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PERSPECTIVE ARTICLE

G6PC2 rs560887 Gene Variant is Associated with Fasting Blood Glucose in the Admixed Mexican Population

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Abstract:

Background:

In Mexico, type 2 diabetes prevalence is 13.7%, which has a huge impact on Mexican public health. There is an urgent need to focus on the prevention of pre-diabetes to decrease the likelihood of type 2 diabetes onset. Gene variants predisposed to increase Fasting Blood Glucose (FBG) and glycosylated hemoglobin (HbA1c) levels could be helpful for prevention purposes. This study aimed to analyze the association of the G6PC2 rs560887 variant with pre-diabetes in a Mexican-Mestizo population.

Methods:

A cross-sectional case-control study was performed in 960 Mexican Mestizos participants. The association of *G6PC2* rs560887 with pre-diabetes was analyzed by logistic regression and with Fasting Blood Glucose (FBG) and glycosylated hemoglobin (HbA1c) by linear regression.

Results:

The *G6PC2* rs560887 variant was significantly associated with FBG (β -1.80, p=0.03), but not with HbA1c or the presence of pre-diabetes.

Conclusion:

The *G6PC2* rs560887 loci could be a potential early marker of type 2 diabetes.

1. INTRODUCTION

Pre-diabetes (pre-DM) is a reversible condition characterized by high blood glucose and/or glycosylated hemog-lobin (HbA1c), but not high enough to diagnose T2D (glucose>126 mg/dl, HbA1c≥6.5%) [1, 2]. About 70% of patients with pre-DM will progress to type-2 diabetes (T2D) [2, 3], a disease whose prevalence in Mexico is 13.7% [4]. Thus, there is an urgent need to focus on the treatment and prevention of pre-DM to decrease the likelihood of T2D onset.

In Mexicans, there are few genes that have been associated with elevated glucose levels: *PPARG* (Pro12Ala), *ASCL1* (rs9997745), *PPARGC1A* (rs8192678), and ADIPOQ (rs82 2396) [5 - 7]. Hence, identifying additional loci influen- cing glucose or HbA1c levels may improve the understanding of the pathogenesis of T2D and contribute to its prevention.

In this framework, the *G6PC2* gene encodes an islet-specific glucose-6-phosphatase catalytic subunit, that hydro-lyzes glucose 6-phosphate; thus, increasing glycolysis and decreasing glycolytic flux [8]. G6pc2- null mice exhibit a mild metabolic phenotype with decreased blood glucose levels but unchanged insulin, glucagon, glycerol, and cholesterol concentrations. In addition, several human related gene variants conferred a decreased G6PC2 protein expression in mice [8].

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The *G6PC2* rs560887 is an intron variant that has been largely associated with T2D and elevated fasting blood glucose (FBG) in Americans (including Hispanics), Europeans, and Asians [8 - 13]. An extensive meta-analysis also showed the association of not only this variant but also rs16856187 and rs573225 with FBG [14]. In addition, the *G6PC2* rs560887 has been associated with FBG in Mexican children [15]; however, it has not been tested in the adult population. This study aimed to analyze the association of the *G6PC2* rs560887 variant with the susceptibility to pre-DM and with FBG and HbAc1 in Mexican Mestizo adults.

2. METHODS

This is a cross-sectional and case-control study consisting of 960 volunteers over 45 years old, non-related, all with Mexican-Mestizo ancestry (at least three not related grandparents born in Mexico), and enrolled in the Cohort for the Study of Complex Diseases [Cohorte para el Estudio de Enfermedades Complejas], carried out at the Hospital Regional Lic. Adolfo López Mateos of the Institute for Social Security for State Workers [Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, ISSSTE]. This study started in 2013 in response to the need for establishing a prospective metabolic epidemiologic study. Participants are ISSSTE right holders who are monitored annually regarding clinical, anthropometric, and biochemical parameters. Participants had no diabetes or cardiovascular disease.

Weight, height, and body mass index (BMI, kg/m^2) were measured. Venous blood samples were drawn after a 10-hour fast to measure FBG, Hb1Ac, and triglycerides. The concentrations were measured in an automated analyzer (Miura 200, Italy) and expressed as mg/dL, except for HbA1c, which was expressed as a percentage.

Genomic DNA was extracted from 500 µL of whole blood, using an automated system InviGenius® and the DNA Mini Kit InviMag Blood (STRATEC Molecular GmbH, Berlin, Germany). For genotyping, a pre-designed 5' exonuclease TaqMan assay was used on a 7500 series Real-Time PCR system, according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

2.1. Data Analysis

Descriptive results are presented as means and standard deviations. Those variables were compared between cases and controls by Student's t-test. The Hardy-Weinberg equilibrium was assessed by using the exact test. Glucose and Hb1Ac mean pairwise comparisons were performed between genotypes by the Mann-Whitney test using the XLSTAT software (Addinsoft 2020, XLSTAT statistical and data analysis solution, New York, USA, https://www.xlstat.com).

The association between the rs560887 variant was evaluated by using logistic and linear regression models. For logistic regression models, participants were classified into two groups (cases and controls) according to their glucose levels: i) pre-DM (case group n=435) when FBG >100 mg/dl, and ii) control group when glucose <100 mg/dl (n=525); or by their Hb1Ac levels: i) pre-DM (case group n=99) when fasting Hb1Ac 35.7%, and ii) control group when Hb1Ac <5.7% (n=861) [2]. For linear regression models, FBG and Hb1Ac were considered as continuous outcome variables. Regression models were adjusted by age, sex, BMI, and triglycerides, under the Additive/Dominant/Recessive model conducted with PLINK 1.7 software version [16] (http://pngu.mgh.harvard.edu/purcell/plink, Harvard University, Cambridge, MA, USA).

The Quanto software version 1.2.4 (http://biostats.usc.edu/Quanto.html, University of Southern California, Los Angeles, CA, USA) was used to perform a power analysis considering prevalence of the disease to be 13.7% [4], and means and standard deviations for glucose (99 ± 10.9 mg/dl) and HbA1c ($4.8\pm0.9\%$) for the case-control and continuous outcome designs, respectively. Power analysis was done for gene only, under additive, dominant and recessive inheritance models using the SNPs allele frequency reported in this study (0.09), obtaining power of 80% to detect $0.6 \leq OR \leq 0.7$ $OR \geq 1.4$ and $\beta \geq 1.5$.

All participants signed informed consent. This study was approved by the Research, Ethics, and Biosafety Committees of the HRLALM (registration number 236.2011).

3. RESULTS

The study included a total of 960 individuals (359 men and 601 women). The descriptive results are shown in Table **1**. All pairwise comparisons between cases and controls resulted in statistically significantly differences (p<0.05), except for BMI and triglycerides when pre-DM classification was based on HbA1c (Table **1**). No departure from Hardy-Weinberg equilibrium was found, being 0.09, the *G6PC2* rs560887 minor allele frequency for the T allele. Comparisons of genotypic frequency are shown in Table **2**.

Table 1. Descriptive results from cases and controls. BMI (Body Mass Index), FBG (Fasting Blood Glucose), Glycosylated Haemoglobin (HbA1c), Standard Deviation (SD).

-	Pre-DM Classification Based on FBG		-	-	Pre-DM Classification Based on Hb1Ac		-	-
-	Cases (n=435)	Controls (n=525)	P- *value*		Cases (n=99)	Controls (n=861)	P- *value*
-	Mean (SD)	Mean (SD)			Mean (SD)	Mean (SD)	-
Age	51 (6.9)	48 (7.4)	0.0001		52 (7.0)	49 (7.3)	0.0001
BMI (kg/m^2)	29.1 (4.7)	28.1 (4.7)	0.001		29.4 (4.6)	28.5 (4.4)	0.1
HbA1c (%)	5.0 (0.9)	4.6 (0.7)	0.0001		6.2 (1.04)	4.6 (0.7)	0.0001
Glucose (mg/dl)	108.6 (6.7)	91.1 (6.3)	0.0001		104.9 (11.7)	98.3 (10.7)	0.0001

(Table 3) contd.....

-	Pre-DM Classification Based on FBG	-	-	Pre-DM Classification Based on Hb1Ac	-	-
Triglycerides (mg/dl)	200.3 (141.3)	165.6 (82.5)	0.0001	184.3(101.0)	181.0 (151.3)	0.77

Table 2. Genotype frequencies of *G6PC2* rs560887. Number of (N) and percentages of participants by genotype. Mean (Standard deviation) of by genotype. Hb1Ac (glycosylated haemoglobin). * *p*-value (P) between CC *vs.* CT, and CC *vs.* CT+TT.

-	CC	CT	TT	CT+TT	P
N	787 (82.0%)	165 (17.2%)	8 (0.8%)	173(18%)	
Glucose	99.4 (10.8)	97.0 (11.1)	98.0 (8.4)	97.2 (11.1)	0.02*
Hb1Ac	4.8 (0.8)	4.7(1.1)	4.8 (0.4)	4.7(1.1)	0.15

Table 3. Logistic and linear regression models for the association Fasting blood glucose (FBG) or glycosylated haemoglobin (Hb1Ac). FBG and Hb1Ac were used to classify individuals as pre-DM or controls for the logistic regression models, and they were considered as continuous for the linear regression models. Odds ratio (OR), and estimates (β), 95% confidence interval (CI 95%), additive model (ADD), dominant model (DOM), recessive model (REC).

-	-	FBG	-	-	-	Hb1Ac	-	-	-
Model	Allele	OR (CI 95%)	P	β (CI 95%)	P	OR (CI 95%)	P	β (CI 95%)	P
ADD	T	0.78 (0.56, 1.08)	0.14	-1.592 (-3.19,0.01)	0.05	0.76 (0.43, 1.33)	0.3442	-0.05(-0.17,0.079)	0.46
DOM	T	0.77 (0.54, 1.08)	0.14	-1.80 (-3.52,-0.09)	0.03	0.78 (0.43, 1.42)	0.4259	-0.05(-0.20,0.08)	0.41
REC	T	0.761 (0.17, 3.39)	0.72	-0.46 (-7.69, 6.76)	0.90	0.001 (0,2)	0.9982	0.04(-0.52,0.60)	0.88

Higher FBG was significantly associated with the T allele under the dominant inheritance model (β=-1.80 p=0.03, Table **3**). None of the other models provided statistically significant associations (Table **3**).

4. DISCUSSION

In this study, the association between the *G6PC2* rs560887 common gene variant and pre-DM, FBG, and HbA1c, in 960 Mexican-Mestizos enrolled in the CPEEC, was evaluated.

It was found that mean BMI was above the standard cut-off for overweight (25kg/m^2) for both cases and controls (Table **1**). Mean triglycerides were also above the level considered normal (150 mg/dl), suggesting that even those subjects classified as controls are at risk of developing pre-DM.

It was also found that *G6PC2* rs560887 was associated only with FBG as a continuous variable, but not with HbA1c or pre-DM (either when the pre-DM classification was based on FBG or HbA1c). The rs560887 T allele seemed to confer protection against high FBG (C/T reported in this study). This is in line with other studies carried out in Europeans; the A allele (complimentary G/A) conferred protection against high FBG in 654 subjects and the results were replicated in other 9,353 subjects [13]. Besides, the G allele was related to the risk of having high FBG in Europeans [12, 17], African Americans, Hispanics [11], and American Indians [12].

A limitation of this study is that it has not enough statistical power to detect a near cero OR (OR≈0.8) or a small β (β<1.5). This could be the reason why a significant statistical association could not be found between G*6PC2* rs560887 and pre-diabetes or Hb1Ac. This means that small effects cannot be discarded before considering larger sample size.

In Mexico, the rs560887 G allele was associated with higher FBG in children [15], suggesting that age could not necessarily be an additional contributor to this association. Additionally, and in agreement with these results, variations in *GCK* and *G6PC2* genes have effects on both FBG and insulin secretion in Mexican Americans [18].

CONCLUSION

Although replications studies in the Mexican population are needed, this fact makes the *G6PC2* rs560887 gene variant a potential early marker of type 2 diabetes in clinical practice.

LIST OF ABBREVIATIONS

T2D	=	Type-2 diabetes
HbA1c	=	Glycosylated haemoglobin
FBG	=	Elevated fasting blood glucose
pre-DM	=	Pre-diabetes

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research, Ethics, and Biosafety Committees of the HRLALM (registration number 236.2011).

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

All participants signed informed consent.

FUNDING

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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