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Research Article

A Case-Control study for Genetic Susceptibility Genes of Type 2 Diabetes with History of Obesity in Jordanian Population of Arab Descent[#]

Laith N AL-EITAN[1, 2]*, Basima A. ALMOMANI[3], Nesreen A. SAADEH[4], Hatem A. AMAN[1]

[1]Department of Applied Biological Sciences, [2]Department of Biotechnology and Genetic Engineering [3]Department of Clinical Pharmacy, [4]Department of Internal Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan

* Corresponding Author : lneitan@just.edu.jo

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Abstract: The aim of this study is to detect and characterize genetic polymorphisms that may influence susceptibility to type 2 diabetes (T2D) patients with history of obesity in Jordanian population. We designed a case-control study involving 600 Jordanian individuals (300 type 2 diabetes cases with history of obesity and 300 healthy individuals with no history of diabetes and obesity). Single Nucleotide Polymorphisms (SNPs) within candidate genes (*ENPP1* (rs7754561), *FTO* (rs9939609), *MC4R* (rs1211913564, rs17782313), and *NPC1* (rs1805081, rs1788799)) were genotyped using a Sequenom MassARRAY system (iPLEX GOLD). The only SNP associated with T2D with history of obesity in this study was rs7754561 in the A/G 3'UTR region of *ENPP1* gene, with overall estimate of *p-value* = 0.025 ($\chi 2$ (2, N = 600) = 9.33). It has been reported that ENPP1, Ectonucleotide Pyrophosphatase/Phosphodiesterase 1, plays a major part in facilitating insulin resistance and in the progression of both diabetes and obesity, signifying that a fundamental molecular genetic mechanism is common to both diseases in Jordanian population.

1. INTRODUCTION

Obesity is considered a major risk factor for a number of chronic diseases, including metabolic disorders (e.g. Type 2 diabetes (T2D)) [1, 2]. It has been reported that Jordan ranked among the worst countries of the world in terms of T2D and obesity, with 33% and 17% of its population being obese and diabetic respectively [1]. To date, the genes responsible for the obese phenotype and type 2 diabetes susceptibility in Arabs are not known. Obesity is a principle factor that contributes to Type 2 Diabetes. There are many important genes (e.g. *ENPP1*, *FTO*, *MC4R*, and *NPC1*), which play a major role in increased the risk of developing T2D [3, 4]. One of the most important of these genes is Ectoenzyme Nucleotide Pyrophosphate Phosphodiesterase 1 (*ENPP1*), which located on chromosome 6 (6q23.2). This gene encodes one of the important proteins which play an essential role in determining insulin sensitivity[5]. A genetic polymorphism in exon 4 of *ENPP1* gene has been described and widely investigated in T2D. Different genetic studies have also been suggested that this genetic variation within *ENPP1* gene associated with insulin resistance in different ethnicities [5, 6]. Consequently, the *ENPP1* gene is considered as a strong candidate gene for insulin resistance and T2D susceptibility [6]. Therefore, this study aimed to detect and characterize genetic polymorphisms in different candidate genes (*ENPP1*, *FTO*, *MC4R*, and *NPC1*) that may influence susceptibility to T2D with history of obesity in Jordanian population of Arab descent.

2. MATERIALS AND METHODS

2.1. Subjects

Patients' samples were collected from King Abdullah University Hospital at Jordan University of Science and Technology. A total of 300 Arab patients diagnosed with T2D with age range 40 to 60 years. In addition, 300 healthy Jordanian individuals were used as control. This study was approved by the

Institutional Review Board of the Jordan University of Science and Technology (RA/23/69/2013). Written informed consent was obtained from all subjects included in the study.

2.2. DNA Genotyping

Genomic DNA was extracted within one week of blood collection via commercially available Wizard® Genomic DNA Purification Kit (Promega Corporation, USA) according to manufacturer's instructions. DNA yield was measured using NanoDrop ND-1000 spectrophotometer. All individuals were genotyped for 6 SNPs within *ENPP1*, *FTO*, *MC4R*, and *NPC1* genes (Table 1) using the Sequenom MassARRAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA). A scatter plot of rs7754561 SNP colored according to genotype calls: GG (blue), GA (yellow) and AA (green) and no call (red) is shown in Figure 1.

Table 1. *List of genes, their SNPs and positions*

Gene	SNP_ID	Chr Position	SNP	Location
ENPP1	rs7754561	6:132212694	A/G	3'UTR
FTO	rs9939609	16:53820527	T>A	Intron 1
MC4R	rs121913564	18:60371403	G/T	Intron-Variant
	rs17782313	18:60183864	C/T	Intron-Variant
NPC1	rs1805081	18:21140432	A>G	Exon 5
	rs1788799	18:23544981	C>G	Exon 12

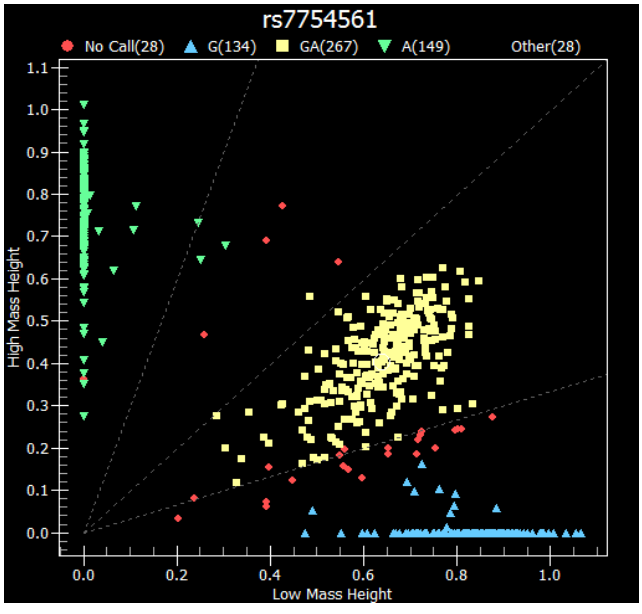

Figure 1. *Sequenom Scatter plot of rs7754561 SNP*

2.3. Statistical Analysis

Hardy-Weinberg Equilibrium (HWE) was used to determine if the population was fulfilling the HWE at each variant locus. A Pearson's Chi squared test was used for genetic association between cases and controls. A p *value* < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS statistical package 19.0 (SPSS Corp., Chicago, Illinois, USA).

3. RESULTS AND DISCUSSION

The present results were based on 300 T2D patients in treatment, all who were Arab descent. The median age of the patients 45 (range: 40 to 60). In addition, a control group of 300 subjects (median: 43, range: 35 to 65) of an ethnically homogenous Jordanian Arab population. DNA Genotyping of the 6 SNPs was highly accurate with average success rate of 98.3%. Genotype discrepancy average rates across the 6 loci were only 0.03% (±0.07%) in the Jordanian Arab samples. No significant deviation from Hardy-Weinberg equilibrium for the polymorphic SNPs was observed in Arab controls and diabetic patients (Table 2). Cases and controls were compared for genotype and allele frequencies across the 6 markers (Table 3). In the Arab population, genotype frequencies at only rs7754561 in the A/G 3'UTR differed significantly between cases and controls with p-*value* = 0.025 ($\chi 2$ (2, N = 600) = 9.33). Allele frequency comparisons between cases and controls were not significant for all tested SNPs (Table 3).

4. CONCLUSION

In conclusion, inconsistent data have been published on the influence of *ENPP1*, *FTO*, *MC4R*, and *NPC1* genes on the increased risk of diabetes [3, 4, 5, 6] but these functional SNPs have not been examined and characterized in Jordanian population of Arab descent in detail. This is the first report of a genetic association between ENPP1 rs7754561 SNP (A/G 3'UTR) and T2D in an Arab population. These findings suggest that the ENPP1 rs775461 may play role in development and progression of T2D in Middle Eastern populations. Currently, these samples are being examined for a possible pharmacogenetic association of these SNPs with glycaemic control and treatment outcomes (Pharmacogenetic/omics). This may allowed to more precisely matching of diabetic patients at genetic personal level (Personalized Medicine) to several choices of treatment and early diagnosis of individuals with and at high risk of T2D. However, our findings require replication in both Arab and other ethnic groups to confirm the results of this study and identify patients at increased risk of diabetes.

Table 2. *List of SNPs, their minor allele frequencies, and HWE p-values for genotypic distribution at each locus based on the cases (300) and controls (300).*

	Cases (n=300)					Controls (n=300)			
Gene	**SNP_ID**	**MA***	**MAF****	***X²***	**HWE*** *p-value***	**MA***	**MAF****	***X²***	**HWE*** *p-value***
ENPP1	rs7754561	G	0.49	3.08	0.08	G	0.48	4.15	0.04
FTO	rs9939609	A	0.48	0.06	0.80	A	0.47	0.04	0.82
MC4R	rs17782313	C	0.29	0.01	0.95	C	0.31	1.65	0.20
NPC1	rs1788799	C	0.35	1.03	0.31	C	0.40	0.59	0.44
	rs1805081	G	0.19	2.86	0.09	G	0.20	0.07	0.79

*MA: Minor allele.

**MAF: Minor allele frequency.

***HWE: Hardy-Weinberg equilibrium.

Table 3. *Allele and genotype distributions of polymorphisms in the ENPP1, FTO, MC4R, NPC1 genes in Jordanian T2D patients and controls.*

Gene	**Markers**	**Allele*/ Genotype***	**Cases**	**Controls**	**Cases vs Controls**	
					Pearson Chi-squared	***p value***
ENPP1	rs7754561	A	280	300	0.830	**0.046**
		G	270	282		
		AA	64	86	9.327	**0.025**
		GA	152	128		
		GG	59	77		
		GC	2	-		
FTO	rs9939609	A	270	270	0.548	0.459
		T	282	308		
		AA	65	64	0.658	0.719
		TA	140	142		
		TT	71	83		
MC4R	rs121913564	T	588	588	n/a**	n/a
		TT	294	294		
	rs17782313	C	175	179	0.047	0.828
		T	409	407		
		CC	26	32	0.919	0.631
		CT	123	115		
		TT	143	146		
NPC1	rs1805081	A	484	468	0.578	0.447
		G	111	120		
		AA	196	187	1.313	0.518
		AG	81	94		
		GG	15	13		
	rs1788799	C	206	233	2.795	0.094
		G	384	355		
		CC	32	43	2.983	0.225
		GC	142	147		
		GG	121	104		

*Allelic and genotypic association *p value*, calculated using Pearson Chi-squared test for a 2 x 3 Contingency Table with 1 df for allelic, 2 df for genotypic; *p value*< 0.05 is significant, in bold.

**n/a: not applicable.

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