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Pyruvate Dehydrogenase Deficiency Due to a 20-bp Deletion in Exon 11 of the Pyruvate Dehydrogenase (PDH) $E_1\alpha$ Gene

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Summary

A 20-bp deletion in the last exon of the pyruvate dehydrogenase (PDH) $E_1\alpha$ gene was found in a severely affected female patient diagnosed with PDH deficiency. PDH-complex activity in the patient's fibroblasts was 22% of that in normal controls. The mutation was characterized using PCR techniques with both patient cDNA and genomic DNA, followed by sequencing of the products. $E_1\beta$ cDNA sequence was found to be the same as that in controls. The deletion causes a frameshift and the occurrence of a premature stop codon. Western blot analysis revealed an extra band migrating just above the PDH $E_1\beta$ band. Northern blot analysis showed normal levels of both $E_1\alpha$ and $E_1\beta$ message when probed with the respective cDNAs. However, a larger intermediate-size transcript was observed for this patient in the $E_1\beta$ blot. The 20-bp deletion was not found in either parent's genomic DNA, and hence we conclude that the mutation must have occurred de novo, either in the germ-line cells or immediately following fertilization.

Introduction

The pyruvate dehydrogenase (PDH) complex plays a central role in energy metabolism. This multienzyme complex is composed of three catalytic enzymes: PDH (E_1), dihydrolipoamide acetyltransferase (E_2), and dihydrolipoamide dehydrogenase (E_3) (Randle et al. 1978). E_1, an $\alpha_2\beta_2$ tetramer, contains the main sites for regulation of the PDH complex, specifically on the $E_1\alpha$ subunit. Regulation is by a phosphorylation-dephosphorylation mechanism (Linn et al. 1969). The $E_1\alpha$ subunit also contains the thiamine pyrophosphate binding site which plays an important role in the decarboxylation of pyruvate (Hawkins et al. 1989).

Genetic defects in the PDH complex are one of the most common causes of congenital childhood lactic acidemia (Robinson 1980). Clinical presentation of patients with PDH-complex deficiency is extremely heterogeneous. Mildly affected patients suffer only from ataxic episodes, while severely affected patients develop fatal lactic acidosis (Robinson et al. 1980, 1987). In addition to the metabolic disturbance, structural central nervous system damage seems to be a characteristic feature of PDH deficiency.

Human PDH-complex deficiency is usually diagnosed by measurement of PDH-complex activity in cultured fibroblasts or tissue, along with immunochemical analysis of the protein subunits. In the majority of cases, the defect seems to lie in the E_1 enzyme, an $\alpha_2\beta_2$ tetramer of subunit M_r 41,000 and 36,000, respectively, for α and β components. The cDNA sequences for both $E_1\alpha$ and $E_1\beta$ have now been elucidated in our own and other laboratories (Dahl et al. 1987; DeMeirleir et al. 1988; Koike et al. 1988; Ho et al. 1989; Chun et al. 1990). The $E_1\alpha$ gene has been localized to the p22.1-p22.2 region of the X chromosome (Brown et al. 1989), and its gene structure has been determined (Maragos et al. 1989). There have been two different frameshift mutations in the PDH $E_1\alpha$ gene described in patients with PDH deficiency (Endo et al. 1989; Dahl et al. 1990). Here, we report on a female patient with PDH deficiency in whom some normal PDH $E_1\alpha$ protein is present, while, because of the presence of a frameshift mutation, some $E_1\alpha$ protein is produced as a truncated smaller version.

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Subject and Methods

Case Report

A female patient, AF, birth weight 1.88 kg, was born at 35 wk gestation and required intubation and ventilation in the immediate postnatal period. The baby continued to be lethargic and hypotonic and showed little psychomotor development in the following months. At age 7 mo she developed some seizure activity and was found to have an abnormal EEG; blood lactate and blood pyruvate were elevated, and she had mild hepatosplenomegaly. When seen at age 12 mo for an episode of pneumonia, she was observed to be profoundly retarded, with some facial dysmorphism, and her weight had increased to 9.3 kg. Blood lactate levels at that time varied between 5.0 and 15.8 mM. Simultaneous measurement of blood lactate (5.46 mM) and blood pyruvate (0.21 mM) gave a lactate-to-pyruvate ratio of 25:1. A computed tomography scan of the head showed dilated ventricles and cortical thinning. At this point, a diagnosis of PDH deficiency was made on the basis of the low activity in cultured skin fibroblasts. In the ensuing 2 years she showed little further development, and she died at the age of 3 years.

Cultured skin fibroblasts were grown from the patient from a forearm skin biopsy in α-MEM culture medium. The activity of the PDH complex in the native and dichloroacetate (DCA)-activated state was determined in fibroblast extracts by the method of Sheu et al. (1981). The activities of the E_1 pyruvate decarboxylase, the E_2 transacetylase, and the E_3 lipoamide dehydrogenase were determined as described previously (Robinson et al. 1980; MacKay et al. 1986). Cytochrome oxidase was measured by the method of Glerum et al. (1987).

mRNA, cDNA, and DNA Preparation

Total RNA was purified from cultured skin fibroblasts of patient AF by the method of Maniatis et al. (1982). First-strand cDNA was synthesized using approximately 20 μg of total cellular RNA and an $E_1\alpha$ gene–specific oligonucleotide, αG17 (5′-GTACAAACTGCATGCAATTACTACC-3′). M-MLV reverse transcriptase (Moloney murine leukemia virus) was purchased from BRL, and RNA Guard® ribonuclease inhibitor and the deoxynucleotides were purchased from Pharmacia. The first strand of the $E_1\beta$ cDNA was synthesized using oligo dT (Pharmacia) as the primer. DNA was prepared from cultured skin fibroblasts by a modified version of the method of Miller et al. (1988).

Western Blot Analysis

Samples of human cultured skin fibroblasts were subjected to digitonin and Triton X-100 extraction, and the Triton extracts were resolved on a 10% polyacrylamide gel. This gel was then electroblotted onto nitrocellulose and probed with rabbit and-bovine heart PDH-complex antibody. Immunoreactive proteins were visualized by goat anti-rabbit IgG and horseradish peroxidase staining.

Northern Blot Analysis

Purified total RNA samples from human skin fibroblasts were run through a 1% agarose gel containing formamide (Davis et al. 1986). The RNA was then transferred onto Hybond membrane (Amersham) and probed with random primer ^{32}P-labeled cDNA, initially for PDH $E_1\alpha$ and later for PDH $E_1\beta$ (DeMeirlier et al. 1988; Chun et al. 1990).

Amplification of DNA

cDNA and genomic DNA were amplified by the PCR (Saiki et al. 1988). Conditions were as recommended by the manufacturer of *Taq* polymerase (Promega). Synthetic oligonucleotide primers used in the $E_1\alpha$ cDNA amplification were αG21 (5′-GTAGAATTCTCGTGCCTCCTGGGTTGTGA-3′), AVA-R (5′-TCTCGGACGGAAAGGCCCCG-3′), AVA-F (5′-TCACGGCTTTACTTTCACCC-3′), and αG17′ (5′-TCTAGAATTCGTACAAACTGCATGCAATTAC-5′). Synthetic oligonucleotide primers used in the $E_1\beta$ cDNA amplification were βG14 (5′-GACCAAGcTtGCGGCGGTGTCT-3′) and βG12 (5′-CCAGGatCcGTGCCAGCAAGTATTT-3′) lowercase letters indicate artificially induced mismatches for ease in subcloning. Synthetic oligonucleotide primers used in the amplification of exon 11 of $E_1\alpha$ in genomic DNA were E11F (5′-TTTAGGAAtTcGATGTGGAAGT-3′) and E11R (5′-CTGACaagCTTAAACTTGATCCAC-3′). A cycle consisted of denaturation at 94°C for 60 s; annealing—at 61°C (for $E_1\alpha$ cDNA fragment A), 55°C (for $E_1\alpha$ cDNA fragment B), 57°C (for $E_1\beta$ cDNA), or 47°C (for genomic DNA)—for 60 s; and extension at 72°C for 90 s (for cDNAs) or 60 s (for genomic DNA). After 30 cycles, the amplified fragments were visualized on ethidium bromide–stained agarose gels (BRL).

DNA Sequence Analysis

Amplified DNA fragments were subcloned into plasmid pSP65 (Boehringer Mannheim) for sequencing. DNA sequencing was performed by the Sanger dideoxy chain-termination method on double-stranded templates, using a T7 polymerase sequencing kit (Pharmacia) (Sanger et al. 1977).

Results

Biochemical investigations (table 1) revealed a defect in the activity of the PDH complex in which the DCA-activated state was 22% of the activity found in controls. The E_1 pyruvate decarboxylase component of the complex was 18% of the activity in controls. E_2 and E_3 values were in the normal range of activities, as was the activity of cytochrome oxidase.

Western and Northern Blot Analysis

A western blot of seven PDH complex–deficient patient fibroblast cell lines (fig. 1, lanes 1–7) and one control cell line (fig. 1, lane 8) was probed with anti-PDH-complex antibody (fig. 1). Two patient cell lines (fig. 1, lanes 5 and 7) had no significant amounts of either $E_1\alpha$ or $E_1\beta$ protein. One patient (fig. 1, lane 2) had two faint $E_1\alpha$ bands, and patient AF (fig. 1, lane 4) had a second protein band immediately above the $E_1\beta$ band. Examination of mRNA levels for the $E_1\alpha$ and $E_1\beta$ subunits, after northern blotting with the respective cDNA probes, revealed normal message levels for both the α and β subunits in the deficient cell lines tested (fig. 2). However, patient AF also displayed an anomolous intermediate-size species of mRNA when probed with the $E_1\beta$ cDNA (fig. 2, lane 2).

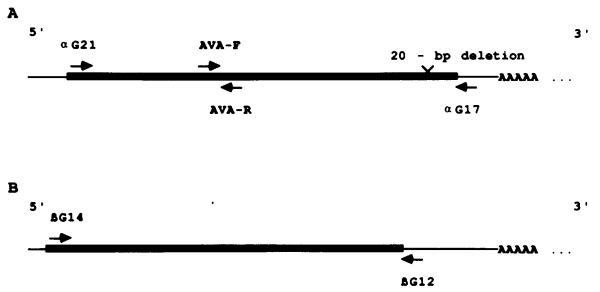

Figure 1 Western blotting of PDH E_1-deficient cultured skin fibroblast extracts with anti-PDH complex antibody. Lanes 1–7, PDH-deficient cell lines 2638, 2653, 2654, 2645 (patient AF), 2641, 2595, and 2583. Lane 8, Control cell line 2491. Above each lane is both the residual percentage PDH complex activity of each cell line (DCA activated) and a code to indicate the clinical presentation of the patient: C = control; A = ataxic episodes; PR = psychomotor retardation, NL = neonatal lactic acidosis.

Characterization of the Mutation

Total RNA was prepared from cultured skin fibroblasts and used as a template for cDNA synthesis of the coding region of PDH $E_1\alpha$. This cDNA was then used to generate two fragments (A and B; fig. 3) by PCR, as described in the Subject and Methods section

Table 1

Activities of Enzyme of PDH Complex in Cultured Skin Fibroblasts

	Mean ± SEM Activity[a] (nmol/mg cell protein)	
	Patient or Mother	Controls
PDH complex:		
Native:		
Patient AF	.313 ± .070 (3)	1.196 ± .137 (5)
Mother	.737 ± .084 (5)	1.170 ± .143 (5)
DCA activated:		
Patient AF	.308 ± .061 (3)	1.386 ± .201 (5)
Mother	.987 ± .113 (3)	1.519 ± .224 (5)
Dihydrolipoyl transacetylase (E_2), patient AF	11.6 (1)	9.2 (1)
Lipoamide dehydrogenase (E_3), patient AF	10.5 (1)	10.8 (1)
Pyruvate carboxylase, patient AF	2.98 ± .32 (2)	1.99 ± 1.03 (2)
Cytochrome oxidase, patient AF	5.06 (1)	4.18 (1)
Pyruvate decarboxylase (E_1), patient AF	.131 ± .045 (3)	.737 ± .499 (3)

[a] Except for pyruvate decarboxylase (E_1) values, which are expressed as activity per hour, data are expressed as activity per minute.

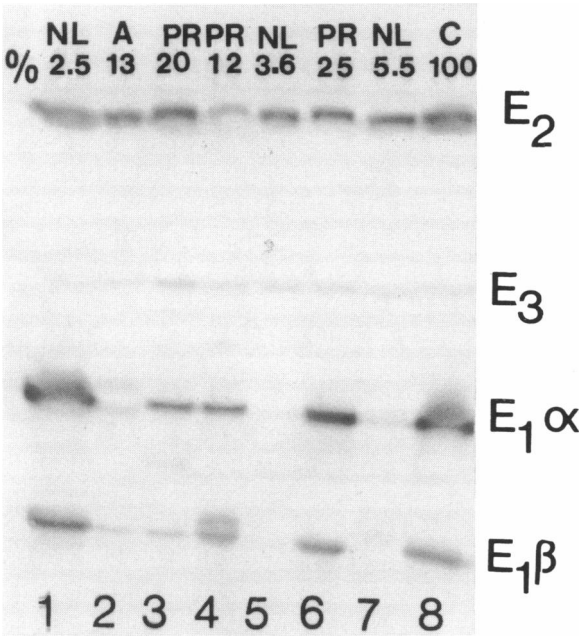

Figure 2 Northern blotting of total cellular RNA from cultured skin fibroblast cell lines of patients with PDH-complex deficiency. The blot was probed with ^{32}P-labeled $E_1\alpha$ cDNA and then with the $E_1\beta$ cDNA, in separate experiments. Lanes 1–4, 7, and 8, PDH-deficient cell lines 2641, 2645 (patient AF), 2554, 825, and 1588, respectively. Lanes 5 and 6, Control cell lines 1206 and 1286, respectively.

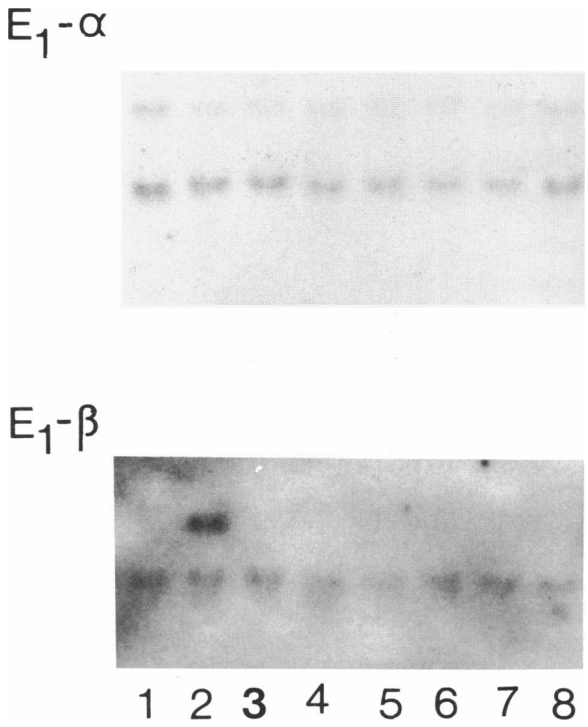

Figure 3 Schematic diagram showing location of oligonucleotide primers used in PCR amplification of PDH $E_1\alpha$ (*A*) and PDH $E_1\beta$ (*B*) cDNAs. The location of the deletion is indicated also, in panel *A*.

above. Subcloning and sequencing made it apparent that the patient had two distinct sequences for the $E_1\alpha$ cDNA, one which was normal and one which contained a 20-bp deletion (fig. 4). Amplification and sequencing of the patient's cDNA for PDH $E_1\beta$ showed only normal sequence, identical to that found in control subjects.

Amplification of exon 11 of the PDH $E_1\alpha$ gene (Maragos et al. 1989) by PCR for patient genomic DNA showed a triple band when the products were run on a 1.5% agarose gel. Along with the normal size band (166 bp) was a second, smaller band (146 bp) and a larger, third band (fig. 5). Subsequent subcloning and sequencing of the two lower bands revealed that the 166-bp fragment contained the normal exon 11 sequence, whereas the smaller fragment contained a 20-bp deletion seen in the cDNA prepared from message. The upper band was thought to be a heteroduplex of the 146- and 166-bp bands. The conclusion was that the patient exhibited heterozygosity at this locus, one allele having a 20-bp deletion starting at nucleotide 1144 (fig. 6) and one allele being normal.

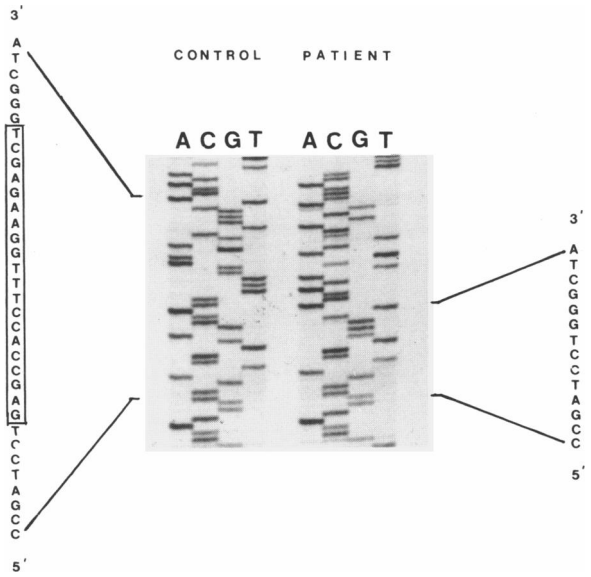

Figure 4 DNA sequence analysis of mutation in patient AF. The DNA sequence of the normal gene of patient AF is shown on the left, and the sequence of the mutant gene is shown on the right. The 20-bp deletion is boxed in the normal sequence.

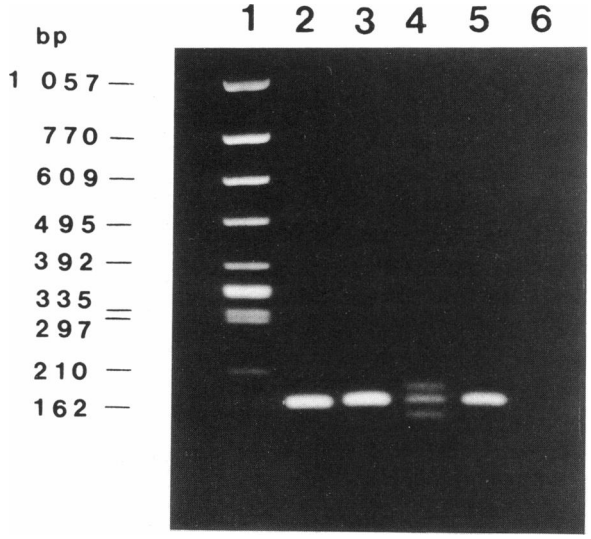

Figure 5 Analysis for presence of 20-bp deletion in exon 11 of PDH $E_1\alpha$ gene in the parents of patient AF. The PCR reaction was performed using oligonucleotide primers E11F and E11R and was analyzed as described in Subject and Methods. The sizes of the PCR products of the normal gene (156 bp) and the mutant gene (136 bp) are shown on the right. Lane 1, DNA size marker. Lane 2, Father. Lane 3, Mother. Lane 4, Patient AF. Lane 5, Control DNA. Lane 6, water blank.

The 20-bp deletion causes a frameshift that a premature stop codon is created at nucleotide 1197. PCR amplification of exon 11 of the parents of the patient revealed that both had only normal versions of the gene present in fibroblast DNA.

Discussion

A mutation was found in the PDH $E_1\alpha$ gene of a female patient who suffered from severe lactic acidosis. cDNA sequence analysis of her PDH $E_1\alpha$ gene revealed a normal sequence and one with a 20-bp deletion, causing a frameshift mutation (fig. 6). Subsequent DNA sequence analysis of exon 11 of the PDH $E_1\alpha$ gene again revealed one normal version and one with the same 20-bp deletion as found in the cDNA. Not only is there a deletion of amino acids that is due to the actual 20-bp deletion, but the frameshift causes a premature stop codon to appear 46 bp upstream of the real stop codon (fig. 6). Hence we would expect the mutant PDH $E_1\alpha$ protein to be 15 amino acids shorter than the normal one of 390 amino acids (including the 29-amino-acid leader sequence). In addition, the frameshift also alters the amino acid sequence from the normal protein (fig. 6), at the C terminus.

This in turn predicts that there are two potential protein products of the PDH $E_1\alpha$ gene—one of normal molecular mass (41 kb) and one of lower molecular mass (~38 kb) that is produced by the deleted $E_1\alpha$ gene. The anomolous band seen in the protein electrophoresis is not, as was originally hypothesized, an abnormal version of the $E_1\beta$ protein but is in fact a shortened version of the $E_1\alpha$ protein. Northern blotting also pointed to $E_1\beta$ as a potential problem because an accumulation of unprocessed higher-molecular-weight mRNA was evident in the total mRNA prepared from the patient cell line. It is possible that some feedback inhibition of mRNA processing is occurring as a result of the formation of nonfunctional PDH complexes. However, there is no evidence of this happening in other PDH $E_1\alpha$–defective cell lines, even when there is little cross-reacting material present in the cells.

After activation, the cultured skin fibroblasts display a PDH-complex activity that is of 22% of that found in controls (table 1). The activity of the pyruvate decarboxylase (E_1) component was found to be 18% of the control value. Clearly, in this particular case

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            340                                 350                                 360
Glu Ile Asp Val Glu Val Arg Lys Glu Ile Glu Asp Pro Ala Gln Phe Ala Ala Asp Pro Glu Pro Pro Leu Glu Glu Leu Gly Tyr
GAA ATT GAT GTG GAA GTG AGG AAG GAG ATT GAG GAT CCT GCC CAG TTT GCC GCG GCC GAT CCT GAG CCA CCT TTG GAA GAG CTG GGC TAC
                                                                                                        GGG CTA
                                                                                                        Gly Leu

            370                                 380                                 390
His Ile Tyr Ser Ser Asp Pro Pro Phe Glu Val Arg Gly Ala Asn Gln Trp Ile Lys Phe Lys Ser Val Ser OC
CAC ATC TAC TCC AGC GAC CCA CCT TTT GAA GTT CGT GGT GCC AAT CAG TGG ATC AAG TTT AAG TCA GTC AGT TAA GGGGAGGAGAAGGAGAGGTT
CCA CAT CTA CTC CAG CGA CCC ACC TTT TGA
Pro His Leu Leu Gln Arg Pro Thr Phe Stop

TACCTTCAGGGGGCTACCAGACAGTGTTCTCAACTTGGTTAAGGAGGAAGAAAACCCAGTCAATGAAATTCAATGAAATTCTTGGAAACTTCCATTAAGTGTGTAGATTGAGCAGGTAGT

AATTGCATGCAGTTTGTACATTAGTGCATTAAAAGATGAATTATTGAGTGCTTAAAGATTAAAAAAAAAAAAAAAAAAA
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Figure 6 DNA sequence of exon 11 and 3′ untranslated region of PDH $E_1\alpha$ gene. The first underlined 20-bp sequence is the deletion; the second underlined 20-bp segment is 75% homologous to the deleted sequence. The change in amino acid sequence caused by the frameshift is shown below the nucleotide sequence.

we do not have the simple 50% reduction in enzyme activity of the complex that would be predicted from the one-gene, one-transcript model of enzyme synthesis. There are two plausible explanations for these findings. The first explanation is based on the fact that the location of the $E_1\alpha$ gene on the X chromosome makes it subject to random inactivation in females (Lyon 1988). It *could* be deduced that some 80% of the cells in this culture had normal $E_1\alpha$ genes that had been randomly inactivated, leaving 80% of the $E_1\alpha$ production in the hands of the defective genes producing short transcripts. There is no evidence from the western blot that this is so. It would seem that complexes formed from the abnormal $E_1\alpha$ translation products are stable, even if inactive. An alternative explanation is that, when stable products are formed in the $\alpha_2\beta_2$ tetramer from the normal gene α and from the abnormal gene α^s, the complexes formed will be $\alpha^s\alpha\beta\beta$, $\alpha^s\alpha^s\beta\beta$, $\alpha\alpha^s\beta\beta$, and $\alpha\alpha\beta\beta$, only the last version being active. The resulting activity would be 25% of normal. However, this would imply that random inactivation had not taken place and that both transcripts were being produced in the same cell.

Since the parents appeared to possess only normal DNA in their skin fibroblast cultures, we can only assume that in this family we are dealing with a germline mutation. However, we could not make a concrete diagnosis, as the clinical presentation of heterozygous females carrying defective X-linked depends on the pattern of X-chromosome inactivation. To check whether either parent carried the same defective PDH $E_1\alpha$ as did the patient, genomic fibroblast DNAs of both parents were amplified in the same set of PCR reactions as was the patient's (fig. 5). No 20-bp deletion was found in either parent's DNA, confirming that the origin of the defect was indeed germ line. In the cases previously reported, the $E_1\alpha$ deletions occurred at the 3′ end of the cDNA sequence (Endo et al. 1989; Dahl et al. 1990). It is interesting that the deletion in patient AF also occurs at the 3′ end of the cDNA sequence. Unlike the two reported cases, however, the 20-bp deletion is not part of a tandem direct-repeat sequence. Nevertheless, 22 bp downstream of the 20-bp deletion is a 20-bp region which is 75% homologous to the deletion (fig. 6). Whether this is just a coincidence or whether there is a significance to it is still unknown.

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