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Identification of Novel Mutations in Cpt1A Gene in a Patient with Carnitine Palmitoyltransferase IA Deficiency Presenting with Cholestatic Jaundice

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Abstract

Background: Carnitine Palmitoyltransferase IA (CPT IA; MIM #255120) deficiency is a rare autosomal recessive inherited disorder of mitochondrial fatty acid oxidation. Patients experience a rapid onset of symptoms which include “Reye-like” hepatic encephalopathy precipitated by fasting or any intercurrent illness, followed by hepatomegaly and hypoketotic hypoglycaemia.

Objective: To perform clinical, biochemical and genetic characterization of a patient with CPT1A deficiency.

Designs and methods: We present a case of a patient with unresolved cholestatic jaundice, failure to thrive and gross developmental delay. Physical examination showed moderate hepatomegaly with mild hypotonia.

Results: Basic laboratory investigations showed moderate transaminitis with cholestasis and compensated metabolic acidosis. Dried blood spot acylcarnitine showed marked elevation of free carnitines (C0) and C0/C16+C18 ratio with suppressed levels of C16, C16OH, C18:1 and C18 Organic acid analysis showed a dicarboxylic aciduria with a prominence of the C12 dicarboxylic (dodecanedioic) acid and increased excretion of 3-methylglutaconic acid. CPT1A mutation analysis identified two novel mutations, c.1244C>A p. (Ala415Glu) in exon 11 and c.1450del p. (Leu484Phefs*47) in exon 12.

Conclusion: Unsuspected CPT1A deficiency was diagnosed in a jaundiced patient from the selective screening for inborn errors and metabolism and confirmed by molecular analysis.

Keywords: Carnitine palmitoyltransferase 1A deficiency • Hepatomegaly • Gene mutation • Newborn screening • Dicarboxylic acid

Abbreviations: CPT1A: Carnitine Palmitoyl Transferase 1A; FAO: Fatty Acid Oxidation; C12: dodecanoic acid; LCMS: Liquid Chromatography Tandem Mass Spectrometry; GCMS: Gas Chromatography Mass Spectrometry

Introduction

The Carnitine Palmitoyl Transferase (CPT) plays an important role as an enzyme and transporter which facilitates the transport of the long chain fatty acid groups from the cytosol into the mitochondrial matrix *via* the action of carnitine acylcarnitine translocase prior to its subsequent beta-oxidation. The first component of the system, Carnitine Palmitoyl Transferase I (CPT 1, EC 2.3.1.21), is located on the outer mitochondrial membrane and catalyzes the conversion of cytosolic long-chain acyl-CoAs into their respective acylcarnitine species, which is the first step in the carnitine shuttle [1,2]. Besides that, CPT I plays an important role as the rate-limiting step of mitochondrial fatty acid oxidation and controls the first few steps in the pathway of mitochondrial fatty acid oxidation. It is a key site for metabolic regulation, being subject to inhibition by malonyl-CoA, the first intermediate in the pathway of beta fatty acid oxidation.

Deficiency of carnitine palmitoyl transferase I hepatic type (CPT IA; EC 2.3.1.21, MIM #255120) is a rare autosomal recessive inherited disorder of mitochondrial fatty acid oxidation characterized by accumulation of long chain fatty acids in the liver due to a mutation in the CPT 1A gene with fewer than 30 reported cases which was first described by Bougneres et al in 19812. There are three CPT I isoforms encoded by distinctive genes which have been identified as liver-type (L-CPT I or CPT IA), muscle-type (M-CPT I or CPT IB) and brain (CPT IC) isoforms. Generally, the three CPT I isoforms are encoded by distinct genes which is derived from a common CPT ancestor gene. Of these, only defects in CPT1A is associated with human disease with a total of 25 mutations being identified by Korman SH, et al. [1], Naher N, et al. [2] and IJlst L, et al. [3]. Children generally manifests as an acute "Reye-like" hepatic encephalopathy which is aggravated by a long fasting or underlying intercurrent illness. Typical clinical features include life-threatening attacks of hypoketotic hypoglycemia and altered consciousness progressive to comatose, hepatomegaly and deranged liver function during the first two years of life [4,5]. The manifestations of cardiac or skeletal muscle involvement in these children is quite rare [4,5]. The CPT1A gene (NM_001876.4) is located on chromosome 11 at the 11q13.1-q13.5 locus and encodes a 773 amino acid polypeptide monomer [4]. The prevalence of this disease is low with less than 100 cases reported in The Human Gene Mutation Database (HGMD). In this

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report, we describe the first case of a Malaysian boy with CPT1A deficiency and provide comprehensive clinical, biochemical and genetic characterization of the patient.

Case Presentation

A 6-month-old boy with no known medical illness and an unremarkable birth history presented with a sudden onset of progressively worsening jaundice with intermittent vomiting for two weeks. He was observed by the parents to have failed to thrive since the age of 3 months. Otherwise, he remained well with no history of recurrent illness or metabolic symptoms until the presentation. He was noted to be less active, however, no fever or excessive crying or abdominal distension were noted. There was no history of neuroimpairment. His blood parameters showed conjugated hyperbilirubinemia with transaminitis. Patient was the youngest born from a non-consanguineous parent with 2 healthy elder siblings aged 9 years old and 6 years old. Both siblings had an uneventful birth history and had no medical illnesses including chronic liver disease. Both of them are attending school and active in their daily routine activities. There was no history of either recurrent maternal miscarriage or early neonatal death. There was also no family history of sudden death or metabolic diseases. At the time of presentation, his physical examination revealed a small for age with weight 5.7 kg (<3rd centile) and length 64 cm (10^{th} centile) and head circumference 42 cm (<3rd centile).

Clinically, he was not encephalopathic, or pale but had a tinge of jaundice. Abdominal palpation revealed a hepatomegaly measuring 4cm span in the right hypochondriac region. There was no facial puffiness, edema, splenomegaly or enlarged lymph nodes. Cardiovascular and respiratory system were unremarkable. Neurological examination showed presence of central hypotonia with a mild head lag. Otherwise, his reflexes were normal with antigravity movement seen over bilateral upper and lower limbs. Upon admission, initial blood investigations showed transaminitis with a cholestatic picture (AST 145 IU/L, Normal range: <40IU/L, ALT 107 IU/L, Normal range: <41 IU/L and ALP 889 IU/L, Normal range: 40-129 IU/L). Blood gas analysis showed a compensated metabolic acidosis with normal lactate and renal profile. Plasma ammonia was not available. A semi quantitative test for ketones in urine was negative and infective screening and viral serology was unremarkable. He was started on ursodeoxycholic acid and 3 days of intravenous vitamin K due to a borderline coagulation profile. Urgent ultrasound of the hepatobiliary system confirmed a hepatomegaly with a mild coarse echotexture, abnormal gallbladder and increased hepatic artery to portal vein ratio with the suspicion of biliary atresia. The child was referred subsequently to the pediatric surgical team to exclude choledochal cyst. He was then treated with five days of antibiotics despite normal inflammatory markers. Throughout the admission, the child remained hemodynamically stable. He was able to tolerate frequent breastfeeding on demand with the occasional weaning diet. No medium chain triglycerides feeding were initiated at that time in view of provisional diagnosis of cholestatic jaundice and cholangitis. There was only one episode of hypoglycemia (blood glucose monitoring 3.6mmol/L) observed at pre-feeding. Otherwise, the glucose monitoring remained stable, ranging from 4.2 to 6 mmol/L since admission. Subsequent echocardiography showed no evidence of cardiomyopathy. Repeat blood investigation after five days courses of antibiotics showed slightly improving liver profile but a persistent compensated normal anion gap metabolic acidosis. In view of the clinical finding, he was investigated further for the possibility of inborn errors of metabolism disorders. Dried blood spot inborn errors of metabolism screening using Tandem Mass Spectrometry (TMS) showed a marked elevation of free Carnitine (C0) (214.81 umol/L) with low levels of C16 (0.14 umol/L), C16OH (0 umol/L), C18:1 (0.07 umol/L) and C18 (0.03 umol/L) and marked elevation of C0/C16+C18 ratio (1265) which was suggestive of Carnitine palmitoyl transferase 1A deficiency (CPT1A deficiency). Urine Organic acid analysis using Gas Chromatography Mass Spectrometry (GCMS) showed dicarboxylic aciduria with a prominence of the C12 dicarboxylic (dodecanedioic) acid with a slight increase in the excretion of lactate with a small pyruvate peak and a moderate increased excretion of 3-methylglutaconic acid with slightly increased excretion of 3-hydroxy 3-methylglutarate and glutarate (Figure 1). Plasma amino acids by High Performance Liquid Chromatography (HPLC) demonstrated non-specific mild elevations of few amino acids (Figure 1).

Mutation analysis was proceeded after a parental informed consent. We identified two novel compound heterozygous mutations, c.1244C>A p. (Ala415Glu) in exon 11 and c.1450del p. (Leu484Phefs*47) in exon 12 (Figure 2). To our knowledge, both variants had not been reported elsewhere including the HGMD database (public). Both variants were also absent in large variant databases such as the Genome Aggregation Database (gnomAD),

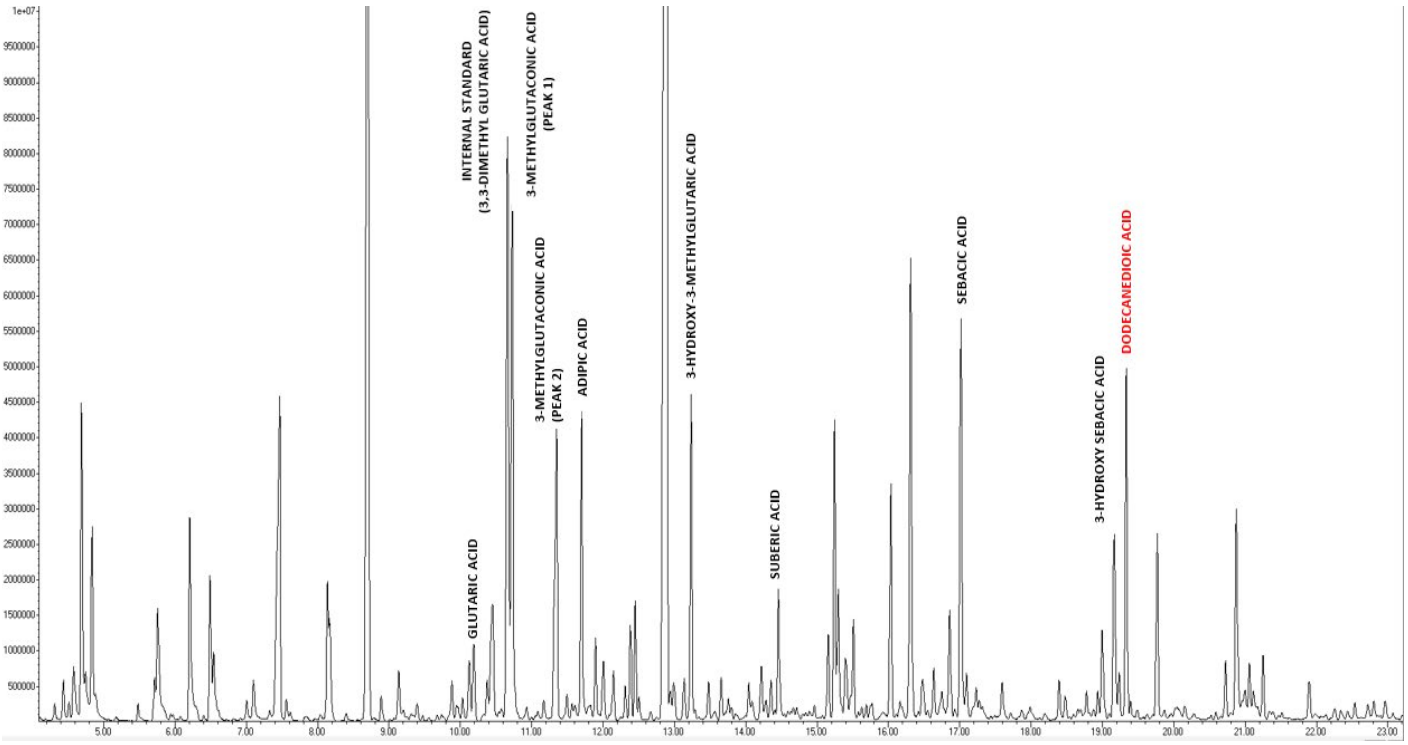

Figure 1. Urine organic acid analysis using gas chromatography mass spectrometry showed dicarboxylic aciduria with a prominence of the C12 dicarboxylic (dodecanedioic acid, in red). In addition, there is a moderate increased excretion of 3-methylglutaconic acid with slightly increased excretion of 3-hydroxy 3-methylglutarate and glutarate.

GenomeAsia and Regeneron Genetics Center (RGC) Million Exome database. Analysis by VarSome predicted the c.1244C>A mutation as Variant 0f Uncertain Significance (VUS), while the c.1450del mutation was reported as a likely pathogenic which extends the genetic heterogeneity in CPT IA deficiency (Figure 3). The c.1244C>A has a Combined Annotation Dependent Depletion (CADD) score of 24.0, suggesting it is likely pathogenic. Next, Frazer J, et al. [6], Cheng J, et al. [7] and Brandes N, et al. [8] employed two deep learning models and one deep protein language model to predict the pathogenicity of this variant, namely the Evolutionary Model of Variant Effect (EVE), Alpha Missense (AM) and ESM1b. All three models predicted the variant as pathogenic, where the score is 0.879 for EVE (pathogenic cutoff >0.5), 0.979 for AM (pathogenic cutoff>0.5), -14.84 for ESM1b (pathogenic cutoff<-10.0).

The child was started on Medium Chain Triglyceride (MCT) oil, supplements in addition to his normal diet which was later changed to portagen as he developed allergic reaction to the MCT oil in addition of supplements. The cholestatic jaundice had resolved and he remained well with no episodes of metabolic crisis. His blood investigations in the ward still showed a transaminitis picture with similar compensated metabolic acidosis. Family counseling and dietary modification education was delivered to the parents. At the subsequent clinic visits, he remained healthy with improved gross motor development. He was tolerating feeding well, thriving along the 3rd-5th centile for his age. His blood parameters showed a resolution of metabolic acidosis with normalization of liver profile (Figures 2 and 3).

Results and Discussion

CPT IA deficiency is a life-threatening fatty acid oxidation disorder whom mostly present as recurrent episodes of hypoketotic hypoglycemic after prolonged fasting or infection. Our patient demonstrated hepatomegaly with transaminitis and cholestatic jaundice picture along with failure to thrive and mild hypotonia as the first manifestation. However, this child presented with a cholestatic jaundice, having only been reported previously in one CPT1A deficiency [9]. CPT1A deficiency led to impaired energy production disrupting Adenosine Triphosphate (ATP) - dependent bile acid secretion which caused accumulation of the toxic metabolites, interfering with the canalicular transport proteins for bile acids or phospholipids leading to hepatocellular and cholestatic picture [9].

Biochemically, DBS and plasma acylcarnitine analysis on TMS showed an increased free carnitine and total carnitine levels and decreased long chain acylcarnitines although rarely, free DBS carnitine concentrations are within the reference interval [5]. Free carnitine in DBS from CPT 1A patients showed a significantly higher concentration than free plasma carnitine. Thus, diagnosis may be missed when perform solely plasma analysis. Diagnostic sensitivity for CPT-1A deficiency could be improved with incorporation of the elevated ratio of free carnitine to the sum of palmitoylcarnitine and stearoylcarnitine [C0/ (C16+C18)] increasing specificity especially in presymptomatic diagnosis [3,10]. Nevertheless, false negative using C16 and C0 / (C16+C18) ratio

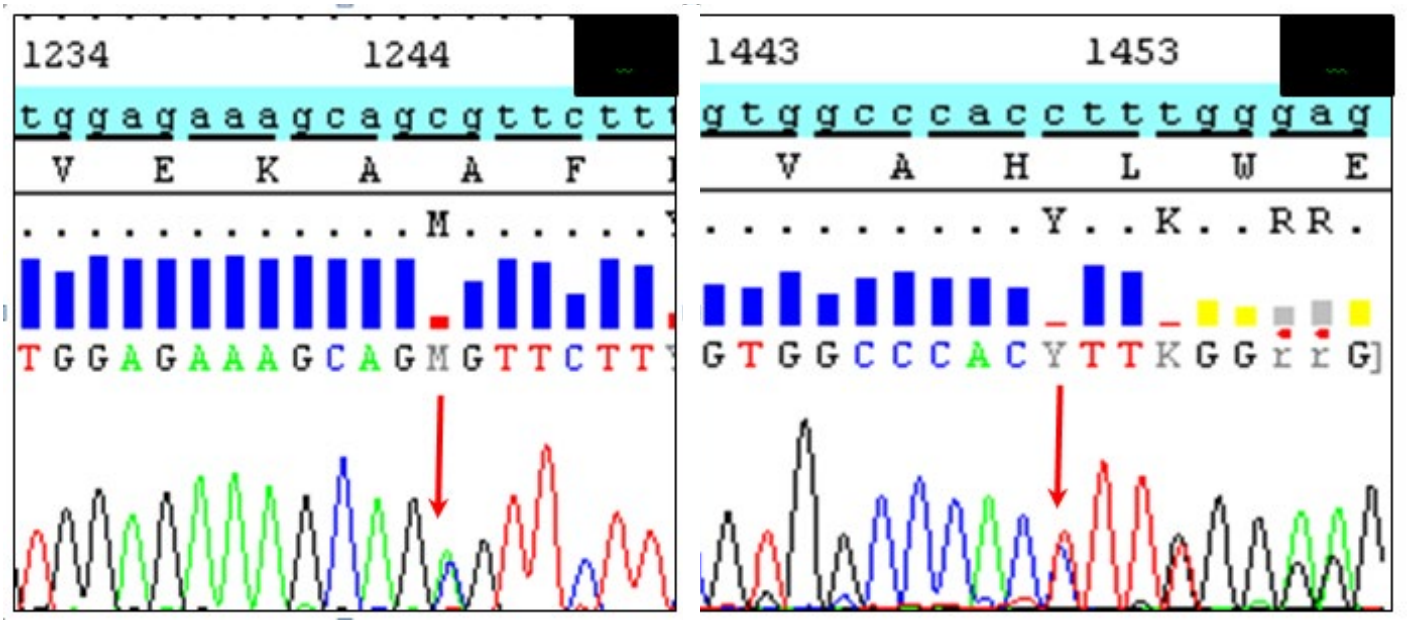

Figure 2. Electropherogram of the mutated sequence in CPT1A. Arrow denotes location of changes. A heterozygous mutation at c.1244C>A causing substitution of amino acid Alanine to Glutamic acid at codon 415 and c.1450del causing a frameshift at codon 484 and introduced a premature stop at 47 codons downstream.

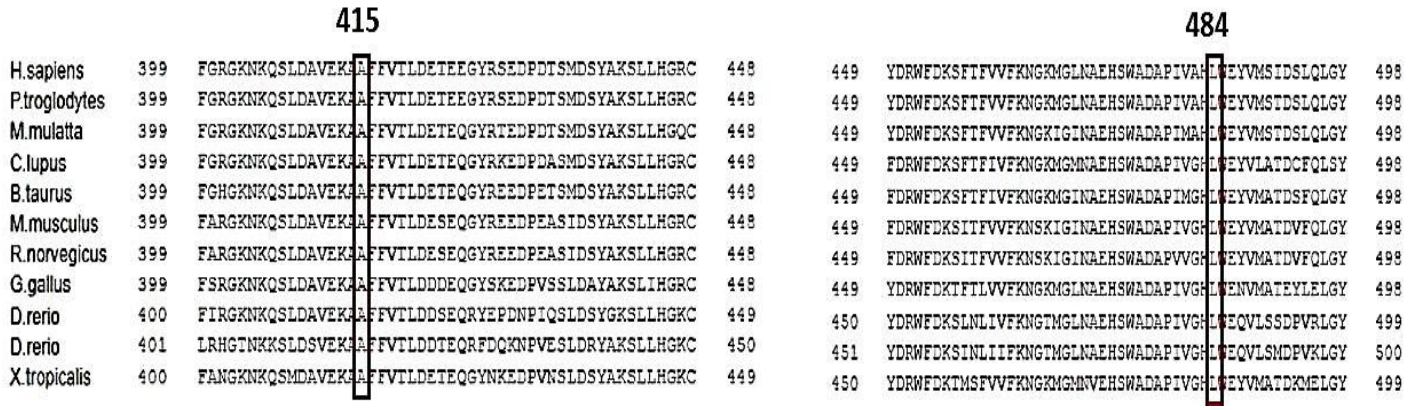

Figure 3. Alignment of the amino acid sequences of human CPT1A showed the amino acid changed in the novel mutations are conserved in various species. Alanine (A) at position 415 and Leucine (L) at position 484 are highly conserved in CPT1A in various species. The box marks the amino acid of interest.

has been reported, however the reliability of the C0/(C16+C18) acylcarnitine ratio is quite dependent on the measurement of low concentrations of long chain acylcarnitine and metabolic status of the patient [11]. An increased C0/(C16+C18) ratio is a more reliable because even in clinically stable patients, there is physiological decline of long acylcarnitine (C16 and C18) during the post-neonatal period [12–14].

Since most fatty acid oxidation disorders demonstrate dicarboxylic aciduria with absence urinary ketones, no specific markers demonstrated for CPT 1A deficiency except mild dicarboxylic aciduria [4,15]. However, our patient had a prominent marked excretion of C12DC (dodecenaoic acid).

The long-chain DCs (unlike medium and short chain DCs) cannot enter the mitochondria for further B-oxidation as their CoA esters are poor substrates for CPT1A may explain the prominent dicarboxylic aciduria and C12DC excretion in the urine [16]. The moderate increased excretion of 3-methylglutaconic acid (3-MG) with increased in excretion of 3-hydroxy 3-methylglutarate (3-OH-3-MG) and glutarate suggests mitochondrial dysfunction in relation to the phospholipid remodeling which may be related to the increased leucine catabolism through the lysine degradative pathway and ketogenic drive in these circumstances, leucine being the ketogenic amino acids [17,18].

Our patient had two compound heterozygous mutations, c.1244C>A, p.(Ala415Glu) in exon 11 and c.1450del, p.(Leu484Phefs*47) in exon 12. The amino acid residue at 415 and 484 in CPT1A is highly conserved among 11 species (Figure 3) suggesting that this region is critical for CPT1A protein function. The frameshift mutation (c.1450del) is predicted to cause a loss of normal protein function through nonsense mediated decay. The missense mutation (c.1244C>A) was absent in large population databases including those containing participants from Malaysia such as GenomeAsia, indicating the extreme rarity of this variant.

Testing of biological parents are recommended to confirm the variant segregation but however the child's parents didn't consent for further investigations. Therefore, we decided to use artificial prediction tools to predict the pathogenecity. This variant was predicted to be pathogenic by both *in silico* tools and deep learning models. AlphaMissense was shown to have 92.9% accuracy at classifying ClinVar variants with 90% precision and it outperformed other methods including those that were trained on ClinVar datasets such as REVEL and gMVP [7]. More importantly, AlphaMissense predictions were found to have good correlation with data from Multiplexed Assay of Variant Effect (MAVE) which provides experimental evidence on the functional impact of missense variants [19].

Conclusion

We recommend screening for metabolic disorders including unsuspected CPT1A deficiency as initial evaluation for patient showing course of cholestatic jaundice to avoid relapses and further complications. We also found two novel mutations in CPT1A gene and predicted that both are pathogenic.

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Conflict of Interest

The authors declare that they have no competing interest.

Authors' Contribution

SA wrote the original draft and organized the research data. NS provide the biochemical data and contributed to the manuscript. AA reviewed the biochemical data and contributed to the manuscript. NAA and M.KN.M.K analyzed the molecular data and contributed to the manuscript. YY provide and reviewed the molecular data and contributed to the manuscript. OFN provide the clinical patient input and contributed to the manuscript. AH reviewed all data and the whole manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author upon request.

Declarations Ethics Approval and Consent to Participate

This study was approved by the MREC, NIH (RSCH ID-23-01160-K4K)

Consent for Publication

This family have given their written consent to be published.

References

1. Korman, Stanley H., Hans R. Waterham, Alisa Gutman and Cornelis Jakobs, et al. "Novel metabolic and molecular findings in hepatic carnitine palmitoyltransferase I deficiency." *Mol Genet Metab* 86 (2005): 337-343.
2. Naher, Nurun, Laila Nurun Nahar, Sabina Sultana and Abdul Matin, et al. "Carnitine palmitoyl tranferase type 1 deficiency in fatty acid oxidation disorder: A case report." *J Shaheed Suhrawardy Med Coll* 6 (2017): 38-40.
3. IJlst, Lodewijk, Hanna Mandel, Wendy Oostheim and J. P. Ruiter, et al. "Molecular basis of hepatic carnitine palmitoyltransferase I deficiency." *J Clin Investig* 102 (1998): 527-531.
4. Bennett, Michael J., Richard L. Boriack, Srinivas Narayan and S. Lane Rutledge, et al. "Novel mutations in CPT 1A define molecular heterogeneity of hepatic carnitine palmitoyltransferase I deficiency." *Mol Genet Metab* 82 (2004): 59-63.
5. Bennett, Michael J. and Avni B. Santani. "Carnitine palmitoyltransferase 1A deficiency." (2016).
6. Frazer, Jonathan, Pascal Notin, Mafalda Dias and Aidan Gomez, et al. "Disease variant prediction with deep generative models of evolutionary data." *Nature* 599 (2021): 91-95.
7. Cheng, Jun, Guido Novati, Joshua Pan and Clare Bycroft, et al. "Accurate proteome-wide missense variant effect prediction with AlphaMissense." *Science* 381 (2023): eadg7492.
8. Brandes, Nadav, Grant Goldman, Charlotte H. Wang, Chun Jimmie Ye, and Vasilis Ntranos. "Genome-wide prediction of disease variant effects with a deep protein language model." *Nat Genet* 55 (2023): 1512-1522.
9. Morris, A. A. M., S. E. Olpin, M. J. Bennett and A. Santani, et al. "Cholestatic jaundice associated with carnitine palmitoyltransferase IA deficiency." *JIMD Rep-Case Res Rep* (2013): 27-29.
10. Prip-Buus, Carina, Laure Thuillier, Nourredine Abadi and Chitra Prasad, et al. "Molecular and enzymatic characterization of a unique carnitine palmitoyltransferase 1A mutation in the Hutterite community." *Mol Genet Metab* 73 (2001): 46-54.
11. Heiner-Fokkema, M. Rebecca, Frédéric M. Vaz, Ronald Maatman and Leo AJ Kluijtmans, et al. "Reliable diagnosis of carnitine palmitoyltransferase type IA deficiency by analysis of plasma acylcarnitine profiles." *IMD Rep 32* (2017): 33-39.
12. Lee, Beom Hee, Yoo-Mi Kim, Ja Hye Kim and Gu-Hwan Kim, et al. "Atypical manifestation of carnitine palmitoyltransferase 1A deficiency: Hepatosplenomegaly and nephromegaly." *J Pediatr Gastroenterol Nutr* 60 (2015): e19-e22.

13. Bellusci, M., P. Quijada-Fraile, D. Barrio-Carreras and E. Martin-Hernandez, et al. "Carnitine palmitoyltransferase 1A deficiency: Abnormal muscle biopsy findings in a child presenting with Reye's syndrome." *J Inherit Metab Dis* 40 (2017): 751-752.

14. Longo, Nicola, Cristina Amat di San Filippo and Marzia Pasquali. "Disorders of carnitine transport and the carnitine cycle." *Am J Med Genet* 142 (2006): 77-85.

15. Ruiz-Sala, Pedro and Luis Peña-Quintana. "Biochemical markers for the diagnosis of mitochondrial fatty acid oxidation diseases." *J Clin Med* 10 (2021): 4855.

16. Schaefer, Jochen, Sandra Jackson, Franco Taroni and Peter Swift, et al. "Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: Implications for diagnosis and therapy." *J Neurol Neurosurg Psychiatry* 62 (1997): 169.

17. Su, Betty and Robert O. Ryan. "Metabolic biology of 3-methylglutaconic acid-uria: A new perspective." *J Inherit Metab Dis* 37 (2014): 359-368.

18. IJlst, Lodewijk, Ference J. Loupatty, Jos PN Ruiter and Marinus Duran, et al. "3-Methylglutaconic aciduria type I is caused by mutations in AUH." *Am J Hum Genet* 71 (2002): 1463-1466.

19. Ljungdahl, Alicia, Sayeh Kohani, Nicholas F. Page and Eloise S. Wells, et al. "AlphaMissense is better correlated with functional assays of missense impact than earlier prediction algorithms." *BioRxiv* (2023): 2023-10.

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