Research Article

Assessment of TCF7L2 Polymorphism in Type II Diabetes in Egyptian Population

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Abstract

Though the genetic susceptibility to T2D is polygenic, with almost 50 loci identified to be associated with T2D risk, single nucleotide polymorphisms (SNP) at the transcription factor 7-like 2 gene (TCF7L2) have been strongly and consistently associated with T2D risk in various populations and ethnic groups. The exact mechanism by which TCF7L2 variations predispose to T2D is not clear. TCF7L2, which spans a 215 863 base-pair region on chromosome 10q25.3, encodes the transcription factor TCF7L2 that is involved in the Wnt signaling pathway, which seems to be critical to pancreatic islet development and adipogenesis through the dysregulation of proglucagon gene expression. Type 2 diabetes is reported to be associated with a systemic low-grade inflammation as indicated by increased levels of circulating acute-phase proteins like CRP and of IL-6. Scientific researchers believe that through exploiting the recent genomic information and better understanding of nutrient-gene interactions, better health outcomes can be achieved if nutritional requirements are customized for each individual taking into consideration the inherited genetic characteristics depending on life stage, dietary preferences and health status.

Subjects: Cases group: 25 patients diagnosed with Type II Diabetes Mellitus selected from the outpatient clinics of the National Nutrition Institute and Ain Shams University Hospitals. Controls group: 25 age and sex matched, apparently healthy patients' relatives, with no known history of Diabetes.

Methods: Full demographic and clinical data will be collected from patients' data base. Then all individuals included in this study were subjected to a peripheral whole blood on EDTA and serum samples will be obtained and the following laboratory investigations will be performed: 1-Random blood glucose. 2-Glycated Hemoglobin (HbA1C). 3- Genotyping of TCF7L2 gene polymorphism by Real time PCR (Polymerase Chain Reaction). 4-Interleukin 6 (IL6) Assay by ELISA.

Results: There was a significant positive correlation between levels of serum IL-6 and dietary fat intake in the case group; however, the same correlation was not statistically significant with caloric or carbohydrate intakes. There was no significant correlations between IL-6 and either caloric intake, carbohydrate intake, or fat intake in the control group. No significant correlation between TCF7L2 genotypes and the levels of IL-6 in the in the cases group. Correlation between levels of IL-6 and the cycle threshold (ct) of the risk (T) allele (indicating allele expression) shows no statistical significance in cases or control group.

Keywords: Type 2 Diabetes; TCF7L2; Cycle Threshold

Introduction

Type 2 diabetes (T2D) is a heterogeneous group of metabolic disorders caused by the interaction between genetic predisposition and amny environmental factors (Freeman and Cox 2006). The incidence of T2D has increased over the last decades, with more than 170 million individuals suffering from T2D all over the world, which imposes a great economic impact on individuals, families, and health systems (Barra., *et al.* 2012.).

The exact mechanism by which TCF7L2 variations predispose to T2D is not clear. TCF7L2, which spans a 215 863 base-pair region on chromosome 10q25.3, encodes the transcription factor

TCF7L2 that is involved in the Wnt signaling pathway, which seems to be critical to pancreatic islet development and the adipogenesis through the dysregulation of proglucagon gene expression, the glucagon-like peptide (GLP-1). TCF7L2 forms heterodimers with betacatenin to induce the expression of various genes, including the gene encoding the insulinotropic hormone GLP-1. the insulin gene, and genes that encode proteins involved in processing and exocytosis of insulin granule [1].

Type 2 diabetes is reported to be associated with a systemic low-grade inflammation as indicated by increased levels of circulating acute-phase proteins like CRP and of IL-6. These changes were found to be associated with patients with impaired glucose tolerance (IGT) as well, which, suggest a role of these mediators in the pathogenesis of type 2 diabetes. Antidiabetic treatment by medication, diet or physical activity results in a significant decrease of systemic immune mediator concentrations (Herder., et al. 2005).

Scientific researchers believe that through exploiting the recent genomic information and better understanding of nutrient-gene interactions, better health outcomes can be achieved if nutritional requirements are customized for each individual taking into consideration, the inherited genetic characteristics depending on life stage, dietary preferences and health status (Fenech., et al. 2011.).

So we aim to explore the relation of TCF7L2 gene polymorphism, inflammatory status, the disease control parameters, and the dietary habit of the diabetic patients type II. We also aim to explore the relation of the genetic variants and the dietary parameters in the patients' relatives and compare the results to those of the patients.

Subjects

The study was conducted on 50 individuals attending the diabetes outpatient clinics of Ain Shams University Hospitals and the National Nutrition Institute. They were classified into two groups.

- Group I included 25 patients obese patients diagnosed as Type 2 diabetes mellitus. There were 18 females and 7 males.
- Group II included 25 of the patients' apparently healthy relatives with no personal history of chronic inflammatory diseases. Twenty one controls had a BMI under 25, and four had a BMI between 30 and 42.5, but with no signs of insulin resistance. There were 14 females and 11 males. Both groups were comparable as regards age and sex.

All individuals included in this study were subjected to:

Nutritional assessment, which included:

- Full history taking focused on history of the present illness (DM), whether controlled or uncontrolled and the patient's lifestyle including diet and exercise habits.
- **Anthropometric measures:** Weight was measured in light clothing without shoes after emptying bladder. Height was measured from the top of the head to the bottom of the feet (no shoes). BMI was calculated as the weight (kg) divided by the square of the height (m^2).
- Food frequency questionnaire (FFQ) and 24 hour dietary recall. The questionnaire is the standard dietary questionnaire designed and used by the National Nutrition Institute for patient management. The questionnaire is localized to include food groups common in the Egyptian population.
- **Thorough clinical examination:** General and local examination for physical signs and symptoms of diabetes.

Laboratory assessment was performed as follows:

- Random blood glucose.
- Glycated hemoglobin (HbA1c)
- Interleukin-6 assay using enzyme-linked immunosorbent assay (ELISA) (Wegner., *et al.* 2013). 131
- Genotyping of transcription factor 7 like-2 gene polymorphism using real-time polymerase chain reaction [1].

Sampling

Six ml of whole venous blood were collected under complete aseptic conditions, while two ml were added to an ethyl diamine tetra-acetic (EDTA) vaccutainer (1.2 mg/mL) as anticoagulant and used for immediate assay of HbA1c.

Methods
Random blood glucose

Random Blood Glucose in serum was measured using Beckman Coulter UniCel DxC 600 Synchron clinical system, (Instruments Inc., Scientific Instruments Division, Fullerton, CA 92634-3100, USA) applying enzymatic colorimetric method.

Glycated Hemoglobin (HbA1c)

HbA1c was measured using Roche cobas b 101 POC system (Roche Diagnostics International Ltd, CH-6343 Rotkreuz, Switzerland).

Interleukin-6 Assay by ELISA

Assay was performed using the commercially available Human IL-6 PicoKine ELISA Kit (Product code: EK0410) supplied by Boster Biological technology (3942 B Valley Ave, Pleasanton, CA 94566, United States).

Principle of the assay

This assay was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody specific for IL-6 has been precoated onto 96-well plates. Standards and test samples were added to the wells, then an enzyme-linked monoclonal antibody specific for IL-6 was added subsequently and then followed by washing with phosphate buffer saline (PBS). A substrate solution was added and unbound conjugates were washed away with PBS buffer. The substrate was used to visualize the enzymatic reaction. A blue color develops then changes into yellow after adding the stop solution. The density of yellow was proportional to the Human IL-6 amount of sample captured in plate.

Genotyping

Genomic DNA extraction and analysis for TCF7L2 gene polymorphisms was done by real-time PCR technique.

This process was done through three steps which included:

- Extraction of genomic DNA from peripheral blood leucocytes of EDTA anticoagulant blood.
- Amplification of the extracted DNA.
- Allelic discrimination.

Extraction of Genomic DNA from Peripheral Blood Leucocytes:

Human genomic DNA was isolated from peripheral blood leukocytes using G-spin total DNA extraction kit from iNtRON Biotechnology, Inc. (Catalog no. 17045).

Amplification of the extracted DNA using polymerase chain reaction (PCR)

The extracted DNA was amplified using TaqMan Universal Master Mix II (Catalog number: 4440043) and ready-made TaqMan SNP genotyping assay for rs7903146 (Catalog number: 4351379) from Applied Biosystems.

The 40XTaqMan® SNP Genotyping Assay contains:

- Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest.

Context Sequence of the tested polymorphism [C nucleotide to be detected by JOE dye/T nucleotide to be detected by FAM dye]

TAGAGAGCTAAGCACTTTTTAGATA[C/T]TATATAATTTAATTGC-CGTATGAGG

Results

Random blood glucose (RBG) showed a highly significant statistical difference between cases and controls (p<0.001) with the mean increased from controls (91.96 ± 16.84 mg/dl) to cases (212.48 ± 86.92 mg/dl), while glycated hemoglobin (HbA1c) shows a highly significant statistical difference between cases and controls (p<0.001) with the mean increased from controls (5.34 ± 0.48%) to cases (8.83 ± 1.77%).

	Cases		Controls		t test	
	Mean	Standard Deviation	Mean	Standard Deviation	p value	sig.
HbA1c	8.83	1.77	5.34	.48	<0.001	HS
RBG	212.48	68.92	91.96	16.48	<0.001	HS

Table 1: Comparison between the two groups as regards glycemic control indicators (HbA1c and random blood glucose).

HS: Highly Significant; HbA1c: Glycated Hemoglobin (%); RBG: random blood glucose (mg/dl).

A highly significant statistical difference between cases and controls (p= 0.003) was found in interleukin-6 serum level, with the mean increased from controls (32.37 ± 24.13 pg/ml) to cases (54.28 ± 25.55 pg/ml), as shown in table.

	Cases		Controls		t test	
	Mean	Standard Deviation	Mean	Standard Deviation	p value	sig.
Interleukin 6	54.28	25.55	32.37	24.13	0.003	HS

Table 2: Comparison between the two groups as regards IL-6.
HS: Highly Significant. IL-6 in pg/ml

There was no statistically significant difference found regarding TCF7L2 gene genotypes between case and control groups (p = 0.183). 36% of all subjects enrolled in the study carried the CC homozygous genotype, while 60% of the subjects carried the heterozygous CT genotype, with only 4% carrying the homozygous TT genotype.

Count		Controls		Cases		Chi square	
		%	Count	%	p value	sig.	
Genotype/ Allele	CC	12	48%	6	24%	0.183	NS
	CT	12	48%	18	72%		
	TT	1	4%	1	4%		
	C allele	36	72%	30	60%		
	T allele	14	28%	20	40%		

Table 3: Comparison between the two groups regarding genotype of TCF7L2 gene and allele frequency.

NS: Non-Significant.

Our results showed a significant positive statistical correlation between HbA1C as a cumulative glycemic control indicator, and BMI in the general sample (p = 0.002) as well as in the cases group (p = 0.036). There was no significant correlation between HbA1c and BMI in the control group (p = 0.509), as shown in table.

Random blood glucose was significantly positively correlated with BMI in both the whole sample and the cases group (p= 0.006 and 0.023 respectively), but not in the control group (p= 0.776).

		All subjects (n=50)	Cases (n=25)	Controls (n=25)
		BMI	BMI	BMI
HbA1c	r	0.433	0.422	0.139
	p value	0.002	0.036	0.509
	Sig.	HS	S	NS
RBG	r	0.384	0.454	0.060
	p value	0.006	0.023	0.776
	Sig.	HS	S	NS

Table 4: Correlation between glycemic control indicators and anthropometric measures.

The results show a highly significant positive correlation between glycemic control indicators (HbA1c and RBG) and IL-6 (p= 0.002 and 0.005 respectively) in the whole sample.

n=50		HbA1C	RBG
Interleukin 6	R	0.437	0.387
	p value	0.002	0.005
	Sig.	HS	HS

Table 5: Correlation between glycemic control and inflammatory markers in the whole sample.

HS: Highly Significant; HbA1c: Glycated Hemoglobin; RBG: random blood glucose.

There was a significant positive correlation between BMI and levels of serum IL-6 in the case group. Meanwhile, the same correlation was not statistically significant in control group, as shown in table.

		Cases	Controls
		BMI	BMI
Interleukin 6	r	0.416	0.005
	p value	0.038	0.981
	Sig.	S	NS

Table 6: Correlation between anthropometric measures and inflammatory markers.

S: Significant; NS: Non significant; BMI: Body Mass Index.

There was a significant positive correlation between levels of serum IL-6 and dietary fat intake in the case group; however, the same correlation was not statistically significant with caloric or carbohydrate intakes. There was no significant correlations between IL-6 and either caloric intake, carbohydrate intake, or fat intake in the control group, as shown in table 7.

		Cases			Controls		
		Caloric intake	Carbo-hydrate	Fat intake	Caloric intake	Carbo-hydrates	Fat intake
IL-6	r	0.286	0.065	0.410*	0.014	0.176	-0.094
	p	0.166	0.756	0.042	0.947	0.399	0.653
	Sig.	NS	NS	S	NS	NS	NS

Table 7: Correlation between dietary habits and inflammatory markers.

T test showed no significant correlation between TCF7L2 genotypes and the levels of IL-6 in the in the cases group, as shown in table 8.

	CC (6)		CT+TT (19)		t test	
	Mean	SD	Mean	SD	p value	sig.
Interleukin-6	57.17	19.78	53.37	27.53	0.758	NS

Table 8: Comparison between different genotypes of TCF7L2 gene regarding levels of inflammatory markers in the case group.

No significant correlation was found between TCF7L2 genotypes and sex distribution in the whole sample or the cases group, as shown.

		CC		CT+TT		Chi square	
		N	%	N	%	p value	sig.
Gender	female	11	61.1%	18	56.3%	0.738	NS
	Male	7	38.9%	14	43.8%		

Table 9

NS; not significant.

		CC		CT+TT		Fisher exact test	
		N	%	N	%	p value	sig.
Gender	Female	4	66.7%	14	73.7%	1	NS
	Male	2	33.3%	5	26.3%		

Table 10

Correlation between levels of IL-6 and the cycle threshold (ct) of the risk (T) allele (indicating allele expression) shows no statistical significance in cases or control group.

		IL-6	
		Cases (19)	Controls (13)
Ct for T allele	r	0.154	0.157
	p value	0.530	0.610
	Sig	NS	NS

Table 11: Correlation between levels of interleukin-6 and expression of the T allele.

NS: Non significant; IL-6: Interleukin-6, Ct: cycle threshold.

Discussion

This case-control study aimed to further explore the TCF7L2 polymorphism interaction with the dietary habits and lifestyle of diabetic patients, and how this affects their inflammatory status, in the Egyptian population.

Fifty subjects were included in this study. Half of them were diabetic patients, while the second half included their healthy relatives. All subjects filled questionnaires regarding their eating habits. Anthropometric data (BMI) were collected, and indicators for glycemic control (RBS and HbA1c) and inflammatory status (IL6) were tested. Then all subjects were genotyped by real-time PCR to identify expression of the C and T alleles of the rs7903146 polymorphism of the TCF7L2 gene.

A highly significant difference in anthropometric measures (weight and BMI) was observed in the case group compared to the control group. A similarly significant difference was observed in the measured glycemic control indicators (random blood glucose and HbA1c).

The study showed a significant increase in IL-6 in diabetic patients compared to the control group. This was in concordance with Pradhan., *et al.* (2001), who found baseline levels of IL-6 (P<.001) and CRP (P<.001) significantly higher in T2D cases than the healthy controls. This is acceptable as T2D is known to be a chronic inflammatory disease.

The present study showed a significant positive correlation between BMI and glycemic control indicators (random blood glucose and HbA1c) in diabetic patients; meaning that the higher the BMI, the poorer the glycemic control.

Sepp., *et al.* (2014) reported that blood glucose level was positively correlated with BMI (p=0.014 and r=0.402). Similar results were found by Norberg., *et al.* (2006), who reported poorer glycemic control indicators (increased fasting glucose and HbA1c) in obese patients, with high Odds ratio (OR) of developing diabetic complications in patients with BMI ≥27.

The present study showed a significant positive correlation between BMI and interleukin-6 levels in the case group (p=0.038). This came in accordance with Khaodhiar., *et al.* (2004) who studied the correlation of inflammatory markers including tumor necrosis factor (TNF), interleukin 6 (IL-6) and C-reactive protein (CRP), with BMI in non-obese, obese, and morbidly obese individuals to explore this relationship across the broad range of obesity. The study concluded that obesity is characterized by rise in inflammatory markers levels. They owed this correlation to the expansion of fat mass particularly in the abdominal region in obese men, which secretes IL-6 in an endocrine manner with a corresponding increase in hepatic production of CRP also. In a similar explanation, Ouchi., et al. (2011) suggested that IL-6 is one of the primary adipokines, which increases with obesity, especially visceral obesity as in men.

A study by Gallist., *et al.* (2001) investigated the changes in interleukin-6 levels in obese children and adolescents during a weight reduction program. They measured indexes of obesity, IL-6, leptin and estradiol at baseline and after 3 weeks. All parameters decreased dramatically during the program (IL-6: 3.9-4.7 vs 2.0-2.2 pg/ml). Changes in IL-6 levels correlated significantly with changes in BMI (r=.0.25 and P <0.05). This concluded that an improved body composition induced by dietary regimens and regular physical activity is obviously associated with lower concentrations of IL-6 in children and adolescents.

The genotyping results of this study found different distribution of the TCF7L2 genotypes in the two groups; cases and controls. As

Cases group included 24% homozygous (CC) and 72% heterozygous (CT), while controls group included 48% homozygous (CC) and 48% heterozygous (CT) equally. Only 2 homozygous (TT) individuals were found among the participants, one in each group, this may be attributed to the small sample size.

The results were similar to the results of Palizban., *et al.* (2012), a study which included 110 patients referring to clinic and 80 healthy controls randomly selected from Persian population. It found a 40% prevalence of the CC genotype, 51% of the CT genotype, and 9% of the TT genotype among the healthy controls group, while a 29% prevalence of the CC genotype, 47% of the CT genotype, and 24% of the TT genotype among the diabetic cases group.

Since there was a limited discrepancy between cases and controls regarding TCF7L2 genotypes in our study, we attempted to enhance our statistics by a comparison between cases and controls in terms of expression of the C and T alleles through the cycle threshold (Ct) results. To avoid extreme or false statistics, we only included in this comparison the subjects having genotypes with T allele expression (CT and TT). Still no significance was found regarding Ct comparison between the two groups with a p value of 0.826, with a mean of 26.37 and 26.18 in controls and cases, respectively, although this meant a slightly higher T allele expression in cases.

The current study found no significant statistical correlation between the levels of serum IL-6 and expression of the risk (T) allele in term of Ct in diabetics or in healthy subjects (P = 0.53 and 0.15 respectively).

The limitations of this study included the small sample number, and also the relatively limited diversity of the patients attending the outpatient clinics in Ain Shams University Hospitals and the National Nutrition Institute. Such limitations can be overcome in further nutrigenetics research [2-22].

Conclusion

No correlations were found between TCF7L2 genotype and the inflammatory markers. Also, no correlations were found between the Ct and the glycaemic control indicators (HbA1c and random blood sugar).

Recommendations

Larger sample size with wider scale of cases are recommended for other studies to give more detailed and conclusive results. We, hereby, advise with a more diverse sample, which includes different classes and groups, to determine the real distribution of TCF7L2 genotypes in the Egyptian population.

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