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RESEARCH LETTER

The associations between polymorphisms in the CD36 gene, fat oxidation and cardiovascular disease risk factors in a young adult Australian population: A pilot study

KEYWORDS
Lipid oxidation;
SNP;
Fatty acid translocase;
Heart rate;
Blood pressure

Summary Our pilot study in a young adult Australian cohort aimed to investigate potential associations between CD36 polymorphisms (rs1527479 and rs1984112), fat oxidation and cardiovascular disease risk. CD36 genotype was associated with fat oxidation during sub-maximal exercise, resting heart rate and blood pressure, indicating increased chronic disease risk in this otherwise healthy cohort.

Introduction

CD36 gene variants have been associated with metabolic and cardiovascular disease (CVD) [1—4]. However, a move from association to causality is required to elucidate the roles of specific genes in the pathogenesis of obesity related diseases [5]. The 88 kDa multifunctional membrane glycoprotein encoded by the CD36 gene is expressed in a number of cells [6], but its facilitation of cellular fatty acid (FA) uptake is of interest when we explore associations with chronic disease [7].

At present, there is no data relating to CD36 gene variants in an Australian cohort. Particular emphasis on a young, healthy cohort in this functional genetic study minimizes secondary phenotypic influence from overt pathology commonly present in older cohorts, allowing for identification of early indicators in a hypothesized ''at-risk'' population.

Our aim was to investigate two CD36 single nucleotide polymorphisms (SNPs) (rs1527479 and rs1984112), previously associated with chronic disease in older populations [2,3], and examine associations with CVD risk factors, as well as whole body fat oxidation (Fat_{ox}) during exercise.

Methods and procedures

Twenty-two (15 men/7 women; 18—30 years) physically active non-smokers, who were capable of performing an incremental cycling test to exhaustion volunteered for the study. This study was approved by the University of Sydney Human Research Ethics Committee.

Phenotyping

Participants reported to the laboratory on two occasions following a 10-h fast (>1-wk apart). Participants abstained from alcohol, caffeine and strenuous exercise for 24-h prior to each session. Women attended during the early-to-mid follicular phase of their menstrual cycle [8].

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Session one involved blood sample collection for DNA extraction, body composition [bio-electrical impedance analysis (BIA)] and maximal oxidative capacity (VO_{2peak}) assessment using an incremental cycling test.

Session two included measurement of resting CVD risk factors, namely resting heart rate (RHR), blood pressure (BP) and rate-pressure product (RPP), and assessment of Fat_{ox} during a sub-maximal cycling task. Participants followed a controlled diet normalized to body weight (175 kJ/kg), on the day prior to assessment [9]. Six 6-min sub-maximal stages representing 30—80% VO_{2peak} were used to assess Fat_{ox}. Ventilatory gas was collected for the final 2-min of each stage. Fat_{ox} was calculated using non-protein respiratory quotient [10].

Genotyping

Genomic DNA was extracted from whole blood. C/T SNP rs1527479 (intron 1B, −3489-bp relative to translation start site) and G/A SNP rs1984112 (5′ flanking exon 1A, −33137-bp relative to translation start site) were genotyped using custom Taqman® real-time polymerase chain reaction (PCR) technology (VIC® and FAM™ labelled-dyes).

Statistical analyses

Data are presented as mean ± SE or median (range). Both SNPs were tested for departure from Hardy—Weinberg equilibrium using chi-square analysis. Sequential one-way ANCOVA models were constructed to determine differences across genotypes at both loci. Dominant allele ANCOVA models were also performed (SNP carriers vs. non-carriers). All ANCOVA models were adjusted for age, sex and VO_{2peak}. Post hoc analysis was conducted using Fisher's LSD test. $p < 0.05$ was considered significant. Post hoc analyses were considered for all ANCOVA models where $p < 0.1$ due to limited sample size. Statistical analyses were performed using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA), and effect sizes (*Cohen's d*) calculated using G*Power 3.1.2 (Kiel, Germany) [11].

Results

Genotype distribution at both SNP loci were in Hardy-Weinberg equilibrium. TT carriers at rs1527479 had significantly lower Fat_{ox} at 40% VO_{2peak} than CC carriers ($p = 0.036$) (Fig. 1). TT participants also tended towards lower Fat_{ox} at 30% VO_{2peak} ($p = 0.088$, $d = 1.3$) during dominant allele analysis (data not shown).

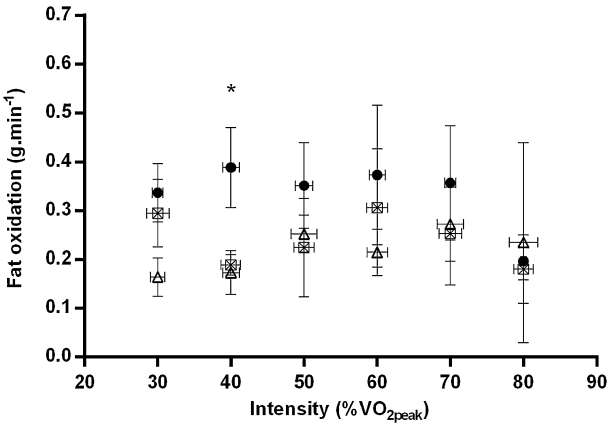

Figure 1 Fat oxidation (Fat_{ox}) rates were obtained during a six stage submaximal cycling test, corresponding to 30—80% VO_{2peak}. Results are stratified by genotype at SNP rs1527479; CC (*circles, n = 6)*, CT (*squares, n = 9*) and TT (*triangles, n = 7*). Results are expressed as mean ± SE, with data at 40% VO_{2peak} log transformed prior to using parametric statistics. All data adjusted for age, sex and VO_{2peak}. * Significance at $p < 0.05$.

TT carriers had significantly higher RHR compared to CC ($p = 0.016$) and CT ($p = 0.008$) carriers (Table 1), as well as C-allele carriers in dominant allele analysis ($p = 0.003$) (Table 2). RPP was significantly higher in TT carriers compared to CT ($p = 0.024$) and C-allele ($p = 0.029$) carriers.

Wild-type GG carriers at rs1984112 showed significantly elevated RHR ($p = 0.005$). DBP was significantly lower in AA carriers at rs1984112 compared to GA ($p = 0.02$) and G-allele carriers overall ($p = 0.013$). No significant difference in Fat_{ox} data was present in rs1984112 using either model (data not shown).

No differences were observed in VO_{2peak} or % body fat at either SNP loci using either model.

Discussion

TT carriers at rs1527479 were observed to have lower rates of Fat_{ox} at 40% VO_{2peak}. Post hoc analysis of both SNPs using both models identified large effect sizes ($d = 0.7—1.4$) at lower exercise intensities (30—40% VO_{2peak}), highlighting the potential influence of CD36 in FA mobilization. A rare CD36 deficiency has been associated with increased serum FA concentration and lower ventilatory threshold during exercise, postulated to be a result of increased glucose oxidation [12].

Analysis of CVD risk factors in our cohort identified interesting associations, in particular RHR. TT

Table 1 Clinical characteristics of all participants according to genotype at both SNP loci.

	rs1527479 variants			rs1984112 variants		
	CC ($n=6$)	CT ($n=9$)	TT ($n=7$)	GG ($n=4$)	GA ($n=9$)	AA ($n=9$)
Age (year)	23.4 ± 1.0	22.1 ± 0.7	21.9 ± 0.7	20.9 ± 0.5	21.9 ± 0.6	23.5 ± 0.8
Body weight (kg)	69.9 ± 5.7	74.5 ± 4.8	70.6 ± 5.2	65.2 ± 5.5	73.7 ± 4.2	73.3 ± 5.3
BF (%)	19.1 ± 1.3	22.6 ± 2.4	18.2 ± 2.4	20.2 ± 3.7	19.2 ± 2.2	21.3 ± 1.9
SBP (mmHg)	127 ± 2*	115 ± 2*	118 ± 4	114 ± 5	119 ± 3	122 ± 3
DBP (mmHg)	77 ± 3	75 ± 3	81 ± 3	79 ± 3	81 ± 3*	74 ± 3*
MAP (mmHg)	93 ± 2	89 ± 3	94 ± 3	90 ± 3	94 ± 3	90 ± 3
RHR (bpm)	55 ± 3*	56 ± 2*,†	69 ± 4*,†	72 ± 5†,‡	59 ± 4†,‡	55 ± 2†,‡
RPP (mmHg.bpm)	6944 ± 422	6431 ± 318*	8135 ± 716*	8207 ± 817	7050 ± 649	6758 ± 305
VO_{2peak} ($L.min^{-1}$)	3.3 ± 0.4	3.0 ± 0.4	3.0 ± 0.3	2.8 ± 0.3	3.0 ± 0.3	3.3 ± 0.4
PO_{peak} (W)	296 ± 26	261 ± 28	286 ± 15	274 ± 18	277 ± 24	281 ± 21

Values are presented as means ± standard error. Non-normally distributed values are represented as median (range). All data adjusted for age, sex and VO_{2peak}. BF, body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; RHR, resting heart rate; RPP, rate-pressure product; VO_{2peak}, maximal oxidative capacity; PO_{peak}, peak power output.

* Significance at $p<0.05$.

† Significance at $p<0{\cdot}01$.

‡ Significance at $p<0.005$.

Table 2 Dominant allele model analyses of clinical characteristics at both SNP loci.

	rs1527479 variants			rs1984112 variants		
	C-Allele ($n=15$)	TT ($n=7$)	Effect size (d)	G-Allele ($n=13$)	AA ($n=9$)	Effect size (d)
Age (years)	22.1 ± 0.8	21.9 ± 0.7		21.6 ± 0.5	23.5 ± 0.8	
Body weight (kg)	72.7 ± 3.6	70.6 ± 5.2	0.2	71.1 ± 3.4	73.3 ± 5.3	0.2
BF (%)	21.2 ± 1.6	18.2 ± 2.4	0.5	19.5 ± 1.8	21.3 ± 1.9	0.3
SBP (mmHg)	120 ± 2	118 ± 4	0.2	117 ± 3	122 ± 3	0.6
DBP (mmHg)	76 ± 2	81 ± 3	0.8	80 ± 2*	74 ± 3*	0.8
MAP (mmHg)	91 ± 2	94± 3	0.4	93 ± 2	90 ± 3	0.4
RHR (bpm)	56 ± 2‡	69 ± 4‡	1.3	63 ± 4	55 ± 2	0.8
RPP (mmHg.bpm)	6340 (3256)*	7000 (4746)*	1.0	7436 ± 516	6758 ± 305	0.5
VO_{2peak} ($L.min^{-1}$)	3.1 ± 0.3	3.0 ± 0.3	0.1	2.9 ± 0.2	3.3 ± 0.4	0.4
PO_{peak} (W)	275 ± 20	286 ± 15	0.2	276 ± 18	281 ± 24	0.1

Normally distributed values presented as means ± SE. Non-normally distributed values are represented as median (range). All data adjusted for age, sex and VO_{2peak}. BF, body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; RHR, resting heart rate; RPP, rate-pressure product; Fat_{ox}, whole body fat oxidation; VO_{2peak}, maximal oxidative capacity; PO_{peak}, peak power output.

* Significance at $p<0.05$.

‡ Significance at $p<0.005$.

carriers at rs1527479, and GG carriers at rs1984112, had significantly elevated RHR values, independent of fitness related variables. Although non-clinical, these participants could be at an increased risk of future CVD due to the linear association between RHR above 60 beats per minute and CVD [13,14].

Additionally, the association between both SNP loci and BP in our young cohort is of particular interest. A model of CD36 deficiency showed that these participants had significantly higher BP [15]. No other data has identified an association between CD36 genotype and altered BP directly.

In summary, CD36 genotype was associated with Fat_{ox}, RHR, RPP and BP, possibly affecting future chronic disease risk in healthy individuals. Validation of these findings in larger cohort studies is warranted.

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