Original Article Open Access

Archives Of Breast Cancer

DOI: 10.19187/abc.20174124-27

The Relationship Between Polymorphic Fibroblast Growth Factor Receptor (FGFR) Gene and Breast Cancer Risk

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ARTICLE INFO

Received:
04 November 2016
Revised:
05 February 2017
Accepted:
25 February 2017

Keywords:
BstNI,
PCR-RFLP,
FGFR4 gene,
polymorphism,
breast cancer

ABSTRACT

Background: Genetic factors associated with cancer have been widely investigated and several polymorphisms have been connected with breast cancer. Breast cancer (BC) can be considered one of the most popular reasons of death among women. BC, likewise, is the second cause of death in Iran. The present research aims at determining the frequency of the fibroblast growth factor receptor (FGFR) gene polymorphism in patients with breast cancer.

Methods: The FGFR family is one of the tyrosine kinase receptors containing 4 members, whose pathway is activated in many tumors. We assessed, for the first time, these polymorphisms and their consequences on the breast cancer risk association in an Iranian sporadic population-based case-control study including 126 patients with breast cancer and 160 controls using a PCR-RFLP-based assay.

Results: The analyses of the experimental and control groups indicated that homozygote genotype FGFR4 Gly/Gly has the highest frequency in experimental and control groups (30.4% and 18.9%). The main genotype FGFR4 Gly/Gly risk factors in our population were: ArgGly /GlyGly, OR = 2.359, 95% CI = 0.208 - 4.621, P = 0.001; ArgArg /ArgGly, OR = 0.412, 95% CI = 0.082 - 0.547, P = 0.078, ArgArg /GlyGly, OR = 0.076, 95% CI = 0.030 - 0.189, P = 0.26.

Conclusions: A significant association was observed between breast cancer risk and FGFR4 GlyGly and ArgGly polymorphism.

Introduction

Of several types of tyrosine kinase receptors, stem cell factor, platelet derived growth factor, epidermal growth factor, macrophage colony stimulating factor, and insulin receptors could be named. The FGFR family is one of the tyrosine kinase receptors containing 4 members, which have a highly conserved structure: extracellular ligand-binding domain, transmembrane domain, and intracellular tyrosine-kinase domain.[1]

There are 4 high-affinity tyrosine kinase FGF receptors (FGFR1-4).[1,2] The 4 FGFRs produce ligand-binding specific isoforms by tissue-specific alternative mRNA splicing of the genes.[3-7] FGFs play a critical role in cell signaling.[8] FGF-FGFR complex activates the intracellular tyrosine kinase, mediating signal transduction through the direct phosphorylation of adaptor proteins.[9] Complex FGF signaling networks are crucial in the multiple cell biological activities like proliferation, differentiation, mitogenesis, migration, and apoptosis, and are, hence, implicated in tumor genesis,[10-12] the development of solid tumors, cancers, and other malignancies. A germ-line polymorphism in the FGFR4 gene (rs351855), resulting in the expression of FGFR4 containing either glycine (Gly388) or arginine (Arg388) at codon 388, and a G to A conversion was discovered, resulting in the substitution of glycine by arginine at position 388

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in the transmembrane domain of the receptor.

On the other hand, a few studies discussed its role and association with breast cancer risk.

Breast cancer is a progressively significant reason of death among women; hence, the present study aimed at clarifying the role of FGFR4 (rs1966265, rs376618, and rs351855) as a high-risk breast cancer using of PCR-RFLP method.

To the best of our knowledge, the present research is the first attempt to investigate the association between the polymorphisms of FGFR4 gene and the risk of breast cancer in an Iranian context.

Methods

Patients data

The analyses were performed for 126 patients and 160 controls genotyped for FGFR4 and aged between 35 to 55.

The Local Ethical Committee of Islamic Azad University approved the present study from patient and control group rights point of view.

Before the onset of the study, the blood samples were collected from patients and controls. The participants were genotyped for the FGFR4 SNP, using genomic DNA obtained from peripheral blood lymphocytes. DNA was separated from peripheral blood, using FelxiGene DNA extraction kit (Qiagen Germany).

Genotyping

The polymorphisms were distinguished utilizing a modified PCR-RFLP method.[13,14] The PCR primers were synthesized by TAG Copenhagen A/S. Primers for each polymorphism is as follow. The primers ofthe FGFR4 were forward 5′GACCGCAGCA GCGCCCGAG GCCAG-3′ and revers 5′-AGAGGGAAGAGGGAGAGCTTCTG-3′ .[15] The cycling conditions were 94°C, 30 sec; 60°C, 30 sec; 72°C, 60 sec (35 cycles). The PCR products were digested with 1 unit of BstNI (New England BioLabs); and the amplified fragment of 168-bp was cut into fragments, the Arg 388 allele by 2 distinctive fragments of 82 and 27 bp, and a single distinctive band of 109 bp was observed for the Gly388 allele and separated on a 6% acrylamide gel.

This method can detect all 3 possible genotypes for the polymorphism: homozygous wild type, heterozygous variant type, and homozygous variant type.

The genotypes and allelic frequencies of FGFR4 polymorphisms in the experimental and control groups were analyzed by $\chi 2$ and Fisher's exact tests.

Results

The present study was the first attempt to clarify the role of FGFR4 as a high-risk breast cancer using PCR-RFLP method.

There was a significant association between FGFR4 polymorphism and breast cancer risk.

The analyses of the experimental (126 patients) and control groups (160 patients) indicate that homozygote genotype FGFR4 Gly/Gly has a high frequency (30.4%, 18.9%) in both groups.

On the other hand, the heterozygote genotype in FGFR4 Arg/Arg has an increase in frequency in control group compared with experimental group (2.1 in patients and 17.1 in control group).

Table 1 showed some information on the Genotype FGFR4 Gly/Gly most common risk factors: ArgGly /GlyGly, OR = 2.359, 95% CI = 0.208 - 4.621, P = 0.001* * *; ArgArg /ArgGly, OR = 0.412, 95% CL = 0.082 - 0.547, P=0.078**; ArgArg /GlyGly, OR = 0.076, 95% CI = 0.030 - 0.189, P = 0.26.

In the present study, we found a significant relationship between the presences of FGFR4 Gly/Gly and breast cancer.

Discussion

The FGFR4, MTHFR, and HFE genes are related to neoplastic diseases development, particularly, FGFR4 or Fibroblast growth factor receptor 4, involved in cancer progression.[16]

The FGFR4 Gly388Arg polymorphism in the transmembrane domain of the receptor contributes to genetic susceptibility to cancer cell invasiveness and

Table 1. FGFR4 genotype frequencies [n (%)] in experimental and control groups

Study group	N	Arg/Arg	Gly/Gly	Arg/Gly
Experimental	126	6 (2.1%)	87 (30.4%)	33 (11.5%)
Control	160	49 (17.1%)	54 (18.9%)	57 (19.9%)
Total	286	55 (19.2%)	141 (49.3%)	90 (31.5%)

Table 2. Comparison of genotypes

Genotype FGFR4	OR	95% CL	P-value
ArgArg /ArgGly	0.412	0.082 - 0.547	0.078*
ArgGly /GlyGly	2.359	0.208 - 4.621	≤0.001
ArgArg /GlyGly	0.076	0.030 - 0.189	0.26

* *P<= 0.05*

clinical chemo-resistance in breast cancer.[17]

The FGFR4 Arg (388) allele was observed to be associated with a poor prognosis according to several studies that showed single nucleotide polymorphism (SNP) at codon 388 (Gly or Arg) of fibroblast growth factor receptor 4 (FGFR4) was related with prognosis in patients with colorectal carcinoma, head and neck SCC, and several types of cancer including breast cancer.[17,18]

In a study in 2011, appeal in the genetic susceptibility to cancers led to a rising attention to the study of polymorphisms of genes engaged in tumor geneses. We had already published a study, in which 2 common functional polymorphisms existed in the *MTHFR* gene, C677T (rs1801133) and A1298C (rs1801131).[19] Thus, we decided to select this SNP FGFR4, because we had already worked upon FGFR2 , even interionic and FGFR1.[20,21]

As a matter of fact, they were enzymes playing a central role in the methyl group metabolic pathway, which were engaged in both DNA methylation and DNA synthesis.

Some scientists challenged the existence of an association between polymorphisms and different cancer; as an example, Nan *et al.* [22] could not find any evidence for associations among these 7 genetic variants and the risks of melanoma and nonmelanocytic skin cancer in Caucasian American women. Bange *et al.*[15] concluded that cancer evolution and tumor cell motility are associated with the FGFR4 Arg (388) allele in German population.

However, many studies discussing other types of cancers found significant association, for example; Marme *et al.*[23] found that FGFR4 Arg388Gly genotype strong context specific prognostic factor in patients with advanced ovarian cancer in German population. Xu *et al.*[24] proposed that the FGFR4 Gly388Arg polymorphism most likely contribute to susceptibility to cancer, particularly in Asians. Besides, Arg (388) allele might be associated with increased risks of prostate cancer. Tanuma *et al.*[25] discovered that the majority of patients with homozygous Arg388 FGFR4 and whereas >90% patients carrying homozygous Gly388 FGFR4 were excellent predictors of the prognosis for oral squamous cell carcinoma for (OSCC) in Japanese patients.

Da Costa Andrade *et al.*[18] demonstrated that the FGFR4 Arg (388) allele was associated with survival in head and neck squamous cell carcinoma in Brazilian population. Wang *et al.* [26] found that the FGFR-4 Arg388 allele was associated with both an increased incidence and clinical aggressiveness of prostate cancer in American population.

In a study conducted by Morimoto *et al.*[27] a significant correlation was found between FGFR4 Gly388 and prognosis in patients with soft tissue sarcoma in Japanese population. Wang *et al.*[26] conclude that Homozygosity for the FGFR-4 Arg allele was strongly associated with prostate cancer in white men with pelvic lymph node metastasis.

Dutra *et al.* [26] found Arg388 genotype and expression, as a novel marker of prognosis in squamous cell carcinoma of the mouth and oropharynx in Brazilian population.

Finally, the results of a research suggested that FGFR-4 induced to breast cancer. Thussbas *et al.* [27] and Xu *et al.* [24] found that FGFR4 Arg388 genotype was a marker for breast cancer progression in German and Asian population. Findings of the Seitzer *et al.* [30] study indicated FGFR4 Arg388 allele as a functional prognostic marker for breast cancer progression in mouse.

In brief, other scholars found best association in FGFR4 Arg, and Arg/Gly, with breast cancer risk.

However, in my research studies, there were a strong association between FGFR4 Gly/Gly, and breast cancer risk.

Hence, in our study, it can be concluded that there is a relation between the presence of FGFR4 Gly/Gly and increase breast cancer risk.

Acknowledgement

The authors of the present research would like to appreciate all patients for their kind collaborations in the project and Islamic Azad University for supporting this research.

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