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


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Abstract

Background: Diabetes mellitus is considered a major public health problem worldwide. Susceptibility to diabetes is influenced by both genetic and environmental determinants.

Aim/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/C210 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 FCGR2A, 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 EXT2rs3740878 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-
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/height² (kg/m²) for all participants. All participants were then classified according to BMI into two groups; diabetic obese patients (N = 26) with BMI ≥ 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 glycylated 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 × 20/(FPG – 3.5) (with serum insulin in mU/l and plasma glucose in mmol/L).

HOMA-IR = Fasting serum insulin × 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); neuropetide 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 Micro-Amp 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 QuantaStudio 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 QuantaStudio 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.
(ORs) and 95% confidence intervals (CI). Multiple linear regression was applied to determine the linear relationship between a dependent variable and a series of independent variables. Multiple logistic regression was used when studying the effect of one nominal and two or more measurement variables. The difference between the two groups was considered statistically significant when \( p < 0.05 \), and considered highly statistically significant when \( p < 0.01 \).

3. Results

The demographics, anthropometric and biochemical findings of the participants are listed in Table 1. Diabetes duration ranged from 0.2 to 22 years. Of diabetic patients 86.5% have positive family history. Of female subjects 44.2% and of male subjects 25.8% were obese with BMI \( \geq 30 \). Of diabetic patients 70.3% were on insulin therapy and 29.7% were on hypoglycemic drugs. Anthropometric indices [WT, WC, HC, and waist to hip ratio (WHR)] were significantly increased in diabetic patients when compared to non-diabetic participants with \( p < 0.001 \). Mean levels of FPG, HbA1c were significantly higher in diabetic patients in comparison to non-diabetic participants with \( p < 0.001 \). Mean levels of FPG, HbA1c were significantly higher in diabetic patients in comparison to non-diabetic participants with \( p < 0.001 \). BMI showed highly significant positive correlation with WHR, WC, HC, WHR showed highly significant positive correlation with BMI, FPG, HbA1c and HOMA-IR (\( r = 0.375 \) and \( p = 0.001 \), \( r = 0.430 \) and \( p = 0.001 \), \( r = 0.234 \) and \( p = 0.004 \) respectively). Also, BMI showed highly significant positive correlation with FPG (\( r = 0.493 \), HbA1c (\( r = 0.606 \) with \( p < 0.001 \). Highly significant negative correlation was detected between BMI and HOMA-B (\( r = 0.430 \) and \( p < 0.001 \), \( r = 0.234 \) and \( p = 0.004 \) respectively). These findings clarified that the gene variants that significantly affect obesity and not shared with T2D were the \( MTHFD1 \) and \( EXT2 \) gene variants. \( GCKR \) and \( PTGS1 \) gene variants showed combined effect on both disease entities.

4. Discussion

In the present study we used a new platform technology on the QuantStudio™ 12K Flex Real-Time PCR System. Custom array was designed to perform genotyping of all selected independent genetic variants in Egyptian patients with T2D with and without obesity. Both T2D and obesity are associated frequently and considered as key components of metabolic syndrome [10]. In this

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diabetic patients (n = 37) Mean ± SD</th>
<th>Control subjects (n = 37) Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>48.7 ± 9.8</td>
<td>45.5 ± 10.3</td>
<td>0.176</td>
</tr>
<tr>
<td>Sex no (%)</td>
<td>Female 21 (56.8)</td>
<td>22 (59.5)</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td>Male 16 (43.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>84.9 ± 10.7</td>
<td>72.8 ± 9.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height</td>
<td>163.1 ± 9.6</td>
<td>168.5 ± 9.3</td>
<td>0.017</td>
</tr>
<tr>
<td>BMI</td>
<td>32.5 ± 4.4</td>
<td>24.9 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC</td>
<td>106.9 ± 11.7</td>
<td>78.7 ± 9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HC</td>
<td>114.0 ± 10.2</td>
<td>88.8 ± 9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.038 ± 0.05</td>
<td>0.086 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG</td>
<td>199.1 ± 66.2 (11.06 ± 3.67)</td>
<td>87.5 ± 12.6 (4.86 ± 0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hba1C</td>
<td>8.7 ± 2.1 (72.6 ± 22.9)</td>
<td>5.1 ± 0.7 (31.1 ± 6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F Ins</td>
<td>11.3 ± 14.9</td>
<td>16.5 ± 9.6</td>
<td>0.087</td>
</tr>
<tr>
<td>HOMA B</td>
<td>16.3 (49.9–59.6)</td>
<td>223.0 (112.3–500.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>5.3 ± 3.6</td>
<td>3.5 ± 2.1</td>
<td>0.182</td>
</tr>
</tbody>
</table>


* Statistical significant difference \( p < 0.05 \).

** Statistical highly significant difference \( p < 0.01 \).
Many previous studies have shown that obesity is associated with increased risk of diabetes [11–13]. In the current work correlation studies revealed significant positive correlation between BMI and central fat accumulation (WHR), and FPG and HbA1c blood levels. Inverse association was detected between BMI and beta cell function (HOMA-B). Also logistic regression analysis showed inverse association between BMI and blood levels. Inverse association was detected between BMI and risk of T2D. These findings suggested that management of BMI could significantly improve beta cell function and reduce the risk of T2D.

Of all tested genetic risk variants seven SNPs showed significant association with the risk of T2D including FTO, PPARG, EXT2 rs3740878, GCKR and PTGS1.

To the best of our knowledge, the FCGRA2 association with T2D has not been previously investigated. FCGRA2 is a member of a family of immunoglobulin Fc receptor genes found on the surface of many immune-response cells. In the current study FCGRA2 risk allele showed significant association with risk of T2D. This finding raises the possibility of inflammatory pathogenic mechanism as a risk factor for T2D.

**Table 2** Genotype frequencies among all the studied groups.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker SNP</th>
<th>Chromosomal Region</th>
<th>Risk Alleles (Bold)</th>
<th>Genotype</th>
<th>Diabetic patients N = 37 n (%)</th>
<th>ControlN = 37 n (%)</th>
<th>X²</th>
<th>P value</th>
<th>Diabetic – obese patients N = 26 n (%)</th>
<th>Non-obese non-diabetic control N = 36 n (%)</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFD1</td>
<td>Rs2236225</td>
<td>14:64442127</td>
<td>G/A</td>
<td>AA</td>
<td>31 (83.8)</td>
<td>34 (91.9)</td>
<td>1.138</td>
<td>0.286</td>
<td>20 (76.9)</td>
<td>34 (94.4)</td>
<td>4.124</td>
<td>0.042</td>
</tr>
<tr>
<td>FCGRA2</td>
<td>Rs1801274</td>
<td>1:161509955</td>
<td>G/A</td>
<td>AA</td>
<td>16 (42.9)</td>
<td>20 (55.3)</td>
<td>0.451</td>
<td>0.709</td>
<td>10 (28.6)</td>
<td>12 (33.3)</td>
<td>10.371</td>
<td>0.006</td>
</tr>
<tr>
<td>STAT4</td>
<td>Rs7574865</td>
<td>2:191099907</td>
<td>T/G</td>
<td>TT</td>
<td>26 (70.3)</td>
<td>19 (51.4)</td>
<td>0.454</td>
<td>0.066</td>
<td>20 (76.9)</td>
<td>18 (50.0)</td>
<td>6.666</td>
<td>0.036</td>
</tr>
<tr>
<td>CFB</td>
<td>Rs547154</td>
<td>6:31943161</td>
<td>T/G</td>
<td>TT</td>
<td>14 (37.8)</td>
<td>31 (81.5)</td>
<td>1.791</td>
<td>0.408</td>
<td>10 (38.5)</td>
<td>12 (33.3)</td>
<td>1.816</td>
<td>0.403</td>
</tr>
<tr>
<td>CELSR2</td>
<td>Rs599839</td>
<td>1:109279544</td>
<td>A/G</td>
<td>AA</td>
<td>5 (13.5)</td>
<td>11 (29.7)</td>
<td>6.815</td>
<td>0.033</td>
<td>10 (38.5)</td>
<td>13 (50.0)</td>
<td>10.502</td>
<td>0.005</td>
</tr>
<tr>
<td>FTO</td>
<td>Rs17817449</td>
<td>16:5379455</td>
<td>T/G</td>
<td>TT</td>
<td>17 (45.9)</td>
<td>26 (70.3)</td>
<td>3.4</td>
<td>0.183</td>
<td>10 (38.5)</td>
<td>23 (63.9)</td>
<td>6.693</td>
<td>0.058</td>
</tr>
<tr>
<td>CDKN2A/B</td>
<td>Rs10811661</td>
<td>9:22134095</td>
<td>T/C</td>
<td>TT</td>
<td>17 (45.9)</td>
<td>26 (70.3)</td>
<td>0.913</td>
<td>0.339</td>
<td>10 (38.5)</td>
<td>13 (38.1)</td>
<td>0.003</td>
<td>0.862</td>
</tr>
<tr>
<td>HFE</td>
<td>Rs1800562</td>
<td>6:26092913</td>
<td>A/G</td>
<td>AA</td>
<td>21 (56.8)</td>
<td>13 (35.1)</td>
<td>1.454</td>
<td>0.144</td>
<td>10 (38.5)</td>
<td>12 (33.3)</td>
<td>0.943</td>
<td>0.333</td>
</tr>
<tr>
<td>PPARG</td>
<td>Rs1801282</td>
<td>3:12351626</td>
<td>G/C</td>
<td>CC</td>
<td>15 (41.0)</td>
<td>18 (48.6)</td>
<td>12.111</td>
<td>0.002</td>
<td>10 (38.5)</td>
<td>13 (38.1)</td>
<td>9.243</td>
<td>0.011</td>
</tr>
<tr>
<td>NPY</td>
<td>Rs16147</td>
<td>7:24283791</td>
<td>C/T</td>
<td>CC</td>
<td>24 (64.9)</td>
<td>23 (62.2)</td>
<td>0.06</td>
<td>0.806</td>
<td>10 (38.5)</td>
<td>12 (33.3)</td>
<td>0.177</td>
<td>0.677</td>
</tr>
<tr>
<td>EXT2</td>
<td>Rs11037909</td>
<td>1:44234064</td>
<td>T/C</td>
<td>CT</td>
<td>16 (43.2)</td>
<td>14 (37.8)</td>
<td>1.162</td>
<td>0.559</td>
<td>10 (38.5)</td>
<td>17 (47.2)</td>
<td>1.234</td>
<td>0.54</td>
</tr>
<tr>
<td>EXT2</td>
<td>Rs3740878</td>
<td>1:44236252</td>
<td>T/C</td>
<td>CT</td>
<td>4 (10.8)</td>
<td>12 (32.4)</td>
<td>9.165</td>
<td>0.011</td>
<td>8 (30.8)</td>
<td>15 (54.5)</td>
<td>9.755</td>
<td>0.008</td>
</tr>
<tr>
<td>GCKR</td>
<td>Rs780094</td>
<td>2:27518370</td>
<td>T/C</td>
<td>TT</td>
<td>11 (29.7)</td>
<td>14 (37.8)</td>
<td>14.457</td>
<td>0.001</td>
<td>6 (21.3)</td>
<td>14 (38.9)</td>
<td>0.113</td>
<td>0.737</td>
</tr>
<tr>
<td>CRP</td>
<td>Rs2808630</td>
<td>1:159711078</td>
<td>T/C</td>
<td>CC</td>
<td>12 (32.4)</td>
<td>22 (59.5)</td>
<td>2.3</td>
<td>0.317</td>
<td>15 (57.7)</td>
<td>22 (61.1)</td>
<td>1.024</td>
<td>0.599</td>
</tr>
<tr>
<td>PTGS1</td>
<td>Rs5788</td>
<td>9:12238153</td>
<td>A/C</td>
<td>AC</td>
<td>17 (45.9)</td>
<td>10 (27.0)</td>
<td>11.937</td>
<td>0.003</td>
<td>12 (46.2)</td>
<td>19 (52.8)</td>
<td>7.854</td>
<td>0.02</td>
</tr>
<tr>
<td>Intergenic</td>
<td>Rs9300039</td>
<td>1:41893816</td>
<td>C/T</td>
<td>CT</td>
<td>15 (40.5)</td>
<td>19 (51.4)</td>
<td>1.982</td>
<td>0.371</td>
<td>10 (38.5)</td>
<td>13 (36.1)</td>
<td>1.358</td>
<td>0.507</td>
</tr>
</tbody>
</table>

* Statistical significant difference p < 0.05.
* Statistical highly significant difference p < 0.01.
* BMI ≥ 30.
that rs7574865 polymorphism was significantly associated with diabetes risk, especially diabetes type 1. However, they did not give consistent results for T2D [15]. They postulated the mechanisms by which chronic inflammation or infections could provoke insulin resistance and thereby contribute to the development of diabetes and its complications. Macrophages release cytokines that cause cells of liver and muscle to be insulin resistant. Another mechanism that is more consistent with the current study is to affect

### Table 3
Risk allele frequencies among all the studied groups.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker SNP</th>
<th>Risk Alleles (Bold)</th>
<th>Diabetic patients N = 37 %</th>
<th>Control N = 37 %</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>Diabetic – obese patients N = 26 %</th>
<th>Non-obese non-diabetic control N = 36 %</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFD1</td>
<td>Rs2236225</td>
<td>G</td>
<td>8.1</td>
<td>4.1</td>
<td>2.1 (0.5–8.9)</td>
<td>0.302</td>
<td>11.5</td>
<td>2.8</td>
<td>4.6 (0.9–23.6)</td>
<td>0.05*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>91.9</td>
<td>95.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2A</td>
<td>Rs1801274</td>
<td>G</td>
<td>18.9</td>
<td>6.8</td>
<td>3.2 (1.1–9.5)</td>
<td>0.027*</td>
<td>17.3</td>
<td>6.9</td>
<td>2.8 (0.9–8.9)</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>81.1</td>
<td>93.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT4</td>
<td>Rs7574865</td>
<td>T</td>
<td>77.0</td>
<td>55.4</td>
<td>2.7 (1.3–5.5)</td>
<td>0.005*</td>
<td>82.7</td>
<td>54.2</td>
<td>4.0 (1.7–9.5)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>23.0</td>
<td>44.6</td>
<td></td>
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<td>&lt;0.001*</td>
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</tbody>
</table>

*a Statistical significant difference p < 0.05.
*b Statistical highly significant difference p < 0.01.

### Table 4
Logistic Regression determining factors affecting diabetes mellitus.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>S.E</th>
<th>Wald</th>
<th>df</th>
<th>Sig</th>
<th>Exp(B)</th>
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<td>.881</td>
<td>34.613</td>
<td>1</td>
<td>&lt;0.001*</td>
<td>178.172</td>
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<tr>
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<td>.859</td>
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<td>.063</td>
<td>4.951</td>
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<td>.640</td>
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<td>.343</td>
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<td>.001*</td>
<td>7.444</td>
</tr>
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<td>PTGS1</td>
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<td>.639</td>
<td>11.582</td>
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<td>.001*</td>
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<td>3.069</td>
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<td>.000</td>
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</table>

*a Statistical significant difference p < 0.05.
*b Statistical highly significant difference p < 0.01.

### Table 5
General Linear Model determining genes affecting BMI.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
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<td>96.51</td>
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<td>116.69</td>
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<td>72.79</td>
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<td>154.72</td>
<td>154.72</td>
<td>7.749</td>
<td>0.006*</td>
</tr>
<tr>
<td>STAT4*PTGS1</td>
<td>102.63</td>
<td>102.63</td>
<td>5.36</td>
<td>0.022*</td>
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</tbody>
</table>

*a Statistical significant difference p < 0.05.
*b Statistical highly significant difference p < 0.01.
insulin secretion through decreasing beta cell mass. This is evi-
denced by the highly significant decrease in the fasting insulin
levels and HOMA-B in association with STAT4 gene variant. Also
STAT4 gene was the only variant that showed significant associa-
tion with insulin resistance (HOMA-IR) in the studied patients.
Additionally STAT4 effect on T2D might be mediated by its impact
on obesity. Several mechanisms were proposed to understand the
links between obesity and inflammation. Obesity increases the
severity of pancreatitis through upregulation of the inflammatory
response. Obesity reduces the number of T-regulatory cells and
increases the number of CD8^T cells, which promotes macrophage
recruitment and increases levels of the proinflammatory hormone
leptin and decreases its anti-inflammatory counterpart, adiponect-
tin [16].

The hemochromatosis gene (HFE) codes for a protein that func-
tions to regulate circulating iron uptake by regulating the interac-
tion of the transferrin receptor with transferrin. This gene has been
suggested to be involved in the etiology of type 2 diabetes mellitus
in previous studies [17,18].

In the present work the AA and AG genotype frequencies were
significantly higher in diabetic patients compared to non-diabetic
participants with p = 0.04. A previous study speculated that the
relatively strong association of increased serum ferritin levels with
abnormal glucose tolerance raises the possibility that progressive
iron accumulation in the pancreas contributes to beta cell dysfunc-
tion and abnormal glucose tolerance [18]. In addition the HFE pro-
tein functions to regulate the expression of hepcidin, a secreted
hepatocyte peptide that is the principal regulator of iron home-
ostasis. Hepcidin production increases with iron loading and
inflammation [19]. These findings added evidence for the link
between inflammation and T2D pathogenesis.

4.2. PPARG

PPARG gene is a target for the hypoglycemic drugs known as thi-
azolidinediones. PPARG is one of the few genes that have been con-
firmed to be associated with insulin resistance [20]. The PPARG
rs1801282 C > G polymorphism also known as Pro12Ala, a SNP in
exon 2 of PPARG, encodes a proline → alanine substitution at amino
acid residue 12. The more common (C) allele encodes the ‘Pro’
amino acid at this SNP position [20,21].

In our results rs1801282 was investigated and PPARG Ala allele
showed highly significant association with T2D risk (p < 0.001). The
GG genotype frequency was highly significantly increased in
diabetic patients (p = 0.002). The presence of Ala allele was signif-
ificantly related to increased blood levels of FPG and HbA1c and
decreased HOMA-B supporting its impact on glucose metabolism
and beta cell function.

These results are in line with the previous Finnish Diabetes
Prevention Study that investigated 522 subjects with impaired glu-
cose tolerance and subjected them to either placebo or a lifestyle
intervention. They reported a twofold increase in risk of developing
T2D among alanine carriers in the placebo arm when compared to
proline homozygotes P/P [22]. On the other hand, the much larger
Botnia Prospective Study (N = 2293) documented a hazard ratio for
developing diabetes of 1.7 among P/P homozygotes, a result which
was statistically significant [23].

In our results the Ala allele bearers had a significantly higher
risk of obesity (p = 0.001) and the presence of the gene variant
was significantly associated with increased BMI.

4.3. EXT2

Exostosin (EXT) proteins may affect beta-cell mass and insulin
secretion capacity in humans, and render subjects at a higher risk
of developing type 2 diabetes when exposed to environmental risk
factors [24,25].

In the current work we studied two SNPs of (EXT2) gene
(rs3740878 and rs11037909). We found an association between
rs3740878 and the risk of T2D which is consistent with previous
studies [26]. The risk allele frequency was highly significantly
increased in diabetic patients compared with non-diabetic individ-
uals. The same was observed when comparing obese diabetic
patients with non-obese non-diabetic individuals. We found a sig-
nificant association between the risk allele of rs3740878 and
increased FPG, HbA1c, F Ins blood levels and BMI and with reduced
HOMA-B. These findings support the impact of this gene variant on
glucose metabolism, beta cell function and obesity. Logistic regres-
sion analysis showed that EXT2 rs3740878 gene variant signifi-
cantly affects obesity only and did not reach significance with T2D indicating that its role in T2D might be mediated by its asso-
ciation with obesity.

Marginal association for the rs3740878 (OR = 1.07, 95%
CI = 0.99–1.16, p = 0.09) was previously reported in a replication
and meta-analysis [27]. Three single nucleotide polymorphism
(SNP), rs3740878, rs11037909 and rs1113132, in the EXT2 gene
were genotyped in a Chinese case control study and suggested that
the EXT2 gene might not have a major role in the development of
type 2 diabetes in the Chinese population. The same was found in
a Lebanese case control study [28,29]. Gene–environment and
genome–gene interactions should be considered for these discrepan-
cies in literature.

4.4. GCKR

The GCKR gene product is a regulatory protein that inhibits glu-
cokinase in liver and pancreatic islet cells by binding non-
covalently to form an inactive complex with the enzyme. In the
current work the GCKR rs780094 risk allele was significantly asso-
ciated with risk of both type 2 diabetes and obesity. The TT and
CT genotypes were significantly more frequent in both disease enti-
ties. The risk variant showed a significant association with
increased FPG and HbA1c blood levels and BMI and reduced
HOMA-B. These are consistent with observations in white Euro-
peans and Han Chinese individuals [30,31]. Conversely Tam et al.
observed significant associations of the minor T-allele of GCKR
rs780094 with decreased FPG (P = 0.013) [32]. Regression analysis
in the present work demonstrated statistical significance with both
T2D and obesity indicating its impact on both disease entities.

4.5. CELSR2

The protein encoded by this gene (Cadherin EGF LAG seven-pass
G-type receptor 2) is a member of the cadherin superfamily. In the current study, the CELSR2 risk
allele frequency was significantly increased in diabetic patients
when compared with non-diabetic individuals. The same was
found when comparing obese diabetic patients with non-obese
non-diabetic individuals. The risk allele showed significant associ-
ation with risk of T2D and obesity. In the present study no impact
of the gene variant could be revealed on both T2D and obesity by
logistic regression analysis and no association could be detected
between CELSR2 risk allele and BMI or glycaemic level. Ahluwalia
et al. identified three loci (CELSR2, HNF1A, and GCKR) significantly
associated with T2D. They stated that the association with the
CELSR2 locus has not been shown previously [33]. Other investiga-
tors previously studied the lipid-metabolizing genetic polymor-
phisms and the BMI. They confirmed their association with
dyslipidaemia, however, they did not find association with higher
BMI [34,35]. From these findings the CELSR2 might be associated
significantly with both T2D and obesity with no recognizable impact on both diseases.

4.6. PTGS1

PTGS1/COX-1 gene was selected for genotyping in the present study based on its role in regulation of angiogenesis in endothelial cells and inflammatory response. It has been suggested that the hypercoagulable state in T2D could be as a result of disturbances of platelet activation, aggregation and lifespan, in addition to the pro-inflammatory state and endothelial dysfunction [36,37]. Endogenous prostanoi and have long been known as inhibitors of insulin secretion [38]. There is evidence that genetic variants in the PTGS1 gene alter the metabolism of arachidonic acid to prostaglandin H2 and prostaglandin F2, the precursors of prostacyclin, thromboxane, and several other prostaglandin metabolites [39].

In the present study the PTGS1 risk allele frequency was highly significantly increased in diabetic patients when compared with non-diabetic individuals. The same observation was obtained when comparing obese diabetic patients with non-obese non-diabetic individuals. The risk allele was significantly associated with increased FPG, HbA1c blood levels, BMI and decreased HOMA-B. Regression analysis indicated the effect of this gene variant on both T2D and obesity that could be related to its impact on glucose metabolism and obesity resulting in reduced beta cell function.

4.7. FTO

Within the FTO gene, many SNPs appear to be co-inherited. We selected one of these SNPs (rs17817449) for genotyping. The risk allele frequency showed increased significance in obese diabetic patients when compared with non-diabetic non-obese individuals. There is significant association with the risk of obesity. The presence of FTO risk allele showed borderline significance with increased BMI. No significant association could be demonstrated with T2D. Thus This gene variant might affect T2D through its relation to obesity and BMI.

A study of Quebec individuals reported that rs17817449 was significantly associated with BMI, weight and waist circumference under an additive model, and in addition, influenced fasting insulin and HOMA-IR [40]. Another study in Mexican women demonstrated that the FTO rs17817449 TT genotype predisposes its carriers to fat deposition in the thoracic and breast region. Individuals carrying this genotype showed a gynoid phenotype in Mexican women [41]. Consistent with our findings Frayling et al. indicated that the diabetes risk associated with the FTO locus is mediated by obesity [42].

4.8. MTHFD1

MTHFD1 is a key gene associated with three sequential enzymatic reactions in folic acid metabolism. Huang et al. stated that subjects with the genotype AA of the MTHFD1 variant (rs2236225) had a significantly lower susceptibility to T2D compared with subjects with the GG or GA + GA genotypes [43]. In the present work the MTHFD1 risk allele was significantly associated with obesity risk but not with T2D. Also the frequency of the GA genotype was significantly increased in obese diabetic patients when compared with non-obese non-diabetic individuals. The risk allele was significantly associated with increased BMI. These findings demonstrated the impact of MTHFD1 gene variant on obesity risk with possible secondary impact on T2D.

4.9. CDKN2A/B

In the current study significant association was found between the risky TT genotype and T2D patients and highly significant association in obese-diabetic patients. A meta-analysis by Li et al. indicated significant association between rs10811661 polymorphism upstream of CDKN2A/B and T2D [44]. Consistent observations have been detected in Japanese and Vietnamese population studies [45,46]. Conversely in Czech Slavonic population study the association between rs10811661 SNP and susceptibility to T2D was not confirmed [47].

5. Conclusion

The inflammatory pathogenic mechanism and obesity are major factors underlying the risk of T2D. 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 EXT2rs3740878 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 preliminary 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.

Contribution statement

The clinical and chemical pathology team (Hanaa R.M.Attia, Solaf A. Kamel, Mona H. Ibrahim, Heba A. Farouk, Amany H.A. Rahman) participated in planning the work and preparing design of the research and final approval of the version to be published. They were responsible for conducting the laboratory work, analysis and interpretation of data and making modifications as appropriate as the work progresses. Nevine I. Musa and Ghada H. Sayed were responsible for proper selection and sample processing of the diabetic cases and controls. Corresponding author was also responsible for drafting the work and revising it critically for important intellectual content and preparing the paper for submission.

Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript

Compliance with ethical standards

The study was an outcome of scientific event held in the National Research Centre (NRC) in April 2015 at the Medical Molecular Research Unit of the Medical Division. The Clinical and Chemical Pathology team organized the workshop including theoretical as well as practical sessions on OpenArray new technology supported by both STDV project ID.17364 and NRC. All the ethical rules of the NRC medical research committee were followed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Informed consent was obtained from all individual participants.

References


