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

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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 Chi²-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

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). Genomewide 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 (Cherdynstseva 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

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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).

**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’-CTTGTTAAGGACACAGGTTG-3’ and reverse, 5’-ATCTGACGTAAGCTGACTTC-3’. Polymerase chain reaction was performed for this polymorphism with the following program: 94˚C for 5min, 94˚C for 30 sec, 98˚C for 30 sec, 72˚C for 30 sec, 72˚C for 10 sec. The resulting 133bp bond was treated by APAF I and second the resulting 133bp bond was treated by APAF I 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.

**Results**

**Single locus frequencies**

The distribution of TP53 rs1042522 and FGFR2 rs1219648 genotypes were in agreement with HW equilibrium (p>0.05).
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 (Susplisin 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 indispensible 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 installment 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 (Shaullian 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 scrutinize 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 early onset sporadic breast cancer susceptibility in Iranian Azeri population. Whereas the rs1042522 GC±CC genotypes have a protective effect on cancer among Saudi women. The p53 codon 72 polymorphism is associated with risk and early onset of breast cancer among Saudi women. Oncol Lett, 3, 875-8.

References


