Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 877–881
Metabolism
Clinical and Experimental
www.metabolismjournal.com

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

Amanda L. Willig[a,b,*], Krista R. Casazza[a,b], Jasmin Divers[a,c], Abigail W. Bigham[a,d,e], Barbara A. Gower[a,b], Gary R. Hunter[a,b], Jose R. Fernandez[a,b,c]

[a]*Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL*
[b]*Department of Nutrition Sciences and the Clinical Nutrition Research Center, University of Alabama at Birmingham, Birmingham, AL*
[c]*Department of Biostatistics, Section on Statistical Genetics, University of Alabama at Birmingham, Birmingham, AL*
[d]*Department of Preventive Medicine, and Physiology and Biophysics, University of Southern California, Los Angeles, CA*
[e]*Department of Physiology and Biophysics, University of Southern California, Los Angeles, CA*

Received 21 August 2008; accepted 23 February 2009

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 2 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 dual-energy x-ray absorptiometry. 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 were obtained from approximately 35 ancestry-informative markers. Uncoupling protein 2 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 ($P < .01$) and independently in EA women ($P = .02$) but not AA women. The exon 8 insertion did not significantly affect AIRg. No interaction effects of the 2 polymorphisms on AIRg were noted. These results indicate that AIRg is associated with the −55C/T UCP2 homozygous mutation and that the presence of this mutation could alter postchallenge insulin concentration.

1. Introduction

Type 2 diabetes mellitus development is associated with failure of the pancreatic β-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 mellitus. Uncoupling proteins (UCPs) located in the mitochondrial membrane of cells "uncouple" oxidative respiration, resulting in decreased adenosine triphosphate (ATP) production. Uncoupling protein 2, 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 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 mellitus and body mass index (BMI) in multiethnic populations [3-5]. However, Bielinski and colleagues [6] recently reported no association of the Ala55Val polymorphism with type 2 diabetes mellitus incidence. Thus, the relationship between UCP2 and type 2 diabetes mellitus risk in humans remains uncertain.

Determining the etiology for diabetes development is complicated by differences in risk factors between ethnic groups. Compared with European Americans (EAs), African Americans (AAs) 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. Admixture

* Corresponding author. Department of Nutrition Sciences, WEBB 429, University of Alabama at Birmingham, Birmingham, AL 35294 3360, USA. Tel.: +1 205 975 9678; fax: +1 205 934 7050.
E-mail address: mandyrd@uab.edu (A.L. Willig).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.02.016

analysis uses 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 (AFADM) with higher AIRg and other diabetes-related comorbidities [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, premenopausal AA and EA women (N = 155) were recruited from the Birmingham, AL, metropolitan area. Subjects had a BMI (kilograms per square meter) between 19 and 33, were nonsmokers, were *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. All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Institutional Review Board–approved informed consent was obtained before admission of the participants to the General Clinic Research Center at the University of Alabama at Birmingham.

2.2. Protocol

Participants were admitted to the General Clinic Research Center for an overnight stay. All meals and snacks before the insulin test were consumed at the center before 7:00 PM. After 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 an FSIGT as described by Gower et al [14]. Because of 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 after glucose administration.

2.4. Genotyping

Genotyping was performed at the Pennsylvania State University using melting curve analysis of single nucleotide polymorphisms 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 AIMs into an estimate of ancestry from 2 parental populations using Admixmap [9].

The UCP2 C→T substitution primers were F 5′-GGCCA-GTGCGCGCTACGG-3′ and R 5′-ATTGTAGAGGCTTC-GGGGGCCC-3′. An A to G mismatch was introduced at position 16 of the forward primer to create a cut site surrounding the substitution. Samples were denatured at 94°C for 5 minutes followed by 30 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds with a final extension at 72°C for 5 minutes. After polymerase chain reaction amplification, polymerase chain reaction product was digested with *Hae*III 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′-CAGTGAGGGAAGTGG-GAGG-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 was 457 bp, and

Table 1
Anthropometric characteristics of the study population by ethnicity

	AA (n = 87)	EA (n = 68)
Age (y)	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)
AFADM	70.5 (10.8)	17.5 (5.9)*
$S_i \times 10^{-4}$ (min μU^{-1} mL^{-1})	3.3 (2.5)	4.4 (2.5)*
AIRg (μU mL^{-1} 10 min^{-1})	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.
* $P < .01$ compared with African Americans.

Table 2
Genotypic frequencies of UCP2 polymorphisms by race

	AA (n = 87)	EA (n = 68)	*P* value
Ala55Val (rs660339)			
C/C	30 (0.35)	31 (0.46)	.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)	.92
ins/del	28 (0.33)	20 (0.30)	
del/del	6 (0.07)	5 (0.07)	

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

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 χ^2 analyses. Lewontin 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, socioeconomic status, AFADM, 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, whereas 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 less than .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 were analyzed using SAS statistical software version 9.1 (SAS Institute, Cary, NC, 2002). Statistical significance was established at *P* less than .05.

3. Results

Descriptive characteristics are shown in Table 1. The AA women had significantly greater levels of AFADM and AIRg, and lower S_i ($P < .01$). Polymorphism frequencies did not differ significantly by race and were in Hardy-Weinberg equilibrium 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 ($r^2 = 0.40$, $P < .01$, Table 3). A nonsignificant trend for greater AIRg was associated with the presence of the T polymorphism in the additive model ($r^2 = 0.37$, $P = .055$). The exon 8 insertion was not significantly related to AIRg levels. African admixture was positively associated with AIRg independently of both the Ala55Val ($P < .01$) and exon 8 ($P < .01$) polymorphisms (Table 3). When analyses were repeated within ethnic groups, the Ala55Val homozygous mutation was associated with higher AIRg in EA women ($r^2 = 0.16$, $P = .02$) but not AA women. The exon 8 insertion was not independently associated with AIRg in either group. African admixture was also not associated with AIRg within ethnic groups. Potential interactions between the 2 polymorphisms and admixture also were evaluated; however, no interactions were observed (data not shown).

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	<.01	−0.64	<.01	−0.33	.0251	−0.59	<.01	−0.68	<.01	−.33	.033
Income	0.01	.42	−0.01	.16	−0.01	.71	0.06	.37	0.01	.15	.01	.95
Test type	−0.09	.41	−0.10	.49	0.03	.86	−0.11	.33	−0.13	.42	.04	.81
AFADM	0.60	.0021	−0.27	.67	−0.31	.78	0.64	.0013	−0.42	.51	−.35	.76
UCP2 Ala55Val	0.40	.0027	0.24	.13	0.46	.02	–	–	–	–	–	–
UCP2 *ins/del*	–	–	–	–	–	–	0.39	.05	0.31	.28	.43	.13

Recessive models shown (CC and CT vs TT; *ins/ins* and *ins/del* vs *del/del*). Results for dominant and additive models not shown. *β* indicates parameter estimate.

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 mellitus risk have been inconsistent [4,6,19]. Our results suggest that the homozygous mutation increases pancreatic postchallenge 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. In addition, 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 with 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 interindividual 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 AFADM have been previously associated with insulin resistance in both EA and AA populations [11-13]. Our results suggest that further investigation into the contribution of AFADM to AIRg within groups is needed.

This study benefited from the 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 small sample size 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.

Acknowledgment

This research was supported by National Institutes of Health grants R01 DK 51684-01, R01 DK 49779-01, and National Institutes of Health CA 47888 Cancer Prevention and Control Training Program; General Clinical Research Center grant M01 RR000032 from the National Center for Research Resources; and Clinical Nutrition Research Unit grant P30-DK56336.

References

[1] Warram JH, Martin BC, Krolewski AS, et al. Slow glucose removal rate and hyperinsulinemia precede the development of type 2 diabetes in the offspring of diabetic parents. Ann Intern Med 1990;113:909-15.

[2] Vauhkonen I, Niskanen L, Vanninen E, et al. Defects in insulin secretion and insulin and action in non–insulin dependent diabetes mellitus are inherited. Metabolic studies on offspring of diabetic probands. J Clin Invest 1998;101:86-96.

[3] Krempler F, Esterbauer H, Weitgasser R, et al. A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans. Diabetes 2002;51:3331-5.

[4] Yu X, Jacobs Jr DR, Schreiner PJ, et al. The uncoupling protein 2 Ala55Val polymorphism is associated with diabetes mellitus: the CARDIA study. Clin Chem 2005;51:1451-6.

[5] Marti A, Corbalan MS, Forga L, et al. Higher obesity risk associated with the exon-8 insertion of the UCP2 gene in a Spanish case-control study. Nutrition 2004;20:498-501.

[6] Bielinski SJ, Pankow JS, Boerwinkle E, et al. Lack of association between uncoupling-protein 2 Ala55Val polymorphism and incident diabetes in the Atherosclerosis Risk in Communities Study (ARIC). Acta Diabetol 2008;45:179-82.

[7] Gower BA, Granger WM, Franklin F, et al. Contribution of insulin secretion and clearance to glucose-induced insulin concentration in African-American and Caucasian children. J Clin Endocrinol Metab 2002;87:2218-24.

[8] Shriver MD, Smith MW, Jin L, et al. Ethnic-affiliation estimation by use of population-specific DNA markers. Am J Hum Genet 1997;60:957-64.

[9] McKeigue PM. Mapping genes that underlie ethnic differences in disease risk: methods for detecting linkage in admixed populations, by conditioning on parental admixture. Am J Hum Genet 1998;63:241-51.

[10] Parra EJ, Kittles RA, Shriver MD. Implications of correlations between skin color and genetic ancestry for biomedical research. Nat Genet 2004;36:S54-S60.

[11] Fernandez JR, Shriver MD, Beasley TM, et al. Association of African genetic admixture with resting metabolic rate and obesity among women. Obes Res 2003;11:904-11.

[12] Gower BA, Fernandez JR, Beasley TM, et al. Using genetic admixture to explain racial differences in insulin-related phenotypes. Diabetes 2003;52:1047-51.

[13] Bonilla C, Shriver MD, Parra EJ, et al. Ancestral proportions and their association with skin pigmentation and bone mineral density in Puerto Rican women from New York City. Hum Genet 2004;115: 57-68.

[14] Gower BA, Ard JD, Hunter GR, et al. Elements of the metabolic syndrome: association with insulin sensitivity and effects of ethnicity. Met Synd Rel Disord 2007;5:77-86.

[15] Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 1981;68:1456-67.

[16] Yang YJ, Youn JH, Bergman RN. Modified protocols improve insulin sensitivity estimation using the minimal model. Am J Physiol 1987; 253:E595-E602.

[17] Walder K, Norman RA, Hanson RL, et al. Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima Indians. Hum Mol Genet 1998;7:1431-5.

[18] Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.

[19] Cho YM, Ritchie MD, Moore JH, et al. Multifactor-dimensionality reduction shows a two-locus interaction associated with type 2 diabetes mellitus. Diabetologia 2004;47:549-54.

[20] De Souza CT, Araujo EP, Stoppiglia LF, et al. Inhibition of UCP2 expression reverses diet-induced diabetes mellitus by effects on both insulin secretion and action. FASEB J 2007;21:1153-63.

[21] Moynihan KA, Grimm AA, Plueger MM, et al. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2005;2:105-17.