Original Article

Investigating the Impact of *miR-21* rs1292037:T>C, *miR-449b* rs10061133:A>G and *PDCD4* rs6585018:G>A Haplotype Polymorphism in Iraqi Breast Cancer Patients

Mohammed Salih Al-Janaby[1], Fadhel M. Lafta[2], Mohammed Q. Al-Ani[1]

[1]Department of Biology, College of Science, University of Anbar, Anbar, Iraq, [2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Background: Breast cancer is considered a hereditary disease characterized by the presence of a large number of genetic changes that have a different effect on its pathogenicity. **Objective:** The present study intended to assess the potential association between micro-ribonucleic acid (miRNA) and *PDCD4* single nucleotide polymorphisms (SNPs) map to different genomic regions (rs6585018:G>A, rs1292037:T>C, and rs10061133:A>G) with breast cancer in Iraqi patients. **Materials and Methods:** A total of 180 peripheral blood samples were collected from 120 breast cancer patients and 60 healthy women as a control group (age range 15–80 years). Following genomic DNA extraction and polymerase chain reaction (PCR) amplification of sequence of interest, PCR products of the participants were genotyped using TaqMan fluorescent oligonucleotide for SNP analysis. **Results:** Significant association was identified for the assessed SNPs with breast cancer. This was evident when the allelic and genotypic patterns T, TT, A, AA and A, AA map to *miR-21* rs1292037:T>C, *miR-449b* rs10061133:A>G, *PDCD4* rs6585018:G>A, respectively seemed to confer breast cancer risk factors in the studied set of patients. While breast cancer protective potential was exhibited for the C, CC, G, GG and G, GG genetic patterns located at the aforementioned genomic regions. Additionally, haplotype analysis of the assessed miRNAs and *PDCD4* genomic regions (map to 17q23.2, 5q11.2, and 10q25.2) suggest that the three chromosomal regions are associated with breast cancer, where the GCA haplotype pattern confers a risk factor, and GCG retains a protective potential of the disease ($P = 0.0038$). **Conclusions:** Overall, the present study findings suggest that breast cancer pathogenicity is significantly influenced by the association between the *miR-21* rs1292037:T>C, *miR-449b* rs10061133:A>G, and *PDCD4* rs6585018:G>A SNPs haplotype.

Keywords: Breast cancer, haplotype, *miR-21*, *miR-449b*, *PDCD4*, polymorphism

Introduction

Breast cancer is a disease marked by a large number of associated-genetic alterations that could disrupt normal cellular functions.[1] However, identifying potential driver or oncogenic events, from passenger ones, is of great interest.[2,3]

Micro-ribonucleic acids (miRNAs) are noncoding RNA molecules (20–24 nt) naturally produced within cells and involved in post-transcriptional regulating of gene expression.[4] A number cancer-associated miRNAs were identified with potential involvement in cancer development and progression. Some of these cancer-associated miRNAs have been investigated for diagnostic and prognostic utility.[5] A notable example of them is *miR-21* that proposed to have key role in the initiation and progression of human malignancies. *miR-21* encodes to the pre-*miR-21*, maps to chromosome 17q23.1, and is expressed at high levels in various tumors.[6] *miR-21* has been reported to involve in several cancer-related

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Address for correspondence: Dr. Mohammed Salih Al-Janaby, Department of Biology, College of Science, University of Anbar, 31001 Anbar, Iraq.
E-mail: sci_mohammedsalih@uoanbar.edu.iq

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processes, including tumor growth, proliferation, anti-apoptosis, and metastasis.[7] Additionally, *miR-21* targets various key cellular proteins implicating in these processes, such as programmed cell death protein 4 (*PDCD4*).[8] Furthermore, functional studies have revealed that *miR-21* exhibits oncogenic properties and can be categorized as an oncomir in solid tumors.[9] While the activity of *miR-449b* is thought to be essential for tumors' development. Some studies showed that *miR-449b* expression was down-regulated in lung cancer tissues.[10]

Single nucleotide polymorphisms (SNPs) are the most common form of human heritable genetic variations, resulting from mutation of a single nucleotide in every 100–300 base pairs of the genome. SNPs can occur in coding or noncoding regions, with around 90% of functional SNPs map to noncoding regions such as promoter and enhancer regions, as well as noncoding RNA sequences.[11] SNPs in miRNA genes can impact the generating, processing, and maturation of miRNAs. They also play significant roles in governing the expression levels of mature miRNAs and the interaction with their target genes.[12] Additionally, since some miRNAs have multiple target genes thus the disruption of complex miRNA networks could significantly impact normal cellular functions and contribute to the development of various diseases, including multiple types of cancer.[13] Furthermore, a groups of SNPs that are inherited as a single block on one or different chromosomes known as haplotypes presented to associate with a number of diseases. These SNPs haplotypes could have important contribution to the field of diseases' prediction and diagnosis.[14]

One of the main contributions of SNPs in clinical research is genome-wide association study. The rs1292037:T>C is one of the highlighted SNPs positioned in the precursor of the *miR-21* microRNA gene and has been linked to the prognosis of cervical cancer.[10,15] While rs10061133:A>G is located in the precursor of the *miR-449b* microRNA gene and have been related to the development of multiple malignancies (including thyroid, esophagus squamous cell carcinoma, and hepatocellular carcinoma[16] and premature ovarian insufficiency).[17] Whereas the rs6585018:G>A SNP is located on *PDCD4* promoter region and supposed to be associated with increased susceptibility to breast cancer. It is thought that G>A substitution at this locus affects the function of a transcription factor such as micro RNA that regulates the expression of *PDCD4* gene which is known to involve in apoptosis, cell growth, and differentiation. Down regulation of such tumor suppresser gene inhibits programmed cell death and promotes the progression of various tumors including breast cancer.[18] Locally in Iraq, breast cancer tops the list of women-effected malignancies, however, no previous study has investigated the impact of *miR-21* rs1292037:T>C, *miR-449b* rs10061133:A>G and *PDCD4* rs6585018:G>A polymorphism in Iraqi breast cancer patients.

Materials and Methods

Subjects and sampling

A total of 180 peripheral blood samples were collected from 120 patients diagnosed with breast cancer at Ramadi Teaching Hospital and Oncology Center, Anbar, Iraq. A group of 60 samples from apparently healthy women aged between 15 and 80 years during the period of January to March 2023. Breast Participants' demographic information and clinicopathological data were obtained from patients' hospital records and a prepared questionnaire for the healthy controls relevant data.

Genomic DNA extraction and quantification

Wizard Genomic DNA Purification Kit, CAT. A1120 Promega Corporation, Wisconsin, USA manufacturing, was utilized for DNA extraction from frozen blood samples. The extracted DNA integrity was initially checked by electrophoresis in agarose gel (1%) for bands quality detection. Quantus Fluorometer procedure was applied to estimate the concentration and quality of extracted DNA before conducting downstream applications. For this method, 1 µL of extracted DNA, 199 µL of diluted QuantyFlour Dye was mixed. After 5 min incubation at 25°C, DNA concentration values were detected.

Genotyping of *miR-21* rs1292037:T>C, *miR-449b* rs10061133:A>G and *PDCD4* rs6585018:G>A

The sequence of *miR-21, miR-449b,* and *PDCD4* SNPs of interest were amplified using TaqMan fluorescent oligonucleotide primers and probes given in Table 1. Primers were provided in a lyophilized form by Macrogen Company, Seoul, Korea. A nuclease-free

Table 1: The primers and probes that were used in this study

SNPs	Primers	Probes
rs1292037:T>C	Forward: ATGGAGGGAGGATTTTATGGAGAA	rs1292037P/C: FamAAGCTGCACTGTGGGT
	Reverse: GAAGGTCAAGTAACAGTCATACAGC	rs1292037-P/T: Hex-ACTAAGCTGCATTGTGGGT
rs10061133:A>G	Forward: GAGAATCGGCAGTGACCTGAA	rs10061133-P/G: Fam-AGGTAGGCAGTGTATCGTTAG
	Reverse: GAAGCAAGTGGCAGGGTAGT	rs10061133-P/A: Hex-AGGCAGTGTATTGTTAGC
rs6585018:G>A	Forward: GCCTGTCCGATTCCTCCTC	rs6585018-P/G Fam-CTGGCCGCTGCTT
	Reverse: AGCATGGGATCTCCAGAAAC	rs6585018-P/A Hex-CTGGCCGTTGCTTT

The primers and probes were designed in this study using NCBI website; Annealing temp (60°C)

water was used to dissolve lyophilized primers to give a final concentration of 100 pmol/μL as a stock solution. This step was followed by adding 10 μL of primer stock solution (stored at freezer −20°C) to 90 mL of nuclease-free water to obtain 10 pmol/μL working primer solution.

DNA samples for breast cancer patients and healthy control were genotyped for the above mentioned three SNPs. According to the author design, the RT-PCR reaction mixture total volume was 20 μL composed 10 μL of TaqMan master Mix, 1 μL of each fluorescence probes, 1 μL from each forward and reverse primer (10 μM), 2 μL of template DNA, and 4 μL nuclease free water. RT-PCR amplification reaction was performed using a programmed thermocycler with one hold cycle of 95°C for 5 min, 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s.

Ethics approval and consent to participate

The current study approved by the Ethical Approval Committee in University of Anbar, College of Science (reference number 22 on January 30, 2023). The participants were interviewed directly by the researcher, and informed consent was obtained from each participant and their parents to participate in the research, and the study was conducted as a result.

Statistical analysis

IBM SPSS statistical software (Version 26.0; IBM SPSS, Armonk, NY) was used to analyzed data along with Chi-square test to assess the deviation from Hardy–Weinberg equilibrium (HWE). Akaike information criterion, Bayesian information criterion (BIC), and ABIC were utilized to analyze the genetic model behavior of loci of interest. Cohen (d) statistic was adapted to test the genetic relationships of SNPs haplotype in the investigated subjects. $P < 0.05$ was considered for statistical significance.

Results

Association of *miR-21* rs1292037:T>C polymorphism with breast cancer pathogenicity

In the current study, frequency of C allele was lower in breast cancer patients compared with healthy control group 27% and 40%, respectively. T allele frequency was significantly higher in breast cancer patients than healthy group (73% and 60%, respectively, OR = 1.83, $\chi^2 = 6.62$, $P = 0.01$, [Table 2]). Results also presented significantly lower frequency of the CC genotype in breast cancer patients than healthy control group (0% and 7%, respectively). By contrast, the frequency of the TT genotype in breast cancer patients higher than that of the healthy controls (47% and 27%, respectively). Results suggested that CC genotype could act as protective factor while TT could confer potential risk factor for breast cancer in the investigated set of Iraqi patients (OR = 16.76, $P = 0.01$).

The allele and genotype frequencies of rs1292037:T>C indicated significant deviation from HWE in the all subjects, patients group, and control group, ($P < 0.0001$, $P < 0.0001$, and $P = 0.0035$, respectively). Genetic model analysis of rs1292037:T>C found that the alleles T and C exhibited over dominant genetic behavior [Table 2].

Association of *miR-449b* polymorphism rs10061133:A>G with breast cancer pathogenicity

The study results presented that frequency of G allele was lower in breast cancer patients compared with healthy group (23% and 43%, respectively), while A allele frequency was higher in breast cancer patients than healthy group (77% and 57%, respectively, OR = 2.51, $\chi^2 = 15.19$, $P = 0.0001$, [Table 3]). Results also exposed significantly lower frequency of GG genotype in breast cancer patients than healthy controls (3% and 20%, respectively), while the frequency of AA genotype in breast cancer patients higher than in healthy group (57% and 33%, respectively). These findings proposed that

Table 2: rs1292037:T>C alleles and genotypes frequency of the breast cancer and healthy group based on Hardy–Weinberg equilibrium test

Alleles and genotypes	All subjects	Healthy	Patients
T	248 (69%)	72 (60%)	176 (73%)
C	112 (31%)	48 (40%)	64 (27%)
T/T	72 (40%)	16 (27%)	56 (47%)
T/C	104 (58%)	40 (67%)	64 (53%)
C/C	4 (2%)	4 (7%)	0 (0%)
P value	<0.0001	0.0035	<0.0001

Genetic model for rs1292037:T>C association with breast cancer

Model	Genotype	Healthy	Patients	AIC	BIC	ABIC
Over dominant	T/T-C/C	20 (33.3%)	56 (46.7%)	160.8	176.8	168.8
	C/T	40 (66.7%)	64 (53.3%)			

the GG genotypes could have protective value while AA genotypes may act as risk factor for breast cancer (OR: 10.2, $P = 0.0002$).

The allele and genotype frequencies of rs10061133:A>G indicated no deviation from HWE in the all subjects, patient group, and control group ($P = 1$, $P = 0.31$, and $P = 0.79$, respectively). Whereas genetic model analysis presented that the A and G alleles in the rs10061133:A>G follow over dominant pattern [Table 3].

Association of *PDCD4* Polymorphism rs6585018:G>A with breast cancer pathogenicity

The results showed that frequency of the G allele was lower in breast cancer patients compared with healthy control group (23% and 33%, respectively), while frequency of A allele was higher in breast cancer patients than healthy group, (77% and 67%, respectively, OR = 1.64, $X^2 = 4.08$, $P = 0.04$, [Table 4]). The present study results showed significantly lower frequency of GG genotype in breast cancer patients than healthy control group (0% and 7%, respectively), while frequency of AA genotype in breast cancer patients higher than in healthy controls (53% and 40%, respectively). The Results suggest that GG genotype act as protective factor from breast cancer potential (OR: 0.07, $P = 0.02$). By contrast, the AA genotype act as risk factor for breast cancer (OR: 13, $P = 0.02$, [Table 4]).

Allele and genotype frequencies showed deviation from HWE in the all subjects and patients group ($P = 0.00055$ and $P = 0.00025$, respectively), While allele and genotype frequencies of healthy control group did not show deviation from HWE. Genetic model analysis for rs6585018: G>A found that alleles A and G in the follow codominance behavior [Table 4].

Association of haplotype for rs6585018:G>A, rs1292037:T>C and rs10061133:A>G with breast cancer pathogenicity

The results showed that the three investigated SNPs are positively correlated with each other, based on D' and *r* values [Table 5].

D' statistic will only be <1 if all possible haplotypes are observed. "r" reviews both recombination and mutational

Table 3: rs10061133:A>G alleles and genotypes frequencies of breast cancer and healthy groups based on Hardy–Weinberg equilibrium test

Alleles and genotypes	All subjects	Healthy	Patients			
A	252 (70%)	68 (57%)	184 (77%)			
G	108 (30%)	52 (43%)	56 (23%)			
A/A	88 (49%)	20 (33%)	68 (57%)			
A/G	76 (42%)	28 (47%)	48 (40%)			
G/G	16 (9%)	12 (20%)	4 (3%)			
P value	1	0.79	0.31			
Genetic model for rs10061133:A>G association with breast cancer						
Model	Genotype	Healthy	Patients	AIC	BIC	ABIC
Over dominant	A/A-G/G	32 (53.3%)	72 (60%)	163	178.9	170.95
	A/G	28 (46.7%)	48 (40%)			

Table 4: rs6585018:G>A alleles and genotypes frequency of the breast cancer and healthy based on Hardy-Weinberg equilibrium test

Alleles and genotypes	All subjects	Healthy	Patients			
A	264 (73%)	80 (67%)	184 (77%)			
G	96 (27%)	40 (33%)	56 (23%)			
A/A	88 (49%)	24 (40%)	64 (53%)			
A/G	88 (49%)	32 (53%)	56 (47%)			
G/G	4 (2%)	4 (7%)	0 (0%)			
P value	0.00055	0.16	0.00025			
Genetic model analysis for rs6585018:G>A association with breast cancer						
Model	Genotype	Healthy	Patients	AIC	BIC	ABIC
Codominant	A/A	24 (40%)	64 (53.3%)	160.7	179.9	170.3
	G/A	32 (53.3%)	56 (46.7%)			
	G/G	4 (6.7%)	0 (0%)			

Table 5: D, D' and *r* values for rs6585018:G>A, rs1292037:T>C and rs10061133:A>G

D statistic				r statistic			
	rs6585018	rs1292037	rs10061133		rs6585018	rs1292037	rs10061133
rs6585018	–	0.084	0.0956	rs6585018	–	0.4101	0.4717
rs1292037	–	–	0.1025	rs1292037	–	–	0.483
rs10061133	–	–	–	rs10061133	–	–	–
D' statistic				*P* value			
	rs6585018	rs1292037	rs10061133		rs6585018	rs1292037	rs10061133
rs6585018	–	0.457	0.5121	rs6585018	–	0	0
rs1292037	–	–	0.4958	rs1292037	–	–	0
rs10061133	–	–	–	rs10061133	–	–	–

Table 6: Haplotype association with breast cancer

	rs6585018	rs1292037	rs10061133	Freq	OR (95% CI)	*P* value
1	A	T	A	0.5075	1.00	–
2	G	C	G	0.1545	0.18 (0.06–0.56)	0.0034
3	A	C	A	0.1025	0.65 (0.18–2.36)	0.51
4	G	T	A	0.0826	0.35 (0.10–1.24)	0.11
5	A	T	G	0.0766	2.17 (0.37–12.88)	0.39
6	A	C	G	0.0407	1.68 (0.10–28.37)	0.72
7	G	T	G	0.0221	0.39 (0.03–5.12)	0.48
8	G	C	A	0.0133	600681831.62 (600681714.32–600681948.93)	<0.0001

Global haplotype association *P* value: 0.0038

history, while D' reviews only history of recombination therefore it is more precise statistic for estimating of recombination differences. Both r and D' statistics are affected by small sizes of samples resulting in highly unpredictable behavior.[19]

Haplotype analysis with three SNPs (rs6585018:G>A, rs1292037:T>C, and rs10061133:A>G) from three chromosomal regions (17q23.2, 5q11.2, and 10q25.2) suggested that three chromosomal regions are associated with breast cancer susceptibility in Iraqi population $P = 0.0038$. The results indicated that one haplotype GCA could be risk factor for breast cancer ($P < 0.0001$), while the other haplotype GCG indicated to be protective factor from breast cancer ($P = 0.0034$, [Table 6]).

Discussion

Genetic alterations in the genome's regulatory elements, including miRNAs' point mutations, have the potential to involve in tumorigenesis. Certainly, miRNAs play an essential role in tumor biology due to their role in the regulation of vital cellular processes including cell proliferation, differentiation.[20] Subsequently, investigating the potential association between panel of miRNAs SNPs and breast cancer could provide a new insight to understand the pathogenicity of such women's life-threating malignancy. A number of miRNA-SNPs (rs6585018:G>A, rs1292037:T>C, and rs10061133:A>G) patterns were examined in the present study have shown significant association with breast cancer with different directionality of effect (risk factor/protective value). This was evident when the genetic patterns T, TT, AA, A (map to the aforementioned genomic regions) seemed to confer breast cancer risk factors in the studied set of patients. While breast cancer protective potential was exhibited by the CC, G, GG genetic patterns located to the studied genomic regions.

Beside the association with cancer, previous studies have reported linked *miR-21* gene polymorphism to ischemic stroke risk. Another study also showed that heterozygous T/C genotype in rs1292037:T>C was more relevant amongst type I diabetes mellitus patients than healthy controls (OR = 2.74, $P < 0.0001$).[14] While Allam and colleagues have highlighted a significant association between rs10061133:A>G and the risk of hepatic cancer. In their study, shown that GG genotype or G allele was significantly associated with increased hepatic cancer risk in Egyptian patients (GG: OR = 2.91, $P = 0.013$; G allele: OR = 1.79, $P = 0.026$) compared with the genotypes of AA or AG or A allele.[16] In study of patients with esophageal squamous cell carcinoma (ESCC), G allele frequency of *miR-449b* rs10061133:A>G was 24.6% and 27.4% in healthy controls while it was the recessive allele in study this population. Individuals carrying GG genotype had an OR of 0.77 compared with individuals with AA genotype. In the recessive model, the GG genotype also showed a

protective association with ESCC (adjusted OR = 0.78).[21] While in diabetes mellitus type 2, rs10061133:A>G did not exhibit associated with the disease occurrence, however, the genotype and allele combination analyses of *miR-449b* polymorphisms showed associations with T2DM prevalence.[22]

The present study results exposed that alleles T/C in the rs1292037:T>C and alleles A/G in the rs1292033:A>G follow over dominant genetic model while the alleles A and G in the rs6585018: G>A follow codominance behavior of inheritance. The observed dominance genetic models (over/codominance) seem to influence the frequency of the investigated alleles. In genetics, overdominance condition is a rare, wherever the heterozygote phenotype lies outside the ranges of phenotypes to both homozygous parents. Also, overdominance can be defined as heterozygote benefit regulated by a single genomic locus, where heterozygous individuals have a higher fitness than homozygous individuals. Nevertheless, not all heterozygote cases advantage is considered overdominance, as they may be controlled by multiple genomic loci. Overdominance has been theorized as an underlying cause for heterosis (increased fitness of hybrid offspring). Codominance refers to a type of inheritance in which two alleles of same gene are expressed independently to produce different characters in an individual.[23]

The allele frequencies and genotype distributions of both rs1292037:T>C and rs6585018:G>A showed significant deviation from HWE in patients with breast cancer, while this was not the case in respect to the rs10061133:A>G. HWE analysis for deviation is essential to detect systematic genotyping errors in case–control studies. Significant violations of HWE or the degree of deviations from HWE may contribute to the association problems of replicating postulated gene disease across different reports.[24]

Overall, these results suggest that the rs6585018:G>A might be a useful biomarker for breast cancer risk assessment, although the direction of the association might differ among populations. Additional studies are required to explain the causal mechanisms and to confirm the clinical relevance of this genetic variant. SNPs alone have relatively small effect sizes and may not be informative for assessing risk of developed breast cancer. Consequently, linking these common SNPs together may be responsible for some comprehension into individual risks of breast cancer. Interestingly, the current study findings have highlighted the potential contribution of GCA-SNPs haplotype as a risk factor of breast cancer. Whereas GCG-SNP haplotype seemed to retains protective potential against breast cancer. This observation fits well with the thought of the contribution of multiple genomic regions in tumorgenesis and provides a new insight for further investigations to assess the impact of SNPs-haplotypes in breast cancer pathogenicity.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. Salman ED, Al Bayyar EA, Ibrahim RK. The relationship between homocysteine, folate, B12 serum level and breast cancer in sample of women in Baghdad city–Iraq. J Univ Anbar Pure Sci 2016;10:1-6.
2. Ali CA, Lafta FM, Al Sayyid MM, Al-Rekabi A-ANG. BRCA1 gene expression is down regulated in both familial and sporadic breast cancer cases in Baghdad-Iraq. Iraqi J Sci 2020;61:34-41.
3. Yousif ES, Fawaz NI, Mohamed BJ. Human breast tissue cancer diagnosis by FT-IR spectroscopy. J Univ Anbar Pure Sci 2015;9:19-24.
4. Fang Z, Li T, Chen W, Wu D, Qin Y, Liu M, *et al*. Gab2 promotes cancer stem cell like properties and metastatic growth of ovarian cancer via downregulation of miR-200c. Exp Cell Res 2019;382:111462.
5. Li C, Dou P, Wang T, Lu X, Xu G, Lin X. Defining disease-related modules based on weighted miRNA synergistic network. Comput Biol Med 2023;152:106382.
6. Feng Y, Chen X, Gao L. Knockdown of miR-21 as a novel approach for leukemia therapy. J Formos Med Assoc 2010;109:621-3.
7. Wei W-J, Wang Y-L, Li D-S, Wang Y, Wang X, Zhu Y-X, *et al*. Association study of single nucleotide polymorphisms in mature microRNAs and the risk of thyroid tumor in a Chinese population. Endocrine 2015;49:436-44.
8. Bayat R, Salehi Z, Dalili S, Bahreini F. Influence of rs1292037 genetic variant on miR-21 gene expression in patients with type 1 diabetes mellitus. Res Sq 2021;9:1-15.
9. Angel CZ, Stafford MYC, McNally CJ, Nesbitt H, McKenna DJ. MiR-21 is induced by hypoxia and down-regulates RHOB in prostate cancer. Cancers (Basel) 2023;15:1291.
10. Zhang Y, Lv J, Wu G, Li W, Zhang Z, Li W, *et al*. MicroRNA-449b-5p targets HMGB1 to attenuate hepatocyte injury in liver ischemia and reperfusion. J Cell Physiol 2019;234:16367-75.
11. Sameer AS, Banday MZ, Nissar S. Mutations and polymorphisms: What is the difference? In: Genetic Polymorphism and Cancer Susceptibility. Singapore: Springer; 2021. p. 1-21.
12. Mir R, Al Balawi IA, Duhier FMA. Involvement of microRNA-423 gene variability in breast cancer progression in Saudi Arabia. Asian Pac J Cancer Prev 2018;19:2581-9.
13. Drury RE, O'Connor D, Pollard AJ. The clinical application of microRNAs in infectious disease. Front Immunol 2017;8:1182.
14. Afzaljavan F, Vahednia E, Barati Bagherabad M, Vakili F, Moezzi A, Hosseini A, *et al*. Genetic contribution of caspase-8 variants and haplotypes to breast cancer risk and prognosis: A case-control study in Iran. BMC Med Genomics 2023;16:1-11.
15. Yang J, Yan Z, Wang Y, Xu J, Li R, Li C, *et al*. Association study of relationships of polymorphisms in the miR-21, miR-26b, miR-221/222 and miR-126 genes with cervical intraepithelial neoplasia and cervical cancer. BMC Cancer 2021;21:1-9.
16. Allam MM, Diab KA, Khalil FO, Khalaf FA, Abdel-Samiee M, Sheble N, *et al*. The association between micro-RNA gene polymorphisms and the development of hepatocellular carcinoma in Egyptian patients. Arch Med Sci 2022;18:62-70.

17. Pan H, Chen B, Wang J, Wang X, Hu P, Wu S, *et al*. The miR-449b polymorphism, rs10061133 A> G, is associated with premature ovarian insufficiency. Menopause 2016;23:1009-11.
18. Sekar D, Krishnan R, Thirugnanasambantham K, Rajasekaran B, Islam VIH, Sekar P. Significance of microRNA 21 in gastric cancer. Clin Res Hepatol Gastroenterol 2016;40:538-45.
19. Rasmussen DA, Guo FE. Espalier: Efficient tree reconciliation and ancestral recombination graphs reconstruction using maximum agreement forests. Syst Biol 2023;72:1154-70.
20. Abd-Allah GM, Ismail A, El-Mahdy HA, Elsakka EGE, El-Husseiny AA, Abdelmaksoud NM, *et al*. miRNAs as potential game-changers in melanoma: A comprehensive review. Pathol Res Pract 2023;244:154424.
21. Zhang P, Wang J, Lu T, Wang X, Zheng Y, Guo S, *et al*. miR-449b rs10061133 and miR-4293 rs12220909 polymorphisms are associated with decreased esophageal squamous cell carcinoma in a Chinese population. Tumour Biol 2015;36:8789-95.
22. Choi Y, Hong S-H. Genetic association between miR-27a and miR-449b polymorphisms and susceptibility to diabetes mellitus. Biomed Rep 2022;16:37.
23. Sameem R, Noordeen N, Praveenan S, Wetthasinghe TK, Dissanayake VHW. The Design and Implementation of a Novel Pharmacogenomic Assay to Genotype the CYP3A53 (rs776746) and CYP3A51E (rs4646453) Genetic Variants. Res Sq 2023;8:1-17.
24. Hao W, Storey JD. Extending tests of Hardy–Weinberg equilibrium to structured populations. Genetics 2019;213:759-70.