International Journal of Cardiology 228 (2017) 507–510

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

The association of uncoupling protein 2 (UCP2) exon 8 insertion/deletion polymorphism and ECG derived QRS duration: A cross-sectional study in an Australian rural population

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ARTICLE INFO

Article history:
Received 7 August 2016
Accepted 6 November 2016
Available online 09 November 2016

Keywords:
UCP2 gene
ECG
QRS duration
QTd
Rural population

ABSTRACT

Background: Associations between inherited mitochondrial disease and cardiac conduction have been previously described. However, there are no available studies exploring the mitochondrial uncoupling protein 2 gene (UCP2) insertion/deletion (I/D) polymorphisms interaction on cardiac electrical conduction. Our aim was to determine if ECG-derived QRS duration is associated with UCP2 DD genotype in a cross-sectional Australian aging rural population.

Methods: A retrospective study design utilizing a rural health diabetic screening clinic data-base containing observational data from September 2011 to September 2014. Inclusion criteria included were having ECG parameters such as QRS duration measures and a DNA sample within the same subject. Genomic DNA was extracted and subjects were genotyped for the 45-bp I/D polymorphism in the 3′-untranslated region of UCP2.

Results: 281 individuals were available for analysis. On the basis of QRS duration >140 ms we found an increased percentage of our population with DD homozygotes, compared to ID heterozygotes and II homozygotes ($p = 0.047$). For other ECG parameters; mean PQ duration, QTc across UCP2 genotypes was not significant ($p =$ NS). QTc using a cut-off >440 ms in contingency table analysis revealed no significant differences across UCP2 I/D genotypes. Mean QT dispersion (QTd) was paradoxically less in the UCP2 DD genotype compared to UCP2 II subgroup ($p = 0.034$).

Discussion: We have demonstrated an association between increasing ECG-derived QRS duration >140 ms and the UCP2 DD polymorphism. The lack of association with ECG derived QTd and UCP2 DD may suggest that gene-related QRS duration prolongation is independent of cardiac hypertrophy.

1. Introduction

Uncoupling proteins (UCPs) are located in the inner mitochondrial membrane and mediate proton leakage, which regulates the production of ATP and reduces oxidative stress [1]. UCP2 is widely expressed in various tissues, including the human heart [2]. The UCP2 insertion/deletion (I/D) polymorphism is a 45 bp insertion in exon 8 in the 3′ untranslated region of the UCP2 gene [3,4]. Polymorphisms in UCP2 I/D genes may regulate UCP2 mRNA stability via post-transcriptional modification of UCP2 protein expression [5]. However, UCP2 45-bp double deletion does not appear to affect muscle UCP2 mRNA expression levels in Prima Indians [5]. Population studies concerning the UCP2 I/D polymorphism have predominantly focused on associations concerning metabolic syndrome, such as high body mass index, insulin resistance and diabetic risk [6,7].

A small number of studies have detailed interactions between mitochondrial inherited disease and electrocardiogram (ECG) parameters. In severe forms of inherited mitochondrial disease affecting the mitochondrial respiratory chain cardiac conduction abnormalities have been identified [8]. For example, electrocardiogram QRS duration prolongation and bundle branch block have been observed commonly in Korean children with inherited rare mitochondrial respiratory chain disorders [9]. On the other hand, it is unknown whether mild oxidative mitochondrial uncoupling, i.e. functional UCP2 activity in the heart, could result in cardiac conduction abnormalities such as QRS prolongation. There is no available data to suggest that UCP2 I/D polymorphisms may influence cardiac electrical function in adult populations. However, associations between the UCP2 I/D gene polymorphism and changes in cardiac

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http://dx.doi.org/10.1016/j.ijcard.2016.11.100
0167-5273/

sympathetic tone and resting metabolic rate (in Japanese men) have been described [10].

We suggest that with aging, UCP2 gene polymorphisms may induce subtle change in cardiac ventricular conduction. Aging is characterized by gradual, though distinct, alterations in myocardial structure and electrical function [11,12]. Healthy cardiac aging appears to require functional expression of mitochondrial UCP2. For example, in an aging mouse mitochondrial DNA mutator model, it has been demonstrated that UCP2 expression is required to enhance lifespan. UCP2 functional expression prevents early onset maladaptive cardiomyopathy that occurs phenotypically in mitochondrial DNA mutator mice [13]. Cardiomyopathy is well known to be associated with conduction delay in both animal models and in humans. Thus, it remains uncertain whether UCP2 I/D polymorphisms may have direct associations with cardiac conduction in the absence of overt cardiovascular remodelling.

Community-based epidemiological studies provide an opportunity to explore interactions between different electrical parameters on the surface ECG and associations with UCP2 I/D genotypes. Our aims are to explore polymorphisms in the 3′-untranslated region of exon 8 of the UCP2 gene (I/D polymorphism) on QRS duration and QT dispersion (QTd) in an Australian aging rural community. We suggest that these ECG conduction bio-markers would be useful to tease out any genetic interactions within population-based cardiac conduction data, concerning slowing (increased QRS duration) and or ventricular heterogeneous conduction QTd.

2. Methods

2.1. Study population and design

A retrospective sub-study for UCP2 I/D polymorphisms on ECG conduction was conducted in 281 subjects from the community health screening program at the Albury-Wodonga campus at Charles Sturt University [14,15]. Briefly, subjects aged 21 years and above were asked to participate in the cardiovascular risk and diabetes screening program. The available subjects with complete UCP2 exon 8 insertion/deletion genotypes were from the Albury-Wodonga area on New South Wales-Victoria border in southeast Australia. Written informed consent was obtained from each subject. Ethical approval for the study was obtained from the institutional review board at Charles Sturt University. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Data collection

A standardized questionnaire was used to collect demographic information such as age, gender, smoking history, alcohol use and previous or pre-existing medical conditions including diabetes, hypertension and any known history of pre-existing cardiovascular disease. Anthropometric and physiologic measurements were taken including body mass index (BMI) and blood pressure. Resting 12-lead electrocardiograms (ECG) were obtained using Welch Allyn PC-Based ECG system which automatically calculated QRS duration and QTd from a 10 s ECG strip [14,16].

2.3. Blood collection and biochemistry assessment

Venous blood samples were collected in EDTA-containing tubes. Fasting blood glucose was measured using Accu-Check Advantage II glucometer (Roche Australia P/L). Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides were processed at South West Pathology, Albury.

2.4. DNA genotyping

Genomic DNA was extracted according to manufacturer's instructions (QIAamp DNA Mini Kit; QIAamp®, Qiagen, Victoria, Australia). The 45-base pair (bp) insertion or deletion flanking region is as follows: Forward and reverse primers (GeneWorks, Adelaide, Australia) for detection of the 45-bp UCP2 polymorphism were 5′ CAG TGA GGG AAG TGG GAG G 3′ and 5′ GGG GCA GGA CGA AGA TTC 3′, respectively [5]. Polymerase chain reaction (PCR) was performed using commercially available PCR master mix, GoTaq® Colorless Master Mix (2X) (Promega Corp., Wisconsin, USA). The final PCR reaction volume is 10 μl, including 500 nM each primer, GoTaq® Colorless Master Mix 1X; 15 ng DNA template. Cycling conditions were: 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 64 °C for 45 s and 72 °C for 5 min. The amplified PCR products were separated on a 6% acrylamide gel for 90 min at 90 V along with a DNA ladder (HyperLadder™ 25 bp; Bioline Ltd., UK). After electrophoresis, RedSafe™ Nucleic Acid Staining Solution was added to the gel according to manufacturer's instructions. Visualization was via G:Box BioImaging (Syngene, Maryland, USA) under ultraviolet light. To confirm the correct DNA amplification, the PCR products from 30 subjects was DNA sequenced, 10 subjects for each genotype.

2.5. Statistical analysis

Data expressed as means and standard deviation (SD). χ^2 test were used to analyse the relationship between categorical variables. ANOVA was used to explore mean ECG parameters and other demographic parameters differences across UCP2 genotype distribution sub-groups. Various statistical models were conducted to evaluate QRS duration and UCP2 I/D genotype interactions. Specifically, we explored continuous QRS durations, using pre-defined categorical cut-offs (80–100 ms, 101–120 ms, >120 ms, >140 ms) and natural QRS duration quartiles across UCP2 I/D genotypes. Statistical analysis was completed using STATA version 13 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

3. Results

The demographic characteristics for the study population of the 281 genotyped subjects for UCP2 45-bp insertion (I) or deletion (D) and are summarized in Table 1. Across demographic factors, diabetic status and physiological blood pressure measurements across UCP2 DD homozygote, ID heterozygote and II homozygote subgroups, no significant differences were found.

ECG parameters (QRS duration, QRS axis, PQ interval, QTd, corrected QT interval (QTc)) interactions across UCP2 I/D polymorphism subgroups are presented in Table 2. 25/281 participants in our study had a QRS duration >120 ms, of which 14/25 (56%) of these participants had a QRS duration ≥ 140 ms, and 9/25 (36%) had a QRS duration of ≥150 ms and 5/25 participants had a QRS ≥ 160 ms. Mean (SD) QRS duration measures across DD homozygotes, ID heterozygotes and II homozygotes were 103.7 ms (18.3), 101 ms (12.9) and 104.5 ms (22.2), respectively. Contingency table analysis for patients stratified on the basis of QRS duration (80–100 ms, 101–120 ms and >120 ms) and UCP2 I/D subgroups, revealed no significant differences ($p = NS$). Further exploratory contingency table analysis for QRS duration across natural quartiles and UCP2 I/D genotype subgroups, revealed no significant differences ($p = NS$).

In contingency table analysis, on the basis of QRS duration ≥ 140 ms we found an increased percentage of subjects who were DD homozygotes, compared to ID heterozygotes and II homozygotes ($p = 0.047$). For other ECG parameters; mean PQ duration across UCP2 genotypes ($p = NS$), mean QTc interval across UCP2 genotypes ($p = NS$). QTc using a cut-off >440 ms in contingency table analysis revealed no significant differences across UCP2 I/D genotypes, similarly contingency table analysis for QRS axis and UCP2 I/D genotypes revealed no significant differences ($p = NS$), whereas QT dispersion (QTd) was significant ($p = 0.034$).

Factors that may have influenced QRS duration (>140 ms) and UCP2 genotypes, were adjusted in multivariate models. Covariates adjusted were age and gender known to influence QRS duration and BMI, and diabetes known to interact with UCP2 genotypes (Table 3). We observed UCP2 I/D heterozygotes had shorter QRS duration compared to UCP2 DD homozygotes (75% UCP2 I/D had QRS < 140 ms, $p = 0.068$) after adjusting for age, gender, BMI and diabetes status.

4. Discussion

We have investigated associations between UCP2 45-bp I/D genotypes and ECG-derived cardiac conduction parameters in a cross-sectional Australian rural population. The primary finding was that the UCP2 45-bp double deletion (DD) genotype was overrepresented in the ≥140 ms QRS subgroup. A QRS duration > 140 ms is suggestive of intra-ventricular conduction delay [17]. In the study population, we have not explored cardiovascular function in terms of pre-existing heart failure, ischemic heart disease or imaging to determine whether the presence of cardiac hypertrophy could account for a QRS duration > 140 ms. Cardiac hypertrophy is likely to be present if QRS > 130 ms or 140 ms (depending on gender) and QTd is increased. Interestingly, we found QTd to be decreased in the population with the UCP2 DD genotype. Our findings suggest a slowed ventricular