The Egyptian Journal of Medical Human Genetics 18 (2017) 341–348

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

# Open-array analysis of genetic variants in Egyptian patients with type 2 diabetes and obesity

Hanaa R.M. Attia [a,*], Solaf A. Kamel [a], Mona H. Ibrahim [a], Heba A. Farouk [a], Amany H.A. Rahman [a], Ghada H. Sayed [b], Nevine I. Musa [c]

[a] National Research Centre, El-Buhouth Street, Dokki, Egypt
[b] National Institute of Diabetes and Endocrinology, Egypt
[c] Ein Shams University, Egypt

ARTICLE INFO

*Article history:*
Received 27 February 2017
Accepted 22 March 2017
Available online 8 May 2017

*Keywords:*
Type 2 diabetes
Obesity
OpenArray®
Real-Time PCR System
Genotyping
Risk gene variants

ABSTRACT

*Background:* Diabetes mellitus is considered a major public health problem worldwide. Susceptibility to diabetes is influenced by both genetic and environmental determinants.
*Aims/hypothesis: The aim of the present study* was to test for 16 independent single nucleotide polymorphisms (SNPs) in established Type 2 diabetes (T2D) and obesity susceptibility loci by GWAS in a sample of Egyptian patients to find out if there is shared genetic background underlying both disease entities.
*Methods:* Genotyping was performed using OpenArray® protocol on the QuantStudio™ 12K Flex Real-Time PCR System. In the present case control study a custom array was designed to facilitate cost-effective analysis of selected SNPs related to glycolysis, gluconeogenesis, inflammation, insulin signalling, and immune function.
*Results:* Seven gene variants showed significant association with the risk of T2D patients including *FCGRA2*, *STAT4*, *CELSR2*, *PPARG*, *EXT2* rs3740878, *GCKR*, *PTGS1*. Factors that significantly affect T2D were obesity ($p < 0.001$) and *GCKR* ($p = 0.001$) and *PTGS1* ($p = 0.001$) gene variants. Gene variants that showed significant or borderline effect on obesity were *MTHFD1*, *EXT2* rs3740878, *GCKR and PTGS1* ($p = 0.03$, 0.017, 0.059, 0.006) respectively.
*Conclusions/interpretation:* Overlapping genetic aspects should be considered and the presence of risk alleles of different genes together could contribute to the risk of T2D or obesity or both. The *MTHFD1* and *EXT2*rs3740878 gene variants significantly affect obesity and not shared with T2D. Gene variants that showed combined effect on both disease entities were *GCKR* and *PTGS1*. These findings provide a basis for future studies on a larger scale. More stress on the risk gene variants that have a combined impact on both diabetes and obesity is recommended to improve risk prediction and preventive strategies.


## 1. Introduction

Diabetes mellitus is considered a major public health problem worldwide. In 2015 the International Diabetes Federation indicated that 415 million adults live with diabetes mellitus in the world. In Egypt over 7.8 million adult cases were reported with 14.9% prevalence [1]. Susceptibility to diabetes is influenced by both genetic and environmental determinants. The major environmental risk factors for Type 2 diabetes (T2D) are obesity and a sedentary lifestyle. Waist-to-hip circumference ratio (WHR), corresponding to fat distribution also has an impact on T2D risk [2,3]. Additionally, chronic inflammation or infections might provoke insulin resistance and thereby contribute to the development of diabetes and its complications [4].

Advances in genotyping technology during the last years have facilitated rapid progress in large-scale genetic studies The large scale genome wide association studies (GWAS) have identified approximately 80 single nucleotide polymorphisms (SNPs) conferring susceptibility to type 2 diabetes (T2D) [5].

The OpenArray®, a new platform technology on the QuantStudio™ 12K Flex Real-Time PCR System accelerates genomic confirmation and screening, enabling unprecedented gene coverage and sample throughput [6]. In the present study a custom array was designed to facilitate cost-effective analysis of independent single nucleotide polymorphisms (SNPs) related to glycolysis, gluconeogenesis, inflammation, insulin signalling, and immune func-

Peer review under responsibility of Ain Shams University.
* Corresponding author.
*E-mail address:* hanarasmy2000@yahoo.com (H.R.M. Attia).

http://dx.doi.org/10.1016/j.ejmhg.2017.03.002
1110-8630/

tion. All of which could provide a rationale for preventive or therapeutic strategies. The common variant associated with T2D, *PPARG*, is a well-established target of thiazolidinedione (TZD) drugs, and used clinically to reduce insulin resistance in T2D patients [7].

Sixteen SNPs at established T2D and obesity susceptibility loci by GWAS were tested using new OpenArray® genotyping protocol among Egyptian patients. Association between genetic variants and the risk of T2D and obesity was analysed to find out if there is shared genetic background underlying both disease entities.

## 2. Subjects and methods

A case - control study of 74 Egyptian participants; 37 patients with type 2 diabetes mellitus (T2D) (mean age: 48.6 ± 9.7 years; M/F: 16/21) and 37 non-diabetics age and sex matched (mean age: 45.4 ± 10.2 years; M/F: 15/22) were investigated. Sample size has been adjusted to the used Open Array platform. Diabetic patients were recruited from Ein-Shams University and National Institute of Diabetes and Endocrinology in 2014–2015. All laboratory investigations and genetic studies were performed at the Medical Molecular Laboratory Research Unit at National Research Centre, Clinical and Chemical Pathology department. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans and was approved by the ethical committee of the National Research Centre. All patients and controls provided informed consent for their participation in the study. Patients were subjected to medical history with special emphasis on demographic and related clinical data, age of onset of T2D, family history, history of drug intake and type of treatment for diabetes. Patients with T2D were diagnosed according to American Diabetes Association guidelines [8].

Body weight (WT), height (HT), waist circumference (WC), and hip circumference (HC) were measured using standard methods, and body mass index (BMI) was calculated as weight/height$^2$ (kg/m$^2$) for all participants. All participants were then classified according to BMI into two groups; diabetic obese patients (N = 26) with BMI $\geq$ 30 and non-diabetic non-obese control group (N = 36).

### 2.1. Patient selection

*Exclusion criteria:*

Patients who receive treatment for inflammatory or chronic infectious disease or malignancy were excluded from the study.

### 2.2. Blood Chemical analyses

Venous blood samples were obtained from all participants for measuring fasting plasma glucose (FPG), fasting insulin (F Ins), and glycosylated haemoglobin (HbA1c) using standard biochemical analysis. The homeostasis model assessment for beta cell function (HOMA-B) and the homeostasis model assessment for insulin resistance (HOMA-IR) were used to assess insulin resistance. The formulas were as follows:

HOMA-B = Fasting serum insulin $\times$ 20/(FPG $-$ 3.5) (with serum insulin in mU/l and plasma glucose in mmol/l).

HOMA-IR = fasting serum insulin $\times$ FPG/22.5.

### 2.3. Genetic analyses

All participants were genotyped for: Methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*) (rs2236225); Fc fragment of IgG, low affinity 2A (*FCGR2A*) (rs1801274); signal transducer and activator of transcription 4 (*STAT4*) (rs7574865); complement factor B (*CFB*) (rs547154); Cadherin, EGF LAG Seven-Pass G-Type Receptor 2 (*CELSR2*) (rs599839); fat mass and obesity-associated gene (*FTO*) (rs17817449); rs10811661 upstream of cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*); hemochromatosis gene (*HFE*) (rs1800562); peroxisome proliferator-activated receptor-gamma (*PPARG*) (rs1801282); neuropeptide Y (*NPY*) (rs16147); exostosin-2 (*EXT2*) (rs11037909, rs3740878); glucokinase (hexokinase 4) regulator (*GCKR*) (rs780094); C-reactive protein (*CRP*) (rs2808630); prostaglandin-endoperoxide synthase 1 (*PTGS1*) (rs5788); the intergenic variant of chromosome 11p12 (rs9300039).

Custom array with TaqMan assays were designed by the Bioinformatics Group at Life Technologies to facilitate cost-effective genotyping using QuantStudio™ 12K Flex OpenArray® genotyping protocol (Custom TaqMan® SNP Genotyping kit including plates and accessories). Selected format of OpenArray™ Plates allowed the performance of the required 16 assays for all the samples.

#### 2.3.1. Preparing the DNA samples

Five milliliters of whole blood were collected from each subject into tubes containing ethylene-diamine-tetra-acetic acid (EDTA). Genomic DNA (gDNA) was purified using the QIAcube automated instrument according to the manufacturer's guidelines (Qiagen, Germany). DNA quality and quantity were adjusted at A260/280 ratio between 1.7 and 1.9 by NanoDrop ND-1000 (PeqLab) and normalized to the recommended working concentration at ~50 ng/μL.

The gDNA samples were stored at −80 °C until further use. The TaqMan PCR reaction was performed using TaqMan® OpenArray™ Master Mix (Life Technologies) according to the manufacturer's recommendation.

#### 2.3.2. Setting up the 96 well plates [9]

The concentration of the thawed normalized genomic DNA samples was reviewed. 5.0 microliter (μL) of the DNA samples was put into the appropriate number of wells of a 96-well MicroAmp Optical Reaction Plate according to the selected format of OpenArray™ Plates. Then Tracking the samples from the 96 well plate to the TaqMan® OpenArray™ 384-Well sample plate was performed using sample tracker software provided by the equipment. 2.5 μL of master mix and 2.5 μl of normalized DNA samples were added to the 384-well sample plate. Then the QuantStudio OpenArray AccuFill Software was initialized for loading onto the OpenArray™ plates. Then the plate was immediately sealed and immersion fluid was injected. At this step the sealed OpenArray plate is ready to be loaded into the QuantStudio 12K Flex System and thermal cycling was performed according to the manufacturer's recommendation. Analysis of the genotyping results was performed using provided TaqMan Genotyper software.

### 2.4. Statistical methods

All test data were converted and manipulated by using SPSS software program version 18.0. Data were analysed, mean and standard deviation were calculated as regards quantitative demographic, anthropometric and biochemical data. The quantitative data were compared and *t* test was applied and p value was established to determine the statistically significant difference between two groups. While numbers and percent were calculated and presented among groups as regards qualitative data. To determine the relationship strength between variables statistical correlation study was performed and coefficient of correlation (r) was measured. Allelic discrimination was analysed using Hardy–Weinberg equilibrium (HWE) analysis tool supplied with the Taqman Genotyper software on the QuantStudio™ 12K Flex Real-time PCR system. Chi-square test was used for testing the association of gene variants with disease entities and for estimating the odds ratios