Molecular Biology Reports (2022) 49:3549–3557
https://doi.org/10.1007/s11033-022-07195-6

ORIGINAL ARTICLE

2q35-rs13387042 variant and the risk of breast cancer: a case–control study

Abolfazl Nesaei[1] · Zari Naderi Ghale-noie[2] · Asma Khorshid Shamshiri[2] · Fahimeh Afzaljavan[2] · Mahdi Rivandi[2] · Amir Tajbakhsh[2] · Fatemeh Homaei Shandiz[3] · Alireza Pasdar[2,4,5]

Received: 4 April 2021 / Accepted: 25 January 2022 / Published online: 20 April 2022

Abstract

Background Breast Cancer is the most frequent neoplasm diagnosed among women worldwide. Genetic background and lifestyle/environment play a significant role in the disease etiology. According to Genome-wide association studies, some single-nucleotide polymorphisms such as 2q35-*rs13387042*–(G/A) have been introduced to be associated with breast cancer risk and features. In this study, we aimed to evaluate the association between this variant and the risk of breast cancer in a cohort of Iranian women.

Methods Demographics and clinical information were collected by interview and using patients' medical records, respectively. DNA was extracted from 506 blood samples, including 184 patients and 322 controls, and genotyping was performed using allele specific-PCR. SPSS v16 was used for statistical analysis.

Result Statistically significant association was observed between AA genotype and disease risk in all patients [$p_{adj} = 0.048$; $OR_{adj} = 2.13$, 95% CI (1.01–4.50)] and also ER-positive breast cancers [$p_{adj} = 0.015$; $OR_{adj} = 2.12$, 95% CI (1.16–3.88)]. There was no association between *rs13387042* and histopathological characteristics of the disease. Furthermore, overall survival was not statistically associated with genotype and allelic models even after adjustment for stage and receptor status ($p > 0.05$).

Conclusion There is a statistically significant association between 2q35-*rs13387042* and breast cancer risk. *rs13387042*-AA genotype might be a risk-conferring factor for breast cancer development in the Iranian population. However, further consideration is suggested to confirm its role in risk assessment and probable association with other genetic markers.

Keywords Breast cancer · Polymorphism · *rs13387042* · 2q35 locus · ER-positive breast cancer

Introduction

Breast cancer is a leading cause of female cancer death globally [1] and has been introduced as the second cause of cancer-related deaths in Iran [2]. Moreover, the high incidence of the disease and low age of diagnosis has been demonstrated among Iranian women during the recent decade [3–5], as it is worth mentioning that most of them are still at the appropriate age for employment [6].

There are heterogeneous risk factors responsible for an increased breast cancer incidence and mortality such as dietary changes, lack of physical activity, late pregnancy, having relatively fewer children as well as commonly used hormone-replacement therapy (HRT) [7, 8]. In addition, the disease may show all the hallmarks of a multistep genetic disease. The recognized genes involved in hereditary breast cancer account only for 16–20% of the familial type. However, 85 to 90% of all cases are non-hereditary, which is the

Abolfazl Nesaei, Zari Naderi Ghale-noie and Asma Khorshid Shamshiri have contributed equally to this work.

✉ Alireza Pasdar
PasdarA@mums.ac.ir

1 Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

2 Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

3 Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

4 Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen, UK

5 Medical Genetics Research Center, Faculty of Medicine, Mashhad University of Medical Scienses, Mashhad, Iran

most common but least known, in terms of genetic predisposition [9, 10]. However, many genetic markers for BC susceptibility have been suggested through Genome-wide Association Studies (GWAS) [11].

rs13387042 on 2q35 locus has been identified as a hotspot for BC susceptibility in GWAS as well as replication studies in different populations [12–15]. It is located near *TNP1* (transition protein 1), *IGFBP5* (insulin-like growth factor binding protein 5), *IGFBP2* (insulin-like growth factor binding protein 2), and *TNS1* (Tensin 1/matrix remodelling-associated protein 6) genes [13, 16]. *IGFBP5* gene is an essential factor in normal mammary epithelial development. Along with 2q35, they have been constantly associated with cancer, although little is known about the nature of their interaction [17]. Moreover, it has been revealed that non-coding regions have a critical role in the regulation of gene expression. Since *rs13387042* is located in this area, it has been at the center of focus of different investigations [16]. The susceptibility of this SNP in breast cancer has been explored in European, African, and Asian populations [18–20]. In the present study, we investigated the association of this genetic marker with the risk and survival of breast cancer in a cohort of North-eastern Iranian patients.

Materials and methods

Subjects

506 women consisting of 184 patients and 322 controls were enrolled in our study. All of the controls were evaluated by routine clinical examinations. They had no sign of breast cancer or a history of malignant breast disease. The breast cancer patients (N = 184) were confirmed cases with available pathological information, including HER2, PR, ER status, stage, and the grade of the tumour.

The study was approved by the local ethical committee of Mashhad University of Medical Sciences (Ethical approval number: IR.MUMS.REC.1394.186). Signed informed consent was obtained from each study participant.

Genotyping method

DNA was extracted from the whole blood samples using the saturated salting-out technique. Allele-specific polymerase chain reaction (AS-PCR) was carried out to detect the genotype of patients and controls. A total of 12 µl of the final PCR reaction volume was used for this purpose. The reaction volume was composed of 2 µl of the DNA template (150 ng), 3 µl distilled water, 1 µl of each of the 2 primers (1 common primer, and 1 mutant or normal primer with the concentration of 10 µM), 5 µl Taq 2 × master mix (AMPLIQON). The 5′ to 3′ sequence of the two allele-specific forward and common reverse primers are listed below: Allele A specific forward 5′-ACAGAAAGAAGGCAAATGGAA-3′ (size band: 220 bp), Allele G specific forward 5′-ACAGAAAGAAGGCAAATGTAG-3′ (size band: 184) and common reverse 5′GGAGAATCACTTGAACCTGGA3′.

PCR condition included 10 min initial denaturation at 95 °C followed by 35 cycles as 15 s denaturation at 95 °C, 15 s annealing at 56 °C for *rs13387042* G and 58 °C for *rs13387042* A, and 15 s extension at 72 °C, and 10 min final extension at 72 °C. PCR was performed in a Veriti 96 well PCR Thermal Cycler (Applied Biosystems, Foster City, California, United States), and then Electrophoresis with 2% agarose gel was done for all the samples. 10% of samples were randomly re-genotyped to confirm the genotyping results.

Statistical analysis

The statistical evaluation was performed using the "SPSS version 16" software package (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including frequencies, mean and standard deviation (SD), were used to describe all variables. Binary logistic regression analysis was performed to assess the association between *rs13387042* and the risk of breast cancer. Moreover, the survival analysis was done using Kaplan–Meier and Cox regression methods and the log-rank test was used to estimate differences between the groups. A p-value of less than 0.05 was considered significant.

Results

Characteristics of the study population

Demographic features of the patients and controls have been summarised in Table 1. Furthermore, tumour characteristics of breast cancer cases have been shown in Table 2. The mean age in patients, 46.03 ± 11.99 years, was higher than controls ($p = 0.016$). Comparison of Body mass index parameter between the two groups showed the cases (28.32 ± 4.80 kg/m^2) were more overweight than controls (25.19 ± 4.17 kg/m^2). Furthermore, the age of the first gestation was 21.36 ± 4.85 and 22.60 ± 4.52 years in patients and healthy women, respectively ($p = 0.009$). The marital status ($p = 0.006$), education ($p < 0.001$), and physical activity ($p < 0.001$) indicated a significant difference between breast cancer cases and controls. This significant difference was also observed between the two groups in the menopause status ($p < 0.001$), and the frequency of menopausal women was higher in patients with 44.7%. A significantly higher

Table 1 Demographic characteristic of the patients and controls

Characteristic	Cases	Controls	p-value	OR (95% CI)
Age (year)[a]	48.69 ± 11.50	46.03 ± 11.99	**0.016**	1.02 (1.00–1.03)
Age of menarche (year)	13.15 ± 1.51	13.23 ± 1.59	0.586	0.97 (0.86–1.09)
Age of menopause (year)	47.45 ± 5.89	48.30 ± 5.30	0.312	0.97 (0.92–1.03)
Age of first gestation (year)	21.36 ± 4.85	22.60 ± 4.52	**0.009**	0.94 (0.90–0.99)
BMI (Kg/m^2)[c]	28.32 ± 4.80	25.19 ± 4.17	**< 0.001**	1.17 (1.12–1.22)
Marital status				
Single	10 (5.5%)	44 (13.7%)	Ref.[b]	
Married	169 (92.9%)	273 (84.8%)	**0.006**	2.72 (1.33–5.56)
Divorced/widow	3 (1.6%)	5 (1.6%)	0.231	2.64 (0.54–12.91)
Menopause status				
Pre/peri-menopause	99 (55.3%)	227 (74.4%)	Ref	
Post-menopause	80 (44.7%)	78 (25.6%)	**< 0.001**	2.35 (1.59–3.48)
Lactation				
No	16 (8.9%)	5 (2.1%)	Ref	
Yes	163 (91.1%)	237 (97.9%)	**0.003**	0.21 (0.08–0.60)
Abortion				
No	120 (68.2%)	163 (66.8%)	Ref	
Yes	56 (31.8%)	81 (33.2%)	0.766	0.94 (0.62–1.42)
Screening				
No	124 (81.6%)	234 (77.5%)	Ref	
Yes	28 (18.4%)	68 (22.5%)	0.314	0.78 (0.48–1.27)
Education				
Non-academic	118 (65.6%)	145 (46.0%)	Ref	
Academic	62 (34.4%)	170 (54.0%)	**< 0.001**	0.45 (0.31–0.65)
BMI				
BMI < 25 (Kg/m^2)	39 (22.8%)	162 (51.9%)	Ref	
BMI ≥ 25 (Kg/m^2)	132 (77.2%)	150 (48.1%)	**< 0.001**	3.65 (2.40–5.57)
Physical activity				
No	54 (33.8%)	30 (12.0%)	Ref	
Yes	106 (66.3%)	221 (88.0%)	**< 0.001**	0.27 (0.16–0.44)

Significant P-values have been shown in bold

[a]Mean ± SD

[b]Reference group

[c]Body Mass Index (BMI)

frequency of no breastfeeding history was observed in the patients (8.9%) than healthy individuals (2.1%) ($p = 0.003$).

The histopathological evaluation showed 51.3% of cases were in stage II, and 58.3% had grade II. 86.1% of the breast tumours were invasive ductal carcinomas, and in most patients (43.6%), cancer had not been spread to the lymph nodes. 30.1% of tumours sizes were between 2 cm or less. Evaluation of oestrogen and progesterone receptors indicated that more than 79.2% of tumours tissue expressed these receptors on their surface, and 9.2% of patients were Triple-Negative Breast Cancer (TNBC).

Association of *rs13387042* with breast cancer risk

Genotypes and alleles frequencies in breast cancer and healthy controls have been shown in Table 3. Based on Backward LR regression, BMI was the only baseline factor with a significant difference between groups. Therefore, all comparisons were adjusted for BMI.

The comparison of allele frequency distribution in the studied population showed that the genotype frequencies for AA, AG, GG were 21.7%, 66.8%, 11.4%, and 11.2%, 75.2%, 13.7% in case and control groups, respectively. More investigation revealed that allele and genotype frequency in cases and controls were not in Hardy–Weinberg equilibrium. Risk assessment was performed under three dominant, recessive and multiplicative models. A two-fold increased breast

Table 2 Frequency of tumor characteristics of Breast cancer cases

Characteristics	Number	Valid percent (%)
Tumor subtype		
Invasive ductal carcinoma	143	86.1
Others	23	13.9
Tumor size		
T1	50	30.1
T2	87	52.4
T3	17	10.2
T4	12	7.2
Lymph node		
Negative	68	43.6
N1	51	32.7
N2	28	17.9
N3	9	5.8
Metastasis		
M0	161	96.4
M1	6	3.6
Stage		
I	25	16.1
II	80	51.3
III	45	38.8
IV	6	3.8
Grade		
I	33	21.9
II	88	58.3
III	30	19.9
ER[a] status		
Negative	37	21.4
Positive	136	78.6
PR[b] status		
Negative	43	24.9
Positive	130	75.1
HER2[c]		
Negative	117	69.2
Positive	52	30.8
Receptor status		
ER/PR+ & HER2±	137	79.2
ER/PR− & HER2+	20	11.6
Triple negative (TNBC)	16	9.2

[a]Estrogen receptor

[b]Progesterone receptor

[c]Human epidermal growth factor receptor

cancer risk was observed in the recessive model for AA genotype [$p=0.003$, OR 2.53, 95% CI (1.31–3.88)]. However, we did not find a significant association under the dominant model between the two groups [$p=0.791$, OR 1.08, 95% CI (0.60–1.97)]. As shown in Table 3 and according to the allele frequency, A allele with 55.2% in cases versus 48.8% in controls, was introduced as the risky allele, however, the p-value was not significant [$p=0.107$, OR 1.26, 95% CI (0.95–1.66)].

The association between the *rs13387042* variant and BC risk for both ER-positive and healthy controls is demonstrated in Table 3. The AA genotype was associated with increased risk in ER-positive breast cancer patients before adjusting for BMI [$p=0.019$, OR 2.53, 95% CI (1.17–5.50)]. There was a statistically significant association in the dominant (AA vs. GG + GA) genetic model [$p=0.015$, OR 2.12, 95% CI (1.16–3.88)] with an increased risk of the disease in ER-positive cases.

Genotype frequencies were evaluated among different subtypes of breast cancer. Based on findings reported in Table 4, no significant association was observed for each genotype among the breast cancer subtypes. The AA genotype frequency was higher than that of the AG and GG genotypes in both the early stage (stage I & II) and ER-negative groups while in the ER/HER positive and PR-negative groups the AG genotype frequency was the highest. Logistic regression did not indicate a significant difference among different subtypes of breast cancer ($p<0.05$).

Association of *rs13387042* with overall survival

According to the findings, *rs13387042* did not indicate any association with the overall survival in the genotype ($p=0.529$) and allelic ($p=0.480$) models before adjustment (Fig. 1A and B, respectively). Furthermore, survival analysis in association with pathological factors showed that overall survival was significantly different in the upper/lower stage groups and the molecular category groups. After adjustment for these two factors, the results did not change. Overall survival information has been shown in Table 5.

Discussion

Recent GWAS have led to the identification of multiple novel genetic variants associated with BC risk, such as 2q35-*rs13387042*, which has been reported in various studies; however, the results have been inconsistent [13, 16, 21, 22]. With recent advances in genomics, elucidating the molecular basis of disease on a personalised level has become an attainable goal. Among them, genetic polymorphisms have a critical role in diseases susceptibility, diagnosis, and therapeutic efficacy of various cancers [13, 23]. Furthermore, the recent studies reported associated SNPs which are located in the noncoding regions, suggesting that the search for functional polymorphisms should extend beyond the gene regions [24, 25]. In the present study, the association between one of the important variants in 2q35 locus, *rs13387042* (located in

Table 3 Distribution of alleles and genotype frequencies of 2q35-*rs13387042* variant in control compared with total and ER-positive patients

Genetic model	Genotype	Breast cancer no. (%)	Healthy no. (%)	p-value	OR (95% CI)	p-value $_{adj}$	OR (95% CI) $_{adj}$
rs13387042	GG	21 (11.4)	44 (13.7)	Ref.			
	GA	123 (66.8)	242 (75.2)	0.827	1.06 (0.61–1.87)	0.829	0.93 (0.51–1.72)
	AA	40 (21.7)	36 (11.2)	**0.016**	2.33 (1.17–4.63)	**0.048**	2.13 (1.01–4.50)
Dominant	GG	21 (11.4)	44 (13.7)	Ref.			
	AA + GA	163 (88.6)	278 (86.3)	0.467	1.23 (0.71–2.14)	0.791	1.08 (0.60–1.97)
Recessive	GG + GA	144 (78.3)	286 (88.8)	Ref.			
	AA	40 (21.7)	36 (11.2)	**0.002**	2.21 (1.35–3.61)	**0.003**	2.53 (1.31–3.88)
Multiplicative	G	165 (44.8)	330 (51.2)	Ref.			
	A	203 (55.2)	314 (48.8)	0.050	1.29 (1.00–1.67)	0.107	1.26 (0.95–1.66)
		ER-positive breast cancer	Healthy				
rs13387042	GG	14 (10.3)	44 (13.7)	Ref.		Ref.	
	GA	93 (68.4)	242 (75.2)	0.568	1.21 (0.63–2.31)	0.884	0.95 (0.47–1.91)
	AA	29 (21.3)	36 (11.2)	**0.019**	2.53 (1.17–5.50)	0.102	2.03 (0.87–4.75)
Recessive	GG	14 (10.3)	44 (13.7)	Ref.			
	AA + GA	122 (89.7)	278 (86.3)	0.323	1.38 (0.73.261)	0.819	1.08 (0.54–2.16)
Dominant	GG + GA	107 (87.7)	286 (88.8)	Ref.			
	AA	29 (21.3)	36 (11.2)	**0.005**	2.15 (1.26–3.68)	**0.015**	2.12 (1.16–3.88)
Multiplicative	G	121 (44.5)	330 (51.2)	Ref.			
	A	151 (55.5)	314 (48.8)	0.062	1.31 (0.99–1.74)	0.190	1.23 (0.90–1.69)

Significant P-values have been shown in bold

intergenic reign), and breast cancer was investigated in the North-eastern Iranian population.

Our findings indicated a significant association between 2q35-*rs13387042* carriers of two A-allele and breast cancer in our population. This genotype increases the risk of the disease by 2.53–folds compared to other genotypes. This result is similar to those in the study by Stacey et al. however, the amount of conferred risk was lower in their study [13]. In spite of the fact that GWASs have revealed novel genetic markers for BC susceptibility in different populations, little is known regarding the risk factors and molecular events associated with BC in the Iranian population.

In our study, we observed that the A-allele (as a risky allele) frequency is higher in cases than controls (55.2% and 48.8%, respectively). In different studies, the A allele has been associated with an increased risk of breast cancer. This allele is the most common in African populations (77%), and has a lower frequency in Europeans (51%) and Mexican Americans (41%), and is less common in Asians (12%) according to the frequencies from the 1000 Genomes Project [21]. Consistent with HapMap data, the A-allele frequency was much more common in Europeans than in the Asian population. Stacey et al. also showed that 25 percent of the European population carry allele A of *rs13387042,* who are estimated to have an increased risk of 1.44-fold in comparison with non-carriers [13]. In a study in the Arab population, the GG genotype of *rs13387042* on 2q35 showed a significant association with the risk of developing distant metastasis. Also, this allele indicated a better prognosis by presenting a considerably higher overall survival rate [26]. A Taiwanese survey revealed A-allele of 2q35 conferred a higher risk for BC risk than allele G. However, in our study, no significant association of the *rs13387042* with breast cancer was found under the multiplicative genetic model.

Although published meta-analysis data on the association between 2q35-*rs13387042* and breast cancer risk has introduced A allele as a risk factor for the disease [27], inconsistent results might be observed in different studies. Campa et al. confirmed the association of 14 SNPs including *rs13387042* with BC risk [13, 28]. This SNP was first identified as a BC susceptibility SNP in two GWASs conducted among Europeans (4554 cases/17,577 controls) [13]. Later studies on African-American (810 cases and 1784 controls), as well as European women (306 cases and 10,393 controls), confirmed a significant association between the mentioned SNP and breast cancer [18, 29]. In Asians the results were inconsistent. For instance, in a study on the Chinese population, the association between this SNP and breast cancer risk varied from having a significant to non-significant association [30, 31]. Additionally, a study by Hutter et al. showed no significant associations between *rs13387042* and BC in African American women [32]. Similar studies and results were reported in the Norwegian series [33]. There are some explanations for such inconsistent results. Importantly,

Table 4 Distribution of alleles and genotype frequencies of 2q35-*rs13387042* variant in different pathological status

Genotype	Pathologic feature		p-value	OR (95% CI)
	Low grade	High grade		
GG	14 (11.6)	1 (3.3)	Ref.	
AG	81 (66.9)	22 (73.3)	0.209	3.80 (0.47–30.52)
AA	26 (11.4)	7 (23.3)	0.236	3.77 (0.42–33.80)
G	109 (45.0)	24 (40.0)	Ref.	
A	133 (55.0)	36 (60.0)	0.482	1.23 (0.69–2.18)
	Early stage	Late stage		
GG	13 (12.4)	4 (7.8)	Ref.	
AG	68 (64.8)	41 (80.4)	0.266	1.96 (0.60–6.41)
AA	24 (29.2)	6 (11.8)	0.777	0.81 (0.19–3.41)
G	94 (44.8)	49 (48.0)	Ref.	
A	116 (55.2)	53 (52.0)	0.586	0.88 (0.55–1.41)
	ER negative	ER positive		
GG	4 (10.8)	14 (10.2)	Ref.	
AG	25 (67.6)	94 (68.6)	0.906	1.07 (0.32–3.55)
AA	8 (21.6)	29 (21.2)	0.960	1.04 (0.27–4.03)
G	33 (44.6)	121 (44.5)	Ref.	
A	41 (55.4)	151 (55.5)	0.987	1.00 (0.60–1.68)
	PR negative	PR positive		
GG	5 (11.6)	13 (10.0)	Ref.	
AG	30 (69.8)	88 (67.7)	0.832	1.13 (0.37–3.23)
AA	8 (18.6)	29 (22.3)	0.615	1.39 (0.38–5.09)
G	40 (46.5)	114 (43.8)	Ref.	
A	46 (53.6)	146 (56.2)	0.666	1.11 (0.68–1.82)
	HER2 negative	HER2 positive		
GG	12 (10.3)	4 (7.7)	Ref.	
AG	80 (68.4)	36 (69.2)	0.623	1.35 (0.41–4.47)
AA	25 (21.4)	12 (23.1)	0.589	1.44 (0.38–5.41)
G	104 (44.4)	44 (42.3)	Ref.	
A	130 (55.6)	60 (57.7)	0.715	1.09 (0.68–1.74)

ethnic differences might give rise to these different results, because of changes in allele frequencies in various ethnic populations. Therefore, it is possible that the association between a genetic marker and one specific subtype would not be replicated in other study populations [34]. In addition, some other risk factors such as lifestyle, environmental exposures, diet schedules, individual health backgrounds, tumour ER/PR status and menopausal status as well as adequate sample size and study design can all play a critical role [35].

Histopathological properties may also influence the results, as the original publications on 2q35-*rs13387042* reported the association with mostly ER-positive BC [13, 36]. In the same way, we could also find a significant association between *rs13387042* with BC for ER-positive diseases. Similarly, in the Stacey et al. study, the risk regarding this variant was observed for ER-positive tumours [13]. In spite of that, this association was observed for both ER+ and ER− in another research [37]. Some studies on genetic markers including *rs13387042* showed stronger associations with ER-positive than with ER-negative tumours for several loci [13, 38]. It has to be mentioned that among various loci, *rs13387042* showed significantly different associations by ER status, although no overall associations were found for this polymorphism in our study as well as in Kim et al. study [39].

We did not find the association between overall survival and 2q35-*rs13387042* alleles and genotypes. Previous studies indicated different results. Studies in UK and Germany did not find prognostic value for *rs13387042* [40,

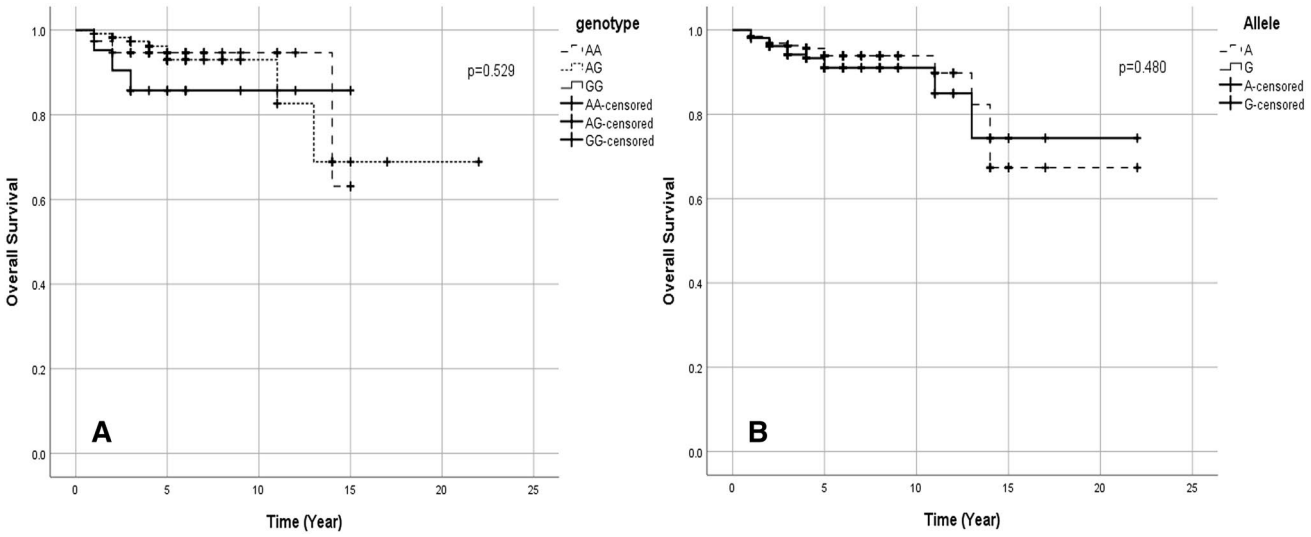

Fig. 1 Kaplan-Meier plots of different genetic models of 2q35-*rs13387042* polymorphism. **A** Genotype model (GG and AG vs. AA), **B** multiplicative model (A vs. G)

Table 5 Multivariable overall survival analysis in association with 2q35-*rs13387042* variant

Variables	P	HR	95.0% CI for HR	
			Lower	Upper
Genotype				
AG vs AA	0.280	0.387	0.069	2.167
GG vs AA	0.280	2.751	0.439	17.226
Late stage vs. early stage	**0.006**	8.018	1.844	34.863
HER2 vs. ER/PR+	0.215	2.437	0.596	9.964
TNBC vs. ER/PR+	**0.003**	11.126	2.233	55.440
Allele				
G vs. A	0.552	1.301	0.582	2.908
Late stage vs. early stage	**0.001**	5.427	2.048	14.380
HER2 vs. ER/PR+	**0.022**	3.104	1.174	8.205
TNBC vs. ER/PR+	**< 0.001**	6.726	2.391	18.921

Significant P-values have been shown in bold

41]. However, the *rs13387042*-A allele was associated with a better prognosis as indicated significantly higher overall survival rates in the Arab population [26].

Finding the potential biological functions of SNPs like 2q35-*rs13387042* can be a significant step towards further studies. Recent studies have shown that two polymorphisms that are in strong linkage disequilibrium (LD) with *rs13387042* (*rs6721996* and *rs4442975*) are associated with decreasing expression of *IGFBP5* (involved in inhibition of cell proliferation via an insulin growth factor (IGF)-dependent mechanism) as well as an increasing number of A allele. Additionally, in the recent years, *IGFBP5* and 2q35 have been consistently implicated in cancer, though little was known about the nature of their interaction [21]. *IGFBP5* contributes to the documented involvement of the IGF signalling axis in mammary density as a risk factor for BC [42, 43]. It has been indicated that the 2q35 plays a role in chromatin architecture, and its functional variation is correlated with gene expression. Since a novel intergenic BC risk locus containing an enhancer copy number variation (enCNV; deletion) is located approximately 400 Kb upstream to *IGFBP5*, which overlaps an intergenic ERα-bound enhancer that loops to the *IGFBP5* promoter, thus 2q35 BC risk loci may be mediating their effect through *IGFBP5* [17]. Consequently, functional studies may lead to a better understanding of the mechanisms of aetiology of BC.

As a limitation, we could not confirm the association of this variant with specific BC subtypes because we did not have a large sample size to evaluate the less frequent molecular categories. Thus, larger sample sizes could help increase the power and ensure the correct conclusion respecting whether this SNP is associated with specific BC subtypes. Therefore, this may warrant the need for more collaborative studies to assess the strength of the risk in association with susceptibility variants. It should be noted that the purpose of our study was to evaluate the variant 2q35-*rs13387042* in the Iranian population, which was confirmed by the risk of breast cancer by the GWA study. In addition, to determine the full role of this genetic locus in the pathogenesis of the disease, it is necessary to consider functional studies as a new project.

Conclusion

Our study demonstrated a slightly significant association of an intergenic SNP with BC risk in an Iranian population. Furthermore, additional investigation of larger data sets along with intrinsic subtypes categorization as well as functional studies are required to conclude how, and to which degree, these variants are influencing BC pathogenesis.

Acknowledgements The authors thank all participants who took part in this study. We would also like to thank Mashhad University of Medical Sciences, Omid and Imam Reza hospitals who supported this project.

Author contributions Design of the research: AN, FA and AP. Data collection: AN, ZNG, FA, AT, MR, FHS and AKS. Laboratory work: AN, ZNG. Statistical analysis: FA and AKS. Manuscript drafting: ZNG, FA, AKS and AP. AP edited and approved the final version of this manuscript. All authors also participated in the finalization of the manuscript and approved the final draft.

Funding This work was based on the Master of Science thesis of Mr. Abolfazl Nesaei and partly was financially supported by Mashhad University of Medical Sciences under Grant 931028.

Data availability The analyzed data sets generated during the present study are not available due to the university rules and regulations, however, upon written request, subjected to approval by the university, some parts may be available.

Code availability Not applicable.

Declarations

Conflict of interest No potential conflicts of interest were disclosed.

Ethical approval The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Ethic No: IR.MUMS.REC.1394.186).

Consent to participate Written informed consent was obtained from all participants included in the study.

References

1. Ghoncheh M, Pournamdar Z, Salehiniya H (2016) Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev 17(S3):43–46
2. Taghavi A, Fazeli Z, Vahedi M, Baghestani AR, Pourhoseingholi A, Barzegar F et al (2012) Increased trend of breast cancer mortality in Iran. Asian Pac J Cancer Prev 13(1):367–370
3. Aghababazadeh M, Dorraki N, Javan FA, Fattahi AS, Gharib M, Pasdar A (2017) Downregulation of Caspase 8 in a group of Iranian breast cancer patients—a pilot study. J Egypt Natl Cancer Inst 29(4):191–195
4. Bagherabad MB, Afzaljavan F, Vahednia E, Rivandi M, Vakili F, Sadr SSH et al (2019) Association of caspase 8 promoter variants and haplotypes with the risk of breast cancer and its molecular profile in an Iranian population: a case-control study. J Cell Biochem 120(10):16435–16444
5. Vahednia E, Shandiz FH, Bagherabad MB, Moezzi A, Afzaljavan F, Tajbakhsh A et al (2019) The impact of *CASP8* rs10931936 and rs1045485 polymorphisms as well as the haplotypes on breast cancer risk: a case-control study. Clin Breast Cancer 19(5):e563–e577
6. Azarkish F, Mirzaii Najmabadi K, Latifnejad Roudsari R, Homaei Shandiz F (2015) Factors related to return to work in women after breast cancer in Iran. Iran Red Crescent Med J 17(9):e19978
7. Tajbakhsh A, Mokhtari-Zaer A, Rezaee M, Afzaljavan F, Rivandi M, Hassanian SM et al (2017) Therapeutic potentials of BDNF/TrkB in breast cancer; current status and perspectives. J Cell Biochem 118(9):2502–2515
8. Shamshiri AK, Afzaljavan F, Alidoust M, Taherian V, Vakili F, Moezzi A et al (2020) ESR1 gene variants, haplotypes and diplotypes may influence the risk of breast cancer and mammographic density. Mol Biol Rep 47(11):8367–8375
9. Lerebours F, Lidereau R (2002) Molecular alterations in sporadic breast cancer. Crit Rev Oncol Hematol 44(2):121–141
10. Alidoust M, Shamshiri AK, Tajbakhsh A, Gheibihayat SM, Mazloom SM, Alizadeh F et al (2021) The significant role of a functional polymorphism in the NF-κB1 gene in breast cancer: evidence from an Iranian cohort. Future Oncol 17(35):4895–4905
11. Zhang H, Ahearn TU, Lecarpentier J, Barnes D, Beesley J, Qi G et al (2020) Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. Nat Genet 52:572–581
12. Gu C, Zhou L, Yu J (2013) Quantitative assessment of 2q35-rs13387042 polymorphism and hormone receptor status with breast cancer risk. PLoS ONE 8(7):e66979
13. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA et al (2007) Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor–positive breast cancer. Nat Genet 39(7):865–869
14. Elematore I, Gonzalez-Hormazabal P, Reyes JM, Blanco R, Bravo T, Peralta O et al (2014) Association of genetic variants at TOX3, 2q35 and 8q24 with the risk of familial and early-onset breast cancer in a South-American population. Mol Biol Rep 41(6):3715–3722
15. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE et al (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 39(7):870–874
16. Liang H, Li H, Yang X, Chen L, Zhu A, Sun M et al (2016) Associations of genetic variants at nongenic susceptibility loci with breast cancer risk and heterogeneity by tumor subtype in Southern Han Chinese women. BioMed Res Int. https://doi.org/10.1155/2016/3065493
17. Wyszynski A, Hong CC, Lam K, Michailidou K, Lytle C, Yao S et al (2016) An intergenic risk locus containing an enhancer deletion in 2q35 modulates breast cancer risk by deregulating IGFBP5 expression. Hum Mol Genet 25(17):3863–3876
18. Zheng W, Cai Q, Signorello LB, Long J, Hargreaves MK, Deming SL et al (2009) Evaluation of 11 breast cancer susceptibility loci in African-American women. Cancer Epidemiol Biomark Prev. https://doi.org/10.1158/1055-9965.EPI-09-0624
19. Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, Cox DG et al (2009) A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet 41(5):579–584
20. Liang H, Yang X, Chen L, Li H, Zhu A, Sun M et al (2015) Heterogeneity of breast cancer associations with common genetic variants in FGFR2 according to the intrinsic subtypes in Southern Han Chinese Women. Biomed Res Int. https://doi.org/10.1155/2015/626948
21. Fejerman L, Stern MC, John EM, Torres-Mejia G, Hines LM, Wolff RK et al (2015) Interaction between common breast cancer susceptibility variants, genetic ancestry, and nongenetic risk

factors in Hispanic women. Cancer Epidemiol Biomark Prev 24(11):1731–1738
22. Dai J, Hu Z, Jiang Y, Shen H, Dong J, Ma H et al (2012) Breast cancer risk assessment with five independent genetic variants and two risk factors in Chinese women. Breast Cancer Research: BCR 14(1):R17
23. Deng N, Zhou H, Fan H, Yuan Y (2017) Single nucleotide polymorphisms and cancer susceptibility. Oncotarget 8(66):110635
24. Tajbakhsh A, Afzal Javan F, Fazeli M, Rivandi M, Kushyar MM, Nassiri M et al (2017) TOX3 Gene polymorphisms and breast cancer; effects and implications of the variations. Tehran Univ Med J TUMS Publications 75(5):323–331
25. Fagny M, Platig J, Kuijjer ML, Lin X, Quackenbush J (2020) Nongenic cancer-risk SNPs affect oncogenes, tumour-suppressor genes, and immune function. Br J Cancer 122(4):569–577
26. Shan J, Mahfoudh W, Dsouza SP, Hassen E, Bouaouina N, Abdelhak S et al (2012) Genome-Wide Association Studies (GWAS) breast cancer susceptibility loci in Arabs: susceptibility and prognostic implications in Tunisians. Breast Cancer Res Treat 135(3):715–724
27. Huang T, Hong J, Lin WL, Yang QQ, Ni KL, Wu QY et al (2013) Assessing interactions between common genetic variant on 2q35 and hormone receptor status with breast cancer risk: evidence based on 26 studies. PLoS ONE 8(8):e69056
28. Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD et al (2011) Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. J Natl Cancer Inst 103(16):1252–1263
29. Reeves GK, Travis RC, Green J, Bull D, Tipper S, Baker K et al (2010) Incidence of breast cancer and its subtypes in relation to individual and multiple low-penetrance genetic susceptibility loci. JAMA 304(4):426–434
30. Dai J, Hu Z, Jiang Y, Shen H, Dong J, Ma H et al (2012) Breast cancer risk assessment with five independent genetic variants and two risk factors in Chinese women. Breast Cancer Res 14(1):1
31. Zheng W, Wen W, Gao YT, Shyr Y, Zheng Y, Long J et al (2010) Genetic and clinical predictors for breast cancer risk assessment and stratification among Chinese women. J Natl Cancer Inst 102(13):972–981
32. Hutter CM, Young AM, Ochs-Balcom HM, Carty CL, Wang T, Chen CT et al (2011) Replication of breast cancer GWAS susceptibility loci in the Women's Health Initiative African American SHARe Study. Cancer Epidemiol Biomark Prev 20(9):1950–1959
33. Heramb C, Ekstrom PO, Tharmaratnam K, Hovig E, Moller P, Maehle L (2015) Ten modifiers of BRCA1 penetrance validated in a Norwegian series. Hered Cancer Clin Pract 13(1):14
34. Wacholder S, Hartge P, Prentice R, Garcia-Closas M, Feigelson HS, Diver WR et al (2010) Performance of common genetic variants in breast-cancer risk models. N Engl J Med 362(11):986–993
35. Slattery ML, Baumgartner KB, Giuliano AR, Byers T, Herrick JS, Wolff RK (2011) Replication of five GWAS-identified loci and breast cancer risk among Hispanic and non-Hispanic white women living in the Southwestern United States. Breast Cancer Res Treat 129(2):531–539
36. Milne RL, Benítez J, Nevanlinna H, Heikkinen T, Aittomäki K, Blomqvist C et al (2009) Risk of estrogen receptor-positive and -negative breast cancer and single-nucleotide polymorphism 2q35-rs13387042. J Natl Cancer Inst 101(14):1012–1018
37. Milne RL, Benítez J, Nevanlinna H, Heikkinen T, Aittomäki K, Blomqvist C et al (2009) Risk of estrogen receptor-positive and–negative breast cancer and single-nucleotide polymorphism 2q35-rs13387042. J Natl Cancer Inst 101(14):1012–1018
38. Garcia-Closas M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA et al (2008) Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet 4(4):e1000054
39. Kim H, Lee J-Y, Sung H, Choi J-Y, Park SK, Lee K-M et al (2012) A genome-wide association study identifies a breast cancer risk variant in ERBB4 at 2q34: results from the Seoul Breast Cancer Study. Breast Cancer Res 14(2):1–12
40. Fasching PA, Pharoah PDP, Cox A, Nevanlinna H, Bojesen SE, Karn T et al (2012) The role of genetic breast cancer susceptibility variants as prognostic factors. Hum Mol Genet 21(17):3926–3939
41. Hein A, Rack B, Li L, Ekici AB, Reis A, Lux MP et al (2017) Genetic breast cancer susceptibility variants and prognosis in the prospectively randomized SUCCESS a study. Geburtshilfe Frauenheilkd 77(6):651
42. Biong M, Gram IT, Brill I, Johansen F, Solvang HK, Alnaes GI et al (2010) Genotypes and haplotypes in the insulin-like growth factors, their receptors and binding proteins in relation to plasma metabolic levels and mammographic density. BMC Med Genom 3:9
43. Woolcott CG, Courneya KS, Boyd NF, Yaffe MJ, McTiernan A, Brant R et al (2013) Longitudinal changes in IGF-I and IGFBP-3, and mammographic density among postmenopausal women. Cancer Epidemiol Biomark Prev 22(11):2116–2120