RESEARCH ARTICLE

# Association of rs1219648 in *FGFR2* and rs1042522 in *TP53* with Premenopausal Breast Cancer in an Iranian Azeri Population

**Zahra Saadatian[1], Jalal Gharesouran[2], Morteza Ghojazadeh[2], Sahar Ghohari-Lasaki[2], Najime Tarkesh-Esfahani[3], Seyyed Mojtaba Mohaddes Ardebili[1]**

## Abstract

**Breast cancer is the most common cancer among women in the world. In Iran, the incidence of breast cancer is on the increase. We here studied the association of rs1219648 in *FGFR2* and rs1042522 in *TP53* and their interaction in development of early onset sporadic breast cancer in Iranian Azeri population to evaluate epistatic effects on the risk of mammary neoplasia. We genotyped the two polymorphisms in 100 women with early onset breast cancer and 100 healthy women by PCR-RFLP. Allele frequency differences were tested using chi$^2$-test with 95% confident intervals. Our results indicated a statistically significant association ($p<0.05$) between rs1219648, but not rs1042522, and risk of breast cancer. We also found that the combination of *FGFR2* major genotype and *TP53* hetero genotype had protective effects against breast cancer , while the hetero allele of *FGFR2* in combination with the minor genotype of *TP53* was associated with a high risk. This study revealed an important crosstalk between two polymorphisms in *FGFR2* and *TP53* in development of breast cancer. These candidates risk variants should be further evaluated in studies with a larger sample size.**

**Keywords:** Early onset breast cancer - *FGFR2* - *TP53* - Single nucleotide polymorphism

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## Introduction

Breast cancer is one of the most common diseases among women in the world with an incidence of more than 1000000 and death rate of 410000 in 2012 (Zhang et al., 2013). In Iran, it is also the most common cancer among women (Rahimzadeh et al., 2014). A number factors such as menopausal states and genetic variants can extend the risk of malignancy (Rai, 2014). Although breast cancer is frequent in postmenopausal women, the presentation of disease is high in premenopausal in undeveloped countries (Rai, 2014).

The role of genetic factors in development of breast cancer is well-documented (Han et al., 2011). Genome-wide association studies have highlighted the role of single nucleotide polymorphisms in non-hereditary breast cancer susceptibility (Marian et al., 2011). As a result, fibroblast growth factor receptor 2 (*FGFR2*), a gene involved in mammary gland development, has been recognized as a prominent candidate in sporadic breast cancer (Sun et al., 2010; Tarkkonen et al., 2012). *FGFR2* codes a transmembrane-type receptor involved in cellular functions (Cherdyntseva et al., 2012). Five single nucleotide polymorphisms (SNP) of *FGFR2* gene are associated with breast cancer (Katoh, 2008). Among other SNPs, the role of rs1219648 (IVS2±7033A>G) in postmenopausal breast cancer is more remarkable (Hunter et al., 2007; Zhang et al., 2010a). Despite this clear evidence some researches stress on the effect of this polymorphism on premenopausal breast cancer (Fu et al., 2012). Genetic alterations of *FGFR2* cause aberrant activation of *FGFR2* signaling in breast cancer (Katoh, 2008). Active *FGFR2* mutants promote DNA-damage signaling and p53-dependent senescence (Ota et al., 2009). In addition it is proven that *FGFR2* is sufficient to protect the cells from apoptosis (4) and apoptosis activation can induce by interaction between *FGFR2* and *TP53* (Ota et al., 2009). *FGFR2* signaling increases the cytoplasmic level of mdm2 which can stop p53-dependent apoptosis. So *FGFR2* has epistatic effect on p53 (Shaulian et al., 1997; Hosokawa et al., 1998). *TP53* gene encodes an antiancogenic homotetrameric protein that acts in control of cell cycle, DNA damage repairing and cell apoptosis (Denisov et al., 2012; Pouladi et al., 2014). rs1042522 (Ex4±119C>G) is the most significant polymorphism, due to its function in the transactivation of the pro-apoptotic target genes of the p53 protein (Li et al., 2005; Khan et al., 2014). After that, the p53 P47S SNP may influence the risk and progression of cancer and the efficiency of therapy differently, depending on

*[1]International Branch of Tabriz University of Medical Sciences (Aras), [2]Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, [3]Department of Biostatistics, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran *For correspondence: mohaddesmo@yahoo.com*

ethnic components (Al-Qasem et al., 2012). Namely, *TP53* rs1042522 polymorphism inspires a protective effect to Mediterranean inhabitants against breast cancer (Hu et al., 2010), but has a predisposing effect in Indians (He et al., 2011). In addition, crosstalk between the *FGFR2* rs1219648 and *TP53* rs1042522 in sporadic breast cancer has been demonstrated in Russian population by Cherdyntseva et.al (2012).

Based on above observations we decided to investigate the association of *FGFR2* rs1219648 and *TP53* rs1042522 polymorphisms with sporadic breast cancer in an Iranian Azeri population. Moreover, we estimated the cooperation of these polymorphisms for breast cancer modifications in both case and control groups.

## Materials and Methods

*Samples*

In this case-control association study, samples included 100 women under 50, with the diagnosis of breast cancer and mean age of 39.32 years old, and 100 healthy women at same age range. All cases were without family history of breast cancer and were in premenopausal status and most of them have histological grade 2 tumors.

*DNA extraction and PCR*

Restriction fragment length polymorphism (RFLP) was our technique in this study. DNA extraction was carried out from blood lymphocytes using salting out protocol with proteinase k (Garner, 2000). The primer set used for rs1219648 amplification was: forward 5'-CACGCCTATTTTACTTGACACGC-3' and reverse, 5'-ATTTGTATGTGGTAGCTGACTTC-3'. Polymerase chain reaction was performed for this polymorphism with the following program: 94˚C for 5min, 94˚C for 30 seconds, 58˚C for 30 sec, 72˚C for 30 sec, 72˚C for 10 sec. The resulting 133bp bond was treated by APAF I and agarose gel (3%) separated 109bp and 133bp fragments. The 24bp digestive fragment was invisible in agarose gel.

The primer set employed for amplification of segment encompassing rs1042522 was 5'-CTGGTAAGGACAAGGGTTGG-3' as forward and 5'-ACTGACCGTGCAAGTCACAG-3' as reverse. A 396bp fragment was amplified and treated with BstuI restriction enzyme, producing two shorter fragments (231 and 165 bp long) on agarose gel (1%). Each PCR reaction was accomplished in a total volume of 15µl containing 4ng genomic DNA, 6.25 mastermix red, 2.25 water and 1.25 from each primer.

*Statistical analysis*

The Chi-square test with 95% confidence intervals was performed using SPSS v.17 software. p-value of less than 0.05 was considered as significant.

## Results

*Single locus frequencies*

The distribution of *TP53* rs1042522 and *FGFR2* rs1219648 genotypes were in agreement with HW equibilirium (p>0.05).

As shown in Table 1, there was a remarkable association between rs1219648 and breast carcinogenesis (p<0.05) and according to table 2 the G allele of this polymorphism was strongly pronounced in breast cancer patients (p<0.05) (OR=1.78 [95%CI: 1.174-2.699], p=0.006). Extraordinarily and in contrast with previous investigation we didn't observe any association between rs1042522 polymorphism and breast cancer liability (p>0.05). However there was an association between heterozygote genotype and disease (OR=0.450 [95%CI: 0.206-0.984], p=0.043).

*Genotype combination frequencies*

Rs1219648 and rs1042522 genotype incorporation handing out is presented in table 3. The combination of rs1219648 AA genotype and rs1042522 GC genotype was protecting from breast cancer (OR=0.512 [95%CI: 0.263-0.997], p=0.047) with the significant enlargement in healthy controls compared to the patients group. Also correlation of *FGFR2* major allele with P53 minor allele presented the similar result powerfully (OR=0.279 [95%CI: 0.088-0.887], p=0.022). In contrast the *FGFR2* AG in combination with *TP53* GG±GC genotypes deviated from association. In spite of that, the significant enhancement in the proportion of rs1219648 AG genotype

**Table 1. *FGFR2* rs1219648 are associated with breast cancer but *TP53* rs1042522 are Not**

| | | Case | Control | p |
|---|---|---|---|---|
| *FGFR2* | Rs1219684 | 100 | 100 | 0.025 |
| Tp53 | Rs1042522 | 100 | 100 | 0.082 |

**Table 2. *FGFR2* and *TP53* Genotype Distribution in Breast Cancer Cases and Controls**

| | Case | Control | p | OR(95%) |
|---|---|---|---|---|
| Rs1219648 allele | | | | |
| A | 117 | 143 | | |
| G | 83 | 57 | 0.006 | 1.78 (1.174-2.699) |
| genotype | | | | |
| AA | 34 | 52 | 1 | |
| AG | 49 | 39 | 0.033 | 1.922 (1.051-3.512) |
| GG | 17 | 9 | 0.02 | 2.889 (1.156-7.222) |
| Rs1042522 allele | | | | |
| G | 92 | 89 | | |
| C | 108 | 111 | 0.763 | 0.941(0.635-1.396) |
| genotype | | | | |
| GG | 22 | 13 | 1 | |
| GC | 48 | 63 | 0.043 | 0.45(0.206-0.984) |
| CC | 30 | 24 | 0.495 | 0.739 (0.309-1.764) |

**Table 3. *FGFR2* and *TP53* Genotype Combination Distribution in Breast Cancer Cases and Controls**

| | Case | Control | p | OR(95%CI) |
|---|---|---|---|---|
| Rs1219648 and rs1042522 | | | | |
| AA and GG | 12 | 9 | 0.489 | |
| AA and GC | 18 | 30 | 0.047 | 0.512(0.263-0.997) |
| AA and CC | 4 | 13 | 0.022 | 0.279(0.088-0.887) |
| AG and GG | 5 | 3 | 0.47 | |
| AG and GC | 24 | 30 | 0.339 | |
| AG and CC | 20 | 6 | 0.003 | 3.917(1.5-10.227) |
| GG and GG | 5 | 1 | 0.097 | |
| GG and GC | 6 | 3 | 0.306 | |
| GG and CC | 6 | 5 | 0.756 | |

combined with CC genotype of rs1042522 was shown in disease caring women compared with control subjects, proposing the importance of this combination in creation of breast tumors (OR=3.917 [95%CI:1.5-10.227], p=0.003). Other genotypes combination didn't show any association with disease.

## Discussion

Breast cancer is one of the most common disorders in women worldwide (Long et al., 2013). Along with multifactorial, genetic background plays a well-established role in cancer etiology (Ayoub et al., 2011). Although a bulk of researches have revealed the role of genetic factors in breast cancer, currently the most of molecular basis of breast carcinogenesis remains unrecognized and conceivably tumor development can be as a result of corporation of genetic variants (Singh et al., 2008).

In the current study we investigated the association of rs1219648 and rs1042522 with breast malignancies separately; we evaluated the risk of early onset breast cancer in cooperation of these variants. Interestingly there was not any noticeable differences in distribution of allele frequencies of rs1042522 or *TP53* codon 72 polymorphism in both case and control groups. However a considerable association was noticed about rs1219648 polymorphism.

Many authors have reported a significant association between rs1219648 and postmenopausal breast cancer (Liang et al., 2008; Raskin et al., 2008; Prentice et al., 2009; Jia et al., 2010). In a genome wide association study, Hunter and his colleagues found a significant association between rs1219648 and postmenopausal breast cancer ($p<0.01$) too (Hunter et al., 2007). However, limited researches have been done on the impact of rs1219648 on early onset breast cancer. Although the recent study by Chun-Lian Liu and coworkers has been refused the impact of rs1219648 in premenopausal breast cancer (Liu et al., 2013), in another investigation carried out on Chines Han woman a great association appeared between rs1219648 and premenopausal breast cancer (Fu et al., 2012). In our study the association between the G allele of rs1219648 *FGFR2* and early onset breast cancer is in agreement with the aforementioned study (p=0.006). In addition, it is most likely that G allele carriers may display a high breast cancer risk and this observation asserts that the role of G allele is considerable in predisposing malignancy than protective effect of A allele in heterozygote's. In contrast to many studies (Papadakis et al., 2000; Buyru et al., 2003; Zhuo et al., 2009; Zhang et al., 2010b), we did not observe any association between the *TP53* codon 72 polymorphism and breast cancer risk. In Cherdyntseva et al work the C allele of the *TP53* gene was significantly linked with an increased cancer risk among young Russian women (Cherdyntseva et al., 2012). According to Abeer Al-Qasem et al the G allele increases the risk of breast cancer (Al-Qasem et al., 2012). Doosti and his colleagues have also confirmed this finding (Doosti et al., 2011). Unlike aforementioned studies, some investigations confirm us (Suspitsin et al., 2003). For instance, Khadang et al didn't observe a significant association between polymorphic alleles of rs1042522 and breast cancer liability (Khadang et al., 2007).

Gene-gene interaction or epistasis is considered as an indispensable component of the genetic factors in multifactorial diseases (Turner and Bush, 2011). Not long past investigation revealed the contribution of cancer-related *FGFR2* mutants with p53 in the installation of DNA-damage signaling and senescence in primary human cells (Ota et al., 2009). Furthermore *FGFR2* activated by bfgf ligand multiply amounts of mdm2 and causes obstruction of p53 dependent apoptosis (Shaulian et al., 1997). In the current study we found *FGFR2* and *TP53* gene variants corporations in breast cancer development. A similar study had been done by Cherdyntseva et al. in Russian population (Cherdyntseva et al., 2012). Their results showed the association of *FGFR2* AG and *TP53* rs1042522 GC±CC genotypes with premenopausal breast cancer liability (OR=2.04[95%CI: 1.14-3.63], p=0.015). Furthermore their study showed the protective effect of the major allele of *FGFR2* in combination with major allele of P53 (OR=0.28[95%CI: 0.13-0.63], p=0.003). However this effect was not seen in our population; we observed the AG genotype of rs1219648 in combination with CC genotype of rs1042522 *TP53* significantly elevates the risk of breast cancer (OR=30917[95%CI: 1.5-10.227], p=0.003), whereas AA genotype of *FGFR2* with GC±CC genotype of *TP53* have a protective effect on cancer development (OR=0.512[95%CI: 0.263-0.997], p=0.047) and (OR=0.279[95%CI: 0.088-0.887], p=0.022).

Regrettably, the number of samples was low in this study and the clinical characteristics of some patients were out of reach. Thereupon we couldn't scrutiny these polymorphisms in relation to some clinical characteristics and this could explain the differentiation between current study and previous studies.

In conclusion, findings of the present study have shown the concerning role of G allele of *FGFR2* rs1219648 in early onset sporadic breast cancer susceptibility in Iranian Azeri population. Whereas the rs1042522 polymorphism has no impact on breast cancer in this population, there is a significant association between *TP53* and *FGFR2* polymorphisms genotype combinations.

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