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Lack of Association between Uncoupling Protein-2 Ala55Val polymorphism and Incident Diabetes in the Atherosclerosis Risk in Communities Study (ARIC)

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Abstract

Type 2 diabetes mellitus (T2DM) is characterized by impaired insulin secretion, peripheral insulin resistance, and increased hepatic glucose production. Genes that contribute to genetic susceptibility to T2DM function in numerous biochemical pathways. Uncoupling protein-2 (*UCP2*) functions as a negative regulator of insulin secretion. Animal studies show induction of *UCP2* plays a pathogenic role in the progression of obesity-induced T2DM, and some human studies have shown an association between a common *UCP2* polymorphism, Ala55Val (rs660339), and T2DM, obesity, and resting metabolic rate with the Val/Val genotype conferring increased risk. We investigated the relationship between the Ala55Val variant and incidence of T2DM among 12,056 participants in the Atherosclerosis Risk in Communities (ARIC) Study ages 45−64 years at baseline. Incident T2DM (n=1,406) cases were identified over 9 years of follow-up. The Val55 allele frequency was 44% in blacks and 41% in whites. The rate of T2DM per 1,000 person-years was 15.0, 15.6, and 15.6 for Ala/Ala, Ala/Val, and Val/Val genotypes respectively. We found no significant association between *UCP2* genotypes and incident T2DM in the whole cohort, in race-gender subgroups, or in categories of body mass index (normal-overweight-obese). The Ala55Val polymorphism of *UCP2* was not associated with incident T2DM in the ARIC cohort.

Keywords

mitochondrial uncoupling protein 2; Diabetes Mellitus; Type 2; Polymorphism; Single Nucleotide; Obesity; genetics

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by impaired insulin secretion, peripheral insulin resistance, and increased hepatic glucose production. T2DM is a heterogeneous disease with a complex etiology including a strong genetic component. Genes that contribute to genetic

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susceptibility to T2DM function in numerous biochemical pathways. Of interest in this study is the uncoupling protein-2 (*UCP2*) gene.

UCP2 is expressed in many tissues including pancreatic islets and β-cells. *UCP2* is thought to uncouple respiration by mediating proton leak thereby altering the mitochondrial membrane potential. The current paradigm is that *UCP2* plays a critical role in the negative feedback loop controlling levels of reactive oxygen species (ROS). *UCP2* is activated by ROS, and the reduction of metabolic efficiency due to *UCP2* uncoupling results in decreased ROS levels. *UCP2* expression also reduces glucose stimulated insulin secretion in pancreatic cells [1]. Therefore, individuals that express higher levels of *UCP2* may be at greater risk for diabetes given that *UCP2* functions as a negative regulator of insulin secretion. Conversely, it has also been suggested in the literature that low levels of *UCP2* may confer increased risk of diabetes through beta cell destruction by high ROS levels [2].

Several studies have found variants of the *UCP2* gene associated with T2DM and obesity, although inconsistent results have been reported[3,4]. Yu et. al. investigated the relationship between diabetes and Ala55Val, in 3,684 individuals participating in the Coronary Artery Risk Development in Young Adults (CARDIA) Study, and observed higher incidence of diabetes in Val/Val compared to Ala/Ala in both black and white subjects [4]. We investigated the relationship between a variant of *UCP2*, Ala55Val (rs660339), and incident T2DM using the Atherosclerosis Risk in Communities (ARIC) Study.

Subjects and Methods

Subjects

The ARIC study is a prospective cohort study investigating the etiology of atherosclerosis. The study randomly selected a sample of 15,792 participants, ages 45−64 years, from four field centers: Forsyth County NC, Jackson MS, northwest suburbs of Minneapolis MN, and Washington County MD. The ARIC study has been described in detail elsewhere [5]. This study had 9 years of follow-up data for incident diabetes from the baseline examination performed in 1987−1989 through three follow-up clinic visits at approximately three year intervals. For the present analysis, individuals were excluded based on the following criteria: race other than black or white (n=48), missing data for *UCP2* genotype (n=1,050), prevalent diabetes at baseline (n=1,723), missing baseline diabetes status (n=99), incomplete data to assess incident diabetes (n=805), missing data on baseline obesity covariates (n=11). The final analysis sample included 12,056 without diabetes at baseline and a mean follow-time of 7.6 years.

Measurements

Height was measured while participants were standing without shoes, heels together against a vertical mounted ruler. A Detecto Platform Balance was used to measure weight. BMI was calculated as weight (kg)/height^2 (m^2). Hip and waist circumferences were measured at the maximal protrusion of the hips and at the level of the umbilicus with the participant standing erect. Medical and personal histories were ascertained via interview. Annual telephone follow-up maintained contact and assessed health status of the participants.

Glucose was measured using a hexokinase/glucose-6-phosphate dehydrogenase procedure. Incident T2DM was defined using the following criteria from data obtained during the follow-up examinations; current diabetic medication use, non-fasting glucose ≥200 mg/dl, fasting glucose ≥126 mg/dl, or self report of diabetes diagnosed by physician. These same criteria were used from data obtained at baseline to determine prevalent diabetes for exclusion.

Genotyping

Genotyping of the *UCP2* Ala55Val polymorphism was performed using the TaqMan assay (Applied Biosystems). Allele detection and genotype calling were performed using the ABI 7900 and the Sequence Detection System software (Applied Biosystems). Sequence information for all oligonucleotide primers used for variant screening is available upon request.

Statistical Methods

We tested for Hardy Weinberg equilibrium using the χ^2 goodness of fit test. Cox proportional hazards models were used to estimate the hazard rate ratios of incident diabetes by genotype. All data were analyzed with SAS, Version 8. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the previous one, as previously described [6]. Follow-up continued for non-diabetics until date of death, the date of last contact, or else a pre-determined censoring date (December 1999).

Results

The study population free of diabetes at baseline included 22% blacks and 78% whites. The mean age at baseline was 54 years and ~55% were women. We observed an allele frequency of 44% in blacks and 41% in whites for Val55, consistent with other published studies[7]. The genotype frequencies were in Hardy-Weinberg equilibrium for all race-sex groups ($p>0.05$). Baseline risk characteristics did not differ by UCP2 genotype (Table 1).

Incidence rates of T2DM by genotype are listed in Table 2 for the total cohort and within levels of BMI adjusting for age, race, and field center. The rate of T2DM per 1,000 person years was 15.0, 15.6, and 15.6 for Ala/Ala, Ala/Val, and Val/Val genotypes respectively. Incidence rates of T2DM were similar across *UCP2* genotypes in the whole cohort and within strata of BMI. T2DM incidence rates also did not differ significantly across genotypes in race-gender subgroups or in BMI-stratified race-gender groups (data not shown).

Discussion

We investigated the association between a polymorphism in the *UCP2* gene, Ala55Val, and T2DM in the ARIC cohort. We found no association between *UCP2* genotypes and incident T2DM in the whole cohort, in race-gender subgroups, or in categories of body mass index: normal, overweight, and obese.

Several hypotheses emerge as we consider why we did not observe an association between Ala55Val and incident diabetes in the ARIC cohort. The *UCP2* gene may be a genetic risk factor in diabetes but the Ala55Val polymorphism may not be an important variant. The Ala55Val polymorphism entails a conservative substitution within coding exon 2 and functional studies for this polymorphism are absent from the literature [7]. Therefore, it remains unknown as to the functional effect, if any, of the Ala55Val alleles.

Another possibility is that other variants in *UCP2* may be important in diabetes risk and differing linkage disequilibrium (LD) patterns between populations for Ala55Val and a functional polymorphism may be responsible for the inconsistent results. Two other *UCP2* polymorphisms have been reported to affect expression levels, −866G/A and a 45bp insertion/deletion polymorphism in the 3' untranslated region (3'UTR I/D) [3,8]. These polymorphisms were not genotyped in the ARIC cohort. However, increased risk of diabetes was observed in homozygotes for the −866A allele in European populations [3,9]. Gable et al reported a significant increased risk of diabetes for Caucasian men homozygous for the *UCP2* −866A allele after 10 years of follow-up compared to men with one or no copies of the −866A allele [9]. In studying a population of subjects with Northern European ancestry, Wang et al reported

strong LD between −866 G/A and Ala55Val ($r^2 = 0.82$) and moderate LD ($r^2 = 0.55$) for Ala55Val and 45bp insertion/deletion. Additionally, three haplotypes were observed that accounted for 93% of all observed haplotypes (−866G, A55, 3'UTR D), (−866A, V55, 3'UTR I), and (−866A, A55, 3'UTR I)[3]. These researchers reported that higher expression of UCP2 was associated with the −866A and 3'UTR D alleles suggesting a counterbalancing effect of these alleles based on the observed haplotypes [3]. This finding contradicts an earlier report by Krempler et al that found the −866A allele to decrease expression levels of UCP2 [8]. However, it is plausible that specific combinations of *UCP2* polymorphisms are associated with diabetes, the effect of which remains elusive when only looking at a single polymorphism despite the strong to moderate LD structure.

The effect of *UCP2* variants on diabetes risk may be overwhelmed by environmental causes of diabetes in some populations. This may explain why an association was seen in younger cohorts like CARDIA (baseline mean age ~25 years) and not in older cohorts like ARIC. Another possibility is that the effect of *UCP2* variants may only be important in specific subsets of T2DM cases or within the context of other gene variation. The contribution of *UCP2* to the development and progression of diabetes may be difficult to detect in a heterogeneous group of cases. To address this issue we looked at the association between Ala55Val and participants who had diabetes at baseline (n =1,723), who were medicated for diabetes at baseline (n = 808), or who developed diabetes that required medication during the follow-up time period (n = 385) compared to non-diabetics. Again, we found no association between *UCP2* genotypes and these three subgroups of diabetes. Alternatively, the *UCP2* gene may not be a genetic risk factor for diabetes.

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Table 1

Baseline Characteristics of ARIC participants free of diabetes by *UCP2* genotype (mean ± standard deviation or percentage)

Characteristic	Ala/Ala	Ala/Val	Val/Val
N	4051	5965	2040
Age, years	54±5.8	54±5.7	54±5.7
BMI,kg/m^2	27.2±5.1	27.2±5.0	27.2±5.0
Waist/hip ratio	0.92±0.08	0.92±0.08	0.92±0.08
Fasting glucose, mg/dL	99±9.1	99±9.2	99±9.5
Fasting Insulin, pmol/L	76±56	78±59	79±56
Current Drinker,%	58	60	60
Current Smoker,%	25	26	26

Table 2

Incidence rate of diabetes per 1,000 person-years and Hazard Rate Ratio by *UCP2* Genotype

	Incidence Rate (n=incident cases) Hazard Rate Ratio (95% CI)		
	Ala/Ala	**Ala/Val**	**Val/Val**
Total Cohort*	15.0 (n=461) 1.0	15.6 (n=706) 1.02 (0.91−1.14) p=0.77	15.6 (n=239) 1.00 (0.86−1.18) p=0.94
■ Normal weight (BMI<25)	5.2 (n=61) 1.0	5.6 (n=97) 1.06 (0.77−1.46) p=0.73	5.4 (n=31) 1.02 (0.66−1.57) p=0.93
■ Overweight (BMI 25−29.9)	13.6 (n=172) 1.0	14.8 (n=266) 1.07 (0.88−1.30) p=0.50	16.4 (n=103) 1.18 (0.92−1.51) p=0.19
■ Obese (BMI≥30)	35.7 (n=228) 1.0	34.2 (n=343) 0.95 (0.81−1.13) p=0.59	31.2 (n=105) 0.86 (0.68−1.08) p=0.19
Black†	22.4 (n=123) 1.0	26.5 (n=246) 1.18 (0.95−1.46) p=0.14	27.5 (n=91) 1.21 (0.92−1.59) p=0.17
White†	13.4 (n=338) 1.0	12.8 (n=460) 0.96 (0.83−1.10) p=0.56	12.3 (n=148) 0.91 (0.75−1.11) p=0.36

*Adjusted for age, sex, race, and field center.

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