The Mouse Gene PDCR Encodes a Peroxisomal Δ^2,Δ^4-Dienoyl-CoA Reductase

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Here we describe the identification and characterization of a novel mouse gene, PDCR, that encodes a peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase. The mouse PDCR cDNA contains an 892-base pair open reading frame and is predicted to encode a 292-amino acid protein with a deduced molecular mass of 31,298 Da that terminates in a consensus type-1 peroxisomal targeting signal. Purified recombinant PDCR protein was generated from Escherichia coli and catalyzed the NADPH-dependent reduction of Δ^2-trans,Δ^4-trans-decadienoyl-CoA with a specificity of 20 units/mg. Enzymatic characterization followed by high pressure liquid chromatography analysis of the products revealed that PDCR converted Δ^2-trans,Δ^4-trans-decadienoyl-CoA to a Δ^2-enoyl-CoA but not to a Δ^2-enoyl-CoA. Kinetic analyses demonstrated that PDCR is active on a broad range of Δ^2,Δ^4-dienoyl-CoAs. Although the observed substrate preference was to Δ^2-trans,Δ^4-trans-decadienoyl-CoA, PDCR was also active on a C_22 substrate with multiple unsaturations, a result consistent with the role of peroxisomes in the oxidation of complex, very long chain, polyunsaturated fatty acids. The presence of a type-1 peroxisomal targeting signal Ala-Lys-Leu-COOH at the C terminus of PDCR suggested that this protein may be peroxisomal. We observed that tagged PDCR was efficiently transported to the peroxisome lumen in normal human fibroblasts but not in cells derived from a Zellweger syndrome patient with a specific defect in peroxisomal matrix protein import. We conclude that this protein resides within the peroxisome matrix and therefore represents the first mammalian peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase to be characterized at the molecular level.

Mammalian peroxisomes contain multiple overlapping and complementary fatty acid β-oxidation pathways that are able to metabolize a variety of different substrates, including saturated long and very long chain fatty acids, branched chain fatty acids, and dicarboxylic fatty acids. In addition, peroxisomes are able to degrade a variety of unsaturated fatty acids and thus contain auxiliary enzymes such as Δ^3,Δ^5-dienoyl-CoA isomerase, Δ^3,Δ^5,Δ^2,Δ^4-dienoyl-CoA isomerase, and Δ^3,Δ^5-dienoyl-CoA reductase (1). The oxidation of fatty acids with an unsaturation at an even positioned carbon eventually leads to formation of a Δ^2,Δ^4-dienoyl-CoA, which cannot be oxidized by β-oxidation. Instead, the further oxidation of Δ^2,Δ^4-dienoyl-CoAs requires the successive action of Δ^2,Δ^4-dienoyl-CoA reductase to generate a Δ^3-enoyl-CoA, and Δ^2,Δ^4-enoyl-CoA isomerase to convert this intermediate to a Δ^2-enoyl-CoA that can re-enter the β-oxidation spiral (see Fig. 1) (2). The oxidation of fatty acids with pre-existing unsaturations at odd-positioned carbons would appear to be even simpler, with one round of β-oxidation converting the Δ^2,Δ^4-dienoyl-CoA to a Δ^2-enoyl-CoA and Δ^3,Δ^4-enoyl-CoA isomerase converting this to a Δ^2-enoyl-CoA substrate for further oxidation (see Fig. 1) (3). However, studies have established that there also exists a Δ^2,Δ^4-dienoyl-CoA reductase-dependent pathway for returning Δ^2,Δ^4-enoyl-CoAs to the core spiral (Fig. 1) (4, 5). Thus, Δ^2,Δ^4-dienoyl-CoA reductases appear to play important roles in the oxidation of virtually all unsaturated fatty acids.

Recent studies have identified mammalian genes that encode the peroxisomal Δ^2,Δ^4-enoyl-CoA isomerase (6) and Δ^3,Δ^5,Δ^2,Δ^4-dienoyl-CoA isomerase (7), but the structural basis for peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase remains to be determined. However, genes encoding mammalian mitochondrial Δ^2,Δ^4-dienoyl-CoA reductases have been identified in both rat and human beings (8, 9), and it is formally possible that the peroxisomal reductase mRNA could be generated from the same gene as the mitochondrial enzyme. In fact, such a mechanism is used to generate both peroxisomal and mitochondrial forms of Δ^3,Δ^5,Δ^2,Δ^4-dienoyl-CoA isomerase (7). Nonetheless, another possibility is that the peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase is encoded by a distinct gene that has yet to be recognized. Here we report the identification and characterization of a novel mouse gene, PDCR, and show that it encodes a peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase.

**EXPERIMENTAL PROCEDURES**

Cloning and Sequencing the Mus musculus PDCR Gene—All sequence searches were performed using algorithms and data bases available at the National Center for Biotechnology Information web site. Mammalian homologues of the putative Caenorhabditis elegans peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase (F53C11.3) were identified by TBLASTN searches of the human and mouse expressed sequence tag data bases. Two cDNA clones from the IMAGE consortium (1824380 and 1888282) corresponded to the mouse ESTs\(^a\) Al314477 and AI286384 (GenBank\(^b\) accession numbers) and appeared to contain the full-length open reading frame of murine PDCR cDNA. This was determined by the ability of these ESTs to encode proteins that (i) shared significant sequence similarities over the N terminus of F53C11.3 and yeast Sps19p and (ii) contained an appropriately positioned ATG codon with a good match to the Kozak consensus for high efficiency translation initiation (10, 11). These two clones were obtained

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\(^a\) The abbreviations used are: EST, expressed sequence tag; HPLC, high pressure liquid chromatography.
from Genome Systems (St. Louis, MO), and the clone with the longer 5' untranslated region (1924380) was sequenced in its entirety on both strands. Amino acid sequence alignments were performed with DNASTAR MegAlign software (Madison, WI) using the PAM250 substitution matrix.

**Plasmids**—The *Escherichia coli* strain DH10B was used for the maintenance and amplification of all plasmids (12). All polymerase chain reactions were performed with a low error rate mixture of polymerases (Expand; Roche Molecular Biochemicals). A full-length form of the mouse *PDCR* open reading frame was generated by polymerase chain reaction using the cDNA clone 1888282 as a template and the oligonucleotides MmPDCR.5 (5'-GAAGTCGACCATGGCCCAGCCGC-GCCCGACGTTG-3') and MmPDCR.3 (5'-GAACGCGGCCGCGAATGCCTTTAATCCCAG-3') as primers. These oligonucleotides append SalI and NotI sites (underlined) at the 5' and 3' ends of the mouse *PDCR* open reading frame, respectively. The product of this polymerase chain reaction was digested with SalI and NotI and subcloned into the corresponding sites of pT7-His6. pT7-His6 is a modified form of pET28A (Novagen, Inc.) that contains XhoI and SalI sites in place of the NheI-HindIII fragment found in the parental vector. The sequence of the mouse *PDCR* open reading frame in pT7-His6 was confirmed by automated fluorescent sequencing, and the resulting plasmid was denoted pT7-His6-PDCR. This plasmid allows for T7-driven expression of a form of mouse *PDCR* bearing an N-terminal hexahistidinyl extension in the *E. coli* strain BL21(DE3) (Novagen). To generate the N-terminal c-myc-tagged form of the mouse *PDCR* open reading frame, the SalI-NotI insert of pT7-His6-PDCR was excised and subcloned into XhoI and NotI sites of pcDNA3-Nmyc (13). This plasmid allows for constitutive expression of an N-terminally c-myc-tagged form of mouse *PDCR* in mammalian cells.

**Purification of Enzymes**—The *E. coli* strain BL21(DE3) was chosen for high level heterologous expression of the His$_6$-PDCR protein. Specifically, several colonies from a plate of freshly transformed BL21(DE3) cells harboring the plasmid pT7-His$_6$-PDCR were used to inoculate a 50-ml preculture of LB medium supplemented with 1% glucose and 25 mg/ml kanamycin sulfate. This preculture was grown 12 h at 30 °C with vigorous shaking (300 rpm), at which time 20 ml of the preculture were used to inoculate 500 ml of 2YT medium supplemented with 25 μg/ml kanamycin sulfate. This culture was grown with vigorous shaking for approximately 7 h at 18 °C until the $A_{600}$ was approximately 0.4, at which time induction of protein expression was begun by the addition of isopropyl-$b$-D-galactothiopyranoside to a final concentration of 1 mM. The induced culture was grown for an additional 18 h under the same conditions. The induced cells were harvested at 5,000 × g for 10 min at 4 °C at the end of this period.

The induced cells were resuspended in 5 ml of binding buffer (20 mM sodium-P$_4$, pH 7.8), 500 mM sodium chloride, and 5 mM benzamidine HCl) with 5 mM 2-mercaptoethanol, and the cells were lysed by sonication (14). Following cell lysis, a clarified extract was prepared by centrifuging the sonicate at 25,000 × g for 30 min at 4 °C. This extract (50 ml) was diluted to 150 ml with binding buffer and was applied at 1.5 ml/min to a 2-ml bed of Chelating Sepharose Fast Flow (Amersham

**Fig. 1. Enzymatic steps specific for unsaturated fatty acid metabolism.** $\beta$-Oxidation of unsaturated fatty acids with double bonds at even-numbered carbons yields $\Delta^2,\Delta^4$-dienoyl-CoAs, which are metabolized as shown at left (2). $\Delta^2,\Delta^4$-Dienoyl-CoAs arising from $\beta$-oxidation of unsaturated fatty acids with double bonds extending from odd-numbered carbons can return to the core spiral through both hydratase/isomerase-pathway (center) (3) and the reductase-dependent (right) shunt (4). Note that $\Delta^2,\Delta^4$-dienoyl-CoA reductase-catalyzed reactions are involved in the metabolism of both dienoyl-CoA species.
PDCR (Δ^2,Δ^4-Dienoyl-CoA reductase) gene searching for mammalian genes that were capable of encoding proteins with significant sequence similarity to yeast peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase, Sps1p (25). However, our searches using the Sps1p sequence as a BLAST query versus the data base of expressed sequence tags identified only ESTs corresponding to the previously identified mammalian mitochondrial Δ^2,Δ^4-dienoyl-CoA reductase gene. Therefore, we altered our search strategy and restricted our search to the recently completed C. elegans genome sequence. Three putative Δ^2,Δ^4-dienoyl-CoA reductase genes were identified in C. elegans, and the sequences of their deduced products (C. elegans proteins T05C12.3, W01C9.4, and F53C11.3) were examined. These three proteins were all highly similar to Sps1p, with BLAST e values of e^-27 to e^-28. However, one of these, F53C11.3, contained a match to the consensus type-1 peroxisomal targeting signal, Ser-Lys-Leu-COOH (26), at its C terminus. The presence of the type-1 peroxisomal targeting signal indicated that F53C11.3 might represent the peroxisomal Δ^2,Δ^4-dienoyl-CoA reductase of C. elegans, and we used the F53C11.3 sequence to rescan the data bases of human and murine expressed sequence tags. Once again, the majority of ESTs we identified corresponded to the previously characterized mammalian mitochondrial Δ^2,Δ^4-dienoyl-CoA reductases. However, we also identified expressed sequence tags representing a second gene (a gene we have designated PDCR) that also shared significant similarity to F53C11.3.

Two murine ESTs encoded proteins that shared sequence similarity to the N terminus of both F53C11.3 and yeast Sps1p and the corresponding cDNA clones were obtained and characterized. Sequence analysis of the longer PDCR cDNA clone revealed the presence of a 2048-base pair cDNA containing a 5'-untranslated region of 121 base pairs, an 876-base pair open reading frame, and a 1451-base pair 3'-untranslated region (Fig. 2). The sequence of the PDCR open reading frame was confirmed by sequencing the second PDCR cDNA clone, which differed only in that it had a slightly shorter 5'-untranslated region. The deduced product of the PDCR gene is 292 amino acids long, has a predicted molecular mass of 31,298 Da, and has a pl of 9.4. Like Sps1p and F53C11.3, murine PDCR terminates in a match to the consensus type-1 peroxisomal targeting signal (26), Ala-Lys-Leu-COOH. Furthermore, an amino acid alignment between mouse PDCR, C. elegans F53C11.3, and S. cerevisiae Sps1p demonstrates that these proteins share extensive sequence similarities across their lengths (Fig. 3), with mouse PDCR showing 32 and 41% identity to the C. elegans and S. cerevisiae proteins, respectively.

Mouse PDCR Encodes a Δ^2,Δ^4-Dienoyl-CoA Reductase—To test the hypothesis that mouse PDCR encoded a protein with Δ^2,Δ^4-dienoyl-CoA reductase activity, we cloned the entire mouse PDCR open reading frame into the prokaryotic expression vector pT7-His6, expressed the His6-PDCR protein in E. coli, and purified the soluble, recombinant enzyme by immobilized metal ion affinity chromatography (Fig. 4). Analysis of the purified protein by SDS-polyacrylamide gel electrophoresis demonstrated that the protein was greater than 95% pure.

As a first assessment of the enzymatic activity of recombinant PDCR, we employed a spectrophotometric method to monitor changes in the absorbance spectrum of Δ^2-trans,Δ^4-trans-decadienoyl-CoA following the addition of PDCR (Fig. 5). The characteristic spectrum of a Δ^2,Δ^4-dienoyl-CoA is shown in Fig. 5, spectrum 1, with major absorbance bands centered near 260 and 300 nm that are attributable to the coenzyme A and dienoyl thioester moieties, respectively. Addition of purified PDCR to the assay mixture resulted in the disappearance of the dienoyl thioester chromophore at 300 nm and a decrease in absorbance at 340 nm that reflects the oxidation of NADPH
The nucleotide sequence of the murine PDCR cDNA is shown along with the deduced translation of the 876-base pair open reading frame contained therein. The deduced PDCR protein is basic (pI = 9.4), has a predicted subunit weight of 31,298, and terminates in the nearly consensus type-1 peroxisomal targeting signal Ala-Lys-Leu-COOH (underlined).
the 10 amino acid c-myc epitope tag at its N terminus and placed it downstream of the cytomegalovirus promoter in the mammalian expression vector pcDNA3. The resulting plasmid, pcDNA3-N\textsuperscript{myc}PDCR, was transfected into human skin fibroblasts. Three days later, the transfected cells were processed for double indirect immunofluorescence microscopy using antibodies specific for the c-myc epitope tag, and for the cytoplasmically exposed C-terminal tail of PMP70, an integral peroxisomal membrane protein (30). Following permeabilization of all cellular compartments, we observed that N\textsuperscript{myc}PDCR colocalized with PMP70 to discrete, punctate structures characteristic of peroxisomes (Fig. 7, A and B). To determine whether

FIG. 3. Amino acid alignment of mouse PDCR with putative \textit{C. elegans} and \textit{S. cerevisiae} homologues. Clustal amino acid alignment of mouse PDCR with the hypothetical \textit{C. elegans} protein F53C11.3 and \textit{S. cerevisiae} Sps19p is shown. Residues that are conserved in all three sequences are shown as white letters on a black background.
any of at least 12 different genes, all of which play roles in peroxisomal biogenesis. Previous studies have established that the cell line PBD100, which was derived from a Zellweger peroxisome biogenesis. Previous studies have established that PBD100 cells expressing the Nmyc-PDCR cDNA were processed for double indirect immunofluorescence after permeabilization with 1% Triton X-100. The subcellular distribution of Nmyc-PDCR was examined using anti-nyc (A) and anti-PMP70 (B) antibodies. Additional Nmyc-PDCR expressing cells were permeabilized with 25 μg/ml digitonin and were stained with anti-nyc (C) and anti-PMP70 (D) antibodies. Cells permeabilized with 25 μg/ml digitonin were also stained with anti-catalase (E) and anti-PMP70 (F) antibodies. Double indirect immunofluorescence was used to examine the distribution of Nmyc-PDCR in pex10-deficient PBD100 cells (permeabilized with 1% Triton X-100) and again with anti-nyc (G) and anti-PMP70 (H) antibodies. Bar, 25 μm.

**FIG. 7. PDCR is a peroxisomal matrix protein.** Human skin fibroblasts expressing the Nmyc-PDCR cDNA were processed for double indirect immunofluorescence after permeabilization with 1% Triton X-100. The subcellular distribution of Nmyc-PDCR was examined using anti-nyc (A) and anti-PMP70 (B) antibodies. Additional Nmyc-PDCR expressing cells were permeabilized with 25 μg/ml digitonin and were stained with anti-nyc (C) and anti-PMP70 (D) antibodies. Cells permeabilized with 25 μg/ml digitonin were also stained with anti-catalase (E) and anti-PMP70 (F) antibodies. Double indirect immunofluorescence was used to examine the distribution of Nmyc-PDCR in pex10-deficient PBD100 cells (permeabilized with 1% Triton X-100) and again with anti-nyc (G) and anti-PMP70 (H) antibodies. Bar, 25 μm.

**FIG. 6. HPLC analysis of the PDCR-catalyzed reaction product.** A, HPLC-purified Δ⁴-trans,Δ⁴-trans-decadienoyl-CoA (8 nmol in 0.2 ml of 60 mM potassium-Pi (pH 7.4)). B, 8 nmol of Δ⁴-trans,Δ⁴-trans-decadienoyl-CoA in 0.2 ml of 60 mM potassium-Pi (pH 7.4) after incubation for 30 s with 0.1 mM NADPH and 0.2 μg of mouse PDCR in the absence or in the presence of 0.1 unit of crotonase from bovine liver. C, 12 nmol of Δ⁴-trans,Δ⁴-trans-decadienoyl-CoA in 0.2 ml of 60 mM potassium-Pi (pH 7.4) after incubation for 30 s with 0.1 mM NADPH, 0.13 μg of rat liver Δ⁴-trans-enoyl-CoA isomerase, and 0.2 μg of mouse PDCR. D, the mixture contained 0.1 unit of bovine liver crotonase in addition to the components listed for C. Δ⁴-trans,Δ⁴-trans-decadienoyl-CoA; Δ³-trans,Δ³-decenoyl-CoA; Δ³,Δ³-decenoyl-CoA; 3HO, 3-hydroxydecanoyl-CoA.

**TABLE 1**

Kinetic parameters of recombinant mouse Δ⁴-trans-Δ⁴-dienoyl-CoA reductase

| Substrate | Vₘₐₓ | Vₘₐₓ/Kₘ | Kₘ |
|-----------|------|---------|-----|
| 2,4-Hexadienoyl-CoA | 108 | 8 | 0.074 |
| 2,4-Decadienoyl-CoA | 155 | 5 | 0.032 |
| 2,4,7,10,13,16,19-docosahexaenoyl-CoA | 6 | 20 | 3.33 |

* Apparent kinetic constant determined at 0.1 mM [NADPH].

**DISCUSSION**

The work presented here describes the first molecular characterization of a mammalian peroxisomal Δ²,Δ⁴-dienoyl-CoA reductase. The mouse cDNA encoding this enzyme (PDCR) was identified on the basis of its potential to encode a protein highly similar to S. cerevisiae Sps19p. Enzymatic characterization of PDCR demonstrated that this enzyme has significant, intrinsic Δ²,Δ⁴-dienoyl-CoA reductase activity and that the product of its action on Δ²-trans,Δ⁴-trans-decadienoyl-CoA was Δ³-decenoayl-CoA. Thus the catalytic mechanism of PDCR can be assumed to be analogous to other eukaryotic Δ²,Δ⁴-dienoyl-CoA reductases, which have previously been shown to reduce Δ²,Δ⁴-dienoyl-CoAs to Δ³-enoyl-CoAs (25, 27, 28). We observed that mouse PDCR displays a substrate preference for Δ²-trans,Δ²-trans-decadienoyl-CoA over both Δ²-trans,Δ⁴-trans-hexadienoyl-CoA and Δ²-trans,Δ³-trans-docosahexaenoyl-CoA based on the ratio of the observed Vₘₐₓ and Kₘ constants for these substrates. The observed preference for Δ²-trans,Δ²-trans-decadienoyl-CoA likely reflects the cellular role of PDCR in peroxisomal β-oxidation, namely in the oxidation of long and very long chain fatty acids that contain unsaturations near the middle of the acyl chain (1). Furthermore the observed activity of PDCR on Δ²-trans,Δ³-trans-docosahexaenoyl-CoA is consistent with the established role of peroxisomes in the metabolism of complex, very long chain, polyunsaturated fatty acids such as arachidonic acid and prostaglandins (1).

Using immunofluorescence microscopy we observed that mouse PDCR is targeted to the peroxisomal lumen. This experimental observation of peroxisomal localization is consistent with several lines evidence presented here and elsewhere. First, the presence of the C-terminal sequence Ala-Lys-Leu-CoOH in PDCR suggested that this enzyme would be targeted
efficiently to peroxisomes. This sequence represents a consensus match to the type-1 peroxisomal targeting signal, an obligatory C-terminal tripeptide motif that is present on the vast majority of peroxisomal matrix proteins (32). Second, we observed a distinct accumulation of PDCR in the cytoplasm of skin fibroblasts derived from a PEX10-deficient Zellweger syndrome patient. The molecular defects in these cells have been characterized at both the genetic and protein level, and it is well established that these cells are deficient in peroxisomal matrix protein import specifically (20). Finally, it has been reported previously that a homotetrameric $\Delta^2,\Delta^4$-dienoyl-CoA reductase isolated from rat liver peroxisomes has a subunit molecular mass of approximately 33,000 Da (24). Based on the deduced protein sequence of PDCR, the calculated molecular mass of this novel enzyme is predicted to be 31,298 Da. Thus, the subunit mass of mouse PDCR is in good agreement with that of an orthologous enzyme isolated from peroxisomes of a highly related species.

Mammalian cells contain differentially compartmentalized pathways for the $\beta$-oxidation of fatty acids and the chemical details of the individual steps in both mitochondrial and peroxisomal pathways appear highly similar. For example, both systems require the action of the auxiliary enzymes shown in Fig. 1 to completely oxidize unsaturated fatty acids. Although the enzymatic steps involved in metabolizing $\Delta^2,\Delta^4$-dienoyl-CoA intermediates are relatively well understood, the presence of two distinct shunts for degrading $\Delta^2,\Delta^4$-dienoyl-CoAs raises questions as to which pathway is responsible for the main flux of carbon skeletons arising from such intermediates. Recent studies on the mitochondrial metabolism of $\Delta^2$-trans,$\Delta^8$-octenoyl-CoA suggest that the hydratase/isoenzyme shunt handles a majority of the metabolic flux for these intermediates (33). A corollary to this finding is that the reductase-dependent pathway is responsible for maintaining coenzyme A homeostasis in the presence of the unavoidable and possibly deleterious action of $\Delta^2,\Delta^4$-enoyl-CoA isomerase on $\Delta^2,\Delta^6$-dienoyl-CoAs (Fig. 1) (33). Currently there is no report in the literature that addresses questions of metabolic flux for peroxisomal $\Delta^2,\Delta^4$-dienoyl-CoAs. Thus, an assessment of these issues is needed to elaborate our general understanding of peroxisomal unsaturated fatty acid metabolism.

These molecular studies of mouse peroxisomal $\Delta^2,\Delta^4$-dienoyl-CoA reductase complete the preliminary characterization of the known auxiliary enzymes of mammalian peroxisomal $\beta$-oxidation. The significance of these studies is underscored by the well established observation that defects in peroxisomal fatty acid metabolism are directly linked to lethal inherited human disorders (34). As of this report four genetically distinct complementation groups of these peroxisomal disorders have been described (34). Although two complementation groups are attributable to defects in acyl-CoA oxidase and the $\delta$-specific multifunctional enzyme, the precise enzymatic deficiency in the remaining complementation groups is unknown. Because $\Delta^2,\Delta^4$-dienoyl-CoA reductase activity is required for complete oxidation of all $\Delta^2,\Delta^4$-dienoyl-CoAs, as well as a portion of $\Delta^3,\Delta^5$-dienoyl-CoAs, the human form of PDCR represents a candidate disease gene for these disorders. In addition to these uncharacterized complementation groups of peroxisomal fatty acid oxidation deficiency, there also exist reports of a lethal human deficiency in $\Delta^2,\Delta^4$-dienoyl-CoA reductase (35, 36). Interestingly, the molecular basis of this deficiency has yet to be resolved. Although one group suggested that the defect lies in a mitochondrial $\Delta^2,\Delta^4$-dienoyl-CoA reductase (35), no report describing patient mutations in a gene encoding a mitochondrial $\Delta^2,\Delta^4$-dienoyl-CoA reductase exists in the literature, and the possibility that this deficiency is peroxisome-associated has not been examined.

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