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**RESEARCH ARTICLE**

# Association of the polymorphisms 292 C>T and 1304 G>A in the *SLC38A4* gene with hyperglycaemia

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## Abstract

**Background** The *SLC38A4* gene is related to system 'A' activity, which seems to be related to impaired gluconeogenesis. The objective of this study was to determine whether the 292 C>T and 1304 G>A polymorphisms of *SLC38A4* gene are associated with hyperglycaemia in humans.

**Methods** A total of 227 individuals were enrolled in a case–control study, in which hyperglycaemia was defined by plasma glucose levels ≥95 mg/dL. Genotyping was carried out by using real-time polymerase chain reaction.

**Results** The frequency of mutant alleles of *SLC38A4* gene for single-nucleotide polymorphism (SNP) 1304 G>A was 23.6% and 30.2% for SNP 292 C>T. The frequency of allele T for the SNP 292 C>T in the case and control groups did not show significant differences, whereas the frequency of allele A for the SNP 1304 G>A was significantly higher in the case group than in the control group ($p = 0.04$). In the logistic regression analysis, the SNP 1304 G>A [odds ratio (OR) 1.78; 95%CI 1.04–3.05, $p = 0.03$] but not SNP 292 C>T (OR 1.41; 95%CI 0.80–2.47, $p = 0.23$) showed a significant association with hyperglycaemia. After adjusting by body mass index, waist circumference and triglycerides, the SNP 1304 G>A remained significantly associated with hyperglycaemia (OR 2.13; 95%CI 1.18–3.83, $p = 0.03$). Pair wise linkage disequilibrium showed correlation ($D' > 0.82$) between 292 C>T and 1304 G>A SNPs. Haplotype association with hyperglycaemia also showed significant association between both homozygous mutant alleles (A/T) and hyperglycaemia (OR 1.68; 95%CI 1.01–2.79, $p = 0.048$).

**Conclusions** Our results suggest that mutant allele A for SNP 1304 G>A of *SLC38A4* gene is associated with hyperglycaemia. Copyright © 2012 John Wiley & Sons, Ltd.

**Keywords** *SLC38A4*; hyperglycaemia; polymorphism; haplotype

## Introduction

In Mexico, type 2 diabetes (T2D) is the main cause of morbidity and mortality in the adult population. An increase in the rates of incidence of T2D (400 000 new cases) and mortality (60 000 deaths) have been reported annually [1]. Data from the Ministry of Health in Mexico showed that Morelos, Coahuila, Durango, Jalisco and Sinaloa are the states with the highest incidence of T2D [1].

Many diseases including T2D begin early in life; accordingly, a study by our group demonstrated the relationship between nutritional deficiencies during early development and phenotypes in adulthood such as glucose metabolism

disorders, insulin resistance, hypertension, cardiovascular disease and obesity (metabolic syndrome) [2].

During fetal life, the appropriate amino acid supplement from the maternal circulation is essential for proper growth and development of the fetus [3]; transport is mediated by transporters located in the placental microvillosities [4].

The SLC38A system, also known as system 'A' amino acid transporter, is a family of transporters comprising the genes *SLC38A1* (*SNAT1*), *SLC38A2* (*SNAT2*) and *SLC38A4* (*SNAT4*). The last one facilitates the cationic amino acid transport in a way independent of $Na^+$ and pH, while the neutral amino acid transport depends on the aforementioned factors [5,6]. The *SLC38A4* gene is subjected to imprinting, a feature that varies between species [7]. It is located on chromosome position 12q13 and contains 34 single-nucleotide polymorphisms (SNPs) in the coding region, of which 19 are missense mutations [8].

The placental expression of *SLC38A4* gene is determined by a paternal allele, suggesting the important role of this gene in promoting fetal growth [7]. Studies by Coan *et al*. [9] show an inverse relationship between birth weight and system A activity. It was long thought that this transport system was liver specific; however, recent studies demonstrate that *SLC38A4* gene is ubiquitously expressed in human tissues including brain, stomach, lung, heart, kidney and placenta [4]. The activity of this system is quite important for hepatic gluconeogenesis through the conversion of amino acids and glucose recycling [10]. It has been hypothesized that alterations in hepatic system A could increase the glucose levels through impairing gluconeogenesis [11]. In this context, the objective of this study was to determine whether polymorphisms 292 C>T and 1304 G>A of the gene *SLC38A4* are associated with hyperglycaemia.

# Material and methods

We conducted a case–control study in which the case group was defined by the presence of hyperglycaemia and was compared with a control group of normoglycaemic subjects. The protocol was approved by the local Ethics Committee of the Ministry of Health at Durango City in Northern México. The study population was enrolled from the clinic Cardio Prevent, the General Hospital and the Security Institute and Social Services for the State Workers of Durango.

The same trained personnel were in charge of handling DNA samples and phenotype measurements. In addition, the same diagnosis criteria and procedures were considered for all patients in the three recruitment centres.

Eligible subjects were men and nonpregnant women aged 35 to 80 years, inhabitants of Durango City in northern México; subjects with hypertension, cancer and polycystic ovaries were excluded.

## Definitions

On the basis of previous results in our population, in order to increase the sensitivity for recognizing hyperglycaemia, the cut-off point for normal glucose levels was lower than 95 mg/dL; so, hyperglycaemia was defined as serum fasting glucose ≥95 mg/dL [12].

Hypertension was defined as systolic/diastolic blood pressure equal to or greater than 140/90 mmHg [13]. Body mass index (BMI) equal to or greater than 30 $kg/m^2$ defined obesity [14]; meanwhile, a waist circumference equal to or greater than 102 cm for men and equal to or greater than 88 cm for women defined central obesity [15].

## Methods

Weight and height were measured with the subject in standing position, relaxed with hands outstretched and resting on the thigh laterally, and in contact with the stadiometer (positioned vertically) [16]. The midpoint between the lower costal margin and the top edge of the iliac crest on the right side was considered to measure the waist circumference [16].

The BMI was determined by using the criterion of the World Health Organization according to the following formula: BMI = weight (kg)/height $(m)^2$ [16].

Triglycerides and high-density lipoprotein (HDL)-cholesterol were measured using enzymatic methods. The glucose-oxidase method was used for determination of plasma glucose levels. The HDL-cholesterol fraction was obtained after precipitation by using phosphotungstic reagent. All measurements were performed by using automated equipment (VITROS® 250, Ortho-Clinical Diagnostics Inc, Raritan New Jersey, USA).

A total of 5 mL of venous blood was collected in EDTA supplemented tubes. The DNA was extracted with the method DTAB/CTAB described by Philips and Simon [17]. The integrity was verified through 1% agarose gel electrophoresis, while purity and concentration were verified by using spectrophotometry at 260/280 nm in a Nanodrop 2000c equipment (ThermoScientific®,Suwanee, GA 30024 USA).

Genotyping was done by using real-time polymerase chain reaction with TaqMan technology in a StepOne (Applied Biosystems®, Carlsbad, CA, USA) equipment. A total of 25 ng of genomic DNA was used under the following reaction conditions: one cycle of initial denaturing at 95 °C/10 min followed by 42 cycles of denaturing (95 °C/15 s), annealing (60 °C/1 min) and extension (60 °C/30 s). The TaqMan probe used to recognize the SNP 1304 G>A (rs11183610) was C_25751555_10, whereas the SNP 292 C>T (rs2429467) was evaluated by using the TaqMan probe C_15797142_10 [18]. Sequences of the probes are shown in Table 1. Both probes amplify fragments of 117 bp. Genotypes were evaluated in duplicate with previously randomized samples.



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**Table 1. TaqMan probes used for the analysis of SNPs 292 C>T and 1304 G>A**

| SNP | Sequence |
|---|---|
| 292 C>T | C_15797142_10 GCTGCCTTTTCTGAATTTCCTATCC**[C/T]**GATGTAGCTATCTGGAGCACTTTCT |
| 1304 G>A | C_25751555_10 CCCCGTGATGGAAATATTTGACACC**[A/G]**TTTGCATTTTTCTCCGGGACCGACT |

Applied Biosystems (2011).
SNP, single-nucleotide polymorphism.

## Statistical analysis

Data are presented as mean ± standard deviation or proportions. Differences between numerical variables were established with the Student's *t*-test for independent samples (Mann Whitney *U*-test for non-parametric data) and $x^2$ test for categorical variables. To determine differences of the allele and genotype frequencies between groups, we used a Pearson's $x^2$ test.

Hardy–Weinberg equilibrium (HWE) was calculated through $x^2$ goodness-of-fit statistic.

The association between polymorphisms and hyperglycaemia was determined using multivariate logistic regression analysis. The model was adjusted with those variables that in bivariate analysis showed significant difference between groups. An additional multivariate linear analysis was carried out to evaluate the association between SNPs and triglyceride levels.

Genotypes from each individual were useful to predict haplotypes and their frequencies; linkage disequilibrium between SNPs was calculated by using $D'$ measurement. Both analyses were carried out by using the program SNPSTATS [19].

Statistical analysis to establish differences for numerical and categorical variables between the groups was performed using SPSS V.15.0.

Statistical significance was established with a 95% confidence interval (CI) and a *p*-value <0.05.

# Results

A total of 227 patients were enrolled, 119 (52.42%) and 108 (47.57%) in the case and control groups, respectively. A total of 32 (26.9%) men and 87 (73.10%) women were enrolled in the case group, while 31 (28.70%) men and 77 (65.74%) women were in the control group, $p = 0.87$. Table 2 shows the anthropometric and biochemical variables in the case and control groups. There were significant differences for waist circumference, BMI and triglycerides, which were higher among women in the case group as compared with the control group; regarding men, significant differences were observed only in systolic blood pressure.

In the case group, 87 (73.1%) individuals were under treatment with metformin, 7 (14.3%) with glibenclamide/metformin, 4 (3.4%) with glibenclamide and 4 (3.4%) with intermediate insulin. Seventeen cases (14.3%) with glucose levels ≥95 to 99 mg/dL were managed with diet and exercise.

The frequency of allele T for the SNP 292 C>T in the case and control groups did not show statistically significant differences, whereas the frequency of allele A for the SNP 1304 G>A was significantly higher in the case than control group ($p = 0.04$; Table 3).

Population in the control group was in HWE determined by calculating the genotype frequencies obtained from the SNPs 292 C>T ($p = 0.82$) and 1304 G>A ($p = 0.53$).

In the logistic regression analysis, the SNP 1304 G>A [odds ratio (OR) 1.78; 95%CI 1.04–3.05, $p = 0.03$] but not SNP 292 C>T (OR 1.41; 95%CI 0.80–2.47, $p = 0.23$) showed a significant association with hyperglycaemia. After adjusting by BMI, waist circumference and triglycerides, the SNP 1304 G>A (OR 2.13; 95%CI 1.18–3.83, $p = 0.03$) remained significantly associated with hyperglycaemia.

The association of SNP 1304 G>A with hyperglycaemia agrees with a dominant inheritance model.

Women in the case group exhibited significantly higher triglyceride levels (231.9 ± 157.2) than women in the control group (185.8 ± 137.3) ($p < 0.047$). The multivariate linear analysis showed a strong association between SNP 292 C>T and hypertriglyceridaemia ($\beta$ 37.5; $p = 0.04$).

**Table 2. Anthropometric and biochemical parameters of the studied population**

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | Case | Control | | Case | Control | |
| | *n* = 32 | *n* = 31 | *p*-value | *n* = 87 | *n* = 77 | *p*-value |
| Age (years) | 59.6 ± 10.8 | 56.1 ± 14.1 | 0.40 | 53.6 ± 9.1 | 52.8 ± 9.4 | 0.59 |
| Waist circumference (cm) | 98.1 ± 10.9 | 94.1 ± 9.3 | 0.22 | 100.9 ± 13.0 | 90.9 ± 9.9 | <0.0005 |
| Body mass index | 29.4 ± 4.3 | 27.7 ± 3.9 | 0.11 | 30.1 ± 5.7 | 28.3 ± 4.1 | 0.001 |
| Systolic blood pressure (mmHg) | 126.0 ± 23.4 | 109.0 ± 6.6 | <0.0005 | 121.5 ± 16.5 | 112.9 ± 36.4 | 0.059 |
| Diastolic blood pressure (mmHg) | 77.7 ± 15.5 | 73.6 ± 8.7 | 0.21 | 75.0 ± 8.7 | 74.7 ± 7.8 | 0.80 |
| Fasting glucose (mg/dL) | 135.5 ± 47.2 | 81.7 ± 8.2 | <0.0005 | 151.7 ± 48.23 | 80.4 ± 8.5 | <0.0005 |
| Triglycerides (mg/dL) | 231.2 ± 123.0 | 190.8 ± 82.8 | 0.13 | 231.9 ± 157.2 | 185.8 ± 137.3 | 0.047 |
| HDL-cholesterol (mg/dL) | 44.2 ± 13.4 | 44.3 ± 18.0 | 0.98 | 48.7 ± 14.4 | 45.7 ± 13.1 | 0.16 |

Values are mean ± standard deviation.
HDL, high-density lipoprotein.



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**Table 3. Allele and genotype frequencies for SNP 292 C>T and 1304 G>A of the gene *SLC38A4***

| | SNP 292 C>T | | | | SNP 1304 G>A | | |
|---|---|---|---|---|---|---|---|
| | Case $n = 119$ | Control $n = 108$ | $p$-value* | | Case $n = 119$ | Control $n = 108$ | $p$-value* |
| C | 162 (68.1) | 155 (71.7) | 0.45 | G | 172 (72.3) | 175 (81.0) | 0.04 |
| T | 76 (31.9) | 61 (28.2) | | A | 66 (27.7) | 41 (19.0) | |
| CC | 55 (46.2) | 56 (51.8) | 0.68 | GG | 63 (52.9) | 72 (66.7) | 0.09 |
| CT | 52 (43.7) | 43 (39.8) | | AG | 46 (38.6) | 31 (28.7) | |
| TT | 12 (10.1) | 9 (8.3) | | AA | 10 (8.4) | 5 (4.6) | |

*N* = individuals; values are *n* (%).
SNP, single-nucleotide polymorphism.
**p*-value between case and control groups.

Pair wise linkage disequilibrium showed correlation ($D' > 0.82$) between 292 C>T and 1304 G>A SNPs. Haplotype association with hyperglycaemia also showed significant association between both homozygous mutant alleles (A/T) and hyperglycaemia (OR 1.68; 95%CI 1.01–2.79, $p = 0.048$; Table 4).

## Discussion

Our results suggest that carrying a mutant allele A for SNP 1304 G>A in the gene *SLC38A4* is associated with hyperglycaemia.

Currently, the American Diabetes Association considers as pre-diabetes the condition of fasting plasma glucose ≥100 and <126 mg/dL and/or post-prandial glucose levels of ≥140 and <200 mg/dL [20], status that confers higher risk to develop diabetes and cardiovascular disease. Furthermore, T2D is a condition that results from the interaction between environmental factors and genetic susceptibility [21]; the knowledge of the latter would allow us to identify susceptible individuals in early stages or even before the disease appears. In this context, the association of alterations *in utero* with development of chronic diseases has been described [2]. Epidemiological studies reveal that malnutrition during pregnancy results in fetal programming, a process that permanently alters the structure and function of tissues that facilitate the emergence of diseases in adulthood [9].

The frequency of mutant alleles of *SLC38A4* gene varies depending on population; in Asian and African individuals, the mutant allele frequency for SNP 1304 G>A is 6.7% and 50%, respectively [22]. In this study, the frequency of allele A was 23.6% similar to that reported by the HAPMAP-MEX in Mexican-American subjects from Los Angeles (29%) [22]. Regarding SNP 292 C>T, a mutant allele frequency of 2.7% in Europeans and 29% in Asian subjects has been reported [9]; the observed frequency in our study was 30.17%, similar to 35% reported by HAPMAP-MEX [23].

To our knowledge, there are no previous reports in the Mexican population that allow comparing our results. So, further research is required to know the allele and genotype frequencies for the analysed SNPs in other sectors of the Mexican population.

Although the analysis of the SNP 292 C>T did not demonstrate association with hyperglycaemia, the SNP 1304 G>A was significantly associated with the phenotype.

In addition, the association with hyperglycaemia was observed in subjects with the haplotype A/T, suggesting that mutant allele A could be related with the increased risk, a finding supported by the fact that other allele combinations did not reach statistical significance. In this regard, the model which better fits an inheritance model was dominant suggesting that a single copy of allele A is enough to increase the risk for hyperglycaemia. Such association persisted in the model adjusted by BMI, waist circumference and triglycerides; so individuals carrying mutant allele A for SNP 1304 G>A have a greater risk to present hyperglycaemia as compared with non-carrying subjects. To the best of our knowledge, there are no previous reports regarding the increased risk of hyperglycaemia related with the presence of SNPs in the gene *SLC38A4*. In this regard, our results strongly suggest that the presence of mutant allele A for SNP 1304 G>A of *SLC38A4* gene is related with glucose metabolic imbalance that contributes to development of hyperglycaemia.

Alterations in the expression of *SLC38A4* gene, and therefore in system A, have been implicated in impaired

**Table 4. Association of *SLC38A4* haplotypes and hyperglycaemia (*N* = 227)**

| SNPs 1304 G>A /292 C>T | Frequency | OR[a] | 95%CI | $p$-value |
|---|---|---|---|---|
| G/C | 66.9 | 1 | – | – |
| A/T | 20.6 | 1.68 | (1.01–2.79) | 0.048 |
| G/T | 9.5 | 0.87 | (0.44–1.70) | 0.68 |
| A/C | 2.9 | 2.83 | (0.71–11.25) | 0.14 |

OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]OR that computes the relationship between haplotypes and hyperglycaemia adjusted by body mass index, waist circumference and triglycerides.



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gluconeogenesis in mice [11]; so it is possible that polymorphisms of the *SLC38A4* gene might be related with elevated glucose levels.

In addition, among the mechanisms related with the increase in glucose levels, it has been hypothesized that hypertriglyceridaemia could be a risk factor (Simental *et al*., unpublished data). This statement was supported in our study by the strong association found between SNP 292 C>T and hypertriglyceridaemia, a finding that suggests that in addition to impairment of gluconeogenesis, other possible indirect mechanisms could be involved in the increase of serum glucose levels induced by polymorphisms of *SLC38A4* gene. Further research is mandatory in order to confirm our finding and to clarify the implications of SNPs in the gene *SLC38A4* on transport protein activity as well as the role of its expression as predictor for hyperglycaemia.

Some limitations of this study deserve to be mentioned: First, the sample size might be small; however, the narrow CIs indicate that the sample size was appropriate to evaluate the association between SNPs 1304 G>A and 292 C>T with hyperglycaemia. Second, because subjects in the control group would later develop T2D, the occurrence of selection bias is possible; however, this is an inherent limitation of the cross-sectional studies.

In conclusion, our results strongly suggest that the presence of mutant allele A for SNP 1304 G>A of *SLC38A4* gene is associated to hyperglycaemia.

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