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RESEARCH ARTICLE

A Genomic Study Of Egyptian Type 2 Diabetics Attending The Outpatient Clinic Of National Institute of Diabetes and Endocrinology.

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Manuscript Info *Abstract*

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Aims: A panel of single nucleotide polymorphisms (SNPs) in patients with type 2 diabetes mellitus (T2DM) is being evaluated compared to a non diabetic population. This study was designed to study the most common SNPs associated with type 2 diabetes to identify genetic markers in Egyptian type 2 diabetics.

Main Methods: 71 subjects were subdivided: Group I: included 49 type 2 diabetic subjects, Group II: included 22 normal subjects. The following SNPs genotypes were studied: (rs10010131, rs7754840 , rs1470579, rs13266634 and rs10923931).

Key Findings: The IGF2BP2 rs1470579 polymorphism showed the highest odds ratio (OR) for type 2 diabetes group (4.714). Odds ratio of other polymorphisms ranged from 1.131 to 4.270. The logistic regression used to assess the contribution of individual SNPs to risk of type 2 diabetes showed that the IGF2BP2 rs1470579 polymorphism showed significant risk of type 2 diabetes, The other polymorphisms are showed insignificant risk of type 2 diabetes. **Significance:** The SNPs (IGF2BP2 rs1470579) was found to be associated with an enhanced risk of future diabetes and prediction of future disease in Egyptian diabetic patients. This finding can be used in the prediction, prevention and early detection of the disease especially if the study is applied to large scale.

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Introduction

A panel of established variant single nucleotide polymorphisms (SNPs) in patient diagnosed with T2DM is being evaluated within many studies compared to a non diabetic control population **[1]**. These results will be used as a basis of comparison to analyze risk – conferring genotypes in T2DM to demonstrate T2DM risk associated factors. The frequency of normal and risk alleles is being evaluated by allelic discrimination to validate ambiguous SNPs genotypes **[2]**. The results of this study will provide a better overall understanding of the genetic epidemiology of T2DM in Egypt. Identifying genetic markers of T2DM will help to reduce disease onset and may ultimately adjust future risk factors through the early detection and healthy life style modification **[3]**. Our study made an attempt to study the most common SNPs genotypes associated with T2DM to identify genetic markers in type 2 diabetic patients attending in the outpatient clinic of National Institute of Diabetes and Endocrinology (NIDE).

Subjects and Methods

This study was conducted on a total number of 71 subjects which were subdivided as follows: Group I: included 49 type 2 diabetic subjects. Group II: included 22 normal healthy subjects (as controls), matching the same age and sex. All patients were selected from the outpatient clinics of NIDE, between September 2011 and October 2012. T2DM

was diagnosed according to the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus **[4]**. The normal control subjects were clinically free from any recognizable disease. They were not receiving any medications. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and normal control subjects.
Inclusion criteria:
-Patient's age between 35-60 years.
-Patient's body mass index is up to 30 Kg/m²

Exclusion criteria:
-Obesity (body mass index is more than 30).
-Other chronic diseases (e.g. Cardiovascular, Liver, or Renal)
Laboratory investigations:
Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers after overnight fasting. First part of collected blood was left to clot. Serum was rapidly separated by centrifugation. It was tested for: Glucose, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase, total bilirubin, albumin, Lipid profile, Creatinine, Potassium levels by using ARCHI TECT 8000 chemistry analyzer (USA, supplied by Abbott, Al kamal company Cairo, Egypt). Second part of collated blood was taken on two EDTA tubes, one for determination of HbA1c level by HPLC technique according to manuifacture's instructions and the other tube for SNPs genotypes analysis by semi- quantitative Real time PCR. Genomic DNA was extracted from peripheral blood (QIAamp DNA blood kit; QIAGEN, Hilden, Germany). All SNPs were analysed by TaqMan probe assay (Applied Biosystems, Forster city, CA) using commercially available primers and probes. The SNPs genotypes which were studied in our study are :

-rs10010131 **[5]**
-rs7754840 **[6]**
- rs1470579 **[6]**
-rs13266634 **[6]**
-rs10923931 **[7]**

Statistical analyses:
Statistical analysis was performed using the statistical package for social sciences (SPSS, USA). Data are expressed as means ± standard error. The SNP genotype was coded as the count of the copy number of the allele (0, 1, 2). The odds ratio for all the studied SNPs was estimated between patient group and control group. Logistic regression also used to assess the contribution of individual SNPs under a log – additive model (1df) to risk of type 2 diabetes studies as a categorical covariate. Statistical differences between groups were evaluated by using the student's t-test.

Results

Clinical and biochemical characteristics of the two studied groups, group (1), which included 49 patients with type 2 diabetes mellitus with male to female ratio30/19 and group (2), which included 22 normal subjects with male to female ratio 7/15 table (1&2) . The mean age of patients group was 46.3±8.1 years versus 43± 5 years in control group table (3) .There was a significant increase in serum level (FBS, AST, ALK, TG, HDL & HbA1C) in patients group compared to normal healthy controls, P value < 0.025, while there was no significant difference between the patients group and normal healthy controls group regarding (Bili, ALT, ALB, CHOL, LDL, Creat & Potassium), P value >0.025 table (4). In our case control subjects, we calculated the odds ratio to every SNPs between the two groups, we found that:
the IGF2BP2 rs1470579 polymorphism showed the highest odds ratio (OR) for type 2 diabetes group (4.714). Odds ratio of other polymorphisms ranged from 1.131 to 4.270 The logistic regression used to assess the contribution of individual SNPs to risk of type 2 diabetes showed that the IGF2BP2 rs1470579 polymorphism showed significant risk of type 2 diabetes, P value is less than 0.05 and this mean that we have evidence against H0 (H0:coefficients = 0). The other polymorphisms (WFS1 rs10010131, CDKAL1 rs7754840, SLC30A8 rs13266634, NOTCH2 rs10923931) present with P- value which is greater than 0.05 and this mean that we have little evidence againstH0, i.e. we accept H0, thus (WFS1 rs10010131, CDKAL1 rs7754840, SLC30A8 rs13266634, NOTCH2rs10923931) are showed insignificant risk of type 2 diabetes table (5). Within positive cases to SNPsIGF2BP2 rs1470579 , there are significant difference in triglycerides between two allele positive cases and one allele positive cases.

Table (1):Sex of patients group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	30	61.2	61.2	61.2
	Female	19	38.8	38.8	100.0
	Total	49	100.0	100.0	

Table (2) :Sex of control group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	7	31.8	31.8	31.8
	Female	15	68.2	68.2	100.0
	Total	22	100.0	100.0	

Table (3): Mean of age in patients and Control groups

		Patient	Control
N	Valid	47	18
	Missing	2	4
Mean		46.361702	43.00
Std. Deviation		8.1358721	5.087
Range		34	16

Table (4):Biochemical characteristic among studied groups

Variables	Patient (N=49)	Control (N=22)	P-value	*Significance.
FBS	244.286±111.3815	84.818 ± 12.117	.000	Sig
Bili	0.508 ± 0.2936	0.468 ± 0.181	.558	Insig
AST	22.51 ± 16.1001	16.667 ± 4.6404	.023	Sig
ALT	27.694 ± 24.4278	19.455 ± 8.4444	.129	Insig
ALK	101.143±31.1789	67.955 ± 18.0725	.000	Sig
ALB	3.829 ± 0.3428	3.968 ± 0.2835	.100	Insig
Chol	190.646±46.9116	195.136±31.3388	.584	Insig
TG	152.792 ±84.6871	98.136 ± 46.9907	.001	Sig
HDL	43.51 ± 11.0983	50.727 ± 10.6422	.012	Sig
LDL	118.735 ±42.8465	121.45 ±22.1157	.789	Insig
Creat	0.786 ± 0.1384	0.732 ± 0.1492	.143	Insig
HBA1C	8.941 ± 1.6053	6.241 ±1.4362	.000	Sig
POTASIUM	4.235 ± 0.3833	4.164 ± 0.292	.442	Insig

***Significant when p-value < 0.025**

Table (5): Logistic regression

Variables	Score	Df	P-value	Significance
rs10010131	2.347	1	0.126	Insig.
rs7754840	.197	1	0.657	Insig.
rs1470579	7.923	1	0.005	Sig.
rs13266634	3.107	1	0.078	Insig.
rs10923931	3.527	1	0.060	Insig.

***Significant when p-value <0.05**

Discussion

With the exception of rare monogenic disorders, most type 2 diabetes results from the interaction of genetic variation at multiple different chromosomal sites with environmental exposures experienced throughout the life span **[8]**. Genetic screening would afford the opportunity for early prevention and control strategies by altering life style and environmental factors that contribute to disease or by medication based treatment. A better understanding of the genetic architecture of T2DM is essential for evaluating the future risk of disease. Genomic-wide association studies analyzing common SNPs have cataloged several loci of human DNA sequence variation conferring risk for T2DM **[9]**. The exact genomic prevalence of T2DM risk association markers in the Egyptian patient's is not exactly known. 5 SNPs associated with T2D identified in previous studies are targeted for analysis in our study. Only one SNPs (IGF2BP2 rs1470579) of the evaluated 5 SNPs was found to be associated with an enhanced risk of future diabetes and prediction of future disease. Our results are in accordance with **Qiong et al, [10]** who investigate whether the insulin – like growth factor 2 mRNA- binding protein 2 (IGF2BP2 rs1470579) polymorphisms are associated with the development of T2DM .**Qiong et al, [10]** conducted a case control study of a total of 350 patients with T2DM and 207 healthy volunteers . Also **Yukio et al, [11]** who genotyped a total of 15 SNPs. Eight SNPs in five loci were found to be associated with T2DM, one of them was (IGF2BP2 rs1470579). **Yukio et al, [11]** conducted a case control study of a total of 1921 subjects with T2DM and 1622 normal controls. **Lyssenko et al, [5]** analyzed 16 known DNA varriants within these 16 variants the 5 variants in our study and reported that genotyping risk - associated SNPs had a minimal but statistically significant effect on the prediction of future T2D. While **Konsta et al, [12]** genotyped a total of 21 tagging SNPs spanning the (IGF2BP2 rs1470579) locus in 3,093 French Caucasian subjects. They found that the SNPs (IGF2BP2 rs1470579) were not associated with T2DM.

The results of our study clearly indicate that currently we have a limited ability to predict the risk of T2DM in general population based on genetic profiles due to the relatively small sample size, so the results is confined to our study and not generalized on wide aspects.

We recommend other studies using large – scale collaborative genome-wide association studies GWAS in Egypt to search for genetic factors controlling T2DM, so we can use their finding to construct susceptibility profiles that will help in the prediction, prevention and early detection of the disease **[13]**.

Conclusion

It can be concluded that SNPs (IGF2BP2 rs1470579), one of the evaluated 5 SNPs was found to be associated with an enhanced risk of future diabetes and prediction of future disease in diabetic Egyptian patients. This finding can be used in the prediction, prevention and early detection of the disease especially if the study applied to large scale.

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