○) and a thiamine-responsive patient (●). *B*, Activity of DCA-activated PDHC in disrupted fibroblasts and activity of native PDHC in biopsied muscle were measured at various concentrations of TPP. Fibroblasts (○) and biopsied muscle (□) of normal subjects, and fibroblasts (●) and biopsied muscle (■) of the thiamine-responsive patient are shown.

The PDHC activities of both fibroblasts and biopsied muscle from this patient strictly depended on the concentration of TPP (Fig. 2B). In normal controls, almost full activities of PDHC were observed in the presence of TPP at concentrations of more than  $1 \times 10^{-4}$  mM. However, the PDHC activities of specimens from the patient in the presence of  $1 \times 10^{-4}$  mM TPP were extremely low, similar to that of his lymphoblastoid cells.

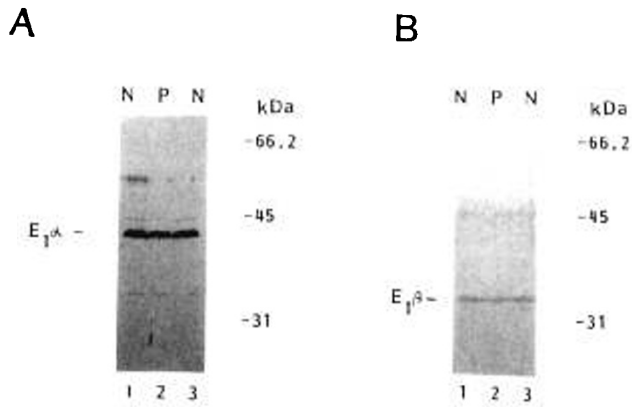
**Affinity of  $E_1$  for TPP.** To investigate the affinity of  $E_1$  for TPP, we measured the activities of DCA-activated  $E_1$  in the presence of various concentrations of TPP (Fig. 3). The activity of DCA-activated  $E_1$  of controls showed approximately 30% of the full activity in the absence of TPP, and addition of  $1 \times 10^{-4}$  mM TPP restored almost full activity. In contrast, the activity of DCA-activated  $E_1$  of the patient was extremely low in the presence of  $1 \times 10^{-4}$  mM TPP, and addition of a high TPP concentration (0.2 mM) was needed to restore 30% of the average activity of controls. Thus, the  $E_1$  of this patient also showed decreased affinity for TPP.

**Immunoblot analysis.** Immunoblot analyses with anti- $E_1\alpha$  and anti- $E_1\beta$  antibodies showed that the levels of  $E_1\alpha$  and  $E_1\beta$  in this patient were similar to those in normal controls (Fig. 4). Immunoblot analysis with anti-PDHC antibody revealed no abnormalities of other components (data not shown).

**Characterization of the mutation.** We sequenced  $E_1\alpha$  cDNA from the patient and a normal subject and found



**Figure 3.** Effect of TPP on the activity of DCA-activated  $E_1$  in disrupted lymphoblastoid cells from normal subjects and the thiamine-responsive patient. The activity of DCA-activated  $E_1$  in disrupted lymphoblastoid cells was measured at various concentrations of TPP. Shown are normal controls (○) and the thiamine-responsive patient (●).

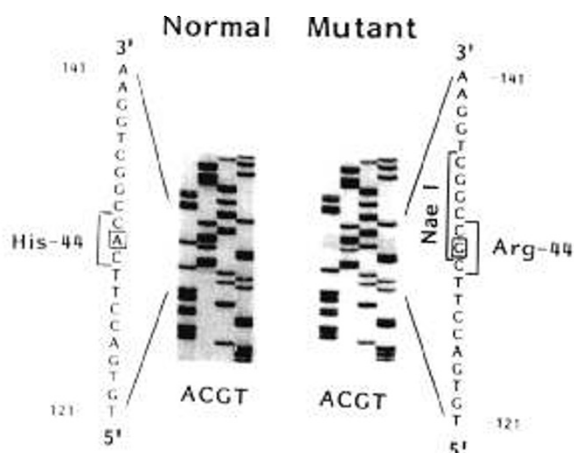


**Figure 4.** Immunoblot analysis of  $E_1$  proteins in disrupted lymphoblastoid cells from normal subjects and the patient.  $E_1$  immunoreactive protein was detected by the immunoblot technique with anti- $E_1\alpha$  antibody (A) or anti- $E_1\beta$  antibody (B). The positions of  $E_1\alpha$  protein (M, 42 kD) and  $E_1\beta$  protein (M, 36 kD) are indicated on the left. The samples were derived from normal controls (lanes 1 and 3) and the thiamine-responsive patient (lane 2).

only one mutation in the second position of codon 44 (Fig. 5). The mutation, substitution of a G nucleotide for an A, changed the amino acid histidine to arginine (CAC→CGC). This mutation was therefore designated as H44R. This nucleotide substitution created a restriction site for *NaeI* (GCCGGC). Subcloning and sequencing of four other clones indicated that the patient had only one amino acid change of H44R for  $E_1\alpha$  cDNA. The amplified segment corresponding to the  $E_1\beta$  cDNA of the patient had a normal length, and no change occurred in its amino acid sequence.

#### Identification of the A→G-131 transition in genomic DNA.

For identification of the A→G-131 transition in genomic DNA, an exon 3 segment of genomic DNA was amplified by PCR with primers PA3F and PA3R as illustrated in

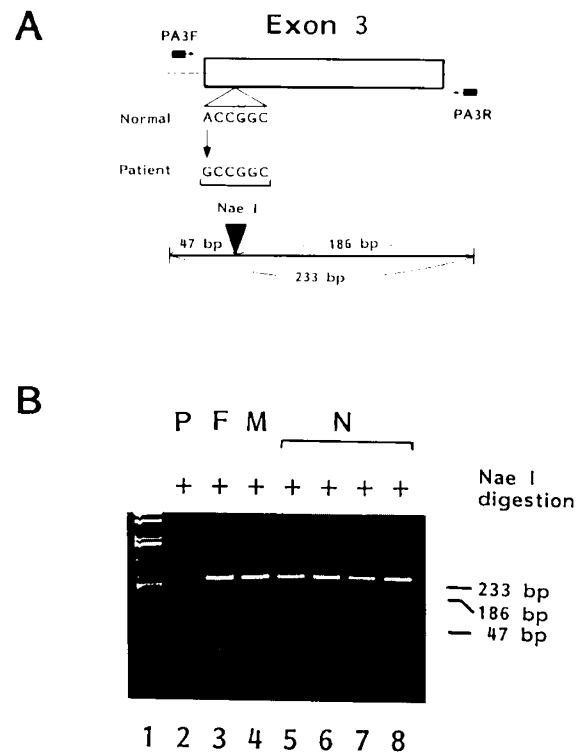


**Figure 5.** Sequence analysis of cDNA synthesized and amplified from mRNA obtained from lymphoblastoid cells of normal subjects and the patient. Results of nucleotide sequencing of the normal cDNA and the mutant cDNA are shown. The nucleotide residue involved in the mutation is boxed. A-131 in the normal cDNA is replaced by G in the mutant cDNA, resulting in His→Arg substitution at the 44th position of the  $E_1\alpha$ . This nucleotide substitution created a restriction site for *NaeI* (GCCGGC) as shown by the solid line.

Figure 6A. The size of the product of this amplification was expected to be 233 bp. When digested with *NaeI*, the 233-bp fragment amplified from the patient's DNA yielded one short band (186 bp) and unclear band (47 bp) (Fig. 6B), whereas the 233-bp fragment amplified from normal genomic DNA remained intact. The 233-bp fragments amplified from the parents of this patient were also not digested with *NaeI*. This exon 3 region has been analyzed in more than 30 individuals, including normal control subjects and other patients with PDHC deficiency, and no other example of this base substitution has been found.

## DISCUSSION

Many reports of clinical response to thiamine in patients with congenital lactic acidemia have been published, but most cases have not been characterized biochemically to be certain that true thiamine dependence exists. In this study, the clinical thiamine response in our patient was well confirmed by *in vitro* studies showing a thiamine-responsive functional PDHC defect in several different cell types. Furthermore, we have obtained the



**Figure 6.** *NaeI* digestion of the PCR-amplified products from genomic DNA from cultured cells of normal subjects and the patient. The open box depicts exon 3 of the  $E_1\alpha$ -gene. Solid boxes with arrows indicate the orientations and regions of primers used for PCR amplification (A). Electrophoretic separation of the restriction fragments from the PCR-amplified products of exon 3 of  $E_1\alpha$  genomic DNA with *NaeI* (B). Shown are PCR-amplified products digested with *NaeI* (lanes 2–8). The DNA samples were derived from genomic DNA of the patient (lane 2), the patient's father (lane 3), the patient's mother (lane 4), and normal controls (lanes 5–8). The molecular weight markers shown in lane 1 were the *PstI*-digested fragments of  $\lambda$  DNA.

first evidence at the molecular level for a mutation of thiamine-responsive PDHC deficiency.

Cultured lymphoblastoid cells derived from our patient showed reduced activities of PDHC and  $E_1$ , decreased affinity of PDHC for TPP, and defective activation of PDHC. The decreased affinity of PDHC for TPP shown in cultured lymphoblastoid cells was confirmed in cultured fibroblasts and biopsied muscle. These results suggested that various organs of this patient might show thiamine-responsive PDHC deficiency resulting in congenital lactic acidemia. In intact cultured lymphoblastoid cells of our patient, addition of a high concentration of thiamine-HCl or TPP markedly increased the rate of [ $1-^{14}C$ ]pyruvate decarboxylation. These results have been related to the observed decreases in blood lactate and CSF lactate after institution of thiamine therapy in this patient, indicating that this patient is indeed thiamine responsive and that therapeutic treatment with a high dose of thiamine is effective for treatment of congenital lactic acidosis caused by thiamine-responsive PDHC deficiency. No effective therapy for congenital lactic acidemia has yet been established. Therefore, diagnosis of thiamine-responsive PDHC deficiency is very important, because it may be possible to control lactic acidemia in this type of PDHC deficiency by a large dose of thiamine. PDHC deficiency has been diagnosed by measuring the activity of PDHC at high concentrations of TPP (0.1–0.7 mM) by several groups (7, 9, 19, 20). However, to diagnose PDHC deficiency caused by the decreased affinity of PDHC for TPP, it is necessary to measure activities of PDHC not only at high concentration but also at low concentration of TPP, especially at lower than  $1 \times 10^{-3}$  mM concentration.

Most of the PDHC deficiencies are due to a mutation of  $E_1\alpha$  gene located in the p22.1 to p22.2 region of the X chromosome (13, 21–25). Most of them have been located near the C-terminus of  $E_1\alpha$ , and little is known about how the mutations affect the structure or function of the PDHC. In our patient, the mutation is located near the N-terminus of  $E_1\alpha$ , and the reading frame remains intact. This mutation was identified at nucleotide position 131 of the  $E_1\alpha$  subunit, resulting in the substitution of Arg-44 for His-44. The following lines of evidence indicate that the H44R mutation found in this case accounts for the thiamine-responsive PDHC deficiency. First, results on enzymatic activity suggested that the mutation might be located on  $E_1$  ( $E_1\alpha$  or  $E_1\beta$ ). Second, point mutation was most likely to be located in the coding region of  $E_1\alpha$  or  $E_1\beta$  because the defect was associated with normal levels and sizes of  $E_1\alpha$  and  $E_1\beta$  proteins. Third, no other mutation was found in the coding region of  $E_1\alpha$  or  $E_1\beta$ . This mutational change was unlikely to be caused by protein polymorphism because no such polymorphism has been found in any multiple human  $E_1\alpha$  cDNA sequenced by us or others (15, 21, 26–28) and because the mutated histidine (amino acid 44) found in this patient is conserved in mammalian  $E_1\alpha$  of the porcine muscle, pig heart, and rat liver (29–31). Fourth, the H44R mutation was not found

in the  $E_1\alpha$  subunit of this patient's parents, who had normal PDHC activity, indicating that this mutational change is not a polymorphism but a *de novo* mutation. These points of evidence support the idea that the H44R mutation results in a decreased affinity of PDHC for TPP and a defective activation of PDHC. Further investigations are required to explain the structure-function implications of the H44R mutant protein.

To our knowledge, until now only three patients with PDHC deficiency clinically improved after supplementation with thiamine (21, 32, 33). The immunochemical analysis in these three cases has demonstrated different patterns; in patient 1 (21), the band of  $E_1\alpha$  subunit was missing, and, instead, an additional band was detected below protein X. However, in patient 2 [patient cited by Old and DeVivo (32)], the amount of  $E_1\alpha$  subunit was comparable with that in control, but its electrophoretic mobility was altered. In patient 3 (33),  $E_1\alpha$  and  $E_1\beta$  were undetectable, and E2 and E3 were decreased. These immunochemical analyses indicated that defects of PDHC in these three patients are due to the different abnormality. Our patient, although responsive to thiamine clinically, may be genetically different from three patients because immunoblot patterns in our patient were essentially the same as in controls, which differs from the previously described cases, and therefore our patient is an original case of thiamine-responsive PDHC deficiency.

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