RESEARCH ARTICLE

Editorial Process: Submission:06/23/2024 Acceptance:01/10/2025

New Genetic Variations in RNA-binding Protein Gene and Breast Cancer Risk: A Case-Control Study

Hosna Sarani[1,2,3], Mohsen Taheri[1,4]*, Danial Jahantigh[5], Mohammad Reza Keramati[6], Seyed Mehdi Hashemi[7], Gholamreza Bahari[2]*, Saba Taheri[5,8]

Abstract

Background: *LIN28*, a highly conserved RNA-binding protein, regulate a wide variety of post-transcriptional cellular processes. The current study aimed to identify genetic variants of five single nucleotide polymorphisms (SNPs) in the *LIN28B* gene (rs221634, rs22163, rs314276, rs9404590, and rs12194974) and their association with Breast cancer. **Method:** 220 patients and 230 controls were genotyped by the RFLP assay for *Lin28B* gene variants. Odds ratio analysis was used to determine the association between *Lin28B* variants and breast cancer. Haplotype analysis was performed to determine the combined impact of the investigated variants on BC. Novel in-silico analysis were performed to predict the potential functions of these polymorphisms, as well. **Results:** Patients carrying all variant genotypes for lin28B rs221634 (codominant, dominant, recessive, and allelic inheritance models), rs221635 (codominant and dominant genotypes), and rs9404590 (codominant, dominant, and inheritance model). Significant associations between reduced cancer risk and rs12194974 and rs314276 were found in codominant, dominant, recessive, and allele inheritance models. According to haplotype analysis of rs9404590, rs12194974, rs314276, rs221634, and rs221635 SNPs ,the GGCTT, GGCAT, TGCAC, TGCTC, GGCAC, GGCTC, and GGAAC haplotypes are associated with an increased risk of BC, whereas the TACAT and TAAAT haplotypes were associated with a decreased risk of BC. The splicing enhancers (ESE) binding site was found to be altered by the SNPs rs9404590, rs12194974, and rs314276, according to in-silico analysis. **Conclusion:** Breast cancer susceptibility appears to be linked to genetic variations in the *Lin28B* gene, and haplotypes in this region have been linked to increased risk.

Keywords: Breast Cancer- *LIN28B*- RNA-binding protein- polymorphism- Gene variation

Asian Pac J Cancer Prev, **26 (1)**, 137-145

Introduction

Breast cancer is introduced as the most frequent malignancy in women worldwide, accounting for 25.1% of all cancers and the most commonly recognized cause of female cancer death [1, 2]. Breast cancer is the commonest cancer among Iranian women, and it strikes them a decade earlier than it does in Western countries [3]. The annual standardized incidence rate (ASR) is around 28 per 100,000 individuals and has been growing in recent years [4]. Breast cancer is caused by a number of confirmed and debatable risk factors; both genetic and environmental variables have a role in the progression of BCa [5]. The role of gene polymorphisms in BCa susceptibility has recently received much attention [6].

LIN28 is one of the most well-defined core "stemness" genes in human cells, with the ability to transform terminally differentiated human fibroblasts into induced pluripotent stem cells [7]. They have been identified as biomarkers for cancer stem cells and involved in the pathophysiology of therapeutic resistance in malignancies [8, 9]. The heterochronic gene *LIN28* is a highly conserved microRNA let-7 family member engaged in crucial tumor suppressor functions [10]. Let-7 suppresses the expression of essential oncogenes such as Ras, Myc, and cyclins by binding to their 3′ end untranslated regions (UTRs) [10, 11]. *LIN28*, one of the several downstream genes of the NF-B signaling pathway, has attracted considerable attention as an inhibitor of let-7 pri-miRNA processing, promoting tumorigenesis by inhibiting let7-miRNA

*[1]Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Iran. [2]Children and Adolescent Health Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan , Iran. [3]Peter Doherty Institute for Infection and Immunity, Melbourne, Australia. [4]Genetic Department, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. [5]Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran. [6]Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. [7]Department of Internal Medicine, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. [8]Department of Biology, Faculty of Science, Shahid Beheshti University, Tehran, Iran. *For Correspondence: Taheri@zaums.ac.ir, ghbahari@zaums.ac.ir*

production [12].

LIN28 encodes two homologs, *LIN28A* and *LIN28B*. *LIN28b* was initially discovered as an RNA-binding protein involved in early development and stem cell differentiation [12], which affects the let-7 miRNA through increased transcriptional factors of HMGA2 and ARID3B [7]. Overexpression of human *LIN28B* has been related to various malignancies, resulting in significantly enhanced malignant aggressiveness and poor prognosis [7, 13-16].

There are a great number of Single Nucleotide Polymorphisms (SNPs) in the human genome, making them the most frequently occurring kinds of genetic variation. Genetic vulnerability to cancer is connected with SNPs that affect DNA mismatch repair, cell cycle regulation, metabolism, and immune system function [17]. Lin28b SNPs have recently been associated with numerous of malignancies, including Neuroblastoma [18], hepatoblastoma [19], colon cancer [20], ovarian cancer [21], and Wilms tumor [22].

Pre-mRNA splicing is a precise and widespread nuclear mechanism that is a natural source of cancer-causing gene expression abnormalities by Exon-skipping events through interionic splice site alterations [23]. Splicing has recently been linked to the development of various cancers, in which aberrant changes in alternative splicing could affect tumor progression [24]. It may also impair protein interaction pathways involved in tumor growth [25]. In this study, we aim to investigate the correlation between breast cancer susceptibility and some *LIN28b* polymorphisms, including rs221634 A>T,rs221635 T>C,rs314276 C>A rs9404590 T>G, and rs12194974 G>A.

Materials and Methods

Patients

In this case-control study, 220 patients with breast cancer who were referred to Department of internal medicine of the Ali ibn Abi Talib hospital (regional referral hospital for cancer cases in the southeast of Iran) and 230 healthy individuals without any clinical symptoms or family records of BC from the same ethnicity as patients were enrolle. The study design and procedure have been defined previously [26]. Ethical approvals for employment were taken from local Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1396.52), and informed consent was obtain from all participants. We take venous blood sample from all participants in NaEDTA tubes and stored them at -20°C until DNA ectraction.

Genotyping

Genotyping of *Lin28B* (rs221634, rs221635, rs314276, rs9404590, and rs12194974) polymorphisms were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers have shown in Table 1. Polymerase chain reaction (PCR) was achieved using commercially available PCR premix (AccuPower PCR Pre-Mix, BIONEER, Daejeon, South Korea) according to the manufacturer's recommended protocol. One µL of template DNA (~ 100 ng/ µL), 1 µL of each primer (10 µM), and 10 µL of master mix, and 7µL of double-distilled water (ddH2O) were added to a 0.2 mL PCR tube.The PCR cycling conditions were: 95°C for 5 min; followed by 35 cycles of 30 s at 95°C annealing temperature for 35 s at 56°C and 72°C for 35 s and a final extension step of 72°C for 10 min for rs221634. For rs 221635: 95°C for 5 min; followed by 30 cycles of 30 s at 95°C annealing temperature 60°C for 30 s; 72°C for 30 s; and a final extension step of 72°C for 5 min. For rs314276: 95°C for 6 min; 30 cycles of 95°C for 30 s; 62°C for 30 s; 72°C for 30 s; and a final extension step of 72°C for 5 min. For rs9404590: 95°C for 6 min; 30 cycles of 95°C for 30 s; 62°C for 30 s; 72°C for 30 s; and a final extension step of 72°C for 5 min. Finally, for rs12194974: 95°C for 6 min; 35 cycles of 95°C for 30 s; 60°C for 35 s; 72°C for 35 s; and a final extension step of 72°C for 10 min. Ten microliters of PCR product were digested by the appropriate restriction enzymes (Table 1) and separated by electrophoresis on an agarose gel. Approximately 20% of random samples were re-genotyped; the results confirmed previous genotyping outcomes.

Computational analysis

An analysis of *Lin28B*-mRNA structures using computational methods evaluated the potential biological effects of these single nucleotide polymorphisms [27]. These intronic SNPs were also subjected to Bioinformatics analysis in order to predict the potential biological functions of these variations on *Lin28B*-mRNA splicing [28, 29]. Furthermore, the conservation of DNA sequences containing *Lin28B* gene polymorphisms (rs221634, rs221635, rs314276, rs9404590, rs12194974) was investigated [30, 31].

Statistical analysis

All statistical analysis was performed by SPSS 20 software (SPSS Inc., Chicago, IL, USA). The data were analyzed by independent sample t-test and χ^2 test. The logistic regession analysis was employed to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs). The Hardy-Wineberg equilibrium (HWE) was calculated for each polymorphism. p-value <0.05 was considered to be statistically significant.

Results

The subjects enrolled in this study are 220 pathologically confirmed BC patients, with a mean age of 48.00 ± 10.67 years, and 230 healthy women, with a mean age of 49.03 ± 12.09 years. No significant difference was found between the groups regarding age (p = 0.339).

Genotypic and allelic frequencies of *Lin28B* (rs221634, rs221635, rs314276, rs9404590, and rs12194974) variants in BC patients and controls are shown in Table 2. The findings showed that all inheritance models of the rs221634 A>T variant of the *Lin28B* gene (codominant, dominant, recessive, and allelic inheritance models) significantly increased the risk of BC. The rs221635 T>C variant significantly increased the risk of BC in codominant and dominant genotypes as well as codominant, dominant, and

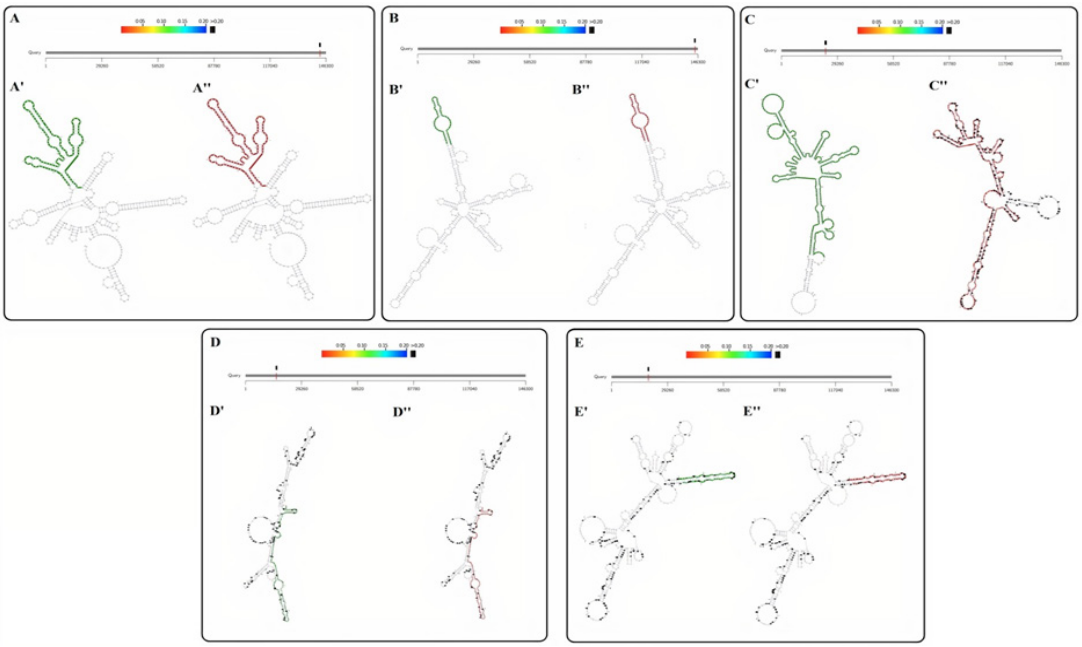

Figure 1. The Effects of SNPs on Local mRNA-Secondary Structure: The effects of rs221634 (A), rs221635 (B), rs314276 (C), rs9404590 (D), and rs12194974 (E) variations on Lin28B-mRNA secondary structure. P-value color direction and graphic summary of the analysis (A, B, C, D and E). The SNP-affected region is not colored in black since of the p-value less than 0.2, which is significant structural change in mRNA structure. The optimal secondary structure of global wild-type sequence depicted in green (A', B', C', D' and E'). The optimal secondary structure of global mutant sequence showed in red (A'', B'', C'', D'' and E'').

allelic inheritance models of rs9404590 T>G. In addition, the genotype analysis demonstrated the rs12194974 G>A (codominant, dominant, recessive, and allelic inheritance models) and rs314276 C>A (codominant, dominant, and allelic inheritance models) variants significantly decreased the risk of BC. The Hardy-Wineberg equilibrium (HWE) was calculated for each polymorphism. The results showed that in controls the distribution of rs221634, rs221635, rs314276 and rs9404590 variants were in HWE (p= 0.114, 0.675, 0.470 and 0.161 respectively), but rs121194974 variant is not in HWE (p<0.001). In BC patients, rs314276 and rs121194974 variants were in HWE (P= 0.474 and 0.339 respectively) but rs221634, rs221635 and rs9404590 deviated significantly from HWE (P= 0.043, 0.017 and <0.001 respectively).

The analysis of SNP combinations elaborated in Table 3 showed evidence of cooperative effects between rs9404590, rs12194974, rs314276, rs221634, and rs221635 of *LIN28B* gene demonstrated the GGCTT, GGCAT, TGCAC, TGCTC, GGC AC, GGCTC, and GGAAC haplotypes were closely associated with a higher risk of BC. In contrast, TACAT and TAAAT haplotypes were linked to decreased risk of BC.

Bioinformatics analysis

The possible effects of *Lin28B* rs221634 A>T, rs221635 T>C, rs314276 C>A, rs9404590 T>G, and rs12194974 G>A single nucleotide polymorphisms were predicted to discover possible significant structural changes on local *Lin28B*-mRNA secondary structures. None of these variations significantly alter the secondary structure of *Lin28B*-mRNA; a p-value of more than 0.2 indicates a non-significant structural change (Figure 1). Using bioinformatics prediction and analysis tools, results

Table 1. The Primers Used for Detection of *Lin28B* (rs221634, rs221635, rs314276, rs9404590 and rs12194974) Polymorphisms

Polymorphism *Lin28B*	PCR primers (5'→3')	Restriction Enzyme	Fragment, bp
rs221634	F: TCTCCCACCAGAGAGCTAGA	SspI	TT= 293
	R: GCACTATAATTAACTGGTACC		AA=213+80
rs221635	F: TTCACAACTGCATGTTTCTGACAA	Hin1I	TT= 457
	R: TAATTCACAGACCTGCTGCCC		CC= 256+201
rs314276	F: TGAATTAAAACATGTAGCTGCTGA	SspI	CC=345
	R: TGAAATCGTCTTGAATTGCAACC		AA=258+87
rs9404590	F: ATCAGGACAGTTTGCCCGAC	BglII	GG=283
	R: AAGTGCGGTCAAAGAGAGGG		TT=232+51
rs12194974	F: AGCTCTTGGGGAACAATCGC	BmrI	AA=289
	R: TAGGAAAAGGCAGAGGCACAT		GG=223+66

Table 2. Association of Lin28B (rs221634, rs221635, rs314276, rs9404590 and rs12194974) Polymorphisms and the Risk of Breast Cancer

polymorphism	Case n (%)	Control n (%)	OR (95%CI)	p-value
Lin28B				
rs221634				
Codominant				
AA	72 (32.7)	115 (50.0)	1	-
AT	120 (54.5)	102 (44.3)	1.88 (1.26-2.79)	0.002
TT	28 (12.8)	13(5.7)	3.44 (1.67-7.07)	0.001
Dominant				
AA	72 (32.7)	115 (50.0)	1	-
AT+TT	148 (67.3)	115 (50.0)	2.06 (1.40-3.01)	<0.001
Recessive				
AA+AT	192 (87.2)	217 (94.3)	1	-
TT	28 (12.8)	13 (5.7)	2.43 (1.22-4.83)	0.014
Allele				
A	264 (60.0)	332(72.2)	1	-
T	176 (40.0)	128 (27.8)	1.73 (1.31-2.29)	<0.001
rs221635				
Codominant				
TT	89 (40.5)	139(60.4)	1	-
TC	114 (51.8)	81 (35.2)	2.20 (1.49-3.25)	<0.001
CC	17 (7.7)	10 (4.4)	2.66 (1.16-6.06)	0.02
Dominant				
TT	89 (40.5)	139 (60.4)	1	-
TC+CC	131 (59.5)	91 (39.6)	2.25 (1.54-3.28)	<0.001
Recessive				
TT+TC	203 (92.3)	220 (95.6)	1	-
CC	17 (7.7)	10 (4.4)	1.84 (0.82-4.12)	0.19
Allele				
T	292 (66.4)	322 (70.0)	1	-
C	148 (33.6)	158 (30.0)	1.03 (0.78-1.36)	0.871
rs9404590				
Codominant				
TT	69(31.4)	146 (63.5)	1	-
TG	130 (59.1)	70 (30.4)	3.93 (2.61-5.91)	<0.001
GG	21 (9.5)	14 (6.1)	3.17 (1.52-6.62)	0.002
Dominant				
TT	69 (31.4)	146 (63.5)	1	-
TG+GG	151 (68.6)	84 (36.5)	3.80 (2.57-5.63)	<0.001
Recessive				
TT+TG	199 (90.5)	216 (93.9)	1	-
GG	21 (9.5)	14 (6.1)	1.63 (0.81-3.29)	0.233
Allele				
T	268 (60.9)	362 (78.7)	1	-
G	172 (39.1)	98 (21.3)	2.37 (1.77-3.18)	<0.001
rs12194974				
Codominant				
GG	141 (64.1)	72 (31.3)	1	-
GA	73 (33.2)	138 (60.0)	0.27 (0.18-0.40)	<0.001
AA	6 (2.7)	20 (8.7)	0.15 (0.06-0.40)	<0.001

Table 2. Continued

polymorphism	Case n (%)	Control n (%)	OR (95%CI)	p-value
Dominant				
GG	141 (64.1)	72 (31.3)	1	-
GA+AA	79 (35.9)	158 (68.7)	0.26 (0.17-0.38)	<0.001
Recessive				
GG+GA	214 (97.3)	210 (91.3)	1	-
AA	6 (2.7)	20 (8.7)	0.29 (0.12-0.75)	0.012
Allele				
G	355 (80.7)	282 (61.3)	1	-
A	85 (19.3)	178 (38.7)	0.38 (0.28-0.51)	<0.001
rs314276				
Codominant				
CC	121 (55.0)	98 (42.6)	1	-
CA	87 (39.5)	108 (47.0)	0.65 (0.44-0.96)	0.031
AA	12 (5.5)	24 (10.4)	0.41 (0.19-0.85)	0.017
Dominant				
CC	121 (55.0)	98 (42.6)	1	-
CA+AA	99 (45.0)	132 (57.4)	0.65 (0.44-0.96)	0.039
Recessive				
CC+CA	208 (94.5)	206 (89.6)	1	-
AA	12 (5.5)	24 (10.4)	0.50 (0.24-1.02)	0.076
Allele				
C	329 (74.8)	304 (66.1)	1	-
A	111 (25.2)	156 (33.9)	0.66 (0.49-0.88)	0.005

show that the mutant alleles A and G of *Lin28B* rs314276 and *Lin28B* rs9404590 SNPs may lose the exonic splicing enhancers (ESE) binding site of serine/arginine-rich (SR) proteins 9G8 and SRp20, respectively. Moreover, it was demonstrated the mutant allele A of *Lin28B* rs12194974 variation can create an ESEs binding sequence for Ser/Arg-rich protein 30c (SRp30c) (Figure 2). Additional bioinformatics techniques revealed that the *Lin28B* rs221635, rs9404590, and rs12194974 are located in a nearly conserved region. While the *Lin28B* rs221634 and rs314276 are positioned in a variable region across multiple mammalian species, as shown in Figure 3.

Discussion

The current population-based case-control study examined the relationships between *LIN28b* polymorphisms and breast cancer susceptibility. The present study was the first to indicate a strong association between Lin28 polymorphisms and breast cancer. Results revealed that rs221634, rs221635, and rs9404590 are linked to a higher risk of breast cancer, while rs314276 and rs12194974 are linked to decreased breast cancer susceptibility.

The *LIN28B* gene, located on chromosome 6q21, was first discovered by Guo et al. [32] and is a member of the RNA binding proteins family. *LIN28* regulates post-transcriptional gene expression by various methods, the most well-known of which is its inhibition of the let-7 miRNA family [33]. Let-7 is a prominent tumor suppressor that targets stemness and oncogenic factors, including MYC, RAS, and Hmga2, by negatively regulating [34]. The role of *LIN28* in a variety of malignancies has been discovered; Overexpression of *LIN28B* has been linked to various malignancies, leading to a significant increase in malignant aggression and a poor prognosis. In a study by Zhang et al. [35], Lin28 expression was shown to be increased in breast cancer tissues. Additionally, suppression of proliferation, migration, invasion and EMT in breast cancer cells were observed when *LIN28b* was silenced. Tummala et al. [36] showed that *LIN28* was substantially expressed in human PCa tissues and PCa cell lines by Tummala et al. [36]. They also found that knocking down *LIN28* stopped tumor cells from proliferating [36]. The correlation between high expression of *LIN28* and ovarian cancer has been illustrated by He et al. [37], which also reported that *LIN28A* expression was a predictor of poor prognosis. Numerous studies indicated the oncogenic role of *LIN28* by detecting high levels of *LIN28* in many cancers such as glioblastoma, ovarian, and gastric cancer [38].

The effect of *LIN28* polymorphisms on cancer susceptibility has only been studied in a few studies. He et al. [18] investigated the relationship between rs221634 A>T, rs221635 T>C, rs314276 C>A, and rs9404590 T>G and Neuroblastoma susceptibility and found that the rs221634 TT genotype is associated with an elevated risk of Neuroblastoma in Chinese children. They discovered no link between cancer susceptibility and rs221635, rs314276, and rs9404590. Consistent with this, in the current study,

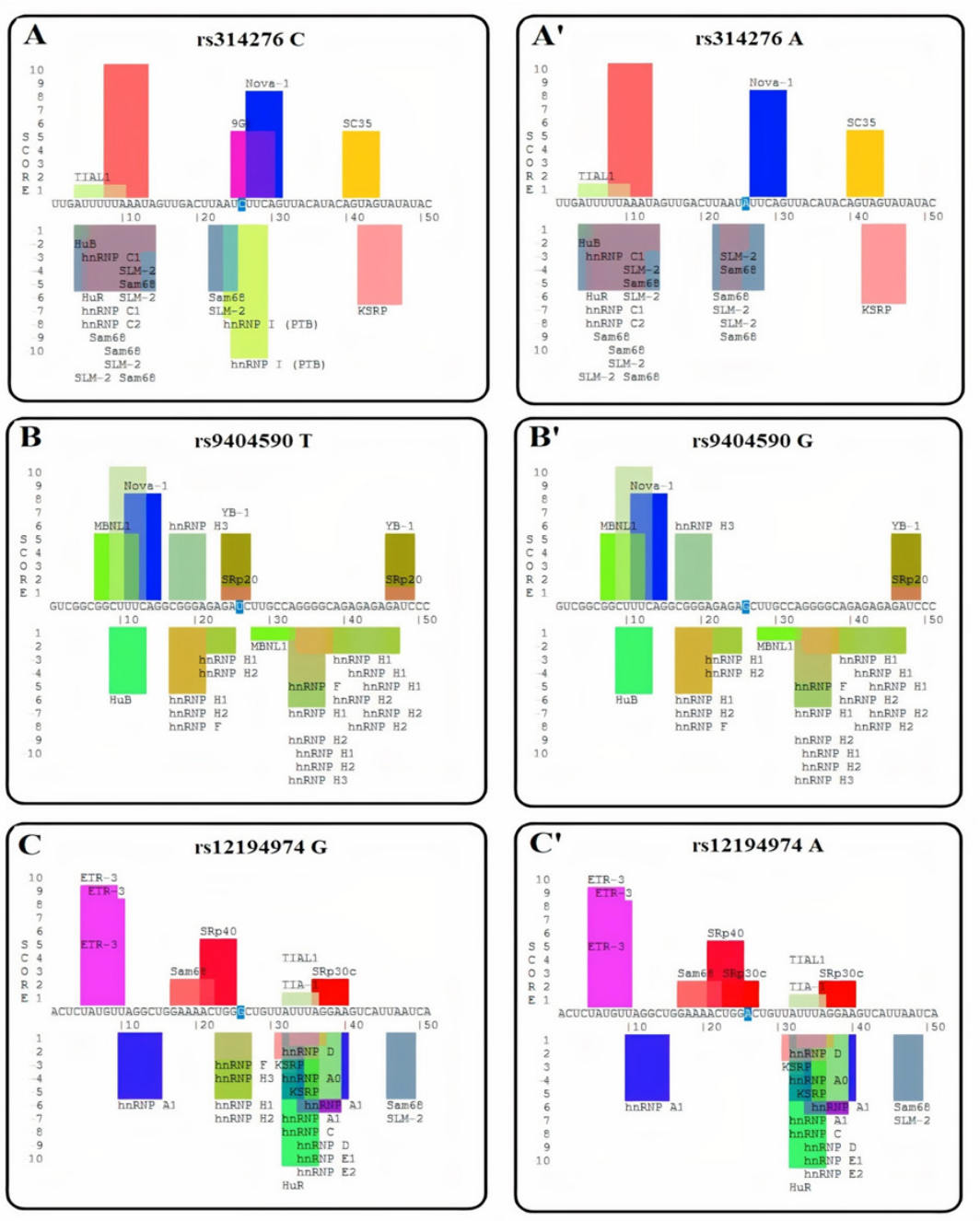

Figure 2. The SNPs Effects on Enhancers and Silencers Motifs, Analyzed by SpliceAid 2 tool: Predicted data of changes in number and type enhancers and silencers motifs for Lin28B rs314276 (A), rs9404590 (B), and rs12194974 (C) gene polymorphisms.

we identified that rs221634 is associated with a higher risk of breast cancer in codominant, dominant, and allelic forms. In a study by Yang [19] on hepatoblastoma patients, no statistically significant correlation with rs221634 was observed in any comparisons. rs314276 (C>A), an intronic polymorphism, has been shown to have a negative correlation with *LIN28B* expression through altering RNA secondary structure [39]. The present study demonstrated that rs314276 (C>A) plays a protective role in breast cancer, probably by decreased expression of *LIN28*. In addition, Fu et al. [22] found that rs314276 was correlated to a lower incidence of Wilms Tumor. Conversely, rs314276 was linked to an increased risk of cancer in cases of hepatoblastoma and non-small cell lung cancer [19, 40]. The discrepancy between these studies could be attributed to allelic heterogeneity varying across ethnic groups, the complexity of gene interaction, the variety of cancer types, or the small sample size. rs12194974 (G>A), located 727 bases upstream from the *LIN28B* transcriptional start site, was investigated by Permuth-Wey [21]; results indicated The A allele of rs12194974 was related to a lower incidence of epithelial ovarian cancer, and it has been proven experimentally to reduce *LIN28B* transcriptional activity and *LIN28B* mRNA expression. Genotype analysis of the current study discovered that all codominant, dominant, and allelic inheritance models were associated with a reduced risk of breast cancer. In contrast, no associations were found between rs12194974

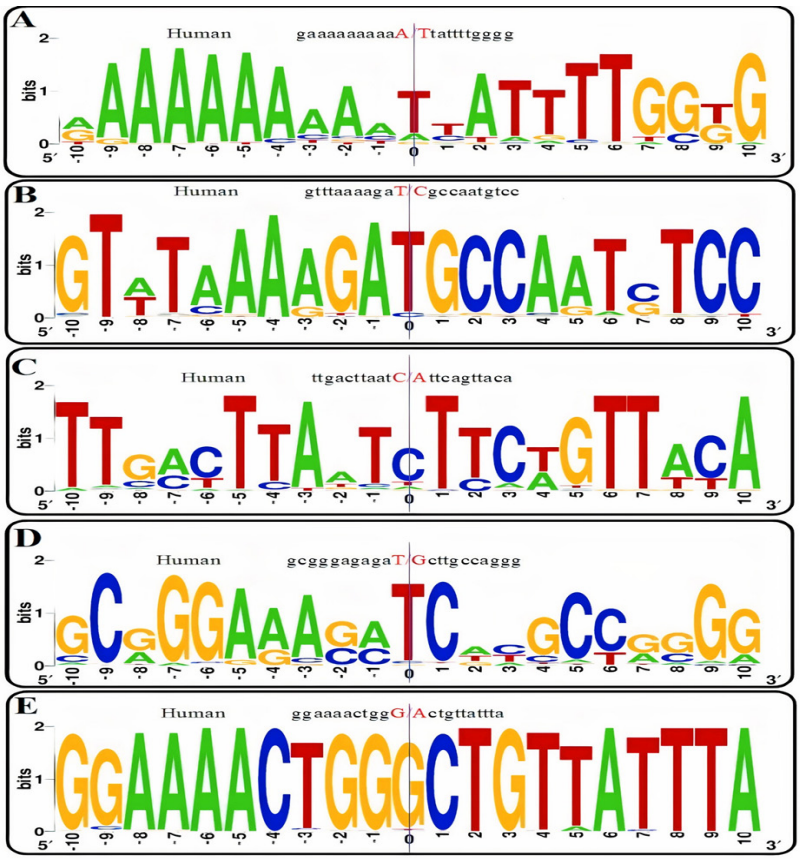

Figure 3. The Conservation of the DNA Sequences around Lin28B rs221634 (A), rs221635 (B), rs314276 (C), rs9404590 (D), and rs12194974 (E) SNPs locus; human DNA sequences around these locus is presented at the top. Blue vertical line indicates the positions of the variant locus SNPs in human and the conservation of wild allele across multiple mammalian species.

Table 3. Linkage Disequilibrium Analysis Lin28B rs221634, rs221635, rs314276, rs9404590 and rs12194974 SNPs.

rs9404590	rs12194974	rs314276	rs221634	rs221635	Case (%)	Control (%)	OR(95%CI)	p-value
T	G	C	A	T	68 (15.1)	87(19.3)	1 [reference]	
T	A	C	A	T	18 (4.0)	50 (11.1)	0.46 (0.25-0.86)	0.0142
G	G	C	T	T	46 (10.2)	15 (3.3)	3.92 (2.02-7.61)	0
G	G	C	A	T	36 (8.0)	23 (5.1)	2.00 (1.09-3.70)	0.0253
T	G	C	T	T	31 (6.8)	26 (5.7)	1.52 (0.83-2.81)	0.1747
T	G	A	A	T	19 (4.2)	33 (7.3)	0.74 (0.38-1.41)	0.3551
T	A	C	T	T	16 (3.5)	27 (6.0)	0.76 (0.38-1.52)	0.4353
T	A	A	A	T	7 (1.5)	32 (7.1)	0.28 (0.12-0.67)	0.003
T	G	C	A	C	25 (5.5)	14 (3.1)	2.28 (1.10-4.73)	0.0241
T	G	C	T	C	24 (5.3)	12 (2.6)	2.56 (1.19-5.48)	0.0139
T	A	C	A	C	15 (3.3)	17 (3.7)	1.13 (0.53-2.42)	0.7561
T	G	A	T	T	14 (3.1)	16 (3.5)	1.12 (0.51-2.45)	0.7784
G	G	C	A	C	18 (4.0)	7 (1.5)	3.29 (1.30-8.33)	0.0091
G	G	A	A	T	14 (3.1)	10 (2.2)	1.80 (0.75-4.28)	0.1869
T	G	A	A	C	11 (2.4)	12 (2.6)	1.17 (0.49-2.82)	0.7223
G	G	C	T	C	18 (4.0)	2 (0.4)	11.51 (2.58-51.34)	0.0001
G	A	C	A	T	8 (1.7)	10 (2.2)	1.02 (0.39-2.73)	0.9631
G	G	A	A	C	13 (2.8)	3 (0.6)	5.54 (1.52-20.24)	0.0044
T	A	A	A	C	4 (0.8)	10 (2.2)	0.51 (0.15-1.70)	0.2689
T	G	A	T	C	8 (1.7)	4 (0.8)	2.56 (0.74-8.85)	0.1277
G	A	A	A	C	4 (0.8)	5 (1.1)	1.02 (0.26-3.96)	0.9731
T	A	A	T	T	3 (0.6)	5 (1.1)	0.77 (0.18-3.32)	0.7238

polymorphism genotypes and non-small cell lung cancer [40].

Previous studies have shown that bioinformatics analysis can efficiently analyze the molecular aspects of genetic mutations [41, 42]. Because the Serine/arginine-rich family of splicing factors binds to exonic splicing enhancers (ESE), they can stimulate splicing efficiency and increase exon recognition and proper splicing. The binding of SR proteins to ESEs can be affected by nucleotide substitution, resulting in splicing mistakes and exon skipping or intron retention [43]. In the present study, the possible biological effects of *Lin28B* rs221634, rs221635, rs314276, rs9404590, and rs12194974 gene polymorphisms were investigated via a novel computational analysis. Bioinformatics investigation indicated that the SNPs rs314276, rs9404590, and rs12194974 might influence the *Lin28B* splicing pattern, which can alter the expression of *Lin28B*. It was also shown that the *Lin28B* rs221635, rs9404590, and rs12194974 loci correspond to a conserved area in mammals, indicating that these gene polymorphisms have related functions. There are some limitations in our study: (a) relatively small sample size (b) we have no data on some known risk factors such as family history, oral contraceptive or hormone therapy use, etc. (c) Some variants did not obey the Hardy-Weinberg equilibrium.

Author Contribution Statement

HS preparation of the first draft of the manuscript and statistical analysis, GHB and ST performed the experiments and analyzed the data, MRK and SMH supervision on data collection, validation of data source and contents, interpretation of data, DJ – statistical analysis, writing statistical components, MT concept and study design, supervision, methodology, formal analysis, review and editing final draft. All authors reviewed and approved the final manuscript.

Acknowledgements

This work was funded by a research grant (#8247) from Zahedan University of Medical sciences. The authors thank to the patients and healthy subjects who willingly participated in the study.

Funding/Support

This work was funded by a research grant from Zahedan University of Medical sciences.

Availability of data and materials

All data of the manuscript will be provided upon reasonable request and approval by the ethics committee.

Ethical Approval

The study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1396.52).

Informed Consent

All patients signed the informed consent form before participation.

Conflict of Interests

All authors declared that they have no conflict of interest.

References

1. Wu J, Eni A, Roussuri E, Ma B. Correlation between zbrk1/znf350 gene polymorphism and breast cancer. BMC Med Genomics. 2021;14(1):7. https://doi.org/10.1186/s12920-020-00862-2.
2. Huang J, Chan PS, Lok V, Chen X, Ding H, Jin Y, et al. Global incidence and mortality of breast cancer: A trend analysis. Aging (Albany NY). 2021;13(4):5748-803. https://doi.org/10.18632/aging.202502.
3. Kolahdoozan S, Sadjadi A, Radmard AR, Khademi H. Five common cancers in iran. Arch Iran Med. 2010;13(2):143-6.
4. Shamshirian A, Heydari K, Shams Z, Aref AR, Shamshirian D, Tamtaji OR, et al. Breast cancer risk factors in iran: A systematic review & meta-analysis. Horm Mol Biol Clin Investig. 2020;41(4). https://doi.org/doi:10.1515/hmbci-2020-0021.
5. Dugandzija T, Sekerija M, Hinic N, Rajcevic S, Kusturica MP. Trend analyses of breast cancer incidence and mortality in vojvodina. J BUON. 2020;25(2):655-61.
6. Wu B, Xing T, Li H, He W, Li B. Association between gene polymorphism of grk5 and breast cancer risk in chinese population. J BUON. 2021;26(3):741-6.
7. Lovnicki J, Gan Y, Feng T, Li Y, Xie N, Ho CH, et al. Lin28b promotes the development of neuroendocrine prostate cancer. J Clin Invest. 2020;130(10):5338-48. https://doi.org/10.1172/jci135373.
8. Cai WY, Wei TZ, Luo QC, Wu QW, Liu QF, Yang M, et al. The wnt-β-catenin pathway represses let-7 microrna expression through transactivation of lin28 to augment breast cancer stem cell expansion. J Cell Sci. 2013;126(Pt 13):2877-89. https://doi.org/10.1242/jcs.123810.
9. Yang X, Lin X, Zhong X, Kaur S, Li N, Liang S, et al. Double-negative feedback loop between reprogramming factor lin28 and microrna let-7 regulates aldehyde dehydrogenase 1-positive cancer stem cells. Cancer Res. 2010;70(22):9463-72. https://doi.org/10.1158/0008-5472.Can-10-2388.
10. Shrivastava G, Aljabali AA, Shahcheraghi SH, Lotfi M, Shastri MD, Shukla SD, et al. Targeting lin28: A new hope in prostate cancer theranostics. Future Oncol. 2021;17(29):3873-80. https://doi.org/10.2217/fon-2021-0247.
11. Chen Y, Xie C, Zheng X, Nie X, Wang Z, Liu H, et al. Lin28/let-7/pd-l1 pathway as a target for cancer immunotherapy. Cancer Immunol Res. 2019;7(3):487-97. https://doi.org/10.1158/2326-6066.Cir-18-0331.
12. Kang M, Lee KH, Lee HS, Jeong CW, Ku JH, Kim HH, et al. Concurrent treatment with simvastatin and nf-κb inhibitor in human castration-resistant prostate cancer cells exerts synergistic anti-cancer effects via control of the nf-κb/lin28/let-7 mirna signaling pathway. PLoS One. 2017;12(9):e0184644. https://doi.org/10.1371/journal.pone.0184644.
13. Qi M, Xia Y, Wu Y, Zhang Z, Wang X, Lu L, et al. Lin28b-high breast cancer cells promote immune suppression in the lung pre-metastatic niche via exosomes and support cancer progression. Nat Commun. 2022;13(1):897. https://doi.org/10.1038/s41467-022-28438-x.
14. Liu X, Lv Q, Jing Z, Long X, Yi R, Yang D, et al. Construction of a prognostic risk model of colorectal adenocarcinoma through integrated analysis of rna-binding

proteins. Transl Cancer Res. 2021;10(5):1962-74. https://doi.org/10.21037/tcr-21-40.
15. Ji W, Diao YL, Qiu YR, Ge J, Cao XC, Yu Y. Linc00665 promotes breast cancer progression through regulation of the mir-379-5p/lin28b axis. Cell Death Dis. 2020;11(1):16. https://doi.org/10.1038/s41419-019-2213-x.
16. Kugel S, Sebastián C, Fitamant J, Ross KN, Saha SK, Jain E, et al. Sirt6 suppresses pancreatic cancer through control of lin28b. Cell. 2016;165(6):1401-15. https://doi.org/10.1016/j.cell.2016.04.033.
17. Sarani H, Mollashahi B, Taheri M, Bahari G, Hashemi SM, Hashemi M, et al. Association between the il-1a, il-1b and il-1r polymorphisms and lymphoma. Nucleosides Nucleotides Nucleic Acids. 2021;40(7):707-19. https://doi.org/10.1080/15257770.2021.1929317.
18. He J, Yang T, Zhang R, Zhu J, Wang F, Zou Y, et al. Potentially functional polymorphisms in the lin28b gene contribute to neuroblastoma susceptibility in chinese children. J Cell Mol Med. 2016;20(8):1534-41. https://doi.org/10.1111/jcmm.12846.
19. Yang Z, Deng Y, Zhang K, Bai Y, Zhu J, Zhang J, et al. Lin28b gene polymorphisms modify hepatoblastoma susceptibility in chinese children. J Cancer. 2020;11(12):3512-8. https://doi.org/10.7150/jca.42798.
20. Ye Y, Madison B, Wu X, Rustgi AK. A lin28b polymorphism predicts for colon cancer survival. Cancer Biol Ther. 2012;13(14):1390-5. https://doi.org/10.4161/cbt.22336.
21. Permuth-Wey J, Kim D, Tsai YY, Lin HY, Chen YA, Barnholtz-Sloan J, et al. Lin28b polymorphisms influence susceptibility to epithelial ovarian cancer. Cancer Res. 2011;71(11):3896-903. https://doi.org/10.1158/0008-5472.Can-10-4167.
22. Fu W, Liu GC, Zhao Z, Zhu J, Jia W, Zhu SB, et al. The correlation between lin28b gene potentially functional variants and wilms tumor susceptibility in chinese children. J Clin Lab Anal. 2018;32(1):e22200. https://doi.org/10.1002/jcla.22200.
23. Venables JP. Aberrant and alternative splicing in cancer. Cancer Res. 2004;64(21):7647-54. https://doi.org/10.1158/0008-5472.Can-04-1910.
24. Zhang Y, Qian J, Gu C, Yang Y. Alternative splicing and cancer: A systematic review. Signal Transduct Target Ther. 2021;6(1):78. https://doi.org/10.1038/s41392-021-00486-7.
25. Agrawal AA, Yu L, Smith PG, Buonamici S. Targeting splicing abnormalities in cancer. Curr Opin Genet Dev. 2018;48:67-74. https://doi.org/10.1016/j.gde.2017.10.010.
26. Hashemi M, Aminiinia S, Ebrahimi M, Hashemi SM, Taheri M, Ghavami S. Association between htert polymorphisms and the risk of breast cancer in a sample of southeast iranian population. BMC Res Notes. 2014;7(1):895. https://doi.org/10.1186/1756-0500-7-895.
27. Sabarinathan R, Tafer H, Seemann SE, Hofacker IL, Stadler PF, Gorodkin J. The rnasnp web server: Predicting snp effects on local rna secondary structure. Nucleic Acids Res. 2013;41(Web Server issue):W475-9. https://doi.org/10.1093/nar/gkt291.
28. Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human splicing finder: An online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009;37(9):e67. https://doi.org/10.1093/nar/gkp215.
29. Piva F, Giulietti M, Burini AB, Principato G. Spliceaid 2: A database of human splicing factors expression data and rna target motifs. Hum Mutat. 2012;33(1):81-5. https://doi.org/10.1002/humu.21609.
30. Speir ML, Zweig AS, Rosenbloom KR, Raney BJ, Paten B, Nejad P, et al. The ucsc genome browser database: 2016 update. Nucleic Acids Res. 2016;44(D1):D717-D25. https://doi.org/10.1093/nar/gkv1275.
31. Crooks GE, Hon G, Chandonia JM, Brenner SE. Weblogo: A sequence logo generator. Genome Res. 2004;14(6):1188-90. https://doi.org/10.1101/gr.849004.
32. Guo Y, Chen Y, Ito H, Watanabe A, Ge X, Kodama T, et al. Identification and characterization of lin-28 homolog b (lin28b) in human hepatocellular carcinoma. Gene. 2006;384:51-61. https://doi.org/10.1016/j.gene.2006.07.011.
33. Ustianenko D, Chiu HS, Treiber T, Weyn-Vanhentenryck SM, Treiber N, Meister G, et al. Lin28 selectively modulates a subclass of let-7 micrornas. Mol Cell. 2018;71(2):271-83.e5. https://doi.org/10.1016/j.molcel.2018.06.029.
34. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and hmga2 enhances oncogenic transformation. Science. 2007;315(5818):1576-9. https://doi.org/10.1126/science.1137999.
35. Zhang Y, Sun Y, Ding L, Shi W, Ding K, Zhu Y. Long non-coding rna linc00467 correlates to poor prognosis and aggressiveness of breast cancer. Front Oncol. 2021;11:643394-. https://doi.org/10.3389/fonc.2021.643394.
36. Nadiminty N, Tummala R, Lou W, Zhu Y, Shi XB, Zou JX, et al. Microrna let-7c is downregulated in prostate cancer and suppresses prostate cancer growth. PLoS One. 2012;7(3):e32832. https://doi.org/10.1371/journal.pone.0032832.
37. He Y, Wang H, Yan M, Yang X, Shen R, Ni X, et al. High lin28a and plk4 co-expression is associated with poor prognosis in epithelial ovarian cancer. Mol Med Rep. 2018;18(6):5327-36. https://doi.org/10.3892/mmr.2018.9562.
38. Balzeau J, Menezes MR, Cao S, Hagan JP. The lin28/let-7 pathway in cancer. Front Genet. 2017;8:31-. https://doi.org/10.3389/fgene.2017.00031.
39. Lu L, Katsaros D, Mayne ST, Risch HA, Benedetto C, Canuto EM, et al. Functional study of risk loci of stem cell-associated gene lin-28b and associations with disease survival outcomes in epithelial ovarian cancer. Carcinogenesis. 2012;33(11):2119-25. https://doi.org/10.1093/carcin/bgs243.
40. Wen J, Liu H, Wang Q, Liu Z, Li Y, Xiong H, et al. Genetic variants of the lin28b gene predict severe radiation pneumonitis in patients with non-small cell lung cancer treated with definitive radiation therapy. Eur J Cancer. 2014;50(10):1706-16. https://doi.org/10.1016/j.ejca.2014.03.008.
41. Teimoori B, Yazdi A, Rezaei M, Mohammadpour-Gharehbagh A, Jahantigh D, Salimi S. The association of the placental caspase-3 gene polymorphisms and preeclampsia susceptibility and in-silico analysis. J Cell Biochem. 2018;119(8):6756-64. https://doi.org/10.1002/jcb.26869.
42. Jahantigh D, Forghani F, Zidanloo SG. Interleukin-23 receptor (il-23r) gene polymorphisms and haplotypes associated with the risk of preeclampsia: Evidence from cross-sectional and in silico studies. J Assist Reprod Genet. 2019;36(7):1523-36. https://doi.org/10.1007/s10815-019-01479-w.
43. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: Exonic mutations that affect splicing. Nat Rev Genet. 2002;3(4):285-98. https://doi.org/10.1038/nrg775.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.