Am. J. Hum. Genet. 67:203–206, 2000

Report

High Incidence of Propionic Acidemia in Greenland Is Due to a Prevalent Mutation, 1540insCCC, in the Gene for the β-Subunit of Propionyl CoA Carboxylase

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Propionyl CoA carboxylase (PCC) is a mitochondrial, biotin-dependent enzyme involved in the catabolism of amino acids, odd-chain fatty acids, and other metabolites. PCC consists of two subunits, α and β, encoded by the *PCCA* and *PCCB* genes, respectively. Inherited PCC deficiency due to mutations in either gene results in propionic acidemia (PA), an autosomal recessive disease. Surprisingly, PA is highly prevalent among Inuits in Greenland. We have analyzed reverse transcriptase–PCR products of the β-subunit mRNA, to characterize the responsible mutation(s). A 3-bp insertion, 1540insCCC, was found in homozygous form in three patients and in compound heterozygous form in one patient. The resulting PCC has no measurable activity, and the mutant β-subunit appears to be very unstable. To test the hypothesis that a common mutation is responsible for PA in the Greenlandic Inuit population, 310 anonymous DNA samples of Inuit origin were screened for 1540insCCC. We found a carrier frequency of 5%, which is very high compared with those of most other autosomal recessive diseases. Analysis of alleles of a very closely linked marker, *D3S2453*, revealed a high degree of linkage disequilibrium between one specific allele and 1540insCCC, suggesting that this mutation may be a founder mutation.

Propionic acidemia (PA [MIM 232000 and 232050]) is an autosomal recessive disorder caused by a deficiency of propionyl CoA carboxylase (PCC [E.C.6.4.1.3]). PCC is a biotin-dependent mitochondrial enzyme involved in the catabolism of odd-chain fatty acids and of the amino acids threonine, methionine, isoleucine, and valine. PCC consists of two nonidentical subunits, α and β, encoded by the *PCCA* and *PCCB* genes, respectively. The native enzyme is believed to have an $\alpha_6\beta_6$ conformation (Fenton and Rosenberg 1995). The subunits are synthesized as larger precursors, imported into mitochondria, processed to mature forms, and assembled (Kraus et al. 1983). Both human αPCC cDNA and human βPCC cDNA have been cloned, and their loci have been mapped (Kraus et al. 1986; Lamhonwah et al. 1986). Patients with a defect in either *PCCA* or *PCCB* present early in life, with a severe, often fatal, metabolic acidosis, hyperglycinemia, and hyperammonemia. Survivors are prone to recurrent attacks of acidemia. Unusual forms with a later onset have been reported for both genes (Fenton and Rosenberg 1995). Recently, 53 mutations in both genes have been reviewed (Ugarte et al. 1999; for up-to-date information, see the Propionyl CoA Carboxylase Page Web site).

The worldwide frequency of PA is unknown, but the disease is considered to be very rare. Our department diagnosed five patients with PA who are of Greenlandic Inuit origin, within the period 1990–96 (the project was conducted in agreement with Helsinki declaration II and was approved by the Science Ethics Committee for Copenhagen and Frederiksberg). This number of patients is much higher than expected, considering the size and the birth rate of the Greenlandic population. The most obvious explanation for this observation is the presence of one common mutation among Inuits in Greenland.

Received March 13, 2000; accepted for publication May 4, 2000; electronically published May 16, 2000.

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0002-9297/2000/6701-0023$02.00

Table 1

PCC Activity in Skin Fibroblasts of Patients and in *E. coli*

Source	PCC Specific Activity[a] (nmol/min/mg protein)	PCC Genotype[a]
Fibroblasts:		
Controls	.42–2.6[b]	Wild type/wild type
Patient 1	.04	1540insCCC/unknown
Patient 2	NA	NA[c]
Patient 3	<.02	1540insCCC/1540insCCC
Patient 4	NA	NA
Patient 5	<.02	1540insCCC/1540insCCC
Patient 6	<.02	1540insCCC/1540insCCC
E. coli:		
Wild type	30	Wild type
513insP	0	1540insCCC

NOTE.—NA = not available.

[a] Assayed by measurement of the fixation of [^{14}C]-HCO_3^- into acid-soluble material (Kalousek et al. 1980). Protein concentration was determined by Bio-Rad assay kit.

[b] Mean activity of control cell lines.

[c] The mother was heterozygous for 1540insCCC.

The family histories of our probands were highly suggestive of previous siblings with PA. The current population size of Greenland is 55,000, of which 50,000 are of Inuit origin, and the birth rate is ~1,000/year. There was no indication of consanguinity among the families, and the birthplaces of the patients were widely scattered over Greenland. Given the birth rate mentioned above, a rough first estimate of the disease frequency at birth is 1:1,000, with a carrier frequency of 1:16.

Assuming that one mutation is responsible for PA among Inuits in Greenland, we used a single patient (patient 6) for our initial investigations. The patient had very low to undetectable activity; however, the activities found in parental fibroblasts were in the normal range. These results suggested a defect in the β-subunit of PCC (Fenton and Rosenberg 1995), and the search for mutations was first directed at *PCCB*. Amplification of overlapping segments of the βPCC cDNA, followed by SSCP, revealed a PCR product with an aberrant pattern. Reamplification of this segment, followed by direct sequencing, identified a homozygous insertion of CCC at nucleotide 1540 (1540insCCC) (results not shown). This mutation predicts an insertion of a proline residue between positions 513 and 514 in the β-subunit (513insP), without disturbance of the reading frame of the mRNA. As expected, both parents were heterozygous for 1540insCCC. Detection of 1540insCCC was performed with the following primers: 5′-FITC-CCTTTTCTGTGCTTCACCAG-3′ forward) and 5′-ACCTTCTTGCTGGCCAAGA-3′ (reverse). The sizes of the normal and mutant alleles were 103 and 106 bp, respectively. The PCR products were analyzed by an ALF-sequenator with Fragment Manager software (Pharmacia).

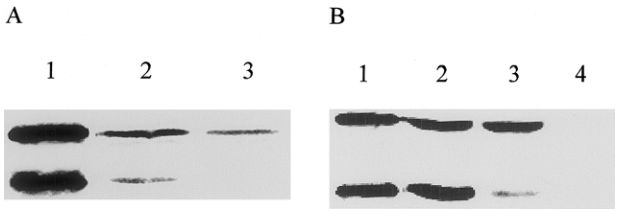

Figure 1 Western blot analysis of PCC α- and β-subunits. *A*, α- and β-subunits expressed in fibroblasts. *Lane 1,* Purified recombinant human PCC (100 ng). *Lane 2,* Control fibroblast cell line 2047 (80 μg). *Lane 3,* Cell line 3173, from the patient homozygous for 1540insCCC (80 μg). *B,* α- and β-subunits expressed in *E. coli*. *Lane 1,* Purified recombinant human PCC (100 ng). *Lanes 2 and 3,* Equal amounts of crude bacterial extracts (30 μg), expressing either wild-type PCC (*lane 2*) or 1540insCCC (513insP) (*lane 3*) protein. *Lane 4,* Bacterial extract from DH5α cells without pPCCAB plasmid (30 μg).

We had access to cell lines from two other patients and to one chorionic villus sample from a PCC-deficient fetus, from the total of six families affected by PA. Two of the samples showed homozygosity for 1540insCCC, and the other sample was compound heterozygous for 1540insCCC and another, yet-unidentified mutation (table 1).

To analyze the PCC polypeptides under steady-state conditions in fibroblasts of the patient homozygous for the 1540insCCC (patient 6, cell line 3173), we performed western blot analysis with rabbit anti-human PCC antiserum. Figure 1*A* shows that the levels of the α-subunit in both cell lines were comparable; however, the β-subunit was not detectable in the patient's fibroblasts. In contrast to the mutations in the α-subunit, which secondarily lead to the complete degradation of the β-subunit, mutations in the β-subunit were found to yield either decreased amounts or a smaller size of the subunit (Lam Hon Wah et al. 1983; Ohura et al. 1989, 1991; Rodriguez-Pombo et al. 1998).

To partially characterize the mutant PCC protein, the 1540insCCC was introduced into a pPCCAB plasmid and was expressed in *Escherichia coli*. The construction of pPCCAB plasmid containing both αPCC cDNA and βPCC cDNA has been described elsewhere (Kelson et al. 1996). The pPCCAB/1540insCCC vector was prepared by insertion of a double-stranded hybrid of two oligonucleotides (82-mer and 86-mer) into pPCCAB cut at unique *Pst* I and *Msc* I sites within exon 15 of the PCCB. After DNA sequencing, DH5α cells were transformed with the construct. PCC activity in *E. coli* extracts was assayed as described elsewhere (Kalousek et al. 1980), with the slight modification of changing the pH of Tris-HCl from 8.0 to 7.0. Protein was determined by the method of Lowry (Lowry et al. 1951).

Bacterial extracts showed no detectable activity of the mutant PCC (table 1). In addition, western blot analysis of these extracts has shown that the mutation, similar to the situation in fibroblasts, leads to degradation of

Table 2

Frequency of *D3S2453* Alleles in Controls and in Individuals Carrying 1540insCCC

Allele Frequencies in 100 Controls		Genotypes in 19 Individuals Carrying 1540insCCC	
Allele (No. of Base Pairs)	Frequency	Genotype	No. Expected[a]/ No. Found
1 (320)	.24	1-1	1/5
2 (324)	.51	1-2	4.65/8
3 (328)	.16	1-3	1.45/4
4 (332)	.09	1-4	.82/4
		2-2	4.94/4
		Other	6.13/0

[a] Calculated on the basis of frequencies found in controls, given linkage equilibrium between 1540insCCC and *D3S2453*.

the β-subunit (fig. 1*B*). This result might indicate that bacteria do not tolerate unassembled βPCC subunits and degrade them intracellularly.

The mutation is in exon 15, the last exon of the β-subunit. Exon 15, together with exon 12, are the sites where approximately half of the PCCB mutations have been identified in patients affected by PA (Ugarte et al. 1999). Moreover, on the basis of results of computer analysis, the segment encoded by exon 15 may be exposed to the surface of the oligomeric structure (data not shown). The introduction of one proline—a strong α-helix and β-sheet breaker—into this region may change the overall conformation of the PCC protein. Taken together, our results indicate that this mutation is clearly a disease-causing mutation rather than a benign variant. This is only the second mutation that has been confirmed, by expression in bacteria, to be pathogenic (Kelson et al. 1996).

At the time of the identification of 1540insCCC, the genomic structure of *PCCB* also was published (Rodriguez-Pombo et al. 1998). Using this information, we were able to design primers flanking the site of 1540insCCC, for direct amplification of genomic DNA, thereby allowing a simple population screening for the mutation. Testing two sets of data—200 anonymous, randomly chosen Guthrie (phenylketonuria) cards on children of Inuit origin who had been born in Greenland and 110 anonymous blood samples of Inuit origin that had been taken for other purposes—we identified 8 heterozygous individuals among the 200 Guthrie cards and 7 heterozygous individuals among the other 110 blood samples. The carrier frequencies were 4% and 6%, respectively ($P = .2$), of a total carrier frequency of 15/310, or 5% (95% confidence interval 1.9%–7.7%). The mutation was not found in 50 samples from Denmark. Although the Inuits from whom the 110 blood samples were obtained lived in the same part of Greenland and therefore might be related, the 200 blood-spot samples were randomly chosen and therefore are expected to be representative of the Greenlandic Inuit population as a whole; however, there was no statistical difference between the carrier frequencies in the two groups. Furthermore, the combined carrier frequency of ~5% was very close to the carrier frequency calculated on the basis of the number of patients observed as affected by PA.

To support the hypothesis of an ancestral mutation, we determined the allele frequencies of the tetranucleotide repeat STR *D3S2453,* which has been mapped to the same YAC (971_c_7) as *PCCB* (Piemontese et al. 1997), using the primers 5′-FITC-AATTACTTGCACTGGCATCC-3′ and 5′-GAGAGGGAGAAAGAAATGTGC-3′ (Genome Database accession number 685179). The fragments were analyzed as described for the detection of the 1540insCCC mutation. The allele frequencies of *D3S2453* among 50 individuals without 1540insCCC, as well as the genotypes of the 4 patients and 15 persons heterozygous for 1540insCCC, are shown intable 2. We found four different alleles of *D3S2453* in the control Inuit population. The patients homozygous for 1540insCCC were all homozygous for an allele of 320 bp (allele 1), and 14 of the 15 persons heterozygous for 1540insCCC had in common at least one copy of allele 1. Using the allele frequencies found in the 50 controls, we calculated, given linkage equilibrium, the expected genotypes for the individuals homozygous or heterozygous for 1540insCCC. We found evidence of strong linkage disequilibrium between allele 1 and 1540insCCC ($P \ll .001$; $\chi^2 = 32.5$, df 5). This finding may indicate that the high frequency of 1540insCCC among Inuits is due to a common ancestor. Unfortunately, we had no access to family members of the patients and were unable to do extended haplotype analyses. An attempt to support the hypothesis, using another polymorphic marker (*D3S3528* [Genome Database accession number 598974]) located on the same YAC but on the other site of *PCCB,* was unsuccessful, since the Inuit population was uninformative for this marker.

The present Inuit population is believed to have descended from Eskimos who settled in Greenland circa year 1200 of the common era, and mtDNA analyses have demonstrated a close relationship between the Greenlandic Inuits and the circumarctic North American Eskimos (Forster et al. 1996; J. Saillard, P. Forster, N. Lynnerup, H.-J. Bandelt, and S. Nørby, personal communication). It is likely that PA is responsible for some of the neonatal morbidity and mortality in the latter population.

Acknowledgments

We are grateful to Novo Nordic Foundation, The Leo Pharmaceutical Company Foundation, and the Dagmar Marshall

Foundation, for financial support. This work was supported in part by National Institutes of Health grant P01HD08315 (to J.P.K.). We would like to thank Ms. Tanya Reed, for growing all fibroblast cultures, and Drs. S. I. Goodman and Viktor Kozich, for helpful discussions.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org (for *D3S2453* [accession number 685179] and *D3S3528* [accession number 598974])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for PA types I [MIM 232000] and II [MIM 232050])

Propionyl CoA Carboxylase Page, http://www.uchsc.edu/sm/cbs/pcc/pccmain.htm (for up-to-date information on PCC mutations)

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