Original Article

Endogenous genetic risk factor for serious heatstroke: the thermolabile phenotype of carnitine palmitoyltransferase II variant

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Aim: In serious heatstroke, elevated body temperature (>40°C) is considered the main cause of illness. Mitochondrial carnitine palmitoyltransferase II (CPT II) plays an important role in adenosine triphosphate (ATP) generation from long-chain fatty acids, and its thermolabile phenotype of *CPT2* polymorphisms leads to ATP production loss under high fever. Whether by heatstroke or influenza, high fever suppresses mitochondrial ATP production in patients with the thermolabile phenotype of *CPT2* polymorphisms. We investigated the relation between *CPT2* polymorphism and severity of heatstroke with a body temperature of over 40°C.

Methods: We analyzed blood chemistry test results, Japanese Association for Acute Medicine Disseminated Intravascular Coagulation (JAAM DIC), Acute Physiologic and Chronic Health Evaluation II, and Sequential Organ Failure Assessment (SOFA) scores, and *CPT2* polymorphisms in 24 consecutive patients with severe heatstroke at two university hospitals.

Results: Eleven patients carried thermolabile CPT II variants (rs2229291; c.1055T>G [p.Phe352Cys]) (F352C), and the genotype frequency was greater in heatstroke patients than in healthy volunteers. There was no significant difference in body temperature or blood chemistry data at emergency room arrival between patients with and without the CPT II variants. However, hospital days were longer and initial antithrombin activity was significantly lower in the variant group, suggesting a possible link with early phase vascular endothelial cell dysfunction. The JAAM DIC diagnostic criteria and SOFA scores were also higher in the group. There were no differences in the serum albumin, serum creatine kinase, and fibrin degradation product levels, and platelet counts.

Conclusions: In addition to known risks (e.g., environmental temperature and old age), the CPT II polymorphism [F352C] can be a predisposing genetic risk factor for serious heatstroke with organ disfunction, and lower antithrombin activity.

Key words: Carnitine palmitoyltransferase II, coagulopathy, energy metabolism, heatstroke, polymorphism

BACKGROUND

REPORTS OF DEATHS caused by heatstroke have been increasing with global warming and the worldwide increase of serious heat waves.[1,2] In Japan, a recent surge of emergency transports due to heatstroke[3] and deaths[4] have become issues of social concern. In serious heatstroke, elevated body temperature of over 40°C is believed to be the main cause of illness and the most serious complication results in the patient's death from multiple organ failure (MOF). The same level of high fever could also occur in influenza patients and occasionally results in MOF.

Recently, this type of MOF with high fever, particularly in influenza pediatrics, has been revealed to be caused by the thermolabile phenotype of carnitine palmitoyltransferase II (CPT II) variant (rs2229291; c.1055T>G [p.Phe352Cys]) (F352C). Carnitine palmitoyltransferase II locates in the inner mitochondrial membranes to import long-chain fatty acids and produce adenosine triphosphate (ATP), and its thermolabile phenotype of *CPT2* polymorphism leads to the suppression of ATP production only under high fever. It is reasonable to understand whether

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high fever is by heatstroke of influenza that high fever suppresses ATP production throughout the body with the thermolabile phenotype of *CPT2* polymorphism, and this should be related to the patient's serious condition induced by high fever. This assumption has never been tested. In this study, the relation between *CPT2* polymorphism and severity of heatstroke with a body temperature of over 40°C was investigated.

METHODS

TWENTY -four consecutive patients diagnosed as having severe heatstroke were enrolled in the study. They were all transported by ambulance directly from the scene and admitted to either of two life-saving emergency centers, Tokyo Medical University Hospital or Tokyo Medical University Hachioji Medical Centre (both Tokyo, Japan).

Severe heatstroke in this study was diagnosed in patients with a core temperature of over 40°C, with loss of consciousness or convulsions. The subjects underwent genetic analysis for *CPT2* after obtaining written informed consent from the closest relative.

A *CPT2* exon 4 region, including F352C and V368I, was screened for mutations or polymorphisms by direct sequencing. We carried out polymorphism screening on the present patients and healthy volunteers, also Japanese, referred from our previous research.[5] Genomic DNA from whole blood (anticoagulated with ethylenediaminetetraacetic acid) was prepared using the QIAamp DNA blood midi kits (Qiagen, Tokyo, Japan). Both strands (sense/antisense) of the *CPT2* exon 4 regions were sequenced at the Dragon Genomics Center (Takara, Mie, Japan) using E4n2F1 (5′-tcgtttacccaaaccagtcg-3′) and E4n2R1 (5′-tcaaacttttccttagcagct-3′) primers.

The χ^2-test and Fisher's exact test were used to compare genotype frequencies. The Mann–Whitney *U*-test was used to compare the durations of hospitalization. Laboratory and scoring data were analyzed using the *t*-test. Japanese Association for Acute Medicine Disseminated Intravascular Coagulation (JAAM DIC), Acute Physiologic and Chronic Health Evaluation (APACHE) II, and Sequential Organ Failure Assessment (SOFA) scores in initial treatment were calculated. We compared age, gender, body temperature at emergency room (ER) arrival, duration of hospitalization, renal replacement therapy, prognosis, complete blood count, and coagulation in the subjects with and without the F352C variant. JMP11 (SAS Institute, Cary, NC, USA) was used for statistical analysis. The significance threshold was set to a *P*-value of 0.05.

This study was pre-approved by the Ethics Committee of Tokyo Medical University, as it involves genetic analysis (Medical Research Ethics Investigation #799).

RESULTS

THE THERMOLABILE CPT II F352C variant with a dominant-negative effect on the enzyme activity was identified in 11 out of 24 patients (45.8%; Table 1). The frequency of the F352C homozygote and heterozygote was significantly higher in the heatstroke group than in the healthy volunteer group (odds ratio = 5.23; 95% confidence interval, 1.91–14.37; $P = 0.001$). The allelic frequency was 0.271, which is 2.68 times greater than the frequency for the healthy volunteer group (0.101).

The characteristics of the patients with and without the F352C CPT II variant are described in Table 2. The groups did not significantly differ in age or gender. Elderly patients (aged ≥70 years) comprised more than 60% of each group. Many had pre-existing conditions and we could not find any difference between the two groups, except that patients with the F352C CPT II variant had longer hospital days. Three patients had cardiac arrest, and one patient died in each group.

Two patients with the F352C CPT II variant had cardiac arrest; the first patient, an 82-year-old woman, developed heatstroke in her living room, and her antithrombin (AT) activity and serum fibrin/fibrinogen degradation product

Table 1. Genotype frequencies of T1055G/F352C in patients with severe heatstroke and matched volunteers

T1055G/F352C genotype	*n* (%)		F352C variant
	Healthy volunteers	Heatstroke	
TT	68 (86.1)	13 (54.2)	–
TG or GG	11 (TG;6, GG;5 13.9)	11 (TG;9, GG;2 45.8)	+
Total	79 (100)	24 (100)	
		$P = 0.003$	

Table 2. Characteristics of patients with severe heatstroke and presence of F352C carnitine palmitoyltransferase II variant

	With F352C variant	Wild type	*P*-value
n	11	13	–
Gender, male	8 (72.7%)	11 (84.6%)	0.630
Age, years; mean ± SD	70 ± 12.6	68 ± 15.8	0.977
≥70 years	7	9	0.772
Underlying disease (with overlaps)			
Hypertension	3	3	0.813
Heart disease	2	2	0.855
Diabetes	1	1	0.953
Malignant disease	2	2	0.867
Dementia	1	2	0.642
Place of onset			0.214
Inside room	7	8	
Outside	3	2	
Bath/sauna	1	3	
Hospital days	21.5 ± 23.0	9.5 ± 13.2	<0.050
Renal replacement therapy	2 (18.2%)	4 (30.8%)	0.649
Death	1 (9.1%)	1 (7.7%)	0.953

SD, standard deviation.

(FDP) levels were 42.2% and 52.3 μg/mL, respectively, at ER arrival. The AT activity was the second lowest among the 24 patients, and this patient had other polymorphisms, namely, CPT II [V368I] which enhances the thermolability of F352C CPT II polymorphism.[5,6] This patient could not be resuscitated.

Another patient with the F352C CPT II variant showed refractory ventricular fibrillation immediately after ER arrival. His AT activity and FDP level were 63% and 222.6 μg/mL, respectively, on arrival. He recovered from unstable hemodynamics and coagulopathy after cooling and was discharged on the 7th hospital day. There was one patient who developed cardiac arrest without the F352C CPT II variant. She was a 90-year-old old woman and her AT activity was well maintained within the normal range (94.3%).

There was no significant difference in the body temperature of patients at ER arrival, but those with the F352C CPT II variant had significantly higher JAAM DIC diagnostic criteria and SOFA scores, and lower AT activity. There were no significant differences in the APACHE II scores, levels of serum albumin, serum creatine kinase, or FDP, nor platelet counts between the two groups (Table 3).

DISCUSSION

THE GENOTYPE FREQUENCY of CPT II [F352C] was greater in heatstroke patients than in healthy volunteers. We also confirmed the reduction of AT activity at ER arrival in patients with the *CPT2* gene polymorphism and their higher JAAM DIC diagnostic criteria and SOFA scores, and longer hospital days.

The presence of this thermolabile polymorphism is not harmful at normal body temperatures; however, due to the elevation of body temperature, CPT II with the F352C variant is heat-inactivated, resulting in mitochondrial energy crisis, particularly in the vascular endothelial cells and heart muscle cells in which fatty acid oxidation is the major energy source.[7,8]

In studies on influenza, it was revealed that the activity of CPT II with F352C decreased to 25%–30% of the wild type at temperatures above 40°C.[5] As CPT II is an enzyme essential for generating ATP through long-chain fatty acid oxidation in the mitochondria, heat-inactivation of CPT II with the F352C variant suppresses β-oxidation, resulting in ATP crisis in vascular endothelial cells.[9]

Heatstroke has been considered to be an exogenous serious condition caused by cellular dysfunction under high temperatures. The cellular damage can be physical, directly caused by heat, or through dehydration.[10] Endothelial cell injury and coagulation disorder are important pathologic mechanisms of heatstroke.[11–15] These pathologic mechanisms could be enhanced by insufficient energy supply to the endothelial cells in patients with the thermolabile polymorphism. In those patients, early phase endothelial dysfunction through ATP crisis induced by the thermolability of CPT II can be severe and could further lower AT activity.

Table 3. Patient data on admission and presence of F352C carnitine palmitoyltransferase II variant

	With F352C variant	Wild type	*P*-value
n	11	13	
Scoring			
JAAM DIC diagnostic criteria score	3.3 ± 1.6	2.0 ± 1.9	<0.05
APACHE II score	25.8 ± 6.3	21.8 ± 8.3	0.19
SOFA score	9.2 ± 2.9	6.6 ± 2.3	<0.05
Laboratory data			
Body temperature (°C)	40.5 ± 0.6	40.6 ± 0.6	0.68
Albumin (g/dL)	2.8 ± 0.7	3.2 ± 0.6	0.09
Creatine kinase (U/L)	7549 ± 12615	1867 ± 4192	0.09
Antithrombin activity (%)	68.7 ± 21.7	91.2 ± 22.4	<0.05
FDP (μg/mL)	39.4 ± 68.7	23.3 ± 13.5	0.26
Platelet ($\times 10^4/mm^3$)	16.1 ± 6.5	17.5 ± 6.9	0.61

APACHE, Acute Physiologic and Chronic Health Evaluation; FDP, fibrin/fibrinogen degradation product; JAAM DIC, Japanese Association for Acute Medicine Disseminated Intravascular Coagulation; SOFA, Sequential Organ Failure Assessment.

This was consistent with the lower AT activity found in the patients with CPT II [F352C] polymorphism in our study.

The other polymorphism of CPT II [V368I] does not cause thermolability of the enzyme; however, a significant reduction of CPT II activity has been reported when this polymorphism is combined with the F352C polymorphism.[6] In our study, only two of the 24 patients had the compound variations for [F352C] and [V368I], and they had the lowest (39.0%) and the second lowest (42.2%) AT activity. The latter patient died. Another cardiac arrest patient with CPT II [F352C] polymorphism had an AT activity of 63% and he was resuscitated. The patient with cardiac arrest among the patients without [F352C] polymorphism had AT activity within the normal range (94.3%), but she was the oldest among our patients.

Higher JAAM DIC diagnostic criteria score and SOFA score in the patients with the F352C CPT II variant could suggest coagulopathy and organ disfunction. Older age made APACHE II scores higher in both groups.

The present results suggest that the pathology of heatstroke cannot be considered solely as an exogenous disease with physical stimuli. This implies that heatstroke has a serious endogenous genetic risk factor associated with thermolabile CPT II polymorphisms. As high fever is the only mechanism for suppressing CPT II activity with a thermolabile phenotype, energy disruption of the endothelial or heart muscle cells can be induced in various types of febrile illnesses, such as sepsis and some infectious diseases. The present results could provide a good foundation for further analysis of genetic risk for the deterioration of a patient's condition as induced by high fever.

As a limitation of this study, the exact duration of exposure to extreme heat in each patient is unknown. Another limitation is that the analysis was confined to Japanese patients. In the future, new single-nucleotide polymorphisms that affect CPT II activity could be identified, and other genetic risk factors for other races might also be discovered.

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DISCLOSURE

Ethical approval: This study was approved by the ethics review committee for human genome analysis at our institutions.
Patient consent: The subjects underwent genetic analysis for *CPT2* after obtaining written informed consent from the closest relative.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.
Conflict of interest: None declared.

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