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RESEARCH ARTICLE

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A genetic variant in CDKN2A/B gene is associated with the increased risk of breast cancer

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Background: Breast cancer is among the leading cause of cancer-related-deaths in women, supporting the need for the identification of novel prognostic and predictive biomarkers. Recent studies have identified common genetic variants in a region on chromosome 9p21 associated with an increased risk of developing different cancers. Here, we explored the association of a genetic variant in CDKN2A/B, rs10811661, for the first time in 564 subjects with/without breast cancer.

Method: Genotyping was performed using TaqMan real time PCR method. The associations of this genetic variant with breast cancer risk and pathological information of patients were assessed.

Results: We observed that patients with breast cancer had a higher frequency of TT genotype (P<.001) than control group, which was associated with advanced TNM classification (P=.04) and larger tumor size (P=.014), as detected by the recessive genetic inheritance model. Moreover, the logistic regression under recessive genetic model revealed that breast cancer patients with TT had higher risk of breast cancer, compared to CC/CT genotypes (eg, OR=4.9, 95% CI:1.9-12, P=.001), after adjusted for potential confounders, age, BMI, and family history.

Conclusion: We demonstrated that patients carrying the TT genotype for CDKN2A/B rs10811661 polymorphism had the increased risk of breast cancer susceptibility. However, further investigations are warranted in a larger and prospective setting to explore the value of this marker as a risk stratification marker in breast cancer.

KEYWORDS
breast cancer, CDKN2A/B, polymorphism, risk marker

1 | INTRODUCTION

Breast cancer is the second leading cause of cancer-related deaths in women with poor prognosis.[1] Since breast cancer is such a dismal disease, any biomarker that can help to better stratify patients might have a substantial impact on the clinical management of patients. This cancer is characterized by progressive multistep processes, interactions of both genetic and environment factors. Several genes have been reported to be associated with poor outcome in patients with breast cancer (eg, Breast cancer susceptibility genes 1 (*BRCA1*),

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Novelty and impact: This study demonstrated the significant association of the CDKN2A/B, rs10811661 polymorphism with outcome in breast cancer patients. This polymorphism can be assessed with a simple blood test, and offers an innovative tool for optimizing palliative chemotherapy in breast cancers. Prospective trials are warranted to validate these findings, which might be applied to the future clinical practice of individualized treatment in selected breast cancer patients.

BRCA2, *HER2*, *P53* and *Ki67*). About 5%-10% of breast cancer cases are supposed to be hereditary [1] and individuals with these mutations have significantly higher susceptibility to developing breast cancer.

Recent genome-wide association (GWA) studies have reported that genetics variants in a region located on chromosome 9p21.3 are associated with multiple cancers [2-5] such as ovarian cancer, acute lymphoblastic leukemia, glioma, malignant melanoma, breast cancer, pancreatic cancer, nasopharyngeal neoplasm, and glaucoma.[5,6] The chromosome 9p21.3 region adjacent to the loci encoding the cyclin-dependent kinase inhibitors *CDKN2A* (ENSG00000147889) and *CDKN2B* (ENSG00000147883) is an important susceptibility locus for various diseases with a complex genetic background. Furthermore, this region is also reported to be linked with several other diseases such as cardiovascular diseases,[6] type-2 diabetes,[7] periodontitis,[8] Alzheimer's disease,[9] frailty in the elderly,[10] and glaucoma.[5] CDKN2A and CDKN2B encode p16 INK4a and p15 INK4b, which inhibit cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 5 (CDK5), respectively. CDK4 and CDK5 play an important role in different cell function and regeneration.[6] Several genetic polymorphisms have been suggested in the upstream of CDKN2A/B, which might influence the expression of these genes and thereby cell cycle.[11] Therefore, the aim of this study was to examine the association of CDKN2A/B rs10811661 polymorphism with breast cancer.

2 | MATERIALS AND METHODS

2.1 | Patient samples

In this study, 564 age-matched Iranian women subjects (92 breast cancer patients and 472 healthy controls) were recruited from Omid or Ghaem hospitals of Mashhad University of Medical Sciences. The patients were histologically confirmed breast cancer between 2013 and 2014. Informed consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences.

2.2 | DNA extraction and genotyping

Genomic DNAs were extracted from peripheral blood leukocytes using QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA, USA) according to the manufacturer's protocol. The concentration and purity of DNA samples were determined using the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, DE, USA). Genotype analysis of CDKN2A/B-rs10811661 polymorphism was carried out using Taqman®-probes-based assay; PCR reactions were carried out in 12.5 μL total volume, using 20 ng of DNA in TaqMan® Universal Master Mix with specific primers and probes (C-901792-10 and C-790057-10; Applied Biosystems, Foster City, CA, USA). The ABIPRISM-7500 instrument equipped with the SDS version-2.0 software was utilized to determine the allelic content of the samples.[12,13]

2.3 | Statistics

Demographic and clinical information were compared across mutations using Pearson's χ^2 tests. The significant prognostic variables in univariate analysis were included in multivariate analyses using Cox's proportional hazards model. This analysis included a step down procedure according to the likelihood ratio test, where Hazard Ratio (HR) was assessed to evaluate the magnitude and the direction of the effect. Appropriate adjustment for false-positive report probability was carried out according to the Wacholder method. The presence of normal distribution within the subgroups was assessed by Kolmogorov-Smirnov tests. Continuous variables were evaluated using Student's *t* tests. The observed genotype frequencies of the CDKN2A/B gen rs10811661 polymorphism were assessed with χ^2 tests. The Hardy-Weinberg equilibrium assumption was assessed by comparing the genotype frequencies by using the Pearson χ distribution. The associations between risk of breast cancer for the CC and CT genotypes, relative to the risk genotype TT homozygote under recessive genetic model were assessed by logistic regression, adjusting for the potential confounders; age, body mass index, family history. Data were analyzed using SPSS-20 software (SPSS Inc., Chicago, IL, USA). All the analyses were two-sided and statistical significance was set at $P<.05$.

3 | RESULTS

3.1 | Association of the genetic variant with clinical characteristics of population

In order to explore whether there was an association between CDKN2A/2B Rs10811661 (C/T) polymorphism and breast cancer, genotyping was performed in all the subjects using DNA extracted from peripheral blood samples. Genotyping was successfully performed in the all DNA samples and no discrepancies were found in the samples analyzed in duplicate. The distribution of the polymorphism was in Hardy-Weinberg equilibrium (HWE; $P>.05$). We then characterized our population based on the genetic information of patients for age, body mass index, SA, tumor size, nodal status, distant metastasis, TNM stage, HER-2/neu status, Ki62, CEA, and CA153 (Table 1). According to the recessive genetic inheritance model, we found that the TT genotype of was associated with advanced TNM classification ($P=.04$) and larger tumor size ($P=.014$), while no association was detected for this genetic variant with distant metastasis, node status, and other clinical features of patients ($P>.05$).

3.2 | Association of the genetic variant with poor prognosis of patients

The distribution of different genotypes (CC, CT, and TT) of CDKN2A/B rs10811661 polymorphism in breast cancer patients and healthy subjects is presented in Table 2. The risk T allele frequency was 85%, and the frequencies of CC, CT, and TT genotypes were 0.03, 22.9, and 74%, respectively, in total population. Patients with breast cancer had a significantly higher frequency of TT genotype ($P<.001$) than control

TABLE 1 Genotype distribution of CDKN2A/B rs10811661 polymorphism with respect to clinicopathological features of breast cancer patients under recessive genetic model

	Genotype	
	CC+CT	TT
Age	48±17	47±11
BMI	27.8±6	28.2±7
Clinical measures		
SA%		
1+2	7.9	92.1
3	0	100
M		
0	8.1	91.9
1	0	100
N, %		
0-1	5.1	94.9
2-3	12	88
T*%		
1-3	5.2	94.8
4	33.3	66.7
Ki62%		
0	60	78
1	40	22
Stage**%		
0	40	60
1	0	100
2	10.3	89.7
3	0	100
4	7.8	92.2
Her2%		
1+2	6	94
3	14.3	85.7

*P=.014, **P=.04.

group (Table 2). Moreover, the logistic regression analysis under recessive genetic model showed that TT carriers had a higher risk of breast cancer (OR=4.9, 95%CI: 1.9-12, P=.001), with respect to CC/CT genotypes, after adjusted for potential confounders, age, BMI, and family history. However, no statistically significant association was detected for the association of this marker in other genetic models (eg, additive and dominant models).

4 | DISCUSSION

To the best of our knowledge, this is the first study showing the prognostic value of a genetic variant in CDKN2A/B with poor prognosis of patients with breast cancer. Our data demonstrated that patients with TT genotype had a significantly higher risk of breast cancer

TABLE 2 Association between the genetic polymorphism in CDKN2A/B rs10811661 with breast cancer

	Genotype distribution					Adjusted model*			
Variant	Risk allele	Gene		Case n (%)	Control n (%)	Additive model OR (95%CI)	Recessive model OR (95%CI)	Dominant model OR (95%CI)	HWE P value
rs10811661	T	CDKN2B	CC	2 (2.2)	16 (3.4)	2.9 (0.4-22)	4.9 (1.9-12)	2.27 (0.3-17)	.2
			CT	7 (7.6)	122 (25.8)	P add.=.3	P res.=.001	P do.=.4	
			TT	83 (90.2)	334 (70.8)				

susceptibility. Moreover, this genotype was related with advanced TNM classification and larger tumor size.

There is growing body of evidence showing the role of CDKN2A/B in breast, ovarian, and bladder and melanoma.[2-5] This link can be explained at least in part by its function in the regulation of cell cycle and apoptosis. It has been shown that the inactivation of CDKN2A by methylation or ANRIL can suppress the activity of tumor suppressor genes encoded by these genes in breast cancer.[12] ANRIL indirectly regulates cell proliferation and senescence via three methylations of lysine 27 in chromosome 9P21 (3meH3K27), upfront of CDKN2A/B.[13] ANRIL is transcribed as a 3.8 kb lncRNA in the opposite direction from the *p15/CDKN2B-p16/CDKN2A-p14/ARF* gene cluster and has been reported to have a direct role in recruiting PRC2 and PRC1 complexes to specific loci.[14] EZH2 (PRC2) and CBX7 (PRC1) are transcriptional repressors of several genes and are specially involved in silencing of the *CDKN2B-CDKN2A-ARF* locus via *ANRIL*.[15] Several genome-wide association studies have identified the ANRIL gene as a shared genetic susceptibility locus in different cancers.[14] However, the potential mechanisms underlying the role of ANRIL in breast carcinogenesis still remained to be elucidated.[16] Dębniak et al.,[3] suggested the value of CDKN2A as a breast cancer susceptibility gene. They showed that the CDKN2A A148T variant might be contributed to early-onset breast cancer in Poland.[17] In line with these studies, Antoniou and colleagues found the association of the rs1011970, near CDKN2A/CDKN2B, with increased breast cancer risk.[18] Another large-scale case-control study conducted by Driver et al.,[19] examined several polymorphisms within 13 genes involved in the cell cycle pathway with breast cancer risk. They illustrated a significant relationship between four genetics variants within the region of CDKN2A/2B and breast cancer risk.

In aggregate, our finding revealed for the first time a correlation between the genetic polymorphism, rs10811661, in CDKN2A/B gene and breast cancer. In particular, our data showed that this genetic marker was related with tumor grade and tumor size, although further studies in a larger population are needed to explore it values as a potential biomarker in risk stratification or prediction a chance of developing breast cancer.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:7-30.
2. Yang XR, Liang X, Pfeiffer RM, et al. Associations of 9p21 variants with cutaneous malignant melanoma, nevi, and pigmentation phenotypes in melanoma-prone families with and without CDKN2A mutations. *Fam Cancer*. 2010;9:625-633.
3. Dębniak T, Gorski B, Huzarski T, et al. A common variant of CDKN2A (p16) predisposes to breast cancer. *J Med Genet*. 2005;42:763-765.
4. Sherborne AL, Hosking FJ, Prasad RB, et al. Variation in CDKN2A at 9p21. 3 influences childhood acute lymphoblastic leukemia risk. *Nat Genet*. 2010;42:492-494.
5. Burdon KP, Macgregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat Genet*. 2011;43:574-578.
6. Hannou SA, Wouters K, Paumelle R, Staels B. Functional genomics of the CDKN2A/B locus in cardiovascular and metabolic disease: what have we learned from GWASs? *Trends Endocrinol Metab*. 2015;26:176-184.
7. Bao XY, Xie C, Yang MS. Association between type 2 diabetes and CDKN2A/B: a meta-analysis study. *Mol Biol Rep*. 2012;39:1609-1616.
8. Schaefer AS, Richter GM, Groessner-Schreiber B, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. *PLoS Genet*. 2009;5:e1000378.
9. Yu J-T, Yu Y, Zhang W, et al. Single nucleotide polymorphism rs1333049 on chromosome 9p21. 3 is associated with Alzheimer's disease in Han Chinese. *Clin Chim Acta*. 2010;411:1204-1207.
10. Melzer D, Frayling TM, Murray A, et al. A common variant of the p16 INK4a genetic region is associated with physical function in older people. *Mech Ageing Dev*. 2007;128:370-377.
11. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*. 2007;316:1336-1341.
12. Nielsen N, Roos G, Emdin S, Landberg G. Methylation of the p16 Ink4a tumor suppressor gene 5′-CpG island in breast cancer. *Cancer Lett*. 2001;163:59-69.
13. Congrains A, Kamide K, Ohishi M, Rakugi H. ANRIL: molecular mechanisms and implications in human health. *Int J Mol Sci*. 2013;14:1278-1292.
14. Yap KL, Li S, Muñoz-Cabello AM, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell*. 2010;38:662-674.
15. Popov N, Gil J. Epigenetic regulation of the INK4b-ARF-INK4a locus: in sickness and in health. *Epigenetics*. 2010;5:685-690.
16. Li CH, Chen Y. Targeting long non-coding RNAs in cancers: progress and prospects. *Int J Biochem Cell Biol*. 2013;45:1895-1910.
17. Dębniak T, Cybulski C, Górski B, et al. CDKN2A-positive breast cancers in young women from Poland. *Breast Cancer Res Treat*. 2007;103:355-359.
18. Antoniou AC, Kuchenbaecker KB, Soucy P, et al. Common variants at 12p11, 12q24, 9p21, 9q31. 2 and in ZNF365 are associated with breast cancer risk for BRCA1 and/or BRCA2 mutation carriers. *Breast Cancer Res*. 2012;14:1.
19. Driver KE, Song H, Lesueur F, et al. Association of single-nucleotide polymorphisms in the cell cycle genes with breast cancer in the British population. *Carcinogenesis*. 2008;29:333-341.

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