ORIGINAL ARTICLE

Clinical and Biochemical Heterogeneity in an Italian Family with CPT II Deficiency due to Ser 113 Leu Mutation

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ABSTRACT: ***Background:*** Carnitine palmitoyltransferase II (CPT II) deficiency is an autosomal recessive disorder which presents with recurrent myoglobinuria. Heterozygotes are usually asymptomatic. ***Methods:*** We correlate the clinical, biochemical and molecular features of a family in which the proband is homozygous for CPT II deficiency, due to the common Ser 113 Leu mutation. ***Results:*** The 20-year-old female proband presented at age three years with episodic myalgia and myoglobinuria, elevated creatine kinase (CK) of 3600 IU/L and had a 33% residual CPT II activity in cultured skin fibroblasts. Her 25-year-old dizygotic twin brothers presented with muscle stiffness following prolonged exercise but no overt pigmenturia and had interictal CKs up to 662 IU/L. Her parents and a 13-year-old brother are asymptomatic. An elder sister, not investigated, had recurrent pigmenturia and died at eight years with myoglobinuria. Molecular analysis revealed that the proband is homozygous for the Ser 113 Leu mutation. Her parents are heterozygotes with CPT II activities of 55% to 70%. Her younger brother is normal with 83% activity. The symptomatic twin brothers are heterozygous but demonstrated unexpectedly low CPT II activities of 40%, which may explain their phenotype. ***Conclusion:*** We postulate that there may be genetic, environmental and sex hormonal factors accounting for this manifesting heterozygosity and biochemical heterogeneity in CPT II deficiency.

RÉSUMÉ: Hétérogénéité clinique et biochimique chez une famille italienne porteuse d'un déficit en CPT II dû à une mutation ser 113 leu. ***Introduction:*** Le déficit en carnitine palmitoyltransférase II (CPT II) est une maladie récessive autosomique ayant comme mode de présentation une myoglobinurie récurrente. Les hétérozygotes sont habituellement asymptomatiques. ***Méthodes:*** Nous décrivons les caractéristiques cliniques, biochimiques et moléculaires d'une famille dont le cas index est porteur d'un déficit en CPT II à l'état homozygote dû à une mutation fréquente, une substitution ser 113 leu. ***Résultats:*** Le cas index, une patiente maintenant âgée de 20 ans, a été vue pour la première fois à l'âge de trois ans parce qu'elle présentait des épisodes de myalgie et de myoglobinurie et un taux élevé de créatine-phosphokinase (CPK) à 3600 U/L. L'activité résiduelle de la CPT II était de 33% dans ses fibroblastes cutanés en culture. Ses deux frères âgés de 25 ans, des jumeaux fraternels, avaient de la raideur musculaire après un exercice prolongé, sans myoglobinurie évidente et un taux interictal de CPK qui atteignait 662 U/L. Ses parents et un frère âgé de 13 ans sont asymptomatiques. Une sœur aînée, qui n'a pas été investiguée, avait une pigmenturie récurrente et elle est décédée à 8 ans d'une myoglobinurie. L'analyse moléculaire a montré une mutation ser 113 leu à l'état homozygote chez le cas index. Ses parents sont hétérozygotes et leur activité CPT II varie de 55% à 70%. Son jeune frère est normal et l'activité CPT II chez-lui est de 83%. Cependant, l'activité CPT II est singulièrement basse à 40% chez ses deux frères jumeaux hétérozygotes qui sont symptomatiques, ce qui peut expliquer leur phénotype. ***Conclusion:*** Nous postulons que ce tableau clinique et cette hétérogénéité chez des hétérozygotes pour un déficit en CPT II pourraient être dus à des facteurs génétiques, environnementaux ou en relation avec les hormones sexuelles.

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Carnitine palmitoyltransferase (CPT) deficiency is one of the most common autosomal recessive inherited disorders of lipid metabolism, affecting skeletal muscle.[1,2] The CPT II enzyme is located on the inside of the inner mitochondrial membrane.[3,4] It participates in the transfer of long chain fatty acids from the cytosol into the mitochondria by converting palmitoylcarnitine and Coenzyme A (CoA) to palmitoylCoA and palmitoylCoA and carnitine. Fatty acid oxidation in the liver is responsible for the generation of ketones, which are essential for cerebral energy metabolism during fasting hypoglycemia.[5] Fatty acid oxidation is important in all tissues with high bioenergetic demands such as muscle, nerve, heart, small bowel and kidney[6] and serves as a primary energy source in resting muscle and during prolonged aerobic exercise[7]. It is also important in shivering thermogenesis.

The CPT II protein is a homotetramer encoded by the CPT II gene, which is located on chromosome 1p32.[8] The gene spans

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approximately 20kb and is composed of five exons.[8,9] More than 39 mutations have been identified; however, the Serine 113 Leucine mutation is the most common, accounting for approximately 60% of the mutant CPT II alleles, and is due to a C-to-T transition at nucleotide 439.[10]

Carnitine palmitoyltransferase II deficiency (OMIM #600650) is clinically heterogeneous and has two well-recognized and distinct clinical phenotypes.[11-13] The benign classic muscular form (OMIM #255110) is the most common presentation and is seen in young adults. It typically presents with recurrent episodes of muscle pain, weakness, and paroxysmal myoglobinuria with elevated serum creatine kinase (CK) and, in some cases, acute renal failure. These crises are triggered by prolonged exercise, cold exposure, or infection with fever and are associated with a decreased residual CPT II activity of approximately 20-25% in cultured skin fibroblasts. The severe infantile, usually lethal, *hepatocardiomuscular form* (OMIM #600649) is uncommon and presents in infants with hepatomegaly, myopathy, cardiomyopathy and episodic hypoketotic, hypoglycemic encephalopathy. There is laboratory evidence of elevated serum CK, low total serum carnitine concentration with elevated long chain acylcarnitines, dicarboxylic aciduria and residual CPT II activity as low as 10% in fibroblasts. Patients are primarily at risk when exposed to precipitating factors that stress fatty acid oxidation, such as fasting, infectious illness, vomiting, prolonged exercise, cold exposure and in some cases, emotional stress. The mainstay of therapy is, therefore, preventative with the avoidance of precipitating factors. In addition, a high carbohydrate, low fat diet and frequent feeding decrease the risk of rhabdomyolysis in CPT II deficient patients. We now report an Italian family, with CPT II deficiency due to the Serine 113 Leucine (Ser 113 Leu) Leu mutation, which presents with clinical and biochemical heterogeneity.

Case Report

The proband is a 20-year-old girl of Italian descent, who presented at the age of three years, with an acute episode of severe myalgia, muscle weakness involving the extremities and neck, as well as respiratory muscles requiring ventilatory assistance and myoglobinuria precipitated by an acute upper respiratory tract infection due to the Epstein Barr virus. On general examination, she appeared pale and ill and was febrile. On neurologic examination, she was alert and oriented. Her cranial nerve examination was normal. On motor examination, she was noted to have significant weakness, with an inability to lift her head from the bed. Manual motor testing (Medical Research Council standard, MRC scale, M1--M5)[14] revealed[14] grade 3/5 weakness in the neck flexors and extensors and grade 4/5 weakness in the remaining muscle groups. Muscle tenderness was noted in all major muscle groups. Muscle tone and deep tendon reflexes were normal in both upper and lower extremities. Plantar reflexes were down going bilaterally. Cerebellar coordination, sensation and autonomic functions were normal.

Her biochemical investigations at that time revealed a serum CK > 3600 IU/L (normal <150), markedly elevated transaminases and myoglobin in the urine. Serum electrolytes, urea, creatinine, glucose, ammonia and lactate were normal. Serum and urine amino acids and organic acids were also normal. Electrocardiogram and chest X-ray were normal. Electromyography revealed mild myopathic discharges. She was managed with bed rest and an intravenous glucose infusion (10mg/kg/min glucose). All her biochemical abnormalities normalized and she recovered fully in 14 days, without residual weakness. Following recovery, muscle biopsy of the right triceps demonstrated type 1 fibre predominance and no evidence of necrosis, degeneration, regeneration, atrophy or hypertrophy. The Oil-Red-O, Gomori trichrome, succinic dehydrogenase (SDH) and cytochrome oxidase (COX) stains were normal. Electron microscopy revealed normal mitochondria and no accumulation of lipid. At the age of 12 years, she suffered another episode of myalgia and myoglobinuria, precipitated by a streptococcal throat infection. Her neurological examination at that time was characterized by muscle tenderness and minimal weakness. Enzyme assay of cultured skin fibroblasts demonstrated a 33% residual CPT II activity, which supported the diagnosis of CPT II deficiency. Molecular analysis identified the proband as homozygous for the Serine 113 Leucine mutation. She has been managed according to standard CPT II management guidelines with a high carbohydrate and low fat diet, frequent feedings, as well as the avoidance of precipitating factors, such as fasting, prolonged exercise, cold exposure and infectious illness with fever and vomiting. Since then she has experienced only minor episodes of myalgia with viral infections, but without overt pigmenturia or weakness. Following the management guidelines with carbohydrate loading prior to and at regular intervals during exercise (e.g. every 15 minutes), her exercise tolerance has improved markedly. She is able to exercise for sequential 15 minute periods of time, followed by brief rest periods accompanied by carbohydrate loads, for a total duration of 30 minutes at a time during gymnastics class at school and has not suffered from subsequent muscle pain, stiffness or weakness. Her neurological examination between acute episodes has remained entirely normal. In addition, her interictal serum CK and plasma carnitine concentrations are normal.

Her parents are consanguineous and originate from Calabria, Italy. Both parents are clinically asymptomatic with normal neurological examinations and are physically active at work with an exercise tolerance for mild-moderate intensity exercise of more than four hours duration at a time. The proband's 25-year-old brothers, who are non-identical twins, are also physically active and participate in regular recreational sports and have experienced milder symptomatology. They have both noticed exertional myalgia, characterized as muscle stiffness in the quadriceps, hamstrings and peroneal muscles, following 1 – 1.5 hours of prolonged exercise, during sports activities such as soccer. There has been no apparent aggravation of their muscle pain with cold exposure or infections. They have also experienced episodes of lightheadedness after periods of fasting in the past. Both had interictal serum CKs up to 662 IU/L. They have had no acute episodes of myoglobinuria or hypoglycemic encephalopathy. Both brothers have normal neurological examinations and have excellent muscle power when compared to their peers. An older sister had experienced recurrent episodes of pigmenturia and myalgia during the first three years of her life, all precipitated by infection with fever. She was then reportedly well between the ages of three to eight years. However, at eight years of age, while on a trip to Europe, she suffered another febrile illness associated with 12 hours of vomiting with fasting, and suffered a final episode of pigmenturia, muscle weakness and acute renal failure and died during this episode. The youngest brother is 13 years old and is entirely asymptomatic with a normal neurological examination.

Materials and Methods

All studies were performed with the approval of the Institutional Review Board of the Hospital for Sick Children in

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Toronto. Cultured skin fibroblasts from the proband and her family and from normal controls were grown to confluence in alpha-MEM medium with 10% fetal calf serum. CPT I and CPT II activities were assayed in cultured skin fibroblasts in the laboratory of Dr. J. Denis McGarry (Southwestern Medical Centre, University of Texas, Dallas) by a modification of the method of Declercq et al.[15]

Genomic DNA was extracted according to standard protocols from cultured skin fibroblasts or peripheral leukocytes of the proband and her relatives and from ten normal controls without CPT II deficiency. The DNA was screened for the four reported missense mutations associated with the typical, adult form of CPT II deficiency, namely the S113L mutation which appears to be common among both European and American patients,[10,16] and three rare mutations R631C, P50H and D553N. The four mutations were screened by polymerase chain reaction (PCR) amplification and restriction enzyme digestion, using primer sets and protocols as previously described.[9,10,16,17]

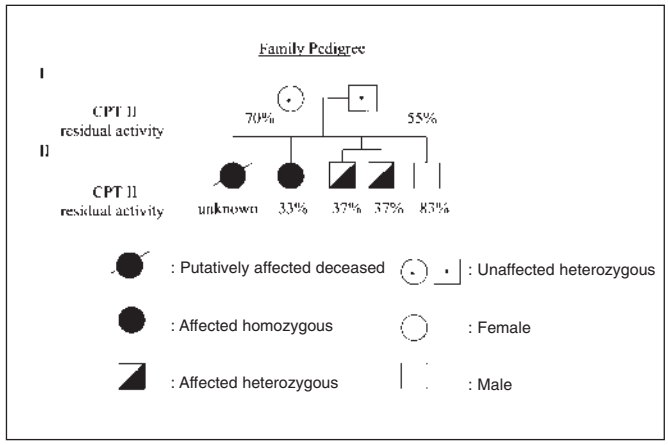

Figure: *Pedigree of the family with CPT II deficiency.*

Results

Enzyme activities: All members of the family had entirely normal fibroblast CPT I activities compared to controls. On analysis of fibroblast CPT II activities, the index patient had a residual CPT II activity of 0.123 nmoles/min/mg fibroblast homogenate (controls = 0.364 ± 0.009; n=20); the 33% residual activity suggests that she is homozygous mutant for the CPT II gene defect. The CPT II activities in nmoles/min/mg fibroblast homogenate were 0.252 in her mother (70% residual), 0.199 in her father (55% residual), 0.146 in her fraternal twin brothers (40% residual), and 0.303 in her youngest brother (83% residual). The 70% and 55% residual activities in her mother and father, respectively, are consistent with their clinically asymptomatic heterozygous state. The 40% residual CPT II activity in her twin brothers is somewhat inconsistent with their mild clinical phenotype. The 83% residual CPT II activity in the youngest brother suggests that he is homozygous normal for the gene.

Mutation Analysis: Molecular analysis of the DNA by the PCR and restriction fragment length polymorphism identified a missense mutation, Serine 113 Leucine, in the CPT II gene of this pedigree. The index patient is homozygous mutant for this mutation. Her asymptomatic parents and her symptomatic twin brothers are heterozygous. Her asymptomatic younger brother is homozygous normal for this gene. Her deceased elder sister was never investigated for CPT II deficiency but was likely homozygous mutant given her early onset and severe presentation (Figure and Table).

Discussion

Carnitine palmitoyltransferase II deficiency is an autosomal recessive disorder of lipid metabolism. The classic adult muscular form of CPT II deficiency manifests in homozygous individuals with episodic muscle pain, weakness and myoglobinuria precipitated by prolonged exercise, fasting, cold exposure or infection with fever.[1] Heterozygotes are usually, though not always, asymptomatic. We found significant biochemical heterogeneity in this Italian family with CPT II deficiency. Biochemical and molecular analysis of the family showed that the index patient, who presented with the typical clinical features of adult CPT II deficiency, is homozygous for the Ser 113 Leu mutation and has a somewhat higher than expected residual CPT II activity of 33% in cultured skin

Table 1: Biochemical Phenotype and Molecular Genotype of Affected Proband and Family

	Female Proband	Twin Brother A	Twin Brother B	Father	Mother	Youngest Brother
CPT II activity (controls = 0.364 ± 0.009 nmoles/min/mg fibroblasts)	0.123	0.146	0.146	0.199	0.252	0.303
% residual CPT II activity	33	40	40	55	70	83
Ser 113 Leu mutation	+/+	+/-	+/-	+/-	+/-	-/-

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fibroblasts. Her parents, who are asymptomatic, are heterozygous for the Ser113Leu mutation and have intermediate residual CPT II activities of 55-70%, consistent with their heterozygous state. Her older dizygotic twin brothers were found to be heterozygous for this mutation but demonstrated unexpectedly low residual CPT II activities of 40% and were mildly symptomatic. Their low residual CPT II activity is similar to that of their homozygous mutant sister (index patient) and may explain their mild clinical phenotype.

Another notable observation is the relatively higher residual CPT II activities in the female members of this family. The index patient is homozygous mutant but the 33% residual fibroblast CPT II activity is higher than expected in the classic adult muscular form of CPT II deficiency, where residual activity is usually 20-25%.[11,12] In addition, though both parents are heterozygotes by molecular analysis, the mother has higher residual CPT II activity (70%) than the father (55%). Conversely, the symptomatic twin brothers have lower residual CPT II activities than expected for their heterozygous mutant state, and this may explain their clinical symptomatology. It is possible that sex-related factors contribute to the relatively lower residual CPT II activities seen in the male members of this family.

Clinical heterogeneity in CPT II deficiency has been previously reported in the literature and has been largely attributed to differences in the severity of the mutations in the CPT II gene, of which at least 39 have been identified to date.[11,16,18] For example, the Ser-113-Leu substitution (adult form) was shown to result in a 20% residual CPT II activity in fibroblasts, without significant impairment of long chain fatty acid (LCFA) oxidation whereas the Tyrosine-628-Serine substitution (severe infantile form) resulted in a 10% residual CPT II activity, markedly impairing LCFA oxidation.[11] Thuillier et al[18] analyzed a cohort of 20 CPT II-deficient patients affected either with the severe infantile (seven patients) or with the adult-onset forms (13 patients) of the disease and identified 13 CPT II mutations, including five novel ones (R124Q, N146T, R161W, D328G and D608H). Based on the consequences of the different mutations on CPT II activity and on LCFA oxidation, they concluded that both the type and the site of the mutations, together with one or more additional genetic factors that remain to be identified, could modulate the LCFA flux and thereby the severity of the clinical presentation.

Compound heterozygosity for different CPT II mutations has also been postulated. However, in the present family all individuals have CPT II deficiency due to the same gene defect. Marked clinical heterogeneity with the same homozygous Ser-113-Leu substitution has been previously reported in a brother and sister, children of a consanguineous marriage, and in their first cousins, also the products of a consanguineous marriage.[19] The proband, a male, suffered from typical adult CPT II deficiency with recurrent myoglobinuria and his sister was less severely affected with muscle stiffness and dark urine following prolonged exercise. One of their homozygous mutant female first cousins was relatively asymptomatic with the exception of reduced tolerance to prolonged exercise and responded well to carbohydrate ingestion. A second female first cousin, who was not tested for CPT II deficiency, died at 16 years of age during a severe episode of myoglobinuria. Different clinical manifestations in three relatives with the same heterozygous gene defect (R503C) have also been reported.[20] The proband in this family, a 54-year-old woman, had a 35-year history of progressive weakness with residual CPT II activities of 47% in lymphoblasts, 43% in cultured skin fibroblasts and 13% in skeletal muscle. Her heterozygous son had a lifelong history of myopathic symptoms while his heterozygous grandfather had only mild weakness during childhood.

Our data suggest that the differences in clinical phenotype in heterozygotes with the same CPT II gene defect, may be due to various factors influencing residual CPT II activity including an additional single gene defect carrier state in another fatty acid oxidation enzyme, additional undetected mutations in the CPT II gene, modifying genes within the same family, a variable exposure to precipitating factors, and perhaps the influence of sex hormones.

We conclude that heterozygotes for the Ser 113 Leu mutation are at risk for clinical symptomatology and that this genotype may be associated with significant biochemical heterogeneity in residual CPT II activity. The clinical phenotype appears to correlate with the degree of residual CPT II activity. Finally, the same genotype may have variable phenotypic expression related to additional genetic or environmental factors.

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