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Amerindian Pyruvate Carboxylase Deficiency Is Associated with Two Distinct Missense Mutations

Mary Anna Carbone,[1,2] Nevi MacKay,[2] Mingfu Ling,[2] David E. C. Cole,[3] Cheryl Douglas,[2] Brigitte Rigat,[2] Annette Feigenbaum,[2] Joe T. R. Clarke,[2] James C. Haworth,[4] Cheryl R. Greenberg,[4] Lorne Seargeant,[5] and Brian H. Robinson[1,2]

[1]Department of Biochemistry, University of Toronto, [2]The Research Institute, Department of Genetics, The Hospital for Sick Children, and [3]The Banting and Best Institute, Toronto; [4]Section of Genetics and Metabolism, Department of Pediatrics, Child Health and Human Genetics, University of Manitoba and Children's Hospital, and [5]Department of Clinical Chemistry, Health Sciences Centre, Winnipeg

Summary

We characterized the pyruvate carboxylase (PC) gene by PCR amplification, subcloning, and sequencing. The coding region has 19 exons and 18 introns spanning ~16 kb of genomic DNA. Screening both the cDNA and the gene of individuals with the simple A form of PC deficiency revealed an 1828G→A missense mutation in 11 Ojibwa and 2 Cree patients and a 2229G→T transversion mutation in 2 brothers of Micmac origin. Carrier frequency may be as high as 1/10 in some groupings. The two point mutations are located in a region of homology conserved among yeast, rat, and human PC, in the vicinity of the carboxylation domain of the enzyme. These data provide the first characterization of the human PC gene structure, the identification of common pathogenic mutations, and the demonstration of a founder effect in the Ojibwa and Cree patients.

Introduction

Pyruvate carboxylase (PC) is a nuclear-encoded mitochondrial enzyme that catalyzes the ATP-driven formation of oxaloacetate from pyruvate and HCO_3^- (Crabtree et al. 1972; Lee and Davis 1979). In mammals, the native enzyme is a homotetramer with each subunit (molecular weight 127,000) containing a covalently bound molecule of biotin, as well as binding sites for pyruvate, ATP, HCO_3^-, and acetyl CoA (Wood and Barden 1977). PC becomes active in conditions in which fatty acids are mobilized and are generating acetyl-CoA (Keech and Utter 1963). Thus, acetyl-CoA is a powerful allosteric activator of human PC, which is virtually inactive without bound acetyl-CoA (Keech and Barritt 1967; Lim et al. 1988). In mammals, PC is located solely in the mitochondrial matrix (Walter and Anabitarte 1973). The roles of PC in intermediate metabolism are tissue specific. In gluconeogenic tissue (liver and kidney) it catalyzes the first step in the synthesis of glucose from pyruvate, whereas in lipogenic tissue (liver, adipose, lactating mammary gland, and adrenal gland) it participates in the export of acetyl groups, as citrate, from the mitochondria to the cytosol. In CNS tissues, PC participates in the replenishment of neurotransmitter pools of glutamate, GABA, and aspartate (Shank et al. 1985). Information from the rat gene indicates that two separate transcripts generated from different promoters 10 kb apart on the same gene are produced with identical coding regions (Jitrapakdee et al. 1997). These two transcripts have, in liver and kidney, 5′ UTR sequences different from those in brain and heart.

PC deficiency in individuals occurs in three distinct forms: a milder form, A, in which the presenting features are lactic acidemia and psychomotor retardation; a more severe form, B, in which severe neonatal lactic acidosis, hyperammonemia, and abnormal redox state result in death at age <3 mo (Robinson 1995); and a third form, which appears to be relatively benign, causing only mild lacticacidemia (Van Coster et al. 1991). Molecular characterization of the A and B forms, by use of [^{3}H]-biotin labeling, immunoprecipitation of [^{35}S]-labeled proteins, [^{35}S]-streptavidin labeling, and northern blot analysis, showed that those with the A form of PC deficiency had a biotin-containing 125-kD protein corresponding to PC (hereafter, this form is designated "CRM$^+$"), whereas those with the B form were missing either biotinylated PC protein (hereafter, this form is designated "CRM$^-$") and/or PC mRNA (Robinson et al. 1984, 1987; Robinson 1989). In the CRM$^-$ patients, the lack of intracellular aspartate and oxaloacetate compromises both the synthetic and the anaplerotic pathways that require

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Address for correspondence and reprints: Dr. Brian H. Robinson, The Hospital for Sick Children, Department of Genetics, 555 University Avenue, Room 9107, Toronto, Ontario, Canada. E-mail: bhr@sickkids.on.ca

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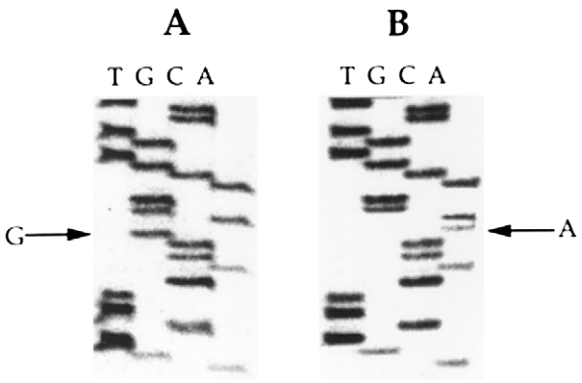

Figure 1 DNA sequence from a small region within the PC gene, showing the G→A transition at nucleotide 1828. *A,* Wild-type sequence from a normal Ojibwa. *B,* Sequence from a PC-deficient Ojibwa homozygous for the mutation.

PC activity. In CRM^+ patients, the gene is expressed in the form of a biotin-containing protein that possesses enough residual catalytic activity to sustain the anaplerotic functions of PC (Robinson et al. 1984).

The Canadian Indian population is strongly represented in our study of CRM^+ PC deficiency, there being cases in the Micmac, Cree, and Ojibwa. This common linguistic group is derived from a founder group in southern Ontario ~300 B.C. It has been suggested that there could be one or more disease-causing mutations in the PC gene that are unique to the "Algonkian-speaking peoples" of North America (Robinson 1989). Having described the cDNA (MacKay et al. 1994; Wexler et al. 1994), we began work on the gene structure of the coding region, so that we could define these inherited defects.

Material and Methods

Patient Samples

Cultured fibroblast cell lines from forearm-skin biopsies were obtained, with informed consent of parents, from patients with a clinical diagnosis of PC deficiency. Diagnosis was based on a PC enzyme assay (Robinson et al. 1985) modified from the method of Utter and Keech (1963), immunoprecipitation of [^{35}S]-labeled proteins, [^{3}H]-biotin labeling, and northern blot analysis (Robinson et al. 1983, 1984, 1987). Table 1 lists several of the clinical features presented by the Ojibwa and Cree from Ontario and Manitoba who have the CRM^+ form of PC deficiency.

Cell Lines and DNA Isolation

Fibroblasts from 5 normal Ojibwa and from 15 PC-deficient patients (11 Ojibwa, 2 Cree, and 2 Micmac) exhibiting the simple A phenotype were used to prepare genomic DNA. Cells were grown at 37°C to 100% confluency in α minimal essential medium/2 × glucose culture medium containing 10% FCS and 1% antibiotic (streptomycin/penicillin). Genomic DNA was extracted by a modified version of the method of Miller et al. (1988). The absorbance (260 nm) was assessed for each sample, and each sample subsequently was diluted to a concentration of 0.100 μg/μl.

Genomic Characterization of the PC Gene

DNA from normal human liver or brain was amplified into 18 different fragments spanning the 16 kb of PC genomic DNA, by use of PC-specific primers (table 2) derived from its cDNA sequence (MacKay et al. 1994). Each PCR product was isolated and subcloned into the pCR 2.1 (3.9 kb) vector, by use of the TA subcloning kit (Invitrogen) designed for PCR products. The nucleotide sequences for several clones of the subcloned fragments were determined by the dideoxynucleotide method (Sanger 1977), by use of the T7 DNA sequencing kit (Pharmacia Biotech). The location and sequence of the introns were identified by alignment of the genomic sequences to the cDNA sequence of human kidney PC (MacKay et al. 1994; Wexler et at. 1994). Table A1, in the Appendix, documents the corrections that, on the

Table 1

Clinical Features of CRM^+ PC Deficiency in a Cree Population and in an Ojibwa Population, in Northwestern Ontario and Manitoba

Category	Distribution or Measurement
Patients	11 Males, 6 females, 11 families
Age at presentation	At birth for 7, at 1–8.5 mo for 10
Presenting signs	Metabolic acidosis in 10, seizures in 5, respiratory distress in 4, pneumonia in 3, hypotonia in 3
Clinical course	Frequent lactic acidosis/severe developmental delay and muscular hypotonia in 17, seizures in 8, hypoglycemia in 4, other CNS involvement (clonus or athetosis) in 4
Outcome	11 Deaths at 3 mo–4.75 years, 6 survivors 3 mo–19 years old
Lactate/pyruvate (control value 10–25)	<15 in 4, 16–25 in 7, >25 in 5
β-OH-butyrate/acetoacetate (control value .5–2.5)	.3–1.6 in 6
PC activity in fibroblasts (control value .4–1.4 nmol/min/mg protein)	1%–4% in 12

Table 2

Sequences of Oligonucleotides (5′→3′) from PC cDNA That Were Used to Amplify and Sequence PC Introns

Intron	Forward Primer	Reverse Primer
1	TAAGCCTATCAAGAAGTAATG	CATCAGCTTTCTGCCTGTGCAT
2	GGAGCAGGACACAGGGCAGATG	GGGCTTCCACCTTGTCTCCCAT
3	CGGTTTATTGGTCCAAGCCCA	GATGATGGGGAAGCCGTAGGT
4	ATCACGTCCCTGCATGAGGCC	TGGCTTCTCGATGAACTTCTC
5	TATGAGGAGTTGGAAGAGAATTA	GTTTAGCGAGTTTCACAGAGTCGC
6	GCGACTCTGTGAAACTCGCTAAAC	CTCTGTGACCGTGTGCTCCACC
7	GGTG GAGCACACGGTCACAGAG	GCCGGTGTCCGGCTGGAACGT
8	ACGTTCCAGCCGGACACCGGC	GGCGAGATGACGGCTCCT
9	ATGAGCAGGGCCCTTGCGGAGTTCCGTGTC	GAGCACATTCTGCAGGAAGGCGATGTTGGT
10	CAGTTCCTGGCAGGCACTGTG	CTGCAGGAAGGCGATGTTGGTCT
11	CAGTTCCTGGCAGGCACTGTG	CGGGTGGTTCCGCACAGCTCGAGC
12	GCTCGAGCTGTGCGGAACCACCCG	CTCCCGGAGCTCCTGCAG
13	GGCATGGATGTCTTCCGTGTG	CTGGGCACAGGCCAGCATGGC
14	ATGACTTCACAGCCCAGCATG	GTACAGTCCCCGAGCCCCCTC
15	AACCTGCACTTCGAGGCCCAC	GGACAGCTCTTCCGCCTGAGC
16	GCTCAGGCGGAAGAGCTGTCC	CCCATGCCGGTCTACCAGCTC
17	TTCAAGGACTTCACTGCCACC	GGACCGCAGCTGCCCATTGAG
18	CTCAATGGGCAGCTGCGGTCC	CGCCCCGATCTGGCCCTTCAC

basis of the sequencing of numerous normal cDNAs, have been made to this sequence since its publication in 1994. Table 3 shows the organization and splice-junction sequences of the gene for human PC.

RNA Extraction and Reverse Transcriptase–PCR (RT-PCR)

Total RNA was extracted from confluent skin fibroblasts by use of the Trizol method (Gibco BRL), as outlined by the manufacturer. cDNA was generated from total RNA by reverse transcription using Superscript II reverse transcriptase (Gibco BRL) and PC-specific primers.

PC Gene–Mutation Screens

PC sequences were amplified from genomic DNA or cDNA templates by use of a final 50-μl PCR reaction composed of 500 ng of each PC-specific primer, 250 μM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 1.5 mM $MgCl_2$, 1 × PCR buffer (Gibco

Table 3

Exon/Intron Splice-Junction Sequences of the Gene for Human PC

Exon Number[a]	Size (kb)	Intron Number	Size (kb)	Sequence		
				Exon	Intron	Exon
1	.136	1	.153	AGA G; Gly (46)	gtgagcac.....ccctctag	Gly (46); GT GAG
2	.185	2	.201	GCC AAG; Lys (107)	gtgagccc.....accgtcag	Glu (108); GAG AAC
3	.166	3	.106	GCG G; Gly (163)	gtgaatat.....cccaacag	Gly (163); GT GTT
4	.146	4	.153	TAC GAG; Glu (211)	gtgagtga.....cctcgcag	Glu (211); GAG CTG
5	.119	5	.480	TTG GG; Gly (251)	gtgagcgg.....cctcccag	Gly (251); G GAC
6	.151	6	1.4	AAA CAG; Gln (301)	gtgaaggg.....cgccacag	Val (302); GTG GGC
7	.119	7	2.4	ACC GA; Asp (341)	gtgagtgt.....ccctacag	Asp (341); C GTA
8	.163	8	2.2	ATT GAG; Glu (395)	gtgggcgg.....cttcccag	Val (396); GTG TTC
9	.183	9	3.0	GTG AAG; Lys (456)	gtgagagg.....ccctgcag	Thr (457); ACC AAC
10	.144	10	.399	TAC CTC; Leu (504)	ggcctggc.....ctccccag	Gly (505); GGC CAT
11	.091	11	.084	ATA G; Gly (535)	gtaggtga.....taccctag	Gly (535); GC CCG
12	.222	12	.770	GGA G; Gly (609)	gtaggctg.....ctgtgcag	Gly (609); GA GCC
13	.400	13	.105	ATC AAG; Lys (741)	gtgcctgg.....tcccccag	Asp (742); GAC ATG
14	.250	14	.207	ACA G; Glu (825)	gtaggaag.....ccctgcag	Glu (825); AG GTG
15	.245	15	.150	ATC AAG; Lys (906)	gtgagccc.....ctcctcag	Val (907); GTG ACG
16	.180	16	.079	TCT AAG; Lys (966)	gtagggaa.....ctccccag	Val (967); GTA CTG
17	.249	17	.600	TTT GAG; Glu (1049)	gtcagtgg.....ccctgcag	Val (1050); GTG GAG
18	.141	18	.081	ATG AAG; Lys (1096)	gtattgtc.....gtcttcag	Glu (1097); GAG ATG
19	.249			GAG TGA; Glu stop (1178)		

[a] Listed in 5′→3′ order.

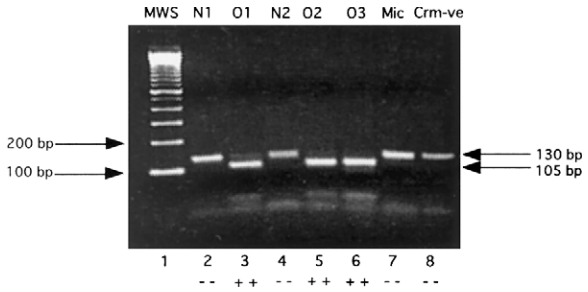

Figure 2 PCR-based diagnosis for detection of G→A transition at nucleotide 1828. A region corresponding to the genomic PC sequence (intron 12 to exon 13) was amplified from cultured skin fibroblasts by use of forward and modified reverse primers. The modified reverse primer contains two altered residues at nucleotides 1833 (G→T) and 1835 (T→C), which creates an *Xmn*I restriction site in mutant DNA (see the Material and Methods section). Lane 1, DNA size marker. Lanes 2 and 4, Normal Ojibwa PCR product. Lanes 3, 5, and 6, *Xmn*I-digested PCR products from CRM^+ PC-deficient Ojibwa patients. Lane 7, PCR product from a CRM^+ PC-deficient Micmac patient. Lane 8, PCR product from a CRM^- PC-deficient non-Indian patient. All PCR products shown were subjected to *Xmn*I restriction-enzyme digestion.

BRL), 1% dimethyl sulfoxide, 0.1 μg human genomic DNA, sterile dH_2O to 50 μl total volume, and 2.5 units of *Taq* polymerase (Gibco BRL). The polymerase was generally added in a manual "hot start" after the first denaturation step (10 min at 94°C). The general cycling parameters were 30–35 cycles of denaturation at 94°C for 0.5–l.0 min, annealing at 55°C–66°C for 0.5–l.0 min, and extension at 72°C for 30 s–l.0 min. A final extension of 72°C for 5–10 min was also included. Cycling parameters depended on the melting temperature of each primer and the length of the PCR product.

The PCR products were screened for mutations either by thermal cycle sequencing (Amersham) or by subcloning the PCR product into the pCR 2.1 vector (Invitrogen), followed by double-stranded dideoxy sequencing (Sanger et al. 1977) by use of [^{35}S]-dATP and the T7 DNA sequencing kit (Pharmacia Biotech). The sequencing reactions were then separated on a 6% acrylamide gel (Diamed) with constant current of 40 amps for 2–3 h. Bands were visualized on x-ray film (Kodak).

Diagnostic PCR for the 1828G→A (A610T) Mutation

Genomic DNA corresponding to the region containing the 1828G→A mutation was PCR amplified by use of 500 ng each of forward (5′-TAT TGG GGC AGA GGC TTG TGC-3′) and modified reverse (5′-GGA AAG CGC ATG GCG ACG TCA GAA GTG G-3′) oligonucleotides. The primer set amplifies a 130-bp fragment that, by the creation of an *Xmn*I restriction-enzyme site, detects the 1828G→A mutation, yielding digestion products of 105 and 25 bp in mutant DNA.

This PCR-based diagnostic method was employed to screen a population of 72 aboriginal controls from Manitoulin Island, 20 aboriginal controls from the White Dog Reserve in Ontario, and 20 nonaboriginal controls.

Results

Genomic Structure of the Human PC Gene

Table 3 shows the detailed structure of the gene for human PC. Exon/intron boundaries were determined by sequencing of the appropriate regions of human liver or brain genomic DNA, as described in the Material and Methods section, and by alignment of these sequences with the PC cDNA sequence (MacKay et al. 1994). The gene for the human PC contains 19 exons and 18 introns, spanning ~16 kb of genomic DNA. As shown in table 3, the 19 exons range in size from 91 bp (exon 11) to 400 bp (exon 13). The 18 introns range in size from 79 bp (intron 16) to 3.0 kb (intron 9). Except for that at the boundary of exon 10 and intron 10, all of the 5′ donor and 3′ acceptor splice sites at each exon/intron junction conform to the GT...AG rule and agree with the consensus sequence compiled for the exon/intron boundaries (Breathnach and Chambon 1981). We report the cDNA and gene structure with the proximal rather than the distal promoter elements shown for the rat as described by Jitrapakdee et al. (1997). We were not able to find upstream introns several kilobases 5′ to the initial ATG, as described for the rat PC gene (Jitra-

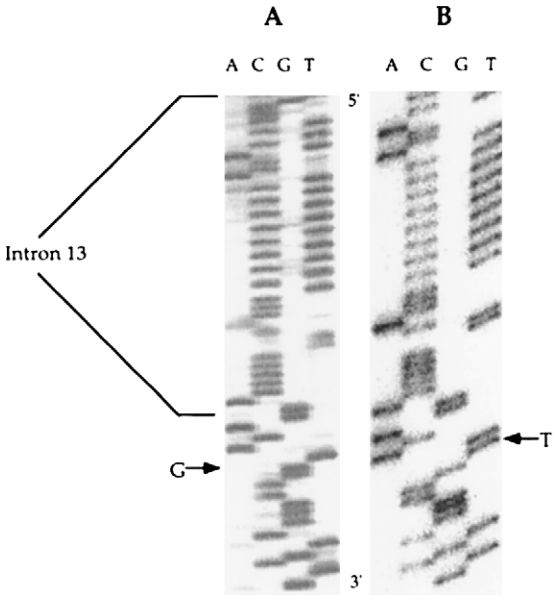

Figure 3 DNA sequence from a small region within the PC gene, showing the G→T transition at nucleotide 2229. The partial sequence of intron 13 is also shown. *A*, Wild-type sequence. *B*, Sequence from a PC-deficient Micmac homozygous for the mutation.

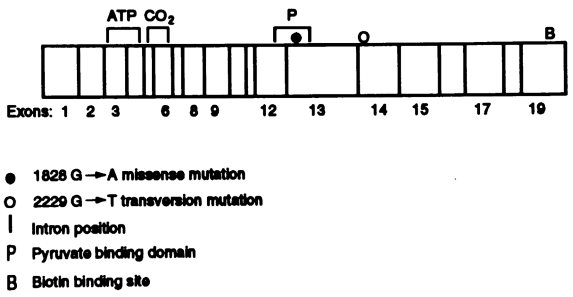

Figure 4 Schematic diagram of PC genomic structure, depicting the location of the two mutations found in the gene. Exons are numbered "1"–"19" and are drawn to scale. Also shown are the ATP, CO_2, pyruvate, and biotin-binding domains encoded by the specific exons.

pakdee et al. 1997). A set of nondistal promoter elements parallel to the one described in rat may give rise to alternatively spliced 5′ UTR versions of the human PC mRNA.

The 1828G→A (A610T) Mutation

All CRM^+ PC-deficient patients of Ojibwa and Cree origin were homozygous for the 1828G→A mutation (figs. 1 and 2). This mutation changes amino acid 610 from an alanine to a threonine in the pyruvate-binding domain of the enzyme. As outlined in the Material and Methods section, several Ojibwa and non-Ojibwa controls were screened by use of the *Xmn*I restriction-digest test. None of the 72 Ojibwa controls from Manitoulin Island and none of the 20 nonaboriginal controls exhibited this mutation. Of 40 alleles tested in 20 nonrelated control individuals from White Dog, Ontario, where many cases of PC deficiency originated, 4 were found to carry the mutation. Although this sample is not large enough to allow estimation of carrier frequencies, the latter may nevertheless be as high as 1 in 10.

The 2229G→T (M743I) Mutation

Further investigation of the CRM^+ PC-deficient patients revealed a 2229G→T mutation (fig. 3), which changes amino acid 743 from a methionine to an isoleucine in the carboxylation domain of the enzyme. This mutation was found only in two Micmac brothers from Nova Scotia.

Discussion

We have characterized the genomic structure, from the initial ATG, of the human PC gene and have identified two point mutations in patients with simple PC deficiency. This is the first report that has identified mutations responsible for PC deficiency.

It has been postulated that biotin carboxylases, including PC, evolved through gene fusion, thus leading to the existence, on one large polypeptide, of three active sites: the biotinyl domain, the transcarboxylation domain, and the biotin carboxylation domain (Obermayer and Lynen 1976). The biotin-binding lysine of PC, encoded by exon 19, is situated 34 amino acids from the C-terminus within the conserved AMKM motif common to most biotin carboxylases (Freytag and Collier 1984; Lamhonwah et al. 1987; Zhang et al. 1993; MacKay et al. 1994). The first 500 amino acid residues from the N-terminus (encoded by exons 1–10), known as the "transcarboxylation domain," are responsible for the ATP-dependent fixation of CO_2 into carboxyphosphate (Lim et al. 1988; Toh et al. 1993). Within this domain, there are a conserved GGGGRG (amino acids 198–203) motif that is known to bind ATP (Samols et al. 1988), and an ERDCSIQRR (amino acids 261–270) motif that

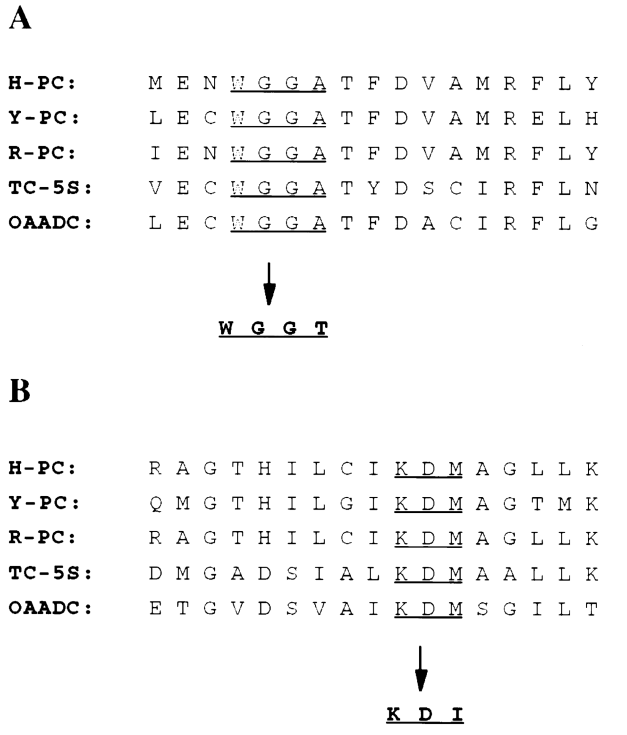

Figure 5 Comparison of the amino acid sequence of human PC (H-PC) (MacKay et al. 1994) and other biotin enzymes, including yeast PC (Y-PC) (Freytag and Collier 1984), rat PC (R-PC) (Jitrapakdee et al. 1996), the 5S subunit of transcarboxylase (TC-5S) from *Propionibacterium shermanii* (Kumar et al. 1982), and oxalacetate decarboxylase (OAADC) from *Klebsiella* (Wood and Barden 1977). *A,* Sequence alignment flanking the alanine→threonine mutation. Shown are residues 604–620 of H-PC, 595–611 of Y-PC, 603–619 of R-PC, 65–81 of TC-5S, and 41–57 of OAADC. *B,* Sequence alignment flanking the methionine→isoleucine mutation. Shown are residues 732–748 of H-PC, 718–734 of Y-PC, 732–748 of R-PC, 188–204 of TC-5S, and 162–178 of OAADC.

plays a role in CO_2 fixation (Li and Cronan 1992). Exon 13, which codes for the conserved motif WGGATATX[5]RXLRXLXXPW (amino acids 607–624) and has 32% and 34% identity to transcarboxylase and oxaloacetate decarboxylase, respectively, is the segment responsible for holding the substrate pyruvate in place for the carboxylation reaction (Samols et al. 1988). This motif is interrupted by intron 12, which is inserted at nucleotide 1830 (amino acid 609), whereas all the other catalytically important domains remain intact. All PCs except for the enzyme from *Pseudomonas citronellolis* have been shown to contain a tightly bound divalent metal. The enzymes from animal liver contain Mn^{2+}, Mg^{2+}, or both, and the enzyme from yeast contains Zn^{2+} (Utter et al. 1975). Exon 14 codes for an HIHTH (amino acids 769–773) motif that satisfies the convention for divalent cation binding.

Figure 4 depicts the organization of the exons, the location of specific domains that the exons encode, and the positions of the mutations in the gene. The gene is composed of 19 exons, ranging in size from 91 bp (exon 11) to 400 bp (exon 13) and spans ~16 kb of genomic DNA. The amino acid sequence of human PC has 98% identity with mouse PC and 96% identity with rat PC. Recently, the rat PC gene structure has been described (Jitrapakdee et al. 1997). The same group of investigators also has identified two alternative forms of human PC cDNA in liver (Jitrapakdee et al. 1996), which differ in their 5′ UTRs but have a common coding region. These two forms have been designated "hUTR A" (class I) and "hUTR B" (class II). The hUTR B form corresponds to that described elsewhere by our group (MacKay et al. 1994), on the basis of their sequence similarity at the 5′ end. Although all the upstream noncoding exons of the human PC gene have not yet been identified, available published sequences (Jitrapakdee et al. 1996) show no sequence similarities to rat 5′ UTRs. To further study similarities among the rat and human PC genes, we compared exon sequences and the positions of intron/exon boundaries. The exon/intron organization was highly conserved between rat and human. Both species of PC contain 19 exons and 18 introns from the start codon. Comparison of the position of the introns with the codons of human and rat PC genes revealed that, in the protein-coding region of the human gene, phase 0 introns occurred in 11 (61%) of the 18 introns; 5 (28%) of these 18 introns were in phase 1, and 2 (11%) of these 18 introns were in phase 2. In the protein-coding region of the rat gene, 16 (89%) of the 18 introns were in phase 0; 2 (11%) of these 18 introns were in phase 1, and 0 of these 18 introns were in phase 2. Each of the protein-coding exons was comparably sized in human and rat, with the exception of exon 12, which, relative to the equivalent exon 13 in the rat gene, was smaller in human. In the human PC gene, an intron is located at nucleotide 1827, thus interrupting the exon portion that encodes the pyruvate-binding domain's conserved motif (amino acids 607–624). In the rat PC gene, the corresponding intron is situated downstream by ~156 nucleotides, such that this motif remains uninterrupted.

Both human and rat PC have a large intron between exons 9 and 10 of human and between exons 10 and 11 of rat, which serves to separate the biotin-carboxylation and -transcarboxylation domains. There is, however, a significant size difference between the two introns. The rat intron is ~10 kb, but in the human form this intron is only ~3.0 kb. The size difference of this intron accounts for the larger size of the rat PC gene relative to the human version.

The coding exon sequences were in agreement with the cDNA sequence that we have reported elsewhere (MacKay et al. 1994). Since that publication, however, some errors in the sequence have been noted; these are listed in table A1, in the Appendix.

The mutations that we report here occur in exons 13 and 14, which include the carboxylation domain (exons 13–20) and the pyruvate-binding site. The 1828G→A missense mutation has the effect of changing amino acid 610 from an alanine to a threonine. The alanine is located in a region of homology among yeast, rat, and human PC, a region that also has homology between other carboxylases, such as transcarboxylase and oxaloacetate decarboxylase (fig. 5*A*). This segment contains the conserved motif (amino acids 607–624), which is thought to be a carboxyl-binding element for substrate binding prior to the carboxylation or decarboxylation reaction.

The 1828G→A (A610T) mutation was identified in all 13 affected patients of Ojibwa and Cree origin but was not identified in the two affected Micmac brothers. Instead, a 2229G→T transversion mutation was identified in these two brothers. This mutation is located in exon 14 of the carboxylation domain of the gene, and it alters amino acid 743, from a methionine to an isoleucine. This methionine is highly conserved among yeast, rat, and human PC, as well as among transcarboxylase and oxaloacetate decarboxylase (fig. 5*B*). The 1828G→A and 2229G→T mutations are located in both the cDNA and genomic DNA of the individuals, and both were identified only in those patients affected with simple PC deficiency. No other mutations were identified in genomic or cDNA sequences from affected individuals. Whether these mutations are deleterious to the functioning of the protein remains to be confirmed, through site-directed mutagenesis and expression studies.

The 1828G→A mutation is not a polymorphism, since, of the 72 aboriginal controls from Manitoulin Island and the 20 nonaboriginal controls, none carried the mutation, as determined by the *Xmn*I diagnostic test. Of

the 20 aboriginal controls from White Dog Reserve, 4 were carriers, on one of their alleles, of this mutation, suggesting that, for this group, the allele frequency for the mutation is possibly as high as 1 in 10. Although these results partially confirm our hypothesis that a single founder mutation might be responsible for PC deficiency in the Algonkian-speaking First Nation groups, the common mutation is present in all Ojibwa and Cree PC-deficient patients but is not present in the two Micmac siblings.

Previous studies in our laboratory (Robinson et al. 1983, 1984, 1987), which examined the levels of protein and mRNA for various patients with the two different clinical manifestations, showed that the North American phenotype displayed evidence of [^{3}H]-biotin protein, immunoreactive protein corresponding to PC, and mRNA, on northern blotting. This is consistent with the idea that the point mutations produce a protein of correct molecular weight but low expression. In contrast, the cell lines with complex PC deficiency consistently showed absence of an mRNA species, absence of immunoreactive protein corresponding to PC, and absence of a biotin-containing polypeptide. If, as is true for the rat PC gene, there are class I– and class II–type transcripts generated from 5′ UTR exons for human PC, then this implies that mutations responsible for human PC deficiency must lie within the coding region of the gene. We have found that lack of expression in liver and kidney directly correlates with a lack of expression in skin fibroblasts, implying that the defect lies in a region common to both transcripts—namely, the coding region. We believe that the complex phenotype is most likely due to deletion mutations influencing splice-site junctions either between exons and introns in the coding region or, possibly, within the introns themselves. Our laboratory is currently investigating these possibilities.

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Appendix

Table A1

Corrections to the cDNA Sequence Published by MacKay et al. (1994)

Original		Corrected		
Amino Acid	Codon	Amino Acid	Codon	Nucleotide
^{385}Pro	CCC	^{385}Arg	CGC	1154
^{486}Asp	GAC	^{486}Glu	GAG	1458
^{487}Val	GTG	^{487}Leu	CTG	1459
^{638}Arg	CGT	^{638}Pro	CCT	1913
^{727}Ala	GCT	^{727}Ala	GCC	2181
^{729}Ala	GCG	^{729}Glu	GAG	2186
^{774}Ala	GCA	^{774}Asp	GAC	2321–2322
^{775}Pro	CCG	^{775}Thr	ACG	2323

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