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Research Article

Assessing the relationship between Iraqi patients' risk of type 2 diabetes and genetic variations in the *CYP17A1* gene

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Abstract

The characteristic feature of type 2 diabetes mellitus is a rise in blood sugar (glucose) because of insufficient insulin secretion from pancreatic beta cells, ineffective insulin, somatic cell resistance to insulin, or a combination of these factors. The purpose of this study is to assess the impact of *CYP17A1* genetic polymorphisms at the rs6163 and rs6162 variant sites on the risk of type II diabetes. The tenth chromosome's mitochondrial *CYP17A1* gene is linked to high blood pressure, insulin resistance, and polycystic ovarian syndrome in addition to encoding a crucial enzyme involved in the manufacture and metabolism of steroid hormones. As the homologous genetic genotype AA and allele A showed an increase in the diabetic group, the study's results demonstrated the presence of genetic heterogeneity at the site of heterogeneity C/A rs6163 in the gene *CYP17A1*. This is a causative factor according to the Fisher's probability, as the probability value (P = 0.026) as there are significant differences between patients and healthy people, and according to the values of the odd ratio, which reached 9.47 and 2.80, respectively. According to the Fisher's probability, the homogeneous genetic type CC and allele C are protective factors against the disease. The probability value was (P = 0.055), indicating that there are significant differences between patients and healthy individuals. The odds ratio values reached 0.24 and 0.36. According to the probability of Frisher, the homogeneous genotype GG and allele G are protective factors; the probability value is (P = 0.226), where there are no significant differences between patients and healthy individuals, and according to the value of the odds ratio, which amounted to (0.44 and 0.42), respectively. The study's results indicated the presence of genetic heterogeneity at the site of the heterogeneity, rs6162 G/A. According to the Fisher's probability, genotype AA and allele A are regarded as causal agents; the corresponding odds ratio values were 2.80 and 9.47, respectively. The p-value was found to be (P = 0.026).

Keywords: Cytochrome P450, Genotype distribution, Molecular genetics, Odd ratio

Introduction

Based on data from the International Diabetes Federation, diabetes mellitus is one of the most prevalent diseases globally. Globally, the monthly prevalence rate is rising, and by 2030, over 550 million individuals are predicted to be affected. Diabetes is known to come in several forms. Among them, type 1 diabetes (T1D) and type 2 diabetes (T2D) are the most well-known (Kene et al., 2021). This could account for over 90% of all diabetes cases globally (Kousar, 2019). According to (Ochoa-Guzmán et al., 2021), type 2 diabetes is characterized by an increase in blood glucose levels because of either a lack of insulin secretion by the pancreatic beta cells, a poor effect of insulin and cell resistance to it, or both. This is known as the HbA1c% cumulative sugar. Given that a high level of cumulative glucose is linked to a reduction in insulin secretion and sensitivity, it is one of the most significant metrics used to track blood glucose levels (Jaacks et al., 2016). It's apparent that genetic predisposition and environmental factors, including nutrition and obesity, interact to generate type 2 diabetes, even though the pathological process and causes of the disease are still unclear (Yaribeygi et al., 2019). The scientific understanding of diabetes has grown significantly in the last few decades (Akhtar et al., 2019). Numerous genetic differences have been connected to the disease's onset and the malfunction of the pancreatic beta cells. More than 140 common genetic variants linked to type 2 diabetes have recently been found through genome-wide genetic association studies (Qin et al., 2020). Despite this success (Regufe et al., 2020), these variants are sadly only able to explain 10-15% of the genetic causes of type 2 diabetes because each genetic variant has a very small effect. Many researchers concur that discovering every genetic variation is unlikely (Sladek et al., 2007). The cytochrome P450 family of mitochondrial genes, which includes the *CYP17A1* gene, is thought to be necessary for the manufacture of steroid hormones. The *CYP17A1* gene has eight exons and seven introns, and it is found on chromosome 10q. This gene is 7.5 kb long (Wang et al., 2019). Type 2 diabetes and polycystic ovarian syndrome are among the disorders caused by mistakes or mutations in the *CYP17A1* gene, which is expressed in a number of tissues, including the gonads and adrenal cortex (Skibola et al., 2005). One of the factors linked to the risk of type 2 diabetes is genetic polymorphism of the *CYP17A1* gene at the rs743572 T>C variant site (Rezgoun et al., 2023).

The goal of this research is to investigate at the relationship between the *CYP17A1* gene's genetic polymorphisms at the rs6163 and rs6162 variant locations and the risk of type 2 diabetes.

Martial and methods

The research was conducted at the Molecular Genetics Lab of the Faculty of Education for Pure Sciences at the University of Diyala in Iraq. The present research focused on patients with type 2 diabetes and healthy individuals who visited the consulting clinics and Baaquba Teaching Hospital. Blood samples were obtained from healthy persons and patients diagnosed with type 2 diabetes between October 2022 and February 2023. There were a total of 80 study samples: 30 were from healthy people and 50 were from those with type II diabetes. The System gDNA Miniprep Blood ReliaPr extraction kit, which was provided by Bioneer in South Korea, was used to extract DNA. Prepare a polymerase chain reaction mixture to amplify the *CYP17A1* gene at the site of the variants rs6162 and rs6163, which consists of 1.5 µl forward primer 5"-CTGGAAGCCCCATTCTAGGC-3" and 1.5 µl reverse primer 5"-TGTGCCCTAGAGTTGCCACA-3 , 3 µl DNA, 5 µl master mix, and 14 µl free nuclease water. The total volume of the reaction product was 25 microliters for each sample. The polymerase chain reaction apparatus was then filled with the reaction mixture for the samples of healthy individuals and diabetic patients. The apparatus was set up with the following reaction conditions: five minutes of initial denaturation at 94°C, thirty seconds of denaturation at 94°C, thirty seconds of primer annealing at 63°C, five minutes of extension at 72°C, and five minutes of final extension at 72°C. This was done because there were thirty-five cycles total of denaturation, primer annealing, and extension. Following the acquisition of the polymerase chain reaction data, the samples were electrophoresed on a 1% agarose gel for 1.5 hours at a voltage of 90. Shipped to Macrogen Company in South Korea, the amplification product allowed for Sanger nucleotide sequencing of the *CYP17A1* gene. Based on the investigation of the nucleotide sequencing data using the Genius application, the Hardy-Weinberg equation was applied to determine which genotype was a protective factor and which genotype was a causal factor.

Results and Discussion

Amplification product of the *CYP17A1* gene in type II diabetic patients and healthy controls for the coding sequence containing both variations, rs6163 and rs6162.

Figure 1 shows the outcomes of *CYP17A1* gene amplification from mitochondrial DNA in both healthy individuals and type 2 diabetic patients. Should the amplification results show that the

variants rs6163 and rs6162 have a molecular weight of 454 bp at the site of the resulting bands in every patient and sample that is healthy individually.

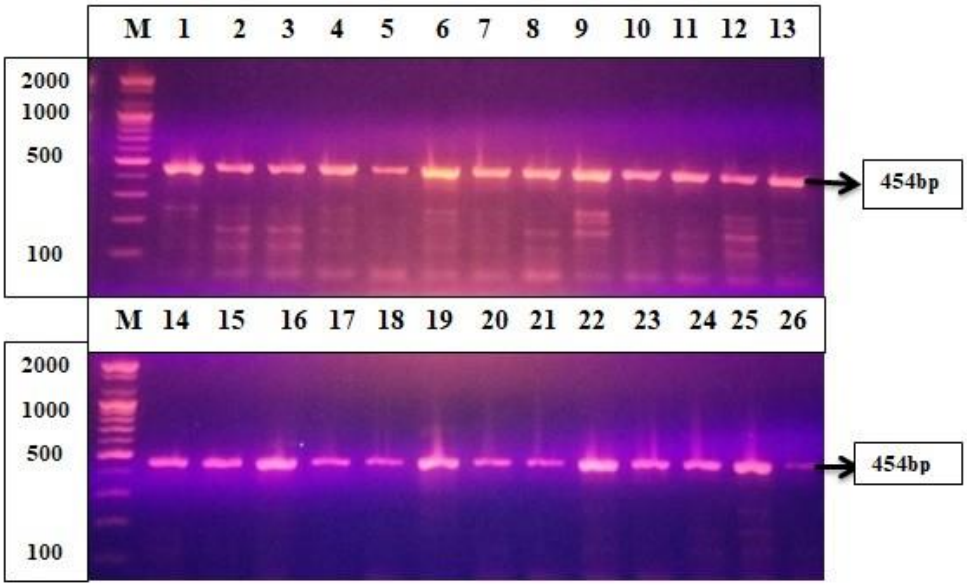

Figure 1. The result of amplification of part of the *CYP17A1* gene for the coding segment that includes the variants rs6163 and rs6162 in type II diabetic patients of the Iraqi population, transferred on agarose gel at a concentration of 1.5% for an hour and a half, at an electrical potential of 90 volts, stained with ethidium bromide dye, and photographed under ultraviolet radiation. The numbers from 1 to 13 represent patient samples, and 14 to 26 represent healthy samples.

When compared to the reference sequence of the gene at the location of the variants, the nucleotide sequence analysis of the *CYP17A1* gene revealed the presence of a point mutation of the transition and transversion type for the rs6163 and rs6162 variants, as shown in Figures 2 and 3, respectively. The results of the current investigation showed a connection between the risk of type 2 diabetes in the Iraqi population and genetic variants in the *CYP17A1* gene. This connection was found as a transition-type point mutation at the rs6163 C/A variant location. The type 2 diabetes patient group had a considerably larger percentage of patients with homozygous genotype AA = 7 than the control group AA = 1 (29.16 and 58.67, respectively, compared to 4.16 and 33.33, respectively). Fisher's probability was also calculated. The genotype AA and the A allele are considered to be causal variables for the disease, as evidenced by the respective likelihood values of 9.47 and 0.36, given the significant differences between patients and healthy persons. The value of probability was P = 0.026.

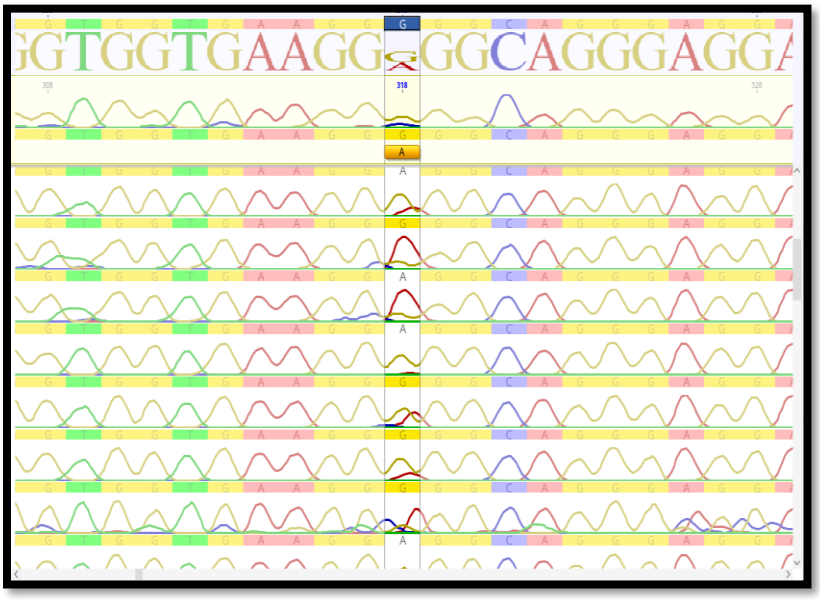

Figure 2. The position of the rs6162 G/A variant and the kind of mutation are displayed by comparing the alignment of the nitrogenous bases of a portion of the *CYP17A1* gene between samples from type II diabetic patients, healthy controls, and the GenBank sample (NCBI, 2023).

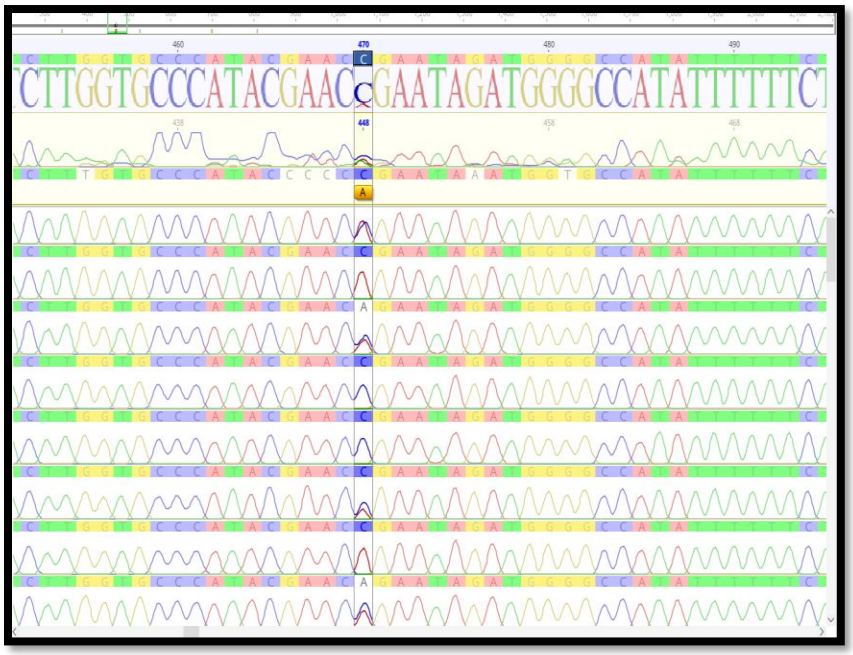

Figure 3. The position of the rs6163 C/A/T variant and the kind of mutation are displayed by comparing the alignment of the nitrogenous bases of a portion of the *CYP17A1* gene between samples from type II diabetic patients, healthy controls, and the GenBank sample (NCBI, 2023).

The Hardy probability values for the patient and healthy groups in Table 1 reached 0.3272 and 0.2167, respectively, indicating that there are no statistically significant differences between the observed and expected values. This suggests that the population is in a state of genetic equilibrium. (Yamada et al., 2013) found a correlation between the risk of prostate cancer and a genetic variation in the *CYP17A1* gene at the rs6163 variant position. Research has demonstrated a correlation between the AA genotype and elevated androgen hormone release, which is indicative of an increased risk of prostate cancer. At an odds ratio for the AA genotype (OR; 1.76-2.43), the GA genotype indicated reduced levels of the androgen hormone. The study disagreed with the findings of the researcher (Al-Rubae'i et al., 2017), who stated that the *CYP17A1* gene's genetic variations are unrelated. Type II diabetes is a possibility at the location of the rs6163 variation.

Three patients with the homozygous CC genotype and twenty eight patients with allele C were detected. The percentages of 12.5 and 58.33, respectively, indicate a significant decline in the type 2 diabetes patient group when compared to the control group (healthy individuals), which recorded nine and sisteen , The percentages of 37.5 and 33.33. Given that there are statistically significant differences between patients and healthy individuals, the homozygous CC genotype and the C allele are thought to be protective factors against the disease, as indicated by the odds ratios of 0.24 and 2.80 in Table 2.

Table 1. Expected frequencies of genotype and alleles of the coding region rs6163 C/A/T for CYP17A1 by using Hardy-Weinberg equilibrium

Genotypes and Alleles Groups		CC	CA	AA	C	A	Hardy P-values
Patients Genotypes	Observed no.	3 12.5%	14 58.33%	7 29.16%	20 41.67%	28 58.33%	0.3272 NS
	Expected no.	4.17 17.36%	11.67 48.61%	8.17 34.03%	Not diagnosed		
Control Genotypes	Observed no.	9 37.5%	14 58.33%	1 4.16%	32 66.67%	16 33.33%	0.2167 NS
	Expected no.	5.51 22.96%	11.98 49.91	6.51 27.13	Not diagnosed		

NS: Not Significant, ** 0.001, * Significant P≤ 0.05

Table 2. Genotype distribution and allele frequency of CYP17A1 rs6163 C/A/T SNPs

Genotype CYP17A1 rs6163 C/A/T	Patients No.(%)	Control No.(%)	Fishers/P-value	O.R. (C.I)

CC	3 (12.5%)	9 (37.5%)	0.055*	0.24 (0.05 - 1.03
CA	14 (58.33%)	14 (58.33%)	0.770NS	1.00 (0.31 - 3.23)
AA	7 (29.16%)	1 (4.16%)	0.026*	9.47 (1.25 - 223.49)
Total	24 (100%)	24 (100%)		
Allele	Frequency			
A	20 (41.67%)	32 (66.67%)	O.R. (C.I.) = 0.36 (0.15 - 0.83)	
C	28 (58.33%)	16 (33.33%)	O.R. (C.I.) = 2.80 (1.21 - 6.49)	

NS: Not Significant, ** 0.001, * Significant P≤ 0.05

Table 3. Expected frequencies of genotype and alleles of the coding region rs6162 G/A for CYP17A1 by using Hardy-Weinberg equilibrium

Genotypes and Alleles Groups		GG	GA	AA	G	A	Hardy P-values
Patients Genotypes	Observed no.	5 20.83%	12 50%	7 29.16%	22 45.83%	26 54.17%	0.9727 NS
	Expected no.	5.04 21.01%	11.92 49.95%	7.04 21.01%	Not diagnosed		
Control Genotypes	Observed no.	9 37.5%	14 58.33%	1 4.16%	32 66.67%	16 33.33%	0.1258 NS
	Expected no.	10.67 44.44%	10.67 44.44%	2.67 11.11	Not diagnosed		

NS: Not Significant, ** 0.001, * Significant P≤ 0.05

Table 3's findings demonstrated that there were no statistically significant variations between the genotype and allele values that were observed and expected for either the group of diabetes patients or the group of healthy individuals. The Hardy probability for the patient group was P = 0.9727, while the group of healthy individuals had P = 0.1258. This suggests that there is genetic equilibrium within the population. (Ragia et al., 2009) investigated the relationship between genetic variations in the *CYP2C9*3* gene and type 2 diabetes. They showed that the homozygous AA genotype at the variant position rs1057910 was associated with a higher risk of developing diabetes with high HbA1c levels. (Yamada et al., 2013) looked at type II diabetes patients' *CYP17A1* gene variants. The results of the study indicate that there is no association between the risk of developing type 2 diabetes and the variant site of a gene's genetic polymorphism.

The information in Table 4. Twenty-two patients had the G allele and five patients had the homozygous genotype GG, according to the mutation at rs6162. Given that the homozygous genotype GG and allele G had odds ratio values of 0.44 and 0.42, respectively, it is believed that they acted as protective factors against the illness. Compared to the healthy and control group, whose percentages reached 37.5 and 66.67, the group of patients with type 2 diabetes showed a reduction, with percentages of 20.83 and 45.83, respectively. Fisher's probability (P = 0.226) indicates that there are no discernible differences between the sick and the well.. The results also showed that type 2 diabetes individuals carried the A allele twenty six, whereas seven had the homozygous genotype AA. In contrast to the one in control group, which had percentages of 29.16 and 4.16, respectively, the type 2 diabetes patient group had a higher percentage. Based on Fisher's probability, P = 0.026 was determined, and the probabilities are 4.16 and 33.33. Based on the significant differences seen between patients and healthy individuals, the A allele and genotype AA are considered to be causative factors for the disease. The odds ratio, which was 2.36 and 9.47, respectively, supports this. The results also showed that 12 type II diabetic patients had the variant genotype GA and 26 its G allele compared to the healthy group of 14 and 32, respectively. Since Fisher's probability P = 0.579 indicates that there are no significant differences between patients and healthy individuals, this genotype GA and its G allele is associated with a lower risk of disease, with odds ratios of 0.71 and 0.42, respectively.

Table 4. Genotype distribution and allele frequency of CYP17A1 rs6162 G/A SNPs

Genotype CYP17A1 rs6162 G/A	Patients No.(%)	Control No.(%)	Fishers/P-value	O.R. (C.I)
GG	5 (20.83%)	9 (37.5%)	0.226 NS	0.44 (0.11 - 1.62)
GA	12 (50%)	14 (58.33%)	0.579 NS	0.71 (0.22 - 2.29)
AA	7 (29.16%)	1 (4.16%)	0.026*	9.47 (1.25 - 223.49)
Total	24 (100%)	24 (100%)		
Allele	Frequency			
G	22 (45.83%)	32 (66.67%)	O.R. (C.I.) = 0.42 (0.18 - 0.98)	
A	26 (54.17%)	16 (33.33%)	O.R. (C.I.) = 2.36 (1.03 - 5.46)	

NS: Not Significant, ** 0.001, * Significant P≤ 0.05

Conclusion

The *CYP17A1* gene's rs6162 and rs6163 variations are linked to genetic polymorphisms that increase the risk of type II diabetes. The homozygous AA genotype was a causative factor for the disease related to the rs6162 variant, but the GG and GA genotypes were protective factors. When it comes to the rs6163 mutation, the homozygous CC genotype confers protection against the condition, while the CA and AA genotypes cause the disease.

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