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ORIGINAL ARTICLE

The role of *DENND1A* and *CYP19A1* gene variants in individual susceptibility to obesity in Turkish population—a preliminary study

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Abstract

Single nucleotide polymorphisms (SNPs), the most common genetic variations in human genome, can manage the predisposition of certain complex diseases or situations such as obesity. Genetic polymorphisms also play an important role as they can impact a population's susceptibility to being overweight or obese and developing related chronic complications such as hypertension, coronary heart disease, diabetes and cancer. The present study comprised of 193 unrelated healthy volunteers (120 females and 73 males) with Turkish origin. Only female adolescents (n = 110) were divided into 2 categories according to their BMI values as overweight (BMI ≥ 25) and normal (18.5 < BMI < 25) according to WHO classification. Genomic DNA was isolated from venous blood samples and genotyping of *DENND1A* rs10818854 and *CYP19A1* rs2414096 variants was performed on Roche Light Cycler 2.0 Real-Time PCR platform. Serum hormone levels were analyzed by Electrochemiluminescent Immunoassay (ECLIA; Roche diagnostics). The genotype distributions were consistent with the Hardy–Weinberg equilibrium for both SNPs in the studied population ($p > 0.05$). The genotype distribution of *DENND1A* rs10818854 was determined for the first time in Turkish population and the variant allele frequency was found as 0.095. According to reduced sex hormone-binding globulin levels and increased free androgen index in the present study, obesity was linked with hyperandrogenism in female subjects. Both polymorphisms were investigated as potential genetic susceptibility markers for obesity and neither *DENND1A* nor *CYP19A1* showed any associations.

Keywords *DENND1A* · *CYP19A1* · Genetic polymorphism · Obesity · Turkish population

Introduction

Obesity is one of the serious health problems worldwide. According to The Organisation for Economic Co-operation and Development (OECD)'s last report, more than one in two adults and nearly one in six children are overweight or obese in OECD countries [1]. More than 1.9 billion adults aged 18 years and older were overweight and over 650 million adults were obese in 2016 [2]. There are many studies about the effects, causes and genetic basis of obesity due to its association with a number of serious diseases. Obesity is associated with several abnormalities in androgen metabolism such as hyperandrogenism which might be a result of reduced conversion of androgens to estrogens by the aromatase enzyme.

Identifying candidate genes and single nucleotide polymorphisms (SNPs) are useful markers of genetic susceptibility to determine the risk of diseases and frame of the effectiveness or safety of drug therapy. The *CYP19A1* gene is located on the long arm of chromosome 15 at position 15q21.1 and encodes aromatase enzyme (P450arom), which catalyzes the final step of estrogen biosynthesis by converting testosterone to estradiol and androstenedione to estrone separately. It has reported that reduced aromatase activity is a risk factor for obesity and more intra-abdominal adipose tissue accumulation observed in aromatase-knockout mice, which suggests the role of *CYP19A1* gene in obesity pathogenesis [3]. Although a few polymorphisms in *CYP19A1* gene have been evaluated in obese or overweight individuals [4], up to date no data is available concerning obesity and its association with rs2414096. However, recently, rs2414096 SNP in the *CYP19A1* gene have been associated

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with hyperandrogenism which has a role in abdominal obesity pathogenesis in women [5].

Genome-wide association studies (GWAS) provide well-replicated data on genetic risk factors for complex diseases such as metabolic syndrome, obesity and Polycystic Ovary Syndrome (PCOS). Recently, the first GWAS was published in a Chinese cohort and three loci were associated with PCOS which is a multifactorial complex disease characterized by hyperandrogenism, insulin resistance and obesity [6]. According to the results of GWAS, one of the genes that PCOS susceptibility loci mapped to is *DENND1A*. It encodes a protein named DENN/MADD domain containing 1A or *DENND1A*, a member of the connecdenn family, was located in the cytoplasm as well as nuclei of theca cells, suggesting a possible role in gene regulation. *DENND1A* transcript variant V2 is overexpressed in PCOS theca cells and is able to increase CYP17 expression and dehydroepiandrosterone (DHEA) production suggesting that *DENND1A* plays a key role in hyperandrogenism [7].

Genetic polymorphisms in *DENND1A* gene were analyzed in a European population and strong correlation was found between rs10818854 SNP of *DENND1A* and PCOS [8]. Although *DENND1A* gene polymorphisms were not correlated with obesity as strong as *CYP19A1* gene in recent studies, it is clear that it has a role in hyperandrogenism [9] and might influence the prevalence of obesity or being overweight since women with the low circulating sex hormone-binding globulin (SHBG) levels and elevated free androgens are related to increased visceral fat accumulation [10].

In the present study, we aimed to determine the genotype and allele frequencies of rs10818854 as one of the *DENND1A* SNPs for the first time in a Turkish population and the frequency of a novel rs2414096 SNP of *CYP19A1* gene in the same population and to evaluate the role of these genetic polymorphisms in obesity.

Materials and methods

Study population

193 unrelated healthy volunteers (120 females and 73 males, mean age 23.25 ± 13.36) with Turkish origin recruited the study. Individuals with any diseases such as metabolic syndrome or PCOS which can alter the hormone levels were excluded from the study. Permission for blood samples of individuals under 18 years of age was taken from their parents. Information about age, gender, weight and height of the subjects were reached by questionnaire and BMI values were calculated for each. 10 of total female subjects excluded from the study due to missing data. Only female subjects ($n = 110$) were divided into 2 categories according to their BMI values as overweight ($BMI \geq 25$) and normal ($18.5 < BMI < 25$) in accordance with WHO classification [11]. This study was approved by the ethics committee of Dr.Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (No: 2015-08/175) and informed consent obtained from each of the participants after a full explanation of the study.

Genotyping

Venous blood samples (~5 ml) were drawn into the EDTA (ethlylenediaminetetraacetic acid) tubes from each individual. Genomic DNA was extracted from whole blood by using High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's protocol. Purified DNA templates were stored at − 20 °C until analyse. Genotyping rs10818854 of *DENND1A* gene and rs2414096 of *CYP19A1* gene was performed by using LightCycler FastStart DNA Master HybProbe and custom designed LightSNiP assay probe (Roche, Germany). Reactions were carried out in a final volume of 12 µl containing 2 µl DNA for each sample on a Lightcycler 2.0 platform (Roche Applied Science, Germany). Genotypes were determined using HybProbe probes, which hybridize on the PCR fragments and emit a fluorescent signal (FRET-fluorescence resonance energy transfer). After 10 min of denaturation at 95 °C, the amplification step was proceeded by 45 cycles running at 95 °C for 10 s, at 60 °C for 10 s and at 72 °C for 15 s. The melting programme consist of 3 steps: denaturalization at 95 °C for 30 s, renaturation at 40 °C for 2 min and then slowly raised to 75 °C to allow monitoring the decline of fluorescence signal as a function of temperature. Subsequently cooling step was performed at 40 °C for 30 s. The same RT-PCR protocol was applied to both genes. Genotyping of the *DENND1A* rs10818854 and *CYP19A1* rs2414096 polymorphisms was based on melting curve analysis and genotypes were identified with the specific melting points (Tm) of the wild/mutant alleles. The wild type *DENND1A* rs10818854 allele (*G*) exhibits a Tm of 60.25 °C, while mutant allele (*A*) had a Tm of 65.49 °C. For the *CYP19A1* gene, the wild type rs2414096 allele (*G*) exhibits a Tm of 52.45 °C, while mutant allele (*A*) had a Tm of 58.72 °C.

Serum hormone measurements

Luteinizing hormone (LH), Follicle-stimulating hormone (FSH) and Sex hormone-binding globulin (SHBG) levels were measured in female subjects by Electrochemiluminescent Immunoassay (ECLIA; Roche diagnostics). Free androgen index (FAI) was calculated by using the following formula;

$$FAI = 100 \times (\text{Total testosterone}/SHBG)$$

Statistics

Genotype and allele frequencies were calculated with the cross tables and data were given as n(%). The Hardy–Weinberg Equilibrium analysis was performed by using the Chi square goodness-of-fit test. The effect of gender on genotype distribution was assessed by Chi square test. The effect of genotypes and alleles on BMI were assessed by Binary logistic regression model. In addition, the odds ratio and 95% confidence intervals for each genotype and allele were calculated. *p value* below 0.05 was considered statistically significant. All statistical analyses were performed by using IBM SPSS Statistics 17.0 software.

Results and discussion

In this study, we determined the genotype and allele frequencies of *DENND1A* rs10818854 and *CYP19A1* rs2414096 and their association with obesity in a Turkish population.

The distribution of genotypes and allele frequencies of genes are given in Table 1. Minor Allele Frequency (MAF), an index that represents the frequency of the second most occurring allele for each polymorphism was also presented in Table 1 with the respective MAFs from the bibliography. Among the studied population, the frequency of heterozygous genotype (AG) in *DENND1A* gene was 19.1% and none of the individuals were having the mutant genotype (AA). The variant allele (A) frequency for *DENND1A* gene was 0.095. The frequencies of *CYP19A1* AA, AG and GG genotypes were 15.3%, 47.0% and 37.7%, respectively and the variant allele (A) frequency was 0.387. No deviation was seen from the Hardy–Weinberg equilibrium for none of the studied genotypes *(DENND1A; $p=0.924$, CYP19A1; $p=0.118$)*. The genotype distributions for *DENND1A* and *CYP19A1* were similar between male and female subjects ($p=0.121$ and $p=0.642$ respectively; data not shown).

The first genome-wide association study (GWAS) for PCOS was published in 2011 and three loci were associated with PCOS in Chinese women [6]. After Chinese study, seven single nucleotide polymorphisms mapping to three Chinese PCOS loci were tested in European cohorts and variation in *DENND1A* was strongly associated with PCOS. Among these variations, rs10818854 were highly correlated with disease risk ($p=0.00014$; OR = 2.08). Therefore, we evaluated the frequency of this variation (rs10818854*)* in *DENND1A* gene for the first time in a Turkish population. There are a few studies regarding to rs10818854 frequency in *DENND1A* gene in Chinese [6], European [8, 12, 13] and Arabian populations [14, 15]. Minor allele frequency in Han Chinese control population was 0.079 and it ranges from 0.038 to 0.073 in European populations. Two studies conducted in Arab control population and MAF ranges between 0.059 and 0.09 according to these studies. The variant allele frequencies in these 3 populations are similar and MAF for *DENND1A* rs10818854 SNP in our study which is 0.095 stays between these results showing similarity with mostly Arabian population (Table 2). A recent meta-analysis about *DENND1A* gene polymorphisms and PCOS risk, reached the conclusion that rs10818854 variant could be

Table 2 Allele frequencies of rs10818854 *SNP* in *DENND1A* gene compared with previously published data*

Race/ethnicity	Study population (n)	Allele frequencies n (%)	References
Arabian	202	A: 70 (9) G: 334 (91)	[14]
Chinese	4964	A: 864 (8.7) G: 9064 (91.3)	[17]
European	957	A: 88 (4.6) G: 1826 (95.4)	[8]
Iceland	16,947	A: 1254 (3.7) G: 32,640 (96.3)	[12]
Boston	477	A: 42 (4.4) G: 912 (95.6)	
Chicago	188	A: 12 (3.2) G: 364 (96.8)	
Turkish	183	A: 35 (10) G: 331 (90)	This study

*Data obtained from control groups

Table 1 Genotype distributions and allele frequencies of rs10818854 *and* rs2414096SNPs in Turkish population

Genes/SNPs (n = 183)	Genotype	Genotype frequency n (%)	Allele frequency	Observed MAF vs Bibl.
DENND1A (rs10818854)	AA AG GG	0 (0) 35 (19.1) 148 (80.9)	A: 0.095 G: 0.904	0.09 vs 0.06[a]
CYP19A1 (rs2414096)	AA AG GG	28 (15.3) 86 (47.0) 69 (37.7)	A: 0.387 G: 0.612	0.38 vs 0.32[b]

[a](NCBI-dbSNP: 10818854)

[b](NCBI-dbSNP: 2414096)

Table 3 Allele frequencies of rs2414096 *SNP* in *CYP19A1* gene compared with previously published data*

Race/ethnicity	Study population (n)	Allele frequencies n (%)	References
African–American	405	A: 191 (24) G: 619 (76)	[18]
	26	A: 13 (25) G: 39 (75)	[19]
European	6920	A: 6373 (46) G:7467 (54)	[20]
Caucasian	802	A: 809 (50) G: 795 (50)	[18]
Chinese	151	A: 141 (47) G: 161 (53)	[18]
	345	A: 312 (45) G: 378 (55)	[21]
	109	A: 100 (46) G: 118 (54)	[19]
	298	A: 303 (51) G: 293 (49)	[22]
Japanese	168	A: 106 (32) G: 226 (68)	[18]
	97	A: 62 (32) G: 132 (68)	[19]
Indian	250	A: 30 (6) G: 470 (94)	[23]
Iranian	70	A: 71 (51) G: 69 (49)	[24]
Turkish	97	A: 78 (40) G: 116 (60)	[25]
	183	A: 142 (39) G: 224 (61)	This study

*Data obtained from control groups

considered as meaningful SNPs significantly in Europeans and Asians [16]. However, more research is needed because of the large heterogenicity of previous studies.In our study, the mutant allele frequency of *CYP19A1* rs2414096 (A) was 0.39, compatible with the genomic databases (Table 1). In previous Eurupean and Caucasian studies, the frequency of rs2414096 variant type (A) was found as 0.46 and 0.50 respectively. It was 0.45−0.51 in Chinese, 0.06 in Indian and 0.51 in Iranian populations. In a previous study conducted in Turkish population by [25], the frequency of the variant allele was reported as 0.40 which is quite similar to our results in the present study (Table 3).

In the present study, we classified female subjects as normal or overweight based on their BMI values according to WHO classification. The aim in this part of the study was to investigate the association between hyperandrogenism biomarkers and obesity in adolescent female subjects. We measured some levels of hormones linked with hyperandrogenism in serum samples of these individuals. On the other hand we evaluated the roles of *DENND1A* rs10818854 and *CYP19A1* rs2414096 polymorphisms in individual susceptibility to obesity.

The age distribution and serum hormone levels of individuals in normal or overweight groups are presented in Table 4. There was no statistically significant difference in terms of age, LH, FSH, LH/FSH and total testosterone levels between normal and overweight groups. However, the overweight group has statistically significantly lower ($p < 0.001$) SHBG and higher FAI levels ($p = 0.004$) compared to the normal group. Since FSH levels vary over age, we adjusted FSH levels for age and there was still no statistically significant difference between groups (p = 0.519; data not shown).

SHBG is a plasma glycoprotein with high binding affinity to testosterone and dihydrotestosterone and lower affinity to estradiol. SHBG is synthesized in the liver, and its plasma level is important in the regulation of plasma free and albumin-bound androgens and estrogens. Obesity and particularly excess visceral fat, known risk factors for

Table 4 Demographic characteristics and serum hormone levels of normal and overweight individuals

	Normal (BMI < 25 kg/m^2)	Overweight (BMI ≥ 25 kg/m^2)	*p* value[¶]
n[#]	74	46	
Age (year)	15.0 (15.0–16.5)	16.0 (14.0–17.0)	*0.928*
LH (IU/L)	5.4 (4.1–7.7)	5.8 (4.0–8.3)	*0.731*
FSH (IU/L)	5.3 (4.1–6.7)	5.1 (3.8−6.0)	*0.389*
LH/FSH	1.0 (0.7−1.7)	1.2 (0.8−1.8)	*0.362*
TotalTestosterone (nmol/L)	0.28 (0.18−0.40)	0.29 (0.20−0.44)	*0.843*
SHBG (nmol/L)	4.8 (3.5–7.1)	2.7 (1.9−3.6)	***< 0.001***
FAI (%)	5.2 (2.6–10.0)	9.1 (5.3–18.0)	***0.004***

LH Luteinizing hormone, *FSH* follicle-stimulating hormone, *SHBG* sex hormone-binding globulin, *FAI* free androgen index

The values are in median (25th−75th percentile); Mann Whitney U test

[¶] p < 0.025 has been considered as statistically significant according to Bonferroni Correction

[#] Only women individuals have been analyzed

cardiovascular and metabolic diseases, are associated with decreased testosterone levels in males and SHBG levels in both sexes [26]. In accordance with this findings, the SHBG levels in overweight individuals in our study have significantly lower SHBG levels ($p < 0.001$; Table 5). Calculating the percentage ratio of total testosterone to SHBG concentration, called free androgen index (FAI), has increasing application as diagnostic tools for hyperandrogenism [27]. As expected, due to possible hyperandrogenic mechanism in obesity, the FAI in our study was significantly higher in overweight individuals ($p = 0.004$; Table 4) compared to normal weighted individuals. These findings in our study supported the positive association between hyperandrogenism and obesity.

To strengthen these findings and to evaluate the role of genetic susceptibility in obesity, we investigated two different SNPs (*CYP19A1* rs2414096 and *DENND1A* rs10818854) that have been previously associated with hyperandrogenism. The frequency distribution of these SNPs in normal and overweight individuals are presented in Table 5. The variant allele (A) frequency of *CYP19A1* rs2414096 in individuals at normal (< 25) or overweight BMI ranges (≥ 25) were 0.43 and 0.38 respectively. The variant allele frequency of rs10818854 in the same groups were 0.096 and 0.107 respectively. For *CYP19A1* the presence of AG, AA or AG + AA genotype did not have a statistically significant effect on the overweight prevalence ($p > 0.05$). Similarly, the presence of G allele vs A allele of *CYP19A1* did not have a statistically significant effect on obesity risk (OR = 0.803; 95%CI = 0.461–1.400). Similar results were obtained for *DENND1A* gene, and no statistically significant effect of variant (A) allele were observed on obesity risk (OR = 1.135; 95% CI = 0.463–2.784).

Table 5 Genotypic distribution and allele frequencies in normal and overweight individuals

	Normal (BMI < 25 kg/m^2)	Overweight (BMI ≥ 25 kg/m^2)	*p* value	OR (%95 CI)
DENND1A (rs10818854)				
GG	55 (%80.9)	33 (%78.6)	–	1.000
AG	13 (%19.1)	9 (%21.4)	*0.769*	1.154 (0.445–2.993)
DENND1A (rs10818854)				
G	123 (%90.4)	75 (%89.3)	–	1.000
A	13 (%9.6)	9 (%10.7)	*0.781*	1.135 (0.463–2.784)
CYP19A1 (rs2414096)				
GG	23 (%33.8)	16 (%38.1)	–	1.000
AG	31 (%45.6)	20 (%47.6)	*0.862*	0.927 (0.396–2.171)
AA	14 (%20.6)	6 (%14.3)	*0.409*	0.616 (0.195–1.945)
AG + AA	45 (%66.2)	26 (%61.9)	*0.649*	0.831 (0.373–1.849)
CYP19A1 (rs2414096)				
G	77 (%56.6)	52 (%61.9)	–	1.000
A	59 (%43.4)	32 (%38.1)	*0.439*	0.803 (0.461–1.400)

OR odds ratio, *CI* confidence interval

Only women individuals have been analyzed; The values are represented as n(%); Univariate logistic regression

There are various SNPs determined in *CYP19A1* gene and rs2414096 is one of the SNPs which is associated with increased risk of some diseases. This is an intronic SNP close to exon 3 in aromatase and was associated with precocious pubarche, post-menarchal ovarian hyperandrogenism and PCOS [18, 28]. There are many studies about causes and effects of obesity and most of them pointed elevated androgen levels in obese individuals [29]. The mechanism via the excess adipose tissue links with hyperandrogenism remains controversial, however the altered levels of aromatase activity which has a key role in converting androgens to estrogens might have a role in obesity risk. *CYP19A1* is a key enzyme for estrogen biosynthesis in the step of aromatization of androgens to estrogens and thus body fat distribution. This enzyme not only plays a rate-limiting role for the conversion of C19 androgens to aromatic C18 estrogens in reproductive organs but it has also a critical role in adipose tissue, muscle, liver, and brain. Due to aromatase's converting ability of androgens to aromatic estrogens, aromatase gene *(CYP19)* polymorphisms, that can modify the aromatase activity [30, 31] have been associated with serum sex hormone levels in several studies [18, 20, 21, 32–34].

There are a few studies about *CYP19A1* rs2414096 polymorphism, and to our knowledge, none of these studies investigated its association with obesity in adolescent girls. In a study by Sowers et al. 3 aromatase gene SNPs (rs936306, rs749292, rs2414096) have been associated with variation in serum androgen concentrations among women, both within and between racial/ethnic groups. In the same study, Caucasian women markedly have lower sex hormone binding globulin (SBGH) levels among those with the AA genotype of the rs2414096 polymorphism compared with other genotypes, after adjusting for age and body mass index [18]. Besides, it has been shown that obese males carrying A allele of the rs2414096 SNP have increased risk of infertility by almost 2 times [35].

Due to limited number of published studies on SNP *DENND1A* rs10818854 and its role in obesity, it is difficult to compare our results with the previous studies. Although, *DENND1A* gene polymorphisms were not correlated with obesity as strong as *CYP19A1* gene in recent studies, it is clear that it has a role in hyperandrogenism. After a large GWAS study that has been strongly associated the *DENND1A* rs10818854 SNP and the risk of PCOS, the

number of studies investigating this association increased recently [15, 36].

Our study is the first that investigated the frequency of variation in *DENND1A* gene and its role in obesity in a Turkish population. Our results showed that this SNP variation in Turkish population is between the range of previous findings in different populations. The small sample size and the lack of a power analysis due to insufficient previous data are the limitations of our study. However, our results might be evaluated as preliminary findings since there is no or limited data for the frequency of *DENND1A* rs10818854 and *CYP19A1* rs2414096 and their association with obesity in Turkish population.

In conclusion, we evaluated the frequencies of *CYP19A1* rs2414096 and *DENND1A* rs10818854 polymorphisms in overweight and normal individuals and didn't find any significant difference between these groups which demonstrates a lack of association between obesity risk and these SNPs from a genetic susceptibility point of view. We think the statistical power should be enhanced with a larger sample size in future studies. Moreover, it might worth to investigate these SNPs and their associations with PCOS or other hyperandrogenism linked diseases in Turkish population since their frequencies were similar with the previous findings in different populations.

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Compliance with ethical standards

Conflicts of interest The authors declare there is no conflict of interest.

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