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SNP rs10800708 within the KIF14 miRNA binding site is linked with breast cancer

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Breast cancer is one of the most frequent malignancies diagnosed in females and one of most common types of neoplasia among all cancers worldwide: its development is the outcome of intricate interactions between the environment and the genome [1–3]. Common variants in low penetrance genes accompanied by lifestyle and environmental factors play key roles in aetiology [4]. Kinesin family member 14 (KIF14) is a motor protein implicated in chromosome segregation, bipolarity regulation, mitotic cell division and cytokinesis [5]. Its expression has been clinically linked with increased breast cancer mortality and invasiveness. Combined data from several studies suggest that KIF14 can play a profound role in oncogenesis, may act as a prognostic factor, and could be a therapeutic target [5–7]. Single nucleotide polymorphisms (SNPs) are the most frequent type of germline variations, and candidate SNPs support the development of multigenic disease models that predict the consequence of a disease [8]. Genome-wide association studies have recognized a relationship between many SNPs, functioning as common low-penetrance variant alleles, and breast cancer in women [1,2,9,10].

mi(cro)RNAs are small endogenous non-coding RNAs that modulate about a third of all human protein-coding transcripts by binding to mRNAs. This usually leads to gene silencing through mRNA degradation or translation inhibition via 2–8 nucleotides known as the 'seed region' in miRNA by base-pairing with a complementary sequence in the 3′-untranslated region (3′-UTR) of the target mRNA [5,11]. SNPs in the miRNA binding site can influence the complementarity between the miRNAs and target mRNAs, directly weaken or strengthen miRNA–mRNA interaction and consequently affect the target gene's expression. However, some have assessed the link between SNPs inside the miRNA binding site of mRNAs with cancer risk [1,11]. Transcripts of human KIF14 include a 3′-UTR of 1.8 kb that includes several SNPs as well as the target site of multiple miRNAs. Although SNPs do not change the KIF14 protein codons, a SNP position at 3′-UTR can affect several miRNA binding sites [5]. Among 30 SNPs recognized in the 3′-UTR of KIF14 [5], we focused on rs10800708, hypothesizing a link with the clinical stage and histological grade of breast cancer.

To test our hypothesis, we recruited 126 patients with recently diagnosed and histologically confirmed breast cancer (mean/SD age = 49.8 (10.9) years) and 114 healthy controls (aged 48.0 (11.9) years) undergoing a regular health check at Sayed-ol-Shohada Hospital, Isfahan, Iran. All participants provided written informed consent: the study was approved by the ethics committee of Sayed-ol-Shohada Hospital. Genomic DNA was extracted from whole venous blood taken into EDTA, using the salting out method. Quality and quantity of the extracted DNA were then checked by electrophoresis on a 1% agarose gel and spectrophotometry at 260/280 nm (NanoDrop 1000, Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). Tetra-primer amplification refractory mutation system PCR (Tetra-primer ARMS PCR) was used for genotyping. Primers (Cinnagen, Iran) were forward outer (FO) primer 5′-TATTTA TCAAAGGCCAGACACGGT-3′, reverse outer (RO) primer 5′-CTGGGATTACAGGCGTGAGTCT-3′, forward inner (FI) primer 5′-AGGTGTACCAAAGCGTGTCTATGAG-3′ and reverse inner (RI) primer 5′-CCCTAGTAGCTGGGA TTACAGGC-3′. PCR reactions were in a 25 µl final volume of 2.5 µl PCR Buffer 10X, 0.75 µl $MgCl_2$ 50 mM, 1 µl dNTP 10 mM, 0.75 µl each primer (10 mM), 0.25 µl Taq DNA pol., 100 ng DNA and ddH20 up to the final volume. The reaction conditions were primary denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58.5 °C for 50 s and extension at 72 °C for 50 s, and a final extension step at 72 °C for 10 min. The PCR products were separated by standard electrophoresis on a 2% agarose gel. The amplicon size produced by outer primers was 413 bp long as an internal control while amplification by A or T allele-specific primers produces 277 or 181 bp long amplicons, respectively. Deviation from Hardy–Weinberg equilibrium (HWE), odd ratios (ORs) with 95% confidence intervals (CIs) and the Cochran-Armitage trend test

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were performed via the DeFinetti program (ihg.gsf.de/cgi-bin/hw/hwa1.pl) to evaluate the possible link between rs10800708 and breast cancer. Links between the SNP and clinicopathological features of breast cancer were assessed by the chi-square test. $P < 0.05$ was considered significant. Statistical analyses were carried out applying SPSS software version 22.0. Since miRNAs typically bind to the mRNAs 3′-UTR, an SNP within the 3′-UTR potentially can cause the binding of miRNAs to be lost or gained. Online tools (MirSNP [12] and miRNA SNP [13]) were used to investigate whether rs10800708 SNP within the 3′-UTR of KIF14 affects its regulation by miRNAs. Data from both tools were classified on the possible effect (gain/create, break/loss or decrease of miRNA-target interaction potential), calculated according to the binding energy change between two variants of SNP.

Genotype distribution of both case and control groups did not deviate from the HWE ($p > 0.05$). In accordance with the allele frequency comparison [OR (95% CI) = 2.16 (1.50–3.12), $p = 0.00003$] and Cochran–Armitage trend test ($p = 0.00004$), the A allele of rs10800708 was significantly linked with breast cancer. The AA, TA and joint AA/TA genotypes were significantly linked with breast cancer compared with the TT genotype (Table 1). Clinical stage was linked to the AA genotype, and histological grade was linked to the AT, AA and joint AA/TA genotypes (Table 2). Our *in silico* study also indicated that SNP in 3′-UTR of KIF14 could alter the binding site of 4 miRNAs. Based on results from MirSNP and miRNASNP, rs10800708 SNP within KIF14 3′-UTR can prevent the miR-892a, miR-4252 and miR-5095 from partially or completely binding to KIF14 mRNA, and so strengthen the binding of miR-2114-3p to KIF14 mRNA.

The most frequently detected mutations in the genome are SNPs occurring once every several hundred base-pairs within the genome. Since miRNA function relies on sequence complementarity between the miRNA and target mRNA, even single nucleotide aberrations can cause significant alterations in miRNA–mRNA interaction. Over the past few years, some studies have identified these functional miRNA-binding-site SNPs as cancer biomarkers via case-control study designs [1,9]. Tchatchou et al. demonstrated that an SNP within the 3′-UTR of ESR1 disrupted miR-453 binding, and the variant allele (A) was associated with elevated oestrogen receptor expression and breast cancer risk [14]. Brendle et al. detected an SNP in miRNA binding site as a prognostic biomarker for breast cancer found in the 3′-UTR of integrin beta-4 (ITGB4), and located within a putative miR-34a binding site that was linked with different aggressive tumour characteristics, including high grade and high stage [15]. Overexpression of KIF14 has been shown in multiple cancers, including breast cancer, and is associated with a poor outcome. Furthermore, knockdown of KIF14 leads to apoptosis and reduced colony formation [7,16]. Taken together, KIF14 may be regarded as an important oncogene, prognostic factor and therapeutic target in the development of breast cancer [5]. As reported by Thériault et al., miR-382 is a regulator of KIF14 mRNA levels in ovarian cancer that could take part in the overexpression of KIF14 mRNA in these tumours [7]. Liang et al. demonstrated that cell-cycle-related genes, specifically KIF14, are upregulated in paediatric high-grade gliomas (pHGGs) and participate in cell proliferation. Moreover, miR-6500-3p directly targets the 3′-UTR of human KIF14 and downregulation of miR-6500-3p promotes cell proliferation via regulating KIF14 in pHGGs [16]. We selected rs10800708 as a candidate target SNP that can potentially break, create or modify miRNA binding sites via a bioinformatics approach. The presence of the variant allele (A) is likely to disrupt binding site of miR-892a, miR-4252 and miR-5095, and create a putative target site for miR-2114-3p. Significant calculated binding energy change (ΔΔG) in two recruited databases suggests rs10800708AA as a potential causative genetic factor for breast cancer development. Here, allele frequency comparison in the case (0.68) and (0.41) control groups highlighted the A allele as a breast cancer risk factor. We support this view with our finding of a significant link between the A allele and the susceptibility to breast cancer through comparing allele frequency and the Cochrane-Armitage trend test. According to genotype distribution data, AA is the minor genotype in the control group, whereas TT is observed at the lowest frequency in the case group. Our data demonstrate a strong relationship between breast cancer and the presence of at least one A allele (AA + AT). Furthermore, analysing the association of rs10800708 with clinicopathological features, we found links between the AA genotype and clinically advanced stage (III + IV). Moreover, significant associations were observed between the AA, TA and AA + TA genotypes with pathologically more malignant breast cancer, i.e. histological grade III.

A large body of epidemiological studies have been recently carried out to explore the relationship between SNPs and the risk of breast cancer. One study suggested the possible importance of SEPP1 rs3877899 and SEP15 rs5859 polymorphisms in susceptibility to breast cancer, reporting that individuals with the AA genotype for

Table 1. Genotype frequencies in case and controls.

Genotype	Controls *N* (%)	Cases *N* (%)	OR (95% CI)	*P* value
AA	20 (18)	45 (36)	4.875 (2.262–10.51)	< 0.0001
TA	55 (48)	63 (50)	2.482 (1.276–4.829)	0.006
AA + TA	75 (66)	108 (86)	3.12 (1.659–5.867)	0.0003
TT	39 (34)	18 (14)	1 (reference)	–

Data presented as number (%). OR: odds ratio; CI: confidence interval.

Table 2. Relationship of rs10800708 with clinical stage and grade of breast cancer.

	TT	AT	AA	AA + AT
Clinical stage				
III+ IV/I+ II	9/6	36/24	36/6	72/30
OR (95% CI)	Reference	1.00 (0.31–3.17)	4.00 (1.04–15.39)	1.6 (0.52–4.89)
P value		1.00	0.036	0.406
Histological grade				
III/I+ II	0/9	21/30	15/21	36/51
OR (95% CI)	Reference	13.39 (1.21–242.8)	13.70 (1.12–253.6)	13.47 (1.211–238.9)
P value		0.017	0.017	0.014

Data presented as number of subjects. OR: odds ratio; CI: confidence interval.

both SNPs have an increased risk of breast cancer [2]. Another assessed the relationship between three microRNA polymorphisms and breast cancer providing evidence of the effects of miR-146a and miR-27a on breast cancer [10]. A small number of studies have investigated the role of KIF family mutations in other disorders: mutations of KIF14 are linked to primary microcephaly by impairing cytokinesis [17], and the Trp719Arg allele of SNP rs20455 in *KIF6* is associated with coronary heart disease [18]. There are several limitations to our study, principally limited statistical power due to the small sample size, and accordingly our findings require confirmation in other populations. The molecular mechanism by which this SNP in KIF14 affects susceptibility to breast cancer is not fully understood and requires further elucidation.

This work represents an advance in biomedical science because it points to the possible importance of rs10800708 in susceptibility to breast cancer, so that this SNP may be used as a marker to identify individuals at high risk for this disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

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