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Evaluating The Frequency of ER2 Polymorphisms in Coronary Heart Disease Patients and Healthy Controls

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ABSTRACT

Background: The leading cause of mortality and rising healthcare costs globally is coronary heart disease (CHD). Interactions between environmental and genetic variables may be the cause of CHD. Research on the role of several genes in the etiology of coronary heart disease is still underway. The estrogen receptor 2 (ER2) gene, which is present in the layers of the vessel wall in the vasculature, is one of the genes thought to be related to CHD. **Aim**: The objective is to evaluate the rs1271572 A/T SNP of the ER2 gene's connection with CHD in a sample of Iraqis and investigate how gender affects the onset of the illness. **Method**: A sample of CHD patients and a group of healthy people were the subjects of a case-control study. Participants' serum lipid levels were assessed, and T-ARMS was used to genotype the ER2 gene, rs1271572 A/T SNP. **Results**: The carriers of the variant allele in the sick group were significantly higher (P: 0.0022-0.015) than in the control group, according to the assessment of genotype frequencies under various inheritance models. It was discovered that the odd ratio was around 2. It was clear that the co-dominant model had the lowest Akaike information criterion (AIC) score. The recruited participants' genotype-gender cross-classification interaction was assessed. With an odd ratio of 2.28, a significant correlation was demonstrated in females with the heterozygous variant genotype. When the biochemical parameter and BMI values were compared to the genotype frequencies, bearers of the variant alleles showed noticeably higher levels. However, in a comparable comparison, HDL-C significantly decreased. It was discovered that the variant alleles were substantially associated with an increase in atherogenic parameters.**Conclusion**: In the Iraqi population, the ER2 gene polymorphism, rs1271572 SNPs, is a risk factor for the development of CHD. Elevated atherogenic lipids are associated with this relationship. Females are more susceptible to the illness than males due to the mutant allele.

KEYWORDS

ER2 polymorphisms, coronary heart disease, rs1271572 A/T SNP

INTRODUCTION

In both industrialized and developing nations, coronary heart disease (CHD) is the leading cause of morbidity and mortality (Safiri et al., 2022). In Iraq, it is the leading cause of disease-related mortality, accounting for around one out of every three fatalities among those over 35. According to age-adjusted mortality rates, Iraq is ranked 23rd in the world (WHO, 2020). The clustering of cardiovascular risk factors is primarily responsible for our nation's concerning CHD rate (Hamid, 2016). According to Buja et al. (2019), atherosclerosis is the main cause of coronary heart disease (CHD). It begins with coronary arterial streaks of fatty tissue lesions that constrict the coronary arteries or their branches, preventing myocardial perfusion. Environmental and genetic factors have equally important roles in the multifactorial aspects of CHD (Li et al., 2019). Polygenic, as identified by Collet et al. (2021) view it; several genes

with different alleles that could play from minor to moderate roles in effect. Several gene polymorphisms have been identified to associate coronary heart disease (Severino et al., 2023; Hussain et al., 2019). The gene that has been identified to be more reactive for CHD is ER2, this gene has alleles located in the layers of vessel wall circulation(Bottner et al., 2014).

ER2 protein contains 530 amino acids (Leung et al., 2006). It is demonstrated by both arterial endothelial cells and vascular smooth muscle cells (VSMCs) (Gaido et al., 2000). The ER2 gene is located at 14q23.2-q23.3. Ogawa et al. (1998) report it as having 16 introns and 17 exons. Chen et al. (2013) also reports that the rs1271572 A>T SNP located near the gene's promoter is one of the interesting polymorphisms in the ER2 gene. The role of ER2 polymorphism in CHD was not well studied, too. In general, few studies reported an association between these polymorphisms and CHD Iorga et al., 2017; Pedram et al., 2016; Schuster et al., 2016). No study has been undertaken in Iraq on the association between ER2 gene polymorphisms and CHD. Besides checking the gender-disease link, this work tries to find out how ER2 variation (rs1271572 A>T) affects coronary heart disease in Iraq.

METHODS

Study subjects: Conduct this case-control study on those having congenital heart disease (CHD) and healthy controls. Serum lipid levels of all participants were evaluated and genotyping of the ER2 gene for rs1271572 SNP was also performed. A detailed questionnaire comprising age, gender, previous family medical history, and other pertinent information was completed by the participants. Measurements of height and weight were used to compute BMI values. It is important to note that the Al-Najaf Centre for Vascular Surgery and Catheterization is a nationwide facility that serves patients from the central, southern, and northern parts of Iraq. The present demographic sample is therefore typical of the Iraqi populace. Every participant gave their informed permission. The Department of Biochemistry laboratory at the College of Medicine in Kufa, Iraq, is where the genetic and biochemical investigations were conducted. The study was approved by the Kufa Faculty of Medicine's Ethical Committee.

Group of patients

150 CHD patients who had received cardiac catheterization at the Al-Najaf clinic were among them. The mean age of the patient was 62.16 ± 6.38 (SD). Patients were diagnosed by specialists. Individuals between the ages of 50 and 75 who had unstable angina or myocardial infarction (MI) and whose angiography showed 70% or more blockage of the coronary arteries or their branches were eligible. Exclusion criteria included a family history of connective tissue disease, cancer, and hypercholesterolemia, as well as the use of hormone replacement therapy in the month before to the trial.

Group under control

There were 150 healthy individuals in it. They were 62.55±6.83 years old and selected from the general population who visited the hospital for a standard checkup. The inclusion criteria included matching individuals based on age, sex, and geographic location, as well as having no prior medical history of diabetes mellitus, CHD, or a family history of the disease.

Measurements of biochemistry

Enzymatic techniques were used to measure HDL-C levels, triglycerides (TG), cholesterol (TC), and fasting blood lipid concentrations. They were measured in accordance with the guidelines provided by the manufacturer. Indirect calculations were made for VLDL-C and LDL-C. Additionally, estimates were made for the atherogenic indices, TG/HDL-C, TC/HDL-C, and LDL/HDL-C.

Measurement of genotype

For DNA analysis, blood samples from CHD patients and control groups were collected in EDTA-anticoagulant tubes. The DNA was extracted using commercial kits (FAVORGENTM Total DNA Extraction). The ER2 gene was genotyped for the rs1271572 G/T polymorphisms using the allele-specific tetra-primer amplification method (TARMS), which was followed by gel electrophoresis.

The primers were selected in accordance with the guidelines provided by Bharathi et al. (2019):

Forward outer primer	5'CCCCTCGTCTTCCTCTATTA3'
Reverse outer primer	5' ACCGGGGAGACCTGTG3'
Forward inner primer	5' GATGTGACACTGGGGGGG3'
Reverse inner primer	5' CCACAGGCCATTGTGAGAA3'

The thermocycler used to perform the PCR reaction was Professional, Biometra, Germany. The GoTaq® G2 Green Master Mix was used to carry out the amplification response. 94°C for 5 minutes, 33 cycles of 95°C for 50 seconds, 54°C for 50 seconds, and 72°C for 50 seconds, followed by 72°C for 10 minutes, were the cycling conditions. The outer forward and outer reverse allele-specific PCR product band widths were 373 bp, 276 bp for the AA genotype, 133 bp for the variant TT genotype, and 276,133 bp for the heterozygous AT genotypes. An investigation using 1.5% agarose gel electrophoresis was performed on the amplification findings.

Statistical analysis:

mean±SD was used to represent continuous variables. ANOVA or a t-test were used to evaluate differences in the numerical parameters between the patient and control groups. The alignment of the genotypes with the Hardy-Weinberg equilibrium (HWE) was analyzed. The study's power was determined using the online sample size estimator [http://osse.bii.a-star.edu.sg/calculation2.php]. The Chi-square test was used to compute the genotype differences between the patients and the control group. The Akaike information criterion (AIC) was estimated in order to choose the final inheritance model.

RESULTS

Table 1 shows the allele frequencies and rs1271572 SNP genotypes of CHD patients and control subjects. In the control group, the frequencies of genotypes AA, AT, and TT were 76%, 23%, and 1%, while in the patients, they were 59%, 36%, and 5%, respectively. A and T allele frequencies were 88% and 12%, respectively, in the control group and 77% and 23%, respectively, in the sick group.

Table 1: Allele and genotype frequencies of rs1271572 SNP in the recruited individuals

	All subjects		**Patients**		**Control group**	
Allele	**No**	**%**	**No**	**%**	**No**	**%**
A	495	82	263	88	232	77
T	105	18	37	12	68	23
Genotype						
A/A	203	68	114	76	89	59
A/T	89	30	35	23	54	36
T/T	8	3	1	1	7	5

The research power was 83%, and the genotype alignment with HWE evaluation showed ultimate compatibility (Table 2)

Table 2: Results of genotype alignment of rs1271572 SNP with HWE in the studied individuals

- N=300						
	N11	**N12**	**N22**	**N1**	**N2**	**P-value**
No of all persons	203	89	8	495	105	0.84
No of Patients	114	35	1	263	37	0.7
No of Control persons	89	54	7	232	68	1
Study power	83%					

When comparing the sick group to the control group, the assessment of genotype frequencies under various inheritance models showed substantial (P: 0.0022-0.015) increases in the carriers of the variant allele. It was discovered that the odd ratio was roughly equal to 2. It was clear that the co-dominant model had the lowest AIC value (Table 3).

Table 3: Frequencies of genotype distributions of rs1271572 SNP in the two studied groups under several inheritance models

Model	**Genotype**	**Control group**	**Patients**	**OR (95% CI)**	**P**	**AIC**
Codominant	A/A	114 (76%)	89 (59.3%)	1.00	0.0029	412.2
	A/T	35 (23.3%)	54 (36%)	1.98 (1.19-3.30)		
	T/T	1 (0.7%)	7 (4.7%)	8.18 (0.98-68.17)		
Dominant	A/A	114 (76%)	89 (59.3%)	1.00	0.0022	412.4
	A/T-T/T	36 (24%)	61 (40.7%)	2.16 (1.31-3.55)		
Recessive	A/A-A/T	149 (99.3%)	143 (95.3%)	1.00	0.032	417.3
	T/T	1 (0.7%)	7 (4.7%)	6.67 (0.80-55.24)		
Over-dominant	A/A-T/T	115 (76.7%)	96 (64%)	1.00	0.015	415.9
	A/T	35 (23.3%)	54 (36%)	1.86 (1.12-3.09)		

The recruited participants' genotype-gender cross-classification interaction was assessed. With an odd ratio of 2.28, a significant correlation was demonstrated in females with the heterozygous variant genotype (Table 4).

Table 4: Gender cross-classification interaction of genotypes of rs1271572 SNP in the recruited individuals

	Females			Males		
	Control	**Patients**	**OR (95% CI)**	**Control**	**Patients**	**OR (95% CI)**
A/A	51	36	1.00	63	53	0.90 (0.43-1.91)
A/T	14	23	2.28 (1.03-5.03)	21	31	1.62 (0.70-3.73)
T/T	0	4	---	1	3	3.12 (0.29-33.35)

When the biochemical measures and BMI were compared to the genotype frequencies, bearers of the variant alleles showed markedly higher levels. In a comparable comparison, HDL-c, however, significantly decreased. It was discovered that the variant alleles were substantially associated with an increase in atherogenic parameters (Table 5).

Table 5: Anthropometric and biochemical characteristics of patients relevant to the genotype distribution of rs1271572 SNP

	AA (N= 87)		**AT (N= 55)**		**TT (N= 8)**		**P value**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (y)	62.74	6.23	63.27	6.55	63.00	6.32	0.54
BMI (kg/m2)	26.99	2.83	30.09	2.52	28.57	2.37	0.001
TC (mg/dl)	262.63	91.71	323.27	113.27	244.29	43.66	<0.001
TG (mg/dl)	265.34	82.89	315.84	53.38	279.57	83.71	<0.001
VLDL-C (mg/dl)	53.07	16.58	63.17	10.68	55.91	16.74	0.001
LDL-C (mg/dl)	171.13	89.07	232.42	107.27	153.09	40.32	<0.001
HDL-C (mg/dl)	38.43	14.36	27.68	6.67	35.29	15.48	0.001
TG/HDL-C	0.84	0.30	1.06	0.15	0.91	0.30	0.001
LDL/HDL-C	5.73	4.49	9.10	4.76	5.29	2.99	0.001
TC/HDL-C	8.42	5.33	12.54	5.38	8.24	4.12	0.001

DISCUSSION

A complex medical condition, coronary heart disease is frequently brought on by a combination of environmental and hereditary factors (Daniel et al., 2012). Numerous genes' roles in the development of coronary heart disease are still being studied. The

primary goals of these research are to better understand the disease's progression, relieve the therapeutic options, and discover more about the disease's etiology (Algenabi et al., 2021). To look into these possible consequences, those who are at a high risk of getting CHD and those who could be genetically immune to the condition will be identified. Therefore, we need to find out more about the different genes that cause CHD and who could be genetically immune to the condition. Under several inheritance models, the study investigated the relationship between CHD in the Iraqi population and the ER2 gene, rs1271572 SNP. Since the genotypes of the examined SNP were clearly in concord with the HWE in the control group, the genotype distribution in our population is acknowledged as constant from generation to generation. Changes in the CHD group might thus be understood correctly. It becomes sense to say that the occurrence of the disease may be connected to these alterations. The obtained odd ratio is around 2, indicating that carriers of the variant alleles have a risk factor of roughly 2 for developing CHD. The model that was thought to suit the data the best was the codominant model. It has been suggested that a predictive performance model performs better if its AIC is less. AIC of Burnham and Anderson (2002) is the measure of relative distance predicted between the fitted model and the actual process that generated the observed data. It is necessary to make a hypothesis on the location of rs1271572 SNP in order to understand how it mechanistically links to an increased risk of CHD incidence.

The rs1271572 polymorphism is located upstream in the promoter region of the gene, where it creates a sequence that can be recognized for binding by various transcription factors (Lurie et al., 2011). Polymorphism may alter binding sites of target genes for ESR2, their transcriptional elements, and activity (Rivadeneira et al., 2006; Shi & Zhou, 2006). As per Chen et al. (2013), results indicated that the TT genotypes of rs1271572 led to lower transcriptional activity in the promoter region of the ER2 gene and deletion of a YY1 binding site. This gene is expressed in layers of vasculature vessel walls (Hilary et al., 2008). Therefore, genetic variation must be included when differences in ER2 function are assayed both inside and outside regions surrounding the ER2 gene. Consequently, alterations might change normal gene expression patterns or protein compositions at tissue or cellular levels (Rexrode et al., 2007).

The findings of this study with respect to the association of ER2 gene polymorphism and CHD are quite in agreement with earlier investigations. The association between ER2 polymorphism and CVD was conducted in the Spanish population through a nested case-control study. This was only reported in men, where an association was made between rs1271572 SNP and increased risk of MI. Study results indicated that the inherited variation of ER2 gene polymorphisms among patients with MI is gender-based (Domingues-Montanari et al., 2008). In the United States, cardiovascular disease and myocardial infarction have been associated with the T allele of the rs1271572 SNP1, but only among women (Rexrode et al., 2007). The noted differences by various studies can however be attuned to population ethnicity and sample sizes of groups under study.

With an odd ratio of 2.28, the gender cross-classification interaction (GCCI) of the rs1271572 SNP with CHD showed a significant association in females only. Investigating this association can provide interesting information on the type of interaction between gender and the SNP under investigation that influences the development of CHD (Yao et al., 2009). Only females having a heterozygous genotype of the rs1271572 SNP have a gender-specific genetic effect on the disease (Batnyam et al., 2013). This indicates that some biological mechanisms linking these two alleles of the SNP to female biology influence CHD in some fashion that is not operative or apparent in males (Griffin et al., 2021). It may thus increase women’s susceptibility to CHD as compared to men.

The rs1271572 SNP and CHD GCCI may be initiated by several pathways. Hormonal factors like progesterone and estrogen could influence the female SNPs on the same gene. The impact of these hormones on CHD might not exist or be starkly different in males due to their different hormonal profiles (Zhou et al., 2007). Perhaps, the variant allele influences gene expression differently across genders. Therefore, genes expressed in females with heterozygous genotypes for a plethora of diseases-related genes differ (Yao et al., 2009). Neither histone acetylation nor DNA methylation is heritable but can result from either genetic or environmental stimuli. From that perspective, an interaction between gender and the

SNP may arise due to this heterozygous variant influencing female-specific epigenetic changes (Fernández et al., 2016).

There may be many clinical implications if the GCCI of the rs1271572 SNP is associated with CHD. In therapy and disease risk causation, the GCCI study makes an exception for simultaneously considering both gender and genotype. For instance, females with heterozygous variation could receive specific preventive or therapeutic interventions that are inappropriate for males or females with other genotypes (Belloy et al., 2023). Elucidating the basic mechanisms of gender-specific interaction would guide in producing gender-specific drugs or targeted therapy for females bearing the heterozygous mutation (Wylie et al., 2017). The development of disease models incorporating gender-specific genetic interactions may help make studies sharper and more insightful to lead to better treatment and diagnostic rationales (Holland et al., 2016). The raised levels of atherogenic lipids, total cholesterol, triglycerides, and low-density lipoprotein; as well as the atherogenicity indices, that is, the ratios of triglycerides to HDL cholesterol, and LDL to HDL cholesterol along with total cholesterol to HDL-C are related to the present association of the rs1271572 single nucleotide polymorphism with coronary heart disease. Results are very promising because they throw light on the role that ER2 gene polymorphism, rs1271572, plays in the pathogenesis of CHD within our cohort. Usategui-Martín et al. (2019) observed an association between a favorable serum lipid profile and polymorphisms in the estrogen receptor gene which concurred with these findings.. In a cohort research, Gomes-Rochette et al. (2017) showed a connection between ER2 and alterations in the lipid profile, namely in serum triglycerides and total lipids. According to Nilsson et al. (2007), extreme obesity cannot be ruled out despite ER2's minimal significance. According to Meng et al. (2023), dyslipidemia may worsen due to compromised estrogen receptor function caused by ER overexpression. According to Kwang (2002), estrogen replacement treatment lowers blood levels of Lp (a), a lipoprotein with structural characteristics similar to LDL. The extraordinary functions of ER2 in regulating serum lipid concentrations were demonstrated by Chen et al. in 2022.

There are many restrictions on the current investigation. It involves examining just one ER2 gene SNP linked to CHD. Together with the genotyping analysis, the ER2 and estradiol concentrations were not ascertained. The absence of gene-environment interaction might reveal additional facets of the rs1271572 SNP's connection to the illness.

CONCLUSIONS

In conclusion, the Iraqi population is at risk for developing CHD due to the ER2 gene polymorphism, namely the rs1271572 SNPs. Elevated atherogenic lipids are associated with this relationship. Females are more susceptible to the illness than males due to the mutant allele.

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