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# Association of *MTHFR* C677T and *ABCA1* G656A polymorphisms with obesity among Egyptian children

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ABSTRACT

*Aim:* Obesity is currently a worldwide public health problem which is associated with a number of polymorphisms of genes involved in its pathogenesis. This study aimed to clarify the association between genetic variations within methylene tetrahydrofolate reductase (*MTHFR*) and ATP-binding cassette transporter A1 (*ABCA1*) genes and obesity in an Egyptian children and adolescents population.

*Method:* Anthropometric parameters along with serum total cholesterol, triglycerides, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol were measured, in addition to genotyping of *MTHFR* C677T and *ABCA1* G656A polymorphisms using allelic discrimination assay in 51 obese child and adolescent and 30 sex and age-matched healthy subjects.

*Results:* Compared to control, obese subjects showed a significant difference in the presence of C677T polymorphism, but not G656A ($p = 0.001$ and $p = 0.214$, respectively). Neither single nucleotide polymorphisms (SNPs) showed significant differences in serum lipid levels among different genotypes in control and obese groups except for high-density lipoprotein-cholesterol which appeared to be influenced by G656A polymorphism ($p = 0.020$ and $p = 0.032$, respectively). C677T polymorphism increased the susceptibility to obesity under all genetic models while G656A polymorphism failed to show any association with the predisposition of developing obesity. SNP-SNP interaction showed that CT/AG carriers are at a higher risk to develop obesity compared to wild type carriers ($p = 0.005$).

*Conclusion:* *MTHFR* C677T polymorphism may contribute to the development of obesity while there is no association related *ABCA1* G656A polymorphism with simple obesity among Egyptian children and adolescents. Joint effects of the two genes revealed that the carriers of the mutant alleles of both *MTHFR* and *ABCA1* are at high risk to develop obesity.

## 1. Introduction

Obesity related co-morbidities are among the leading public health problems worldwide. Over the last decades, the prevalence of obesity has been in rise in adults as well as in children in many countries (Atay and Bereket, 2016). Obesity is a major contributor to the development of dyslipidemia, insulin resistance, and hypertension and it is associated with chronic diseases such as type 2 diabetes, cardiovascular diseases (CAD), metabolic syndrome, stroke, sleep disorders, osteoarthritis, and increased incidence of certain forms of cancer (Kopelman, 2000).

Many genes and polymorphisms have been hypothesized to be involved in the pathogenesis of obesity in the past few decades (Bell et al., 2005). Among them, the C677T polymorphism in the methylene tetrahydrofolate reductase (*MTHFR*) gene was assessed as a potential candidate (Thawnashom et al., 2005; Settin et al., 2009; Yin et al., 2012). *MTHFR* gene is located on 1p36.3 and its protein product irreversibly catalyzes the conversion of 5, 10 methylene tetrahydrofolate to 5-methyl tetrahydrofolate, which serves as a methyl donor in the remethylation of homocysteine (Hcy) to methionine. The *MTHFR* C677T polymorphism can affect the activity of its corresponding enzyme and ultimately lead to hyperhomocysteinemia (Alam et al., 2008).

Previous epidemiological studies have reported higher

*Abbreviations:* ABCA1, ATP-binding cassette transporter A1; AD, allelic discrimination; Apo-A1, apolipoprotein A1; BMI, body mass index; CAD, cardiovascular diseases; CI, confidence interval; EDTA, ethylene diamine tetra-acetic acid; Hcy, homocysteine; HDL-C, high-density lipoprotein-cholesterol; HWE, Hardy-Weinberg equilibrium; LDL-C, low-density lipoprotein-cholesterol; MENA, Middle East and North Africa; MTHFR, methylene tetrahydrofolate reductase; OR, odds ratio; OSSE, Online Sample Size Estimator; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein

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homocysteine level in obese subjects compared to normal weight controls (Marchesini et al., 2002; Martos et al., 2006). The mechanisms underlying these findings remain unclear; however, some investigators have postulated that elevated homocysteine level might affect the development of obesity since the metabolism of methyl group and homocysteine are interrelated processes and are closely related to the methylation of cytosine residues in DNA and amino acid residues in histones, both of which are involved in epigenetic control of the expression of some genes participate in the regulation of fat storage and food intake (Williams and Schalinske, 2007; Lewis et al., 2008). Data from animal experiments and cell and genetic studies seem to support this hypothesis (Cooney et al., 2002).

ATP-binding cassette transporter A1 (*ABCA1*) is another gene that its polymorphisms had been correlated to overweight/obesity (Yao et al., 2016). It is located on 9q31 and encodes for a key protein that affects the efflux of excess lipids from the peripheral cells into lipid-poor apolipoprotein A1 (Apo-A1) particles and facilitates the formation of high-density lipoprotein (HDL) (Hayden et al., 2000). *ABCA1* gene mutations may affect the transcription and expression of the protein product, thereby causing a disturbance in lipid metabolism which can lead to obesity according to the fact that cholesterol imbalance in adipose tissue is postulated to be one of the causes of the adipose tissue dysfunction which is at the center of metabolic dysfunctions associated with obesity (Vincent et al., 2017). Further, *ABCA1* gene mutations can lead to a variety of diseases, such as coronary artery diseases, atherosclerosis, type 2 diabetes mellitus, Alzheimer's disease, familial HDL deficiency, and Tangier disease in various populations (Oram and Heinecke, 2005; Brunham et al., 2006; Saleheen, 2005; Sundar et al., 2007; Mott et al., 2000; Negi et al., 2013).

Based on these findings, information on these naturally occurring polymorphisms is essential to identify the risk/cause relationship in obesity with the *MTHFR* and *ABCA1* genes. Therefore, the main goal of the present study was to assess the association of simple childhood obesity with the genetic variants of *MTHFR* (single nucleotide polymorphism [SNP]: C677T, SNP ID: rs1801133) and *ABCA1* (SNP: G656A, SNP ID: rs2230806) as potential risk factors for obesity.

## 2. Subjects and methods

### 2.1. Study population

The examined group consisted of 51 obese children and adolescent (30 girls and 21 boys) with non-complicated obesity (body mass index [BMI] > 95$^{th}$ percentile for age and sex reference values) who were aged between 7 and 15 years with a mean age of 10.82 years. They were sequentially recruited from the outpatient clinic of Pediatrics Department, National Nutrition Institute, The General Organization for Teaching Hospitals and Institutes in the period from August 2015 to February 2016. Thirty healthy children and adolescent (13 girls and 17 boys) with normal body weight (BMI between 5 and < 85$^{th}$ percentile for age and sex reference values) and aged between 7 and 16 years with mean age of 10.87 years were enrolled as a control group.

There were no significant differences in age and sex among obese and non-obese children. A complete history was obtained and a physical examination was carried out on all the participants. All included children were free from any allergic disease, immune or hematological disorders.

Informed consent was obtained from the legal guardians of each subject before enrolment in the study. This work was carried out in accordance with The Declaration of Helsinki for experiments involving humans and the study protocol was approved by the ethical committee of Faculty of Medicine, Ain Shams University, Cairo, Egypt.

All subjects in the study group presented with normal fasting and postprandial blood glucose levels (78.48 ± 6.78 and 97.92 ± 15.05 mg/dl, respectively), and were thus considered to be non-diabetics.

Five ml of 12 h fasting venous blood were collected from each participant. A part of the sample (3 ml) was collected into dry tubes, left to clot and centrifuged at 1000 × *g* for 5 min to obtain sera in which lipid levels were determined. Another part of the sample (2 ml) was collected into ethylene diamine tetra-acetic acid (EDTA) coated tubes for molecular analyses.

### 2.2. Anthropometric and biochemical parameters

Height (cm) and weight (kg) were measured using standardized equipment. The BMI was calculated as weight/height$^2$ (kg/m$^2$) and gender-specific BMI-for-age percentile was calculated using gender-specific growth charts. Obesity was defined as a BMI percentile > 95 adjusted for age and sex according to the Expert Committee (Barlow and Dietz, 1998).

Serum triglycerides (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) concentrations were determined enzymatically using available commercial kits provided by Biodiagnostics (Giza, Egypt) on Roche Hitachi 912 chemistry analyzer (Roche Diagnostics, In, USA) and low-density lipoprotein-cholesterol (LDL-C) concentration was calculated using the Friedewald's formula (Friedewald et al., 1972).

### 2.3. Molecular analyses

The rs1801133 polymorphism of the *MTHFR* gene and rs2230806 polymorphism of *ABCA1* gene were genotyped using allelic discrimination (AD) assay. Briefly, after isolating mononuclear cells from the whole blood using Ficoll-Paque™ density gradient media (GE Healthcare UK Ltd, Buckinghamshire, UK) in order to increase the yield of DNA, cells were resuspended in phosphate buffered saline (pH 7.4) and finally, genomic DNA was extracted from the cell suspension using QIAamp® DNA blood mini kit (Qiagen, Hilden, Germany).

Real-Time PCR reactions were performed in a final volume of 25 µl with ~20 ng of DNA, 12.5 µl of 2× TaqMan® universal PCR master mix (Applied Biosystems), 1.25 µl of 20× working stock solution of SNP genotyping assay which contained the primers and probes for the gene of interest as part of the kit (Cat. # 4351379, assay ID: C_1202883_20 for *MTHFR* and C_2741051_1 for *ABCA1*, respectively) (Applied Biosystems), the reaction's volume was then completed with water. PCR conditions were as follows: 10 min at 95 °C for AmpliTaq Gold enzyme activation and initial denaturation followed by 40 amplification cycles of denaturation at 92 °C for 15 s, annealing at 60 °C for 1 min and extension at 60 °C for 1 min. PCR reactions were carried out in MicroAmp® fast optical 96-Well reaction plate with MicroAmp® optical adhesive film (Applied Biosystems) The plate was loaded into the 7500 Real-Time PCR system (Applied Biosystems, CA, USA) and the fluorescence data were analyzed in genotyping mode by the instrument's software.

### 2.4. Statistical analysis

The distribution of data was tested using the Shapiro-Wilk test, normally distributed data are expressed as mean ± SD, non-normally distributed data are expressed as median and interquartile range (25$^{th}$ and 75$^{th}$ percentile) and categorical variables are expressed as frequencies (percentages). Continuous variables were compared between two or more groups using the unpaired Student's *t*-test, Mann-Whitney *U* test, one-way ANOVA followed by Tukey's *post hoc* for multiple comparisons or Kruskal-Wallis test as appropriate. $\chi^2$ test was used to compare the differences between categorical variables and to assess the departures from Hardy-Weinberg equilibrium (HWE) ensuring a lack of a significant difference between observed and expected frequencies. For each polymorphism, homozygous for the major allele (the allele with greater frequency among controls) was used as the reference genotype. To investigate the strength of the associations between the *MTHFR* and

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