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Single nucleotide polymorphism in CPT1B and CPT2 genes and its association with blood carnitine levels in acute myocardial infarction patients

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ABSTRACT

Ischemic and reperfusion injuries in acute myocardial infarction (AMI) lead to mitochondrial dysfunction in heart cells. Lipid metabolism takes place in mitochondria where carnitine palmitoyltransferase (CPT) enzyme system facilitates the transport of long-chain fatty acids into matrix to provide substrates for beta-oxidation. We sequenced the coding regions of CPT1B and CPT2 genes to identify the single nucleotide polymorphism (SNP) in 23 AMI patients and 23 normal subjects. We also determined blood carnitine levels in these samples to study the impact of these SNPs on carnitine homeostasis. The sequencing of coding regions revealed 4 novel variants in CPT1B gene (G320D, S427C, E531K, and A627E) and 2 variants in CPT2 gene (V368I and M647V). There were significant increases in total carnitine (54.18 ± 3.11 versus 21.49 ± 1.03 μmol/l) and free carnitine (37.78 ± 1.87 versus 10.06 ± 0.80 μmol/l) levels in AMI patients as compared to normal subjects. CPT1B heterozygous variants of G320D and S427C among control subjects showed significantly higher levels of total and free carnitine in the blood. The homozygous genotype (AA) of CPT2 variant V368I had significantly less blood carnitine in AMI patients. Serum troponin T was significantly less in GG genotype of CPT1B variant S427C whereas the genotype AA of CPT2 variant V368I showed significantly higher serum troponin T levels. Further studies on large number of patients are necessary to confirm the role of CPT1B and CPT2 polymorphism in AMI.

1. Introduction

Acute myocardial infarction (AMI) is a key component of the burden of cardiovascular disease due to its associated complications and mortality (Roger, 2007). AMI is the consequence of the chronic development of atherosclerosis lesions. Ischemic and reperfusion injuries in AMI lead to mitochondrial dysfunction in heart cells (Misra et al., 2009). Carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is an essential cofactor in fatty acid metabolism. Carnitine transfers and regulates long-chain fatty acid transport across the inner mitochondrial membrane for beta-oxidation. Carnitine has been shown to protect against ischemic insult (Zhang et al., 2012). Studies on the role of carnitine administration to patients with AMI, angina, and congestive heart failure generally have been positive (Davini et al., 1992; Pauly and Pepine, 2003; Rizzon et al., 1989). L-carnitine therapy led to a reduction in early mortality without affecting the risk of death and heart failure at six months in patients with anterior AMI (Tarantini et al., 2006). L-carnitine adjunct therapy appears to be associated with a reduced level of cardiac markers in patients with NSTEMI (Xue et al., 2007). L-carnitine has been found to induce an immediate recovery of myocardial contractility in experimental animals, affected by very brief and repeated coronary occlusions (Hernandiz Martínez et al., 1997).

Carnitine acyltransferase I (synonym; carnitine palmitoyltransferase I, CPT1) in the outer surface of inner mitochondrial membrane transfers acyl group from acyl CoA to carnitine forming acylcarnitine. There are 3 isoforms of CPT1 including liver isoform (CPT1A), muscle isoform (CPT1B) and brain isoform (CPT1C). CPT1B is highly expressed in heart and skeletal muscle cells. Another enzyme, CPT2, located in the inner surface of the inner mitochondrial membrane, removes the acyl group from acylcarnitine and transfers it to CoA to form acyl CoA in the mitochondrial matrix. Administration of L-carnitine in rats significantly increases the activities of cardiac CPT1 and CPT2 by approximately 50% above those of the control animals (Yoon et al., 2003). Recently, an association has been reported between CPT1B coding polymorphisms and metabolic syndrome, with a deleterious effect of the CPT1B I66V and a protective impact of the CPT1B K531E SNP, though

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; CoA, coenzyme A; CPT1B, carnitine palmitoyltransferase IB; CPT2, carnitine palmitoyltransferase II; dbSNP, database of single nucleotide polymorphism; DNA, deoxyribonucleic acid; dNTP, deoxyribonucleotides; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NSTEMI, non-ST-elevated myocardial infarction; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; STEMI, ST-elevated myocardial infarction.

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haplotype analysis indicates a relevance of the E531K polymorphism only (Auinger et al., 2012). In the present investigation, we sequenced the coding regions of CPT1B and CPT2 genes to identify single nucleotide polymorphism (SNP) in AMI patients and normal subjects. We also determined blood carnitine levels in these samples to study the impact of these SNPs on carnitine homeostasis.

2. Materials and methods

2.1. Patients and controls

This study was conducted on 23 AMI patients (18 males and 5 females, aged 61.34 ± 13.69 y) admitted to King Khalid University Hospital and Prince Sultan Cardiac Center, Riyadh, Saudi Arabia. We also included 23 age-matched normal subjects (19 males and 4 females) to serve as control group. All the subjects used in this study were Saudis. The AMI patients were classified as STEMI (N = 12) or NSTEMI (N = 11). The diagnosis of MI required the presence at least two of the following criteria: (i) history of characteristic prolonged (≥30 min) pain or discomfort, (ii) abnormal troponin T levels, and (iii) presence of new Q waves or new abnormal ST–T features. Patients with STEMI were classified on the basis of (i) continuous chest pain upon presentation, refractory to nitrates, and lasting ≥30 min, (ii) presence of ST-segment elevation of ≥0.2 mV in ≥2 contiguous precordial leads, or ≥0.1 mV in ≥2 contiguous limb leads, or new left bundle branch block on admission electrocardiogram, and (iii) abnormal troponin-T levels. Patients with NSTEMI were classified on the basis of (i) angina-like chest pain at rest during the last 24 h lasting ≥5 min, (ii) absence of ST-segment elevation, and (iii) abnormal troponin-T levels. The exclusion criteria included recent surgery, active infection, chronic inflammatory diseases, significant hepatic or renal dysfunction and malignancy. The patients' characteristics are summarized in Table 1. Most of the patients were non-smokers; only 3 patients were smoker and ex-smokers. The protocol of this study was approved by our Institution Ethics Review Board for human studies and the patients signed an informed consent.

2.2. Gene sequencing for SNP analysis

DNA was extracted from the blood samples using DNeasy Blood and Tissue Kit (Qiagen GmbH, Germany) according to manufacturer's instructions. The isolated DNA was dissolved in 200 μl of elution buffer and stored at −20 °C.

We obtained the sequences of *Homo sapiens* CPT1B (accession, NG_012643) and CPT2 (accession, NG_008035) genes from the GenBank and primers were designed for the coding regions of these genes using Primer Primier version 5.0 software. Targeted regions of both the genes were amplified using specific primers (Table 2).

The total PCR reaction volume (30 μl) contained 10× rTaq buffer (3 μl), dNTP (2 μl), each primer (1 μl), rTaq (0.2 μl), DNA (2 μl) and double distilled water (20.8 μl). The conditions of PCR amplification were as follows: denaturation of DNA at 95 °C for 5 min followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 50 s at 72 °C. At the end of PCR, a final extension step for 5 min at 72 °C followed by maintaining the temperature at 4 °C was used. Before sequencing, all the PCR products were subjected to purification using PCR Cleanup Filter Plates (Millipore). We used a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) for sequencing of PCR products on a 3130XL genetic analyzer (Applied Biosystems).

2.3. Biochemical analysis

Serum troponin-T was analyzed using a commercially available sandwich ELISA kit (Roche Diagnostics, Germany). For carnitine analysis, a single drop of venous blood sample was placed on filter paper cards (No. 903; Schleicher and Schuell, Dassel, Germany), dried overnight at room temperature and then stored in brown color paper envelopes. The levels of total and free carnitine in blood spots were determined using a NeoBase non-derivatized MS/MS kit (Perkin Elmer, USA). The internal standards of carnitine were reconstituted with 1 ml of NeoBase extraction solution followed by their dilution to 1:110 to get the daily working solution of internal standards. Punch outs (3.2 mm diameter, equivalent to 3.1 μl of blood) of control and patient samples were individually placed in assigned wells of a clear microplate and 100 μl of working solution of internal standard were added to each well. The plate was sealed with an adhesive cover to minimize evaporation and then shaken (750 rpm) at 45 °C for 45 min. The cover was removed and the aliquots (75 μl) of well contents were transferred to a V-bottomed microplate before injecting to API 3200 LC-MS/MS (Applied Biosystems, USA).

2.4. Statistics

Statistical comparisons were performed using Fisher Exact Test for SNP data and Student's t-test for biochemical data. Pearson test was

Table 1
Patient's characteristics.

Patient no.	Gender	Age (y)	BMI (kg/m^2)	Diagnosis	Associated disease(s)	Family history
1	Female	40	35.09	NSTEMI	Diabetes, hypertension	Hypertension
2	Male	58	24.71	STEMI	Diabetes, hypertension	–
3	Female	65	36.32	STEMI	Diabetes, Hypertension, dyslipidemia	–
4	Male	60	30.44	NSTEMI	Diabetes, dyslipidemia	–
5	Male	66	26.10	STEMI	Diabetes, hypertension	–
6	Male	79	39.00	STEMI	Hypertension	–
7	Female	51	30.62	STEMI	Diabetes, hypertension, dyslipidemia	–
8	Male	52	30.96	STEMI	Diabetes, hypertension, dyslipidemia	–
9	Male	72	28.36	NSTEMI	Diabetes, hypertension	–
10	Female	83	31.64	NSTEMI	Diabetes, hypertension	–
11	Male	55	40.07	NSTEMI	Diabetes, hypertension	–
12	Male	63	27.57	NSTEMI	Diabetes, hypertension	–
13	Male	58	22.49	STEMI	Diabetes, hypertension	–
14	Male	85	21.27	NSTEMI	Diabetes, hypertension	–
15	Male	35	26.59	STEMI	Dyslipidemia	Hypertension
16	Male	56	27.20	NSTEMI	Diabetes, hypertension	Diabetes, CAD
17	Male	72	21.91	NSTEMI	Diabetes, hypertension	–
18	Female	86	21.36	STEMI	Diabetes, hypertension	–
19	Male	67	25.89	NSTEMI	Diabetes, hypertension, dyslipidemia	–
20	Male	52	28.02	STEMI	Diabetes, hypertension, dyslipidemia	–
21	Male	48	23.87	STEMI	Hypertension, dyslipidemia	–
22	Male	60	22.49	STEMI	Diabetes, hypertension	Diabetes, CAD
23	Male	48	35.64	NSTEMI	Hypertension	Diabetes, CAD