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Special communication

Primary carnitine deficiency in the Chinese

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Primary carnitine deficiency (CDSP, OMIM: 212140) was first described in 1975 by Karpati et al.[1] The patient had a very low blood carnitine level, in addition, the carnitine level was also reduced inside cells. Therefore, this condition is named systemic carnitine deficiency. At that time, there was little insight into the pathogenesis of this disease. With intensive researches carried out in last two decades, a defect in carnitine transport across the cell membrane was established and the molecular genetic basis of the disease was just revealed with discovery of the responsible gene, OCTN2, early this year. This review focuses on the pathogenesis and recent advances in the understanding of molecular genetics of this disease, which was also found in Chinese patients.

CLINICAL FEATURES

CDSP may manifest in two different clinical presentations. During infancy, these patients are at risk of acute episodes of lethargy and biochemical derangement. It could simulate Reye's syndrome, sudden infant death or acute encephalopathy.[2-5] These episodes usually occur subsequent to acute stress, like infection or a period of poor feeding. During this period, hypoglycemia develops as a result of a prolonged stress and depletion of body carbohydrate store, and therefore, the body starts to utilize fatty acid as energy source. However, carnitine, which plays a key role in the transportation of long chain fatty acids into the mitochondria to undergo oxidation, is deficient in these patients and thus fatty acid oxidation cannot proceed normally. In addition, other metabolic pathways in the carnitine depleted mitochondria were also affected.[6,7] Therefore, these patients may respond poorly even after correction of hypoglycemia.[3] A proportion of patient died acutely despite active resuscitation.[5]

The other manifestation of CDSP occurs in childhood, typically around the age of three to four years. They present with endocardial fibroelastosis.[8,9] Hypertrophic cardiomyopathy has also been reported.[10]

As the disease is inherited in an autosomal recessive mode, carrier of a single defective allele is mostly asymptomatic. Although the carriers have a subnormal blood carnitine level, it is believed that the level is sufficient for cellular functions. There was a report of cardiomyopathy found in one carrier.[11] Furthermore, carriers are also at higher risk of induced hypocarnitinemia. There are a number of drugs, which inhibit carnitine uptake/reabsorption. They include sodium valproate and pivampicillin or its pro-drugs.[12-17] It was found that heterozygote carrier of CDSP were at a high risk of becoming carnitine deficient when they were put on valproate.[18] Therefore, blood carnitine level should be frequently monitored in pediatric patients and particularly CDSP heterzygotes while they are on long-term drug treatment which are known to induce carnitine deficiency.[14]

CDSP, or primary carnitine deficiency has to be differentiated from secondary carnitine deficiency, which results from defects in fatty acid oxidation or organic aciduria.[7,19] In these conditions, free carnitine binds to the CoA derviatives of accumulated metabolites, such as acyl-CoAs in medium-chain acyl-CoA dehydrogenase deficiency (MCAD), which is the most common defect in fatty acid oxidation pathway among Caucasians and these acyl-carnitine is excreted through the kidney.

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CLINICAL DIAGNOSIS OF CDSP

In a patient with suggestive clinical features, serum or plasma carnitine should be measured. CDSP patients have very low level of serum carnitine, both total and free carnitine will be low in the range of less than 10 μmol/L (ref range: 26 – 60 μmol/L). The free to acyl carnitine ratio is not decreased. This differentiates CDSP from secondary carnitine deficiency, which will have a large amount of acyl-carnitine. In addition, plasma carnitine levels in the parent would be around the lower limit of reference range which reflect their partially reduced renal reabsorption capacity.

Although defective cellular uptake could be revealed in renal clearance experiment, this procedure is time-consuming and the loading test requires a continuous 4 to 6 hours of intravenous infusion which may not be feasible for young children. Uptake experiment with radioisotope labeled carnitine is used as the diagnostic functional assay. This was first performed in cultured fibroblast and the experiment was later modified to allow assay on cultured peripheral lymphoblasts.[20,21]

CDSP IN THE CHINESE

CDSP had been reported in the Chinese. In the literature, we could find three Chinese CDSP families.[4,22,23] Christodoulou et al[22] reported their experience of prenatal diagnosis of this condition and the proband in their family presented with cardiac symptoms at 6 year-of-age. We reported a Hong Kong Chinese family with two affected children.[4] In addition to a deficiency of carnitine, the fibroblasts culture from the patient also showed an impaired activity for long-chain fatty acid oxidation. This could be related to the fact that carnitine was required for the transport of acyl group across the mitochondria membrane before beta-oxidation of the acyl-CoA could proceed inside the mitochondria compartment. Another family was reported in Macau and the patient presented with cardiomyopathy at age of 6 years.[23]

Thus, the whole spectrum of CDSP could be found in the Chinese (Table 1). In two of the families, there were more than one affected child. The diversity of age of presentation and clinical features may make the condition difficult to diagnose. For example, the elder sib of the Macau family presented with acute cardiac symptoms at 18 month-of-age, which could be one of the youngest CDSP case presenting with cardiac symptoms alone.[9] Therefore, a high clinical suspicion and facilities for measurement of serum carnitine would be required to identify these patients who may have a potentially treatable condition.

Table 1. Summary of Chinese CDSP cases

Reference	Age of first presentation	Presenting illness	Diagnosis made by
Christodoulou et al[22]	6 years	Acute cardiac decompensation after viral illness	Fibroblast uptake assay
Tang et al[4]	6 months	Metabolic derrangement, hypoglycemia, and died shortly after admission	Fibroblast uptake assay
Tang et al[4]	11 months (elder sister of the above case)	Reye-like illness and died on the day of admission in another hospital	Suggested from history and clinical presentation
Marques[23]	6 years	Ieart failure, dilated cardiomyopathy	Fibroblast uptake assay
Marques[23]	18 months (elder brother of the above case)	Sudden death, presented with acute dilated cardiomyopathy	Suggested from history and clinical presentation

Treatment of CDSP with oral carnitine supplement is a highly effective intervention. This condition is one among the rare incidences of inborn error of metabolism in which treatment could completely restore a homeostasis and prevent adverse clinical outcome. This feature adds the clinical value of identification of patient with this condition.

CARNITINE AS A NUTRIENT

Carnitine, 3-hydroxy-4-N, N, N-trimethylaminobutyrate, has been described as a "conditionally essential" nutrient.[24] In study of mealworm, it has a vitamin-like property and the name vitamin BT was given.[25] Carnitine intake from diet comes mainly from food of animal origin. Plant contains very little of this amino acid. However, amount of carnitine in egg is also small. Children on a vegetarian diet had a lower plasma carnitine level than those on omnivorous diet. Soy-based formulae without carnitine supplement could also lead to an insufficient carnitine intake in infants.[24]

PATHOGENESIS OF LOW BODY CARNITINE

At first, it was uncertain which of the two limbs of carnitine turnover (insufficient synthesis or excessive loss) was responsible for the deficient status. Rebouche and Engel[26] investigated the in vivo synthesis of carnitine in CDSP patients. Although the de novo synthesis of carnitine

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accounted for the majority of carnitine supply in primitive species, it only accounted for 25% of daily carnitine requirement in man.[24,27,28] It was concluded that the 75% of our carnitine requirement was derived from dietary intake. However, dietary carnitine absorption from the gut is unaffected in CDSP patients[26] and therefore, primary carnitine deficiency is unlikely due to a defect in carnitine biosynthesis or its absorption from the gut.

Chapoy et al[3] performed the first loading test of carnitine in a CDSP patient. The serum carnitine level of the proband increased to a peak level around 500 μmol/L after an intra-venous injection and then decreased to a very low level within 24 hours while the blood carnitine level in control subjects were maintained at a normal level after 24 hours. This experiment suggested that CDSP was related to an inability to maintain a normal level of carnitine.

Six months later, Engel et al[29] demonstrated in a intravenous loading experiment that CDSP patients had a markedly reduced renal reabsorption capacity for carnitine resulting in an extensive renal loss which explained the very low carnitine level in the circulation.

The basic functional defect was revealed by Treem et al.[20] They showed a markedly deficient cellular uptake of carnitine in multiple tissues of patients with CDSP, including kidney, muscle and fibroblasts. In normal subjects, the physiological level of carnitine in plasma is 30 to 60 μmol/L while the level in various tissue is higher by 20 to 40 times. Therefore, it suggested that the high intra-cellular carnitine required an active transport mechanism to maintain and the transporter mediated an inward transportation of carnitine against a concentration gradient. Expression of this transporter in kidney would mediate reabsorption of carnitine in the renal tubules. Renal clearance study during intravenous infusion of carnitine demonstrated the existence of a high affinity reabsorption mechanism in the kidney tubule which reabsorbs almost all the carnitine passed into the kidney tubule through glomerular filtration.[29] The fractional reabsorption of carnitine in the renal tubule approached 95% in normal subjects. But, the fractional reabsorption in CDSP patients was only about 50% and resulted in a severe renal wastage of carnitine. A partially depressed renal reabsorption capacity for carnitine was also demonstrated in parents of CDSP patients, who were obligated carrier of the disease.

Further functional assay delineated that this high affinity plasmalemmal carnitine transporter operated in the presence of a sodium gradient.[30,31]

MOLECULAR GENETICS OF CDSP

The gene of this transporter was mapped to chromosome 11 in a mouse model of this condition.[32] This corresponded either to chromosome 5q or chromosome 17q in human by comparative mapping. Evidence of linkage to 5q31-32 was reported recently.[33] Wu et al[34] and subsequently another group[35] cloned an organic cation transporter gene, OCTN2. This transporter mediates the uptake of both organic cations and carnitine. The OCTN2 cDNA encodes a 557-amino acid protein and its genomic structure consists of 10 exons and spans 26 kb.

Based on the mapped location of OCTN2, which was located on 5q, the same location as the putative carnitine transporter identified by linkage study in the Japanese family,[33] we and others sequenced the gene in probands with CSDP. Three independent studies confirmed that OCTN2 was the defective genes in CDSP in early 1999.[36-38] Altogether, 11 mutations, which caused a defect in transport activity of OCTN2, were identified at present (Table 2).

Table 2. Summary of OCTN2 mutations/variants

Mutations*	Location in OCTN2 cDNA	Consequence of mutation	Functional assay of expressed mutant	Clinical phenotype	Reference
113 bp deletion	Exon 1 (including initiation codon)	Loss of first 176 amino acid residues in the N-terminal	Not done	Hypertrophic cardiomyopathy at 3 years old	37
254 del1395	Exon 1	Large deletion	Not done	Cardiomyopathy	43
225 insC	Exon 1	Frameshift	Not done	Hypertrophic cardiomyopathy at 8 years old	37
506 C/T	Exon 1	Silent mutation			38
W132X (TGG→TGA)	Exon 2	Non-sense	Not done	Hypertrophic cardiomyopathy at 8 years old	37
W132X (TGG→TGA)	Exon 2	Non-sense	0% activity	SIDS at 6 months old	38
1028 G/A	Exon 4	Silent mutation			38
R282X	Exon 5	Non-sense	0% activity	Cardiomyopathy at 2 years old	36
1423 insA	Exon 7	Frameshift	0% activity	Clinical details not given	36
P478 L	Exon 8	Missense	0% activity	SIDS at 6 months old	38
1524 delG	Exon 8	Frameshift	0% activity	Clinical details not given	36
1672-1G→A	Splice site at 3' end of intron 8	Splice site error; loss of exon 9 and 10	Not done	Reye-like illness since 2 years old	37

* Nucleotide positions are according to OCTN2 cDNA (GenBank accession No. AF057164).

Among them, ten mutations led to a truncation in the translation of the coding region. One of them Trp132→Stop was found in both Japanese and Chinese patients. Otherwise, there was no other recurrent mutation known up to present. Only one missense mutation was known so far in human, Pro478→Leu, which substituted proline by leucine at codon 478. The encoded proline residue is highly conserved across various organic cations transporters.[38] This amino acid residue is located in one of the twelve transmembrane domain, which suggests that it may be responsible for a key conformation of the transporter and substitution of this residues leads to a complete loss of transport function. Another missense mutation was found in the mouse model of CDSP, it was Leu352→Arg.[39] Again, codon 352 is located inside one of the transmembrane domain. It suggests that the transmembrane domains of OCTN2 may be particularly important for maintaining its function.

Carnitine in fact existed in the form of organic ion under physiological pH. Similar compounds also exist as an organic cations, which also include xenobiotics and a number of drugs routinely used in clinical setting. Among the families of organic ion transporters, some are responsible for cellular uptake and some secrete these compounds. Well-characterized transporters included OCT1 and OCT2.[40,41] Based on gene homology, two new members of an organic ion transporter subfamily was identified and was called OCTN1 and OCTN2.[34,42]

Identification of the responsible gene will enable detailed molecular study on the function of the transporter. In addition, carrier identification and early post-natal diagnosis or pre-natal diagnosis could be performed by simple molecular techniques, which could very much improve the clinical management of the patients and their families.

CONCLUSION

CDSP is a good example to illustrate the potential clinical heterogeneity of phenotype due to a single defect. It is still uncertain why some patient present with severe metabolic derangement during early childhood while others presented with cardiomyopathy later. Diagnosis of the condition is now become straightforward. Blood carnitine levels from the proband and parents serve as an efficient screening test. Definitive diagnosis could by now be established either by functional assays or at molecular level. Early diagnosis of the affected child at birth and confident identification of carriers would allow carnitine treatment to be started at an early age which may prevent both life-threatening situations and the development of cardiomyopathy.

REFERENCES

1. Karpati G, Carpenter S, Engel AG, et al. The syndrome of systemic carnitine deficiency: clinical, morphologic, biochemical, and pathophysiologic features. Neurology 1975;25:16-24.

2. Cruse RP, Di Mauro S, Towfighi J, et al. Familial systemic carnitine deficiency. Arch Neurol 1984;41:301-305.

3. Chapoy PR, Angelini C, Brown WJ, et al. Systemic carnitine deficiency: a treatable inherited lipid-storage disease presenting as Reye's syndrome. N Engl J Med 1980;303:1389-1394.

4. Tang NL, Hui J, Law LK, et al. Primary plasmalemmal carnitine transporter defect manifested with dicarboxylic aciduria and impaired fatty acid oxidation. J Inherit Metab Dis 1998;21:423-425.

5. Boles RG, Buck EA, Blitzer MG, et al. Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life. J Pediatr 1998;132:924-933.

6. Bremer J. The role of carnitine in intracellular metabolism. J Clin Chem Clin Biochem 1990;28:297-301.

7. Pons R, de Vivo DC. Primary and secondary carnitine deficiency syndromes. J Child Neurol 1995;10(Suppl 2):S8-S24.

8. Tripp ME, Katcher ML, Peters HA, et al. Systemic carnitine deficiency presenting as familial endocardial fibroelastosis. New Engl J Med 1981;305:385-390.

9. Stanley CA, DeLeeuw S, Coates PM, et al. Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake. Ann Neurol 1991;30:709-716.

10. Matsuishi T, Hirata K, Terasawa K, et al. Successful carnitine treatment in two siblings having lipid storage myopathy with hypertrophic cardiomyopathy. Neuropediatr 1985;16:6-12.

11. Garavaglia B, Uziel G, Dworzak F, et al. Primary carnitine deficiency: heterozygote and intrafamilial phenotypic variation. Neurology 1991;41:1691-1693.

12. Stadler DD, Bale JF Jr, Chenard CA, et al. Effect of long-term valproic acid administration on the efficiency of carnitine reabsorption in humans. Metabolism 1999;48:74-79.

13. Castro-Gago M, Eiris-Punal J, Novo-Rodriguez MI, et al. Serum carnitine levels in epileptic children before and during treatment with valproic acid, carbamazepine, and phenobarbital. J Child Neurol 1998;13:546-549.

14. de Vivo DC, Bohan TP, Coulter DL, et al. L-carnitine supplementation in childhood epilepsy: current perspectives. Epilepsia 1998;39:1216-1225.

15. Melegh B, Pap M, Molnar D, et al. Carnitine administration ameliorates the changes in energy metabolism caused by short-term pivampicillin medication. Eur J Pediatr 1997;156:795-799.

16. Abrahamsson K, Eriksson BO, Holme E, et al. Pivalic acid-induced carnitine deficiency and physical exercise in humans. Metabolism 1996;45:1501-1507.

17. Holme E, Greter J, Jacobson CE, et al. Carnitine deficiency induced by pivampicillin and pivmecillinam therapy. Lancet 1989;2:469-473.

18. Tein I, DiMauro S, Xie ZW, et al. Heterozygotes for plasmalemmal carnitine transporter defect are at increased risk for valproic acid-associated impairment of carnitine uptake in cultured human skin fibroblasts. J Inherit Metab Dis 1995;18:313-322.

19. Duran M, Loof NE, Ketting D, et al. Secondary carnitine deficiency. J Clin Chem Clin Biochem 1990;28:359-363.

20. Treem WR, Stanley CA, Finegold DN, et al. Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. N Engl J Med 1988;319:1331-1336.

21. Tein I, Xie ZW. The human plasmalemmal carnitine transporter defect is expressed in cultured lymphoblasts: a new non-invasive method for diagnosis. Clin Chim Acta 1996;252:201-204.

22. Christodoulou J, Teo SH, Hammond J, et al. First prenatal diagnosis of the carnitine transporter defect. Am J Med Genet 1996; 66:21-24.

23. Marques JS. Dilated cardiomyopathy caused by plasma membrane carnitine transport defect. J Inherit Metab Dis 1998;21:428-429.

24. Rebouche CJ. Carnitine function and requirements during the life cycle. Faseb J 1992;6:3379-3386.

25. Fraenkel G, Friedmann S. Carnitine. Vitam Horm 1957;15: 73-118.

26. Rebouche CJ, Engel AG. Primary systemic carnitine deficiency: Ⅰ. Carnitine biosynthesis. Neurology 1981;31:813-818.

27. Stanley CA. Carnitine disorders. Adv Pediatr 1995;42:209-242.

28. Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. Annu Rev Nutr 1998;18: 39-61.

29. Engel AG, Rebouche CJ, Wilson DM, et al. Primary systemic carnitine deficiency. Ⅱ. Renal handling of carnitine. Neurology 1981;31:819-825.

30. Prasad PD, Huang W, Ramamoorthy S, et al. Sodium-dependent carnitine transport in human placental choriocarcinoma cells. Biochim Biophys Acta 1996;1284:109-117.

31. Roque AS, Prasad PD, Bhatia JS, et al. Sodium-dependent high-affinity binding of carnitine to human placental brush border membranes. Biochim Biophys Acta 1996;1282:274-282.

32. Nikaido H, Horiuchi M, Hashimoto N, et al. Mapping of jvs (juvenile visceral steatosis) gene, which causes systemic carnitine deficiency in mice, on chromosome 11. Mamm Genome 1995;6:369-370.

33. Shoji Y, Koizumi A, Kayo T, et al. Evidence for linkage of human primary systemic carnitine deficiency with D5S436: a novel gene locus on chromosome 5q. Am J Hum Genet 1998;63:101-108.

34. Wu X, Prasad PD, Leibach FH, et al. cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family. Biochem Biophys Res Commun 1998;246:589-595.

35. Tamai I, Ohashi R, Nezu J, et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 1998;273:20378-20382.

36. Wang Y, Ye J, Ganapathy V, et al. Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. Proc Natl Acad Sci U S A 1999;96:2356-2360.

37. Nezu J, Tamai I, Oku A, et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat Genet 1999;21:91-94.

38. Tang NL, Ganapathy V, Wu X, et al. Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency. Hum Mol Genet 1999; 8:655-660.

39. Lu K, Nishimori H, Nakamura Y, et al. A missense mutation of mouse OCTN2, a sodium-dependent carnitine cotransporter, in the juvenile visceral steatosis mouse. Biochem Biophys Res Commun 1998;252:590-594.

40. Zhang L, Brett CM, Giacomini KM, et al. Role of organic cation transporters in drug absorption and elimination. Organic cation transporters in intestine, kidney, liver, and brain. Ann Rev Pharmacol Toxicol 1998;38:431-460.

41. Koepsell H. Organic cation transporters in intestine, kidney, liver, and brain. Annu Rev Physiol 1998;60:243-266.

42. Tamai I, Yabuuchi H, Nezu J, et al. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. Febs Lett 1997;419:107-111.

43. Lamhonwah AM, Tein I. Carnitine uptake defect: frameshift mutations in the human plasmalemmal carnitine transporter gene. Biochem Biophys Res Commun 1998;252:396-401.

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