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Mitochondrial Uncoupling Protein Gene Cluster Variation (*UCP2-UCP3*) and the Risk of Incident Type 2 Diabetes Mellitus: The Women's Genome Health Study

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Abstract

Objective—Uncoupling protein 2, mitochondrial, (*UCP2*) gene variation has recently been implicated in type 2 diabetes Mellitus (T2DM). To date, no prospective epidemiological data are available.

Methods—The association between 14 UCP (*UCP2-UCP3*) gene cluster tagging-SNPs and incident T2DM was investigated in 22,715 Caucasian participants of the prospective Women's Genome Health Study. All were free of known cardiovascular disease and diabetes at baseline. During a 13-year follow-up period, 1,445 participants developed an incident T2DM. Multivariable Cox regression analysis was performed to investigate the relationship between genotypes and T2DM risk assuming an additive model. Stratified analysis by smoking status, and haplotype analysis were also performed.

Results—No evidence for an association of any of the tagging-SNPs tested with T2DM risk. Further investigation using stratified analysis, and haplotype-based approach showed similar null findings.

Conclusion—The present investigation suggests that *UCP* gene cluster variation may not be useful predictor for T2DM risk assessment.

1. Introduction

Heightened inflammatory responses and oxidative stress are noted in patients with diabetes [1–3]. Genetic variation of uncoupling protein 2, mitochondrial [4] (encoded by UCP2 on chromosome 11q13) --an important regulator of mitochondrial oxidative stress damage induced by reactive oxygen species (ROS)-- has recently been implicated in T2DM [5]. In addition, a *UCP2* gene variant dbSNP rs659366 (−866G>A) has been shown to interact with smoking to increase oxidative stress in T2DM patients [6]. As *UCP2* is part of a *UCP2-UCP3* gene cluster locus, this cluster locus represents an important candidate in the pathogenesis of T2DM.

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Conflict of Interest
None declared

However, to date, no prospective epidemiological data are available on examining the importance of *UCP2-UCP3* gene cluster locus as a risk marker for T2DM. We evaluated the potential association of 14 *UCP* gene cluster-tagging single nucleotide polymorphisms (SNPs) with (i) baseline glycosylated hemoglobin (HbA1c) and C-reactive protein (CRP) levels, and (ii) T2DM risk, in a large prospective cohort of 22,715 initially healthy women.

2. Material and Methods

2.1. Study design

Details of the study design have been previously described [7]. In brief, participants in the Women's Genome Health Study (WGHS) – a genetic substudy of the Women's Health Study [8,9]– included initially healthy North American women aged 45 or older with no previous history of cardiovascular disease, cancer, diabetes or other major chronic illness. A baseline blood sample was collected between 1992 and 1995. All participants gave an informed consent for blood-based analyses related to risks of incident chronic diseases. All study participants were followed up through March 2007 for incident events that were adjudicated by an endpoints committee using standardized criteria and full medical record review. Only confirmed end points were included in this analysis. During a 13-year follow-up period, 1445 newly diagnosed T2DM cases of 22,715 Caucasian participants of the WGHS were identified. Genotyping was performed using the Illumina Infinium II assay to query a genome-wide set of 318,237 single nucleotide polymorphisms (SNPs) (Human HAP300 panel) as well as an additional focused panel of 45,751 SNPs thought to be of relevance to metabolic, lipid, inflammatory, and other biological functions [10,11]. The Brigham and Women's Hospital Institutional Review Board for Human Subjects Research approved the study protocol.

2.2. Statistical analysis

Genotype frequencies were compared with values predicted by Hardy-Weinberg equilibrium using the chi-square test. Multivariable linear regression analysis, adjusting for age, body-mass index (BMI), current smoking status and current hormone use, was performed to assess the relationship of the tagging-SNPs with baseline glycosylated hemoglobin (HbA1c) and C-reactive protein (CRP) levels. Hazard ratios (HRs) of T2DM associated with each of the individual SNPs were calculated separately by Cox regression analysis adjusting for age, current smoking status, and further adjusting for BMI, randomized treatment assignment, history of hypertension, and hyperlipidemia, and current (any) hormone use, assuming an additive model for genetic effects. In addition, the relationship between genotypes and current smoking status was examined by multivariable Cox regression analysis, with genotype-by-smoking interaction terms. Stratified analysis by smoking status was also performed.

Haplotype estimation and inference were determined by expectation-maximization algorithm [12]. Haplotype blocks were defined using the software Haploview v4.1. In addition, the relationship between haplotypes and T2DM was examined by a referent haplotype-based Cox regression analysis, adjusting for the same potential covariables. All analyses were carried out using SAS v9.1 package (SAS Institute Inc). A 2-tailed p-value of 0.05 was considered a statistically significant result. Genotyping call rates were >99% per SNP.

3. Results

The baseline characteristics of the 22,715 initially healthy Caucasian women are shown in Table 1. All SNPs were in Hardy-Weinberg equilibrium (HWE) with p-values >0.08, except

rs1626521 (p=0.0212), rs1800849 (p=0.0345), and rs1685333 (p=0.0251), respectively (Supplementary Data Table 1). In the multivariable linear regression analysis, a modest negative relationship of rs622064 with baseline HbA1c (beta-estimate=−0.010, S.E.=0.004, t=−2.32, p=0.020), and with CRP (beta-estimate=−0.118, S.E.=0.059, t=−2.01, p=0.045) levels were observed. However, none of these remained significant after Bonferroni correction for multiple testing or false discovery rate (data not shown). Results from the Cox regression analysis showed no evidence for an association with T2DM risk (Table 2), although the direction of effect estimates was concordant for rs622064 with its effect on HbA1c, and CRP. Additional adjustment for baseline HbA1c and CRP levels did not materially change our results (data not shown). Supplementary Data Figure 1 presents the LD relationship of the SNPs tested. Results from the haplotype-based analysis, genotype-by-smoking interaction, stratified analysis by smoking status as well as non-additive (dominant or recessive) genetic models again showed virtually identical null findings (data not shown).

4. Discussion

The present prospective investigation found no evidence for any association of the *UCP* SNPs/haplotypes thereof tested with T2DM risk. Recent findings have implicated common polymorphisms of *UCP2,* in particular rs659366 and rs660339, in diabetes, in obesity, and with changes in UCP2 mRNA levels [13]. Furthermore, the *UCP2-UCP3* gene cluster variation has also been implicated in the metabolic aberrations observed in obesity [14,15] and type 2 diabetes [15]. A more recent study by Salpea *et al*. further suggested the importance of *UCP2* gene variation and oxidative stress in T2DM [4]. In their study, the authors found an association of the *UCP2* functional promoter variants (rs659366) with the leukocyte telomere length and a trend to lower plasma total antioxidant status, implicating a link between mitochondrial production of ROS and shorter telomere length in T2DM [4]. As no prospective epidemiological data of the *UCP* gene cluster variation on the risk of incident T2DM are available, a cross-reference comparison cannot be made on the present null findings. Of note, as mitochondrial uncoupling represents only one source of ROS generation which may or may not play a role in T2DM, other candidate genes/loci (not in LD with *UCP* cluster locus) within the ROS pathway warrant further investigation.

Strengths of the present study are the overall sample size, the prospective design and the complete long-term follow-up. Nonetheless, some potential limitations of our study require discussion. Our sample population was limited to Caucasian female healthcare professionals from the US. Thus, our results may not be generalizable to other racial/ethnic or socio-economic groups, geographical regions, or to males. Further information on UCP gene cluster variation relating to functional implication was explored via public databases including the NCBI PubMed, the UCSC Genome Bioinformatics-Browser and the Gene Ontology Database. Because of limited information available, no relevant or constructive implications can be drawn. Furthermore, the possibility that an uncontrolled (masking) confounders/covariables could account for the present findings with T2DM cannot be excluded, especially when few risk factors' association with T2DM risk or diabetes-associated intermediate phenotypes was examined (for example, geographical location of residence, dietary preferences, and glucose and insulin levels were not included). In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, an effect estimate of greater than 1.15 if the minor allele frequency is 0.45, and of greater than 1.90 if the minor allele frequency is 0.01.

5. Conclusion

The present prospective data from a large cohort of apparently healthy Caucasian US females provide no evidence for an association between *UCP* cluster variants tested and

T2DM risk. If corroborated in other large prospective studies, our data further suggest that *UCP2-UCP3* locus is not informative for risk assessment of T2DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics.

Variable	N=22,715
Age, years	52 (48, 58)
Body-mass index, kg/m^2	24.80 (22.40, 28.28)
History of hyperlipidemia ≥ 240 mg/dL, %	29.29
History of hypertension ≥140/90 mmHg, %	23.72
Smoking status	
current	11.62
past	37.42
never	50.96
Current hormone use, %	44.15
Aspirin use, %	49.82
Beta-carotene use, %	49.81
Vitamin E use, %	50.13
C-reactive protein, mg/L	1.98 (0.79, 4.27)
HbA1c, %	4.98 (4.083, 5.16)

Data are median and interquartile range for continuous, and percentages for categorical variables.

Table 2

Cox regression analysis of incident T2DM.

SNP	HR	95%CI	$p_{Uncorrected}$
UCP2-UCP3			
rs622064	0.969	0.886–1.059	0.4864
rs2306820	0.991	0.914–1.076	0.8354
rs655717	0.997	0.926–1.074	0.9429
rs660339	1.004	0.931–1.082	0.9241
rs17132534	1.045	0.877–1.245	0.6224
rs659366	0.991	0.917–1.070	0.8105
rs668514	0.938	0.859–1.024	0.1528
rs3741135	1.030	0.944–1.124	0.5042
rs1626521	0.951	0.874–1.034	0.2392
rs2734827	0.984	0.910–1.063	0.6768
rs3781907	1.015	0.935–1.102	0.7248
rs1800849	1.045	0.959–1.139	0.3183
rs1685333	1.032	0.947–1.125	0.4693
rs826071	0.871	0.651–1.164	0.3495

Adjusted for age, body-mass index, randomized treatment assignment, history of hypertension, hyperlipidemia, current smoking, and current hormone use.