Research on Relation between SNPsin BRCA1 Gene Promoter Region and Sporadic Breast Cancer Susceptibilit*

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ABSTRACT Objective: To investigate the relationship between rs11655505 and rs73625095 SNPs of BRCA1 gene promoter region and the susceptibility of sporadic breast cancer. Methods: ASA-PCR method was used to analyze the rs11655505 (A/G), rs73625095 (A/G) SNPs of BRCA1 gene promoter region in 200 patients with breast cancer (all confirmed diagnosis) and nomal women, and their PCR products were all sequenced. Results: The A/G genotype frequency of BRCA1 gene promoter region rs11655505 site of patients with breast cancer (75%)was higher than that of the nomal person (40%);The A/A genotype frequency (7%)and the G/G genotype frequency (18%)were both lower than that of the nomal person(30%); There was no difference in the A or G alleles in this site between the cases and the controls (x^2=2.427,P=0.119). The A/G genotype frequency in rs73625095 site of patients with breast cancer (68%)was higher than that of the nomal person (15%), while the G/G genotype frequency (32%)was lower than that of the nomal person (84%); Comparing the A and the G alleles between the cases and the controls, it showed statistically significant differences (x^2=80.107,P=0.000) ; According to further analysis of the cases, there was statistically significant differences comparing A/G genotype in BRCA1 gene promoter region rs11655505, rs73625095 sites and lymph node metastases or not. (x^2=7.321,P=0.026, x^2=4.782,P=0.029) . Conclusions: A/G genotype in BRCA1 gene promoter region rs11655505, rs73625095 sites may have relationship with the sporadic breast cancer and lymph node metastases. A and G alleles may be the dangerous genetic factors of the sporadic breast cancer.

Key words: Sporadic breast cancer; BRCA1 gene; Single nucleotide polymorphisms (SNP); ASA - PCR; Genotype

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Introduction

BRCA1 gene is a suppressor gene of breast and ovarian cancer, which is located in 17q12-21. It plays a very important role in the cell cycle signaling process [1]. Currently, research on the BRCA1 gene single-nucleotide polymorphisms focuses on its exon area. And related reports showed SNPs in its exon area increased or reduced breast cancer susceptibility. There was few related reports on the relation between SNPs in the promoter region and the occurrence of breast cancer. In our study, first, the DNA of peripheral intravenous blood was extracted and PCR amplification was proceeded by using special primers; Second, the distribution of the single-nucleotide polymorphisms of rs11655505 site, rs73625095 site in BRCA1 gene promoter region was studied, and the relationship between the two sites and pathological characters of sporadic breast cancer was analyzed.

1 Material and Methods

1.1 Material

The experimental group includes the peripheral intravenous blood of 200 women with breast cancer accepted by Department of Breast Surgery, Affiliated Hospital of Qingdao University Medical College from October 2009 to April 2010. Every patient has been diagnosed confirmly; They are from 23 to 71 years old with the average age of 47. There are 181 cases of infiltrating ductal carcinoma, 8 cases of invasive lobular carcinoma and 11 cases of other types. The diameters of the tumor are from 0.2 to 10 cm with the the average of (3.25± 2.47)cm. Histologic grade: grade I 12 cases,grade II 116 cases,grade III 72 cases (American Joint Committee on Cancer Staging the sixth edition, 2003); The number of period Ⅰ and period Ⅱ is 197. The number of period III is 3. The number of patients with axillary lymph node metastasis are 84. There are 129 cases with positive estrogen receptor, 117 cases with positive progesterone receptor, 35 cases with strong positive HER-2 and 125 cases with positive P53. The control group includes the peripheral intravenous blood of 200 normal women , who are examined by Department of Physical Examination Center, Affiliated Hospital of Qingdao University Medical College from March 2010 to April 2010. The specimens are stored at ?80℃ immediately.

1.2 Experimental methods

1.2.1 ASA－PCR was used to examine single nucleotide polymorphisms in the BRCA1 gene promoter of 200 normal controls and 200 patients with sporadic breast cancer.

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Extraction of genomic DNA: using E.Z.N.A.™ SQ Blood DNA Kit to extract DNA in lymphocyte of Peripheral blood quickly and testing concentration and purity of DNA by ultraviolet spectrophotometry,stored in -20℃ environment.

The SNPS sites of the BRCA1 gene promoter sequence were searched according to NCBI, rs11655505 and rs73625095 were chosen, using the Primer Premier 5.0 software to design primers. The base sequences, annealing temperature and the length of PCR products were shown in table 1. PCR reaction system is 10μ L, and the ingredients of reaction liquid include DDW (2.7μ l), $MgSO_4$ (3mmol/l), dNTP (400 μ mol/l) , each primers (10mol/l), double-stranded DNA (1μ l), Taq template DNA polymerases(1.25 U) (Guangzhou enterprise biological technology Co.LTD). In the process of PCR amplification,the conditions of reaction are as follows: (1) rs11655505 (A/G) :94℃ 5min, 94℃30 s, 67℃30s, 72℃ 30 s, 72℃7 min, 30 cycles ;(2) rs73625095 (A/G) :94℃ 5min, 94℃30 s, 63℃30s, 72℃30 s, 72℃7 min, 30 cycles. 2 % agarose gel electrophoresis, EB dyeing, gel imaging analysis system.

Table 1 The sequence table of primers

Name of primers	base sequence	Annealing temperature	The length of PCR products
rs11655505-F	5'-TTCCAGTTGCGGCTTATTGC-3'		
rs11655505-R1	5'-GTGGGGTGAATCTAACATGGCGGAC-3'		
rs11655505-R2	5'-GTGGGGTGAATCTAACATGGCGGAT-3'	67℃	746bp
rs73625095-F	5'-CAGAGCAGAGGGTGAAGGC-3'		
rs73625095-R1	5'-AATACGAAAACATAACACTCCAGTC-3'	63℃	728bp
rs73625095-R2	5'-AATACGAAAACATAACACTCCAGTT-3'		

Note :the primers are all designed by Primer Premier 5.0 software, compound by Shanghai.sunny biology limited company

1.2.2 GG, AA genotypes segments recycling, purification and sequencing

The PCR reaction system of sequencing analysis is 50μ L, the ingredients of reaction liquid are DDW (13.5μ l), $MgSO_4$ (3mmol/l), dNTP (400 μ mol/l), each primer (10mol/l), double-stranded DNA (1μ l), Taq template DNA polymerases(1.25U) (Guangzhou enterprise biological technology Co.LTD). In the process of PCR amplification, The conditions of reaction are the same as above. The specimens are sent to Shanghai Sunny Biology Limited Company to sequence.

1.3 Statistical Methods

The representativeness of the study sample is tested by the Hardy-Wein-berg law of genetic equilibrium, The relationship between the distribution of genotype and allelethe and clinicopathological features are tested by test.

2 Results

PCR products included A/A, A/G and G/G genotypes accooding to SNPs. The cases and the controls were both in conformity with the Hardy-Wein-berg law.

2.1 The genotype frequency and allele frequency of rs11655505、rs73625095 sites in BRCA1 gene promoter area

The A/G genotype frequency of rs11655505 site in patients with breast cancer (75%)was higher than that of the nomal person (40%) (x^2=50.128, P=0.000); The A/A genotype frequency (7%) and the G/G genotype frequency (18%) were both lower than that of the nomal person (30%) (x^2=35.085,P=0.000, x^2=7.895,P=0.005). The A or G alleles in this site was no difference between the cases and the controls. The A/G genotype frequency of rs73625095 site in patients with breast cancer (68%) was higher than that of the nomal person (15%), which showed statistically significant differences (x^2=115.704,P=0.000), while the G/G genotype frequency(32%) was lower than that of the nomal person (84%) and showed statistically significant differences(x^2=111.002, P=0.000). Comparing the A and G alleles between the cases and the controls , it showed statistically significant differences (x^2=80.107,P=0.000) .They were showed at figure 1,2 and table 2.

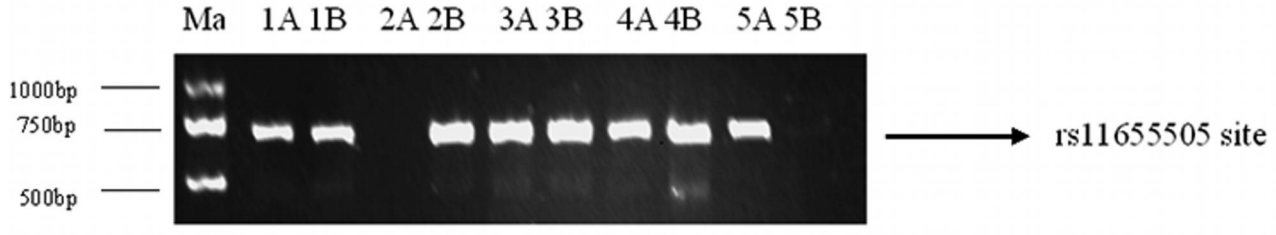

Fig.1 Genotype electrophoresis figure of rs11655505 site in BRCA1 Gene promoter region: the Marker was DS2000(100bp), the length of rs11655505 site produet was 746bp, lane 1A was amplification of sample 1 and rs11655505-F, rs11655505-R2 of rs11655505 site in BRCA1 gene; lane 1B was amplification of sample 1 and rs11655505-F, rs11655505-R1 of rs11655505 site in BRCA1 gene; the genotype of sample 1,3,4 was A/G, the genotype of sample 2 was G/G, the genotype of sample 5 was A/A

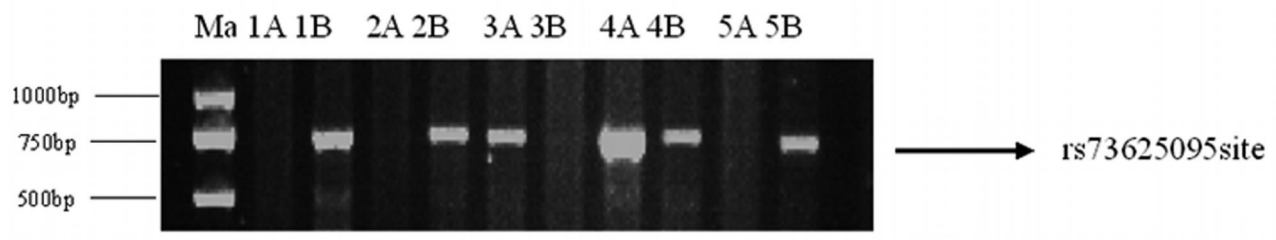

Fig.2 Genotype electrophoresis figure of rs7362505 site in BRCA1 Gene promoter region: the Marker was DS2000(100bp), the length of rs73625095 site product was 728bp, lane 4A was amplification of sample 4 and rs73625095-F, rs73625095-R2 of rs73625095 site in BRCA1 gene; lane 4B was amplification of sample4 and rs73625095-F, rs73625095-R1 of rs73625095 site in BRCA1 gene; the genotype of sample 4 was A/B, the genotype of sample 1,2, 5 was G/G, the genotype of sample3 was A/A

Table 2 The distribution of rs11655505、rs73625095 genotypes and alleles [% (cases)]

	cases	controls	x^2	Pvalue
Frequence of rs11655505 genotype (%)				
A/A	7.00 (14)	30.00 (60)	35.085	0.000*
A/G	75.00 (150)	40.00 (80)	50.128	0.000*
G/G	18.00 (36)	30.00 (60)	7.895	0.005*
Frequence of alleles (%)				
A	44.50 (178)	50.00 (200)	2.427	0.119
G	55.50 (222)	50.00 (200)	2.427	0.119
Frequence of rs73625095 genotype (%)				
A/A	0.00 (0)	1.00 (2)	2.010	0.156
A/G	68.00 (136)	15.00 (30)	115.704	0.000*
G/G	32.00 (64)	84.00 (168)	111.002	0.000*
Frequence of alleles (%)				
A	34.50 (136)	8.50 (34)	77.714	0.000*
G	65.50 (264)	91.50 (366)	80.107	0.000*

Note :the criterion of having a statistically significant difference is Pvalue <0.05

2.2 The sequencing results of PCR products of rs11655505、rs73625095 site was in line with that suplied by NCBI,they were showed at figure 3,4,5,6

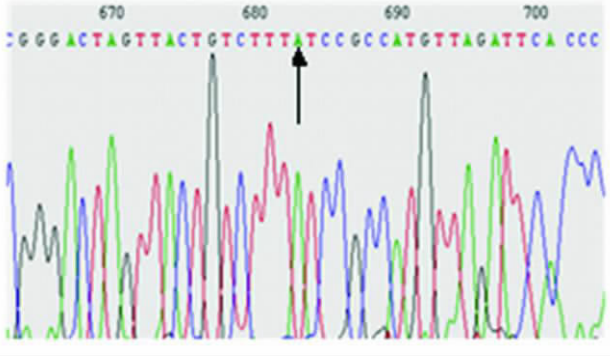

Fig 3 the A/A genotype sequencing of rs11655505 site in BRCA1 gene promotor area

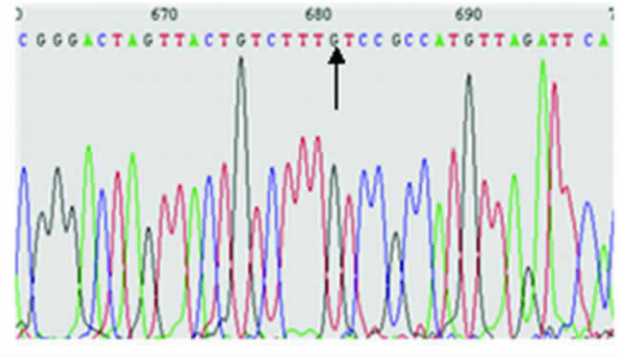

Fig 4 the G/G genotype sequencing of rs11655505 site in BRCA1 ene promotor area

2.3 Clinicopathological feature of all genotypes of rs11655505、rs73625095 site in sporadic breast cancer

The difference of the A/G genotype of rs11655505、rs73625095 site and lymphatic metastasis or not in the cases was significant (x^2=7.321，P=0.026、x^2=4.782，P=0.029). However, there were no significant differences compared to immunohistochemical features of ER、PR、Herb-2、P53 (Table 3,4).

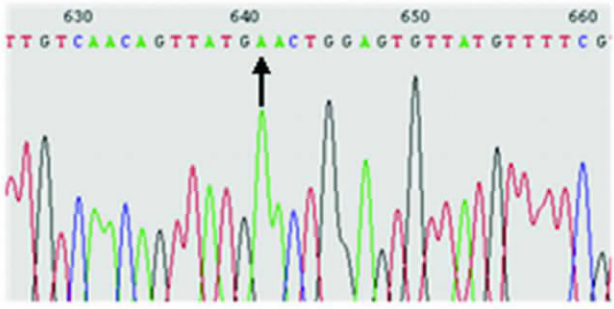

Fig 5 the A/A genotype sequencing of rs73625095 site in BRCA1 gene promotor area

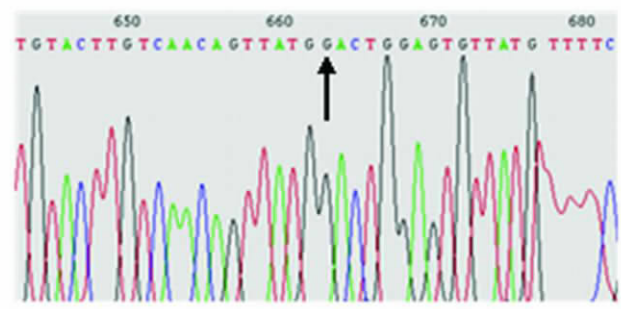

Fig 6 the G/G genotype sequencing of rs73625095 site in BRCA1 gene promotor area

Table 3 the relation between rs11655505 genotype in BRCA1 gene promoter area and clinical features[cases (%)]

		A/A	A/G	G/G	x^2	Pvalue
Diameter of tumor	≤ 2cm	11 (8.90)	90 (72.60)	23 (18.50)		
	>2cm	3 (3.90)	60 (78.90)	13 (17.20)	1.941	0.379
histological grade	grade I	0 (0.00)	11 (91.70)	1 (8.30)		
	grade II	8 (6.90)	85 (73.30)	23 (19.80)		
	grade III	6 (8.30)	54 (75.00)	12 (16.70)	2.424	0.658
Clinical stage	stage stageI+ II	14 (7.10)	147 (74.60)	36 (18.30)		
	stage III	0 (0.00)	3 (100.00)	0 (0.00)	1.015	0.602
lymphatic metastasis	(+)	9 (10.70)	55 (65.50)	20 (23.80)		
	(-)	5 (4.30)	95 (81.90)	16 (13.80)	7.321	0.026*
ER	(+)	7 (5.40)	100 (77.50)	22 (17.10)		
	(-)	7 (9.90)	50 (70.40)	14 (19.70)	1.774	0.412
PR	(+)	8 (6.80)	86 (73.50)	23 (19.70)		
	(-)	6 (7.20)	64 (77.10)	13 (15.70)	0.525	0.769
Herb-2	(-~++)	12 (7.30)	123 (74.50)	30 (18.20)		
	(+++)	2 (5.70)	27 (77.20)	6 (17.10)	0.143	0.931
P53	(+)	7 (5.60)	94 (75.20)	24 (19.20)		
	(-)	7 (9.30)	56 (74.70)	12 (16.00)	1.202	0.548

Note :the criterion of having a statistically significant difference is Pvalue <0.05

Table 4 The relation between rs73625095 genotype in BRCA1 gene promoter area and clinical features[cases (%)]

		A/A	A/G	G/G	x^2	Pvalue
diameter of tumor	≤ 2cm	0 (0.00)	86 (69.40)	38 (30.60)		
	>2cm	0 (0.00)	50 (65.80)	26 (34.20)	0.275	0.600
histological grade	grade I	0 (0.00)	6 (50.00)	6 (50.00)		
	grade II	0 (0.00)	81 (69.80)	35 (30.20)		
	grade III	0 (0.00)	49 (68.00)	23 (32.00)	1.965	0.374
Clinical stage	stage I+II	0 (0.00)	135 (68.50)	62 (31.50)		
	stage III	0 (0.00)	1 (33.33)	2 (66.67)	1.682	0.195
lymphatic metastasis	(+)	0 (0.00)	50 (59.50)	34 (40.50)		
	(-)	0 (0.00)	86 (74.10)	30 (25.90)	4.782	0.029*
ER	(+)	0 (0.00)	88 (68.20)	41 (31.80)		
	(-)	0 (0.00)	48 (67.60)	23 (32.40)	0.008	0.929

PR	(+)	0 (0.00)	80 (68.40)	37 (31.60)		
	(-)	0 (0.00)	56 (67.50)	27 (32.50)	0.018	0.892
Herb-2	(-~++)	0 (0.00)	116 (70.30)	49 (29.70)		
	(+++)	0 (0.00)	20 (57.10)	15 (42.90)	2.298	0.130
P53	(+)	0 (0.00)	89 (71.20)	36 (28.80)		
	(-)	0 (0.00)	47 (62.70)	28 (37.30)	1.569	0.210

Note :the criterion of having a statistically significant difference is Pvalue <0.05

3 Discussion

Breast cancer is induced by the common effect of heredity, immune and environmental factors. Nowadays, the research on breast cancer has reached the genetic level [2]. Moreover, the BRCA1 gene is a specific tumor suppressor gene of breast cancer and ovarian cancer, its change is highly related with occurrence of breast cancer and ovarian cancer. With the development of science, reseach on breast cancer has done further, not only confined to gene mutation, gene loss, and DNA methylation and so on. Recently, research on BRCA1 gene mostly concentrates on its mutation and SNPs. Mutation in the BRCA1 gene has been widely researched both at home and abroad. It is found that there are more than 300 mutation sites in BRCA1 gene, 48.5 % of which located in the exon 11[3]. The research of European genealogy showed that there are the hot regions of BRCA1gene mutation (the exon 2、5、16、20) [4,5]. Though there was relationship between the mutations of BRCA1gene and occurrence of breast cancer, its mutation could not be the only reason of occurrence of breast cancer. Recently, it is found that SNPs are highly related to survival rate and occurrence of breast cancer [6], especially related to DNA repair, hormone metabolism and oncogene metabolism [7]. Jing Cui has found that there are some SNP sites in the BRCA1 gene exon, which are associated with occurrence of breast cancer [8]. The data shows that SNPs in the exon could strengthen or weaken the susceptibility. Durocher has reported that polymorphism in the rs799917 site may be related with occurrence of breast cancer[9]. The research of Rao NY showed that the SNPs of rs16942 G and rsl060915 C in the BRCA1 code area was independent dangerous factor of breast cancer. rs16942G could prevent the occurrence of breast cancer, while rsl060915 C increased possibilities of occurrence of breast cancer. The occurrence risk of person with rsl060915 CC is 4.3 as much as that of person with rsl060915TT and it is 2.2 as much as that ofperson with rsl060915CT. There is linkage disequilibrium among BRCA1 rs16942 site , rsl060915 site and rsl799966 site.The haplotype GCG composed by three SNP is a dangerous factor of occurrence of breast cancer [10]. However, there was few research on the SNPs in the BRCA1 promoter region both at home and abroad. Kelvin YKC has found that c.-2265C>T (rs11655505:C>T) is highly related with the occurrence of breast cancer in a crowd of more than 3000 Asian population, it would largely increase the activity of BRCA1 promoter region and decrease the susceptibility of breast cancer further[11]. In the experiment, the rs11655505 site's A/G genetype frequency of BRCA1 promoter region (75%) was larger than that of controls (40%) (x^2=50.128，P=0.000) , which showed rs11655505 A/G genetype might have relationship with occurrence of breast cancer. The research also showed the lymphaden's transfer or not was closely associated with rs11655505 A/G genetype. Moreover, rs11655505 A/G genetype was inversely proportion to the lymphaden's transfer. These all confirmed that rs11655505 A/G genetype was related to occurrence of breast cancer, and it could decrease the susceptibility of breast cancer. These results were in accordence with foreign research [11]. The A/G genotype frequency in rs73625095 site (68%) was higher than that of the nomal person(15%),which has highly statistics significance(x^2=115.704，P=0.000). It showed no statistical significance comparing A/G genetype in rs11655505 site and rs73625095 site with the immunohistochemical features of ER, PR, Herb-2, P53. SNPs in the BRCA1 promoter region are related with occurrence and development of sporadic breast cancer. This study also proved some SNP sites in the promoter region could decrease the susceptibility of sporadic breast cancer, but there was no evidence to prove that SNP sites in the promoter region could increase the susceptibility of sporadic breast cancer, so it should be studied further. In summary, breast cancer is induced by the common effect of heredity ,immune and environmental factors. The research provided new reference for etiology study of sporadic breast cancer, research material for clinical treatment and prevention and reliable basis for the breast cancer's early screening.

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BRCA1 基因启动子区 SNPs 与散发性乳腺癌易感性的相关研究 *

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摘要 目的：探讨 BRCA1 基因启动子区 rs11655505、rs73625095 位点单核苷酸多态性与散发性乳腺癌易感性的关系。**方法：**采用 ASA-PCR 方法对 200 例乳腺癌患者（均经病理确诊）及 200 例正常女性 BRCA1 基因启动子区 rs11655505（A/G）、rs73625095（A/G）位点单核苷酸多态性(SNP)进行分析，并将其 PCR 产物进行测序。**结果：**乳腺癌患者 BRCA1 基因启动子区 rs11655505 位点的 A/G 基因型频率为 75%，显著高于正常人的 40%；A/A 基因型频率为 7%，G/G 基因型频率为 18%，分别低于正常人的 30%、30%。此位点的 A 或 G 等位基因在乳腺癌病例组及对照组中均无差别(x^2=2.427，P=0.119)；rs73625095 位点的 A/G 基因型频率为 68%，显著高于正常人的 15%；G/G 基因型频率为 32%，低于正常人的 84%；乳腺癌病例组中 BRCA1 基因启动子区 rs11655505、rs73625095 位点的 A/G 基因型与淋巴结转移与否相比，差别均有统计学意义（x^2=7.321，P=0.026、x^2=4.782，P=0.029）。**结论：**BRCA1 基因 rs11655505 位点、rs73625095 位点的 A/G 基因型可能与散发性乳腺癌的发生相关，而且与有无发生淋巴结转移密切相关。rs73625095 位点 A 和 G 等位基因可能为散发性乳腺癌发生的遗传危险因素。

关键词：散发性乳腺癌；BRCA1 基因；单核苷酸多态性；ASA-PCR；基因型

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