

# Methylene tetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms related to overweight/obesity among Saudi subjects from Qassim Region

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**Abstract.** *Background:* This work was planned to check for the association of polymorphisms related to methylenetetrahydrofolate reductase (MTHFR) and angiotensin converting enzyme (ACE) genes with overweight/obesity among Saudi subjects from Qassim region.

*Methods:* This work included 130 subjects having overweight or obesity and 111 normal controls. Their age mean  $\pm$  SD was  $27 \pm 9.8$  and  $24 \pm 8.8$  years respectively. Their DNA was analyzed for polymorphisms of MTHFR; 677C/T and 1298 A/C and ACE; I/D genes using real-time PCR.

*Results:* Genotype and allele frequencies of studied polymorphisms in cases of overweight/obesity showed no significant statistical difference compared to that of controls. However, on analysis of body mass index (BMI), cases showed slightly higher but statistically nonsignificant mean  $\pm$  SD values among those carrying the mutant MTHFR 677 T allele (CT + TT vs. CC,  $30.7 \pm 4.5$  vs.  $29.9 \pm 4.9$ ), 1298 C allele (AC + CC vs. AA,  $29.9 \pm 4.1$  vs.  $29.7 \pm 5.5$ ) and ACE D allele (ID + DD vs. II,  $30.0 \pm 5.1$  vs.  $29.1 \pm 2.8$ ). In addition controls having the DD and ID genotypes showed higher statistically significant values of BMI than those of the II genotype ( $22.0 \pm 1.9$ ,  $21.7 \pm 2.6$  and  $19.5 \pm 2.3$  respectively,  $p < 0.05$ ).

*Conclusion:* There is no solid association of polymorphisms related to MTHFR and ACE genes with non-complicated overweight or obesity among Saudi subjects from Qassim Region.

Keywords: Obesity, Saudi population, MTHFR, ACE, gene polymorphism, Qassim Region

## 1. Introduction

Obesity is an excessive accumulation of body fat and in its gross manifestation poses a real threat to health [1, 2]. It is the most prevalent, chronic medical condition in the developed, as well as in developing countries [3].

There are a number of etiological factors producing obesity and these include both genetic and environmental factors and hence it is classified as a multifactorial disorder. Endocrine alterations are also an important cause of obesity but are rare, even though obesity influences the functions of the endocrine system [4,5].

Methylenetetrahydrofolate reductase enzyme (MTHFR) plays a central role in folate metabolism by irreversibly catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and a co-substrate for homocysteine (Hcy) methylation to methionine. Its involve-

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ment in the regulation of homocysteine concentration turns it out to be a risk factor for cardiovascular disorders (CVD) [6]. In the MTHFR gene located in chromosome 1, a common single nucleotide polymorphism is associated with reduced enzyme activity: C → T in exon 4 at nucleotide 677, leading to Ala222Val, with the T allele frequency being around 44% in Caucasians; and A → C in exon 7 at nucleotide 1298, leading to Glu429Ala, with the C allele frequency around 20% in Caucasians [7,8]. Individuals who are homozygous for the MTHFR 677 less frequent variant (TT) have 30% of the expected enzyme activity with a reduced folate status and higher serum Hcy levels, while heterozygous carriers have 65% activity [9]. On the other hand, individuals carrying the 677CC together with the 1298CC genotypes have 60 percent activity compared to subjects carrying the 1298AA variant [8].

Epidemiological studies have shown that low folate levels are associated with a high body mass index (BMI) [10]. This finding has potentially important health implications and warrant further investigation particularly for MTHFR gene polymorphisms to determine whether a causal relationship exists and the direction of this relationship [11].

Angiotensin converting enzyme (ACE) catalyzes the formation of angiotensin I to angiotensin II. A polymorphism has been identified in intron 16 in which a 287 base-pair alu sequence was found to be present (insertion or I) or absent (deletion or D) in the population. This enzyme is involved in adipocyte growth and function and the ACE-processed angiotensin II inhibits adipocyte differentiation. Although associations between BMI and ACE polymorphisms have been reported in general populations, the contribution of this gene to severe obesity is generally unknown [12,13].

During the last three decades Saudi Arabia has seen a considerably rising rates for both overweight and obesity [14–18]. Qassim region is a tribal area in the middle zone of Saudi Arabia characteristically having high rate of consanguinity and familial diseases as obesity with diabetes and cardiovascular disorders [19]. This work was planned to check for the association of non-complicated obesity with genetic polymorphisms of MTHFR and ACE genes as potential risk factors for obesity in this population.

## 2. Subjects and methods

This work was designed as a cross sectional case control study. In order to test the direct association

of overweight/obesity with MTHFR and ACE genetic polymorphisms, we included healthy subjects after exclusion of all possible endocrinologic or genetic disorders through thorough history taking, examination and relevant investigations. Cases also were selected as being free from all probable complications that might be related to obesity or metabolic disorders like hypertension, diabetes, hyperlipidemias and coronary heart disease. They included 241 unrelated subjects ethnically belonging to Qassim Region taken from those attending Qassim University affiliated Clinics for routine check-ups. Of them 130 subjects were affected with simple overweight or obesity with a body mass index (BMI)  $\geq$  25% including 66 (50.8%) males and 64 (49.2%) females with an age mean  $\pm$  SD of 27 (9.8) and a median of 23 years. The others, 111 subjects were taken as a control group having a normal BMI < 25% including 57 (51.4%) males and 54 (48.6%) females with an age mean  $\pm$  SD of 24 (8.8) and a median of 20 years. An informed consent was obtained from all subjects in addition to an authorized approval that was obtained from the Scientific and Ethical Committees of Qassim University. For all subjects, the BMI or Quetelet's index was conventionally calculated as weight in kg/(height in meters)<sup>2</sup>. The classifications of BMI were those used by the WHO Expert Committee, 2000: normal BMI = 18.5–24.99 kg/m<sup>2</sup>; overweight BMI = 25.00–29.99 kg/m<sup>2</sup>; grade I (obesity) BMI = 30.00–34.99; grade II (obesity) BMI = 35.00–39.99 kg/m<sup>2</sup>; grade III (obesity) BMI > 40 kg/m<sup>2</sup> [20]. Based on that classification, our sample included 76 subjects having overweight, 43 subjects with grade I obesity and 11 subjects with grade II and III obesity.

For all subjects, DNA was extracted from peripheral blood using the MagNA Pure LV blood reagent set (Roche Diagnostics) according to the manufacturer's instruction. Real-time PCR amplifications for C677T, A1298C gene polymorphisms of MTHFR and I/D polymorphism of ACE genes were done using Light Cycler apparatus (Roche Diagnostics). Amplification mix including specific primers and probes (Light Mix, TIB MOLBIOL and Light Cycler Fast Start DNA Master hybridization probes) were purchased from Roche Diagnostics and used in an optimized PCR conditions recommended by the manufacturer.

## 3. Statistical analyses

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 10.0).

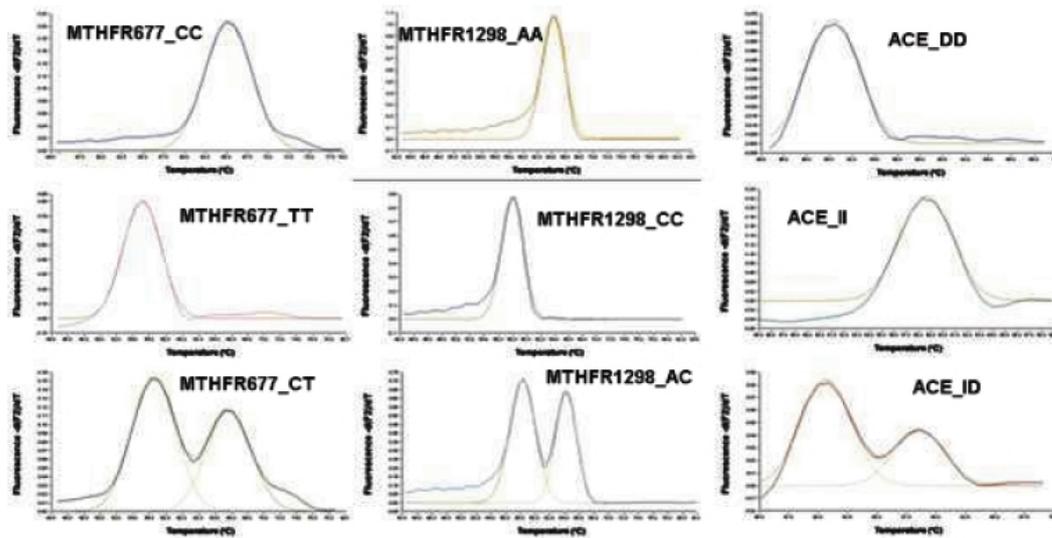


Fig. 1. Real-time PCR tracing showing bands of amplified genes with melting temperatures characteristic for each band.

The frequency of studied allelic polymorphisms among cases was compared to that of controls and tested for positive association using Fisher's exact tests and odds ratio (OR) with 95% confidence interval (95% CI). A minimum level of significance is considered if  $P \leq 0.05$ . Furthermore, the distribution of alleles in studied groups was tested for fitting to the Hardy-Weinberg equilibrium assuring no significant difference between observed and expected frequencies using  $\chi^2$  test.

#### 4. Results

Comparing cases having overweight/obesity to controls, no statistical significant difference was observed between both groups in terms of frequencies of all studied MTHFR and ACE genotypes and alleles ( $p > 0.05$ ) (Table 1). However, on analysis of body mass index (BMI), slightly higher mean values were observed (nonsignificant) among those carrying the MTHFR 677 T allele (CT + TT) compared to those with CC genotype ( $30.7 \pm 4.5$  vs.  $29.9 \pm 4.9$ ), carriers of the 1298 C allele (AC + CC) compared to those with AA genotype ( $29.9 \pm 4.1$  vs.  $29.7 \pm 5.5$ ) and among carriers of ACE D allele (ID + DD) compared to those with II genotype ( $30.0 \pm 5.1$  vs.  $29.1 \pm 2.8$ ). In addition controls having the DD and ID genotypes showed statistically significant higher values of BMI than those of the II genotype ( $22.0 \pm 1.9$ ,  $21.7 \pm 2.6$  and  $19.5 \pm 2.3$  respectively,  $p < 0.05$ ) (Table 2).

Further analysis comparing controls to higher grade obese subjects (having higher values of BMI) using

various combined genotypes and/or haplotypes failed to reveal any significant difference between the two studied group. Analysis of BMI values related to sex in both groups showed non-significant statistical difference except for males in the control group who were ACE D allele carriers who showed significant higher BMI values than females (data not shown).

#### 5. Discussion

In this work we attempted testing the association of three genetic polymorphisms pertinent to two important enzymes (MTHFR and ACE) claimed by many authors to have a link to obesity and related complications like hypertension, diabetes, dyslipidemia and cardiovascular disorders [21–25].

In order to assess this association we have selected our cases with simple overweight/obesity without complications and compared them to unrelated matched controls of a same ethnic background. Although no significance could be retrieved comparing the genotype and allele frequencies of studied genes in overweight/obese cases and controls, we could observe a slightly statistically non-significant higher BMI values among subjects carrying the mutant forms of these polymorphisms particularly that of the ACE DD and ID genotypes. This was markedly manifested in male subjects in the control group. This finding is in agreement with the finding of El-Hazmi and Warsy of high frequency of DD genotype and D allele in Saudi over-

Table 1  
Genotypes and allele frequencies of studied genes polymorphisms among overweight/obese cases compared to controls

Genotypes and alleles	Overweight/obese n (%)	Control n (%)	P ( $\chi^2$ )	OR (95% CI)
<b>MTHFR 677</b>				
CC	89 (69.5)	69 (62.7)	0.33	1.4 (0.8–2.3)
TC	34 (26.6)	36 (32.7)	0.37	0.7 (0.43–1.3)
TT	5 (3.9)	5 (4.5)	0.94	0.85 (0.24–3.03)
C	212 (82.8)	174 (79.1)	0.36	1.3 (0.81–2.02)
T	44 (17.2)	46 (20.9)	0.36	0.79 (0.5–1.2)
<b>MTHFR 1298</b>				
AA	70 (54.7)	51 (46.4)	0.25	1.4 (0.84–2.3)
AC	45 (35.2)	47 (42.7)	0.29	0.73 (0.43–1.23)
CC	13 (10.2)	12 (10.9)	0.98	0.92 (0.42–2.12)
A	185 (72.3)	149 (67.7)	0.33	1.2 (0.84–1.84)
C	71 (27.7)	71 (32.3)	0.33	0.81 (0.54–1.19)
<b>ACE I/D</b>				
II	12 (9.5)	5 (4.5)	0.21	1.23 (0.76–6.55)
ID	54 (42.9)	44 (39.6)	0.71	1.14 (0.68–1.92)
DD	60 (47.6)	62 (55.9)	0.26	0.72 (0.43–1.2)
I	78 (31.0)	54 (24.3)	0.13	1.4 (0.93–2.1)
D	174 (69.0)	168 (75.7)	0.13	0.72 (0.48–1.08)

Table 2  
Body mass index (mean and SD) related to different polymorphic genotypes among studied overweight/obese cases compared to controls

Genotypes	Overweight/obese BMI, M $\pm$ SD	Controls BMI, M $\pm$ SD
<b>MTHFR 677</b>		
CC	29.9 $\pm$ 4.9	21.7 $\pm$ 2.5
CT	31.1 $\pm$ 4.5	21.8 $\pm$ 1.8
TT	28.4 $\pm$ 4.6	23.5 $\pm$ 0.66
CT + TT	30.7 $\pm$ 4.5	21.97 $\pm$ 1.8
<b>MTHFR 1298</b>		
AA	29.7 $\pm$ 5.5	21.7 $\pm$ 1.9
AC	30.3 $\pm$ 4.1	21.8 $\pm$ 2.5
CC	28 $\pm$ 3.9	22.2 $\pm$ 2.6
AC + CC	29.9 $\pm$ 4.1	21.8 $\pm$ 2.5
<b>ACE</b>		
II	29.1 $\pm$ 2.8	19.5 $\pm$ 2.3
ID	30.2 $\pm$ 4.2	21.7 $\pm$ 2.6*
DD	29.5 $\pm$ 5.9	22.0 $\pm$ 1.9*
<b>ID + DD</b>	30.0 $\pm$ 5.1	21.9 $\pm$ 2.2*

\* Significantly different from the II genotype in the control group ( $p < 0.05$ )

weight and obese individuals in a previous study in Riyadh region [26].

Similar finding suggesting that ACE gene polymorphisms may influence the development of weight gain with a sex difference in males and females was previously reported [13,27].

On the other hand, researchers recently reported that there is a trend towards association of ACE I/D polymorphism with hypertension but not with obesity [28]. Likewise, in a rigorous, large-scale French study, authors stated that functionally relevant sequence variation in ACE, whether it is defined at the level of SNPs, haplotypes, or clades, is not associated with obesity [12].

It was reported that MTHFR Ala222Val polymorphism characterized individuals with metabolic obesity with normal weight; and is associated with elevated BMI and waist hip ratio in healthy postmenopausal women [29–31]. Previous researches could also elicit positive association of obesity and hypertension, coronary artery disease or liver diseases with MTHFR C677T mutations [32–34]. Also Thwnashom et al. have reported that folic acid and MTHFR gene polymorphism were found to be significantly related to the overweight/obese and control groups in logistic regression analysis [35].

However, in a recent wide scale study in UK, Lewis et al. denied the initial findings in the British Women's

Heart and Health Study and concluded a negative association between the MTHFR TT genotype and obesity [11]. Liu et al studying a large sample of Chinese families have also reported no association between the MTHFR gene polymorphism with fat body mass or obesity [36].

From the above mentioned data, it is obvious that these genetic polymorphisms are mostly associated with complicated obesity rather than obesity alone. It may be also related to ethnic background and genetic makeup of each particular population.

Although most of our cases were in the group of overweight rather than the higher grades of obesity, we could presume that there is no solid association of polymorphisms related to MTHFR C677T and A1298C and ACE I/D genes with non-complicated overweight or obesity among Saudi subjects from Qassim Region. However, we recommend a further wider scale and longitudinal study for follow up and genotyping of cases that might develop obesity complications.

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