Received: 7 March 2020 | Revised: 13 July 2020 | Accepted: 15 July 2020

DOI: 10.1002/jcla.23529

RESEARCH ARTICLE

WILEY

# A case-control study on the SNP309T → G and 40-bp Del1518 of the *MDM2* gene and a systematic review for *MDM2* polymorphisms in the patients with breast cancer

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## Abstract

**Objective:** The current research was conducted to study the association between the SNP309 and del1518 polymorphisms with the breast cancer in the patients with the Kurdish ethnic background from western Iran. Also, a systematic review of the relevant case-control studies on the *MDM2* polymorphisms in the patients with breast cancer was performed.

**Methodology:** Two mL of peripheral blood was taken from 100 patients with breast cancer and 100 healthy individuals. The frequencies of *MDM2* SNP309 and del1518 genotypes and alleles were determined using the PCR-RFLP and PCR methods, respectively.

**Results:** The frequency of the TT, TG, and GG of *MDM2*-SNP309 genotypes in the patients was obtained as 23%, 52%, and 25%, and they were equal to 22%, 40%, and 38% in the control group, respectively. Also, considering the *MDM2*-del1518 polymorphism, the frequencies of ins/ins, ins/del, and del/del genotypes were equal to 52%, 41%, and 7% in the breast cancer group and they were equal to 62, 30, and 8% in the control group, respectively. Analysis of the results indicated that the GG genotype plays a protective role for the breast cancer in the recessive model (GG vs TT + TG) of SNP309 ($\chi^2 = 3.916$, $P = .048$, and OR = 0.54).

**Conclusion:** Our findings revealed that the GG genotype of *MDM2*-SNP309 can play a protective role in the breast cancer disease. Also, our systematic review indicated that the SNP309, SNP285, and del1518 of *MDM2* gene in different populations mostly did not have a significant association with the risk of breast cancer.

KEYWORDS

Breast cancer, Del1518 variant, Iranian population, *MDM2* gene, SNP309 polymorphism, Systematic review

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## 1 | INTRODUCTION

Breast cancer (BC), as the most common type of cancer in the women, is a multi-factorial and heterogeneous malignancy in the world.[1-3] The crude incidence rate of BC in the Iranian women has raised from 24 cases in 2004 to 33.8 cases per 100 000 in 2018.[1,4,5] This cancer is caused by the interaction effects of the genetic, hormonal, and environmental factors.[1,6,7] Exposure to the carcinogen agents, ionizing radiation, diet, physical activity or exercise, gender, woman's hormonal history, age, and obesity are the main effective risk factors for the breast cancer.[8,9] Single nucleotide polymorphisms (SNPs) in the functional genes could influence the incidence of breast cancer and its progression.[10-12] Results of a recent meta-analysis and research studies have confirmed a correlation between the SNPs of candidate genes with the risk of breast cancer.[11]

Main cellular regulatory mechanisms for the arrest of cell cycle and apoptosis in the stressed or damaged cells are involved in the cancer susceptibility.[13] The p53 is a primary regulatory factor that can be expressed in response to the increased expression of oncogenic proteins in the stressed conditions of the cells.[14-16] Under normal conditions, it is important to keep the p53 at the low level, so human homologue of mouse double minute 2 (MDM2) is a negative regulator of the p53 reducing the p53 level in the cell.[17] The MDM2 controls the expression level of the p53 using two mechanisms: Firstly, it prevents the transcriptional activity of p53 through direct binding as a physical blocker. Secondly, it can be degraded by the proteasome through the ubiquitination of the p53.[16,18-21] Mono-ubiquitination causes the p53 to transfer from the nucleus to the cytoplasm and ultimately to be degraded by the proteasome, while poly-ubiquitination targets p53 cause the degradation with the proteasome in the nucleus.[17]

There are controversial findings regarding the association of the SNP309 and del1518 of the *MDM2* gene with the breast cancer in different populations. On the other hand, there are big ethnicities in the Iran's population. Also, the frequency and association of these two main polymorphisms of the *MDM2* gene together have not been investigated in the patients with breast cancer among the Kurdish population so far. Therefore, the current study was conducted for the first time to investigate the SNP309T>G and del1518 of the *MDM2* gene in the patients with breast cancer and healthy individuals with the Kurdish ethnic background from western Iran. Also, a systematic review was designed and the case-control studies on evaluating the effect of all the investigated *MDM2* variations on the breast cancer risk were perused for the first time.

## 2 | PATIENTS AND METHODOLOGY

### 2.1 | Patients

Two mL of the blood samples in tubes was obtained from every 100 patients with breast cancer (mean age ± SD of 48 ± 11 years old, with the age range of 24-71 years old) and 100 age-matched (≤5 years) healthy individuals as the control group, with no history of cancer. The sampling was carried out from September 2017 to May 2018. Patients with a history of other types of cancer and fibroadenoma were excluded from the study. Also, all the cases and controls had the Kurdish ethnicity and were from the western Iran. Information on the age onset of the disease, first-degree family history of breast cancer, and a first-degree family of other types of cancer, estrogen receptor (ER), HER's-2 expression, progesterone receptor (PgR), P53, Ki-67, lymph node metastasis, and tumor grade was obtained from the patients′medical history. Tumor grading was carried out according to the pathologists′report. HER-2 was tested by the immunohistochemistry (IHC) method, and the samples with score of 2+ were then checked by the FISH protocol. Scores of 3+ and 2+ with the positive FISH report were recorded as the HER-2 positive.[22] The sample size of the study was defined based on the *MDM2* genotype frequency data presented in the studies by Akisik et al (2011)[11] and Alshatwi et al (2012)[23] using the following formula:

$$n=\frac{z_1-\alpha/2+z_1-\beta)^2(p_1q_1+p_2q_2)}{(P_1-P_2)^2}$$

A sample size of ~80 was calculated for each study group considering the alpha value of 0.01 and beta value of 0.05, but to ensure regarding the sample adequacy, a sample size of 100 was studied in each group.

### 2.2 | Selection of the SNPs and bioinformatics analysis

The SNPs were selected based on the reported studies on the main polymorphisms in the promoter region of the *MDM2* gene in the different populations of breast cancer worldwide. Also, the selected SNPs were checked in some web servers and online prediction tools. SNP309T>G (rs2279744) and del1518 (rs3730485) were checked by the Variant Effect Predictor (Ensembl Tools) (https://www.ensembl.org/Homo_sapiens/Tools/VEP), rSNPBase 3.1 (http://rsnp3.psych.ac.cn/), SNP Function Prediction (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), TFBIND (http://tfbind.hgc.jp/), and MethPrimer 2.0 (http://www.urogene.org/methprimer2/).

### 2.3 | DNA extraction

DNA was extracted from the blood samples using the DNA extraction kit based on the manufacturer's instructions (Zagros Bioidea Co., Iran). Concentration and purity of the extracted DNA were evaluated using a NanoDrop (Thermo-2000/2000c, USA).[24]

### 2.4 | Genotype analysis

The PCR-RFLP and PCR techniques were carried out to investigate the distribution of *MDM2* SNP309 and del1518 genotypes,

respectively. The final volume was equal to 25 μL for each PCR process, containing 12 μL of Master Mix PCR (SinaClon Co., Iran), 100-500 ng of the extracted DNA and 400 nmol/L of each specific primer.[14,25] The sequences of the primers were checked and confirmed using the UCSC In-Silico PCR (http://genome.ucsc.edu/).[26] Amplification conditions of the SNP309 were as follows: first denaturation cycle at 95°C and 5 minutes, 40 cycles: 35 seconds at 94°C, 35 seconds at 59°C and 30 seconds at 72°C, followed by a final extension (72°C for 10 minutes). Also, PCR parameters for del1518 were as follows: initial denaturation cycle for 5 minutes at 94°C, then 35 cycles: 30 seconds at 94°C, 30 seconds at 58°C, 30 seconds at 72°C, followed by a final extension cycle for 10 minutes at 72°C. The amplified fragment of *MDM2*-SNP309 with 352 bps was digested by two units of restriction enzyme, MspA1I (New England Biolabs, Ipswich, MA) for 15 hour of incubation period at 37°C. Then, the obtained fragments of *MDM2*-SNP309 and amplified products of *MDM2*-del1518 were electrophoresed on the 2% and 2.5% agarose gels and were stained by the GelRed, respectively. An amount of 5% of *MDM2*-SNP309 and *MDM2*-del1518 genotypes in the patients was randomly selected and identified by the DNA sequencer (BiONEER, South Korea).

## 2.5 | Statistical analysis

Frequencies of the genotypes in the studied SNPs were analyzed using the online Hardy-Weinberg equilibrium calculator.[27] The genotype frequencies were compared using the Pearson's chi-squared

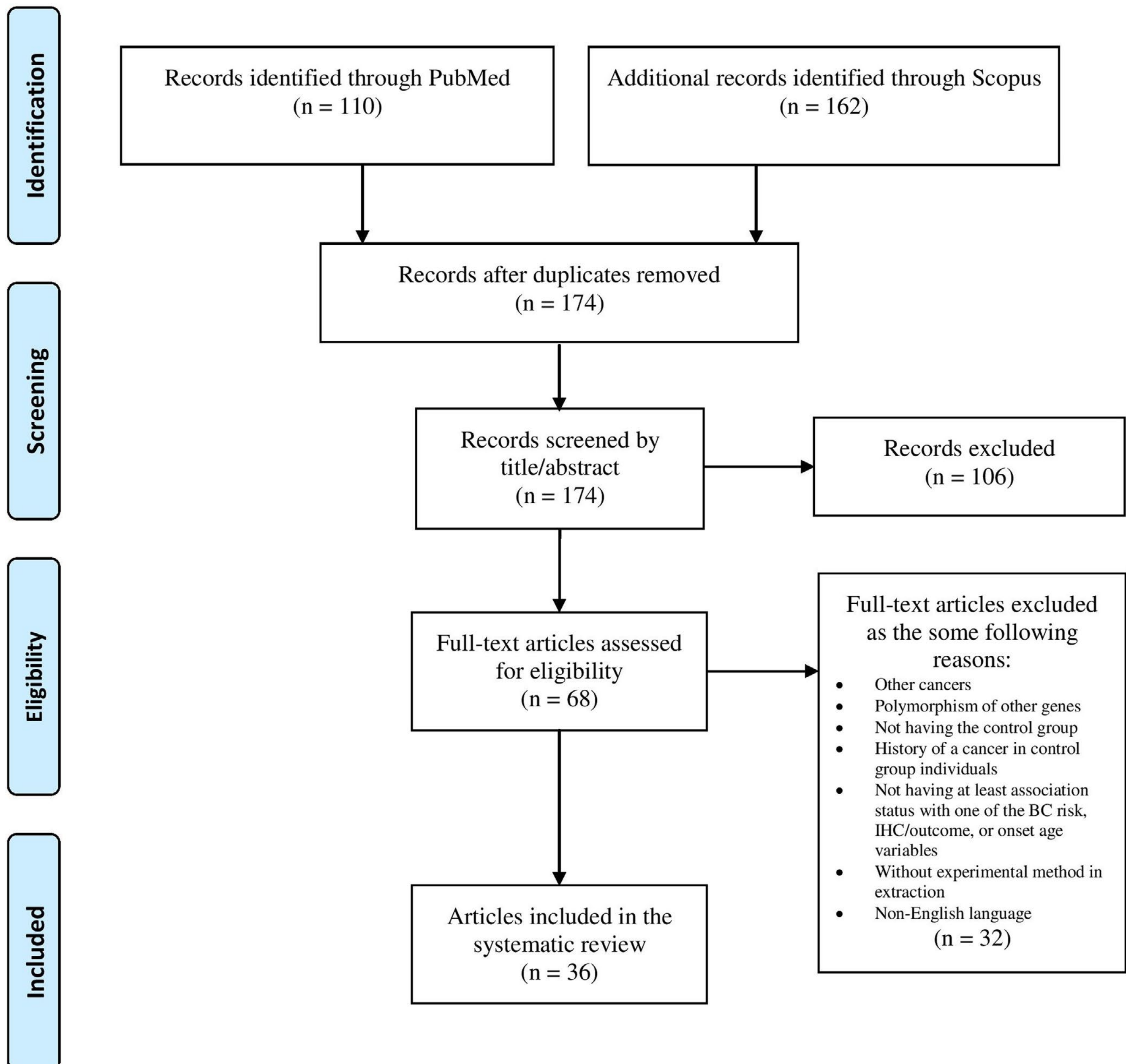


**FIGURE 1** PRISMA flow diagram of article selection for systematic review on the MDM2 variations in breast cancer patients

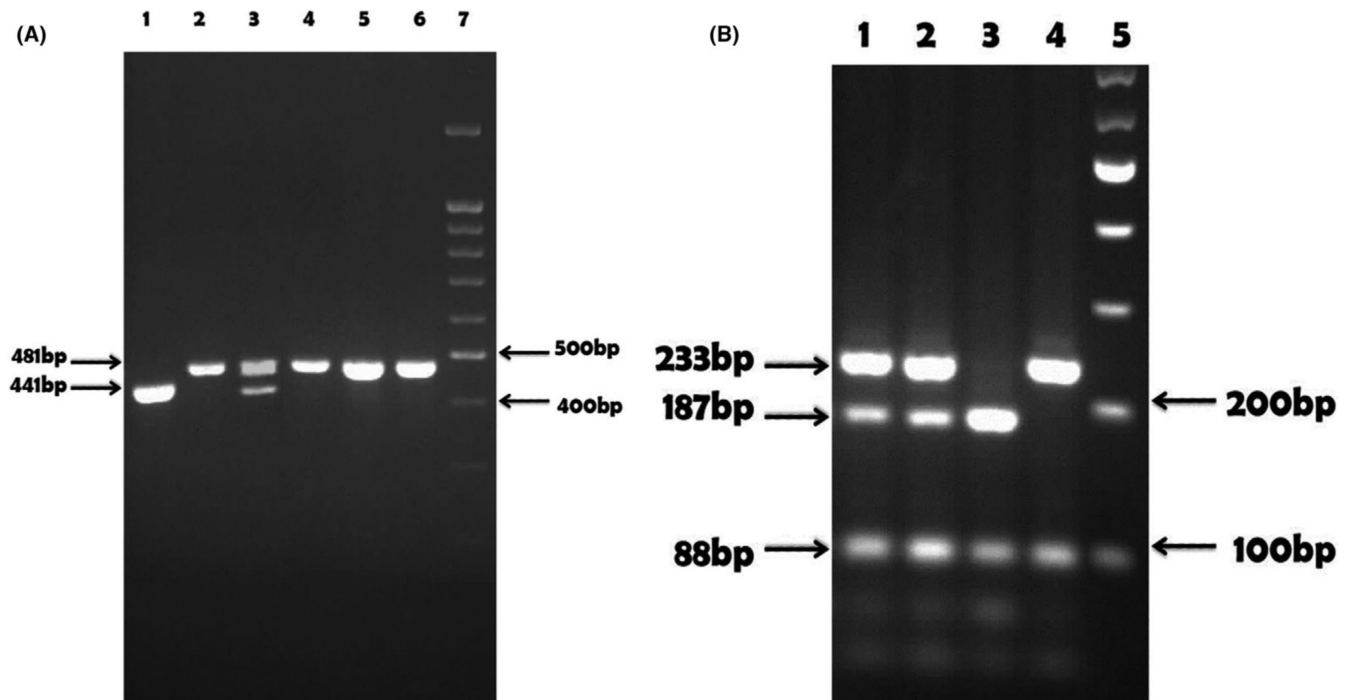


**FIGURE 2** Gel (agarose) electrophoresis of PCR products for del1518 and PCR-RFLP for SNP309 of the MDM2 gene. (A) Lane 1 shows del/del genotype; lanes 2, 4, 5, and 6 show ins/ins genotypes; and lane 3 shows ins/del genotype. (B) Lanes 1 and 2 show TG genotypes, and lanes 3 and 4 demonstrate GG and TT genotypes, respectively. Lane 7 in (A) and lane 5 in (B) show 100 bp DNA ladder. The map showed the 100-bp upstream and downstream of the SNP309

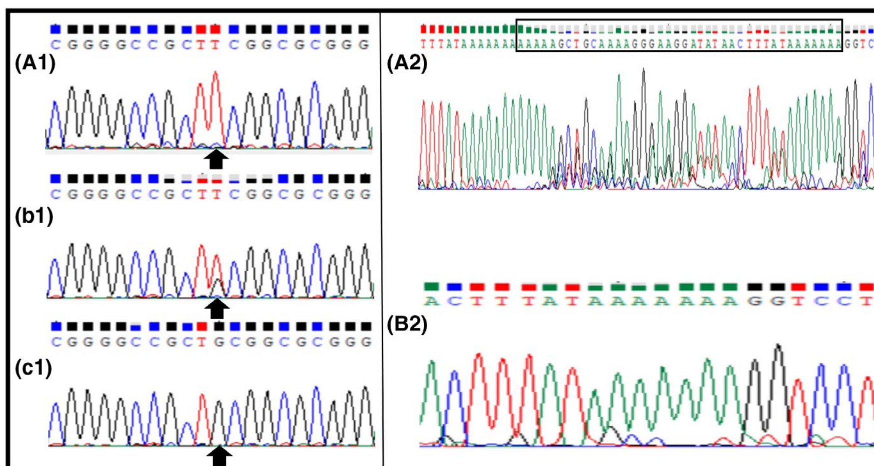


**FIGURE 3** Sequencing results of SNP309 and del1518 in case patients. 1. SNP309 positions were marked with the arrows for TT (1.A), TG (1.B), and GG (1.C) genotypes. 2. The sequence of 40-bp del1518 was showed in the box in ins/ins genotype (2.A). The Figure 2.B shows the del/del genotype of 40-bp del1518. Sequencing results were viewed in Chromas (Version 2.6.6)

test in the IBM SPSS software (version 16). Analysis of the odds ratios and 95% confidence intervals were done by the online MedCalc statistical software (MedCalc, Ostend, Belgium). Haplotype analysis was carried out using a web-based application.[28] The analysis of the age onset of the disease was done by the one-way analysis of variance (ANOVA).

## 2.6 | Search strategy, inclusion and exclusion criteria, and data extraction

The relevant papers were selected from the PubMed and Scopus databases. A time limit was set in the searches up to August 7, 2019 (last updated search). The terms of (MDM2 OR "mouse double minute 2" OR "murine double minute 2" OR HDM2) and (breast) and (polymorphism OR SNP OR variant) were used to search in the Scopus database, and the used terms in the PubMed database were (MDM2 OR mouse double minute 2 homolog OR human homolog of mouse double minute 2 OR HDM2 OR murine double minute 2) AND (breast cancer OR breast carcinoma OR breast neoplasm) AND (single nucleotide polymorphism OR SNP OR polymorphism OR variant).

Inclusion criteria in this systematic review were as follows: (a) original studies investigated the *MDM2* gene variations by the experimental methods for genotyping of the patients with breast cancer, (b) using the blood or tissue of the human samples, and (c)

**TABLE 1** Association of SNP309 with HER2, P53, and family history of other cancers' characteristics in breast cancer patients

| | SNP309 | | | SNP309 | | |
|---|---|---|---|---|---|---|
| Variable | TG | TT + GG | *P*-value* ($\chi^2$) | GG | TT + TG | *P*-value* ($\chi^2$) |
| Family history of other types of cancer cancer | | | | | | |
| No | 43 | 31 | .142 (2.155) | 13 | 61 | .049 (3.869) |
| Yes | 7 | 11 | | 7 | 11 | |
| P53 | | | | | | |
| Positive | 23 | 12 | .018 (5.595) | 7 | 28 | .152 (2.054) |
| Negative | 14 | 23 | | 13 | 24 | |
| HER2 | | | | | | |
| Positive | 25 | 14 | .031 (4.677) | 8 | 31 | .268 (1.226) |
| Negative | 15 | 23 | | 12 | 26 | |

**P*-values were calculated with the chi-square test.

evaluating both case and control groups. Exclusion criteria were as follows: (a) cell line surveys; (b) studies that did not report the association of SNP status at least with one of the BC risk, IHC/outcome, or onset age variables; (c) review and meta-analysis papers; (d) lack of reporting the frequency of each genotype; (e) papers that had the full text in a non-English language; (f) the conference, letter, note, and editorial papers; and (g) history of cancer in the individuals of control groups (Figure 1). Full text of the eligible papers was checked. Information on the studied variation, country, geographic area of the population, ethnicity, sample size, main case background, matching in case-control studies, source of samples, and the associated risk with BC, IHC/outcome and onset age was obtained from the selected papers. In some included studies, the corresponding author or co-authors were contacted (email) to complete the study information.

## 3 | RESULTS

### 3.1 | Population specifications

Among the patients, there were 98 females and 2 males. A family history (breast cancer) was detected among 7.4% of the patients. Among 94 patients with the recorded breast cancer history in the first-degree relative, 80.4% of them were positive. Also, the frequency of IHC markers including ER+, PgR+, and P53+ was equal to 69.4%, 62.4%, and 48.6%, respectively. Patients were classified into four groups of Luminal A, Luminal B, HER2-enriched, and Basal-like based on the tumor markers with the values of 14.6%, 54.9%, 24.4%, and 6.1%, respectively. These molecular subclasses were classified according to the presentations provided in the IMPAKT Breast Cancer Conference in Brussels, Belgium, on May 2012.[29]

**TABLE 2** Properties of included studied polymorphisms in the systematic review

| RsID | Name | Polymorphism | Risk allele[*,a] | Position[b] | Consequence in *MDM2* | Main TF-related SNP | Frequency of minor allele[c] |
|---|---|---|---|---|---|---|---|
| Rs150550023[d] | Del1518 | 40-bp ins/del | Deletion | chr12:68806996-68807065 | 2KB Upstream | RORA, MEF2A, MIZF | 0.37[e] |
| Rs2870820 | SNP55 | C → T | T | chr12:68808546 | Intron 1-2 | Sp1 | 0.23 |
| Rs117039649 | SNP285 | G → C | C | chr12:68808776 | Intron 1-2 | Sp1 | 0.01 |
| Rs2279744 | SNP309, G2580T | T → G | G | chr12:68808800 | Intron 1-2 | Sp1 | 0.36 |
| Rs1196333 | SNP344 | T → A | A | chr12:68808835 | Intron 1-2 | TFAP2A | 0.04 |
| ND | SNP443 | G → T | T | chr12:68808934 | Intron 1-2 | NR | NR |
| Rs769412 | SNP354 | A → G | G | chr12:68839435 | Exon 11[f] | NR | 0.07 |
| Rs937283 | G2164A | A → G | G | chr12:68808384 | 5 Prime UTR | NR | 0.30 |

[a]Minor alleles were considered as the risk allele.
[b]Based on GRCh38.p12 assembly.
[c]1000 genome.
[d]Rs3730485 based on GRCh38.p7 assembly.
[e]Based on the frequency of GenomAD.
[f]Synonymous mutation.

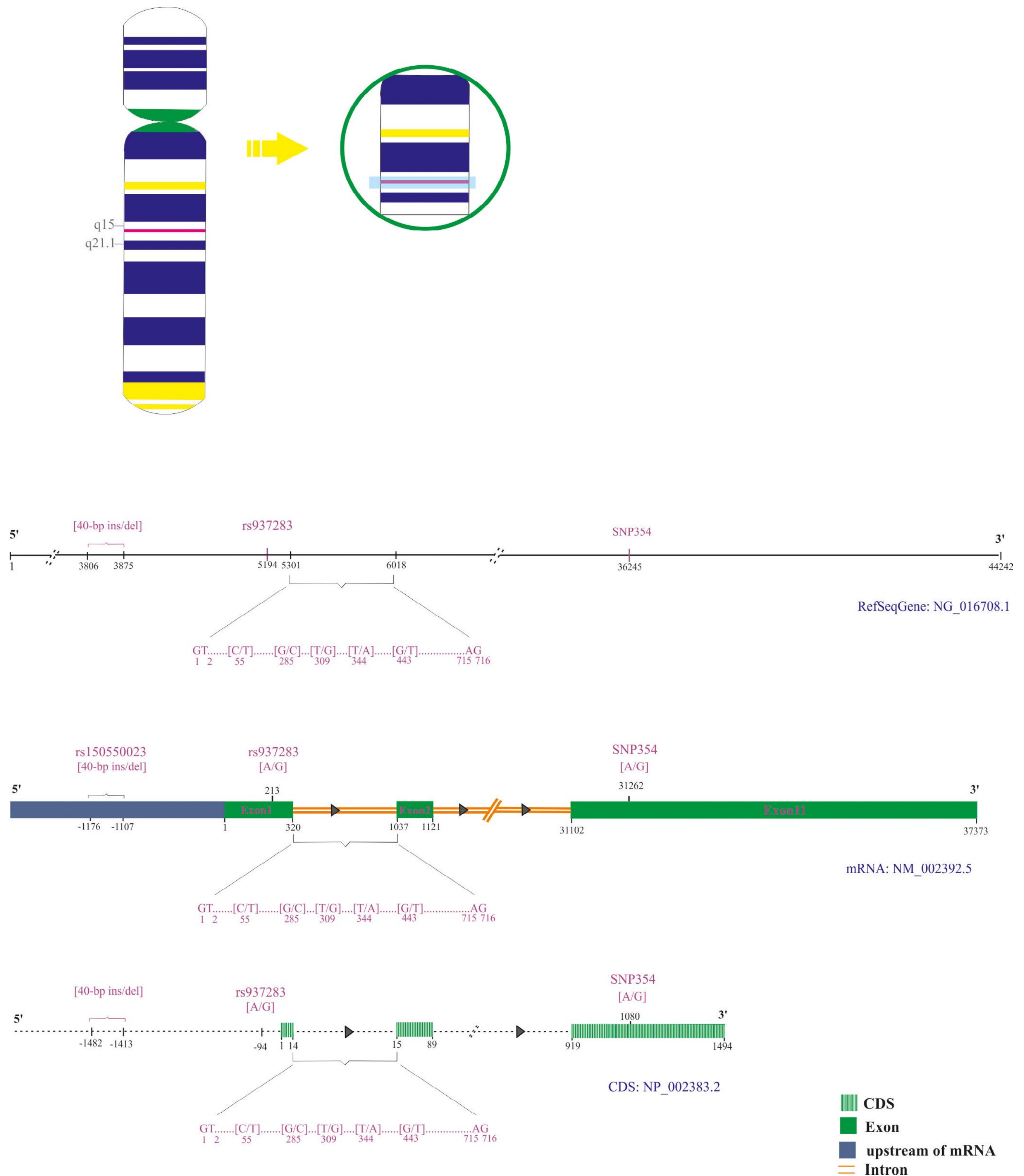


**FIGURE 4** The positions of the all assessed MDM2 variations in the current systematic review on breast cancer patients

## 3.2 | Bioinformatics analysis

In the rSNPBase database, SNP309 and del1518 polymorphisms were introduced as rSNPs, and the presence of TF binding region as the related regulatory element was confirmed. The performed analysis by the VEP tool indicated the rs2279744 as a risk factor in the ClinVar database, and a high score was reported for rs3730485 in the transcript support level. SNP Function Prediction and TFBIND tools confirmed a new site region for the SNP309G allele and del allele in del1518. The CpG island predictions of two polymorphisms

were checked in the MethPrimer 2.0 tool, and the results indicated that the rs2279744 is located in the CpG island region.

### 3.3 | SNP309 and Del1518 frequencies in the control and breast cancer groups

Figure 2 shows the electrophoresis pattern of the RFLP fragments of *MDM2*-SNP309 and amplified products of *MDM2*-del1518. The frequencies of SNP309 genotypes were as follows: TT (23%), TG (52%), and GG (25%) among the patients, and they were obtained as follows: TT (23%), TG (52%), and GG (25%) in the controls (*P*-value = .118). Also, the rate of del1518 genotypes in the patient group was as follows: ins/ins (52%), ins/del (41%), and del/del (7%), and they were obtained as follows: ins/ins (62%), ins/del (30%), and del/del (8%) in the control group (*P*-value = .266). The frequencies of SNP309 and del1518 genotypes were evaluated among all the individuals in the two study groups using the Hardy-Weinberg equilibrium by the available software,[27] and the results showed no significant deviation (*P*-values > .05). Frequency distribution of *MDM2* SNP309 and del1518 genotypes was analyzed in the patients and control subjects, and the results indicated no positive difference in the genotype frequencies of the co-dominant, dominant, recessive, and over-dominant models between the two study groups. Also, no significant difference was found for *MDM2* SNP309 and del1518 allele frequencies between the patients and controls. However, in SNP309 polymorphism, the GG genotype in the recessive model was positively different among the patients and controls (*P* = .048, OR = 0.54, 95% CI = 0.30-1.00) indicating its protective role against the breast cancer. Then, genotyping results were confirmed by the DNA sequencing. Figure 3 shows the results obtained from each genotype for SNP309 and homozygous genotypes for del1518. Haplotype study of *MDM2* gene polymorphisms showed a strong linkage disequilibrium between the SNP309 and del1518 variants (D' = 0.9995) in our studied population.

### 3.4 | Polymorphisms, clinicopathological features and demographic factors

The possible association between the clinicopathological features of the patients with SNP309 and del1518 genotypes was analyzed in the co-dominant, dominant, recessive, and over-dominant models. Our results indicated a significant association between the *MDM2*-SNP309 genotypes in an over-dominant model with HER2 and p53 status and also in a recessive model with the family history of other types of cancer (Table 1). Also, the association of the SNP309 and del1518 genotypes of the *MDM2* gene with the age onset of the disease was evaluated in the patients with breast cancer. Our results demonstrated no significant association between the genotypes of the SNP309 and del1518 and onset age of disease (Data not shown).

## 4 | DISCUSSION

The *MDM2* gene is located on the chromosome 12 q14.3-q15.1.[30] The *MDM2* gene (*HDM2*) is transcribed through two promoters: basic promoter (p1) and alternative promoter (p2).[19,31-33] Some functional and regulatory sequences in the promoter regions of the *MDM2* (rSNP) can change the gene expression level.[19,34] It has been indicated that the rSNPs of the genes associated with DNA damage and apoptosis mechanisms can influence the individual's sensitivity to the cancer progression.[19] Several types of research have studied the SNPs in the MDM2 gene and their association with the risk of breast cancer in different populations. Common studied polymorphisms are located in the first intron of the *MDM2* gene as P2 promoter. The SNP309T>G (rs2279744) is located at position 309 (IVS1 + 309) in the P2 region so that T to G transversion enhances the binding affinity of specificity protein 1 (Sp1) to a specific sequence in the promoter and, thus, increases the transcriptional level of the *MDM2* gene.[35-37] Rs3730485 (GRCh38.p7 assembly), also known as del1518 polymorphism (merged into rs150550023 in GRCh38.p12 assembly), is located in the P1 region of the *MDM2* gene having a putative TATA motif.[25] In the del1518 polymorphism, del-allele can increase the binding of some transcription factors, such as Myocyte Enhancer Factor 2A (MEF2A), RAR-Related Orphan Receptor A (RORA), and MBD2-Interacting Zinc Finger protein (MIZF) to the regulatory sequence in the promoter regions. The del1518 has high linkage disequilibrium (LD) with SNP309 locus.[37,38] High-level expression of MDM2 can influence the p53 signaling pathway allowing the damaged cells to escape from the control point of the cell cycle leading to the increase in the carcinogenesis.[6,18,19,39]

Risk of BC is influenced by the environmental, hormonal, and genetic factors.[1,6,7] The MDM2 protein is a main negative regulator in controlling the p53 expression.[17] It has been found that the MDM2 protein binds to the p53 and induces the ubiquitination and therefore degradation of the p53 by proteasome.[18] Therefore, high-level expression of the *MDM2* gene contributes to the reduced p53 activity and results in escaping from the checkpoints in the cell cycle.[6,18,19] The P53, as a tumor suppressor protein, activates the cellular processes, such as halt of the cell cycle, autophagy, and apoptosis process in response to the genotoxic stresses and damages.[17,30,40] Studies have shown that the genotypes of SNP309GG and del/del in 40-bp ins/del polymorphisms in the *MDM2* gene could influence its expression and may play a significant role in the cancer susceptibility. The association of *MDM2*-SNP309 and del1518 with different types of cancer has been investigated in relation to the breast,[15,22] esophageal,[41,42] uterine leiomyomas,[43] endometrial,[44] bladder,[45] lung,[39] and colorectal cancers [46]; however, the reported results were contradictory. There are no available reports related to the T309G and del1518 of the *MDM2* gene among the Iranian population in the west of Iran; therefore, this study was conducted to investigate the relationship between these *MDM2* polymorphisms with the breast cancer for the first time.

Our results showed a negative association between the *MDM2*-del1518 polymorphism and development of the breast cancer.

**TABLE 3** All investigated MDM2 variations in the case-control studies on the BC patients

| rs# (variation name) | Country (population[1]) | Ethnicity | Sample source | No. of case/ control | Patients | | Control group |
|---|---|---|---|---|---|---|---|
| | | | | | Type | Gender | Match with BC |
| rs3730485 (del1518) | China (Nanjing And Southeast) | Chinese, Unrelated Ethnic Han | Blood | 366/605 | BC | Female | Age, gender |
| | Iran (Kermanshah Province, West) | Kurdish | Blood | 100/100 | BC | 98 Females, 2 Males | Age, Area Of Residence |
| | Iran (Southeast) | NR | Blood | 236/203 | BC | NR | Age |
| | Mexico (Guadalajara City) | NR | Blood | 742/345 | BC | Female | Gender, Area Of Residence |
| | Norway (CONOR) Study | NR | Blood | 1717/1872 | BC | Female | Age, Gender, Area Of Residence, Same Cohort |
| rs2870820 (SNP55) | Norway (CONOR) Study | NR | Blood | 1707/1858 | BC | Female | Age, Gender, Same Cohort |
| rs117039649 (SNP285) | Austria | Austrian | Blood | 406/254 | BC | Female | Gender, Geography |
| | Mixed (Norway And Netherlands) | Mixed (Norwegian And Dutch) | Blood & Cancerious Breast Tissue | 1973/2518 | 1. BC 2. BC, ER+ | NR | Same Countries |
| | Norway (CONOR) Study | NR | Blood | 1717/1872 | BC | Female | Age, Gender, Area Of Residence, Same Cohort |
| | Poland (Wielkopolska) | Caucasian | Blood | 468/550 | BC | Female | Gender, Geography, Ethnicity |
| | Scotland | Scottish Caucasian | Blood | 299/275 | BC | Female | Gender, Geography, Ethnicity |
| rs2279744 (SNP309) | Austria | Austrian | Blood | 406/254 | BC | Female | Gender, Geography |
| | Brazil | NR | Blood/ Non-Tumoral Tissue | 39/186 | BC, R337H Mutation Carriers | NR | NR |
| | Canada | Caucasian | Blood | 38/379 | BC, Pre-Menopasual | Female | NR |
| | China (Shanghai) | NR | Blood | 1. 402/84 2. 402/605 | BC | Female | 1. Gender, Geography 2. Gender |
| | China (Nanjing And Southeast) | Chinese, Unrelated Ethnic Han | Blood | 366/605 | BC | Female | Age, Gender |
| | Czech Republic | NR | Tissue/ Blood | 158/149 | BC | NR | NR |
| | England (WCGS) | NR | NR | 59/102 | BC/ With BRCA1 Mutations | Female | NR |
| | England (Anglo-Saxon Population) | British | NR | 351/258 | BC | Female | Gender, Geography |
| | German | NR | Blood | 549/1065 | Familial BC, Lacking BRCA1&2 Mutations | Female | Ethnicity |
| | India (North) | NR | Blood | 100/100 | BC, IDC | NR | Age |

| Properties | Genotyping method(s) | Association with | | | References |
|---|---|---|---|---|---|
| | | Risk[2] | IHC & outcome[3] | Age of onset[4] | |
| Cancer-free | PCR | Not significant | NR | Negative | [1] |
| Healthy, Cancer-Free | PCR | Not Significant | Negative | Negative | Present Study |
| Cancer-Free | PCR | Higher Risk | Negative | Negative | [72] |
| Healthy | PCR | Higher Risk | NR | NR | [73] |
| Healthy, Cancer-Free | PCR | Not Significant | NR | NR | [74] |
| Healthy | LightSNiP | Not Significant | NR | Negative | [75] |
| benign gynecological lesion/ Healthy | Real-Time PCR | Not Significant | Negative | Negative | [76] |
| Healthy | Sequencing | NR | NR | 1. NR<br>2. Negative | [77] |
| Healthy | LightSnip | Not Significant | NR | NR | [78] |
| Healthy | Sequencing | Lower Risk | Negative | NR | [79] |
| Cancer-Free | Sequencing | NR | NR | Negative | [80] |
| Benign gynecological lesion/ Healthy | Real-Time PCR | Not Significant | P53, Ki67 | Positive | [76] |
| Cancer-Free, No Family History Of Cancer, Without The R337H Mutation | Real-Time PCR | NR | Negative | NR | [81] |
| NR | Sequencing | Not Significant | NR | NR | [82] |
| Healthy, Cancer-Free | Sequencing | 1. Higher Risk<br>2. Higher Risk | Negative | Positive | [83] |
| Cancer-Free | PIRA-PCR, Sequencing | Not Significant | NR | Negative | [71] |
| Mixed Of Healthy And Ischemic Disease (Cancer-Free) | PCR-RFLP | Not Significant | Negative | Negative | [84] |
| NR | Pyrosequencing | NR | NR | Negative | [85] |
| Cancer-Free | Allele Specific PCR | Not Significant | NR | Negative | [86] |
| Healthy | Real-Time PCR, Sequencing | Not Significant | NR | Negative | [87] |
| Healthy, Cancer-Free | Allele Specific PCR | Not Significant | HER2, Distant Metastasis | NR | [88] |

(Continues)

**TABLE 3** (Continued)

| rs# (variation name) | Country (population[1]) | Ethnicity | Sample source | No. of case/ control | Patients | | Control group |
|---|---|---|---|---|---|---|---|
| | | | | | Type | Gender | Match with BC |
| | India (Lucknow, North) | NR | Blood | 104/105 | BC | Female | Gender, Geography, Ethnicity |
| | Iran (Kermanshah Province, West) | Kurdish | Blood | 100/100 | BC | 98 Females, 2 Males | Age, Area of residence |
| | Iran (Mashhad City, Southeast) | NR | Blood | 128/126 | BC | Female | Age, Gender |
| | Israel | Ashkenazi–Jewish (AJ) Origin | NR | 187/138 | BC, BRCA1/2 Mutation Non-Carrier | Female | Gender |
| | KSA | Arabian | Blood | 100/100 | BC | Female | Gender, Ethnicity |
| | Kyrgyzstan | Kyrgyz | Blood | 117/102 | BC | Female | Age, Gender |
| | Mixed (Norway And Netherlands) | Mixed (Norwegian And Dutch) | Blood & Cancerious Breast Tissue | 1973/2518 | 1. BC<br>2. BC, ER+ | NR | Same Countries |
| | Mixed (UK) | Scottish Caucasian | Blood | 299/182 | BC | Female | Gender, Geography, Ethnicity |
| | Netherlands (South–West) | NR | Blood | 343/126 | Familial BC | 340 Females, 3 Males | Geography |
| | Norway (CONOR) Study | NR | Blood | 1717/1872 | BC | Female | Age, Gender, Area Of Residence, Same Cohort |
| | Poland (Wielkopolska) | Caucasian | Blood | 468/550 | BC | Female | Gender, Geographically, Ethnicity |
| | Sweden (South-East Sweden Health Care Region) | NR | Blood/ Normal Lymph Node Tissues | 123/146 | BC, Young Women | Female | Gender, Geography |
| | Taiwan | Asian Taiwanese, Not Immigrants From America Or Europe. | Blood | 255/324 | BC | 254 Females, 1 Male | - |
| | Taiwan | Taiwanes | Blood | 124/97 | Sporadic BC | Female | Gender, Ethnicity |
| | Turkey | NR | Blood | 110/138 | BC | Female | Age, Gender |
| | Turkey | NR | Blood | 147/120 | Familial BC | Female | Gender |
| | Turkey | Turkish | Blood | 223/149 | BC (Ductal Carcinoma) | Female | Age, Gender, Ethnicity |
| | US (NHS Study) | NR | Blood | 1519/2271 | BC | Female | Age, Menopausal Status, Recent Postmenopausal Hormone (PMH) use |
| | US (Baltimore) | 1. African American Descent<br>2. Caucasian White Descent (Not Hispanic White) | Blood & Cancerious & Noncancerious Breast Tissue | 1. 165/178<br>2. 125/136 | BC | Female | Age, Gender, Geography, Race |
| | US (Carolina Breast Cancer Study) | 1. African-Americans<br>2. Whites | Blood | 1. 767/680<br>2. 1270/1133 | BC | Female | NR |

| | | Association with | | | |
|---|---|---|---|---|---|
| Properties | Genotyping method(s) | Risk[2] | IHC & outcome[3] | Age of onset[4] | References |
| Tumor/Cancer-Free | ARMS-PCR | Not Significant | NR | NR | 89 |
| Healthy, Cancer-Free | PCR | Lower Risk | HER2, P53, Family history of cancer | Negative | Present Study |
| Healthy | ARMS-PCR | Not Significant[5] | Negative | Positive | 90 |
| Cancer-Free | MALDI-TOF | Not Significant | NR | Negative | 91 |
| Healthy | Real-Time PCR | Higher Risk | Negative | Negative | 92 |
| BC-Free | PCR-RFLP | Not Significant | NR | NR | 93 |
| Healthy | Sequencing | Not Significant | NR | 1. NR<br>2. Negative | 77 |
| Cancer-Free | Sequencing | Not Significant | Tumour Grade, Lymph Node, NPI | Negative | 80 |
| Heterozygous carriers of cystic fibrosis gene mutations | Sequencing | Not Significant | NR | Positive | 94 |
| Healthy | Lightsnip | Not Significant | NR | NR | 78 |
| Healthy | PCR-RFLP, Sequencing | Not Significant | Negative | NR | 79 |
| Healthy | Pyrosequencing | Not Significant | Negative | NR | 95 |
| Healthy, Cancer-Free | PCR-RFLP, Sequencing | Higher Risk | NR | NR | 96 |
| Healthy, Cancer-Free | PCR-RFLP, Sequencing | Higher Risk | NR | Positive | 97 |
| Healthy | PCR-RFLP | Higher Risk | Negative | NR | 98 |
| Healthy | PCR-RFLP | Higher Risk | NR | NR | 99 |
| Healthy, Cancer-Free | PCR-RFLP | Not Significant | NR | NR | 100 |
| Healthy | PCR-RFLP | Not Significant | NR | NR | 101 |
| Cancer-Free | Real-Time PCR | Not Significant | Tumor P53 Expression | Negative | 102 |
| NR | Real-Time Pcr | Not Significant | ER, PgR | Negative | 103 |

(Continues)

**TABLE 3** (Continued)

| rs# (variation name) | Country (population[1]) | Ethnicity | Sample source | No. of case/control | Patients | | Control group |
|---|---|---|---|---|---|---|---|
| | | | | | Type | Gender | Match with BC |
| rs1196333 (SNP344) | Mixed (Norway And Netherlands) | Mixed (Norwegian And Dutch | Blood | 1271/2954 | BC | Female | NR |
| | Scotland | Scottish Caucasian | Blood | 299/275 | BC | Female | Geography, Ethnicity |
| rs769412 (SNP354) | US (Baltimore City) | 1. African American Descent 2. Caucasian White Descent (Not Hispanic White) | Blood & Cancerious & Noncancerious Tissue | 1. 166/176 2. 127/134 | BC | Female | Age, Gender, Race |
| (SNP443) | Scotland | Scottish Caucasian | Blood | 299/275 | BC | Female | Geography, Ethnicity |
| rs937283 | China (Central Chinese Population) | NR | Blood | 480/500 | BC | Female | Age, Gender, Smoking Status, Drinking Status |

*Note:* 1. Region of the hospital-based studies was not reported. 2. Combined effect with other SNPs and classified groups (based on demographic characteristics and clinical features) were not reported. 3. Association between IHC reports and clinical/outcome features with distribution of alleles and genotypes in BC patients. 4. Association with age of incidence in breast cancer patients was considered for each variation alone, and it was not reported in cases of haplotypes or in the subgroup of other variants. Also, age similarity was not included. 5. According to the stated result in the article, no significant association is reported ($P = .048$).

Abbreviations: ARMS, Amplification Refractory Mutation System; BC, Breast Cancer; CONOR, Cohort of Norway; ER, Estrogen Receptor; IDC, Invasive Ductal Carcinoma; IHC, Immunohistochemistry; MALDI-TOF, Matrix Assisted Laser Desorption Ionization-Time of Flight; NPI, Nottingham Prognostic Index; NR, Not Reported; PgR, Progesterone Receptor; PIRA, Primer-Introduced Restriction Analysis; RFLP, Restriction Fragment Length Polymorphism; WCGS, Wessex Clinical Genetics Service.

Limited studies have been done with the contradictory results on the correlation of *MDM2*-del1518 polymorphism and BC.[15,37,47,48] Consistent with our results, Ma et al found no correlation between the *MDM2*-del1518 polymorphism and BC in the Chinese population.[15] Gansmo et al (2016) in a meta-analysis concluded that the *MDM2*-del1518 polymorphism was not associated with the BC.[37] Also, Hua et al (2017) performed a meta-analysis study to investigate the role of *MDM2* del1518 polymorphism associated with the cancer susceptibility. Their results provided a significant support for the lack of association between the *MDM2* del1518 polymorphism and cancer risk.[30] In other studies conducted in the Chinese population, no association has been reported between the *MDM2*-del1518 polymorphism with esophageal squamous cell carcinoma and uterine leiomyomas.[41,43] However, inconsistent with the results of our study, Hashemi et al (2014) revealed an association between the *MDM2*-del1518 polymorphism with the risk of BC in the Zahedan population, Sistan and Baluchestan province (southeast of Iran).[47] Also, a significant correlation has been observed between the *MDM2*-del1518 polymorphism and the BC in the Mexican population.[48] Contradictory results in the reported studies can be due to the differences in the sample size and ethnicity of the populations. It is suggested that the 40-bp ins/del in the promoter of the *MDM2* might not play a major role in the risk of BC.[15]

Herein, no positive association was found between the frequencies of *MDM2*-SNP309 alleles with the risk of BC. Consistent with our results, Hosein Pour et al and Tavakkol Afshari et al reported no positive association between the SNP309 with the BC across populations of the northwest[49] and northeast of Iran.[50] Different studies in the various populations failed to confirm a significant association between the MDM2-SNP309 and an increased risk of BC.[15,22,39,51-55] Also, Zhao et al (2012) in a meta-analysis reported no significant correlation between the *MDM2*-SNP309 and the incidence of BC in the European populations.[12] A negative association has been confirmed between the *MDM2*-SNP309 and other types of cancer, such as endometrial,[44] bladder,[45] esophageal squamous cell,[41] Kaposi's sarcoma,[56] and uterine leiomyomas.[43] However, in several studies, an association has been reported between the SNP309 polymorphism and the incidence of BC worldwide.[10,11,14,18,23,57-59]

Our results indicated a protective role of the GG genotype in SNP309 against the BC ($P = .048$, OR = 0.54, 95%CI = 0.30-1.00). Consistent with our findings, some studies have revealed the protective role of GG genotype of *MDM2*-SNP309 in different cancers including the lung,[39] colorectal,[46] and esophageal[42] cancers. Protective role of GG genotype in *MDM2*-SNP309 may be due to the formation of haplotypes with other unknown effective rSNPs.[19] It can reduce the positive effect of SNP309 and highly influences the expression level of the *MDM2* gene and decreases it.

The strong linkage disequilibrium was observed between the SNP309 and del1518 variants in the studied population. In fact,

| Properties | Genotyping method(s) | Association with | | | References |
|---|---|---|---|---|---|
| | | Risk[2] | IHC & outcome[3] | Age of onset[4] | |
| Healthy | Sequencing | Not Significant | NR | Negative | [104] |
| Cancer-Free | Sequencing | Not Significant | NR | Negative | [80] |
| BC-Free | Real-Time PCR | 1. Higher Risk<br>2. Not Significant | NR | Negative | [102] |
| Cancer-Free | Sequencing | Not Significant | NR | Negative | [80] |
| Healthy, Cancer-Free | PCR-RFLP, Sequencing | Higher Risk | NR | NR | [105] |

when the alleles are in the linkage disequilibrium condition, haplotypes do not occur with the expected frequencies. It can be used to improve the power of the cancer-genetic association studies, and it helps to detect the true associations in the case-control studies. So, the LD results suggested the homogeneity of these polymorphisms in the Kurdish population.

The contradictory results for the function of *MDM2*-SNP309 in different types of cancer may be due to several reasons: (a) The effect of this variant is related to the changes in the binding of tissue specific-transcription factors (TF) in the promoter [60]; (b) malignancies in various tissues can have different molecular mechanisms, and even in one type of cancer because of heterogeneity across different individuals [30]; (c) the different sample size of the studied populations [12]; (d) differences in the ethnicity and lifestyle-related factors in various studied populations may influence the effect of *MDM2*-SNP309 on the incidence of BC [12,61]; (e) function and expression level of the *MDM2* gene or TFs-associated genes may be related to the DNA methylation as a main epigenetic mechanism so that the methylation status of the regulatory sequence in the promoter is different in various populations based on the specific environmental and ethnic conditions [62,63]; (f) polymorphism effect can be related to the interaction with the haplotypes and other SNPs in the *MDM2* gene [64]; and also (g) variable of minor allele frequency (MAF) can show up in the demographic layers of the populations, and as a result, the risk of BC may change with respect to the ethnicity because of their allele frequencies.[12]

Some diseases, such as cancer, are complex, meaning that they are caused by the multiple genes and environmental factors. Studying a few genetic polymorphisms in such small populations is like only a piece of the large puzzle presenting their ability to influence the cancer risk in different populations, especially ethnicities. Also, it can provide an overall perspective for the subsequent studies on the genes involved in the desired pathway in the ethnic groups.

In the next step, our research was expanded with a systematic review of all the variations of the studied *MDM2* gene in the patients with breast cancer for the first time. There were several case-control studies on assessment of the functional variations of the *MDM2* and the susceptibility to BC. As indicated in Table 2, seven single nucleotide variations (SNVs) and an ins/del were investigated in the eligible reviewed studies. Commonly studied polymorphisms are located in the first intron (Intron 1-2) as P2 promoter of *MDM2* gene.

Totally, 46 studies on the SNVs and one ins/del reported in 36 papers and the current research had the eligibility to be included in the systematic review (Figure 4). The status of eight variants in the patients with BC was included in the populations from 23 countries in the systematic review. Table 3 shows the characteristics of the eligible studies.

As demonstrated in Table 3, the main sample sources in the reviewed studies were taken from the blood. Two studies[55,64] have used the samples including the cancerous tissue for evaluation of two variations in *MDM2* gene that may change the genotype

frequency of the target polymorphisms and influence the association of SNP with the studied outcome and, therefore, intervening in the interpretation of the results. Most studies have been conducted on the female patients with BC. In most studies, the control groups were matched with the patients in terms of age, sex, and geographical location parameters. BC-free and healthy individuals were mostly considered as the control group. Also, rs2279744 known as SNP309 and G2580T was the well-studied SNP in the *MDM2* gene. This polymorphism occurs as a result of T to G transversion that has a higher affinity to the transcription factor of Sp1 in its mutant allele. Our review revealed that there are contradictory results for this SNP. Six studies conducted in Asia have confirmed the positive result of this polymorphism in increasing the BC incidence. In overall, in accordance with our case-control research, studies conducted worldwide until 2019 have shown that this SNP did not increase the BC risk. Also, a recent meta-analysis performed in 2018 showed that this polymorphism could not have a significant intervention in the carcinogenesis of BC.[6] Results of some studies on the correlation between the SNP309 with onset age of disease indicated no positive association in this regard.[65-67]

SNP285(G>C) is located in intron1-2 and the upstream of the SNP309 (Figure 4). Some studies have demonstrated that the C allele plays a role as an antagonist factor for SNP309 and reduces the Sp1 affinity for binding to the gene promoter and decreases the transcription activity.[39,64] In the literature review, reported results did not confirm the role of SNP285 as a risk factor for BC susceptibility.

Rs3730485 (GRCh38.p7 genome assembly), known as del1518, is located in the promoter region of the 2kb upstream. Deletion allele can make a higher potential binding site for the RORA, MEF2A,and MIZF TFs.[37] This 40-bp ins/del is located near the 40-bp ins/del (rs150550023) so that, in the GRCh38.p12 genome assembly, these polymorphisms are merged into rs150550023 with three alleles: 40-bp double insertion, 40-bp insertion, and 40-bp deletion. The current review revealed controversial results regarding the lack of significant association and positive correlation for an increasing role of del1518 on the BC susceptibility in different populations.

Limited number of studies have been conducted on the relationship between the SNP55,[68] SNP344,[22,69] SNP354,[55] SNP443,[22] and rs937285[70] with the risk of BC. SNP443 does not have the RefSeq ID and is not validated as a variation in the National Center for Biotechnology Information (NCBI) and Ensemble databases. SNP55 and Rs1196333 have a higher affinity to bind to the Sp1[68] and TFAP2A[69] TFs in the mutant allele, respectively, leading to the increase in the expression level of *MDM2* in the cell.

In conclusion, our results indicated that the GG genotype of SNP309-*MDM2* plays a protective role in the BC in our studied population. However, our findings indicated no positive association between the *MDM2* del1518 polymorphism and the risk of BC in the Kurdish population from western Iran. Also, our systematic review indicated that the SNP309, SNP285, and del1518 polymorphisms of *MDM2* gene in different populations mostly were not associated with the BC risk. Thus, there is a need to evaluate other novel rSNPs in the *MDM2* gene in the future researches as well as the expression of the *MDM2* gene and its associated transcription factors, such as RORA, Sp1, MEF2A, and MIZF.

There were some limitations in the present study including a small number of included studies with the contradictory results for 40-bp del1518 polymorphism to calculate the sample size, obtaining the patients′consent to participate in the study, achieving a maximum number of the included new cases, time constraint, and obtaining a maximum data from the patients' medical history in new cases. Therefore, more time should be taken to collect the sample of new cases, increase the sample size, and obtain the complete IHC characteristics of the patients for the future studies.

## ACKNOWLEDGMENT

We thank our colleagues in Molecular Genetics Laboratory, Medical Biology Research Center, Kermanshah, Iran, for technical assistance.

## AUTHOR CONTRIBUTIONS

AJ collected the samples, performed experiments, extracted the systematic review data, and wrote the first draft of the manuscript. KY and MA designed the case-control and systematic review studies and analyzed the data. ZR and IS edited the final version of the manuscript. PM draw the schematic figure. All authors reviewed the final version of the manuscript and approved it for publication.

## ETHICAL APPROVAL

Ethics Committee in Kermanshah University of Medical Sciences, Iran, approves the current study. All control and patient's individuals agreed to participant in the study and signed the form of informed consent (Helsinki II declaration).

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**How to cite this article:** Jalilvand A, Yari K, Aznab M, Rahimi Z, Salahshouri Far I, Mohammadi P. A case-control study on the SNP309T → G and 40-bp Del1518 of the *MDM2* gene and a systematic review for *MDM2* polymorphisms in the patients with breast cancer. *J Clin Lab Anal*. 2020;34:e235299. https://doi.org/10.1002/jcla.23529