RESEARCH ARTICLE

Analysis of Some Candidate Genes of Polycystic Ovary Syndrome for Iraqi Women

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the multifunctional disorders related to endocrinopathy in females of reproductive age. Most of the family studies reported that PCOS is related to a genetic basis by noted the high number of female affected between the relatives and PCOS patients. The relation between inheritance and PCOS still unknown, and the most recent studies indicate that this syndrome could be a multifunctional trait.

The isolated DNA by using a special reagent kit DNA-express, from the peripheral blood leukocytes of women were diagnosed with PCOS their age from 25 to 44 years old, and 23 healthy women as a control group, the amplification reaction used (SNP-express) by PCR and specific kits for three candidate genes (MTHFR, CYP17 and CYP21). The detected the produced by horizontal electrophoresis method and then visualized into identified the alleles in different genes its used.

The present results showed a significant difference between the candidate genes and polycystic ovary disorder under two level $p < 0.05$ and $p < 0.01$. The significant difference in MTHFR(C677T) gene was the polymorphic marker T < C and the $p < 0.05$, $p < 0.01$ values and carriers Genotype *C/T* alleles are increased the risk of this disorder. In comparison, the significant difference in CYP17 P450-C17 gene was C < P in the significance level $p < 0.05$ and the patients carriers heterozygote P/C more candidate to development the PCOS and the patients whose carriers CYP21 R341P gene also candidates by effect by this syndrome whose carriers heterozygote R/P under the $p < 0.05$, $p < 0.01$ significance levels.

For known the interaction between the genes used the multifactor dimensionality reduction method (MDR method) and shown the estimation between the polymorphic all the candidate genes and associated with PCOS and development with this disorder.

Keywords: CYP17 and CYP21 genes, MTHFR gene, PCOS.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the multifunctional disorder which related with endocrinopathy in females of reproductive age.[1] PCOS could be defined as the relation between of chronic anovulation and hyperandrogenism.[2] Most family studies reported that PCOS was related with a genetic basis by noted a high number of female affected between the relatives and PCOS patients.[3] The relation between the inheritance and PCOS still unknown, and the most recent studies indicate that this syndrome could be a multifunctional trait.[4] This means that many genes interact with many other factors such as environmental factors to happened the phenotype and given biochemical parameters, like fasting insulin levels or hyperandrogenism, this parameter, suggesting that many clinical signs could be transmitted as X-linked or maybe mendelian autosomal dominant.[5] The MTHFR gene is considered one of POCS's most important genes because this gene plays an important role in established methylenetetrahydrofolate reductase (MTHFR) enzyme responsible for folates metabolism.[6] This enzyme's activity related with a mutation in MTHFR gene; this mutation from missense type by substitution between cytosine and thymine at nucleotide 677 and the result was a conversion of alanine to valine.[7] There are many alleles belong the CYP genes which carry from people, there are simple variation in sequences of it due to change in nucleotide, these variation carry out people difference in the tolerance against toxins or drugs.[8] The pathways of Cytochrome P450 are classified according to the gene sequences; they are classified into a family number (e.g., CYP1, CYP2) and a given some of the subfamily letter (e.g., CYP1A, CYP2D) and are then according to the personal enzyme (e.g., CYP1A1, CYP2D6). Both of the CYP17 and

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CYP21 genes belong to cytochrome P450 Family 17 and 21. These genes coding proteins are monooxygenases which catalyze involve many reactions in drug metabolism and synthesis of lipids, cholesterol, and steroids. This protein localizes to the endoplasmic reticulum. These enzyme activities consider as a key to a steroidogenic pathway that produces progestins and estrogens. any mutations in this gene lead to enzyme deficiency and adrenal hyperplasia.[9,10]

SUBJECTS AND METHODS

Collection of Blood Samples

There were about thirty eight women surveyed with difficult in delivery, their age from 25 to 44 years old, DNA extracted from peripheral blood leukocytes of women were diagnosed with PCOS, and 23 healthy women as control group, the Blood samples were collected from different Hospitals in Baghdad-Iraq.From September to October 2019.

EXTRACTION OF DNA

from peripheral blood (white blood cells) done the isolated the DNA by using a special reagent kit DNA-Express, which is used to isolate the DNA from different natural materials. By taking about 1000 uL whole blood with mix very well this necessary until the blood notice smooth.then centrifugation the tubes at 3000 rpm. At room temperature for 5 minutes. Remove the pipette plasma which contained the leukocytes. After that frozen and then extract the DNA by used reagent "DNA Express according to the standard procedure. The amplification reaction used (SNP-express) by PCR and specific kits for genes. The detected the produced by horizontal electrophoresis method and then visualized into identified the alleles in different genes its used.

The assay is based on the simultaneous conduction of two amplification reactions with two pairs of allele- specific primers, one pair of primers complementary to the "normal" allele, the second complementary to the modified allele. This analysis allows us to identify both heterozygous carriers of the polymorphism and the homozygous state. The statistical treatment of results

When evaluating the data researched, the X- square criterion: $X2 = \Sigma (P-E)\ 2/E$, where P is the exponent in the study group, E - data control group. If the calculated criterion does not exceed the table for the significance level of 0.05, the data on the frequency of genotypes, we obtained the corresponding control.

The formulas calculated frequency of alleles:

$$p + q = 1$$

where q - the frequency of a polymorphic variant of the gene.

Determining the ratio of genotype frequencies were performed using the Hardy-Weinberg equilibrium, according to which:

$$p2AA + 2pq\ Aa + q2aa = 1$$

The multifactor Dimensionality Reduction method (MDR) used this program to estimate the combinations of polymorphic variants between three candidate genes associated with PCOS development.

RESULTS AND DISCUSSION

The difference between polymorphic markers of three candidate genes shown in this results was The present results showed a significant difference between the candidate genes and polycystic ovary disorder under two-level $p < 0.05$ and $p < 0.01$. the significant difference between the cases and controls in MTHFR(C677T) gene shown the value was the polymorphic marker T<C and the $p < 0.05$, $p < 0.01$ values which mean the MTHFR gene related with the development the PCOS and carriers Genotype *C/T* alleles have increased the risk of this disorder (Table 1). While the significant difference in CYP17 P450-C17 gene was C < p in the significance level

Table 1: Distribution of allele and genotype of MTHFR(C677T) gene

	Frequency of alleles and genotypes		*value χ2*		*Level of*
Alleles and Genotypes	*Cases (No. = 48)*	*Controls (No = 23)*	*(p)*	*OR[CI 95%]*	*significance*
Allele *C*	0.604	0.804	5.63(0.02)*	0.16–0.86	0.05, 0.01
Allele *T*	0.396	0.196		1.17–6.21	
Genotype *C/C*	0.292	0.652		0.08–0.63	
Genotype *C/T*	0.625	0.304	6.84(0.009)**	1.32–11.03	
Genotype *T/T*	0.083	0.043		0.21–18.98	

*(p <0.05), **(p <0.01), NS: Non-significant.

Table 2: Distribution of allele and genotype of CYP17 P450-C17 gene

	Frequency of alleles and genotypes				
Alleles and genotypes	*Cases (No. = 48)*	*Controls(No = 23)*	*value χ2 (p)*	*OR[CI 95%]*	*Level of significance*
Allele *P*	0.552	0.739	4.58(0.03)*	0.20–0.94	
Allele *C*	0.448	0.261		1.06– 4.97	
Genotype *P/P*	0.208	0.565		0.07–0.60	0.05
Genotype *P/C*	0.688	0.348	5.85(0.02)*	1.44–11.82	
Genotype *C/C*	0.104	0.087		0.22–6.82	

* (p <0.05), ** (p <0.01), NS: Non-significant.

Table 3: Distribution of allele and genotype of CYP21 R341P gene

Alleles and genotypes	*Frequency of alleles and genotypes*		*value χ2 (p)*	*OR[CI 95%]*	*Level of significance*
	Cases (No. = 48)	*Controls (No = 23)*			
Allele *R*	0.500	0.696	4.84(0.03)*	0.21 – 0.92	
Allele *P*	0.500	0.304		1.09 – 4.81	
Genotype *R/R*	0.146	0.435		0.07 – 0.70	0.05, 0.01
Genotype *R/P*	0.708	0.522	7.09(0.008)**	0.80 – 6.22	
Genotype *P/P*	0.146	0.043		0.43 – 32.52	

* ($p < 0.05$), ** ($p < 0.01$), NS: Non-significant.

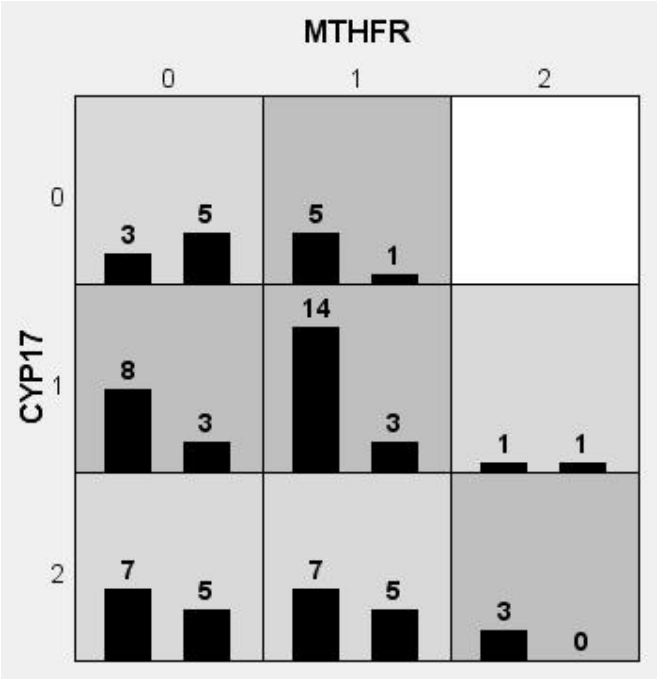

Figure 1: Comparison between two-locus genotypes MTHFR and CYP17 genes (dark gray mean high-risk genotype, light gray mean low risk)

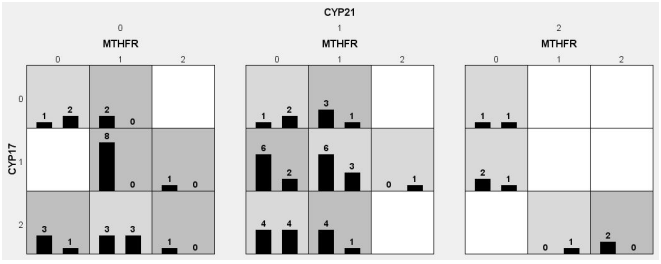

Figure 2: Comparison between three locus genotypes CYP21, MTHFR, and CYP17 genes (dark gray mean high-risk genotype, light gray mean low risk)

$p < 0.05$ and the patients carriers heterozygote P/C more candidate to development the PCOS and the patients whose carriers CYP21 R341P gene also candidates by effect by this syndrome whose carriers heterozygote R/P under the $p < 0.05$, $p < 0.01$ significance levels Tables 2, and 3.

The interaction between the genes used MDR method and shown the estimation between the polymorphic all the candidate genes and associated with PCOS and development with this disorder. The combination between MTHFR and CYP17 genes by two-locus model which indicate the significance and increase the risk development in heterozygote's alleles (dark gray) about 14 patients and in 3 healthy women Figure 1, while in three loci model shown the interaction between three candidate genes MTHFR, CYP 21 and CYP17 the most ratio in the interaction between heterozygote alleles MTHFR, CYP 21 and CYP17 about 8 patients Figure 2.

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