Implication of the TNRC9 rs12443621A/G Polymorphisms in Breast Cancer in Chinese Women

JIA Lei[1] , LI Yang[2] , SUN Mei[2], CUI Wei[2], SONG ZH-Di[2]

(1 Qingdao University Qingdao 266003, China ;2 Department of General Surgery, Qingdao municipal HospitalQingdao 266003, China)

ABSTRACT Objective: To investigate the relationship between the rs12443621 polymorphisms of TNRC9/ LOC643714 and breast cancer risk and clinico-pathological characteristics in Chinese women. Methods: Genomic DNA was extracted from peripheral blood. The Single Nucleotide Polymorphisms of the TNRC9/LOC643714 rs12443621, from breast cancer of 321 cases and 340 controls, were detected by polymerase chain reaction (PCR) and direct DNA sequencing. Results: The genotypes of rs12443621 can not increase the risk for breast cancer(X2=1.43,P＞0.05). There was no relationship between the genotypes and pathological category, lymph node metastases, ER status or PR status or Her-2 status (X2=2.90,P＞0.05; X2=2.25,P＞0.05; X2=1.671,P＞0.05; X2=1.34,P＞0.05; X2=3.24, P＞0.05). Conclusion: There was no relationship between three genotypes of rs12443621A/G and individual susceptibility or clinic pathological characteristics of breast cancer.

Key Words:Breast cancer; Liability; Single Nucleotide Polymorphisms; TNRC9 gene ;rs12443621

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1 Materials and methods

1.1 materials

Genotyping was performed in 321 Chinese women with breast cancer and 340 Chinese healthy age-matched women. During January 2009 and October 2010, the blood samples of all breast cancer patients were consecutively recruited from the General surgery Ward at Qingdao Municipal Hospital. Cancer-free women controls were randomly selected from the same Hospital. There are 217 cases of infitrating ductal carcinoma, 91 cases of infiltrating lobular carcinoma and 13 cases of other category. And there are 194 cases with positive estrogen receptor, 189 cases with positive progesterone receptor, 150 cases with strong positive HER-2 and 124 cases with positive lymph node metastases. All blood samples were collected before surgery or therapy and preserved in -80℃. According to the kit for extracting human genomic DNA From whole blood (Bioteke Corporation, Beijing), we extracted genomic DNA.

1.2 experimental methods

The SNP, rs12443621, were genotyped by Polymerase chain reaction and direct DNA sequencing. Forward primer was CAGAAACCTTGGCTTGGA. Reverse primer 1 was ATAGTA-ATACCTACCTCAAGTTCAC and reverse primer 2 was ATAG-TAATACCTACCTCAAGTTCAT. Reactions were performed in 25ul of a mixture containing 1ul genomic DNA, 12.5ul 2 × Master Mix (Bioteke Gorporation 2 × Power Taq PCR Master Mix), 1ul forward primer, 1ul Reverse primer 1 and 1ul Reverse primer2, 9.5ul ddH_2O , and the PCR were run on an TC9600-G-230V MultiGene Gradient thermocycler (Labnet corporation, USA). After denaturing for 5 min at 94℃, the DNA was amplified for 35 cycles at 94℃ for 30s, 63℃ for 30s, and 72℃ for 45 s, followed by a 10-min extension at 72℃. The length of PCR products was 154bp. After the PCR reaction, the PCR products1 or 2 were subjected to direct DNA sequencing in BGI Corporation (Beijing Genomics Institute) (Fig. 1).

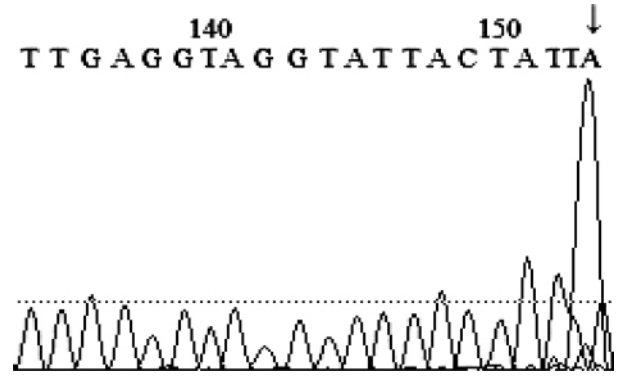

Fig.1 The consequence of direct DNA sequencing: the A/A genotype sequencing of rs12443621, the length of the products was 154bp.

1.3 statistical methods

SPSS 17.0 was used (SPSS Software, Munchen, Germany) to process the data. Differences in demographic characteristics, selected variables, and frequencies of the genotypes of TNRC9 variants between the cases and controls were evaluated for the Hardy Weinberg equilibrium using the X^2 test. The associations between TNRC9 genotypes and the risk of breast cancer were also estimated by the test. The allele distributions in cases and controls were consistent with the Hardy Weinberg equilibrium (data not shown). The relationship between the genotype frequencies and clinico-pathological parameters was also analyzed by X^2 test.

Author：Jia Lei（1983- 01）,Male, Longnan of Gansu province, Postgraduate students, Mainly engaged in General Surgery, Tel:15605323936 E- mail : jialei0201@163.com

Corresponding author：Li Yang（1954- 3）, Male, Qingdao, Docter, Chief Physician，Postgraduate Tutor ,Mainly engaged in General Surgery Tel:13808976899 E- mail: jialei0201@163.com

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2 Results

The samples of the SNPs AG, GG, and AA genotypes were 171, 62, and 88 in cases and 182, 76, and 82 in controls, respectively. There were no relationship between rs12443621A/G of TNRC9 and risk of breast cancer (table1, X^2=1.43 P＞0.05). And there were no interactions between gene polymorphisms and pathological category, ER status or PR status, Her-2 status or lymph node metastases, (table2,X^2=2.90,P＞0.05; X^2=2.25,P＞0.05; X^2=1.671,P＞0.05; X^2=1.34,P＞0.05; X^2=3.24, P＞0.05).

Table 1 The association between rs12443621A/G of TNRC9 and risk of breast cancer

Genotype	AG	GG	AA
Cases (321)	171	62	88
controls (340)	182	76	82
X^2	1.43		

Table 2 The interactions between rs12443621A/G of TNRC9 and pathological category, ER status, PR status, HER-2 status, or lymph node metastases.

Genotype	AG	GG	AA	X^2
infitrating ductal carcinoma	121	42	54	
infiltrating lobular carcinoma	45	17	29	
other category	5	3	5	2.90
ER (+)	97	39	58	
ER (-)	74	23	30	2.25
PR (+)	95	39	55	
PR (-)	76	23	33	1.67
HER-2 (+)	78	33	39	
HER-2 (-)	93	29	49	1.34
lymph node (+) metastases	61	22	41	
lymph node (-) metastases	100	40	47	3.24

3 Discussion

In this study, SNP rs12443621, which was associated with breast cancer risk in the European GWAS [1], was not correlated with breast cancer. At the same time, the SNP had not relationship with ER, PR, Her-2 status, or lymph node metastases of breast cancer in the Chinese population [3].

TNRC9, likewise called TOX3, is a gene located at chromosome 16q12, of uncertain function. The gene contains a putative high-mobility group box motif, suggesting its potential to play the role of transcription factor. It has been implicated in breast cancer metastasis of the bone[4]. Some newly investigations described as a risk factor for breast cancer [5, 6]. But some reports found that the allele and genotype frequencies of the SNPs did not confer an increased risk on women afflicted with breast cancer in the Chinese population. Thus, this meta-analysis was performed [7, 8].

Rs12443621 is located in an LD block containing the 5’ end of TNRC9. This study found that the LD pattern for this gene region was also different for European Caucasians and Chinese populations through Hap Map databases. Rs12443621 is in the same block in Europeans, while which is divided into three blocks in Chinese. Easton reported that some SNPs showed a positive association with breast cancer risk by a large-scale GWA study [1]. Rs12443621 is one of the most important [9, 10]. But other research papers found that the allele and genotype frequencies of the SNP did not confer an increased risk on women afflicted with breast cancer [11, 12]. Some research papers indicated that there have an intimately association between the rs12443621 polymorphisms and breast cancer clinico-pathological characteristics, some genotypes can significantly increased onset risk of ER-positive breast cancers [13, 14, 15, 16].

This investigation has not found the association between the rs12443621 polymorphisms and breast cancer risk and clinico-pathological characteristics in a hospital-based Chinese population. These results provide supporting evidence that the rs12443621 allele showed no association with breast cancer risk and clinico-pathological parameters, and can not be a diagnostic criterion of breast cancer in Chinese women.

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TNRC9 基因 rs12443621A/G 多态性与中国女性乳腺癌易感性及临床病理关系的研究

贾 雷[1] 李 扬[2△] 孙 梅[2] 崔 伟[2] 宋振迪[2]

(1 青岛大学 山东 青岛 266071;2 青岛市东部市立医院普外科 山东 青岛 266071)

摘要 目的:研究 TNRC9/LOC643714 基因 rs12443621A/G 多态性与乳腺癌易感性及临床病理之间的关系。**方法:**DNA 试剂盒提取 321 例乳腺癌患者和 340 例正常女性静脉血全基因组 DNA,PCR 扩增目的基因片段,提取扩增样本进行 DNA 测序检测分析 rs12443621 多态性。应用 SPSS17.0 软件对实验结果进行统计学分析。**结果:**应用 SPSS17.0 软件对 TNRC9/LOC643714 基因 rs12443621A/G 多态性 AA、AG、GG 进行卡方检验分析,结果显示三种基因型分布在病例组及对照组中无统计学意义(X^2=1.43, P>0.05),与乳腺癌易感性无关,与乳腺癌病理分型、ER、PR、HER-2 状态以及淋巴结是否转移无相关性(X^2=2.90,P>0.05; X^2=2.25, P>0.05; X^2=1.671,P>0.05; X^2=1.34,P>0.05; X^2=3.24, P>0.05)。**结论:**TNRC9 基因 rs12443621A/G 多态性与乳腺癌易感性及临床病理特征无关,不能作为独立的基因标志物对乳腺癌进行早期检测和诊断。

关键词:乳腺癌;易感性;单核苷酸多态性;TNRC9 基因;rs12443621

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作者简介:贾雷(1983-),男,硕士研究生,研究方向:普通外科,电话:15605323936,E-mail: jialei0201@163.com

通讯作者:李扬(1954-),男,硕士、研究生导师、主任医师,研究方向:普通外科。电话:13808976899

E-mail: jialei0201@163.com

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