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WRAP53 Polymorphism, rs2287498: A Case Study in Northwest of Iran?

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Abstract

Background: Non-coding RNAs apply regulations on expression or function of a gene. A class of non-coding RNAs, natural antisense transcripts, might overlap with their flanking genes and emerge a new complexity upon regulation. WRAP53, is a natural antisense transcript overlapped in a head-to-head manner on the opposite strand of TP53. It has 3 transcripts of which WRAP53β produces a protein and is needed for RNP biogenesis. Single nucleotide polymorphisms in this gene are associated with cancer susceptibility.

Methods: In this study, we investigated the impact of WRAP53 Ex2+19 C>T polymorphism (rs2287498) in breast cancer susceptibility in Iranian-Azeri women, by tetra-primer amplification-refractory mutation system-polymerase chain reaction (tetra-ARMS PCR) method, in 222 patients women with breast cancer. We analyzed our data using javastat statistics package (http://statpages.org/ctab2x2.html) online software for allele and genotype frequencies and SPSS v.24 for evaluating rs2287498 association with clinicopathological features. Also, in silico experiments were carried out for predicting the second RNA structure with mfold v3.6, and for prediction of amino acid substitution effect with online software polyphen2.

Results: Our results show a statistical significance of tumor size with the risk of breast cancer (p-value=0.036) but no significant genotype frequencies of rs2297498 and clinicopathological features with breast cancer susceptibility. in silico analysis estimated no significant changes in RNA or protein for this polymorphism.

Conclusion: In conclusion, these findings suggest no relationship between rs2287498 and breast cancer susceptibility except with tumor size which confers a possible implication as a prognostic marker in relation to the size of the tumor.

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Introduction

Most of the genome consists of non-coding regions and with a 200-nt threshold can divide into short and long ncRNAs (1). From this category, lncRNAs have emerged new insights into the gene regulations. LncRNAs are categorized into sense, antisense, bidirectional, intronic and intergenic RNAs according to their relative positions from their nearby protein-coding or non-protein coding genes (2). Besides, they regulate gene expression through cis/trans acting mechanism (3). An important subclass of lncRNAs is natural antisense transcripts (NATs) which are complementary to or overlap with flanking sequence of their neighbor genes (2). Even though there have

been wide and deep investigations into gene regulations applied by ncRNAs, the exact mechanism by which these transcripts are exerted is not elucidated yet, but three possible mechanisms have been proposed; decoy mechanism, tethering mechanism, and generation of endogenous siRNA and miRNA (4).

The first exon of tumor suppressor gene, *TP53*, is overlapped by its flanking cis-acting gene, *WRAP53*, in a head to head fashion on the opposite strand (5, 6). The existence of the NAT for *TP53* provides a more complex regulatory pathway by which *TP53* expression and its protein level are controlled (7). WD40-encoding RNA antisense to p53, *WRAP53* (also known as *TCAB1* or *WDR79*), is located on chromosome 17p13.1 and has three alternative start exons which result in three different transcripts (8). *WRAP53*α, which is expressed upon exon 1α usage, is overlapped by 227bp of *TP53* first exon. Also, *WRAP53*β, which is transcribed by exon 1β, does not overlap with p53 and produces a protein-coding RNA. There is still no specific role and function elucidated for *WRAP53*ϒ to have an impact on cell destination (9).

RNP biogenesis (10), telomerase and survival of motor neuron (SMN) trafficking to the cajal bodies (CBs) and telomeres (8, 11) require WRAP53 protein, which is also known as TCAB1 or WDR79. These scaRNAs have a conserved element, C/D or H/ACA CAB box in which WRAP53 protein recognizes and binds (12-14). Upon DNA damage, WRAP53β is needed for p53-dependent apoptosis induction (15). In a DNA damage site, TCAB1 accumulates in an ATM, H2AX and MDC1 dependent manner and by directing ubiquitin ligase RNF8 to the breaks (DSBs), repairs DNA breaks through homologous recombination (HR) and non-homologous end joining (NHEJ) pathways (16-18).

The role of this protein overexpression and single nucleotide polymorphisms (SNPs) in nasopharyngeal carcinoma (19), non-small cell lung cancer (NSCLC) (20, 21), esophageal squamous cell carcinoma (22), head and neck carcinoma (23) and dyskeratosis congenital by TCAB1 mutations (24) has been elucidated.

In this study, we have investigated the role of *WRAP53* Ex2+19 C>T polymorphism (rs2287498) in breast cancer susceptibility in Iranian-Azeri women.

Material and Methods

We investigated 222 women with breast cancer and 202 healthy women without any cancer history in their family as controls. We used the salting-out method for DNA extraction from tumor tissues of patients and the peripheral blood of controls. Informed consent was granted from all participants. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences research center

The tetra-primer Amplification Refractory Mutation System (tetra-ARMS) was used for this purpose. Four primers were designed with online software batchprimer3. The outer primers amplify a 368bp DNA target, forward inner primer amplifies the "C" allele and reverse inner primer amplifies the "T" allele, which generate 226 and 196 amplicons, respectively. Their sequences are listed in Table 1. The PCR-amplification was carried out in a total volume of 20µl reaction mixture including 3.13µl PCR buffer (10x), 1.2 µl $MgCl_2$ (50 µM), 1 µl of each outer primers (1 µM), 0.3 µl of each inner primers, 0.62 µl dNTPs (10 µM), 12.22 µl sterile distilled H_2O, 0.23 µl Taq DNA polymerase (5 unit/µl) and 1 µl template DNA (20-25 ng), all purchased from Takapouzist, Iran.

Table 1. Primer sequences for rs2287498 amplification

Name	Sequence	Amplicon size (bp)	SNP
Forward-outer	5′AGACACGTAAGTGGTGATGGCAGTGGAG3′	368	-
Reverse-outer	5′TCTAAAAGGGCTACGTGCCGCTTCTCCC3′		
Forward-inner	5′CCTTTCAGCGCTTGGAACTACAGCGTC3′	226	C
Reverse-inner	5′ACCACTGAGAAATCGAGGCAGCTGGTAA3′	196	T

PCR was conducted in a thermal cycler (Sensoquest, GmbH, Germany) with the following cycling program: an initial denaturation step for 10 minutes at 95˚c, 30 seconds at 95˚c for denaturation, 30 seconds at 58˚c for primer annealing, 35 seconds at 72˚c for extension and also a final extension for 10 min at 72˚c, with the number of 40 cycles in PCR program. The amplification products and a 50-bp DNA ladder, as a molecular size marker, were loaded on 2% agarose gel and electrophoresis was conducted for 30 minutes at 100 V/cm. After genotyping, the sequences were confirmed by Sanger sequencing method using forward primer.

For allele and genotype frequencies, we used javastat statistics package online software (25). To investigate the relationship of rs2287498 with breast cancer risk and genotype frequencies between cases and controls as well as evaluating rs2287498 association with clinicopathological features such as age, side of breast involvement, tumor size, stage of cancer and histologic subtypes, Pearson's χ^2 test and Fisher's exact test were applied accordingly. SPSS software version 24 was used for data analysis. $P<0.05$ was considered as statistically significant.

To further investigate the polymorphism effect, *in silico* analysis was conducted. For predicting the second RNA structure, mfold v3.6 software was used and for estimating the effect of amino acid substitution, we used the online software PolyPhen-2.

Results

The distinct pattern of the target sequences on the gel (Figure 1) was confirmed by direct sequencing (Figure 2). Genotype frequencies of patients were 26.1% CT, and 73.9% CC. Frequencies of C and T alleles were 86.93 and 13.06, respectively. In healthy women, genotype frequencies were 23.3% CT and 76.7% CC and C and T frequencies were 88.36% and 11.63%, respectively (Table 2). These data were in accordance with Minor Allele Frequency (MAF) reported by ExAC. Noticeably, we observed no TT homozygote sample, which might be related to the genetic diversity of our assessed population.

Table 2. Genotype and allelic frequencies in patients and controls

Genotype	Patients % (n=222)	Controls % (n=202)	p-value	OR (CI=95%)
CC	73.9	76.7	0.496	0.537
CT	26.1	23.3	0.496	0.731
TT	-	-	-	-
Alleles				
C	86.93	88.36	0.759	0.348
T	13.06	11.63		

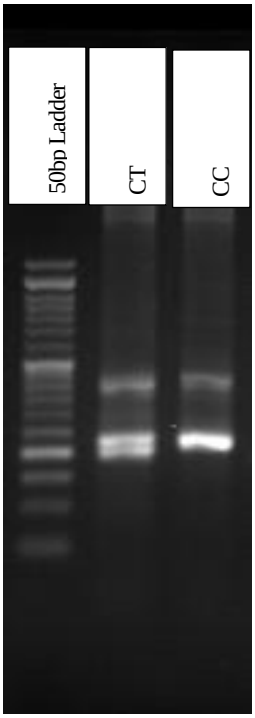

Figure 1. Tetra-ARMS PCR bands on the 2% agarose gel. The right well is loaded with CC (homozygote) sample with the exact size of 226bp. The middle well is loaded with CT (heterozygote) sample with the exact size of "C" and "T" alleles with 226 and 196bp, respectively. The left well is loaded with 50bp DNA ladder.

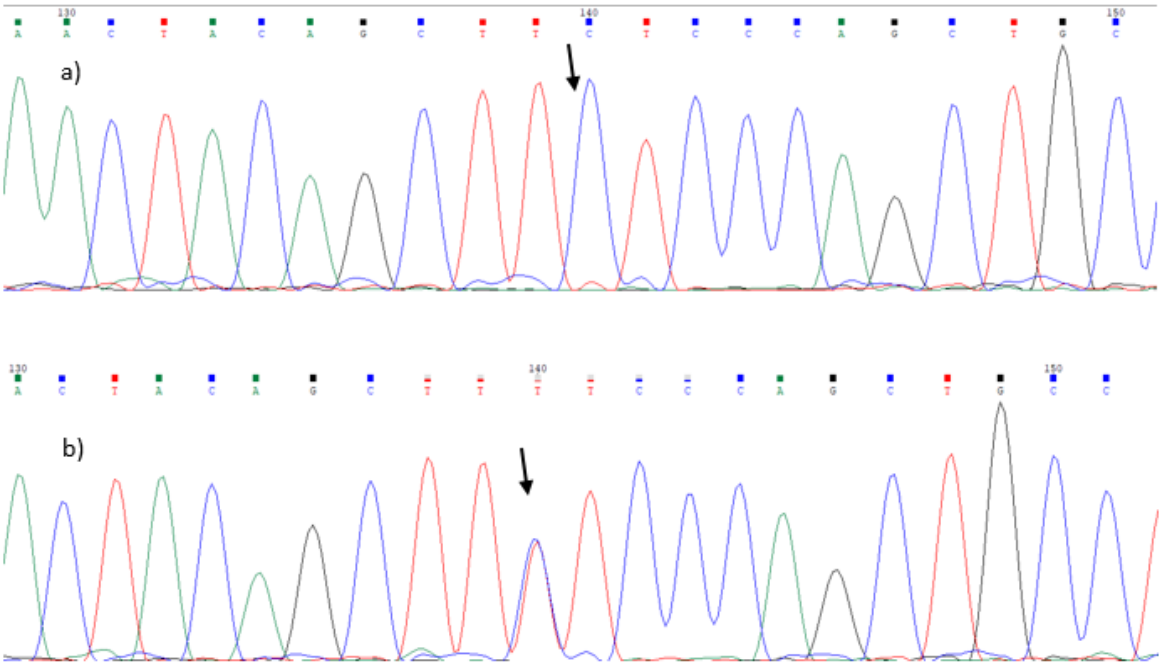

Figure 2. Sanger sequences of a) CC and b) CT genotypes. The SNPs are indicated by black arrows.

Correlation between genotypes and clinicopathological properties of patients (Table 3) revealed no statistically significant results. Whereas, there was a significant frequency distribution of tumor sizes bigger than 3cm (p-value=0.036).

Altogether, these findings suggest no relation between rs2287498 with breast cancer susceptibility in Iranian-Azeri population, but there was a relationship between SNP with tumor size which confers a possible application as a prognostic marker.

In silico analysis with mfold revealed an estimation delta-G of -22.90 for T allele and -22.50 for C allele and showed no significant changes for this substitution (Figure 3). The polyphen 2 showed an estimation of healthy protein with F150F substitution.

Table 3. Correlation between genotypes and clinicopathological properties of patients

	C/C	C/T	p-value
Histological subtype			
DCIS	7	0	0.093
Fibroadenoma	4	2	
IDC	126	51	
ILC	8	0	
Tumor stage			
I to IIA	64	17	0.806
IIB to IV	82	35	
Age			
=<40	48	111	0.785
>40	18	38	
Side of involvement			
L	83	26	0.840
R	75	30	
Tumor size			
>3cm	66	30	0.036
<=3cm	69	15	

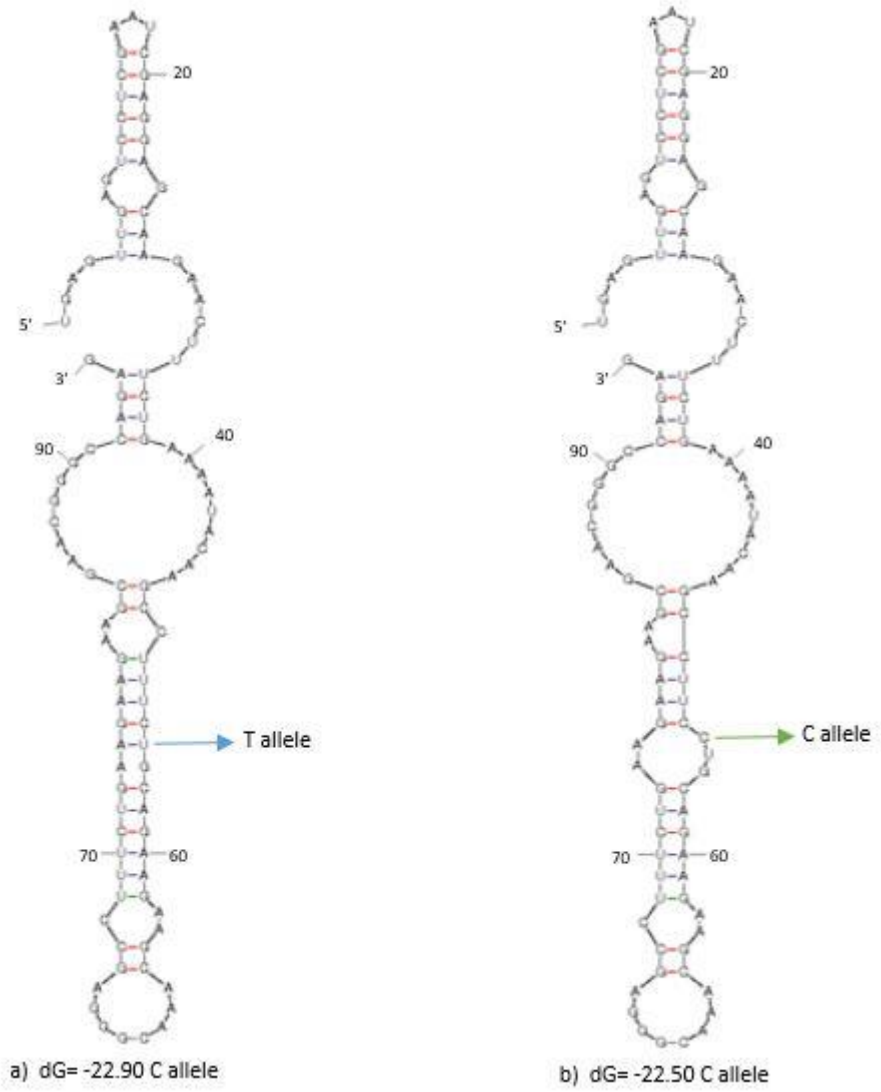

Figure 3. Second RNA structure of WRAP53 polymorphism with a) T allele (indicated by blue arrow) and b) C allele (indicated by green arrow).

Discussion

WRAP53 SNPs may affect the protein structure and stability and help the malignancy to progress and develop metastasis (26). Sedaie Bonab et al. evaluated the possibility of the association of rs2287499 with breast cancer susceptibility in Iranian-Azeri population and their results showed no significant association (27). Some studies have been conducted to investigate the impact of rs2287498 on the risk of diseases and cancers (24, 28). Lv et al. conducted a study on 96 men with laryngeal cancer and did not find an association between rs2287498 and laryngeal cancer occurrence (29) which is in line with our results.

Huang et al. reported a 46% increased risk of male infertility in rs2287498 TT homozygotes (30). In 626 cases of ovarian cancer and 1045 controls in Polish population, the TT genotype of rs2287498 was associated with ovarian cancer

susceptibility (31). In the case of breast cancer, Garcia-Closas et al. investigated Norwegian and Polish populations and showed a significant association of rs2287498 with breast cancer risk (32). This finding is not in agreement with our result, but we showed a statistically significant association between rs2287498 and tumor size (p-value=0.036) which might be helpful as a prognostic marker for patients harboring breast cancer.

Also, *in silico* studies have been conducted for this SNP. F150F substitution caused no harmful effects on *WRAP53* and 100% healthy protein has been predicted by Polyphen2 prediction. We also estimated the effects of SNP on secondary RNA structure by mfold software v3.6, which revealed very slight, not significant changes by different alleles with dG of -22.90 and -22.50 for T and C alleles, respectively. So, no differences in RNA stability was revealed.

Discussion

There is an urgent need to find a specific and sensitive prognostic and predictive marker for breast cancer. In this regard, we need further studies to get comprehensive insights into the probable causes of breast cancer and to design a suitable therapeutic agent.

In conclusion, this study did not report any association of breast cancer susceptibility with rs2287498 single nucleotide polymorphism, but showed a relation between the variant and the size of the tumor in Iranian-Azeri women.

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Conflict of interest

The authors have no conflicts of interest to declare.

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