

Genetic association between two Estrogen Receptor 2 (ER2) Gene Polymorphism (rs4986938 and rs1256049) and SYNTAX Score in Patients with Coronary Artery Disease: A Report from Southern Iran

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Abstract

Background

One of the most common causes of death in the world is coronary artery disease (CAD). Estrogen, the most important early sex hormones in women, plays an important role in the risk reduction of cardiovascular disease (CVD). Expression of estrogen as well as its receptors—including estrogen receptor alpha (ER1) and estrogen receptor beta (ER2) might have an association with the severity or complexity of CAD. Since most articles have focused on the relationship between ER1 gene polymorphism and CAD, in this study we aimed to evaluate the association of two ER2 gene polymorphisms, AluI and RsaI, with severity of CAD.

Methods

148 patients with confirmed CAD who underwent elective percutaneous coronary intervention (PCI) were included in this study. Blood samples were collected before coronary angiography and ER2 gene polymorphisms were analyzed by the PCR-RFLP method. The STNTAX Score (SS), grading system for CAD complexity, was evaluated by an interventional cardiologist who was blinded to other data.

Results

110 men and 38 women were participated in this study. Our results revealed a statistically significant relationship between SS and RsaI polymorphism of ER2 ($p = 0.01$). In contrast, there was no association between AluI genotypes and SS.

Conclusions

Besides to estrogen level, the genetic variation of its receptors might play an important role in the severity or complexity of CAD. According to our results, RsaI polymorphism of ER2 gene may assert a pivotal role in the severity of CAD; however this assumption needs to be proved in studies with a larger population.

1. Background

Cardiovascular disease (CVD) is considered as one of the major causes of death worldwide. Coronary artery disease (CAD) and cerebrovascular diseases are among the most common causes of deaths due to CVD [1]. According to the epidemiologic studies, premenopausal females have less risk for CVD in comparison with males with the same age that may be because of the protective effects of estrogen in preventing CVD. Estrogen can serve its effect by binding to estrogen receptor α (ER1), estrogen receptor β (ER2), and G-protein-coupled estrogen receptor 30 (GPR30). The possible protective mechanism of

estrogen against CVD would be related to its role on angiogenesis, fibrosis, vascular function, and oxidative stress processes [2].

Estrogen could induce its cardioprotective effects through a receptor-based biological mechanism. Recent researches revealed that genetic variants have an important role in cardio- and cerebro-vascular diseases. For instance, ER1 is an important indicator of atheroprotective effect of estrogen on cardiovascular system. [3]. A study evaluated the association between ER1 single nucleotide polymorphism (SNP) and Intima-media thickness (IMT) in carotid artery, an important predictor of CVD, in female Taiwanese population. This research demonstrated a significant association between ER1(NM_001122740.1):c.1782G > A [rs2228480] and ER1(NM_001122740.1):c.*1753C > A [rs3798758] of female sex with the IMT of carotid artery. According to this article, women with c.1782G > A polymorphism had an increased IMT of carotid artery and were more prone to the risk of atherosclerotic disorders such as ischemic stroke [4]. A case-cohort design on evaluation of the relationship between ER1 gene polymorphism and the risk of coronary heart disease (CHD) and stroke in Finnish population, revealed the significant association of PvuII NM_000125.3:c.453 – 397 T > C [rs2334693] polymorphism with the higher risk of CHD in men. On the other hand, this study failed to confirm any significant association between ER1 gene polymorphism and the occurrence of ischemic stroke [5]. In addition, there was a report for a significant association between ER1 gene polymorphism and low density lipoprotein (LDL) metabolism in women which is an important predictor of atherosclerosis and CVD [6].

Arterial stiffness enhancement and increased wave reflection are the other risk factors for CVD. It has been hypothesized that there is an association between arterial stiffness and genetic variance of ER1, ER2, and CYP19A1 (aromatase). The results of the American population study showed a strong relation between ER1 and ER2 gene polymorphisms and enhanced wave reflection but it is failed to show any association respected to CYP19A1 [7].

The SYNTAX Score (SS) is an angiographic tool using to predict the coronary vasculature characteristics based on the number of lesions and also their complexity, location, and function. SS would be calculated through a computer program using 12 main questions which are about the dominance, number of lesions, segments involved per lesion, total occlusion, trifurcation, bifurcation, aorto-ostial lesion, severe tortuosity, length > 20 mm, heavy calcification, thrombus, and diffuse disease/small vessels. Higher SS would be a predictor of higher level of disease complexity, more challenging pharmacotherapy, and worse prognosis [8].

Since most of studies have focused on ER1 and few studies evaluated the role of ER2 in atherosclerotic diseases, in this study, we aimed to evaluate the association between two major SNPs of ER2 gene containing AluI [rs4986938] and RsaI [rs1256049] and the severity of CAD expressed as SS.

2. Methods

The study protocol was assessed and approved by the Ethics Committee of Shiraz University of Medical Sciences (SUMS, Iran) (No: IR.SUMS.REC.1397.369). Written consent form and permission for DNA

analyses was obtained from all participants.

2.1. Subjects

Inclusion criteria were age of 18-80 years old and confirmed diagnosis of CAD ($\geq 50\%$ luminal stenosis in at least one major coronary artery in angiography). Exclusion criteria include consumption of oral contraceptives and hormone replacement therapy during one month prior to the study, malignancy, schizophrenia, connective tissue disease, or chronic inflammatory disease.

Patients who fulfilled the criteria were included into this project. Demographic and clinical data were documented for each patient according to patients' hospital record.

2.2. Coronary angiography

Patient who were candidates for invasive strategy with coronary angiography were enrolled into the study. Patients received Aspirin, clopidogrel, statins, and anticoagulation according to current guidelines prior to coronary angiography. Procedure was performed with local anesthesia and via radial or femoral approaches. Ultravist[®] or Visipaque[®] was used as contrast agents.

Subjects with $\geq 50\%$ stenosis in at least one major epicardial artery greater of 1.5 mm were included into the study. Coronary angiographic data, including vessels' involvement, percentage of stenosis and SS was evaluated by an interventional cardiologist who was blinded to other data. SS was calculated using the official calculator from SYNTAX score website [9].

The final plan of the management for patients were varied from medical therapy (11%) to percutaneous coronary intervention (PCI) (67%) and coronary artery bypass grafting in the remaining (22%).

Severe CAD is defined as $\geq 70\%$ luminal stenosis in one major epicardial vessel or $\geq 50\%$ stenosis in the left main coronary artery (LMD) and Multivessel disease (MVD) is defined as more than one coronary artery involvement with $\geq 70\%$ stenosis [10, 11].

2.3. Biological samples and genotyping

Blood samples were collected in EDTA vacutainer tubes from all patients in order to further assessment of ER2 gene polymorphism. Whole blood was stored at -20°C freezer until use. Genomic DNA for PCR was extracted from whole blood by DNA extraction kit (Yekta tajhiz, Iran) according to manufacturer protocol.

Selected regions of ER2 gene were amplified by polymerase chain reaction (PCR) using specific primers listed in Table 1. All primers used for amplification of RsaI and AluI polymorphisms regions in ER2 gene were designed by the Primer 3 software and were obtained from metabion (metabion international AG, Martinsried, Germany). The reaction program for RsaI and AluI polymorphisms amplification was as follows: initial denaturation for 4 min at 94°C , followed by 35 cycles of 94°C for 50 s, 66°C for 50 s, 72°C

for 50 s and final elongation at 72°C for 7 min. The PCR reactions were performed in Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA).

For RFLP, the PCR products of RsaI and AluI polymorphisms were digested with RsaI (#ER1121, 5U at 37°C for 16h) and AluI (#ER0011, 5U at 37°C for 16h) (Fermentas), respectively. DNA fragments from RFLP were electrophoresed on 2% agarose gel to determine the RsaI and AluI polymorphic patterns.

Table 1. Primers used in this study.

Gene	Sequence	Band size (fragments obtained after digestion)
RsaI Forward	TTCTGAGCCGAGGTCGTAGT	582 bp
RsaI Reverse	TGAATCCTTGGACCCAACCTC	(A: 293 bp + 289 bp; G: 582 bp)
AluI Forward	GTGTGTGGTGGGACACAGAG	646 bp
AluI Reverse	AGGCCATTGAGTGTGGAAC	(A: 445 bp + 201 bp; G: 646bp)

2.4. Biostatistical analysis

Collected information of this study was analyzed using Statistical Package for Social Sciences (SPSS) for Windows software (version 16, Chicago, USA). All continuous variables were reported as the mean \pm SD and the differences between male and female were analyzed by independent samples t test. Categorical variables were showed as absolute number and percentages and were tested by using the chi-square test.

For comparison between ER2 genotypes and allele frequencies and SS or sex, chi-square test and Fischer's exact test were applied. P-value of <0.05 was considered as significant.

3. Results

Totally, 148 patients were enrolled into the study. The mean age of all patients was 59.06 ± 11.42 . 110 (74.7%) male with the mean age of 57.62 ± 11.75 and 38 (25.3%) female with the mean age of 63.29 ± 9.28 were participated in our study. The same gender distribution was found in previous investigation studied Iranian patients undergoing elective PCI [12].

Clinical baseline characteristics of patients were described in Table 2, dividing participants in two groups according to the sex. Both groups were similar in all variables with exception of age ($p = 0.01$), weight ($p = 0.001$), glomerular filtration rate (GFR) ($p = 0.001$) and frequency of hypertension ($p = 0.02$), diabetes mellitus (DM) ($p = 0.01$), and active smoking ($p = 0.001$). In addition, no significant difference was found between sex and endpoints such as SS, severe CAD, and the number of diseased vessels.

Table 2
Patients' demographic and clinical characteristics

Variables	Total(n = 148)	Male(n = 110)	Female (n = 38)	p-value
Age, years, mean \pm SD	59.06 \pm 11.42	57.62 \pm 11.75	63.29 \pm 9.28	0.01
weight, kg/m ² , mean \pm SD	77.19 \pm 17.27	80.93 \pm 15.28	66.18 \pm 18.26	\leq 0.001
DM, N(%)	60(40)	3(33.6)	22(57.9)	0.01
Hyperlipidemia, N(%)	68(45.3)	46(41.8)	21(55.3)	0.15
Hypertension, N(%)	74(49.3)	48(43.6)	25(35.8)	0.02
Current smoker, N(%)	68(45.3)	64(57.1)	4(10.5)	\leq 0.001
Previous MI, N(%)	6(4)	5(4.5)	1(2.6)	NA
BB-treated, N(%)	109(72.7)	80(71.4)	29(76.3)	0.66
ACE inhibitor-treated, N(%)	93(62)	68(60.7)	25(65.8)	0.66
GFR (MDRD), ml/min/1.73 m ² , mean \pm SD	77.16 \pm 20.28	81.41 \pm 18.79	64.61 \pm 19.53	\leq 0.001
Creatinine, mg/dL, mean \pm SD	1.04 \pm 0.22	1.06 \pm 0.21	1.00 \pm 0.25	0.12
BUN, mg/dL, mean \pm SD	16.31 \pm 6.38	15.86 \pm 5.22	17.64 \pm 8.9	0.15
SYNTAX Score, mean \pm SD	18.61 \pm 11.85	17.86 \pm 10.85	19.79 \pm 13.76	0.38
SYNTAX Score, N(%)	41(27.3)	28(25)	13(34.2)	0.20
\geq 23	109(72.7)	84(75)	25(65.8)	
\leq 23				
Severe CAD, N(%)	122(82.4)	90(81.1)	32(84.2)	0.74
Diseased vessel, N(%)	51(34.5)	36(33)	15(39.5)	0.66
One vessel	56(37.8)	44(40.4)	12(31.6)	
Two vessel	40(27)	29(26.6)	11(28.9)	
Three vessel				
MVD, N(%)	74(50)	56(50.9)	18(47.4)	0.71
Notes: DM Diabetes mellitus; MI Myocardial infarction; BB Beta blocker; ACEI Angiotensin converting enzyme inhibitor; GFR Glomerular filtration rate; BUN Blood urea nitrogen; CAD Coronary artery disease; MVD Multi-vessel disease				

All samples were examined for ER2 gene polymorphisms (RsaI and AluI polymorphisms). The gel electrophoresis results after enzymatic digestion are shown in Fig. 1.

Of 148 samples analyzed for RsaI polymorphisms, a statistically significant relationship was found between SS and RsaI genotypes of ER2 gene ($P = 0.01$) but not with RsaI alleles ($P = 0.05$). Of note, AG genotype was found to be the more prevalent ones in patients with $SS \geq 23$. In contrast, there was no association between AluI genotypes and alleles and SS (Table 3). The same results were reported for the association between sex and RsaI and AluI polymorphisms of ER2 gene.

Table 3
Association of sex and SYNTAX score with ER2 gene polymorphisms in patients with CAD.

Receptor gene	SYNTAX score		P-value	Gender		P-value
		≤ 23 (N = 109)	≥ 23 (N = 41)	Male	Female	
AluI, N (%)	GG	58(53.2)	25(61)	61(54.5)	22(57.9)	0.9
	AG	37(33.9)	15(36.6)	40(35.7)	12(31.6)	
	AA	14(12.8)	1(2.4)	11(9.8)	4(10.5)	
Alleles, N (%)	A	63(28.9)	19(23.2)	64(28.6)	18(23.7)	0.41
	G	155(71.1)	63(76.8)	160(71.4)	58(76.3)	
RsaI, N (%)	GG	101(92.7)	32(78)	98(87.5)	35(92.1)	0.82
	AG	7(6.4)	9(22)	13(11.6)	3(7.9)	
	AA	1(0.9)	0(0)	1(0.9)	0(0)	
Alleles, N (%)	A	9(4.1)	9(11)	15(6.7)	3(3.9)	0.53
	G	209(95.9)	73(89)	209(93.3)	73(96.1)	

4. Discussion

In this study the association between ER2 gene polymorphisms, RsaI and AluI, and SS was evaluated in 148 Iranian patients with CAD. According to our results, CVD risk factors such as hypertension and active smoking were more prevalent in males compared to females. Higher frequency for history of smoking in males is completely predictable since smoking is not a common habit among Iranian women due to cultural issues. Unfortunately, it is in a growing manner in recent years. In contrast, older age, more cases with diseases such as diabetic mellitus and chronic kidney disease (CKD) were reported to be more prevalent in studied females than males. Older ages in studied women is congruent with the fact that women experienced CVD at higher ages than men. In addition, more rates of CKD involvement in female gender may be assumed due to higher rate of DM among women than men in this study. Although there was significant differences regarding some cardiovascular risk factors between genders, it dose not interpreted to difference in SS among these two groups.

Results of polymorphisms analysis revealed that there was neither significant association between AluI gene polymorphism nor their alleles with SS, on the other hand; a significant association between RsaI genotypes and SS was found. Even though several researches evaluated the association between ER gene polymorphism and CVD prognosis, most of them worked on ER1 gene polymorphisms [13–16] and only few evidences investigated the association between ER2 gene polymorphisms and CVD progress [17].

A recently published case-control study performed on Chinese Han women population evaluated the association of SNPs of ER2 genes, RsaI and AluI, with CAD. Results of this study revealed that there was no significant association between the genotypes and/or haplotypes of these two SNPs and risk of CAD. Further subdivided age-based analysis in this study revealed significantly lower risk of CAD in AG genotypes of AluI in comparison to homozygotes GG carriers in patients younger than 40 years of age. In contrast, homozygotes AA carriers had higher risk of CAD. They concluded that A allele of AluI SNP would be an important predictor of CAD risk in Chinese women younger than 40 years of age. These correlations were not found in RsaI SNP [18]. Another case-control study on Brazilian population with premature CAD revealed a statistically significant association between AluI SNP and CAD and it was introduced as one of the most important independent risk factors for CAD as well as dyslipidemia, elevated levels of triglycerides and apolipoprotein B and low levels of HDL in this research. The homozygote AA genotype was more prevalent in case group in comparison to control group [19]. Results of these studies are not similar to ours since no significant association was found between AluI genotypes and SS in our study. In contrast, AG genotype of RsaI polymorphism was associated to more severe coronary artery stenosis (SS ≥ 23).

There are some other researches worked on other SNPs of ER2 gene, rs1271572. A nested case-control study on Spanish population which was assessed the relationship between three SNPs of ER2 and myocardial infarction (MI) revealed that there was a significant association between rs1271572 SNP and enhanced risk of MI occurrence. This finding was limited to the male sex, therefore; this study suggested the potential role of gender in genetic variance of ER2 gene polymorphisms in MI patients [20]. Another study performed on American population confirmed a significant association between T allele of rs1271572 SNP and higher risk of MI and CVD, however; this association was just seen in female gender. Because of these controversial results on the association of ER2 polymorphisms and risk of CVD occurrence and severity, further studies with larger sample sizes and assessments of patients with different ethnics and genetics would be necessary [17].

The exact mechanism by which ER signaling and ER gene polymorphism play a role on CAD pathogenesis is uncertain. Recent studies revealed that estrogen could induce anti-fibrotic effects in the heart that would be occurred through the ER2 [2]. Results of an *in vivo* animal study revealed that activation of ER2 could stop the effects of angiotensin II (Ang-II) and endothelin-1 (ET-1) pro-fibrotic signaling thus prevent further cardiac fibrosis. Ang-II and ET-1 could induce cardiac fibrosis through the inversion of fibroblasts to myofibroblasts and also through the induction of transforming growth factor- β 1 (TGF β 1) which is a known cardiac fibrosis inducer. Estrogen and ER2 agonists could stop TGF β 1

action via cAMP and protein kinase A pathway [2, 21]. Also previous studies revealed that estrogen administration could enhance ER2 transcript expression and therefore induce its anti-fibrotic effects [22]. It has been reported that ER2 gene overexpression would be a predictive factor of heart function improvement and survival in patients who had a recent history of MI both in males and females [23]. Results of another study on the role of ER1 and ER2 gene expression on the occurrence of neovascularization after ischemic heart disease revealed that both ER1 and ER2 (with the dominance of ER1) might have a pivotal role in estrogen-induced endothelial progenitor cells (EPCs) mobilization and further protection of cardiac function following a recent MI [24]. Another suggestive cardioprotective mechanism of ER2 gene would be through the induction of eNOS expression and further vasodilation in cases of ischemia/reperfusion (I/R) injury, especially in female gender [25]. These researches emphasize the fact that in addition to the estrogen plasma level and the extent of ER1 and ER2 gene expression, evaluation of ER1 and ER2 genetic variance and polymorphisms would be helpful as predictive measure for identification of high risk patients for CVD and also their prognosis.

Because of the controversies around the role of ER2 SNPs in CVD in different populations, further larger investigations would be necessary in order to confirm our observed association between RsaI polymorphism and SS.

Study Limitations

The possible limitations of our study were small sample size and unequal number of participants according to their gender (males and females). Also, it is suggested to evaluate the plasma estrogen levels of patients in combination with investigation of ER2 gene polymorphisms in future studies.

5. Conclusions

Besides to estrogen level, the genetic variation of its receptors, ER1 and ER2, might play an important role in the pathogenesis, severity and complexity of CAD. According to our results, RsaI polymorphism of ER2 gene may assert a pivotal role in the severity of CAD; however this assumption needs to be proved in higher population studies.

Abbreviations

ER1: Estrogen receptor alpha; ER2: Estrogen receptor beta; SS: SYNTAX score; CVD: Cardiovascular disease; CAD: Coronary artery disease; CHD: Coronary heart disease; MVD: Multi-vessel disease; LMD: Left main coronary artery disease; MI: Myocardial infarction; CKD: Chronic kidney disease; DM: diabetes mellitus; Ang-II: Angiotensin II; ET-1: Endothelin-1; TGFβ1: Transforming growth factor-β1; EPCs: Endothelial progenitor cells; ACE: Angiotensin converting enzyme; GFR: Glomerular filtration rate; MDRD: Modification of diet in renal disease; GPR30: G-protein-coupled estrogen receptor 30; SNP: Single nucleotide polymorphism; IMT: Intima-media thickness; LDL: low density lipoprotein; PCR: Polymerase chain reaction.

Declarations

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Authors' contributions:

FF participated in study design, acquisition of data, statistical analysis, interpretation of results, manuscript preparation, and critical manuscript revisions. PD contributed to data collection and critical manuscript revisions. MD contributed to data collection and critical manuscript revisions. AA contributed to data collection and manuscript preparation. IJ contributed to data collection and manuscript preparation. PG contributed to data collection, manuscript preparation, and critical manuscript revisions. All authors approved the final version of the manuscript.

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Availability of data and materials:

All data generated or analysed during this study are included in this published article

Ethics approval and consent to participate:

All procedures performed in this study were in accordance with the ethical standards of the institutional research Committee of Shiraz University of Medical Sciences and with the 1964 Helsinki declaration and its later amendments. All participants signed the written informed consent.

Competing Interests:

All authors declared that they have no conflict of interest.

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Figures

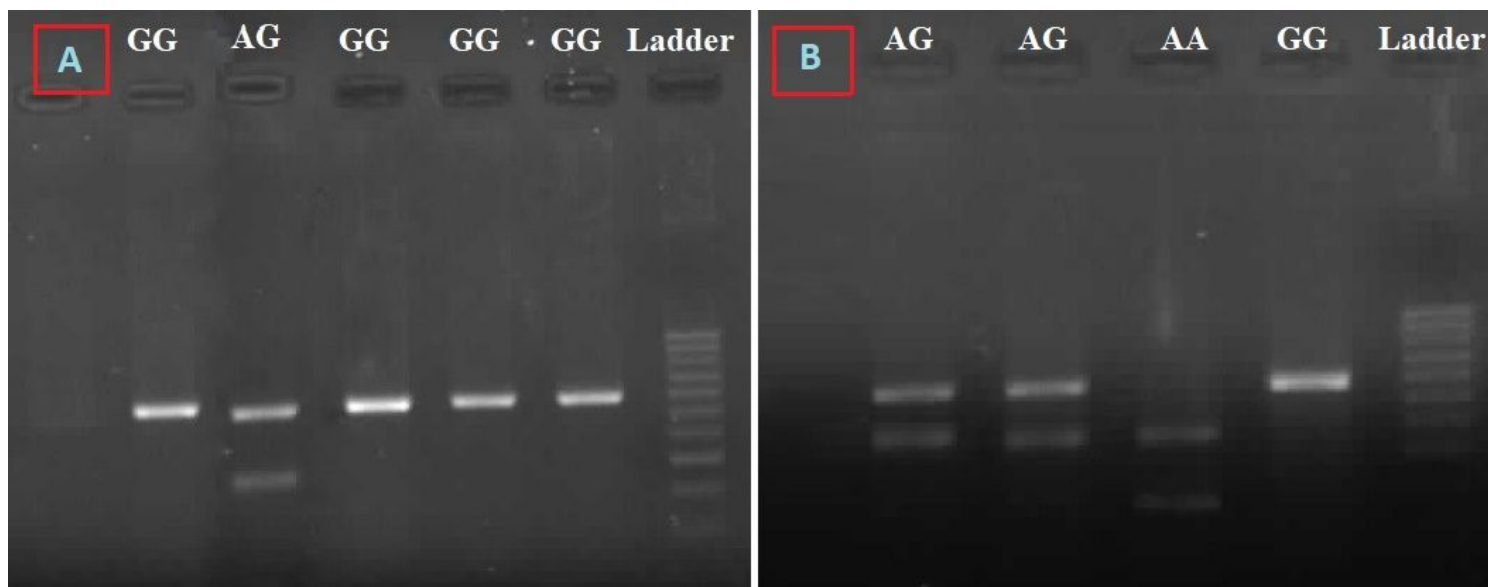


Figure 1

The gel electrophoresis results after enzymatic digestion. A) Genotypes of RsaI polymorphisms. B) Genotypes of AluI polymorphisms. 100 bp ladder used.