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Homozygous carnitine palmitoyltransferase 1a (liver isoform) deficiency is lethal in the mouse

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Abstract

To better understand carnitine palmitoyltransferase 1a (liver isoform, gene = *Cpt-1a*, protein = CPT-1a) deficiency in human disease, we developed a gene knockout mouse model. We used a replacement gene targeting strategy in ES cells that resulted in the deletion of exons 11–18, thus producing a null allele. Homozygous deficient mice (CPT-1a −/−) were not viable. There were no CPT-1a −/− pups, embryos or fetuses detected from day 10 of gestation to term. FISH analysis demonstrated targeting vector recombination at the expected single locus on chromosome 19. The inheritance pattern from heterozygous matings was skewed in both C57BL/6NTac, 129S6/SvEvTac (B6;129 mixed) and 129S6/SvEvTac (129 coisogenic) genetic backgrounds biased toward CPT-1a +/− mice (>80%). There was no sex preference with regard to germ-line transmission of the mutant allele. CPT-1a +/− mice had decreased *Cpt-1a* mRNA expression in liver, heart, brain, testis, kidney, and white fat. This resulted in 54.7% CPT-1 activity in liver from CPT-1a +/− males but no significant difference in females as compared to CPT-1a +/+ controls. CPT-1a +/− mice showed no fatty change in liver and were cold tolerant. Fasting free fatty acid concentrations were significantly elevated, while blood glucose concentrations were significantly lower in 6-week-old CPT-1a +/− mice compared to controls. Although the homozygous mutants were not viable, we did find some aspects of haploinsufficiency in the CPT-1a +/− mutants, which will make them an important mouse model for studying the role of CPT-1a in human disease.

Keywords: Carnitine palmitoyltransferase-1; Inborn error of metabolism; Mouse model; Gene targeting; Lethal trait

Introduction

Carnitine palmitoyltransferase 1 (CPT-1) deficiency has been identified as a potentially fatal human inborn error of metabolism. CPT-1 exists in at least two isoforms, CPT-1a, often called the liver isoform, and CPT-1b, also known as the muscle isoform. These two isoforms appear to have different expression levels depending on tissue, developmental stage, hormonal regulation, and species. In adult mice, rats, and humans, CPT-1a is the predominant isoform expressed in liver, kidney, lung, spleen, brain, intestine, pancreatic islets, and ovary [1,2]. Alternatively, CPT-1b is the primary isoform expressed in heart, skeletal muscle, testis, and both brown and white adipose tissue, except in mice where CPT-1a predominates in white adipose tissue [1,2]. CPT-1a and CPT-1b have conventionally been considered the only two isoforms of CPT-1; however recently, a third isoform, CPT-1c, has been described and is expressed predominantly in brain [3].

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Unlike medium- and short-chain fatty acids, long-chain fatty acids cannot enter mitochondria via simple diffusion. Both isoforms of CPT-1 (CPT-1a and CPT-1b) catalyze the transfer of the acyl group of long-chain acyl-CoAs to a carnitine, forming an acylcarnitine product. The resulting acylcarnitine is translocated across the mitochondrial membrane via carnitine-acylcarnitine translocase. Finally, carnitine palmitoyltransferase 2 catalyzes the conversion of acylcarnitine back to acyl-CoA, allowing it to proceed with the succeeding steps of the mitochondrial β-oxidation pathway. CPT-1, but not CPT-2, is sensitive to inhibition by malonyl-CoA. Thus, CPT-1 has a pivotal regulatory role in the inward mitochondrial flux of fatty acid substrates, as well as serving as a key point for regulation of mitochondrial β-oxidation [4].

β-oxidation of fatty acids is a vital cellular process responsible for supplying 80–90% of cellular energy requirements during prolonged fasting [5]. Although most tissues are reliant on fatty acid oxidation to provide cellular energy during the fasted state, long-chain fatty acid oxidation is the primary source of energy for heart and skeletal muscle in the fed and fasting state [6]. Furthermore, infants, who generally have limited glycogen stores, are heavily reliant on long-chain fatty acid oxidation for energy [7].

CPT-1a deficiency has been found in more than 30 children [8]. Adults and children with inborn errors of fatty acid oxidation exhibit severe metabolic disturbances including hypoketotic-hypoglycemia, and fatty liver [9]. Disease occurs most commonly in children from birth to 2 years of age, although adult patients have presented as well. CPT-1a deficient patients may have residual CPT-1a activity, measured in fibroblasts, with values ranging from 0 to 65% of controls [8,10]. Although overall pathogenesis is complex, impaired β-oxidation of fatty acids is an essential part of the disease. As a consequence of its pivotal role in fatty acid oxidation, CPT-1a deficiency can be fatal. The acute effects of CPT-1a deficiency occur primarily in young patients as a result of catabolic stress and are reported to recede with aging. To better understand mechanisms in acute disease, we developed a gene knockout mouse model for CPT-1a deficiency.

Materials and methods

Construction of targeting vector and gene targeting in ES cells

The *Cpt-1a* targeting vector was constructed from genomic DNA fragments derived from a mouse 129X1/SvJ genomic P1 clone, PV1. The P1 clone was identified by screening a mouse 129X1/SvJ strain genomic library by PCR (Genome Systems, St. Louis, MO) (Fig. 1). Exons 11–18 were deleted by a replacement gene targeting strategy (Fig. 1) by gene transfer into ES cells. The targeted ES cells were used to generate mice with a null allele (*Cpt-1a*$^{tm1\,Uab}$). ES cells (TC-1) were originally derived from 129S6/SvEv mice. Screening for recombinant ES cell clones was done by G418 selection (350 μg/ml) for 7 days. Surviving colonies were picked and expanded for Southern blot analysis.

Mice

Chimeric mice were produced by microinjection of gene targeted ES cells into C57BL/6NTac (B6) embryos. The chimeric founders were bred to 129S6/SvEvTac

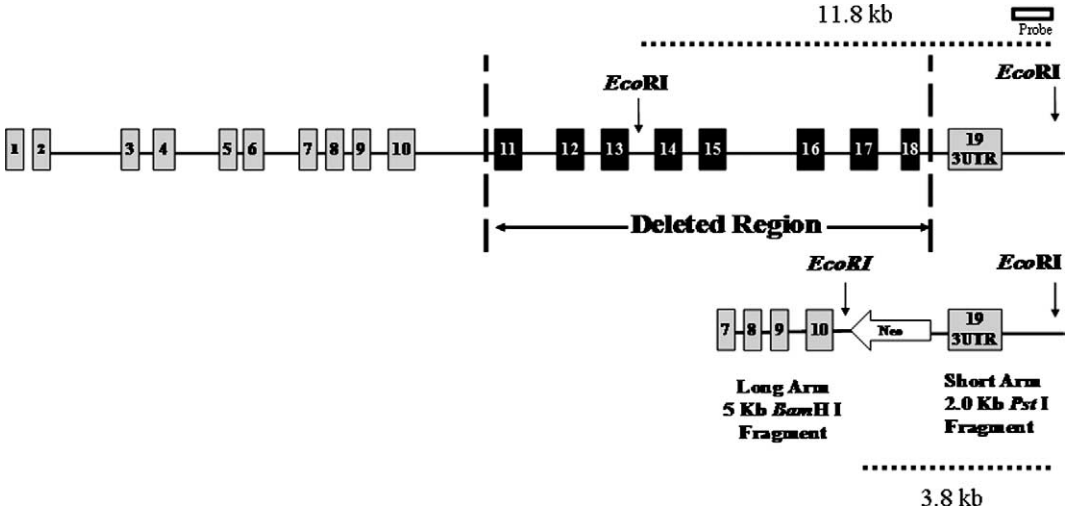

Fig. 1. Gene targeting strategy consisted of replacement of exons 11–18 with a 1.8 kb Neo insertion cassette containing a phosphoglycerate kinase promoter and a bovine poly(A) signal derived from pNTK vector [34]. A genomic probe located 3′ to exon 19 was used to detect both restriction fragments as indicated. *Note*. Not drawn to scale.