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Lack of Association between Polymorphisms in rs2981582, rs2420946, rs17102287, rs1219648, rs2981578, and rs17542768 Sites of *FGFR*2 Gene with Breast Cancer in the Population of Kazakhstan

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Abstract

Worldwide, breast cancer (BC) is the most common invasive cancer in women. Fibroblast growth factor receptor 2 (FGFR2) is a tyrosine kinase receptor that is a member of the family of individually distinct fibroblast growth factor receptors involved in tumorigenesis. *FGFR*2 gene is amplified and over expressed in breast cancer (1 - 3). The aim of the study was to determine whether polymorphisms in rs2981582, rs2420946, rs17102287, rs1219648, rs2981578, and rs17542768 in *FGFR*2 gene are associated with breast cancer susceptibility in the population of Kazakhstan. The statistically significant associations between SNPs analyzed and breast cancer risk according χ^2 and $p < 0.05$ criterions were not evaluated. The information describing the association of SNPs in *FGFR*2 with BC risk in the world populations could not be unambiguously used for Kazakhstan population.

Keywords

Fibroblast Growth Factor Receptor 2 (FGFR2), Single Nucleotide Polymorphism (SNP), Association, Breast Cancer (BC), Kazakhstan

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1. Introduction

Worldwide, breast cancer (BC) is the most common invasive cancer in women. Fibroblast growth factor receptor 2 (FGFR2) is a tyrosine kinase receptor that is a member of the family of individually distinct fibroblast growth factor receptors involved in tumorigenesis. *FGFR2* gene is amplified and overexpressed in breast cancer [1]-[3].

A meta-analysis of 37 studies of rs2981582, rs2420946, rs17102287, rs1219648, rs2981578, and rs17542768 polymorphisms demonstrated that these *FGFR2* SNPs are a risk factor associated with increased BC susceptibility, but these associations vary significantly in different racial and ethnic groups [4].

Significant associations between breast cancer risk and SNPs in rs11200014, rs2981579, rs1219648, rs2420946 of *FGFR2* (P_{trend} for all SNPs < 0.0001) were found in Jewish and Arab Israeli population [5].

Kazakhstan is situated in the middle of Central Asia. The multinational population of Kazakhstan totaled 17.2 million in 2013 with the major ethnic groups represented by mongoloid Asian Kazakhs (65%) and Caucasian Russians (22%), according to the Agency of the Republic of Kazakhstan on Statistics data [6].

The aim of the present work was to determine the association of individual SNPs in rs2981582, rs2420946, rs17102287, rs1219648, rs2981578, and rs17542768 sites of *FGFR2* with BC in Kazakh and Russian ethnic groups of Kazakhstan.

In studies performed in Russia, the associations of *FGFR2*'s SNPs with BC risk were shown for rs1219648, especially in combination with polymorphisms in *TP53* [7]; rs2981582 in the population of West Siberia [8]; and rs2981582, particularly in genetically-enriched BC patients versus elderly tumor-free women [9]. In Kazakhstan the presented research devoted to the evaluation of association of SNPs of *FGFR2* with BC is performed at a first time.

2. Materials and Methods

2.1. Patients and Controls

Informed consent was received from all individuals prior to study inclusion. Ethical permissions were obtained from the ethical committees of the medical organizations listed below. Venous blood samples (5 ml) were collected from 495 women of Asian Kazakh (311 Kazakh) and Russian Caucasian (184 Russian) descent with diagnosed and histologically confirmed BC from the Kazakh Research Institute of Oncology and Radiology and Regional Oncological Dispensary (Almaty, Kazakhstan). Samples obtained from 190 healthy Kazakh and 170 Russian female blood donors (Almaty City Blood Center) without clinical symptoms or family history of cancer according to a questionnaire were used as a control. The average age of BC patients was 49.58 ± 8.70 (Kazakhs) and 53.40 ± 9.97 (Russians) years, while that of control donors was 49.84 ± 6.09 (Kazakhs) and 50.43 ± 6.56 (Russians) years old.

2.2. DNA Extraction and Genotyping

Genomic DNA was isolated from blood using commercially available DNA Blood & Tissue extraction kits (Qiagen, USA) according to the manufacturer's instructions. Genotyping was performed by restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) products. Deoxyribonucleoside triphosphates (dNTP), restriction endonucleases, and *Taq*DNA-polymerase were purchased from SibEnzyme (Russia).The PCR reaction mixture (10 µl total volume) contained 67 mM Tris-HCl, (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.01% Tween-20, 0.15 mg/ml bovine serum albumin, 2 pM primers, 0.25 mM dNTPs, 100 ng template DNA, and 1 unit of *Taq*DNA-polymerase using a Mastercycle gradient (Eppendorf, Germany). PCR-amplified products were separated by 8% polyacrylamide gel electrophoresis at 50 mA and 300 V for 2 - 3 h. RFLP products were visualized by 0.05% ethidium bromide staining and analyzed using GelDoc-Imager (BioRad, USA). Primers were designed by Primer3 (v. 0.4.0) [10], and *FGFR2* nucleotide sequences of interest were obtained from the Ensemble data base [11]. Amplification conditions and primers for each FGFR2 nucleotide sequence are presented in **Table 1**.

Each PCR product was digested with the appropriate restriction endonuclease according the manufacturer's recommendations (SibEnzyme, Russia). PCR fragment and restriction product sizes and endonucleases used are presented in **Table 2**.

Table 1. Sites analyzed, primers sequences, amplification conditions.

Site	Primers	Amplification conditions
rs2981582	F 5'-CAGGCACCAGGTGGACTC-3' R 5'-CGAGGACTACATGAGGCTGA-3'	95˚C - 5 min, 35 cycles (95˚C - 30 s, 64.5˚C - 30 s, 72˚C - 40 s), 72˚C - 5 min
rs2420946	F 5'-AAGCCCTCAGACGACAGAAA-3' R 5'-CTGCTCAACCTGGGATCTGT-3'	94˚C - 7 min, 35 cycles (94˚C - 30 s, 57˚C - 30 s, 72˚C - 40 s), 72˚C - 7 min
rs17102287	F 5'-CCTCTGCTGGTGCCCTATAA- 3' R 5'-TGGCTTTGTGCAATATCGTATC-3'	95˚C - 3 min, 35 cycles (95˚C - 30 s, 63˚C - 35 s, 72˚C - 35 s), 72˚C - 5 min
rs1219648	F 5'-CACGCCTATTTTACTTGACACGC-3' R 5'-ATTTGTATGTGGTAGCTGACTTC-3'	95˚C - 2 min, 35 cycles (95˚C - 30 s, 58˚C - 30 s, 72˚C - 30 s), 72˚C - 5 min
rs2981578	F 5'-AATGCTGCTTTGGAGGATTG-3' R 5'-CCAGAGGACTGAAACCCACA-3'	95˚C - 4 min, 35 cycles (95˚C - 30 s, 56.8˚C - 35 s, 72˚C - 40 s), 72˚C - 5 min
rs17542768	F 5'-CAGACCCCCAGAGGAATCTT-3' R 5'-CTGGGTGGGCTTGTAGGTAG-3'	95˚C - 3 min, 36 cycles (95˚C - 30 s, 60˚C - 40 s, 72˚C - 30 s), 72˚C - 5 min

Table 2. Sites analyzed, PCR product size, restriction fragments size, restriction endonucleases.

Site	PCR product size, bp	Restriction fragments size	Endonuclease
rs2981582	233	T allele - 233 bp, C allele - 211 bp, 22 bp	*BspAC*I
rs2420946	269	T allele - 269 bp, C allele - 244 bp, 25 bp	*AspLE*I
rs17102287	237	T allele - 237 bp, C allele - 212 bp, 25 bp	*Fat*I
rs1219648	133	A allele - 133 bp,G allele - 109 bp, 24 bp	*BstHH*I
rs2981578	173	A allele - 173 bp, G allele - 89 bp, 84 bp	*BspAC*I
rs17542768	206	A allele - 206 bp,G allele - 159 bp, 47 bp	*BstC*8I

Table 3. The allele frequencies and genotypes distribution of *FGFR2* gene rs2420946, rs2981578, rs1219648, rs1281582, rs17102287, rs17542768 polymorphic sites in Kazakh ethnic groups of patients (cases) and controls.

Alleles/genotypes	Cases % (n)	Controls % (n)	P	χ^2	OR (95% CI)
		Kazakhs			
rs2420946					
C	59.2 (366)	54.4 (196)	0.14	2.13	1.22 (0.93 - 1.58)
T	40.8 (252)	45.6 (164)			0.82 (0.63 - 1.07)
CC	34.0 (105)	26.7 (48)			1.42 (0.94 - 2.12)
TC	50.5 (156)	55.6 (100)	0.24	2.85	0.82 (0.56 - 1.18)
TT	15.5 (48)	17.8 (32)			0.85 (0.52 - 1.39)
rs2981578					
A	45.4 (334)	46.4 (231)	0.73	0.12	0.96 (0.76 - 1.21)
G	54.6 (402)	53.6 (267)			1.04 (0.83 - 1.31)
AA	19.0 (70)	23.3 (58)			0.77 (0.52 - 1.15)
AG	52.7 (194)	46.2 (115)	0.24	2.83	1.30 (0.94 - 1.79)
GG	28.3 (104)	30.5 (76)			0.90 (0.63 - 1.28)
rs1219648					
A	59.4 (366)	60.3 (216)	0.78	0.08	0.96 (0.74 - 1.26)
G	40.6 (250)	39.7 (142)			1.04 (0.80 - 1.36)
AA	34.7 (107)	36.3 (65)			0.93 (0.64 - 1.37)
AG	49.4 (152)	48.0 (86)	0.94	0.12	1.05 (0.73 - 1.52)
GG	15.9 (49)	15.6 (28)			1.02 (0.62 - 1.69)
rs1281582					
C	61.8(465)	61.2(350)	0.81	0.06	1.03 (0.82 - 1.28)
T	38.2(287)	38.8(222)			0.97 (0.78 - 1.22)
CC	38.6(145)	35.3(101)			1.15 (0.84 - 1.58)
CT	46.5(175)	51.7(148)	0.41	1.81	0.81 (0.60 - 1.10)
TT	14.9(56)	12.9(37)			1.18 (0.75 - 1.84)
rs17102287					
T	75.3 (521)	76.7 (437)	0.57	0.32	0.93 (0.72 - 1.20)
C	24.7 (171)	23.3 (133)			1.08 (0.83 - 1.40)
TT	56.1 (194)	57.2 (163)	0.63	0.92	0.96 (0.70 - 1.31)

Continued

TC	38.4 (133)	38.9 (111)			0.98 (0.71 - 1.35)
CC	5.5 (19)	3.9 (11)			1.45 (0.68 - 3.09)
rs17542768					
A	92.3 (696)	89.5 (501)	0.07	3.20	1.41 (0.97 - 2.07)
G	7.70 (58)	10.5 (59)			0.71 (0.48 - 1.03)
AA	84.6 (319)	78.9 (221)			1.47 (0.98 - 2.19)
AG	15.4 (58)	21.1 (59)	0.17	3.55	0.68 (0.46 - 1.02)
GG	0 (0)	0 (0)			0.74 (0.01 - 37.56)

P—Fisher's exact test p-value; OR—Odds Ratio; CI—Confidence Interval.

Table 4. The allele frequencies and genotypes distribution of *FGFR*2 gene rs2420946, rs2981578, rs1219648, rs1281582, rs17102287, rs17542768 polymorphic sites in Russian ethnic group of patients (cases) and controls.

Alleles/genotypes	Cases % (n)	Controls % (n)	P	χ^2	OR (95% CI)
		Russian			
rs2420946					
C	60.9 (223)	60.2 (200)	0.85	0.03	1.03 (0.76 - 1.39)
T	39.1 (143)	39.8 (132)			0.97 (0.72 - 1.32)
CC	32.2 (59)	34.3 (57)			0.91 (0.58 - 1.42)
TC	57.4 (105)	51.8 (86)	0.4948	1.48	1.25 (0.82 - 1.91)
TT	10.4 (19)	13.9 (23)			0.72 (0.38 - 1.38)
rs2981578					
A	56.4 (246)	55.5 (234)	0.77	0.08	1.04 (0.79 - 1.36)
G	43.6 (190)	44.5 (188)			0.96 (0.73 - 1.26)
AA	31.2 (68)	31.3 (66)			1.00 (0.66 - 1.50)
AG	50.5 (110)	48.3 (102)	0.85	0.33	1.09 (0.75 - 1.59)
GG	18.3 (40)	20.4 (43)			0.88 (0.54 - 1.42)
rs1219648*					
A	64.0 (233)	64.1 (209)	0.98	0	1.00 (0.73 - 1.36)
G	36.0 (131)	35.9 (117)			1.00 (0.74 - 1.37)
AA	37.4 (68)	41.1 (67)			0.85 (0.55 - 1.32)
AG	53.3 (97)	46.0 (75)	0.33	2.21	1.34 (0.88 - 2.05)
GG	9.30 (17)	12.9 (21)			0.70 (0.35 - 1.37)
rs1281582					
C	63.5 (284)	62.0 (306)	0.63	0.23	1.07 (0.82 - 1.39)
T	36.5 (162)	38.0 (188)			0.94 (0.72 - 1.22)
CC	39.2 (87)	38.6 (95)			1.02 (0.71 - 1.49)
CT	48.6 (108)	46.7 (115)	0.73	0.63	1.08 (0.75 - 1.55)
TT	12.2 (27)	14.6 (36)			0.81 (0.47 - 1.38)
rs17102287					
T	83.6 (373)	80.5 (401)	0.21	1.54	1.24 (0.88 - 1.73)
C	16.4 (73)	19.5 (97)			0.81 (0.58 - 1.13)
TT	69.1 (154)	65.1 (162)			1.20 (0.82 - 1.76)
TC	29.1 (65)	30.9 (77)	0.31	2.36	0.92 (0.62 - 1.36)
CC	1.8 (4)	4.00 (10)			0.44 (0.13 - 1.41)
rs17542768					
A	84.2 (401)	87.3 (433)	0.17	1.86	0.78 (0.54 - 1.12)
G	15.8 (75)	12.7 (63)			1.29 (0.90 - 1.85)
AA	69.7 (166)	75.4 (187)			0.75 (0.50 - 1.12)
AG	29.0 (69)	23.8 (59)	0.36	2.03	1.31 (0.87 - 1.96)
GG	1.30 (3)	0.8 (2)			1.57 (0.26 - 9.48)

P—Fisher's exact test p-value; OR—Odds Ratio; CI—Confidence Interval.
*In the group of patients alleles frequencies did not corresponded to Hardy-Weinberg equilibrium.

2.3. Statistical Analysis

The Pearson χ^2 and Fisher's exact tests were used to compare differences between allele frequencies and genotypes distribution between groups of BC patients and control. Cancer risk associated with genotype was calculated with odds ratios (ORs) and 95% confidence intervals (CI). All BC cases and controls were tested to be in Hardy-Weinberg equilibrium. Statistical analyses were performed using STATISTICA v. 5.0 software (StatSoft, USA). Fisher's exact test and Chi-square criterion were estimated using Free Statistics Calculator v. 3.0 [12].

3. Results and Discussion

To investigate whether SNPs in rs2981582, rs2420946, rs17102287, rs1219648, rs2981578, and rs17542768 of *FGFR2* gene are associated with BC in Kazakhstan population, we performed PCR-RFLP based assay. The results of genotyping in Kazakh ethnic group (311 BC patients and 190 controls) are presented in **Table 3** and the results of genotyping in Russian ethnic group (184 BC patients and 170 controls) are presented in **Table 4**. Allele frequencies in all groups corresponded to Hardy-Weinberg equilibrium with the exception of site rs1219648 in the group of BC patients. The differences neither in allele's frequency nor in genotypes distribution were evaluated in both Kazakh and Russian ethnic groups.

The information describing the association of SNPs in *FGFR2* with BC risk in the world populations could not be unambiguously used for Kazakhstan population.

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