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# A Mutation in the $E_1\alpha$ Subunit of Pyruvate Dehydrogenase Associated with Variable Expression of Pyruvate Dehydrogenase Complex Deficiency

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**ABSTRACT. Defects in pyruvate dehydrogenase, the first catalytic component of the pyruvate dehydrogenase complex, are the most common cause of pyruvate dehydrogenase complex deficiency. A family with variable pyruvate dehydrogenase complex deficiency had been described in which cultured skin fibroblasts of affected family members had normal pyruvate dehydrogenase complex activity, but different tissues and blood lymphocytes had significantly diminished activities. Enzymatic activity and immunoblot studies indicated that pyruvate dehydrogenase was affected. Further evidence is presented here showing that the defect affecting pyruvate dehydrogenase complex activity is posttranscriptional. Sequencing of the coding region of the $\alpha$-subunit of pyruvate dehydrogenase revealed a point mutation in the codon for amino acid 234 resulting in a substitution of glycine for arginine. Study of other members of the family suggested that this mutation is inherited in a sex-linked mode. The point mutation is located in a highly conserved region of the pyruvate dehydrogenase $\alpha$-subunit gene that contains both hydrophobic and positively charged amino acid residues. Variable expression of pyruvate dehydrogenase complex deficiency in this case may be due to instability of the pyruvate dehydrogenase heterotetramer in specific tissues because of a disruption in subunit-subunit interaction. (*Pediatr Res* 32: 169–174, 1992)**

**Abbreviations**

**$E_1$, pyruvate dehydrogenase**
**$E_1\alpha$, $\alpha$-subunit of $E_1$**
**$E_1\beta$, $\beta$-subunit of $E_1$**
**$E_3$, dihydrolipoamide dehydrogenase**
**PDC, pyruvate dehydrogenase complex**
**PCR, polymerase chain reaction**

The PDC is a nuclear-encoded mitochondrial enzyme complex that catalyzes the conversion of pyruvate to acetyl-CoA. PDC consists of three catalytic components: $E_1$ (pyruvate:lipoamide 2-oxidoreductase, EC 1.2.4.1.), dihydrolipoamide acetyltransferase, and $E_3$ as well as a protein X that forms part of the complex. The $E_1$ component consists of two subunits encoded by different genes, $\alpha$ and $\beta$, which combine as a heterotetramer. Regulation of the complex is via phosphorylation by $E_1$-kinase and dephosphorylation by phospho $E_1$-phosphatase of three specific serine residues on $E_1\alpha$ (1, 2).

PDC deficiency, an inborn error of pyruvate metabolism, is manifested by elevated serum pyruvate and lactate. Individuals with PDC deficiency have varying levels of neurologic dysfunction ranging from mild ataxia to neuroanatomical lesions incompatible with life (3, 4). Most PDC mutations affect the $E_1$ component of PDC (5, 6). Recently, identification of mutations affecting $E_1\alpha$ has been facilitated by the cloning and characterization of cDNA (7–10) and genomic DNA for $E_1\alpha$ (11, 12). By *in situ* hybridization, the $E_1\alpha$ gene has been localized to the X chromosome (13). Mutations involving deletions or single base changes have been found on the $\alpha$-subunit (14–17).

Previously, we described a family with PDC deficiency in which variable PDC enzymatic activity was observed in different tissues (18). The proband was found to have minimal PDC activity in his lymphocytes, heart, liver, muscle, and brain, whereas cultured skin fibroblasts had normal activity. These findings were confirmed in lymphocytes and cultured skin fibroblasts from a similarly affected male sibling of the proband. Additional family studies suggested a sex-linked pattern of inheritance for variable expression of PDC deficiency. Enzymatic assay of the catalytic components of PDC indicated that the defect only affected the $E_1$ component. Immunoreactivity studies using antibodies to specific catalytic components demonstrated that tissues deficient in PDC activity lacked immunoreactive material to both $E_1$ subunits, whereas cultured skin fibroblasts that had normal activity also had normal levels of immunoreactive material for $E_1\alpha$ and $E_1\beta$. In this report, we present evidence that a point mutation located in the coding region of $E_1\alpha$ causes PDC deficiency in this family.

## MATERIALS AND METHODS

*Human subjects.* The case history and biochemical analyses of two brothers with variable PDC deficiency in cells and tissues has been previously described (18). For the proband, autopsy specimens of heart, liver, kidney, brain, and skin fibroblasts were obtained. Skin biopsies were performed on the affected brother as well as on the mother and father. All studies were carried out

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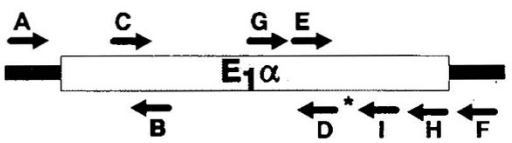
with informed consent according to protocols approved by the Institutional Review Board of University Hospitals of Cleveland.

*Isolation of RNA and genomic DNA.* RNA was isolated from cells and frozen tissue by guanidine isothiocyanate and separated by alcohol precipitation or cesium chloride density centrifugation (19). The amount of RNA in each sample was determined by UV absorbance at 260 nm. Genomic DNA was prepared from cultured skin fibroblasts by harvesting cells in PBS followed by boiling the cells for 2 min in a solution of 0.1 M NaOH, 2 M NaCl (20) and centrifuging the cells at 16 000 × *g* for 10 min. Aliquots of the supernatant containing genomic DNA were used directly for PCR experiments described below.

*RNA blot analysis.* Equal amounts of total RNA from different samples were separated by electrophoresis in an agarose gel containing 1% formaldehyde and transferred to a membrane (Genescreen; Dupont-New England Nuclear, Wilmington, DE). RNA on the membrane was hybridized (21) with $^{32}$P-labeled probes prepared from denatured double-stranded cDNA fragments of $E_1\alpha$, $E_1\beta$, and $E_3$ (6) using the random priming method (22, 23). After autoradiography and removal of previously used radioactive probes (following the protocol recommended in the manufacturer's instructions), the same membrane was rehybridized with successive cDNA probes.

*Reverse transcription and DNA amplification.* Oligodeoxynucleotide primers were synthesized on a 380A DNA synthesizer (Applied Biosystems, Foster, CA). The sequence and location of the primers used for reverse transcription and DNA amplification are shown in Figure 1. Reverse transcription of 1 to 4 μg of total RNA was performed as previously described (10, 24). The products of this reaction were amplified by PCR using Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) and a DNA thermal cycler (Perkin Elmer Cetus). cDNA fragments were purified by agarose gel electroelution. Conditions for PCR were denaturing at 94°C for 60 s, annealing at 52°C for 45 s, and extension at 72°C for 90 s for a total of 35–40 cycles. For the fragment using primers E and F (Fig. 1), an annealing temperature of 45°C was used. Genomic DNA fragments were amplified using the same PCR protocols.

**A**




**B**

| | | |
|---|---|---|
| A: | TCTGCTGGGGCACCTGAAGGA | 5′ UPSTREAM |
| B: | GTAGGCTGTGATGAGATGGT | 397 - 378 |
| C: | CTGTACGCCGAATGGAGTTGA | 252 - 272 |
| D: | CAGACGTTCCCATTCCATAGCGAT | 740 - 717 |
| E: | GGCAGCTTTGTGGAAATTACC | 673 - 693 |
| F: | CTACCTGCTCAATCTACACAC | 1356 - 1336 |
| G: | GCCAGATATTCGAAGCTTACAACA | 648 - 671 |
| H: | GTCACTCATAGCGTGTCCGTGGTA | 931 - 908 |
| I: | TTCCCAGATCTACAATAGGCAGCA | 873 - 850 |
| * W: | GTTGCCTCTCGGACGCACAGG | 840 - 820 |
| * M: | GTTGCCTCTCCGACGCACAGG | 840 - 820 |

Fig. 1. Deoxynucleotide primers and sequencing strategy. *Panel A* depicts the location of deoxyoligonucleotide primers used for reverse transcription and PCR amplification of $E_1\alpha$ mRNA. *Panel B* lists the deoxyoligonucleotide primers and their sequence (numbering based on Ref. 10). The *capitalized letters* in panel A mark the location of primers listed in panel B. Those primers shown above the diagram are sense primers and those shown below are antisense. The *asterisk* denotes the location of the wild type (*W*) and mutant (*M*) probes used for dot-blot studies. Boxed-in nucleotides differ from the wild type sequence. Primer sets used to generate patient-specific cDNA included A-B, C-D, E-F, C-F, and C-H.

*DNA sequencing.* Amplified cDNA fragments were subcloned into a plasmid vector (pBluescript; Stratagene, La Jolla, CA) at convenient restriction sites. DNA was sequenced by the dideoxy method (25) using T7 DNA polymerase (Sequenase; US Biochemical, Cleveland, OH). Nucleotide sequences were analyzed with DNAsis and deduced amino acid sequences with PROsis software (Hitachi America, Brisbane, CA). Comparison of $E_1\alpha$ sequences of both the PDC and the branched-chain keto acid dehydrogenase complex from various species was done by searching the National Biomedical Research Foundation data banks using the FastA program by the Genetics Computer Group Analysis Software Package for VAX computers, University of Wisconsin, Madison, WI. The sequence for PDC $E_1\alpha$ for nematodes was kindly provided by Dr. Keith Johnson, University of Toledo, Toledo, OH.

*Dot-blot analysis.* Genomic DNA fragments generated by the PCR were dot-blotted onto a nylon membrane according to the manufacturer's directions (Bio-Rad, Richmond, CA). The membranes were then hybridized as described (20) with the hybridization mixture containing either the T4 polynucleotide kinase radiolabeled wild type or mutant oligodeoxynucleotide probe (Fig. 1) for several hours at 60°C. After hybridization, the membranes were washed at temperatures ranging from 65 to 75°C and autoradiographed.

## RESULTS

In previous studies, biochemical and immunologic experiments demonstrated that the proband had variable PDC activity in cells and tissues and that the level of activity corresponded to the amount of $E_1$ subunit proteins detected by immunoblot analysis (18). Whereas fibroblasts had normal activity for PDC and its catalytic components, lymphocytes, heart, and liver had minimal PDC and $E_1$ activity. Kidney had partial activity for PDC and $E_1$. The catalytic activity and immunoreactivity of dihydrolipoamide acetyltransferase and $E_3$ components of PDC were normal, and exogenous phospho $E_1$-phosphatase failed to activate PDC activity in tissues with low PDC activity. The latter finding indicated that the lack of $E_1$ activity was not due to a defect in phospho $E_1$-phosphatase (18). Similar to a subgroup of patients with $E_1$ deficiency (4, 6), the proband was missing cross-reactive material for both the $\alpha$- and the $\beta$-subunit. To determine whether the defect interfered with transcription or posttranscriptional processing, RNA blot analysis was performed on total RNA isolated from different tissue sources using radiolabeled $E_1\alpha$ and $E_1\beta$ cDNA probes (Fig. 2). The results show that mRNA for $E_1\alpha$ and $E_1\beta$ were present in liver and heart and were of normal size even though these tissues had greatly diminished levels of enzymatic activity. Although the amounts of both $E_1\alpha$ and $E_1\beta$ mRNA appear to be greater relative to the control, this is nonspecific because the mRNA for $E_3$ is also increased relative to the control. There is some variation in the relative intensity of signals for the $E_1\alpha$, $E_1\beta$, and $E_3$ mRNA between the patient and control in various tissues, but these are qualitative data obtained from postmortem samples analyzed by sequential hybridization. The relative amounts of $E_1\alpha$, $E_1\beta$, and $E_3$ mRNA were similar in fibroblasts (data not shown). These RNA blot findings, taken as a whole, suggest that the effect of the mutation causing PDC deficiency in this family is posttranscriptional.

Family studies suggested that the defect might be sex-linked (18) because the proband's brother was affected, the sister and father were normal, and the mother had heterozygote levels of PDC activity. For this reason, the coding region of $E_1\alpha$ was sequenced because the gene for this subunit had been localized to the X chromosome (13). To identify the specific mutation,

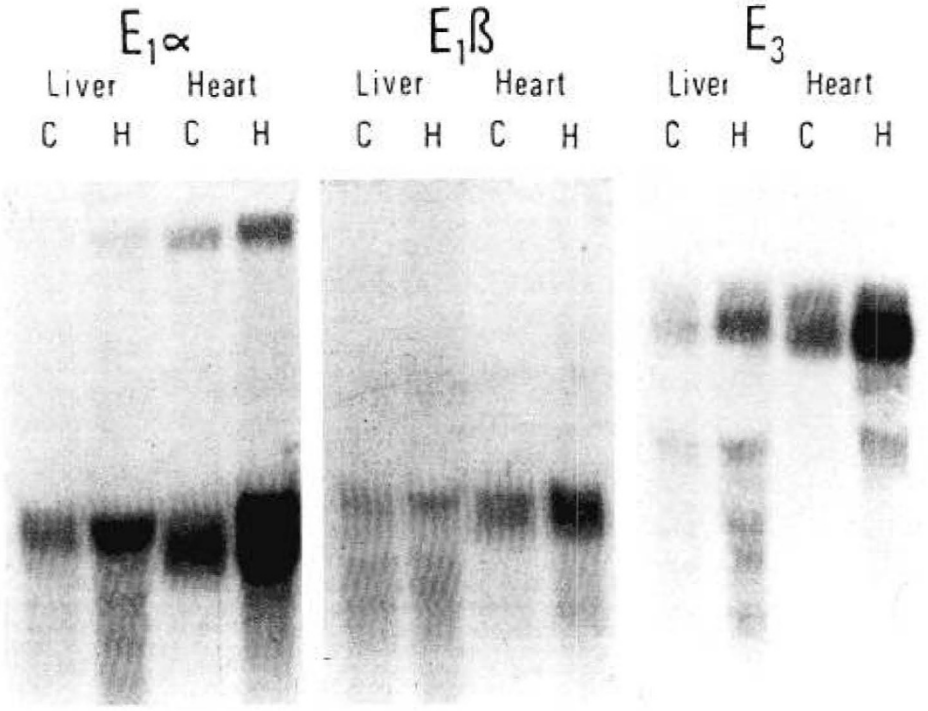



Fig. 2. RNA blot analysis. Equal amounts of total RNA isolated from liver or heart from the proband (*H*) and a unrelated human control (*C*) were hybridized with radiolabeled $E_1\alpha$ (*left panel*), $E_1\beta$ (*middle panel*), and $E_3$ (*right panel*) cDNA fragments. The same membrane was used for all of the hybridizations. The sizes of $E_1\alpha$, $E_1\beta$, and $E_3$ are 1.6, 1.5, and 2.2 kb, respectively. Hybridization with $E_3$ cDNA was used as an internal control for determining the amount of RNA loaded onto the gel.

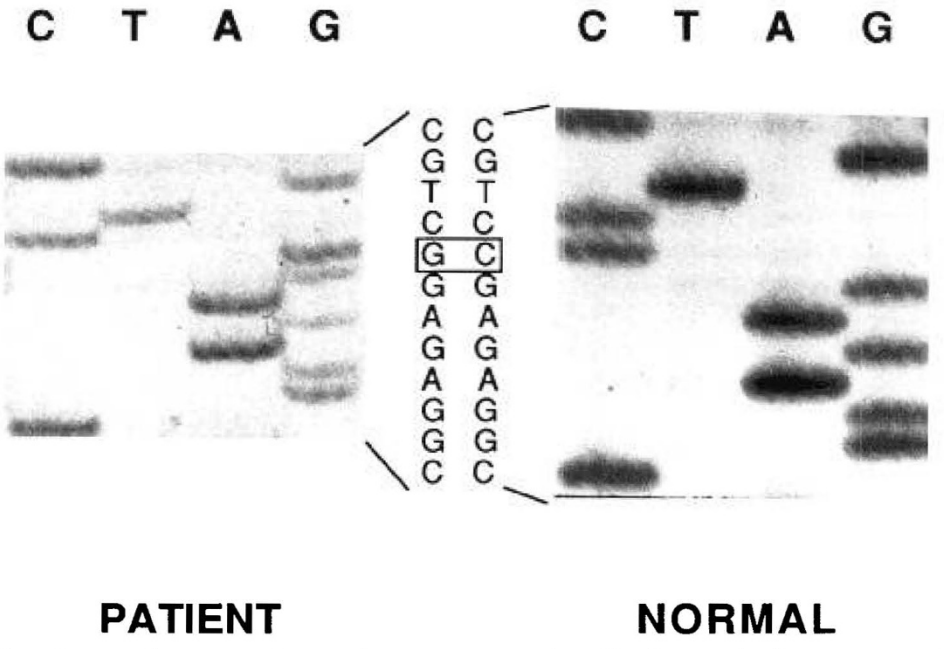



Fig. 3. DNA sequencing gel. The patient had a point mutation at nucleotide 829 resulting in a guanine for cytosine substitution. The nucleotide location of the mutation is enclosed in a box. In the normal sequence, there is a doublet in the cytosine lane, whereas in the mutant this doublet is replaced at a corresponding position by a doublet in the guanine lane. *C*, cytosine; *T*, thymidine; *A*, adenine; and *G*, guanine.

patient-specific $E_1\alpha$ cDNA fragments from the proband's heart tissue were generated using reverse transcription and PCR amplification. Repeated sequencing of PCR-generated fragments spanning the coding region and flanking 5′ and 3′ sequences (Fig. 1) indicated that the proband's $E_1\alpha$ was identical to the wild type human $E_1\alpha$ except for a single guanine for cytosine substitution (Fig. 3) at the first nucleotide encoding amino acid 234 of the mature peptide (Fig. 4). This change causes arginine to be replaced by glycine in exon 8 (11), which contains a stretch of 24 amino acids separating phosphorylation sites 3 and 1 (Fig. 4).

To ensure that this mutation was not an artifact of either the reverse transcription or the PCR reactions, multiple samples of heart RNA were amplified, subcloned, and sequenced. All of these samples were found to have the mutation, whereas concurrent controls prepared in the same manner had the wild type sequence. To exclude the possibility that activity in the fibroblasts was due to the presence of a normal allele, $E_1\alpha$ cDNA generated

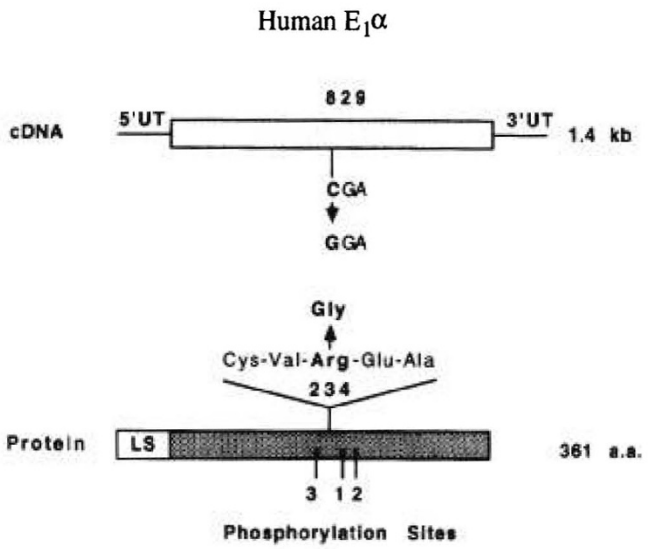



Fig. 4. Location of the mutation in $E_1\alpha$. The diagram shows the location of the mutation (numbering based on Ref. 10) in the nucleotide sequence and the resulting change in the deduced amino acid sequence. The nucleotide and amino acid affected by the mutation are in bold. The position of the mutant amino acid residue relative to the phosphorylation sites is also shown. *UT*, untranslated region; *LS*, leader sequence.

from cultured skin fibroblasts shown previously to have normal activity was also sequenced repeatedly and found to contain only the mutant sequence (Fig. 5B).

Sequencing of $E_1\alpha$ cDNA obtained from cultured skin fibroblasts of other family members (Fig. 5*C-F*) showed the presence of the mutation in the affected brother and the mother (Fig. 5*C* and *D*). However, $E_1\alpha$ cDNA from the mother's skin fibroblasts also had the normal sequence (Fig. 5*E*), as would be expected for a heterozygous female who carried both the normal and the mutant allele. Because the maternal grandmother had normal enzymatic activity in lymphocytes (18), it is probable that the mutation arose in her germ cell line. The father's $E_1\alpha$ cDNA (Fig. 5*F*) had only the normal sequence.

A processed $E_1\alpha$ gene (intronless) that has been localized to chromosome 4 has a nucleotide sequence that is highly homologous to the $E_1\alpha$ cDNA derived from the $E_1\alpha$ gene located on the X chromosome (26). To avoid the possibility of amplifying cDNA from the processed gene, a fragment of genomic DNA from the proband that spanned intron 7 and exon 8 was generated using primers I and G (Fig. 1) (11). The size of the amplified DNA (400 bp) generated was consistent with the fragment being from the $E_1\alpha$ genomic DNA located on the X chromosome. Dot-blot analysis of this genomic DNA fragment using an oligonucleotide containing the mutant sequence (M primer) (Fig. 1) hybridized only with DNA from the two brothers and mother (data not shown). Comparison of the human $E_1\alpha$ processed gene sequence (26) with the patient-specific cDNA generated from the proband indicated that the latter was from the $E_1\alpha$ gene on chromosome X because the codon encoding the arginine residue is CGT for the processed gene (26) in contrast to CGA for the same amino acid on the $E_1\alpha$ gene located on the X chromosome (Figs. 4 and 5).

## DISCUSSION

In this report, we have identified a mutation in the coding region of $E_1\alpha$ that is associated with variable expression of PDC deficiency. All previously identified mutations affecting $E_1$ have been located on the $\alpha$-subunit (14–17). Most of these mutations have been deletions located near the C-terminus of $E_1\alpha$. In the present case, the mutation is located closer to the center of $E_1\alpha$ and the reading frame remains intact. There are several lines of evidence indicating that the mutation found in this case accounts for PDC deficiency. First, previous results suggested that the mutation might be located on $E_1\alpha$ because levels of enzymatic activity among family members were consistent with a sex-linked pattern of inheritance (18). Second, the mutation is most likely located in the coding region of $E_1\alpha$ because the defect was associated with normal levels and normal-sized $E_1\alpha$ mRNA. Third, no other mutation was found in the coding region. Fourth, a glycine for arginine substitution is a significant change in both size and charge of an amino acid residue, thereby affecting the secondary structure of $E_1$ (27, 28). This is especially significant in this case, in which the mutation is located in a critical region (see below). It is unlikely that this mutational change is a protein polymorphism because such polymorphisms have not been found in any of the multiple human $E_1\alpha$ cDNA sequenced by us or other groups (7–10, 14, 15) and this particular residue is highly conserved (see below).

It is surprising that a single point mutation would result in the absence of both the $\alpha$- and the $\beta$-subunit in specific tissues, yet

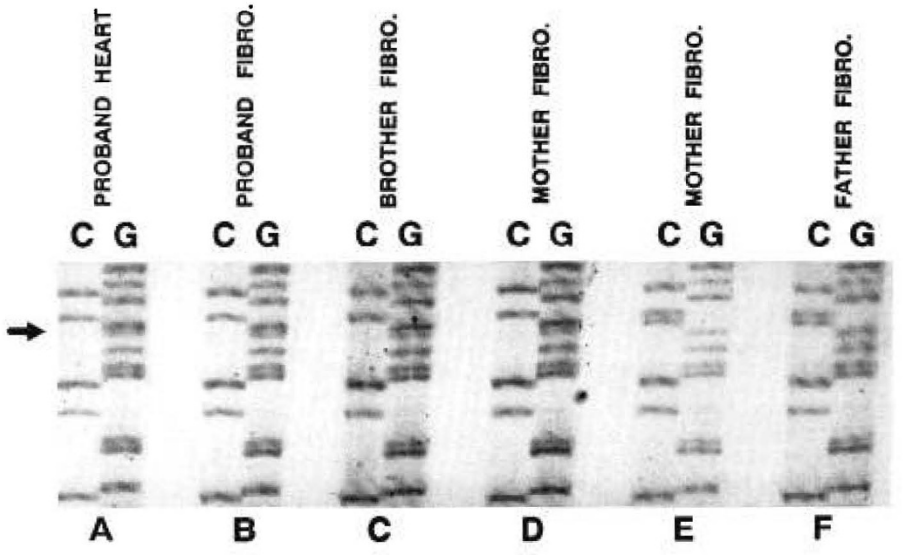



Fig. 5. DNA sequencing gel. Reverse-transcript ion, PCR-amplified cDNA from different family members were sequenced. Only the cytosine and guanine dideoxy nucleotide reactions were run on the gel. The *arrow* shows the location of the mutation. *C*, cytosine; *G*, guanine; and *FIBRO*, fibroblasts.

not affect catalytic activity when both subunits were present. This would suggest that the mutation is in a critical location, which is essential for the stability of the $E_1$ heterotetramer. This mutation occurs in exon 8 of $E_1\alpha$, which is situated between phosphorylation site 3 and phosphorylation site 1 (11). The identified mutation might alter the secondary structure in this region, thereby affecting the phosphorylation-dephosphorylation of $E_1\alpha$, but this would not account for the lack of immunoreactivity of the two subunits. In addition, the stretch of amino acids encoded by this exon and the contiguous phosphorylation site 1 is highly conserved both in length and in amino acid homology when a comparison is made between the $\alpha$-subunits from multiple species, both eukaryotic and prokaryotic, of the $\alpha$-keto acid dehydrogenase complexes (Fig. 6*A*) (10, 26, 29–36). The structure of this region is also interesting. There are multiple nonpolar residues as well as conserved positively and negatively charged amino acids. Based on Chou-Fasman analysis (37), there is a strong tendency for regions within this stretch of amino acids to form $\alpha$-helical structures. Positively charged amino acids in yeast, nematode, and mammalian $E_1\alpha$ tend to be spaced seven or 14 amino acid residues apart (Fig. 6*A*) so that these residues would be positioned on the same side of an $\alpha$-helix. Graphic representation of potential $\alpha$-helical structures shows that an amphipathic helix (38) is formed, with one side of the helix containing predominantly hydrophobic residues and the other side having positively charged residues (Fig. 6*B*).

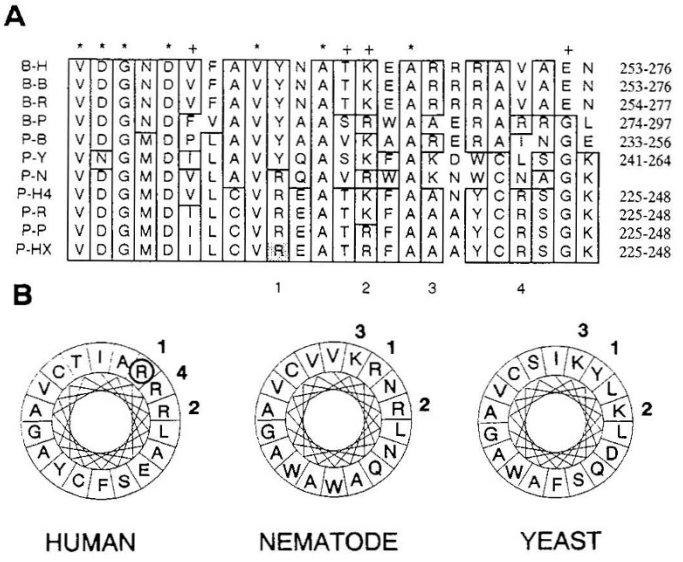



Fig. 6. Regional homology of $E_1\alpha$ in the area of the mutation. *Panel A* shows the alignment of nine $E_1\alpha$ sequences from PDC and branched-chain keto acid dehydrogenase complex (BCKDC) corresponding to exon 8 of human $E_1\alpha$. An *asterisk* over a column denotes identity for at least 10 of 11 amino acids, and a *plus sign* signifies homology between at least nine of the amino acid residues in a column. Amino acids are boxed in if four or more residues are identical in a column. The *shaded arginine residue* is the site of the mutation in the patient. *B-H*, BCKDC human $E_1\alpha$ (29); *B-B*, BCKDC bovine $E_1\alpha$ (30); *B-R*, BCKDC rat $E_1\alpha$ (31); *B-P*, BCKDC *P. putida* $E_1\alpha$ (32); *P-B*, PDC *Bacillus stearothermophilus* $E_1\alpha$ (33); *P-Y*, PDC yeast $E_1\alpha$ (34); *P-N*, PDC nematode *Ascaris suum* $E_1\alpha$; *P-H4*, PDC human processed gene $E_1\alpha$ located on chromosome 4 (27); *P-R*, PDC rat $E_1\alpha$ (36); *P-P*, PDC porcine $E_1\alpha$ (35); and *P-Hx*, PDC human $E_1\alpha$ located on chromosome X (10). The numbering on the right side provides the location of the amino acid residues. The numbers below the human sequence mark the location of basic amino acids as they appear in an $\alpha$-helix shown in *panel B*. The sequence for nematode *Ascaris suum* is unpublished data provided by Dr. K. Johnson, University of Toledo, Toledo, OH. Panel B is a graphic representation of a potential $\alpha$-helical structure for human, nematode, and yeast PDC $E_1\alpha$. Amino acid residues from 230 to 247 of the human $E_1\alpha$ (X chromosome) and the corresponding amino acid residues of the nematode and yeast are depicted as they would appear in helical form. Amino acid 230 is at the top of the helix. *Lines within the circles* connect contiguous amino acid residues. The location of the mutation found in the patient is *circled.*

The mutated arginine (amino acid 234) found in this patient is conserved in mammalian $E_1\alpha$ including the human $E_1\alpha$ processed gene and nematode, but not in yeast or other $E_1\alpha$ genes from either the PDC or the branched-chain $\alpha$-keto acid dehydrogenase complex. However, despite the apparent lack of conservation in the primary structure among the different $E_1\alpha$ subunits at this site, the structure of a potential $\alpha$-helix is conserved. For example, all $\alpha$-keto acid dehydrogenase complexes (except *Pseudomonas putida* branched-chain keto acid dehydrogenase complex $E_1\alpha$) have a basic amino acid residue seven amino acids downstream (at position 241 of the human sequence) that is not found in mammalian $E_1\alpha$. Thus, the positively charged residue at amino acid 238, which is conserved in all species, is flanked by another positive residue either above or below it in the helical structure so that there are at least two positively charged residues in close proximity on the same side of the helix (Fig. 6*B*). It is interesting that the nematode has positive residues at amino acid residues 234 and 241 (Fig. 6*A*), suggesting that it was at this stage of evolutionary development that the hydrophilic residues shifted in position (Fig. 6*B*). In this patient, the substitution of a glycine for arginine would mean the loss of a conserved structural feature found in almost all $\alpha$-keto acid $E_1\alpha$ subunits. Furthermore, glycine could increase the instability of a potential $\alpha$-helical structure because of enhanced conformational entropy due to the flexibility of this residue (27, 28).

Based on sequence comparison of the $E_1$ subunits of $\alpha$-keto acid dehydrogenases from different species, we have speculated that this region is involved in subunit-subunit interaction because this highly conserved region is found only in $\alpha$-keto acid dehydrogenase complexes in which the $E_1$ catalytic component has $\alpha$- and $\beta$-subunits (39). The interaction between the $\alpha$- and $\beta$-subunits is known to be ionic (40), and it has been shown that the presence of both the $\alpha$- and the $\beta$-subunit is required for protein stability so that when one subunit is missing, the other subunit is also absent (6, 41, 42). Thus, the presence of a mutation in this region may disrupt the ionic interaction between the $\alpha$- and $\beta$-subunits, thereby resulting in the absence of both subunits because a stable heterotetramer cannot form. In this patient, the effect of the mutation may depend on the intracellular environment, thereby causing variable expression of PDC deficiency. Depending on intracellular conditions, the substitution of a glycine for arginine may not be sufficient to destabilize the $E_1$ heterotetramer. The possibility that intracellular conditions might affect the stability of the $E_1$ heterotetramer is suggested by the fact that the $\alpha$- and $\beta$-subunit interaction of yeast pyruvate decarboxylase, an enzyme closely related to PDC $E_1$, has been shown to be pH dependent (43).

There are other possibilities for variable expression of PDC deficiency in this patient. A glycine in place of arginine might make $E_1\alpha$ more susceptible to sequence-specific proteases, which may be present in certain tissues (27). Tissue variable expression has also been described in cytochrome oxidase deficiency (44, 45), and the explanation in those cases was isozymic variation. In the case of $E_1\alpha$, there is no evidence of isozymes except for the processed $E_1\alpha$ gene on chromosome 4 (26). It may be possible that in our patient this gene was both transcribed and translated in cultured skin fibroblasts and that this gene product replaced the mutant $E_1\alpha$, thereby restoring activity. However, Dahl *et al.* (26) have shown that this gene is only transcribed in postmeiotic spermatogenic cells. Another possibility is that there was allelic variation in different tissues of the patient due to mosaicism of the X chromosome such that tissues with normal activity had a normal $E_1\alpha$ gene. Similar mosaicism has been described with other X-linked disorders such as ornithine transcarbamylase deficiency (46). However, this appears unlikely in this case because only the mutant allele was identified in cultured skin fibroblasts with normal activity.

Further investigation will be required to determine the intracellular factors responsible for tissue variable PDC deficiency. Expression of the mutant phenotype would ordinarily be useful in determining the significance of this mutation in causing variable PDC deficiency. However, introduction of this mutation into $E_1$-deficient cultured fibroblasts by transfection would in all likelihood restore normal activity because this mutation is not expressed in the patient's cultured fibroblasts. Therefore, it will be necessary to establish an $E_1$-deficient animal model and introduce a transgene carrying the mutant $E_1\alpha$ into the animal's genome. It would then be possible to determine the specific factors responsible for variable PDC deficiency.

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