Genetic variants of two-pore calcium channel 2 rs1551305 and its association with type 2 diabetes risk

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Context
The prevalence of diabetes is highest in the Eastern Mediterranean Region, with Egypt leading the region (11% for both sexes) and lowest in the European Region (7% for both sexes). Genome-wide association studies of single-nucleotide polymorphisms (SNPs) have identified a number of variants that are associated with β-cell function and insulin resistance. Two-pore calcium channel 2 (TPCN2) localizes to the lysosome and is a likely receptor for the calcium-mobilizing agent nicotinic acid adenine dinucleotide phosphate. Several studies have indicated that nicotinic acid adenine dinucleotide phosphate may play a role in the insulin signaling of β-cells.
Aim
of the study The aim of this study was to investigate the association between *TPCN2* rs1551305 SNPs and the development of type 2 diabetes.
Patients and methods
A sample of 158 Egyptian participants was divided into two groups. Group one included 79 type 2 diabetic patients and group two included 79 healthy controls. *TPCN2* rs1551305 SNPs were determined by the real-time PCR technique.
Results
A significant increase in the frequency of G/G genotype in diabetic patients was found. A/A genotype was significantly more frequent in the control group (P=0.001). G allele was also significantly higher in diabetic patients (P=0.008). The G/G genotype showed a 21.37-fold increase in the risk of developing diabetes mellitus.
Conclusion
The previous findings suggest that *TPCN2* rs1551305 SNP is associated with the risk of type 2 diabetes development.

Keywords:
single-nucleotide polymorphism, two-pore calcium channel 2 rs1551305, type 2 diabetes

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Introduction

Diabetes mellitus (DM) has high prevalence, morbidity, and mortality rates with multiple social and economic consequences of its complications compromising the quality of life and productivity of the affected patients [1]. It is believed that physical inactivity and the pattern of diet may lead to type 2 diabetics in the presence of a permissive genetic background [2].

A region of rat chromosome 1 that maps both fasting and postprandial glucose was discovered [3]. This region overlaps a larger region that maps fasting insulin and insulin sensitivity [4]. Expression and sequence analysis in the heterogeneous stock rats identified a two-pore calcium channel 2 (*TPCN2*) as a possible candidate gene within this region.

The role of *TPCN2* in glucose homeostasis was confirmed by demonstrating that *TPCN2* knockout mice exhibit altered fasting glucose and insulin in response to a glucose challenge, when studied in a sample of Chinese population. *TPCN2* single-nucleotide polymorphism (SNP) rs1551305 showed altered glucose and insulin levels in a *TPCN2* variant [5].

Patients and methods

This cross-sectional study was carried out between April 2016 and January 2017 at Kasr El-Aini Hospital, Cairo University. The ethical committee approved the study. It was conducted on a total number of 158 patients who were divided into two groups:

Group 1: included 79 patients recruited from the Outpatient Endocrinology and Diabetic Clinic with established diagnosis of type 2 DM for at least 2 years

with one of the following criteria repeated at least on two successive settings:

A hemoglobin A1c (HbA1c) level of 6.5% or higher. A fasting plasma glucose level of 126 mg/dl or higher. A 2-h plasma glucose level of 200 mg/dl or higher during a 75-g oral glucose tolerance test.

Group 2: 79 apparently healthy volunteers, age-matched and sex-matched with patients as the control group included in the study after being tested negative for diabetes (fasting plasma glucose <100 mg/dl).

Exclusion criteria

(1) Patients less than 18 years old.
(2) Recently diagnosed cases of type 2 diabetes.
(3) Cases presenting with type 1 DM, tumors, Cushing's syndrome, acromegaly, pheochromocytoma, hyperthyroidism, primary hyperaldosteronism, chronic pancreatitis, pancreatectomy, cystic fibrosis, hemochromatosis, pregnancy, and polycystic ovary syndrome.
(4) Usage of medications (corticosteroids, beta-blockers, and thiazide diuretics) and women taking oral contraceptives.

An informed consent form was signed by each patient before participating in the study and all patients were subjected to:

(1) History taking.
(2) Clinical examination including sphygmomanometric measurement of the current blood pressure, anthropometric measurements: height, weight, and waist circumference and BMI, which was calculated as weight (kg) divided by height (m) squared,
(3) Laboratory investigations for the patients and controls,

Calculations

(1) Insulin resistance status was determined using the homeostatic model assessment (HOMA-IR) calculation from the following equation: fasting insulin in (uU/ml)×fasting glucose in (mg/dl) divided by 405 [6].
(2) β-cell function was measured using the HOMA-B calculation from the following equation: (fasting insulin in μU/ml×360) divided by (fasting glucose in mg/dl–63) [7].
(3) Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation from the measured values of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol according to the relationship: LDL=total cholestrol–HDL–triglycerides/5, where triglycerides/5 is an estimate of very low-density lipoprotein cholesterol and all values are expressed in mg/dl. This equation is not valid in TG higher than 400 mg/dl [8].
(4) Fasting insulin was assessed using electrochemiluminescence immunoassay performed on cobas e411 (Roche Diagnostics, North America).
(5) Analysis of *TPCN2* polymorphisms by real-time PCR technique using TaqMan probes (Applied Biosystems, Foster City, CA) and primers [9].

Statistical analysis

Data were coded and entered using the statistical package for the social sciences, version 22. Data were summarized using mean and SD or median and interquartile range for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired *t* test when comparing two groups and analysis of variance with multiple comparisons post-hoc test when comparing more than two groups in normally distributed quantitative variables while nonparametric Kruskal–Wallis test and Mann–Whitney test were used for non-normally distributed quantitative variables [10]. For comparing categorical data, χ^2 test was performed. The exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Pearson's correlation coefficient. *P* values less than 0.05 were considered as statistically significant [11].

Results

The study was conducted on 158 patients who were divided into two groups. Group one includes 79 patients with type 2 diabetes while group two includes 79 healthy controls.

No statistical significant difference was found in sex distribution between the diabetic group and the control group (P=0.999). Also no statistical difference was observed in age distribution among both groups.

A significant increase in BMI, waist circumference, and weight was observed in DM patients in comparison to the healthy controls (P<0.001, P<0.001, P=0.001, respectively). Systolic and

Table 1 Comparison between the studied groups fasting insulin, homeostatic model assessment-insulin resistance, and homeostatic model assessment-B

	DM (*N*=79)				Control (*N*=79)				*P* value*
	Mean	SD	Median	25th–75th	Mean	SD	Median	25th–75th	
Fasting insulin (μU/ml)	19.00	16.0	13.80	7.30–20.90	6.00	2.00	5.40	3.70–7.20	<0.001
HOMA-IR	8.00	7.00	5.9	3.10–10.80	1.00	1.00	1.10	0.70–1.60	<0.001
HOMA-B	73.42	85.4	44.04	27.59–91.38	101.3	35.69	97.33	(75.60–114.86)	<0.001

DM, diabetes mellitus; HOMA-IR, homeostatic model assessment-insulin resistance. **P* value was done by Mann–Whitney.

Table 2 Frequency distribution of two-pore calcium channel 2 rs1551305 genotypes and alleles between the two studied groups

rs1551305	DM (*N*=79) [*n* (%)]	Control (*N*=79) [*n* (%)]	*P* value
G/G (*N*=15)	14 (17.7)	1 (1.3)	0.001
A/A (*N*=48)	19 (24.1)	29 (36.7)	
A/G (*N*=95)	46 (58.2)	49 (62.0)	
G allele (*N*=125)	74 (46.8)	51 (32.3)	0.008
A allele (*N*=191)	84 (53.2)	107 (67.7)	

DM, diabetes mellitus.

Table 3 Odds ratio of the genotypes in all participants

rs1551305	DM (*N*=79) [*n* (%)]	Control (*N*=79) [*n* (%)]	OR (95% CI)	*P* value
G/G (*N*=15)	*14* (*17.7*)	*1* (*1.3*)	21.37 (2.59–176.19)	0.004
A/G (*N*=95)	*46* (*58.2*)	*49* (*62.0*)	*1.433* (*0.708–2.899*	*0.317*
GG+AG (*N*=110)	60 (75.9)	50 (63.3)	*1.832* (*0.919–3.65)*	0.084
A/A (*N*=48)	*19* (*24.1*)	*29* (*36.7*)	*Reference*	
Allele G	*74* (*46.8*)	*51* (*32.3*)	*1.848* (*1.17–2.92)*	*0.008*
Allele A	*84* (*53.2*)	*107* (*67.7*)	*Reference*	

CI, confidence interval; DM, diabetes mellitus; OR, odds ratio.

diastolic blood pressure values were also higher in DM patients ($P<0.001$), while the controls were significantly taller ($P<0.001$).

A significant increase in the median levels of fasting insulin and HOMA-IR ($P<0.001$ for both parameters) in the diabetics than the control group. HOMA-B was found to be significantly lower in the diabetics group ($P<0.001$) (Table 1).

A significant increase in the levels of triglycerides, total cholesterol, and LDL was observed in diabetic patients ($P<0.001$) for each parameter, while HDL cholesterol showed no significant difference between the studied groups ($P=0.190$).

A significant increase in the frequency of G/G genotype in diabetic patients was found, while the A/A genotype was significantly more frequent in the control group ($P=0.001$). A significant increase in the frequency of G allele in the diabetic group ($P=0.008$) was observed in all participants (Table 2).

When taking the A/A genotype as a reference, the G/G genotype showed a 21.37-fold increase in the risk of developing DM with a confidence interval of 2.59–176.19 and a *P* value of 0.004. The G allele also showed a 1.85-fold increase in the risk of diabetes development in comparison to A allele with a confidence interval of 1.17–2.92 and a *P* value of 0.008 (Table 3).

A significant increase in body mass and weight was found in patients with the G/G genotype compared with A/A and A/G genotypes, *P* value=0.019, 0.017, respectively. No significance was found regarding waist circumference, height, systolic, and diastolic blood pressure among the three genotypes in all participants (Table 4).

A significant increase in fasting plasma glucose and HbA1c ($P=0.002$, 0.014, respectively) was observed in patients with the G/G genotype compared with A/A and A/G genotypes in all participants (Table 5).

A significant increase in HOMA-IR (*P* value=0.011) was observed in patients with the G/G genotype compared with A/A and A/G genotypes in all participants. Although fasting insulin level was higher in G/G genotype participants, this did not reach significance ($P=0.160$). HOMA-B was found to be higher in A/A and A/G genotypes compared with the G/G genotype, but it has just failed to reach significance ($P=0.088$) (Table 6).

A significant increase in total cholesterol and LDL cholesterol ($P=0.002$, 0.022), respectively, was observed in patients with the G/G genotype compared with A/A and A/G genotypes, but no significant difference was found regarding triglycerides and HDL between the three genotypes ($P=0.151$, 0.116), respectively, in all participants (Table 7).

Table 4 Comparison of the clinical data of the three genotypes among all studied population

	rs1551305						*P* value
	G/G (*N*=15)		A/A (*N*=48)		A/G (*N*=95)		
	Mean	SD	Mean	SD	Mean	SD	
BMI (kg/m^2)	32.17[a]	5.10	28.05[b]	4.39	28.25[b]	5.52	0.019
Waist circumference (cm)	109.73	9.15	103.15	8.42	105.54	10.38	0.066
Weight (kg)	87.53[a]	15.83	78.75[b]	11.04	78.41[b]	10.96	0.017
Height (cm)	164.73	8.93	168.02	8.70	167.43	9.13	0.463
Systolic blood pressure (mmHg)	124.00	12.42	123.96	17.23	125.79	17.66	0.807
Diastolic blood pressure (mmHg)	82.00	8.62	80.83	8.21	82.74	11.80	0.597

Different symbols indicate significant difference.

Table 5 Comparison of fasting plasma glucose and hemoglobin A1c of the three genotypes among all studied participants

	rs1551305						*P* value
	G/G (*N*=15)		A/A (*N*=48)		A/G (*N*=95)		
	Mean	SD	Mean	SD	Mean	SD	
Fasting glucose (mg/dl)	186.73[a]	88.21	120.29[b]	54.76	129.71[b]	63.58	0.002
HbA1c (%)	8.51[a]	2.13	6.75[b]	2.25	6.88[b]	2.03	0.014

HbA1c, hemoglobin A1c. Different symbols indicate significant difference.

Table 6 Comparison of fasting insulin, homeostatic model assessment-insulin resistance, and homeostatic model assessment-B of the three genotypes among all studied participants

	rs1551305						*P* value
	G/G (*N*=15)		A/A (*N*=48)		A/G (*N*=95)		
	Median	25th–75th	Median	25th–75th	Median	25th–75th	
Fasting insulin (μU/ml)	10.50	6.40–20.9	6.85	4.90–12.85	7.30	5.00–13.60	0.160
HOMA-IR	5.00[a]	3.10–8.9	1.60[b]	1.00–4.20	1.90[b]	1.00–6.20	**0.011**
HOMA-B	26.35	19.93–108.00	79.27	62.06–114.86	78.35	50.40–110.12	0.088

HOMA-IR, homeostatic model assessment-insulin resistance. Different symbols indicate significant difference.

Table 7 Comparison of the lipid profile parameters of the three genotypes among all studied participants

	rs1551305						*P* value
	G/G (*N*=15)		A/A (*N*=48)		A/G (*N*=95)		
	Mean	SD	Mean	SD	Mean	SD	
Triglycerides (mg/dl)	167.13	58.42	124.71	81.07	138.89	73.11	0.151
Total cholesterol (mg/dl)	225.53[a]	38.43	182.90[b]	38.98	187.84[b]	43.11	0.002
HDL cholesterol (mg/dl)	40.53	9.62	40.19	12.20	37.21	6.79	0.116
LDL cholesterol (mg/dl)	145.93[a]	41.41	117.67[b]	31.63	127.16[ab]	35.06	0.022

HDL, high-density lipoprotein; LDL, low-density lipoprotein. Different symbols indicate significant difference.

No significant difference was observed in the clinical data, fasting glucose, HbA1c, fasting insulin, HOMA-IR, HOMA-B, and the lipid profile between the three genotypes in the DM group.

No significant difference was found in the clinical data, fasting glucose, HbA1c, fasting insulin, HOMA-IR, HOMA-B, and the lipid profile between A/A and A/G genotypes in controls.

Discussion

Diabetes is an enormous, growing clinical and public health problem. In 2015, the International Diabetes Federation estimated that 415 million adults had diabetes and that, by 2040, the number will increase to 642 million. The financial burden of diabetes is huge. In 2015, the International Diabetes Federation estimated that most countries devote 5–20% of the total health-care expenditures to diabetes [12].

Genome-wide association studies of SNPs have identified a number of genetic variants that are associated with β-cell function and insulin resistance. Some of these SNPs appear to increase the risk for type 2 diabetes [13].

TPCN2 localizes to the lysosome and is a likely receptor for the calcium-mobilizing agent nicotinic acid adenine dinucleotide phosphate. Several studies

have indicated that nicotinic acid adenine dinucleotide phosphate may play a role in the insulin signaling of β-cells [14]. Its involvement in glucose homeostasis has been suggested [15].

Another study demonstrated that *TPCN2* was differentially expressed in heterogeneous stock rats with glucose intolerance relative to those with normal glucose regulation and demonstrated that *TPCN2* expression levels negatively correlated with fasting glucose. The data have pointed to *TPCN2* as a new gene contributing to glucose and insulin regulation. Variants within and near TPCN2 have been significantly associated with fasting glucose as well as TPCN2 expression levels in heterogeneous stock rats. Therefore, it is possible that variants within *TPCN2* may be associated with diabetes in humans [16].

Up to our knowledge, only one study was conducted in China to identify the association between genetic variants of *TPCN2* rs1551305 and type 2 diabetes risk in humans [17]. Our study was conducted to examine this potential association in Egyptian patients and to study *TPCN2* rs1551305 in the context of other laboratory findings of diabetic patients.

The diabetic group was compared with an age-matched and sex-matched healthy control (P=0.08, P=1, respectively).

In this study, there is a significant increase in the levels of fasting plasma glucose and HbA1c, fasting insulin levels and HOMA-IR in diabetic patients in comparison to the controls (P<0.001 for each parameter), while HOMA-B was significantly decreased (P<0.001). There was also a significant increase in levels of triglycerides, total cholesterol, and LDL observed in the diabetic group (P≤0.001).

In our study, the G/G genotype was found to be significantly increased in diabetic patients, while the A/A genotype was significantly more frequent in the control group (P=0.001). Similarly, G allele was significantly increased in the diabetic group (P=0.008). Opposite results were observed by Fan *et al.* [17] with a higher prevalence of A allele in type 2 diabetes individuals compared with those carrying the G allele in the study conducted in the Chinese population, on rs1551305 (P<0.05). Ethnic variations can serve as an explanation for this contradiction, as the genetic makeup in Chinese people who are characteristically nonobese remarkably differs from that of Western patients with type 2 diabetes [18] as well as Egyptian patients of our study and other studies [19].

When taking the A/A genotype as a reference, the G/G genotype showed a 21.37-fold increase in the risk of developing DM with a confidence interval of 2.59–176.19 (P=0.004). G allele also showed a 1.85-fold increase in the risk of diabetes development in comparison to A allele (P=0.008). Similarly, *TPCN2* knockout mice have been shown to exhibit significantly decreased insulin response to glucose challenge relative to wild-type mice [16].

A significant increase in BMI and weight was found in all studied patients with the G/G genotype compared with A/A and A/G genotypes, P value=0.019 and 0.017, respectively; however, when dividing the participants, this significance disappeared in diabetic patients, probably due to the small sample size.

Similarly, no association was observed between *TPCN2* polymorphisms and BMI in the diabetic patients in the Fan *et al.* [17] study. They suggested that the effects of the *TPCN2* gene on the development of type 2 diabetes may be independent of the effects of BMI.

In all participants a significant increase in fasting plasma glucose, HbA1c and HOMA-IR, was observed in patients with the G/G genotype compared with A/A and A/G genotypes (P=0.002, 0.014, and 0.011, respectively). HOMA-B was found to be lower in the G/G genotype compared with A/A and A/G genotypes; however, it showed no statistical significance (P=0.088). Although fasting the insulin level was higher in G/G genotype participants, it did not reach statistical significance (P=0.160).

In addition, no statistical difference could be observed in diabetics when comparing the three genotypes regarding the means of the fasting plasma glucose, HbA1c, fasting insulin, HOMA-IR, or HOMA-B.

In the study of Fan *et al.* [17], only HOMA-B of patients having the AA genotype was less than that for the GG genotype, with a statistically significant difference (P<0.05). However, fasting plasma glucose and HbA1c were not significantly different among the three genotypes in their diabetic patients, even when controlling for sex and age. They suggested that the genetic variation may affect the incidence of diabetes, but not its severity [17].In all studied patients a significant increase in total cholesterol and LDL cholesterol was observed in patients with the G/G genotype compared with the A/A genotype

(P=0.002, 0.022, respectively). No significant difference was found regarding triglycerides and HDL between the three genotypes (P=0.15 and 0.116, respectively). Endolysosomal organelles, controlled by *TPCN2*, play a key role in trafficking, breakdown, and receptor-mediated recycling of different macromolecules such as LDL cholesterol, which in turn affects the total cholesterol [20].

When comparing A/A and A/G genotypes in the control group, there was no statistically significant difference in fasting glucose, fasting insulin, HOMA-A, HOMA-B, and lipid profile parameters. Because only one case with the G/G genotype was found, statistical comparison for the different biochemical parameters could not be done.

Conclusion

(1) TPCN2 rs1551305 variants can be considered as a potential risk factor for type 2 DM development. The GG genotype is the risky genotype in Egyptian population and G allele is the risky allele.
(2) Ethnic differences account for the discrepancy in risky genotypes and alleles and different clinical pictures of DM worldwide.
(3) HOMA-IR correlates well with diabetic profile parameters and is a good indicator of insulin resistance.
(4) HOMA-B can be a good predictor of B-cell function and correlates inversely with the diabetic profile parameters.

Recommendations

(1) Larger cohort studies on different ethnic populations are recommended to emphasize these results and discover ethnic differences in different areas of the world.
(2) Study of other SNPs on TPCN2 and their relationship to the development of DM.
(3) Extensive studies on other two-pore channel genes in different types of DM.
(4) Further studies are recommended to evaluate the relationship between TPCN2 rs1551305 and diabetic complications development.
(5) Evaluation of the cost/benefit relationship of conducting genetic screening with the discovered genetic risk factors for the development of diabetes and lifestyle modulation.

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Conflicts of interest
There are no conflicts of interest.

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