Heliyon 5 (2019) e02542

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Association analysis between genomic variants within advanced glycation end product specific receptor (*AGER*) gene and risk of breast cancer in Iranian women

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ARTICLE INFO

Keywords:
Epidemiology
Genetics
Cancer research
Bioinformatics
Oncology
AGER
Advanced glycation end-product
Breast cancer

ABSTRACT

The advanced glycation end product specific receptor (*AGER*) gene codes for a cell surface receptor which is one of the immunoglobulin superfamily members. This gene has a number of single nucleotide polymorphisms (SNPs) whose variants are associated with altered function of the encoded protein. In the current project, we examined association between rs184003 and rs1800625 SNPs and susceptibility to breast cancer in an Iranian population. The current study excludes participation of rs184003 *AGER* variant in conferring cancer risk. However, for the rs1800625, based on the calculated P value, the results should be assessed in larger cohorts. Primarily, the rs1800625 SNP was associated with breast cancer risk in dominant model (OR (95% CI) = 1.79 (1.03–3.11)), but after correction for multiple comparisons it did not reach the level of significance (adjusted P value = 0.07). The other SNP was not associated with breast cancer risk in any inheritance model. Haplotype analyses revealed a trend toward association between the GC haplotype (rs184003 and rs1800625 respectively) and risk of breast cancer (OR (95% CI) = 1.77 (1.09–2.88), adjusted P value = 0.08)). The current study excludes participation of rs184003 *AGER* variants in conferring cancer risk. However, for the rs1800625, based on the calculated P value, the results should be assessed in larger cohorts.

1. Introduction

The advanced glycation end product specific receptor (*AGER*) gene codes for a cell surface receptor namely RAGE which is one of the immunoglobulin superfamily members. Being a multiligand receptor, it chiefly binds to glycation products of proteins or lipids which are called advanced glycation end products (AGE). The interaction between RAGE and its ligands induces some signaling pathways including proinflammatory routes [1]. Decreasing AGE amounts suppresses development of breast cancer in mice model [2]. Moreover, lack of RAGE expression diminishes chemically-induced inflammation in animal models [3]. The RAGE protein has a soluble form (sRAGE) which reverses the effects of RAGE by acting as a decoy. A previous study in Chinese population has shown elevated serum levels of AGEs, while decreased levels of sRAGE in breast cancer patients compared to normal persons [4]. Another study in the same population has reported association between rs184003 single nucleotide polymorphism (SNP) within *AGER* gene and breast cancer risk. Moreover, a certain haplotype within this gene containing four SNPs frequency including rs1800625 and rs184003 has been demonstrated to confer risk of this malignancy [5]. A single study in Iranian population reported no association between three *AGER* SNPs namely –374T/A, –429T/C, and 63 bp Ins/del polymorphisms in a cohort of 71 breast cancer cases and 93 normal subjects [6].

Based on the provided clues regarding the role of RAGE in the pathogenesis of breast cancer and unavailability of comprehensive data regarding the effects of its SNPs in this malignancy in Iranian population, we performed the present work to genotype two *AGER* SNPs (rs184003 (c.870 + 49G > T) and rs1800625 (-429C > T)) in a cohort of Iranian breast cancer patients. The reasons for selection of these SNPs were the presence of evidences regarding their contribution in cancer risk [5] and their functions in modulation of sRAGE levels. *In vitro* studies have shown that the rs1800625 SNP enhances the transcription of *AGER* about

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https://doi.org/10.1016/j.heliyon.2019.e02542
Received 3 April 2019; Received in revised form 4 May 2019; Accepted 26 September 2019

twofold [7]. Moreover, this SNP is associated with higher expression of sRAGE and endogenous secretory RAGE (esRAGE) [8]. The rs184003 has been identified in a certain haplotype in immaculate linkage disequilibrium with two other SNPs. Notably, the mentioned haplotype was linked with a reduction in esRAGE levels [9].

2. Material and methods

2.1. Enrolled individuals

One hundred and six breast cancer patients with mean age (standard deviation (SD)) of 39.3 (1.2) and 120 healthy individuals with mean age (SD) of 38.9 (1.5) participated in the study. All study participants were female. Patients were admitted in Department of surgery, Hamadan University of Medical Sciences, Iran in the period of February 2014 to December 2016. Diagnosis was confirmed by a pathologist. Samples were gathered before chemo/radiotherapy. Control group consisted of females referred for a routine health workup during the same period. Informed consent was obtained from all participants. The study protocol was approved by ethical committee of Hamadan University of Medical Sciences.

2.2. Genotyping

Routine salting out method was used for DNA extraction. The genotypes of the rs184003 and rs1800625 were assessed using tetra primer-amplification refractory mutation system-PCR strategy. The detailed information about PCR conditions, sequences of inner and outer primers and PCR product sizes are shown in Table 1. The results of the mentioned method were confirmed by sequencing of 10% of the amplified reactions.

2.3. Statistical methods

Agreement of genotypes rates with Hardy-Weinberg equilibrium (HWE) principle was judged by $\chi 2$ test using SNPStats [10]. The association between rs184003 and rs1800625 variants and occurrence of breast cancer was assessed using recessive, dominant and co-dominant models through measuring odds ratios (ORs) and 95% confidence intervals (CIs). The occurrence of anticipated *AGER* haplotypes was calculated in both cases and controls. D′ and r values were quantified for appraisal of the linkage disequilibrium (LD) between rs184003 and rs1800625. P values were corrected using Bonferroni method considering the number of genotyped SNPs. Corrected P values less than 0.05 were regarded as significant. Difference in mean age between case and control groups were assessed using t-test. Other numerical variables including body mass index (BMI), menarche age, menopause age and breast feeding duration were analyzed using Mann–Whitney-U test. Chi-square test was applied for categorical variables. Moreover, Binomial Logistic Regression model was used with including genotypes, age, BMI, menarche age, menopause status, pregnancy history, and breast feeding duration variables. Nagelkerke R Square was calculated to understand how much variation in the dependent variable can be explained by the model. Wald test was used to determine statistical significance for each of the independent variables.

3. Results

Table 2 summarizes the features of enrolled cases and controls. There was no difference in age, marriage status or history of pregnancy between cases and controls. However, significant difference was found between patients and controls in BMI and breast feeding duration.

Accordance of genotypes frequencies with HWE was approved in both study groups. Table 3 shows the results of $\chi 2$ test.

The frequency of C allele of the rs1800625 was more among breast cancer patients compared with normal subjects (OR (95% CI) = 1.77 (1.11–1.82), P-value = 0.015). Primarily, this SNP was associated with breast cancer risk in dominant model (OR (95% CI) = 1.79 (1.03–3.11)), but after correction for multiple comparisons it did not reach the level of significance (adjusted P value = 0.07). The other SNP was not associated with breast cancer risk in any inheritance model (Table 4). Binomial Logistic Regression model was performed. The calculated Nagelkerke R Square was 0.79. The Wald test ("Wald" column) was used to determine statistical significance for each of independent variables. The statistical significance of the test is found in Table 5. Statistical analyses showed

Table 2
The features of enrolled cases and controls.

Characteristics	Cases	Controls	P values
Number of individuals	106	120	-
Age (mean ± SD)	40.2 ± 18.0	39.5 ± 12.0	0.762
BMI (kg/m^2) (mean ± SD)	26.4 ± 1.0	22.6 ± 1.8	<0.05
Menarche age (mean ± SD)	12.05 ± 0.3	12.9 ± 3.1	0.918
Number of individuals entered menopause	16	16	-
Menopause age (mean ± SD)	54.16 ± 6.5	52.7 ± 4.2	0.210
Status of marriage (married) (%)	89.2%	85.2%	0.300
Positive history of pregnancy (%)	80.32%	73.5%	0.225
Breast feeding duration (months) (mean ± SD)	15.5 ± 6.2	17.2 ± 2.1	<0.05
Positive family history of breast cancer	None	None	-
Alcohol use	None	None	-
Smoking	None	None	-

Table 1
Primers, PCR conditions and product sizes.

SNP	Primer sequence	Tm	Annealing temperature	PCR product size (bp)
rs184003	Forward inner primer (C allele): GATGAGAGGTAGGGTGAACCATAACCAG	75 °C	61 °C	211 bp (C allele)
	Reverse inner primer (A allele): GTTAGCACTCTGCACTTCCTGTCGA	75 °C		267 bp (A allele)
	Forward outer primer: ATGACCAATGGGATCACTCACAAGTG	75 °C		422 bp (two outer primers)
	Reverse outer primer: TCAGGGAGGATTAGCACAGGGCTG	77 °C		
rs1800625	Forward inner primer (T allele): AGATAATAAATGATTGTCTTTCACGACGT	65 °C	60 °C	203 bp (T allele)
	Reverse inner primer (C allele): GCGAATAGGAGAGAAACCTGTTTGTAG	65 °C		293 bp (C allele)
	Forward outer primer: TTTTACAAGACTGTCAGAGTGGGGA	65 °C		440 bp (two outer primers)
	Reverse outer primer: CGATGCTATATATTCCATCAGTCCATCA	65 °C		

Table 3
Exact test for Hardy-Weinberg equilibrium.

SNP	rs184003				P-value	rs1800625				P-value
	Minor Allele (T) Number (Frequency in %)	GG	GT	TT		Minor Allele (C) Number (Frequency in %)	TT	TC	CC	
Patients	49 (23)	65	33	8	0.201	53 (25)	61	37	8	0.476
Controls	69 (29)	64	43	13	0.169	38 (16)	85	32	3	0.763

Table 4
Associations between *AGER* SNPs and risk of breast cancer in Iranian population (The two-sided mid-P-values are shown).

SNP	Model		Cases (%)	Controls (%)	OR (95% CI)	P-value	Adjusted P-value
rs184003	Allele	T vs. G	49 (23)	69 (29)	0.74 (0.49–1.14)	0.173	0.347
			163 (77)	171 (71)			
	Co-dominant	TT vs GG	8 (7.5)	13 (10.8)	0.61 (0.23–1.56)	0.438	0.875
		GT vs GG	33 (31.2)	43 (35.9)	0.76 (0.43–1.33)		
	Dominant	GT + TT vs GG	41 (38.7)	56 (46.7)	0.72 (0.42–1.22)	0.226	0.452
			65 (61.3)	64 (53.3)			
	Recessive	TT vs GT + GG	8 (7.5)	13 (10.8)	0.67 (0.27–1.69)	0.396	0.792
			98 (92.5)	107 (89.2)			
rs1800625	Allele	C vs T	53 (25)	38 (16)	1.77 (1.11–2.82)	0.015	0.031
			159 (75)	202 (84)			
	Co-dominant	CC vs TT	8 (7.5)	3 (2.5)	3.70 (0.94–14.28)	0.057	0.114
		TC vs TT	37 (35)	32 (26.7)	1.61 (0.91–2.86)		
	Dominant	TC + CC vs TT	45 (42.5)	35 (29.2)	1.79 (1.03–3.11)	0.037	0.074
			61 (57.5)	85 (70.8)			
	Recessive	CC vs TC + TT	8 (7.5)	3 (2.5)	3.18 (0.82–12.33)	0.078	0.157
			98 (92.5)	117 (97.5)			

Table 5
Results of Binomial Logistic Regression model.

Independent variables	Wald	Significance
rs1800625 genotypes	5.04	0.08
BMI	58.61	<0.001
Age of start Menarche	2.74	0.09

significance of BMI in conferring risk of breast cancer. However, other variables had no significant effect on breast cancer risk.

The assessed SNPs were not linked with each other (D' = 0.25, r = 0.006). Haplotype analyses revealed no associations between any estimated haplotype and risk of breast cancer (Table 6). However, there was a trend toward association between the GC haplotype (rs184003 and rs1800625 respectively) and risk of breast cancer (OR (95% CI) = 1.77 (1.09–2.88), adjusted P value = 0.08)).

4. Discussion

In the current study, we examined association between two *AGER* polymorphisms and risk of breast cancer in Iranian population. Our data excluded participation of rs184003 in conferring cancer risk. However, for the rs1800625, based on the calculated P value, the results should be assessed in larger cohorts. Primarily, the rs1800625 SNP was associated with breast cancer risk in dominant model, but after correction for multiple comparisons it did not reach the level of significance (adjusted P value = 0.07). The other SNP was not associated with breast cancer risk in any inheritance model. Haplotype analyses revealed a trend toward association between the GC haplotype (rs184003 and rs1800625 respectively) and risk of breast cancer.

Breast cancer as a leading cause of cancer-related death in female has encouraged investigators to find genetic markers that predict susceptibility to this malignancy. Among putative susceptibility loci for breast cancer are SNPs within *AGER* gene which codes a protein with multiple functions in carcinogenesis especially metastatic process. RAGE protein enhances cell survival in the situations of nutrients or oxygen deprivation through induction of autophagy and suppression of apoptosis. This process is involved in development of epithelial malignancies cancers [3].

In this investigation, we appraised associations between two *AGER* SNPs and susceptibility to breast cancer in Iranian population. The association between rs184003 and rs1800625 SNPs and hepatocellular carcinoma has been previously assessed in Taiwanese [11]. Researchers have found association between rs1800625 and higher risk of this malignancy. However, rs184003 was not associated with hepatocellular carcinoma [11]. While the rs1800625 was correlated with gastric cancer risk, both SNPs were associated with tumor stage in these patients [12]. However, we could not find any association between mentioned SNPs and risk of breast cancer. The rs1800625 has not been associated with risk of pancreatic cancer in Czech population [13] or breast cancer in Iranian population [6]. Based on the relative small sample size, we could not assess associations between genotypes and patients' characteristics. So, we recommend conduction of upcoming research projects with larger sample sizes to appraise putative correlations.

Statistical analyses showed no LD between the assessed SNPs. Haplotype analyses revealed a trend toward association between the GC haplotype (rs184003 and rs1800625 respectively) and risk of breast cancer which is in agreement with the observed higher frequency of the C allele of the latter SNP in breast cancer patients. The current study excludes participation of rs184003 *AGER* variants in conferring cancer risk. However, for the rs1800625, based on the calculated P value, the results

Table 6
Frequencies of estimated *AGER* haplotypes in cases and controls (Adjusted P values were calculated by multiplying the P values by the number of estimated haplotypes).

rs184003	rs1800625	Frequency in cases	Frequency in controls	Total frequency	OR (95% CI)	P-value	Adjusted P-value
G	T	0.56	0.59	0.58	0.89 (0.61–1.29)	0.544	1.00
T	T	0.19	0.25	0.22	0.70 (0.45–1.09)	0.117	0.466
G	C	0.21	0.12	0.16	1.77 (1.09–2.88)	0.020	0.079
T	C	0.04	0.04	0.04	1.42 (0.38–5.38)	0.851	1.00

should be assessed in larger cohorts. Associations between other SNPs within this gene and risk of breast cancer should also been assessed in future studies. Meta-analyses of the obtained data from different populations would provide more conclusive results in this regard. However, it should be emphasized that certain SNPs might have distinct roles in the tumorigenesis based on the ethnic-based factors.

Finally, assessment of relative expressions of RAGE and sRAGE in patients and controls might provide further clues for understanding the role of SNPs in conferring risk of cancer.

Declarations

Author contribution statement

Mohammad Taheri, Soudeh Ghafouri-Fard: conceived and designed the experiments; wrote the paper.

Rezvan Noroozi: analyzed and interpreted the data.

Mehrnoush Mousavi: performed the experiments.

Funding statement

This work was supported by Shahid Beheshti University of Medical Sciences.

Competing interest statement

The study was financially supported by Shahid Beheshti University of Medical Sciences.

Additional information

No additional information is available for this paper.

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