AL-Bahir
Journal for Engineering and Pure Sciences
BJEPS

Volume 1 | Issue 2 Article 5

Association of Breast Cancer with Single Nucleotide Polymorphism of CYP2C9 Gene for Women Patients in Baghdad, Iraq

Mohammed R. Al-Lami
Department of Chemistry / College of Science / Al-Mustansiriyah University / Baghdad-Iraq

Falah S. Al-Fartusie
Department of Chemistry / College of Science / Al-Mustansiriyah University / Baghdad-Iraq

Dheaa SH. Zageer
DNA Center for Research and Training / Al-Nahrain University / Baghdad-Iraq

Recommended Citation

Al-Lami, Mohammed R.; Al-Fartusie, Falah S.; and Zageer, Dheaa SH. (2022) "Association of Breast Cancer with Single Nucleotide Polymorphism of CYP2C9 Gene for Women Patients in Baghdad, Iraq," *Al-Bahir*: Vol. 1: Iss. 2, Article 5.
Available at: https://doi.org/10.55810/2313-0083.1012

ORIGINAL STUDY

Association of Breast Cancer with Single Nucleotide Polymorphism of CYP2C9 Gene for Women Patients in Baghdad, Iraq

Mohammed R. Al-Lami [a,*], Falah S. Al-Fartusie [a], Dheaa SH. Zageer [b]

[a] Department of Chemistry, College of Science, Al-Mustansiriyah University, Baghdad, Iraq
[b] DNA Center for Research and Training, Al-Nahrain University, Baghdad, Iraq

Abstract

Breast cancer (BC) is a very varied disease, with several subtypes that exhibit different biological, molecular, and clinical characteristics. Standard information on tumor biology and clinicopathology can be identified by gene expression profile; have provided enhanced prognostic and predictive information. This study included 37 patients suffering from breast cancer and 15 healthy subjects. DNA has been extracted from all samples and then real-time PCR has been performed to analyze the genotyping of Rs1799853. The results showed higher frequency of CT genotype in the control subjects than the patients (73.3 and 43.2, respectively) and this difference was significant (0dd = 0.28, p-value = 0.038). The CC genotype showed a non-significant higher frequency in patients than in control (24.3 and 6.7, respectively. P-Value = 0.247). The TT genotype also showed higher frequency in patients than in control (32.4 and 20, respectively. P-Value = 0.506). In conclusion, the genotype CT of the SNP Rs1799853 for CYP2C9 gene may have a great defense role against the breast cancer.

Keywords: Breast cancer, CYP2C9, SNP, Real −time PCR

1. Introduction

One of the most prevalent malignancies in women globally, breast cancer caused 570,000 fatalities in 2015. Every year, almost 1.5 million women worldwide—25% of all cancer patients—are given a breast cancer diagnosis. Because breast cancer is a metastatic disease that frequently spreads to distant organs such the bone, liver, lung, and brain, it is essentially incurable [1]. A favorable prognosis and a high survival rate can result from early detection of the illness. Due to early diagnosis of the disease, the 5-year relative survival rate of breast cancer patients in North America is above 80% [2]. Most breast cancers begin as ductal hyperproliferation and progress into benign tumors or even metastatic carcinomas when they are repeatedly stimulated by numerous carcinogenic factors [3]. BC development and progression are significantly influenced by the tumor microenvironment, including stromal effects and macrophages [4]. When just the stroma of the rat mammary gland was exposed to carcinogens—not the extracellular matrix or the epithelium—neoplasms might be produced [5]. Research suggests that single nucleotide polymorphism (SNP) genotyping could help to more precisely predict a person's likelihood of getting BC and offer therapeutic suggestions [6]. The CYP (Cytochrome P450 proteins) gene produces the enzyme aromatase, which aromatase inhibitors (AI) target in BC treatment because of its crucial role in the biosynthesis of estrogen [4]. The effects of CYP19A1 polymorphisms on circulating estrogen levels, tumor features, arthralgia brought on by AI, bone loss brought on by AI, and letrozole effectiveness in BC patients have all been discovered [5].

Received 14 November 2022; revised 4 December 2022; accepted 4 December 2022.
Available online 10 January 2023

* Corresponding author.
E-mail address: mohammdry@gmail.com (M.R. Al-Lami).

https://doi.org/10.55810/2313-0083.1012

Previous research has demonstrated that the CYP enzyme gene that possess with altered genotypes has a higher risk of developing cancer [7]. According to earlier studies, this study intends to examine the association between the CYP2C9 single nucleotide polymorphism and the development of breast cancer.

2. Materials and methods

This study is a case control study which was conducted on 37 patients with breast cancer along with age- and sex-matched 15 apparently healthy subjects have been recruited from Oncology Teaching Hospital (Baghdad-Iraq) between January and December 2021. About 2 ml of venous blood were collected in the EDTA tube. All participants have subjected to history taking and physical examination.

2.1. Genotyping of reference SNP 1799853 (Rs1799853)

The included samples (patients and control) have been subjected to DNA extraction by following the instructions provided by the commercial kit (Quick-gDNA™ Blood MiniPrep/Cat.No.3073) manufactured by (Zymo Research/USA). The concentration of DNA sample is estimated using Nanodrop device, were a (1) μl of the extracted DNA was placed in the lens of the device in order to detect the concentration in ng/μl at 260 nm wavelength, while the ratio of the absorbance at 260/280 nm was used to assess the purity of DNA.

This study utilized the TaqMan SNP Genotyping Assay, which aids in the accurate and efficient detection of SNPs. This kit contains two TaqMan minor groove binder (MGB) probes that are differentially labeled and specific for each allele, as well as a pair of PCR primers that amplify each allele in a distinct manner and offer the allele of interest unmatched specificity. The frozen materials that had thawed were briefly centrifuged after being vortexed to re-suspension. Then it was determined how many reactions would need to be carried out for each test. For each experiment, the total amount of each component required was determined as shown in Table 1 below:

Table 1. Total amount of required components.

Component	20 μl (Final volume)
2X TaqMan® Master	12 μl
20X Detection Assay	0.50 μl
Nuclease-free	2.50 μl
DNA	5 μl

After that, tubes were moved to the plate for thermal cycling. The components were momentarily combined by a vortex before the tube was sealed. To spin down the contents and remove air bubbles from the solution, the tube was quickly centrifuged. The thermal cycler has been designed to do 40 cycles as it is shown in Table 2:

2.2. Statistical analysis

Microsoft Excel and SPSS version 24.2 were both used in the statistical analysis of this study. Two categorical parameters have been used with chi-square. The Wnipepi software has also been used to analyze SNP odds and fisher test results.

3. Results

The results of the real time-PCR are shown in Fig. 1, results have appeared in two different channel as logarithmic increment curves each curve represent one sample. The FAM channel showing the C allele and Hex channel showing the T allele.

After genotyping each sample, the resulted genotypes frequencies are shown in Table 3. The results showed higher frequency of CT genotype in the control subjects than the patients (73.3 and 43.2, respectively) this difference accompanied with a significant odd ratio (0dd = 0.28, p-value = 0.038). The CC genotype showed a non-significant higher frequency in patients than in control (24.3 and 6.7, respectively. P-Value = 0.247). The TT genotype also showed higher frequency in patients than in control (32.4 and 20, respectively. P-Value = 0.506).

The results of hardy Weinberg equilibrium are shown in Table 4 showed the frequency of both patients and control no significant departure from Hardy–Weinberg equilibrium.

4. Discussion

A number of recent studies have focused on identifying the genes that increase the risk of developing BC. It has been claimed that up to 10% of BC cases globally are caused by pathogenic

Table 2. Steps and durations of thermal cycler.

Steps	Predesigned SNP		
	Temp.	Duration	Cycles
Enzyme activation	95 °C	10 min	HOLD
Denaturation	95 °C	15 s	40
Annealing/Extension	60 °C	1 min (scanning)	

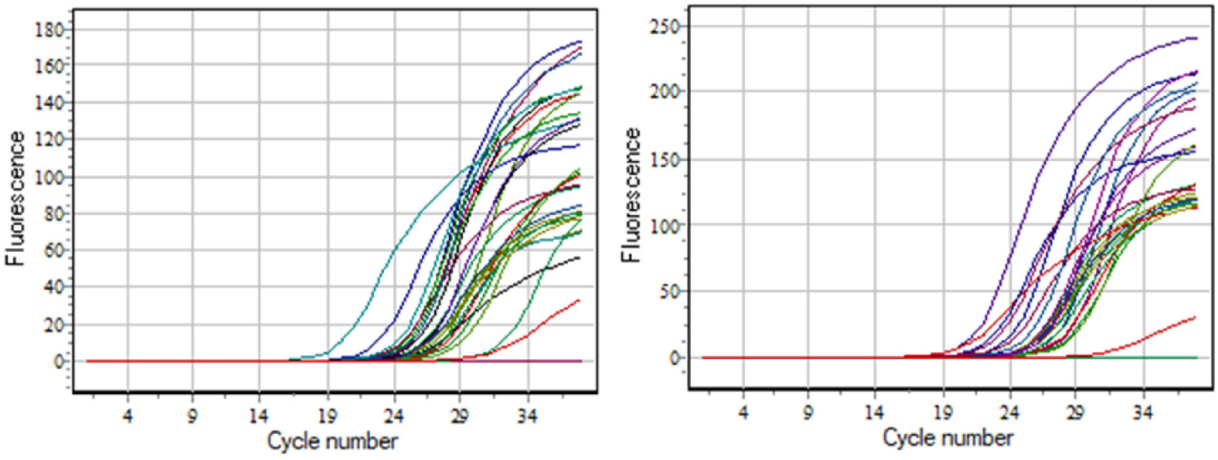

Fig. 1. Amplification curves of real time- PCR for the genotyping of Rs1799853. A; represent the FAM channel. B; represent the Hex channel.

mutations [8]. The bulk of metabolic and clearance processes include the cytochrome P450 (CYPs) genes, which also have key roles in drug metabolism and encode for hemoproteins [9]. The CYP19A1 gene produces the enzyme aromatase, which aromatase inhibitors (AI) target in BC treatment because of its crucial function in estrogen production [10].

According to research, variations in the CYP genes can affect a person's susceptibility to developing BC, how quickly it spreads, and how they react to anticancer medications [11]. When examined as a component of a haplotype, the rs1799853 SNP for CYP2C9 was shown to be strongly linked to cyclophosphamide toxicity [12]. However, neither the rs1799853 SNP nor the higher BC risk in women receiving menopausal hormone treatment were connected to BC in Asian Singaporeans [13]. A case–control research with a small sample size might result in selection bias, which was the primary drawback in our investigation. In a recent study [5], none of the studied CYP2C9 SNPs were substantially linked with BC or its prognostic variables in Jordanian females. Other possible drawbacks, however, were avoided; as the Jordanian Arab community is a very homogeneous group, demographic stratification does not lead to any prejudice. Additionally, there were no appreciable differences in terms of fundamental demographic traits between the case and control groups.

Table 3. Differences among genotypes and allele frequencies of patients and controls.

Genotype Frequency					
Genotype	Control	Patients	P-value	Odds	95% Cl
CC	1(6.7)	9 (24.3)	0.247	4.50	0.55 to 36.58
CT	11 (73.3)	16 (43.2)	0.038	0.28	0.08 to 0.99
TT	3 (20)	12 (32.4)	0.506	1.92	0.47 to 7.77
Chi-squared	4.163				
P Value	0.125				
Allele frequency (%)					
Allele	**Control**	**patient**	**P-value**	**Odds Ratio**	**95% Cl**
C	13	34	0.831	1.11	0.48 to 2.58
T	17	40			

Table 4. Observed numbers alleles frequencies (Hardy–Weinberg equilibrium) at Rs1799853 SNP in breast cancer patients and Control.

Group			Genotype			H–W (p ≤)
			CC	CT	TT	
Patients	Observed	No.	9	16	12	0.619
		%	24.3	43.3	32.4	(0.733)
	Expected	No.	7.1	19.2	10.7	
		%	19.2	51.9	28.9	
Controls	Observed	No.	1	11	3	3.648
		%	6.7	73.3	20	(0.161)
	Expected	No.	2.9	7.8	4.3	
		%	19.3	52	28.7	

5. Conclusion

In this study it can conclude that the SNP Rs1799853 for CYP2C9 gene has a significant impact on the susceptibility to breast cancer. The heterogeneous CT genotype may have a role in the defense against the breast cancer.

Acknowledgments

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad, Iraq, for its support in the present work.

References

[1] Sonnenschein C, Soto AM. Carcinogenesis explained within the context of a theory of organisms. Prog Biophys Mol Biol 2016;122(1):70–6.

[2] Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, et al. Global patterns of breast cancer incidence and mortality: a population-based cancer registry data analysis from 2000 to 2020. Cancer Commun 2021 Nov;41(11):1183–94. https://doi.org/10.1002/CAC2.12207.

[3] Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & Diseases 2018 Jun 1;5(2):77–106. https://doi.org/10.1016/J.GENDIS.2018.05.001.

[4] Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal 2020 Dec;18(1):1–9. https://doi.org/10.1186/S12964-020-0530-4. 2020 18:1.

[5] Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C. The stroma as a crucial target in rat mammary gland carcinogenesis. J Cell Sci Mar. 2004;117(8):1495–502. https://doi.org/10.1242/JCS.01000.

[6] Van Veen EM, Brentnall AR, Byers H, Harkness EF, Astley SM, Sampson S, et al. Use of single-nucleotide polymorphisms and mammographic density plus classic risk factors for breast cancer risk prediction. JAMA Oncol 2018 Apr 1;4(4):476–82. https://doi.org/10.1001/JAMAONCOL.2017.4881.

[7] Nsaif GS, Al-Mualm M, jaber faisal A. The effect of e-cigarettes smoking on expression and methylation of CYP1A1 and CYP1B1 genes and other biochemical parameters. Mater Today: Proceedings Jul. 2021. https://doi.org/10.1016/J.MATPR.2021.07.403.

[8] Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. BioMed Res Int 2013;2013.

[9] Guengerich FP. Cytochrome p450 and chemical toxicology. Chem Res Toxicol 2008;21(1):70–83.

[10] Chumsri S, Howes T, Bao T, Sabnis G, Brodie A. Aromatase, aromatase inhibitors, and breast cancer. J Steroid Biochem Mol Biol 2011;125(1–2):13–22.

[11] Hrycay EG, Bandiera SM. Involvement of cytochrome P450 in reactive oxygen species formation and cancer. Adv Pharmacol 2015;74:35–84. https://doi.org/10.1016/BS.APHA.2015.03.003.

[12] Tulsyan S, Agarwal G, Lal P, Mittal B. Significant role of CYP450 genetic variants in cyclophosphamide based breast cancer treatment outcomes: a multi-analytical strategy. Clinica chimica acta; international journal of clinical chemistry Jul. 2014;434:21–8. https://doi.org/10.1016/J.CCA.2014.04.009.

[13] T. M.-G. C. on G. S. for M. H. T. R. B. C. Risk. Polymorphisms in the BRCA1 and ABCB1 genes modulate menopausal hormone therapy associated breast cancer risk in postmenopausal women. Breast Cancer Res Treat 2010;120(3):727–36. https://doi.org/10.1007/s10549-009-0489-8.