Original Article

Insulin receptor substrate 1 (*IRS1*) variants confer risk of diabetes in the Boston Puerto Rican Health Study

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Objective: Published data concerning associations between *IRS1* variants and type 2 diabetes and related traits have been inconsistent. We examined the relationship between common variants in *IRS1*, type 2 diabetes, and related traits including insulin resistance, hyperglycemia and DNA damage in the Boston Puerto Rican Health Study. Methods: We genotyped six common *IRS1* variants in an adult Puerto Rican population (n=1132) and tested for association with risk of type 2 diabetes and related traits. Results: SNPs rs934167 and rs1801123 showed significant association with fasting glucose concentrations ($p = 0.005$ and $p = 0.016$, respectively) and rs934167 showed significant association with plasma insulin levels ($p = 0.005$). Carriers of the rs934167 minor allele had significantly higher HOMA-IR and lower QUICKI ($p = 0.001$ and $p = 0.001$, respectively), and a 40% and 58% greater likelihood of being hyperglycaemic or hyperinsulinemic (OR = 1.40 and 1.58; $p = 0.013$ and 0.002, respectively). However, they exhibited only a marginally significant trend towards having type 2 diabetes (OR=1.27, $p = 0.077$). Furthermore, carriers of the haplotype C-T of the rs934167 and rs1801123 minor alleles showed consistent patterns of associations after correction for multiple testing. In addition, the G972R (rs1801278) minor allele was significantly associated with higher urinary 8-OHdG concentrations ($p = 0.020$) and plasma CRP levels ($p = 0.035$). Conclusions: Our results support *IRS1* variants associated with type 2 diabetes risk in adult Puerto Ricans. Moreover, we report the novel finding that *IRS1* variant G972R (rs1801278) may contribute to oxidative DNA damage and inflammation.

Key Words: genetic association, *IRS1*, type 2 diabetes, insulin resistance, DNA damage

INTRODUCTION

While multiple genetic factors and complex interactions between genetic and environmental factors affect onset and progression of type 2 diabetes, incidence of the disease differs across regions and ethnicities, being higher in African-American, Asian, Native-American, and Hispanic populations.[1,2] Insulin receptor substrate 1 (*IRS1*) is a ligand of the insulin receptor tyrosine kinase and is central to the insulin receptor signal transduction pathway. Deregulation in *IRS1* expression and function has been reported in insulin-resistant states such as obesity and type 2 diabetes.[3] Skeletal muscle and adipose tissue from *IRS1* gene knockout mice showed diminished insulin-induced glucose transport.[4,5] Furthermore, pancreatic beta cells from *IRS1* knockout mice showed defects in the insulin secretion response to glucose.[6] Hence, it has been proposed that altered regulation and or function of the *IRS1* gene or protein might be causal, in part, for insulin resistance and type 2 diabetes.[7,8]

Many polymorphisms in *IRS1* have been identified and examined for their associations with type 2 diabetes and related traits in European populations. In many cases, the associations are not reproduced across other populations, even for the most extensively studied variant is Arg^{972} (Glycine → Arginine, G972R, rs1801278),[9] a nonsynonymous and potentially functional mutation. Recently, rs2943641, a genetic variant about 495 kbp down

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stream of the *IRS1* gene or 570 kbp downstream of the transcription start, was found to be associated with type 2 diabetes, insulin resistance and hyperinsulinemia in 14,358 European individuals.[10] It remains to be demonstrated, however, if rs2943641 is a genetic risk factor for diabetes in Hispanic populations.

In addition to classical disease biomarkers, current evidence suggests that elevated levels of oxidative DNA damage and the inflammatory biomarker C-reactive protein (CRP) are associated with multiple risk factors for diabetes including obesity and insulin resistance.[11-13] Recent studies have provided novel aspects of the contribution of the insulin signaling pathway, including IRS proteins, to oxidation and inflammation in diabetes. In this pathway, tyrosine phosphorylation of IRS proteins links the insulin receptor tyrosine kinase to activation of the PI3K-Akt cascade, which phosphorylates and inactivates regulatory proteins, such as the forkhead transcriptional factors (FoxO). FoxO family members counter DNA damage and growth-factor withdrawal by suppressing cell-cycle progression and increasing expression of *GADD45A* and *DDB1* to facilitate DNA repair.[14] Meanwhile, FoxO factors can activate peroxisome proliferator-activated receptor-γ coactivator-1 α (PPARGC1A), a well characterized positive regulator of mitochondrial function and oxidative metabolism.[15] Therefore, it is plausible to hypothesize that, beyond its associations with type 2 diabetes, genetic variation of *IRS1* could be associated with oxidative DNA damage and oxidation-induced inflammation through multiple pathways.

Adult Puerto Rican Hispanics differ from other Hispanic populations and have been identified as a vulnerable group with an increased risk for age-related chronic diseases.[16] Yet, little is known about links between *IRS1* gene variation and diabetes risk in this population. The high prevalence of diabetes, obesity, hypertension, physical impairment in this population underscores the importance of exploring the correlation between *IRS1* genetic variation and the risk factors of diabetes, including insulin resistance and oxidative DNA damage. The goal of this study was to replicate some of findings at the *IRS1* locus that were previously reported [17-19] and to determine if *IRS1* contributes to risk of diabetes and related phenotypes in a Puerto Rican population.

MATERIALS AND METHODS

Subjects

The study population consisted of 340 men and 792 women who were self-identified Puerto Ricans living in the greater Boston metropolitan area and for whom full data records for demographics, biochemical characteristics and genotypes were collected. Participants were recruited from the Boston Puerto Rican Health Study (BPRHS), a longitudinal cohort study on stress, nutrition, health, and aging, as described.[16] Written informed consent was obtained from each participant and the protocol was approved by the Institutional Review Board at Tufts University.

Data collection and variable definition

Information from participants regarding socio-demographics, health status, and behavior was collected by home interview administered by bilingual interviewers. Anthropometric measurements were collected using standard methods. Physical activity was estimated as a physical activity score based on the Paffenbarger questionnaire of the Harvard Alumni Activity Survey.[20] Physical activities are divided into five categories, which are sleeping and lying down, vigorous activity, moderate activity, light activity, and sitting. Different activities have different scores of strength. A physical activity score was calculated as the sum of the products of strength of each activity and hours a subject spends on such activity.

Using the American Diabetes Association (ADA) criteria, subjects were classified as having type 2 diabetes when fasting plasma glucose concentration was ≥7.0 mmol/L [21] or use of insulin or diabetes medication was reported. Hyperglycemia was defined as fasting plasma glucose concentration was ≥5.6 mmol/L. While there is no recognized cut-off value for hyperinsulinemia, we set the median value of the population as the threshold because over half of the participants of this population have type 2 diabetes.

Fasting plasma glucose and insulin were analyzed using standard procedures. Insulin resistance by homeostasis model assessment (HOMA-IR) was calculated as: [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/ 22.5, and quantitative insulin sensitivity check index (QUICKI) was calculated as: 1/[log (fasting insulin, μU/mL) + log (fasting glucose, mg/dL). To convert glucose (mmol/L) to mg/dL, multiply by 18; to convert insulin (pmol/L) to μU/mL, divide by 6.945].

C-reactive protein (CRP) in serum was analyzed using an immunoturbidimetric reaction in a Cobas Fara II Centrifugal Analyzer with DiaSorin CRP SPQ test system antibody reagent set II (AM-0039; Atlantic Antibodies, Stillwater, MN). Participants were also instructed to provide a 12-h urine sample, which was retrieved at the home the following morning. Oxidative DNA damage and the whole-body repair of DNA were estimated by measuring 8-hydroxydeoxyguanosine (8-OHdG) in urine samples with a monoclonal antibody ELISA kit from Assays Designs (Ann Arbor, MI) as described.[22] Concentrations of urinary 8-OHdG were calculated by the multiplication of the measured concentration by the total volume of 12-h urine, and then normalized by urinary creatinine concentrations.

SNP selection

Although many genetic variants at the *IRS1* locus have been reported in European populations, genetic variants, allele frequency distribution, and linkage disequilibrium at *IRS1* are not known in Puerto Rican populations. Thus, six SNPs were initially selected for genotyping based on a determination of tag SNPs across the *IRS1* region, minor allele frequency, and literature reports in the European and African populations. Tag SNPs were selected by running TAGGER[23] separately on the CEU (White) and YRI (African) populations at HapMap with parameters set to "pair-wise" and r^2 >0.8. Minor allele frequencies above 0.05 in CEU or substantially different between CEU and YRI populations were preferred.[24] The selected six tag SNPs capture all major LD blocks at the *IRS1* region of 100 kbp in European and African populations. The char-

acteristics of *IRS1* SNPs are presented in Supplemental Table 1.

DNA isolation and genotyping

Genomic DNA was isolated from buffy coats of the peripheral blood using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) according to the vendor's recommended protocol. SNPs were genotyped with Applied Biosystems TaqMan SNP genotyping system.[22] For all genotyping, blinded no-template controls and replicates of DNA samples were incorporated in each DNA sample plate, which were routinely checked by laboratory personnel. Based on our internal quality control and that estimated independently by external laboratories, the genotyping error rate was <1%.

Statistical analysis

Statistical analyses were performed using SPSS 13.0 (Chicago, IL) and SAS 9.2 (Cary, NC, USA). Continuous dependent variables, such as 8-OHdG and plasma glucose concentrations that were not normally distributed, were Box-Cox transformed to achieve normality before fitting statistical models. We assessed the relationship between *IRS1* variants and urinary 8-OHdG, fasting plasma glucose concentration, plasma glucose concentration, HOMA-IR, and QUICKI by analysis of covariance. For type 2 diabetes, we used logistic regression. With a rare minor allele, homozygotes and heterozygotes were combined to increase statistical power. In these analyses, dependent variables were DNA damage, plasma glucose concentration, type 2 diabetes, and hyperglycemia status. Independent variables were genotypes of the individual *IRS1* SNPs. Analyses were adjusted for potential confounders (age, sex, smoking, alcohol intake, medications, and physical activity) using a linear or logistic regression model. Men and women were analyzed together, as well as separately, to examine sex-specific effects. A nominal p-value ≤ 0.05 was considered statistically significant.

Linkage disequilibrium and haplotype analysis

Pair-wise linkage disequilibria among all six SNPs were estimated as correlation coefficients (r^2) using the HelixTree program (GOLDEN Helix, Bozeman, MN, USA). For haplotype analysis, we estimated haplotype frequencies using the expectation-maximization algorithm for a subset of SNPs selected on the basis of individual association at a nominal significance (p-value ≤ 0.05) with a given trait. To determine the association between haplotypes and phenotypes, we used haplotype trend regression analysis with the option of composite haplotype estimation implemented in HelixTree. Analyses were adjusted for potential confounders and population admixture (see below). p-values were further adjusted for multiple testing by a permutation test.[25]

Population admixture

For BPRHS participants, population admixture was estimated using Principle Component Analysis based on 100 ancestry informative markers.[26] All analyses were adjusted for the estimated population admixture using the first major principal component with linear regression models.[26]

RESULTS

Characteristics of participants

BMI, the percentage of participants who were obese (BMI ≥ 30 kg/m^2), fasting plasma glucose and insulin concentrations and CRP levels were significantly higher in participants with diabetes than in those without diabetes ($p < 0.001$, Table 1). In addition, diabetes subjects were more likely smokers and alcohol users compared to non-diabetes subjects. Other demographic characteristics did not differ significantly by diabetic status.

The frequencies of minor alleles of the six selected SNPs ranged from 0.05 to 0.31 (Supplemental Table 1). No significant difference in genotype frequency was observed between men and women. All six SNPs at *IRS1* were in Hardy-Weinberg equilibrium. Two SNP pairs

Table 1. Characteristics of participants according to type 2 diabetes status

	Diabetes	Non-diabetes
n	444	688
Men	135	205
Women	309	483
Age (year) †	59.4±7.1 (45-75)	56.7±7.9 (42-75)
BMI (kg/m^2)*	33.7±7.1 (18.1-63.8)	29.7±6.1 (17.0-59.9)
Obesity*	209 (47.1)	237 (34.4)
Drinkers*	148 (33.3)	299 (43.4)
Smokers*	92 (20.7)	184 (26.7)
Glucose (mmol/L)*	8.8±3.7 (2.6-32.6)	5.4±0.6 (4.0-6.9)
CRP (mg/L)*	7.33±10.61 (0.1-127.0)	5.26±6.50 (0.00-53.7)
Insulin (pmol/L)*	158.4±167.9 (13.2-1593.8)	105.3±71.0 (10.4-571.3)
Physical activity score	30.8±4.3 (24.3-56.0)	32.0±4.9 (25.0-62.6)
On diabetes drug‡	436	0
On depression drug	161 (36.3)	227 (33.0)

†Data are means ± SD (range) or n (%).
*Statistical significance at $p \leq 0.05$. n, sample size.
‡Six types of anti-diabetes drugs: metformin (319), sulfonylureas (180), insulin (156), glitazones (112), meglit (1). Some subjects used multiple types of anti-diabetes drug.

Table 2. Fasting glucose concentrations (mmol/L) according to *IRS1* genotypes and type 2 diabetes status

SNP	Genotype	MAF[a]	All (n=1132)			Diabetes (n=444)			Non-diabetes (n=688)		
			n	Mean±SE	*p* value*	n	Mean±SE	*p* value*	n	Mean±SE	*p* value*
rs12053536	CC	0.2	718	6.8±0.1	0.155	294	8.8±0.2	0.839	424	5.5±0.1	0.264
	CT+TT		402	6.6±0.1		147	8.8±0.3		255	5.4±0.1	
rs13306465	CC	0.15	814	6.7±0.1	0.589	319	8.8±0.2	0.908	495	5.4±0.1	0.943
	CT+TT		308	6.8±0.2		125	8.7±0.3		183	5.4±0.1	
rs1801123	CC+CT	0.27	527	6.9±0.1	0.016	215	9.1±0.3	0.104	312	5.5±0.1	0.17
	TT		588	6.5±0.1		224	8.4±0.2		364	5.4±0.1	
rs1801278	CC	0.05	1007	6.7±0.1	0.399	395	8.7±0.2	0.328	612	5.4±0.1	0.629
	CT+TT		117	7.0±0.3		47	9.4±0.7		70	5.5±0.1	
rs934167	CC	0.18	756	6.5±0.1	0.005	287	8.4±0.2	0.24	469	5.4±0.1	0.005
	CT+TT		356	7.1±0.2		153	8.9±0.4		203	5.6±0.1	
rs2943641	CC	0.31	508	6.8±0.3	0.801	209	8.9±0.3	0.328	299	5.4±0.1	0.749
	CT+TT		575	6.7±0.3		225	8.7±0.2		350	5.5±0.1	

*All means and *p*-values were calculated by ANCOVA using general linear models adjusted by age, sex, smoking status, alcohol use, physical activity, and population admixture. n = sample size.
[a]MAF=minor allele frequency.

exhibited intermediate linkage disequilibrium (LD): SNPs rs1801123 and rs13306465, and SNPs rs934167 and rs1801123 (r^2 = 0.68 and 0.59, respectively), whereas pair-wise LD measures for other SNPs were weak ($r^2 \leq$ 0.4, data not shown).

Association of IRS1 variants and glycaemic quantitative traits

After adjustment for smoking, age, sex, alcohol use, physical activity, and population admixture, two SNPs (rs934167 and rs1801123) showed significant association with fasting plasma glucose concentrations (p = 0.005 and p = 0.016, respectively; Table 2). Carriers of rs934167 minor T allele had a higher fasting plasma glucose level than CC homozygotes (7.1 vs 6.5 mmol/l), and carriers of rs1801123 minor C allele had a higher fasting plasma glucose level than TT homozygotes (6.9 vs 6.5 mmol/l). To exclude the influence of diabetes and diabetic medications on plasma glucose, we analyzed the data by dividing subjects into two groups: participants with diabetes (most subjects with diabetes used anti-diabetes drugs) and those without diabetes (non-diabetes). Among the non-diabets, we found that rs934167 showed significant associations with fasting plasma glucose concentrations, whereas among the diabets no SNP showed significant associations (Table 2).

For insulin sensitivity and resistance status, we examined association between *IRS1* variants and fasting insulin concentration and derived HOMA-IR (insulin resistance index) and QUICKI (insulin sensitivity index). Consistent with the above results, rs934167 showed significant association with fasting plasma insulin levels (p = 0.005, Table 3) when both diabetes and non-diabetes subjects were combined, and significant (p=0.034 for non-diabetes) or marginally significant (p = 0.077 for diabetes) when both were analyzed separately. Because the results based on all subjects (with diabetes and non-diabetes) are similar to those of the non-diabetes subjects alone, we combined both diabetes and non-diabetes subjects for HOMA-IR and QUICKI analysis to increase statistical power. Similar results were observed for HOMA-IR and QUICKI index (data not shown). In particular, carriers of the rs934167 minor T allele had a higher insulin resistance index, HOMA-IR, and a lower insulin sensitivity index, QUICKI, than CC homozygotes (p = 0.001 and p = 0.001, respectively; data not shown). In addition, a trend of association, but non-statistically significant, was observed for SNP rs1801123, whereas no association was observed for the other variants (data not shown).

Association of IRS1 variants and hyperglycaemia, hyperinsulinemia, and type 2 diabetes

We examined next the risk of being hyperglycaemic or hyperinsulinemic or having type 2 diabetes in relation to *IRS1* genotypes (Table 4). Carriers of the rs934167 minor T allele had a 40% greater likelihood of being hyperglycaemic than CC homozygotes (OR = 1.38; p = 0.013). For the analyses examining association between *IRS1* variants and hyperinsulinemic status, we dichotomized fasting insulin concentration to corresponding median value. Again, we found that carriers of the rs934167 minor T allele had a 58% greater likelihood of being hyperglyinsulinemic than CC homozygotes (OR = 1.58; p = 0.002). In addition, carriers of the rs1801123 minor C allele also had significantly greater likelihood of presenting hyperglycaemia than those with the TT genotype (OR 1.41, p = 0.007). Carriers of the minor allele rs934167 exhibited a trend towards having type 2 diabetes over CC homozygotes (OR 1.27, p = 0.077). A similar non-statistically significant association was observed for SNP rs1801123 (OR 1.19, p = 0.171). Furthermore, we failed to find any significant association between rs2943641 and hyperglycemia, hyperinsulinemia, and type 2 diabetes in this Puerto Rican population.

Association of IRS1 variants with DNA damage and CRP level

As the insulin signaling pathway, including IRS and PPARGC1A proteins, may influence oxidation and inflammation.[13-15] we examined the association between

Table 3. Fasting insulin concentrations (pmol/L) according to *IRS1* genotypes and type 2 diabetes status

SNP	Genotype	All (n=1132)			Diabetes (n=444)			Non-diabetes (n=688)		
		n	Mean±SE	*p* value*	n	Mean±SE	*p* value*	n	Mean±SE	*p* value*
rs12053536	CC	718	121±4.2	0.897	294	154±8.3	0.665	424	99.3±2.8	0.443
	CT+TT	402	124±6.2		147	155±16.0		255	107±4.9	
rs13306465	CC	814	120±3.5	0.260	319	151±8.3	0.416	495	101±2.8	0.448
	CT+TT	308	126±7.6		125	156±16.7		183	105±5.5	
rs1801123	CC+CT	527	126±5.6	0.235	215	157±11.8	0.650	312	104±4.2	0.248
	TT	588	119±4.2		224	151±10.4		364	100±3.5	
rs1801278	CC	1007	122±3.5	0.523	395	153±8.3	0.155	612	103±2.8	0.688
	CT+TT	117	121±9.0		47	159±18.7		70	96.5±6.9	
rs934167	CC	756	115±3.5	0.005	287	144±8.3	0.077	469	97.9±2.8	0.034
	CT+TT	356	136±7.6		153	169±16.0		203	112±5.5	
rs2943641	CC	508	120±4.6	0.961	209	148±9.7	0.691	299	101±3.9	0.978
	CT+TT	575	123±5.7		225	159±13.1		350	101±3.8	

*All means and *p*-values were calculated by ANCOVA using general linear models adjusted by age, sex, smoking status, alcohol use, physical activity, depression medication, and population admixture. n = sample size.

Table 4. Association of *IRS1* variants with hyperglycemia, hyperinsulinemia, and diabetic status

SNP	Genotype (n)	Fasting hyperglycemia		Fasting hyperinsulinemia		Type 2 diabetes	
		OR (95%CI)*	*p* value*	OR (95%CI)*	*p* value*	OR (95%CI)*	*p* value*
rs12053536	CC (716) vs CT+TT (402)	1.31 (1.02-1.69)	0.039	0.90 (0.70-1.12)	0.863	1.21 (0.93-1.57)	0.158
rs13306465	CC (812) vs CT+TT (308)	0.86 (0.65-1.15)	0.311	0.98 (0.74-1.28)	0.874	0.88 (0.66-1.17)	0.387
rs1801123	CC+CT (527) vs TT (587)	1.41 (1.10-1.80)	0.007	1.08 (0.83-1.40)	0.578	1.19 (0.93-1.53)	0.171
rs1801278	CC (1007) vs CT+TT (117)	0.95(0.64-1.42)	0.816	1.01(0.68 -1.48)	0.977	0.95(0.64-1.42)	0.800
rs934167	CT+TT (358) vs CC (755)	1.40 (1.07-1.82)	0.013	1.58 (1.21-2.07)	0.002	1.27 (0.97-1.66)	0.077
rs2943641	CC (508) vs CT+TT (575)	1.01 (0.78-1.30)	0.951†	0.95 (0.74-1.21)	0.664†	1.03 (0.79-1.33)	0.823†

*Odds ratio, 95% interval, and *p*-value were calculated by logistic regression models, and adjusted for age, sex, smoking, alcohol use, physical activity, and population admixture.
†Under a recessive model (CC+CT vs TT), these *p* values are 0.166 (OR=1.36), 0.128 (OR=0.41), 0.103 (OR=1.47), respectively.

IRS1 genotypes and DNA damage. SNP G972R (rs1801278) showed significant association with urinary 8-OHdG concentration, an easily measurable metabolite of DNA damage ($p = 0.020$; Table 5). Carriers of the minor T allele had significantly more DNA damage than non-carriers (156 vs 138 ng/mg creatinine). Furthermore, this SNP showed significant association with plasma CRP levels ($p = 0.035$; Table 5). Carriers of the rs1801278 minor T allele had significantly higher CRP levels than non-carriers (7.91 vs 5.86 mg/L). Other SNPs showed no significant association with DNA damage or CRP level.

***IRS1* haplotype analysis**

To explore the combined effects of *IRS1* variants on glyacemic traits, we conducted haplotype analysis with two SNPs, rs1801123 and rs934167 (Supplemental Table 2). Four haplotypes T-C, C-T, C-C, and T-T were identified with frequencies of 0.70, 0.15, 0.12, and 0.03, respectively. At a global level, *IRS1* haplotypes were significantly or marginally associated with fasting plasma glucose, insulin, HOMA-IR, and QUICKI ($p = 0.025$, 0.074, 0.015, and 0.017, respectively) after multiple-test correction by permutation. Carriers of the haplotype C-T showed significantly higher fasting plasma glucose and insulin levels ($p = 0.001$ and 0.012, respectively) and significantly higher HOMR-IR and lower QUICKI ($p = 0.0005$ and 0.0005, respectively). After Bonferroni correction ($p = 0.05/8 = 0.0063$), both associations with HOMR-IR and QUICKI are still statistically significant.

We also examined the association of these haplotypes (based on rs1801123 and rs934167) in relation to hyperglycaemia. All four haplotypes showed a globally significant association with hyperglycaemia after correction of permutation test ($p = 0.029$ after correcting for multiple testing). Carriers of the C-T haplotype, representing 15% of the population, correlated significantly with increased risk of hyperglycaemia (OR = 1.44, $p = 0.016$). However, association between these haplotypes and hyperinsulinemia and type 2 diabetes did not reach significance (data not shown).

For haplotype analysis and DNA damage, we selected the two SNPs rs1801278 and rs1801123. Three haplotypes (C-T, C-C, and T-T) were identified with frequencies of 0.67, 0.27, and 0.05, respectively. These haplo-

Table 5. DNA damage 8-OHdG and plasma CRP concentrations according to *IRS1* genotypes

SNP	Genotype	n	Urinary 8-OHdG (ng/mg creatinine)		Plasma CRP (mg/L)	
			Mean±SE	*p* value*	Mean±SE	*p* value*
rs12053536	CC	715	141±3.0	0.845	5.88±0.29	0.987
	CT+TT	402	139±3.7		6.42±0.48	
rs13306465	CC	812	141±2.7	0.577	6.20±0.31	0.206
	CT+TT	307	139±4.4		5.67±0.42	
rs1801123	CC+CT	525	139±3.3	0.861	5.75±0.34	0.058
	TT	587	142±3.3		6.32±0.36	
rs1801278	CC	1004	138±2.4	0.020	5.86±0.26	0.035
	CT+TT	117	160±8.9		7.91±0.84	
rs934167	CC	754	141±2.8	0.871	6.13±0.31	0.513
	CT+TT	354	141±4.1		6.01±0.44	
rs2943641	CC	508	142±3.8	0.613	6.19±0.41	0.604
	CT+TT	575	138±3.4		6.17±0.35	

*All means and *p*-values were calculated by ANCOVA using general linear models, and adjusted for age, sex, smoking, alcohol use, physical activity, and population admixture.

types were significantly associated with 8-OHdG at the global level ($p = 0.033$) after correction for multiple testing by permutation test. Subjects with the haplotype T-T had significantly higher DNA damage compared to non-carriers ($p = 0.020$; data not shown).

DISCUSSION

In the present study, we examined the association of six common variants in *IRS1* with type 2 diabetes and glycaemic traits in a cohort of Puerto Rican adults living in the Boston metropolitan area of Massachusetts.[24] Our results showed carriers of the *IRS1* variant rs934167 minor T allele had elevated fasting plasma glucose and insulin levels compared to CC homozygotes, and this variant was also associated with insulin resistance index, HOMA-IR, and insulin sensitivity index, QUICKI. Furthermore, carriers of the rs934167 minor T allele had a 40% and 58% greater likelihood of hyperglycaemia and hyperinsulinemia than CC homozygotes. In addition, haplotype analysis after correction for multiple testing further demonstrated the combined effects of this variant and rs1801123, which showed consistent and stronger associations with fasting plasma glucose, HOMA-IR and QUICKI. However, since these four traits are not totally independent from each other, Bonferrroni correction was not applied for multiple testing. Furthermore, SNP rs934167 showed only a trend but not statistically significant association with type 2 diabetes, which is supported by Florez *et al.*'s similar findings in North American and Polish case control samples.[27]

Type 2 diabetes is highly prevalent in the Boston Puerto Rican population and most of those individuals with diabetes were under treatment to control plasma glucose. As both medication and the disease state may affect fasting plasma levels of both glucose and insulin, we thus analyzed the data by dividing subjects into two groups: diabetes and non-diabetes. In this respect, rs934167 was significantly associated with plasma glucose, and insulin concentrations only among participants without diabetes. The overall results among the non-diabetes are consistent with those based on the analysis undertaken when combining diabetes and non-diabetes, but no variants showed association with these traits among participants with diabetes. As most of the diabetes subjects (98%) were taking diabetic medication, the observed difference between diabetes patients and non-diabetics could be because IRS1 protein functions as a responder to insulin signaling and observed insulin resistance is simply a response signal to uncontrolled glucose. Therefore, drug treatment may weaken the correlation between the *IRS1* rs934167 variant and glycemic traits among those patients using diabetes drugs.

Our observations suggest that the rs934167 variant is associated with hyperglycaemia and insulin resistance, which increased the risk of type 2 diabetes in Puerto Ricans. Although this non-coding SNP is located 2.5 kbp downstream of the *IRS1* gene, it may be a good proxy for other SNPs within the 3'UTR, which might affect *IRS1* mRNA function. Large independent studies with adequately powered samples would be required to confirm whether this variant influences the susceptibility to type 2 diabetes and affects glycaemic traits. The common variant rs2943641, a functional variant at *IRS1* identified by GWAS,[10] is associated with type 2 diabetes and insulin resistance and hyperinsulinemia based on 14,358 Europeans. In the present study, however, the association between this variant and type 2 diabetes or other related traits did not reach significance. This is likely due to small sample size of our population and difference in genetic background and environments.[16, 26]

The common missense variant glycine → arginine at codon 972 (Gly972Arg, rs1801278) has demonstrated functional consequences, but data concerning the association of this variant with type 2 diabetes in population studies are conflicting.[9] The current study has examined *IRS1* G972R (rs1801278) with respect to genetic susceptibility to type 2 diabetes in adult Puerto Ricans. In this population, we were unable to observe an association of G972R with type 2 diabetes. We also detected no association of G972R with glycaemic quantitative traits. Based on our power calculation, the main reason that we did not detect association between G972R and type 2 diabetes,

glucose, and insulin, is the small sample size and a low MAF (0.05 in this population vs 0.10 in Europeans) of this variant in this population. A meta-analysis of all case-control studies available to date also indicated that G972R was not significantly associated with type 2 diabetes and illustrated the difficulties of ascertaining the contribution of 'low-frequency-low-risk' variants to type 2 diabetes.[28] It was estimated that a total of ~40,000 and ~200,000 study individuals would have been required to attain 80% power at nominal significance ($\alpha = 0.05$) and genome-wide significance ($\alpha = 5 \times 10^{-8}$), respectively.

Interestingly, although we also could not detect an association of G972R (rs1801278) with type 2 diabetes in Puerto Ricans, our report provides the first supporting evidence that this *IRS1* variant is associated with oxidative DNA damage, as measured by concentrations of urinary 8-OHdG, and inflammation, as measured by plasma CRP. Carriers of the rs1801278 minor T allele had significantly higher 8-OHdG concentrations and higher CRP levels than non-carriers. Haplotype analysis also demonstrated a consistent and stronger association with DNA damage. IRS proteins may affect oxidative stress and its induced DNA damage by regulating the transcriptional activity of FoxO proteins via the insulin signaling pathway.[14,29] FoxO factors can also counter DNA damage by inducing cell-cycle inhibition through regulating factors such as CDK2 and increasing *GADD45* gene family expression to facilitate DNA repair.[14]

Another interpretation is that IRS proteins could regulate PPARGC1A activity via IRS→ PI3K→Akt→FoxO branch of insulin signaling cascade. The transcriptional co-activator PPARGC1A regulates mitochondrial function, oxidative phosphorylation, and cellular energy metabolism.[15] Alteration in PPARGC1A levels or activity has been demonstrated in metabolic diseases.[30] Our previous study also showed that *PPARGC1A* genetic variation is associated with DNA damage, diabetes, and cardiovascular diseases in this same adult Puerto Rican population.[22] FoxO factors, especially FOXO3, induce *PPARGC1A* expression and interact with PPARGC1A directly to regulate the expression of oxidative stress protection genes.[31] Thus, through the *IRS* → PI3K → Akt → FoxO → PPARGC1A pathway, *IRS1* genetic variation or functional alteration may affect the balance of ROS production and oxidative DNA damage. An imbalanced superoxide production can activate intracellular production of advanced glycation end products, leading to inflammation and increased plasma CRP.[32]

In summary, we have observed that *IRS1* variants (rs934167) showed a strong association with hyperglycemia and insulin resistance in Puerto Ricans. Our results are consistent with experimental studies showing that *IRS1* knockout mice are mildly hyperinsulinemic and insulin resistant but do not develop diabetes.[33] Our study is the first to report that *IRS1* variant G972R (rs1801278) may contribute to oxidative DNA damage and inflammation. This novel genetic association requires replication with multiple measurements in different populations.

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AUTHOR DISCLOSURES

There are no financial relationships or other potential conflict of interest to disclose.

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APPENDIX

Supplemental Table1. Characteristics of *IRS1* SNPs genotyped in 1132 subjects

SNP name[†]	rs #	HGVS name[‡]	Distance from TSS (bp)[§]	Gene region	Minor allele frequency
m7069	rs12053536	NT_005403.16:g.77879991C>T	-7069	Upstream	0.20
m395	rs13306465	NT_005403.16:g.77873317C>T	-395	Upstream	0.15
Ala804Ala	rs1801123	NM_005544.2:c.2464A>G	2464	Exon 1	0.27
Gly972Arg	rs1801278	NP_005535.1:p.Gly971Arg	2963	Exon 1	0.054
d70986	rs934167	NT_005403.16:g.77801937C>T	70986	Downstream	0.18
d570084	rs2943641	NC_000002.11:g.227093745T>C	570084	Downstream	0.31

[†]m indicates a SNP is located in the promoter or upstream of the gene; d is in the downstream of the gene; and others are substitution changes in exons.
[‡]HGVS names of SNPs are based on nomenclature recommendations of the Human Genome Variation Society (http://www.hgvs.org/rec.html).
[§]TSS, transcription start site.

Supplemental Table 2. Association between *IRS1* haplotypes and glycemic traits

Haplotype[†]	Frequency			Fasting insulin (pmol/l)		Fasting glucose (mmol/l)		HOMA-IR[‡]		QUICKI[§]	
			n	Mean ± SE	*p* value[*]	Mean ± SE	*p* value[*]	Mean ± SE	*p* value[*]	Mean ± SE	*p* value[*]
T-C	0.7	Carrier	1015	126±4.2	0.472	7.2±0.1	0.620	0.62±0.01	0.191	0.313±0.001	0.434
		Non-carrier	98	142±12.5		7.2±0.3		0.64±0.04		0.310±0.004	
C-T	0.15	Carrier	312	144±6.9	0.012	7.6±0.2	0.001	0.67±0.02	<0.001	0.307±0.002	<0.001
		Non-carrier	801	122±4.9		7.0±0.1		0.60±0.01		0.315±0.001	
C-C	0.12	Carrier	250	116±7.6	0.124	7.1±0.2	0.519	0.59±0.02	0.076	0.316±0.002	0.139
		Non-carrier	863	132±4.9		7.2±0.1		0.63±0.01		0.313±0.001	
T-T	0.03	Carrier	55	155±16.0	0.418	7.4±0.4	0.964	0.65±0.05	0.010	0.311±0.005	0.542
		Non-carrier	1058	126±4.2		7.2±0.1		0.62±0.01		0.314±0.001	

[†]*IRS1* haplotypes were estimated based on two SNPs in the order: rs1801123 and rs934167.
[*] *p*-values were calculated by linear regression models, and adjusted for age, sex, smoking, alcohol use, physical activity, medications for type 2 diabetes, and population admixture, the sample size *n*=1085.
[‡] Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: [fasting insulin (μU/ml) × fasting glucose (mg/dl)]/405.
[§] Quantitative insulin sensitivity check index (QUICKI) was calculated as: 1/[log(fasting insulin, μU/ml) + log(fasting glucose, mg/dl)].

Original Article

Insulin receptor substrate 1 (*IRS1*) variants confer risk of diabetes in the Boston Puerto Rican Health Study

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胰岛素受体底物 1（*IRS1*）多态性与糖尿病危险因素有关（波士顿波多黎各人群健康研究）

目的：目前关于 *IRS1* 多态性与 2 型糖尿病及危险因素的关联研究结果并不一致。在波士顿波多黎各人群健康研究的对象中研究 *IRS1* 基因多态性与 2 型糖尿病及危险因素，包括胰岛素抵抗、高血糖血症和 DNA 损伤等的关联作用。对象与方法：测定 1132 例波多黎各成人中 *IRS1* 基因 6 个 SNP 并分析其与 2 型糖尿病及危险因素之间的关联。结果：SNP rs934167 和 rs1801123 与空腹血糖含量有显著性关联(p=0.005 和 p=0.016)，rs934167 与血浆胰岛素水平也呈显著性关联(p=0.005)。rs934167 小等位基因携带者的 HOMA-IR 指数显著较高，而 QUICKI 指数显著较低(p=0.001 和 p=0.001)；他们发生高血糖血症和高胰岛素血症的危险性分别增加了 40%和 58%(OR=1.40 和 1.58，p=0.005 和 p=0.016)。然而，他们罹患 2 型糖尿病的危险性只是轻度增高(OR=1.27，p=0.077)。进一步发现，rs934167 和 rs1801123 小等位基因单倍型 C-T 携带者显示了类似的关联(经过多重检验校正后)。此外，G972R(rs1801278)小等位基因与较高的 DNA 损伤水平-尿 8-OHdG(p=0.020)和血浆 CRP 水平(p=0.035)有显著性关联。结论：在成年波多黎各人中，*IRS1* 基因多态性与 2 型糖尿病危险因素有关。同时有新的发现，即 *IRS1* 多态性位点 G972R(rs1801278)可能与 DNA 氧化损伤和炎症有关。

关键词：基因多态性、*IRS1*、2 型糖尿病、胰岛素抵抗、DNA 损伤