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Uncoupling Protein 2 Ala55Val polymorphism is associated with a higher acute insulin response to glucose

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Abstract

Recent evidence suggests that mitochondrial uncoupling protein 2 (UCP2) in pancreatic β cells plays a crucial role in insulin production and secretion. We hypothesized that two UCP2 polymorphisms, a −55C/T (Ala55Val) substitution in exon 4 and an exon 8 insertion, would alter the acute insulin response to glucose (AIRg). Subjects were 155 African American (AA) and European American (EA) women. Body composition was determined by DXA. Insulin sensitivity and AIRg were measured with an intravenous glucose tolerance test and minimal modeling. To account for the confounding effects of population stratification, estimates of African admixture (AFADM) were obtained from approximately 35 ancestry informative markers. UCP2 genotyping was conducted with gel electrophoresis. Information was analyzed using mixed linear models. A positive association between the −55C/T homozygous mutation and AIRg was identified in the total sample ($P < 0.01$) and independently in EA women ($P = 0.02$) but not AA women. The exon 8 insertion did not significantly affect AIRg. No interaction effects of the two polymorphisms on AIRg were noted. These results indicate that AIRg is associated with the −55C/T UCP2 homozygous mutation, and that presence of this mutation could alter post-challenge insulin concentration.

1. Introduction

Type 2 diabetes development is associated with failure of the pancreatic beta-cells. Thus, it is possible that genes coding for proteins involved in insulin production and/or secretion by pancreatic β-cells predispose individuals to type 2 diabetes. Uncoupling proteins (UCP) located in the mitochondrial membrane of cells “uncouple” oxidative respiration, resulting in decreased ATP production. Uncoupling protein 2 (UCP2), the only UCP located in the mitochondria of pancreatic β-cells, is hypothesized to affect insulin secretion and hence the acute insulin response to glucose (AIRg) by decreasing ATP available for insulin production [1,2]. Genetic

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polymorphisms in the human UCP2 gene on chromosome 11q13, including the variant Ala55Val (−55C/T) in exon 4 and an exon 8 deletion, have been associated with increased incidence of type 2 diabetes and body mass index in multiethnic populations [3–5]. However, Bielinski and colleagues [6] recently reported no association of the Ala55Val polymorphism with type 2 diabetes incidence. Thus, the relationship between UCP2 and type 2 diabetes risk in humans remains uncertain.

Determining the etiology for diabetes development is complicated by differences in risk factors between ethnic groups. Compared to European-Americans (EA), African-Americans (AA) have greater AIRg, independent of age, diet, physical activity levels and individual fasting insulin [7]. To evaluate these racial differences, ancestral genetic admixture (ADM) analysis is used to evaluate the contribution of biological ancestry on disease risk. ADM analysis utilizes DNA Ancestry Informative Markers (AIMs) that differ among the West African, European, and AmerIndian groups that intermingled to produce current ethnic/racial groups in the United States. [8–10]. This approach has previously revealed an association of greater African admixture with higher AIRg and other diabetes-related co-morbidities [11–13].

The goals of this study were thus to determine if the UCP2 variant Ala55Val (rs660339) and a 45 base pair (bp) exon 8 insertion/deletion were associated with changes in insulin secretion (AIRg), and to evaluate whether these polymorphisms accounted for the effect of ethnic population stratification by the use of ADM analysis. Hence, this investigation represents a novel approach to the study of human insulin dynamics.

2. Patients and methods

2.1. Subjects

Healthy, pre-menopausal AA and EA women (n=155) were recruited from the Birmingham, Alabama metropolitan area. Subjects had a body mass index (BMI; kg/m^2) between 19 and 33, were nonsmokers, sedentary (defined as exercising less than once per week during the previous year), and had regular menstrual cycles. They were not taking any medications known to alter body composition or affect insulin sensitivity. Racial classification was determined by self-reported AA or EA ancestry in both parents and grandparents. Self-reported income was used as a proxy for socioeconomic status (SES). All procedures were conducted in accordance with the principles of the declaration of Helsinki. Institutional Review Board-approved informed consent was obtained prior to admission of the participants to the General Clinic Research Center (GCRC) at the University of Alabama at Birmingham.

2.2. Protocol

Participants were admitted to the GCRC for an overnight stay. All meals and snacks prior to the insulin test were consumed at the center before 7:00 $_{PM}$. Following an overnight fast, patients participated in a frequently sampled intravenous glucose tolerance test (FSIGT). Testing was conducted during the follicular phase of menstruation or during the estrogen phase for women using oral contraceptives.

2.3. Frequently sampled intravenous glucose tolerance test

After fasting, measures of AIRg were obtained during a FSIGT as described by Gower et al. [14]. Due to a change in university-wide FSIGT test protocol, the women underwent either a tolbutamide-modified or insulin-modified bolus test, or an insulin-infusion-modified test. Preliminary analysis indicated that differences in test type did not predict changes in insulin outcomes, specifically AIRg, among participants, and protocol use did not differ by UCP2 genotype classification. Serum samples were analyzed for glucose and insulin concentrations. The independent variable insulin sensitivity (S_i) was determined using the MINMOD computer

program (version 3.0) [15,16]. The dependent variable AIRg was calculated as the incremental area under the curve for insulin during the first 10 minutes following glucose administration.

2.4. Genotyping

Genotyping was performed at the Pennsylvania State University using melting curve analysis of single nucleotide polymorphisms (McSNP) and agarose gel electrophoresis. Markers and techniques used for the identification of the ancestry-informative DNA sequences have previously been described by Parra et al. [10] and are available through dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) using the handle PSU-ANTH. Ancestral genetic makeup was measured by combining information of approximately 85 ancestral informative markers (AIMs) into an estimate of ancestry from 2 parental populations using Admixmap [9].

The UCP2 C→T substitution primers were F 5′-GGCCAGTGCGCGCTACGG-3′ and R 5′-ATTGTAGAGGCTTCGGGGGCCC-3′. An A to G mismatch was introduced at position 16 of the forward primer to create a cutsite surrounding the substitution. Samples were denatured at 94°C for 5 min followed by 30 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s with a final extension at 72°C for 5 min. Following PCR amplification, PCR product was digested with *Hae111* at 37°C overnight. C/C homozygotes consisted of 1 fragment: 95 bp, T/T homozygotes consisted of 2 fragments: 77 and 18 bp, and T/C heterozygotes consisted of 3 fragments, 95, 77, and 18 bp. The UCP2 ins/del polymorphism primers were F 5′ – CAGTGAGGGAAGTGGGAGG-3′ and R 5′ – GGG GCAGGACGA AGA TTC – 3′ as previously reported by Walder *et al* [17]. The product size for fragments containing the 45 bp ins/del were 457bp and fragments without the ins/del were 412 bp. Both variants were genotyped using 2% agarose gel electrophoresis and visualized with ethidium bromide staining.

2.5. Statistical Analyses

Descriptive data for AA and EA groups were compared using 2-tailed *t*-tests and chi-square analyses. Lewontin's D' and r^2 were used to evaluate linkage disequilibrium with the method developed by the Broad Institute for Haploview software [18]. A mixed linear modeling approach was used to analyze the relationship of AIRg with the independent variables S_i, SES, African admixture, and the UCP2 polymorphisms. To adjust for the differences in FSIGT protocols, test type was also included as an independent variable in all models. Previous investigations have found this to be an acceptable control for the study population [14]. Orthogonal coding of the polymorphisms was used to test recessive, dominant and additive models for each polymorphism. For example, when the genotype CC was present for the Ala55Val variant, the CC variant received a value of 1 in the dominant model, while the CT and TT variants were assigned values of 0. For the recessive model, the TT variant was assigned a value of 1 with the remaining variants assigned a 0 value. In the additive model, the following values were assigned to each variant: TT = 0, CT = 1, and CC = 2. This coding was repeated for the exon 8 insertion/deletion. Values were log-transformed to improve normality of the data. Multivariate regression analysis was used to identify potential interactions between the polymorphisms on AIRg in the total sample and within each ethnic group. Permutation tests were run to ensure adequate power of hypothesis testing by ethnic group. Results indicated that for any p value < 0.047, the results remained significant after controlling for the effect of multiple comparisons via permutation tests (1000 simulations) to generate empirical P values under the null hypothesis of no association between genotype and/or admixture and other traits. Data was analyzed using SAS statistical software version 9.1 (SAS Institute, Cary, NC, 2002). Statistical significance was established at $P < 0.05$.

3. Results

Descriptive characteristics are shown in Table 1. The AA women had significantly greater levels of African admixture (AFADM) and AIRg, and lower S_i ($P < 0.01$). Polymorphism frequencies did not differ significantly by race, and were in Hardy-Weinberg equilibrium (HWE) by race (Table 2). Linkage disequilibrium was similar in EA ($D' = 0.98$; $r^2 = 0.06$) and African ($D' = 1.0$; $r^2 = 0.34$) groups. Levels of AIRg did not differ by test type (results not shown). After controlling for covariates, the Ala55Val homozygous mutation (TT; recessive model) was positively associated with greater AIRg in the total sample ($r^2 = 0.40$; $P < 0.01$; Table 3). A nonsignificant trend for greater AIRg was associated with presence of the T polymorphism in the additive model ($r^2 = 0.37$; $P = 0.055$). The exon 8 insertion was not significantly related to AIRg levels. AFADM was positively associated with AIRg independently of both the Ala55Val ($P < 0.01$) and exon 8 ($P < 0.01$) polymorphisms (Table 3). When analyses were repeated within ethnic groups, the Ala55Val homozygous mutation was associated with higher AIRg in EA women ($r^2 = 0.16$; $P = 0.02$) but not AA women. The exon 8 insertion was not independently associated with AIRg in either group. AFADM was also not associated with AIRg within ethnic groups. Potential interactions between the two polymorphisms and admixture also were evaluated; however no interactions were observed (data not shown).

4. Discussion

Our results indicate that the Ala55Val UCP2 polymorphism is associated with higher AIRg levels, particularly in EA women. Previous findings regarding the Ala55Val polymorphism and type 2 diabetes risk have been inconsistent [4,6,19]. Our results suggest that the homozygous mutation increases pancreatic post-challenge insulin concentration, inferring a decrease of UCP2 activity in pancreatic β-cells. Down-regulation of UCP2 activity in the β-cells has previously been associated with increasing mitochondrial ATP production and increased insulin secretion [20], suggesting one possible mechanism for the effects of this polymorphism on insulin dynamics.

No association was noted between the exon 8 insertion/deletion polymorphism and insulin secretion. It is possible that either this polymorphism or Ala55Val could interact with additional UCP2 polymorphisms to influence insulin secretion. Additionally, potential regulation of UCP2 activity by compounds such as sirtuin proteins (SIRT1 gene) highlights the need to investigate interactions between multiple genes affecting pancreatic β-cell activity [21]. Mutations within the UCP2 gene, such as those investigated here, could affect UCP2 interactions with other regulatory proteins.

Such interactions could also explain the disparate relationship of the Ala55Val polymorphism with AIRg among ethnic groups. Of note, the TT variant was associated with increased AIRg in EA, but not AA women. Previous investigations of this polymorphism have studied predominately EA population groups [3,12,19]. It is possible that other genes or proteins associated with African ancestry compensate for the effects of this particular UCP2 variant. However, AA women also exhibited greater variability of AIRg within polymorphism subgroups compared to EA women (Table 1), and it is possible that a larger sample size would be needed to detect an effect of UCP2 in this group. This finding highlights the importance of considering inter-individual biological differences when evaluating disease etiologies. The current study revealed that genetic admixture is associated with AIRg in AA and EA women, with greater levels of AFADM independently associated with higher AIRg. However, AFADM was not associated with AIRg within ethnic groups. It is possible that the lower variability of AFADM among our sample of EA resulted in AFADM serving as an ethnic identifier in the total population. Levels of African admixture have been previously associated with insulin

resistance in both EA and AA populations [11–13]. Our results suggest further investigation into the contribution of AFADM to AIRg within groups is needed.

This study benefited from inclusion of multiple UCP2 polymorphisms that allowed for testing of interaction effects within a multiethnic population. Furthermore, genetic admixture allowed us to control for population stratification during analysis. The smaller sample size prohibited prohibited further analysis to identify specific haplotypes affecting AIRg, and revision of FSIGT testing protocol resulted in participants undergoing slightly different methods during this test; however, no differences in AIRg or insulin sensitivity by test type were noted. Overall, our results indicate a potential role of the Ala55Val polymorphism in altering insulin secretion, particularly in EA women. They suggest that genetic admixture is also associated with AIRg, and that use of genetic admixture when evaluating UCP2 may prove useful in future studies. Additional work should evaluate the interaction of these polymorphisms with others in the UCP2 gene in the presence of genetic admixture.

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Table 1

Anthropometric characteristics of the study population by ethnicity

	African Americans (*n* = 87)	**European Americans (*n* = 68)**
Age (years)	35.3 (4.5)	36.3 (5.3)
Height (cm)	163.2 (6.7)	164.4 (6.4)
Weight (kg)	71.4 (9.4)	74.4 (9.3)
BMI (kg/m^2)	27.1 (2.8)	27.4 (2.4)
African Admixture	70.5 (10.8)	17.5 (5.9) *
$S_i \times 10^{-4}$ ($min \cdot \mu U^{-1} \cdot ml^{-1}$)	3.3 (2.5)	4.4 (2.5) *
AIRg (μU/ml per 10 min)	960.6 (614.1)	486.8 (261.9) *
AIRg by Ala55Val:		
CC	912.5 (645.3)	450.3 (262.0)*
CT	921.3 (586.7)	466.8 (195.1)*
TT	1137.5 (385.1)	691.65 (404.6)*
AIRg by Insertion/Deletion:		
ins/ins	931.8 (615.2)	498.4 (267.2)*
ins/del	1029.8 (656.4)	442.3 (250.8)*
del/del	1043.4 (412.3)	555.4 (286.3)*

Means ± SD are given.

* $P < 0.01$ compared with African Americans.

Table 2

Genotypic frequencies of UCP2 polymorphisms by race

	African Americans (*n* = 87)	European Americans (*n* = 68)	*P* value
Ala55Val (rs660339)			
C/C	30 (0.35)	31 (0.46)	0.11
C/T	35 (0.41)	29 (0.43)	
T/T	21 (0.24)	8 (0.11)	
Insertion/Deletion			
ins/*ins*	52 (0.60)	43 (0.63)	0.92
ins/*del*	28 (0.33)	20 (0.30)	
del/*del*	6 (0.07)	5 (0.07)	

Data are *n* (frequency). *P* value = distribution of genotype between groups.

Table 3

Independent predictors of AIRg determined from multiple linear regression analysis (n = 155)

Phenotype	**Genotype**											
	Ala55Val						**Exon 8 *ins/del***					
	All		**AA**		**EA**		**All**		**AA**		**EA**	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
S_i	− 0.56	< 0.01	− 0.64	< 0.01	−0.33	0.0251	− 0.59	<0.01	− 0.68	<0.01	−0.33	0.033
Income	0.01	0.42	−0.01	0.16	−0.01	0.71	0.06	0.37	0.01	0.15	0.01	0.95
Test type	−0.09	0.41	−0.10	0.49	0.03	0.86	−0.11	0.33	−0.13	0.42	0.04	0.81
AFADM	0.60	0.0021	−0.27	0.67	−0.31	0.78	0.64	0.0013	−0.42	0.51	− 0.35	0.76
UCP2 Ala55Val	0.40	0.0027	0.24	0.13	0.46	0.02	--	--	--	--	--	--
UCP2 *ins/del*	--	--	--	--	--	--	0.39	0.05	0.31	0.28	0.43	0.13

Recessive models shown (CC and CT versus TT; *ins/ins* and *ins/del* versus *del/del*). Results for dominant and additive models not shown. All = total sample. AA = African-American. EA = European-American. AFADM = African Admixture. β = Parameter Estimate.