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ORIGINAL ARTICLE

Association between gene polymorphism of GRK5 and breast cancer risk in Chinese population

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Summary

Purpose: *Potential influences of GRK5 polymorphism on cancer risks have been reported. This study aimed to explore the distribution of GRK5 genotypes and alleles in Chinese breast cancer (BCa) patients, and to analyze the association between GRK5 and BCa risk.*

Methods: *Blood samples were collected from 412 BCa patients and 533 healthy individuals for isolating genomic DNA. GRK5 polymorphisms of Gln41Leu A > T and Arg304His G > A, and their alleles were detected using PCR-RFLP. Their influences on BCa susceptibility and pathological indexes were analyzed using Logistic regression model.*

Results: *No significant differences in age, body mass index (BMI) and smoking status were found between BCa patients and healthy persons, while significant differences were detected in drinking status, family history of cancer, hypertension and diabetes. GRK5Gln41Leu A > T and its allele frequency distribution were correlated to BCa susceptibility, while GRK5 Arg304His G > A was not. Higher risks of GRK5 Gln41Leu A > T and Arg304His G > A indicated a higher susceptibility to BCa. Compared with people carrying 0-1 risk allele, those carrying 2-4 risk alleles of GRK5 Gln41Leu A > T and Arg304His G > A of had a higher susceptibility to BCa, manifesting as worse tumor staging and grading, and higher rates of estrogen receptor (ER) (-), progesterone receptor (PR) (-) and HER2 (-).*

Conclusions: *Gln41Leu A > T and Arg304His G > A fusion gene polymorphisms of GRK5 are vital genetic susceptibility genes to BCa. Our findings require to be validated in a multicenter study with a high-quality large sample size.*

Key words: *GRK, polymorphism, breast cancer, genetic susceptibility*

Introduction

Breast cancer (BCa) is a highly prevalent tumor in females [1,2]. There are more than 1.67 million new cases of BCa worldwide, placing BCa first among all malignancies in females [3,4]. In recent years, the incidence and mortality of BCa have been constantly on the rise. It is estimated that the global number of BCa in females will reach 2.64 million in 2030, and the number of deaths will be up to 1.7 million [4,5]. In our country, the incidence of BCa increases at an annual rate of 2-3% [6,7]. Morbidity and mortality of BCa differ from countries, regions, and races [8]. According to literature reports, the incidence of BCa in the Chinese population is much higher than the national average [6-8]. Therefore, screening risk population of BCa is of significance [9,10]. A large number of epidemiological studies have shown that both genetic and environmental factors are involved in the development of BCa [4]. Very recently, the role of gene polymorphisms in BCa susceptibility has been well concerned [11,12].

G protein-coupled receptor kinases (GRKs) are a family of serine/threonine protein kinases that specifically recognize agonists and activate

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G protein-coupled receptors as substrates [13,14]. There are 6 GRKs distributed differently in mammal tissues. GRK4, 5, and 6 contain a unique subgroup of GRKs, which are anchored on the membrane by a unique mechanism [15]. All GRKs present conventional cellular functions as desensitized G protein-coupled receptors in their own manners [15,16]. In recent years, the role of GRK5 SNPs (Gln41Leu A > T and Arg304His G > A) has been well studied in multiple chronic diseases [17,18].

In the present study, we recruited BCa patients and healthy individuals for detecting GRK5 phenotypes and alleles. Potential influences of GRK5 Gln41Leu A > T and Arg304His G > A, and their alleles on BCa susceptibility and pathological indexes were mainly explored.

Methods

Study population

All participants were Chinese females with similar daily diet, living environment and climate. Age and BMI of participants were not restricted. A total of 412 BCa patients and 533 healthy individuals undergoing physical examinations were randomly recruited.

BCa patients aged 46.7±10.8 years were pathologically confirmed by two pathologists independently and they did not have cancer history or anti-cancer treatment. Healthy controls aged 47.2±9.7 years did not have any biological connection with the recruited BCa patients.

In addition, non-smokers were defined as less than one cigarette a day. Drinkers were defined those who has a drinking habit for more than six months. Family history of cancer was defined as the presence of cancer in any first-degree relatives (parents, siblings or offspring). This study was approved by the Ethics Committee of our hospital. Signed written informed consents were obtained from all participants before the study entry.

Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP)

Genomic DNA was isolated and purified from peripheral blood lymphocytes by proteinase K digestion and phenol-chloroform method. Gln41Leu A > T and Arg304His G > A polymorphisms of GRK5 and its alleles were detected using PCR-RFLP (Applied Biosystems, Foster City, CA, USA). Single nucleotide polymorphism (SNP) primers were amplified at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. PCR products were cleaved by BccI, and loaded on 1%DNA agarose gel containing $C_{21}H_{20}BrN_3$. Their positive expressions were finally analyzed.

Table 1. Distribution of selected variables in breast cancer cases and healthy controls

Variables	*Cases (n=412)* *n (%)*	*Controls (n=533)* *n (%)*	*p value**
Age, years (mean ± SD)	46.7±10.8	47.2±9.7	0.843
<45	193 (46.84)	275 (51.59)	
≥45	219 (53.16	258 (48.41)	
BMI), kg/m^2 (mean ± SD	24.8±3.3	24.0±2.7	0.231
<24	200 (48.54)	283 (53.10)	
≥24	212 (51.46)	250 (46.90)	
Smoking status			0.914
Never	322 (78.16)	415 (77.86)	
Ever	90 (21.84)	118 (22.14)	
Drinking status			<0.001
Never	362 (87.86)	385 (72.23)	
Ever	50 (12.14)	148 (27.77)	
Family history of cancer			<0.001
No	380 (92.23)	435 (81.61)	
Yes	32 (7.77)	98 (18.39)	
Hypertension			0.017
No	307 (74.51)	359 (67.35)	
Yes	105 (25.49)	174 (32.64)	
Diabetes			<0.001
No	295 (71.60)	472 (88.56)	
Yes	117 (28.40)	61 (11.44)	

*Student's t-test for age and BMI distributions between cases and controls; two-sided x^2 test for other selected variables between cases and controls.

Table 2. Summary of the clinicopathologic features of breast cancer studied

Variables	*Cases (n=412) n (%)*
Clinical stage	
I	246 (0.60)
II	103 (0.25)
II	39 (0.09)
IV	24 (0.06)
Grade	
I	179 (0.43)
II	144 (0.35)
II	52 (0.13)
IV	37 (0.09)
Histology	
Ductal carcinoma *in situ*	100 (0.24)
Infitrating ductal carcinoma	253 (0.61)
Unclassified	59 (0.14)
ER	
Negative	148 (0.36)
Positive	264 (0.64)
PR	
Negative	180 (0.44)
Positive	232 (0.56)
HER2	
Negative	301 (0.73)
Positive	111 (0.27)

Statistics

SPSS 22.0 software (IBM, Armonk, NY, USA) was utilized for statistical analyses. Enumeration data were expressed as frequency (%). The Hardy-Weinberg equilibrium (HWE) of control genotype distribution, and comparison of enumeration data were evaluated using the x^2 test. Risk factors for BCa were assessed by performing univariate Logistic regression model, and data were expressed as odds ratio (OR) and 95% CI. $P<0.05$ was considered as statistically significant.

Results

Baseline characteristics of participants

A total of 412 BCa patients and 533 healthy subjects were recruited. No significant differences in age, BMI and smoking status were found between BCa patients and healthy controls, while significant differences were detected in drinking status, family history of cancer, hypertension and diabetes (Table 1). Furthermore, pathological indexes of BCa patients were analyzed. Classified by tumor staging, the number of stage I-IV BCa patients was 246, 103, 39 and 24, respectively. Classified by tumor grading, there were 179, 144, 52 and 37 cases of grade I-IV BCa patients, respectively. Histologically, 100 cases were ductal carcinoma *in situ*, 253 were infiltrating ductal carcinoma and 59 were unclassified. In addition, 148 patients were ER (-), 180 were PR (-) and 301 were HER2 (-) (Table 2).

Table 3. The basic information of the genotyped polymorphisms in two SNPs in GRK2 associated with the risk of breast cancer

Polymorphisms	*Cases (n=412) n (%)*	*Controls (n=533) n (%)*	*p**	*Adjusted OR (95% CI)**
Gln41Leu A > T				
AA	224 (54.4)	272 (51.0)		1.00(reference)
AT	157 (38.1)	200 (37.5)	0.025	1.09 (1.02-1.94)
TT	31 (7.5)	61 (11.5)	0.005	1.32 (1.21-2.33)
AT+TT	188 (45.6)	261 (49.0)	0.032	1.19 (1.07-1.82)
A allele	605 (73.4)	744 (69.8)		1.00 (reference)
T allele	219 (26.6)	322 (30.2)	0.043	1.11 (1.26-1.91)
Arg304His G > A				
GG	279 (67.7)	359 (67.4)		1.00 (reference)
GA	80 (19.4)	137 (25.7)	0.123	1.10 (0.82-1.79)
AA	53 (12.9)	37 (6.9)	0.432	1.47 (0.93-2.15)
GA+AA	133 (32.3)	174 (32.6)	0.292	1.11 (0.76-1.91)
G allele	638 (77.4)	855 (80.2		1.00 (reference)
A allele	186 (22.6)	211 (19.8)	0.372	1.53 (0.84-1.67)

*Adjusted for age, sex, BMI, smoking status, drinking status, diabetes and hypertension in logistic regression model. CI: confidence interval; OR: odds ratio.

Association between BCa susceptibility and genetic polymorphisms of GRK5

GRK5Gln41Leu A > T and its allele frequency distribution were correlated to BCa susceptibility (Table 3). Compared with GRK5 Gln41Leu AA, people carrying AT, TT and AT+TT genotypes of GRK5 were at higher risk of BCa [OR=1.09 (1.02-1.94), 1.32 (1.21-2.33) and 1.19(1.07-1.82), respectively]. In addition, a higher susceptibility to BCa was predicted in people carrying GRK5 Gln41Leu T in comparison with those carrying Gln41Leu A [OR=1.11(1.26-1.91)] (Table 3).

Combined analysis between GRK5 polymorphisms and BCa susceptibility

Logistic regression analysis concluded that higher risks of GRK5 Gln41Leu A > T and Arg304His G > A indicated a higher susceptibility to BCa (Table 4). Compared with subjects carrying 0-1 risk allele, those carrying 2-4 risk alleles of GRK5 Gln41Leu A > T and Arg304His G > A had a higher susceptibility to BCa, manifesting as worse tumor staging and grading, and higher rates of ER (-), PR (-) and HER2 (-) (OR=1.55, 1.47, 1.20, 1.42 and 1.36, respectively) (Table 5).

Table 4. Analysis between combined risk alleles and the susceptibility of breast cancer

Gln41Leu A > T and Arg304His G > A	*Cases (n=412)* n (%)	*Controls (n=533) n (%)*	*p**	*Adjusted OR (95% CI)**
Number of risk alleles				
0	208 (50.5)	293 (55.0)		1.00 (reference)
1	85 (20.6)	120 (22.5)	0.437	0.96(0.65-1.44)
2	95 (23.1)	98 (18.4)	0.067	1.87(0.94-1.99)
3	16 (3.9)	12 (2.3)	0.009	1.43(1.95-2.12)
4	8 (1.9)	10 (1.9)	0.023	1.52(1.03-3.24)
Recombined groups				
0–1	293 (71.1)	413 (75.3)		1.00 (reference)
2–4	119 (28.9)	120 (24.7)	0.026	1.54(1.14-1.97)

*Two-sided x^2 test for either genotype distributions or allele frequencies between the cases and controls. The 0–4 represents the numbers of risk alleles within the combined genotypes; the risk alleles used for the calculation were the Gln41Leu A > T and Arg304His G > A alleles.

Table 5. Association between the combined genotypes of GRK2 polymorphisms and breast cancer in stratified analysis

Variables	*Risk allele*				*p**	*Adjusted OR (95% CI)**
	0-1		*2-4*			
	Case n (%)	*Control n (%)*	*Case n (%)*	*Control n (%)*		
Clinical stage	293	413	119	120		
I+II	166 (56.7)	241 (58.4)	77 (64.7)	74 (61.7)	0.001	1.55 (1.05-1.86)
III+IV	127 (43.3)	172 (41.6)	42 (35.3)	46 (38.3)	0.064	1.43 (0.66-2.43)
Grade						
I+II	150 (51.2)	232 (56.2)	92 (77.3)	81 (67.5)	0.001	1.47 (1.10-1.97)
III+IV	143 (48.8)	181 (43.8)	27 (22.7)	39 (32.5)	0.114	1.65 (0.89-3.08)
ER						
Negative	177 (60.4)	283 (68.5)	64 (53.8)	79 (65.8)	0.008	1.20 (1.07-1.58)
Positive	116 (39.6)	130 (31.5)	55 (46.2)	41 (34.2)	0.077	1.43 (0.93-1.58)
PR						
Negative	135 (46.1)	245 (59.3)	87 (73.1)	70 (58.3)	0.043	1.42 (1.20-1.63)
Positive	158 (53.9)	168 (40.7)	32 (26.9)	50 (41.7)	0.324	0.74 (0.40-1.87)
HER2						
Negative	201 (68.6)	264 (63.9)	88 (73.9)	66 (55.0)	0.001	1.36 (1.13-1.67)
Positive	92 (31.4)	149 (36.1)	31 (26.1)	54 (45.0)	0.105	1.33 (0.94-1.35)

*Two-sided x^2 test for number of risk alleles in cases and controls; 95% CI: 95% confidence interval.

Discussion

BCa can severely harms women's health and life, and its onset is gradually seen in younger ages [1-4]. According to the report of National Cancer Center (2018), the incidence of female BCa in China is 156/100,000 and the number of BCa patients in the past 5 years had reached 1.02 million [3-6]. BCa is a malignant disease composed of neoplastic complexity and tumor cells. It is highly heterogeneous in clinical manifestations, pathological classifications, immunohistochemistry, and tumor marker expressions [7,8]. Epidemiological studies have shown that the interaction of genetic and environmental factors exerts a crucial role in the pathogenesis of BCa [9,10]. With the rapid development of genome-wide association studies, SNP has a certain correlation with BCa incidence [11,12].

GRK family contains 7 members, including rhodopsin family members (GRK1 and GRK7), β-Adrenergic receptor kinases (GRK2 and GRK3) and GRK4 family members (GRK4, GRK5 and GRK6) [13-15]. GRK1/7 are only partially expressed in the retina. GRK2/3 and GRK4/6 are generally present in all tissues of the body. GRK5 is only expressed in a few tissues, like the kidney, testis, brain, and myometrium [15,16]. Previous studies have shown that GRK5 SNPs may affect human cancer progression [17,18]. Our findings demonstrated that variations of GRK5 Gln41Leu A > T remarkably enhanced susceptibility to BCa, especially the population carrying AT, TT or AT+TT genotypes. However, we did not obtain any correlation between GRK5 Arg304His G > A and BCa susceptibility. In addition, because Gln41Leu A > T and Arg304His G > A genotypes posed statistically different risks of BCa, they were combined for co-evaluation. Our analysis identified that the population carrying 2-4 risk alleles had a significantly increased susceptibility to BCa. To our knowledge, this study is the first to evaluate the role of GRK5 polymorphism in the etiology of BCa.

Compared with GRK5 Gln41Leu AA, people carrying AT, TT and AT+TT genotypes of GRK5 were at higher risk of getting BCa. Besides, higher susceptibility to BCa was predicted in people carrying GRK5 Gln41Leu T than others carrying Gln41Leu A. Logistic regression analysis concluded that higher risks of GRK5 Gln41Leu A > T and Arg304His G > A indicated a higher susceptibility to BCa. Compared with subjects carrying 0-1 risk allele, those carrying 2-4 risk alleles of GRK5 Gln41Leu A > T and Arg304His G > A had a higher susceptibility to BCa, manifesting as worse tumor staging and grading, and higher rates of ER (-), PR (-) and HER2 (-).

Finally, some limitations of this study should be noted. First of all, this was a retrospective case-control study. Selection bias of subjects related to specific genotypes should not be neglected. Second, the lack of detailed survival data from all participants limited our ability to explore the relationship between GRK5 SNPs and BCa prognosis. Third, the small sample size reduced the statistical power of combined analysis and hierarchical analysis, especially environment-gene interaction analysis. Our findings require to be further validated in multicenter studies with a high-quality large sample size.

Conclusions

Gln41Leu A > T and Arg304His G > A fusion gene polymorphisms of GRK5 are vital genetic susceptibility genes in BCa.

Conflict of interests

The authors declare no conflict of interests.

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