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Review

Uncoupling protein 2 −866G/A and uncoupling protein 3 −55C/T polymorphisms in young South African Indian coronary artery disease patients

Alisa Phulukdaree [a], Devapregasan Moodley [a], Sajidah Khan [b], Anil A. Chuturgoon [a,*]

[a] Discipline of Medical Biochemistry, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella, 4013 Durban, South Africa
[b] Department of Cardiology, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella, 4013 Durban, South Africa

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ABSTRACT

Background: Uncoupling proteins (UCPs) 2 and 3 play an important role in the regulation of oxidative stress which contributes to chronic inflammation. Promoter polymorphisms of these genes have been linked to chronic diseases including heart disease and type II diabetes mellitus in several populations. This is the first investigation of the UCP2 −866G/A rs659366 and UCP3 −55C/T rs1800849 polymorphisms in young South African (SA) Indians with coronary artery disease (CAD).

Methods: A total of 300 subjects were recruited into this study of which 100 were SA Indian males with CAD, 100 age- (range 24–45 years), gender- and race-matched controls and 100 age-matched black SA males. The frequency of the UCP2 −866G/A and UPC3 −55C/T genotypes was assessed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Results: The heterozygous UCP2 −866G/A and homozygous UCP3 −55C/C genotypes occurred at highest frequency in CAD patients (60% and 64%, respectively) compared to SA Indian controls (52% and 63%) and SA Black controls (50% and 58%). The UCP2 −886G/A (OR = 1.110; 95% CI = 0.7438–1.655; p = 0.6835) and UCP3 −55C/T (OR = 0.788; 95% CI = 0.482–1.289; p = 0.382) polymorphisms did not influence the risk of CAD.

The rare homozygous UCP3 −55T/T genotype was associated with highest fasting glucose (11.87 ± 3.7 mmol/L vs. C/C:6.11 ± 0.27 mmol/L and C/T:6.48 ± 0.57 mmol/L, p = 0.0025), HbA1c (10.05 ± 2.57% vs. C/C:6.44 ± 0.21% and C/T:6.76 ± 0.35%, p = 0.0006) and triglycerides (6.47 ± 1.7 mmol/L vs. C/C:2.33 ± 0.17 mmol/L and C/T:2.06 ± 0.25 mmol/L, p < 0.0001) in CAD patients.

Conclusion: The frequency of the UCP2 −866G/A and UCP3 −55C/T polymorphisms was similar in our SA Indian and SA Black groups. The presence of the UCP2 −866G/A and UCP3 −55C/T polymorphisms does not influence the risk of CAD in young South African Indian CAD patients.

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Abbreviations: UCP, Uncoupling protein; UCP2, Uncoupling protein 2; UCP3, Uncoupling protein 3; SA, South Africa/n; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; OR, Odds ratio; CI, confidence interval; mmol/L, millimoles per litre; HbA1c, glycosylated haemoglobin; CAD, coronary artery disease; T2D, type 2 diabetes mellitus; ATP, adenosine triphosphate; SNP, single nucleotide polymorphism; CRP, C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; EDTA, ethylenediaminetetraacetic acid; bp, base pair; $MgCl_2$, Magnesium chloride; DNA, deoxyribose nucleic acid; RE, restriction endonuclease; BMI, body mass index; IL-6, interleukin 6; hsCRP, high sensitivity C-reactive protein; ROS, reactive oxygen species.

* Corresponding author. Fax: +27 31 260 4785.
E-mail address: chutur@ukzn.ac.za (A.A. Chuturgoon).

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1. Introduction

Cardiovascular disease is amongst the major causes of death worldwide (Mathers and Loncar, 2006). The underlying mechanism of coronary artery disease (CAD) is atherosclerosis. Atherosclerosis progresses gradually from an early age into the fifth and sixth decades. Atherosclerosis is exacerbated by type II diabetes mellitus (T2D) amongst other factors. The symptoms of CAD include angina, pain in the chest and radiating toward the back and down the left arm, shortness of breath, fatigue after exertion and weakness. The clinical indications of CAD include abnormal electrocardiogram and elevation of cardiac enzymes. These symptoms and clinical indications of fully blown CAD usually manifest on average in the sixth decade of an individual's lifespan. In South African Indians, the age of onset is at least 15 to 20 years earlier (http://www.lifeahead.net/atherosclerosis.htm). Originally thought to be a disease of first world countries, the prevalence of heart disease is increasing in developing countries, and particularly within populations of Indian ethnicity (Rajeshwari et al., 2005). This is true for the increase in incidence of CAD and T2D in South Africa.

Atherosclerosis is a chronic inflammatory vessel disorder characterized by lipid deposition and formation of fibrous plaques on the artery wall (Nordestgaard and Zacho, 2009). The process of atherogenesis activates the immune response that involves a host of inflammatory cells and cytokines (Nordestgaard and Zacho, 2009) and is augmented by oxidative stress (Hulsmans and Holvoet, 2009).

The roles of uncoupling proteins (UCPs) 2 and 3 are not clearly defined but have been linked for protection against oxidative damage; ageing and degenerative diseases (Affourtit et al., 2007). Uncoupling proteins are members of the superfamily of anion carrier proteins present in the inner mitochondrial membrane. They regulate the mitochondrial membrane potential by discharge of the proton gradient generated during oxidative phosphorylation and negatively regulate mitochondrial ATP synthesis. UCP2 and UCP3 can reduce the production of superoxide radicals at complex I of the mitochondrial respiratory chain by reducing the electrical potential across the inner mitochondrial membrane (Laskowski and Russell, 2008).

A recent review describes the role of UCPs in regulating oxidant stress and implications with respect to the pathogenesis of heart failure (Laskowski and Russell, 2008). Two other studies showed that UCP2 in macrophages is compulsory for efficient mitochondrial oxidation of glutamine which is a strong inducer of UCP2 expression (Hurtaud et al., 2007; Nübel et al., 2008). UCP2 increases fatty acid oxidation and promotes the metabolic shift from glucose oxidation to fatty acid oxidation in mouse embryonic fibroblasts (Pecqueur et al., 2008).

Changes in mitochondrial function and skeletal muscle fatty acid oxidation lead to increased triglyceride synthesis and ectopic lipid deposits (Roberts and Sindhu, 2009; Rogge, 2009). The accumulation of cellular triglycerides has been implicated in increased lipid peroxidation, nitric oxide synthase activity and pro-inflammatory cytokine production due to oxidative stress (Roberts and Sindhu, 2009). Furthermore, oxidative stress has emerged as the underlying mechanism for pathology in T2D and CAD. Several studies show that UCP2- and UCP3-deficient mice overproduce reactive oxygen species (ROS) and hyper-secrete insulin (Blanc et al., 2003; Brand et al., 2002; Vidal-Puig et al., 2000; Zhang et al., 2001). A number of studies have linked UCPs with disease prevalence, particularly, diabetes (Xu et al., 2011), obesity (Salopuro et al., 2009) and metabolic syndrome (de Luis et al., 2012a) which is associated with the development of CAD. The distribution of UCP single nucleotide polymorphisms (SNPs) has been identified in several ethnic groups at different frequencies (Xu et al., 2011).

A few studies assessed the UCP2 −866G/A SNP (located in the *cis*-regulatory site of the promoter region) and the UCP3 −55C/T SNP (located 6 base-pairs upstream from the TATA box in the core promoter region) and found a functional link with disease severity, progression and occurrence (Salopuro et al., 2009; Srivastava et al., 2010). A recent study showed a significant association of C-reactive protein (CRP) levels with the UCP2 −866G/A SNP in a group of diabetic patients (Lapice et al., 2010).

Premature CAD has been observed previously, but is still not well understood. The aim of this study was to explore whether a relationship between UCP2 −866G/A and UCP3 −55C/T SNPs influences the risk of CAD in young South Africans of Indian descent.

2. Materials and methods

2.1. Patient recruitment

One hundred young Indian male CAD patients (age range: 24–45 years), one hundred age, race and sex matched controls and 100 age- and sex-matched Black controls were enrolled in the study following institutional ethical approval (BE154/010). A full pathology report clinical marker was assessed by routine laboratory testing at Global Clinical and Viral Laboratory (Durban, South Africa), a fully accredited national pathology laboratory. The following parameters were tested: Haematology (Roche Sysmex 1800XT), Chemistry (Beckman Coulter DXC600), Endocrinology (Siemens Centaur XP), Serology (BD Biosciences FACS Calibur) as per international standards to obtain levels of total cholesterol, HDL, LDL, triglycerides, glucose, insulin and glycosylated haemoglobin in a fasted state as well as 2 h glucose levels. The inclusion criteria for CAD patients were: Indian ancestry and unrelated, adults below 45 years old and stable CAD confirmed at angiography. The exclusion criteria for controls were: an acute coronary syndrome/revascularization procedure in the preceding 3 months, chronic renal or liver disease, malignancy and known active inflammatory or infectious disease. All investigations conform to the principles outlined in the Declaration of Helsinki 2008.

2.2. DNA isolation

Genomic DNA was extracted from whole blood. Cells were transferred to 500 μL lysis buffer (0.5% sodium dodecyl sulphate, 150 mM NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris–HCl (pH 8.0)). To this RNase A (100 μg/mL; DNase-free) was added and incubated (37 °C, 1 h). Subsequently proteinase K (200 μg/mL) was added and incubated (3 h, 50 °C) and a 0.1% volume 5 mM potassium acetate was added before centrifugation (5000 ×*g*; 15 min). Supernatants containing genomic DNA were transferred to fresh tubes and extracted with 100% isopropanol and washed with 70% ethanol. DNA samples were solubilised in 10 mM Tris and 0.1 mM EDTA (pH 7.4, 4 °C). Concentration of DNA was determined spectrophotometrically and standardised.

2.3. Polymerase chain reaction–restriction length fragment polymorphism (PCR-RFLP)

Evaluation of SNPs in the UCP2 −866(G/A) rs659366 and the UCP3 −55(C/T) rs1800849 promoter region was done using PCR-RFLP. The UCP2 promoter region was amplified using 10 pmol of both forward 5′CACGCTGCTTCTGCCAGGAC3′ and reverse 5′AGGCGTCAGGAGATGGA CCG3′ primers that resulted in a 363 bp PCR product, the UCP3 region