## Research Article

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Turk J Biochem 2022; 47(4): 417-423

# MAP3K1 SNP rs889312 potential risk and MAP3K9 SNP rs11628333 menopause dependent association for breast cancer

https://doi.org/10.1515/tjb-2021-0161 Received July 11, 2021; accepted November 15, 2021; published online December 27, 2021

#### Abstract

**Objectives:** Breast cancer is the leading cause of mortality in today's world. An alarming rise in cancer incidence has been observed in the South Asian region. The aberrant molecular mechanisms regulating cell proliferation and development contribute to cancer development. A better understanding of the detailed molecular mechanisms at genetic and epigenetic levels can help to treat breast cancer more efficiently. The present study is aimed to identify the possible association of MAP3K1 SNP rs889312 and MAP3K9 rs11628333 in breast cancer in the South Asian region.

**Methods:** Female breast cancer patients were recruited in the study. DNA was isolated from the blood samples collected from the patients. PCR-RFLP was used for genotyping, and data analysis was done by SPSS software.

Results: Genotyping data for MAP3K1 SNPrs889312 showed statistically significant association with breast cancer, while MAP3K9 SNPrs11628333 showed characteristic association of rare allele heterozygote's and homozygotes in pre and post-menopausal patients, respectively.

**Conclusions:** The study concludes a strong association of the rs889312 with breast cancer in the Pakistani population and a characteristic association of unique genotypes TC and CC in pre- and post-menopausal breast cancer patients. These findings can provide a ready tool as a breast cancer marker in south Asian populations.

**Keywords:** breast cancer; *MAP3K1*; *MAP3K9*; rs11628333; rs889312.

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# Introduction

Breast cancer is one of the widespread cancer worldwide, with an estimated 2.08 million cases diagnosed in 2018, accounting for 24.2% of all cancer cases. Breast cancer has been responsible for 15% of total cancer deaths, especially in less developed countries [1]. The incidence of breast cancer in Pakistan is 2.5 times more than in the neighboring countries [2]. Therefore, it is necessary to find the underlying causes of breast genetic cancer in the Pakistani population. Variations in a few important genes such as BRCA1 and BRCA2 are coupled with a high risk of breast cancer, but the majority of the breast cancer cases are related to the low penetrance genes that are often altered in the whole population.

Genetic studies have shown that breast cancer is a multifaceted and genomically complex disease. Deregulated signaling pathways e.g. competitive endogenous RNAs, non-coding RNAs, NEDD4 family of E3 Ubiquitin

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ligases and mTOR1 and mTOR2 play fundamental role in carcinogenesis and metastasis [3–6].

Understanding the molecular basis of breast cancer can help early tumor detection and prevention [7]. One of the genetic causes of breast cancer is MAPK (mitogenactivated protein kinase) pathway. It consists of a family of protein-serine/threonine kinases, which are highly preserved in protein structures from unicellular eukaryotic organisms to multicellular organisms, including mammals [8]. The MAPK pathway is a vital bridge in the switch from extracellular signals to intracellular responses. The *MAPK* pathway controls various cell functions, including cell proliferation, differentiation, migration, apoptosis and responds to a diverse variety of signals inclusive of physiological origin for instance cytokines, growth factors and hormones, in addition to environmental signals and endogenous stress. Abnormalities in MAPK signaling influence most if not all these processes and play an important role in the progression and development of cancer. Classically, they are categorized as a mitogen and stress-activated MAPK, with ERK being the representative of mitogen, JNK and p38 of stressresponsive MAPKs [9–11]. These pathways are clinically important targets for cancer therapy by targeting; ERK pathway. Though, stress-induced MAPK pathways such as JNK play modulatory roles that can alter the feedback of cancer cells to both chemotherapies & targeted therapies [7]. MAPK pathway has a functionally essential role in many cancers including breast cancer and the role of MAPK pathway in breast cancer has been established therefore the purpose of the current study was to investigate the genetic predisposition of MAPKSNPs in breast cancer. We analyzed two hotspot SNPs of MAPK i.e.

rs11628333 (*MAP3K9*, C>T) and rs889312 (*MAP3K1*, C>A) for their association analysis.

# Materials and methods

The study was approved by the Ethical Review Committee, PMAS-Arid AAU, Rawalpindi, Pakistan. All the protocols, methods, and experiments used in the study were as per guidelines and approved by IBGE, Islamabad, Pakistan. The patients and controls were requested to participate in this study after informed consent.

#### Inclusion and exclusion criteria

Cases from the Pakistani population clinically diagnosed with breast cancer were taken for the study. Only female cases with breast cancer including invasive & non-invasive, ductal, lobular and nipple areola carcinoma, were selected in this study. All stages of breast cancer, metastasis & non-metastasis cases included. The female cases with the early onset of breast cancer i.e. before menopause and cases with the disease onset after menopause were also selected.

Breast cancer cases not willing to participate and cases unable to sign the consent form were not targeted in the study. Practicing ethical consideration of the study, pregnant females and cases with any psychological illness were not requested to participate. To eliminate factors of biases, cases with comorbidities were excluded from this study. Furthermore, individuals with parents of gross ethnic differences were also not considered in this research.

#### **Subjects**

Two hundred female patients of breast cancer, along with age and gender matched healthy individuals were included in the study (Table 1). These patients were clinically diagnosed with breast cancer at tertiary care hospitals. Venous blood samples from patients as well as control

Table 1: Demographic characteristics of case and healthy controls.

Parameter		Cases	Healthy controls	Chi square
Age		48.46 ± 15	46.23 ± 15.63	
Ethnicity		Pakistani	Pakistani	
Tumor status	M0 (metastasis stage 0)	67%	Nil	
	M1 (metastasis stage 1)	33%	Nil	
Menopausal status	Pre-menopause	38.41%	42.5%	p-value 0.55581629 (>0.05)
	Post-menopause	61.59%	57.5%	
Tobacco smoking	Smoker	13.10%	2%	p-value 0.00296969 (<0.05)
-	Non smoker	86.90%	98%	•
Alcohol drinking	Drinker	0%	0	Not applicable
ū	Non drinker	100%	100%	p-value
Age at first delivery (mean age $\pm$ ) e.g. $30 \pm 10$		22.09 years	22.88 years	p-value 1 (>0.05)
Family history of breast	Family history	16.28%	0.41%	p-value 0.00004948 (<0.05)
	No family history	83.70%	99.59%	

were collected by trained personal in a 5 mL ACD vacutainer (BD, USA). The blood samples were stored at 2–8  $^{\circ}$ C for further processing.

#### **DNA** isolation

DNA was extracted from the blood samples by QIAamp® DNA Mini Kit (Qiagen, Germany). Isolated DNA was checked for quantity and quality by Nanodrop 2000C (Thermo Scientific, USA).

### Genotyping

Genotyping of the SNPs rs11628333 (*MAP3K9*, C>T) and rs889312 (*MAP3K1*, C>A) was performed by PCR-RFLP. Initially, amplification of the genetic region harboring the polymorphism for SNPs rs11628333 and rs889312 was done by PCR. Forward primer 5'GTGGGAGAAGGGGAAGAAGG'3 and reverse primer 5'TCCTGCATTCAAGCGATTCT'3 for rs11628333, forward primer 5'TGGCTTGTAGCTTTGTGGTG'3 and reverse primer 5'TTAGGCCTTCTTTGGCTTC'3 for rs889312 at a concentration of 1 mM was used for SNP specific PCR in a final volume of 25  $\mu$ L containing 10  $\mu$ g of genomic DNA, 1X PCR buffer (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 U *Taq* DNA polymerase, 1.5  $\mu$ L of 1 mM MgCl<sub>2</sub>, 0.5mM dNTPs and PCR water (Thermo Scientific, USA). PCR product was analyzed on 2% (w/v) agarose gel by electrophoresis.

RFLP of the PCR product of rs11628333 and rs889312 was done by incubation with restriction enzymes HpyF10VI and NlaIII (Thermo Fisher Scientific, USA) respectively at 37 °C for 16 h. The digested product was visualized on 2.5% (w/v) agarose gel by electrophoresis.

The digested PCR products are shown in Figure 1. The gels were analyzed by gel documentation system (Syngene USA Inc.).

## Statistical analysis

The SNP polymorphism data for patients and controls were statistically analyzed using SPSS software (version 20). The likelihood of SNP association was determined by *Chi*-square test. p-value <0.05 for *Chi*-square was considered as significant. Odds ratios (OR) were calculated with a 95% Confidence interval (CI) to further affirm the statistical significance of the genotypes with the disease.

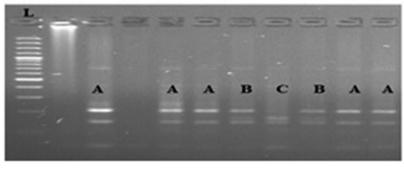
# **Results**

Breast cancer patients (mean age  $48.46 \pm 15$  years) along with gender-matched healthy individuals (mean age  $46.23 \pm 15.63$ ) as control was analyzed. Breast cancer patients developed cancer before and after the onset of menopause. The majority of the patients were in stage II of breast cancer; 33.87% stage IV; 27.41% stage III and 3.22% stage I. As an anatomical categorization, the majority of the patients had invasive ductal carcinoma (85.18%) and lobular carcinoma (14.82%), while of these two categories one fourth (24.77%) patients suffered metastasis (Table 2). Furthermore, tumor was manifested in the upper outer



rs889312 RFLP gel pic

Key:
Lanes:
L: 100bp Ladder
A: AA- 102bp, 392bp
B: AC- 102bp, 145bp, 247bp, 392bp
C: CC- 102bp, 145bp, 247bp



rs11628333 RFLP gel plc
Key:
Lanes:
L: 100bp Ladder
A: TT- 44bp, 142bp,200bp
B: TC- 40bp, 44bp, 142bp,160bp,
200bp
C: CC- 40bp, 44bp, 142bp,160bp

Figure 1: Digested PCR products for the SNPs.

Table 2: Clinical data of breast cancer patients.

Variables	Patients (n=226)
Stage	
1	3.22%
II	35.48%
III	27.41%
IV	33.87%
Undetermined	164
Metastasis	
Absent	69.02%
Present	24.77%
Undetermined	6.19%
Menopause status	
Pre-menopausal	35.39%
Post-menopausal	41.59%
Undetermined	23%

quadrant of the mammary gland, while nipple-areola was the least affected.

# Genotyping analysis

The genotyping data showed that all the genotype frequencies were in Hardy-Weinberg equilibrium (Table 3).

Genotyping data for the polymorphism of rs889312 and rs11628333 were analyzed statistically for likely association with the disease. The observed frequency of SNP *MAP3K1* rs889312 major allele AA was 39.82% and 54.60%, heterozygous genotype AC was 50.48% and 43.90% and rare allele genotype CC was 9.70% and 1.50% in patients and controls, respectively. The chi-square calculations showed a strongly significant p-value 0.01 (p<0.05).

Moreover, the rs889312 risk allele heterozygous genotype AC was considerably more frequent in postmenopausal women (50%) as compared to premenopausal women (38.80%). However, the frequency (47.22%) of wild-type homozygotes was greater in premenopausal patients.

The SNP data for rs11628333 showed the frequency of the homozygous dominant genotype TT was 56.19% and 64.52%, while for heterozygous genotype TC the frequency was 37.17% and 30.10%, and for minor allele homozygotes, CC frequency was 6.64% and 5.38% in patients and controls respectively. The *Chi*-square test for the SNP showed non-significant p-value 0.48 (p>0.05).

## **Odds** ratio

To define the strength of association, odds ratio (OR) with 95% Confidence interval (CI) for both SNPs was calculated (Table 2). The SNP rs889312 OR value was 0.55 (95% CI 0.31–0.96) for homozygous wild type AA, 1.30 (95% CI 0.74–2.26) inheterozygous AC and 7.06 (95% CI 1.23–40.34) observed in rare allele homozygous CC. The strength of association with an odds ratio for rare allele homozygous genotypes was statistically significant p-value 0.01 (p<0.05). However, the wild-type homozygous genotype OR value revealed a significant prevalence p-value 0.03 (p<0.05) in the control group. The heterozygous AC genotype behavior remained non-significant (Table 2). The forest plot for the OR results are shown in Figure 2(A).

In the case of rs11628333 OR value for wild-type genotype TT was 0.70 (95% CI 0.39–1.24), rare allele CC homozygotes OR was 1.25 (95% CI 0.38–4.04), and for heterozygote TC the value was 1.37 (95% CI 0.76–2.47).

**Table 3:** Genotype frequency distributions of MAPK rs11628333 and rs889312 polymorphisms along with their correlation by OR between patients and controls.

Genotypes	Patients observed frequency, %	Expected H-W frequency, %	Control observed frequency, %	Expected H-W frequency, %	p-Value for the risk assessment between patients & controls	(95% CI) between	p-Value
rs11628333	_	_	_	_	0.48	_	
π	56.19	55.91	64.52	63.31		0.70 (0.39-1.24)	0.22
TC	37.17	37.72	30.10	32.49		1.37 (0.76-2.47)	0.29
CC	6.64	6.37	5.38	4.20		1.25 (0.38-4.04)	0.70
	p-value 0.	.98	p-value 0.	.76		_	-
rs889312	-	_	_	_	0.01 <sup>a</sup>		
AA	39.82	42.30	54.60	58.56		0.55 (0.31-0.96)	$0.03^{a}$
AC	50.48	45.50	43.90	35.93		1.30 (0.74-2.26)	0.35
CC	9.70	12.20	1.50	5.51		7.06 (1.23-	$0.01^a$
						40.34)	
	p-value 0.	54	p-value 0.	.08			

aSignificant.

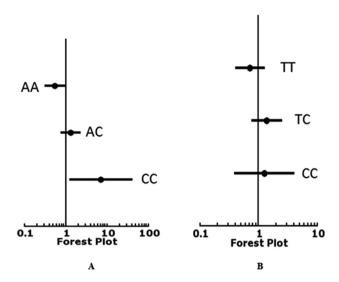


Figure 2: Forest plots for odds ratio values. (A) Forest plot rs889312 (MAP3K1). (B) Forest plot rs11628333 (MAP3K9).

The OR with 95% CI was non-significant p value >0.05. The forest plot for the OR results are shown in Figure 2(B).

# Risk allele genotype assessment

Risk allele genotypes i.e., the genotypes carrying the mutant allele for the SNP were analyzed statistically. MAP3K1 SNP rs889312 results showed a statistically significant (p-value 0.03, <0.05) prevalence of risk alleles genotypes AC/CC in patients as compared to controls. While no statistically significant demarcation was observed for MAP3K9 SNP rs11628333 in either patients or controls (Table 4).

## **Discussion**

Breast cancer is among the life-threatening causes in developing countries. Compromised health care facilities and rapid urbanization in these countries, especially in the south and east Asia, there has been a rapid increase in the incidence of breast cancer.

A prompt and targeted strategy for assessing the likelihood of disease can help to lower the burden of breast cancer development. Therefore, the present study was designed in which only female patients with breast cancer having invasive or non-invasive breast cancer, along with lobular, ductal, or nipple-areola carcinoma, were selected for the study. Patients presenting with all stages of cancer, metastasis, and pre and post-menopause disease development were genetically screened.

MAPK pathway is multi-layered and composed of a three-tier kinase module in which a MAPK is activated by phosphorylation by MAPKK (mitogen-activated protein kinase), which consequently is activated upon phosphorylation by MAPKKK. MAP3K1-targeting therapeutic miRNA suppressed the invasion of breast cancer cells in experimental mice [12].

Depletion of MEKK1 inhibited the invasion and migratory ability of pancreatic cancer cells [13].

Tumor suppressor microRNAs have been shown to directly target MAP3K1 to inhibit the proliferation of nonsmall cell lung cancer cells [14].

Involvement of MAP3K1 and MAP3K9 genes SNP as candidate molecular marker for breast cancer association in Pakistani population was identified.

The involvement of MAP3K1 gene SNP rs889312 in association with breast cancer defined a mechanistic association with breast cancer in the Pakistani population. A strong association (p-value-0.01, <0.05) of the SNP has been observed with breast cancer, which was further evaluated for concrete evidence by OR 7.06 (1.23-40.34) at 95% CI.

The disease association strength for rs889312 has been well documented in East Asia, North Africa, and Northern Hemisphere populations (Table 4). These studies were conducted on breast cancer patients for the likelihood of SNPs associated with the disease development and or

Table 4: Risk assessment of MAPK polymorphism, rs11628333 and rs889312 with combined genotype frequency calculation and OR correlation of combined genotype effect its association with risk of breast cancer.

Genotypes	Patients observed frequency, %	Control observed frequency, %	p-Value for the risk assessment between patients & controls	Correlation by OR (95% CI) between patient & control	p-Value
rs11628333			0.22		
TT	56.19	64.52	-	0.74 (0.38-1.44)	0.37
TC/CC	43.81	35.48	-	1.3 (0.69-2.62)	0.37
rs889312			0.03 <sup>a</sup>		
AA	39.82	54.60	-	0.57 (0.30-1.06)	0.07
AC/CC	60.18	45.40	-	1.75 (0.94–3.25)	0.07

<sup>&</sup>lt;sup>a</sup>Significant.

progression. Strong association of the SNP was observed in GWAS study conducted by Easton et al. 2007 [15] and the study included 3,882 breast cancer patients from the European network of excellence Cancer control using Population-based Registries and Biobank (CCPRB) [16]. A previous meta-analysis conducted to check the association of rs889312 with the hazard of breast cancer published that rs889312 was associated with a higher risk of breast cancer in small and large models of populations [17]. Another review indicated that rs889312 showed a relationship with the risk of breast cancer in Latin America [18]. In the Chilean population MAP3K1 rs889312 was associated with increased risk of familial breast cancer & in early-onset of non-familial breast cancer [19]. The present study of the Pakistani population representing the south Asian region also found a statistically significant association of rs889312 with breast cancer patients.

Contrastingly no statistically significant association of the *MAP3K9* gene SNP rs11628333 (p-value 0.48), >0.05, OR 1.25 (0.38–4.04) at 95% CI was found for breast cancer. But it was surprising to find a higher frequency (9.57%) of risk allele homozygotes in breast cancer that developed breast cancer after the onset of menopause. However, the heterozygous TC was more prevalent (41.25%) in premenopausal breast cancer patients (Table 5).

The available literature on rs11628333 has been documented for its association with breast cancer at different

levels of indigenous American ancestry by Slattery et al. 2015 [20]. The raw; adjusted p-value 0.018; 0.087 was reported. Our study also did not find statistically significant interaction of the SNP despite high-risk allele genotype frequency in patients compared to controls.

The higher frequency of rs889312 risk allele heterozygous AC and rs11628333 risk allele homozygous genotype in post-menopause patients (Table 5) can be considered as a prophylactic target with hormonal, lifestyle therapies.

*MAP3K1* SNP rs889312 has been widely documented in strong association with breast cancer in different populations of the world (Table 6). The present study of the South Asian region Pakistan also strengthens previously reported scientific literature. But unlikely the association documented for *MAP3K9* SNP rs11628333 with the risk of breast cancer [21], we have been unable to find a significant concrete association of the SNP with breast cancer etiology in the present study.

The present study presents MAP3K1 and MAP3K9 SNPs data for the first time from the south-east Asian region. Ethnicity, lifestyle, dietary habits, and environmental differences may provoke characteristic association of the SNPs with the risk of breast cancer which may not be significant in other parts or populations of the world. Secondly, in the present demographic data; there has been a family history of cancer.

Table 5: Genotype frequency of pre and post-menopausal breast cancer patients for rs889312 and rs11628333 SNPs.

Disease onset time	rs889312 genotype	Frequency, %	rs11628333 genotype	Frequency, %
Pre-menopausal	СС	13.80	сс	3.75
	AC	38.80	TC	41.25
	AA	47.22	π	55
Post-menopausal	CC	11.11	CC	9.57
	AC	50	TC	30.85
	AA	38.80	Π	59.57

Table 6: Population based comparative analysis of rs889312 SNP.

Study	OR homozygous wild type AA	OR heterozygous AC	OR homozygous mutant type CC	p-Value
Present	0.55 (0.31-0.96)	1.30 (0.74-2.26)	7.06 (1.23–40.34)	$3.6 \times 10^{-2}$
				$3.5\times10^{-2}$
				$\textbf{1.1}\times\textbf{10}^{-2}$
Easton et al. 2007 [15]	1.27 (1.19-1.36)	1.13 (1.09-1.18)	1.13 (1.10-1.16)	$7\times10^{-20}$
Harlid et al. 2012 [16]	1.13 (1.09-1.18)	1.27 (1.19-1.36)	1.13 (1.10-1.16)	$7.0\times10^{-20}$
Zheng et al. 2010 [21]	1.15 (0.99-1.33)	1.02 (0.89-1.16)	1.07 (0.99-1.15)	$5.3\times10^{-3}$
Shan et al. 2012 [22]	1.33 (0.92-1.92)	0.95 (0.55-1.66)	1.07 (0.83-1.37)	$3\times10^{-3}$
Han et al. 2011 [23]	1.13 (1.02-1.26)	1.22 (1.06-1.41)	1.15 (1.02-1.31)	$\boldsymbol{2.25\times10^{-2}}$
				$5.16\times10^{-3}$
				$\textbf{2.14}\times\textbf{10}^{-2}$

# **Conclusions**

In conclusion, the study summarizes a strong association of the MAP3K1 SNP rs889312 with the risk of breast cancer in this demographic region. Interestingly MAK3K9 SNP rs11628333 risk allele genotypes presented with the characteristic association of heterozygous genotype TC in premenopause breast cancer patients, while rare allele homozygous CC genotype was significantly prevalent in post-menopause patients. Thus rs889312 SNP can be regarded as a genetic marker for scaling the likely risk of breast cancer. And the female life hallmark menopause event may be regarded as provoking some epigenetic at its onset triggering the breast cancer as seen in the case with risk allele genotypes of rs11628333.

## **Research funding:** None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest. Informed consent: Informed consent was obtained from all individuals included in this study.

**Ethical approval:** The study was approved by the Ethical Review Committee, PMAS-Arid AAU, Rawalpindi, Pakistan.

# References

- 1. Gulzar F, Akhtar MS, Sadig R, Bashir S, Jamil S, Baig SM. Identifying the reasons for delayed presentation of Pakistani breast cancer patients at a tertiary care hospital. Cancer Manag Res 2019;11:1087-96.
- 2. Liede A, Malik IA, Aziz Z, Rios-PdPde L, Kwan E, Narod SA. Contribution of BRCA1 and BRCA2 mutations to breast and ovarian cancer in Pakistan. Am J Hum Genet 2002;71:595-606.
- 3. Liu PF, Farooqi AA, Peng SY, Yu TJ, Dahms HU, Lee CH, et al. Regulatory effects of noncoding RNAs on the interplay of oxidative stress and autophagy in cancer malignancy and therapy. Semin Cancer Biol 2020:1-14. https://doi.org/10.1016/ j.semcancer.2020.10.009.
- 4. Farooqi AA, Legaki E, Gazouli M, Rinaldi S, Berardi R. MALAT1 as a versatile regulator of cancer: overview of the updates from predatory role as competitive endogenous RNA to mechanistic insights. Curr Cancer Drug Targets 2021;21:192-202.
- 5. Butt G, Yaylim I, Attar R, Aras A, Romero MA, Qureshi MZ, et al. NEDD4 family of E3 Ubiquitin ligases in breast cancer: spotlight on SMURFs, WWPs and NEDD4. Adv Exp Med Biol 2019;1152: 365-75.
- 6. Butt G, Shahwar D, Qureshi MZ, Attar R, Akram M, Birinci Y, et al. Role of mTORC1 and mTORC2 in breast cancer: therapeutic targeting of mTOR and its partners to overcome metastasis and drug resistance. Adv Exp Med Biol 2019;1152:283-92.

- 7. Velloso FJ, Bianco AF, Farias JO, Torres NE, Ferruzo PY, Anschau V, et al. The crossroads of breast cancer progression: insights into the modulation of major signaling pathways. OncoTargets Ther 2017;10:5491-524.
- 8. Huang P, Han J, Hui L. MAPK signaling in inflammation-associated cancer development. Protein Cell 2010;1:218-26.
- 9. Slattery ML, Lundgreen A, Wolff RK. MAP kinase genes and colon and rectal cancer. Carcinogenesis 2012;33:2398-408.
- 10. Braicu C, Buse M, Busuioc C, Drula R, Gulei D, Raduly L, et al. A comprehensive review on MAPK: a promising therapeutic target in cancer. Cancers 2019;11:1-25.
- 11. Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signaling pathways in cancer. Oncogene 2007;26:3279-90.
- 12. Liu C, Wang S, Zhu S, Wang H, Gu J, Gui Z, et al. MAP3K1-targeting therapeutic artificial miRNA suppresses the growth and invasion of breast cancer in vivo and in vitro. SpringerPlus 2016;5:11.
- 13. Su F, Li H, Yan C, Jia B, Zhang Y, Chen X. Depleting MEKK1 expression inhibits the ability of invasion and migration of human pancreatic cancer cells. J Cancer Res Clin Oncol 2009;135:1655-63.
- 14. Chang Y, Yan W, Sun C, Liu Q, Wang J, Wang M. miR-145-5p inhibits epithelial-mesenchymal transition via the JNK signalling pathway by targeting MAP3K1 in non-small cell lung cancer cells. Oncol Lett 2017;14:6923-8.
- 15. Easton DF, Pooley KA, Dunning AM, Pharoah PDP, Thompson D, Ballinger DG, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087-93.
- 16. Harlid S, Ivarsson MIL, Butt S, Grzybowska E, Eyfjord JE, Lenner P, et al. Combined effect of low-penetrant SNPs on breast cancer risk. Br J Cancer 2012;106:389-96.
- 17. Zheng Q, Ye J, Wu H, Yu Q, Cao J. Association between mitogenactivated protein kinase Kinase Kinase 1 polymorphisms and breast cancer susceptibility: a meta-analysis of 20 case-control studies. PLoS One 2014;9:1-9.
- 18. Zavala VA, Serrano-Gomez SJ, Dutil J, Fejerman L. Genetic epidemiology of breast cancer in Latin America. Genes 2019;10: 1-25.
- 19. Jara L, Gonzalez-Hormazabal P, Cerceño K, Di Capua GA, Reyes JM, Blanco R, et al. Genetic variants in FGFR2 and MAP3K1 are associated with the risk of familial and early-onset breast cancer in a south American population. Breast Cancer Res Treat 2013;
- 20. Slattery ML, Lundgreen A, John EM, Torres-Mejia G, Hines L, Giuliano AR, et al. MAPK genes interact with diet and lifestyle factors to alter risk of breast cancer: the Breast Cancer Health Disparities Study. Nutr Cancer 2015;67:292-304.
- 21. Zheng W, Wen W, Gao Y, Shyr Y, Zheng Y, Long J, et al. Genetic and clinical predictors for breast cancer risk assessment and stratification among Chinese women. JNCI 2010;102:972-81.
- 22. Shan J, Mahfoudh W, Dsouza SP, Hassen E, Bouaouina N, Abdelhak S, et al. Genome-Wide Association Studies (GWAS) breast cancer susceptibility loci in Arabs: susceptibility and prognostic implications in Tunisians. Breast Cancer Res Treat 2012;135:715-24.
- 23. Han W, Woo JH, Yu J, Lee M, Moon H, Kang D, et al. Common genetic variants associated with breast cancer in Korean women and differential susceptibility according to intrinsic subtype. Cancer Epidemiol Biomarkers Prev 2011;20:793-8.