Polymorphisms in CPT1B and CPT2 have no significant effect on plasma carnitine levels in Japanese cancer patients

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ABSTRACT

Treatment of cancer patients undergoing chemotherapy with L-carnitine (LC) supplementation is becoming increasingly popular in the clinic. The present study aimed to examine the possible effects of polymorphisms in CPT1B and CPT2 (CPT1B G320D, S427C, c.282-18 C>T, and p.E531K, and CPT2 V368I) on the plasma concentration of carnitine in humans. The subjects were the 218 participants of the Iga Cohort Study. Differences in plasma-free carnitine levels by genotype were examined. Genotyping was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP). The plasma carnitine levels were significantly higher in males (P<0.001; Student’s t-test), and there was no significant difference in plasma carnitine levels between the age groups (P=0.202; ANOVA). One-way ANOVA revealed the plasma levels of carnitine were neither significantly different by CPT1B G320D, S427C, c.282-18 C>T, or p.E531K, nor by CPT2 V368I genotypes (P=0.133, P=0.538, P=0.636, P=0.509, and P=0.398, respectively). When analysis of covariance (ANCOVA) adjusted for age and sex was applied, the plasma levels of carnitine were not statistically significantly different according to these genotypes (P=0.299, P=0.715, P=0.980, P=0.851, and P=0.674, respectively). The present study did not identify any statistically significant differences in plasma carnitine levels between subjects with different CPT1 and CPT2 genotypes, suggesting that there may be no need to tailor treatments to patients’ genotypes when determining the dose/amount of LC to be administered to cancer patients undergoing palliative care.

Keywords: cancer palliative care, carnitine, CPT1B, CPT2, genetic polymorphisms

INTRODUCTION

Cancer is becoming the biggest public health burden in Japan, with the incidence rate estimated to reach 50% in a few decades.¹ Despite the recent achievements in the field of cancer chemotherapy as well as the advent of molecular targeted agents, as represented by the BCR–ABL kinase inhibitor in chronic myeloid leukemia (CML), many cancer patients remain incurable and require clinical support with palliative care. In the palliative care of cancer patients at advanced
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clinical stage, weight loss with muscle wasting, namely cachexia or sarcopenia, is recognized as a critical clinical challenge. Although the actual biological mechanism under which this muscle wasting develops in cancer patients has not yet been fully clarified, imbalances between protein catabolism and metabolism due to the existence of cancer are considered to have important roles. Some molecules such as inflammatory cytokines (e.g., interleukin [IL]-6, IL-8, or tumor necrosis factor [TNF]-α), or tumor-derived factors (e.g., proteolysis-inducing factor [PIF]) have been reported to have roles in its genesis.

Meanwhile, loss of quality of life (QOL), fatigue, or anorexia, and the resultant reduced adherence to palliative chemotherapy in patients with cancer cachexia is also a clinically important issue. Efforts have been made to improve these accompanying complications, of which oral/intravenous (i.v.) administration of carnitine is one of the most promising approaches. L-carnitine (LC) is a water-soluble quaternary amine with essential functions in all tissues, the main one being to import long-chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation. Clinically, supplementation with LC has been also reported to improve hepatic encephalopathy in patients with liver cirrhosis. Control of such symptoms in cancer patients, especially in those receiving chemotherapy, will help to improve their compliance with chemotherapy administration, and treatment with LC supplementation is becoming a popular treatment option as a complementary and alternative medicine (CAM) in the clinic. There are two crucial molecules in the catalyzation and transportation of carnitine, which is an important process in the β-oxidation of long chain fatty acids. One is carnitine palmitoyltransferase 1 (CPT1), which exists in the outer surface of the outer mitochondrial membrane and catalyzes the transfer of acyl groups from acyl CoA to carnitine, forming acylcarnitine. Another critical enzyme is carnitine palmitoyltransferase 2 (CPT2), which resides in the inner surface of the inner mitochondrial membrane, playing a vital role in catalyzing the reconversion of acyl groups from acylcarnitine to acyl CoA.

Recent advances in the fields of molecular genetics and epidemiology have clarified the possible roles of genetic variations in drug response or predispositions to various diseases. For carnitine metabolism, however, little is known regarding the roles of genetics. Among them, one study in French-Canadians and another in Saudi-Arabians clarified the functional polymorphisms of CPT1B and CPT2. The present study aimed to examine the possible effects of these polymorphisms in CPT1B and CPT2 (CPT1B G320D, S427C, c.282-18 C>T, and p.E531K, and CPT2 V368I) on the serum/plasma concentration of carnitine in humans.

MATERIAL AND METHODS

Study subjects

The subjects were the 218 participants of the Iga Cohort Study, which was conducted as part of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study), one of the largest genome cohort studies in Japan, which was launched in 2005 to investigate the gene–environment interactions for the possible establishment of the personalized prevention of human chronic diseases based on the genetic information of Japanese patients. All the participants included in this study provided written informed consent for the analysis of their questionnaire data and serum/plasma samples as well as that for genotyping. The blood samples were drawn from the participants when they were fasting.

Measurement of plasma carnitine and creatinine concentration

After deproteinization using a Pall Nanosep® spin column (Pall Corp, Port Washington, NY),
plasma free carnitine levels were measured using the L-Carnitine Colorimetric/Fluorometric Assay Kit (BioVision, Milpitas, CA) in accordance with the manufacturer’s instructions. Briefly, 50 μl of plasma samples and 50 μl of reaction mix were applied to 96-well plates, which were incubated at room temperature for 30 min, and the ODs were measured at 570 nm. The measured ODs were corrected for background levels and the carnitine concentrations were finally obtained from the standard curves. The reference range of plasma free carnitine is 26–76 μmol/L. The coefficients of variation (CV) at low and high levels within the reference range were 13.9% and 23.1% for inter-assay CV (n=2), and 19.2% and 3.7% for intra-assay CV (n=2), respectively. The serum creatinine levels were also measured using the enzymatic method by BML, Inc. (Tokyo, Japan).

Genotyping
Genotyping for CPT1B G320D (rs2269383), S427C (rs8142477), c.282-18 C>T (rs131759), and p.E531K (rs470117), and CPT2 V368I (rs1799821) was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).12 The primers used (and the thermal cycler conditions) for each SNP were as follows: F1: CAC TCG GAT CCC GGA, R1: GCC AGC ATC ATG CCT GTA AAC A, F2: AGT TGC GTC AGG GTT GAA GGT G and R2: GCC TCA GTT ACC TGT GTC CTT G (initial denaturation at 95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 64°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min) for CPT1B G320D; F1: CTC ATC TTC GGG GTC ATA GC, R1: CAT CTC CAG GTG CTA CCT AAG A, F2: TAT CTT TAA TTC TCT AGG GAT GTT GTC TGT CTT TGT C (95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 5 min) for CPT1B S427C; F1: AAG AGG ACC CCC TGC AGC CT, R1: CAG GCA CCA GTG CCC TGT CT, F2: GTT CCT GAG CTG GGC AGA TAC AGG and R2: CAC ACC TAT TGG AGT AGG GCA AG (95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min) for CPT1B c.282-18 C>T; F1: CAG TGC CAG GTC ATC A, R1: CGC TGG TAC AGG AAC GCA CAG T, F2: CCT CTT ACC CAC AAA GCT GAG CC and R2: CTT GGC AAC CTC GTG GTA GGA ACT CTG C (95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min) for CPT1B p.E531K; and F1: CAA GGA TGG CTC TAC TGC CA, R1: GAA ACT GGA CCG AGTCAA TAG TG, F2: CTG AGG AAA GTG GAC TCG GC and R2: CCA AGA GTG CTC AAA GTG GAC (95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 59°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min) for CPT1B c.282-18 C>T. Representative gels for genotyping are shown in Figure 1.

Statistical analysis
The differences in plasma carnitine levels between two groups were tested by Student’s t-test, and the differences in plasma carnitine levels by genotype were tested by analysis of variance (ANOVA), analysis of covariance (ANCOVA), and linear regression. All calculations were conducted using STATA Ver. 13 (STATA Corp, TX).
RESULTS

The characteristics of the studied subjects are detailed in Table 1. The genotype frequencies for CPT1B G320D, S427C, c.282-18 C>T, and p.E531K, and CPT2 V368I were in accordance with Hardy-Weinberg’s equilibrium (P=0.946, P=0.812, P=0.613, P=0.517, and P=0.867, respectively).

The plasma carnitine levels were significantly higher in males (P<0.001; Student’s t-test), and there was no significant difference in plasma carnitine levels between the age groups (P=0.202; ANOVA). We further examined the effects of BMI and body weight on plasma carnitine levels, which did not reveal any statistically significant associations (P=0.075 for BMI; P=0.499 for body weight; one-way ANOVA) (Table 2 and Fig. 2).

Using one-way ANOVA, the plasma levels of carnitine were neither statistically significantly different by CPT1B G320D, S427C, c.282-18 C>T, or p.E531K, nor by CPT2 V368I genotypes (P=0.133, P=0.538, P=0.636, P=0.509 and P=0.398, respectively). When ANCOVA adjusted for age and sex was applied, the plasma levels of carnitine were not statistically significantly different by these genotypes (P=0.299, P=0.715, P=0.980, P=0.851, and P=0.674, respectively) (Table 3 & Fig. 3). As the renal functions of the participants may affect plasma carnitine levels, analysis with
a linear regression model adjusted for age, sex, and serum creatinine levels were also conducted, the results of which were not significant by genotype (Table 3), although plasma carnitine levels were significantly correlated with serum creatinine levels (Pearson’s r=0.2516, P=0.0002).

### Table 1  Characteristics of the study subjects

| Variables                           | Sex [n (%)] | Age Group [n (%)] | Genotype Frequency |
|-------------------------------------|-------------|-------------------|--------------------|
|                                     | Male        | 35–39             | CPT1B G320D        |
|                                     | Female      | 40–49             | **G/G**            |
|                                     |             | 50–59             | 216 (99.1)         |
|                                     |             | 60–69             | **A/G**            |
|                                     |             |                   | 2 (0.9)            |
|                                     |             |                   | **A/A**            |
|                                     |             |                   | 0 (0.0)            |
|                                     |             |                   | CPT1B S427C        |
|                                     |             |                   | **G/G**            |
|                                     |             |                   | 63 (28.9)          |
|                                     |             |                   | **C/G**            |
|                                     |             |                   | 110 (50.5)         |
|                                     |             |                   | **C/C**            |
|                                     |             |                   | 45 (20.6)          |
|                                     |             |                   | CPT1B c.282-18 C>T |
|                                     |             |                   | **C/C**            |
|                                     |             |                   | 62 (28.4)          |
|                                     |             |                   | **T/C**            |
|                                     |             |                   | 105 (48.2)         |
|                                     |             |                   | **T/T**            |
|                                     |             |                   | 51 (23.4)          |
|                                     |             |                   | CPT1B p.E531K      |
|                                     |             |                   | **G/G**            |
|                                     |             |                   | 62 (28.4)          |
|                                     |             |                   | **A/G**            |
|                                     |             |                   | 104 (47.7)         |
|                                     |             |                   | **A/A**            |
|                                     |             |                   | 52 (23.9)          |
|                                     |             |                   | CPT2 V368I         |
|                                     |             |                   | **A/A**            |
|                                     |             |                   | 110 (50.5)         |
|                                     |             |                   | **A/G**            |
|                                     |             |                   | 89 (91.3)          |
|                                     |             |                   | **G/G**            |
|                                     |             |                   | 19 (8.7)           |

Plasma carnitine [mean (sd)]

35.9 (10.4)

Serum creatinine [mean (sd)]

0.782 (0.172)

sd: standard deviation
### Table 2  Plasma carnitine levels by demographic factors

| Variable      | n  | Plasma carnitine | P (ANOVA) |
|---------------|----|------------------|-----------|
| **Gender**    |    |                  |           |
| Male          | 139| 38.4 + 9.9       | < 0.001*  |
| Female        | 79 | 31.5 + 9.9       |           |
| **Age Group** |    |                  |           |
| 35–39         | 42 | 33.0 + 10.4      | 0.202     |
| 40–49         | 64 | 36.4 + 10.2      |           |
| 50–59         | 72 | 36.1 + 10.0      |           |
| 60–69         | 40 | 37.7 + 11.3      |           |
| **BMI**       |    |                  |           |
| < 18.5        | 19 | 31.4 + 10.6      | 0.075     |
| 18.5– < 25    | 148| 36.4 + 10.4      |           |
| 25– <30       | 42 | 37.4 + 9.6       |           |
| 30–           | 9  | 30.7 + 11.6      |           |
| **Body Weight** |   |                  |           |
| < 50kg        | 32 | 34.9 + 11.5      | 0.499     |
| 50kg– < 70kg  | 126| 35.5 + 10.2      |           |
| 70kg–         | 60 | 37.2 + 10.4      |           |

*Student’s *t*-test
CPT1B and CPT2 SNPs and plasma carnitine in Japanese

Fig. 2: Plasma carnitine levels according to demographic factors

Fig. 2A: Plasma carnitine levels by sex.
Fig. 2B: Plasma carnitine levels by age.
Fig. 2C: BMI.
Fig. 2D: Body weight.

*The horizontal bar represents the plasma carnitine concentration (unit: μmol/L).
**The box plots indicate the medians and the inter-quartile ranges (IQR). The upper (lower) limits of the whisker plots represent the most extreme values within 1.5 IQR from the nearer quartiles (i.e., 75 percentiles or 25 percentiles).
### Table 3  Plasma carnitine levels by genotype

| SNP          | genotype | n  | plasma carnitine | P ANOVA | P ANCOVA* | P regress** |
|--------------|----------|----|------------------|---------|-----------|------------|
|             |          |    |                  |         |           |            |
| **CPT1B G320D** |          |    |                  |         |           |            |
| G/G         | 216      |    | 36.0 + 10.4      | 0.133   | 0.299     | 0.348      |
| A/G         | 2        |    | 24.9 + 2.3       |         |           |            |
| A/A         | 0        |    | -                |         |           |            |
| **CPT1B S427C** |          |    |                  |         |           |            |
| G/G         | 63       |    | 37.0 + 11.0      | 0.538   | 0.715     | 0.498      |
| G/C         | 110      |    | 35.2 + 10.2      |         |           |            |
| C/C         | 45       |    | 36.2 + 10.2      |         |           |            |
| **CPT1B c.282-18 C>T** |          |    |                  |         |           |            |
| C/C         | 62       |    | 36.1 + 10.1      | 0.636   | 0.980     | 0.449      |
| T/C         | 105      |    | 35.3 + 10.4      |         |           |            |
| T/T         | 51       |    | 37.0 + 10.9      |         |           |            |
| **CPT1B p.E531K** |          |    |                  |         |           |            |
| G/G         | 62       |    | 36.1 + 10.1      | 0.509   | 0.851     | 0.356      |
| A/G         | 104      |    | 35.2 + 10.4      |         |           |            |
| A/A         | 52       |    | 37.2 + 11.0      |         |           |            |
| **CPT2 V368I** |          |    |                  |         |           |            |
| A/A         | 110      |    | 35.4 + 10.4      | 0.398   | 0.674     | 0.582      |
| A/G         | 89       |    | 37.0 + 10.5      |         |           |            |
| G/G         | 19       |    | 34.0 + 10.5      |         |           |            |

*P ANCOVA: analysis of covariance with age and sex input as covariates.

**P regress: P-values for the regression slope (β-coefficient) of genotype against plasma carnitine.
*The horizontal bar represents the plasma carnitine concentration (unit: μmol/L).
**The box plots indicate the medians and the inter-quartile ranges (IQR). The upper (lower) limits of the whisker plots represent the most extreme values within 1.5 IQR from the nearer quartiles (i.e., 75 percentiles or 25 percentiles).

**Fig. 3** Plasma carnitine levels by genotype

Fig. 3A: CPT1 G320D
Fig. 3B: CPT1 S427C
Fig. 3C: CPT1B c282-18C>T
Fig. 3D: CPT1B p.E531K
Fig. 3E: CPT2 V368I
DISCUSSION

The present study revealed that polymorphisms in CPT1B and CPT2 were not significantly associated with serum carnitine levels, suggesting that genetic factors may have little role in its expression.

CPT1 resides in the outer surface of the outer mitochondrial membrane, and transfers acyl groups from acyl CoA to carnitine-forming acylcarnitine. Three isoforms of CPT1 exist: the liver isoform (CPT1A), the muscle isoform (CPT1B), and the brain isoform (CPT1C). CPT1B expression is shown to be high in heart and skeletal muscles.

CPT2, which exists in the inner surface of the inner mitochondrial membrane, has essential roles in removing the acyl group from acyl CoA to acylcarnitine. A recent report that investigated the influence of CPT1B polymorphisms on the risk of metabolic syndrome (MetS), indicated the E531K polymorphism of the CPT1B gene as the relevant polymorphism for the risk of MetS. In a Saudi-Arabian study, CPT1B G320D, CPT1B S427C, and CPT2 V368I were identified as polymorphisms significantly associated with serum troponin T levels in acute myocardial infarction (AMI) patients, suggesting that these polymorphisms are biologically functional in humans. Another study in French-Canadians revealed the significant associations of CPT1B c.282-18C>T and p.E531K variants with obesity phenotypes, together with the interaction between dietary fat intake and the CPT1B p.E531K variant on weight, BMI, and waist circumference, supporting the functionality of this CPT1B polymorphism.

That being said, our study investigated the associations of all these polymorphisms with serum carnitine levels in 218 healthy Japanese subjects, and did not identify any statistically significant associations, suggesting that the effects of these polymorphisms in carnitine transporter genes (CPT1B and CPT2) on carnitine concentration in blood in humans are limited.

In the present study, we observed significantly higher plasma carnitine levels in males than in females, which was the same trend reported in previous studies. Meanwhile, one previous study demonstrated significant age-related decreases in muscle carnitine levels, whereas the serum carnitine levels were not significantly changed, suggesting that serum carnitine levels do not reflect the carnitine reservoirs in humans. Muscle carnitine levels, rather than blood levels, are considered to be a good surrogate for human carnitine reservoirs; such studies that take into consideration carnitine levels in muscles (and also other organs) are worth conducting. Aside from this, given all the results observed, the clinical implications of the present study are that there is no need to customize treatments to patients’ genotypes when determining the dose/amount of LC to be administered for palliative care.

In summary, the present study could not identify any statistical difference in plasma carnitine levels between subjects with different CPT1 and CPT2 genotypes. As the sample size of the present study was relatively limited, further studies with a larger number of subjects, as well as a study design, aimed to examine the roles of these polymorphisms in muscle carnitine levels would improve our understanding of the effects of genetics in human carnitine metabolism.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare that they have no conflict of interest to disclose with regards to this manuscript.

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