LncRNA MEG3 rs3087918 was associated with a decreased breast cancer risk in a Chinese population: a case-control study

Yi Zheng
Xi'an Jiaotong University Second Affiliated Hospital
Meng Wang
Xi'an Jiaotong University Second Affiliated Hospital
Shuqian Wang
Zhejiang University School of Medicine First Affiliated Hospital
Peng Xu
Xi'an Jiaotong University Second Affiliated Hospital
Yujiao Deng
Xi'an Jiaotong University Second Affiliated Hospital
Shuai Lin
Xi'an Jiaotong University Second Affiliated Hospital
Na Li
Xi'an Jiaotong University Second Affiliated Hospital
Kang Liu
Xi'an Jiaotong University Second Affiliated Hospital
Yuyao Zhu
Xi'an Jiaotong University Second Affiliated Hospital
Zhen Zhai
Xi'an Jiaotong University Second Affiliated Hospital
Ying Wu
Xi'an Jiaotong University Second Affiliated Hospital
Zhi-Jun Dai (✉ dzj0911@126.com)
https://orcid.org/0000-0001-5209-8626
Gaixia Zhu
Xi'an Jiaotong University Second Affiliated Hospital

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Abstract

Background

LncRNA MEG3 expressed abnormally in various cancers including breast cancer, but no studies reported the correlation between MEG3 SNPs and breast cancer susceptibility.

Methods

This study is aimed to explore the association between three SNPs of MEG3 (rs3087918, rs7158663, rs11160608) and breast cancer. The study is a population-based case-control study including 434 breast cancer patients and 700 healthy controls. Genotyping was performed using Sequence MassArray technique. Function prediction of rs3087918 were based on RNAfold and lncRNASNP2 databases.

Results

Pooled analysis indicated that rs3087918 was related to a decreased risk of breast cancer (GG vs. TT: P = 0.042; GG vs. TT + TG: P = 0.046), especially for women aged 49 and above (GG vs. TT: P = 0.02). Comparison between case groups showed genotype GG and TG/GG of rs3087918 were correlated with her-2 receptor expression (GG vs. TT: P = 0.010; TG + GG vs. TT: P = 0.045). We didn't find statistical significance for rs11160608, rs7158663 and breast cancer. Structure prediction based on RNAfold found rs3087918 may influence the secondary structure of MEG3. The results based on lncRNASNP2 indicated rs3087918 may gain the targets of hsa-miR-1203 to MEG3, while loss the target of hsa-miR-139-3p and hsa-miR-5091 to MEG3.

Conclusions

MEG3 rs3087918 was associated with a decreased risk of breast cancer. MEG3 haplotype TCG (SNP sequence: rs3087918, rs11160608, rs7158663) may increase the risk of breast cancer. And the protest effect of rs3087918 on breast cancer may owe to its effect on the structure and function of MEG3.

Introduction

Breast cancer (BC) is a serious threat to women's health. According to Global cancer statistics 2018[1], there will be an estimated 2.1 million new BC cases and 630 thousand BC related death in 2018. For females, BC is the most common diagnosed cancer (24.2% of the total cases) and the leading cause of cancer death (15.0% of the total cancer death). Although epidemiological studies have identified several risk factors that may be involved in BC, such as age, hormonal state, and family history[2], the pathogenesis of BC is still unclear. BC is a complex and genetically heterogeneous disease in which genetic changes such as abnormal amplification of oncogenes, or deletion/mutation of tumor suppressor genes, play a substantial role.

Maternally expressed gene 3 (MEG3) is an imprinted gene located at chromosome 14q32.3 in humans, encoding a long non-coding RNA (lncRNA) belonging to the imprinted DLK1-MEG3 regions [3]. This region contains at least three paternally expressed protein coding genes and numerous maternally expressed noncoding RNAs [4]. The imprinted expression of these genes was related to cell development and growth [5], and experiments in vitro indicated MEG3 can suppress the proliferation of human cancer cells lines [6]. Researchers found loss of MEG3 related to a variety of human cancers, such as gastric [7], cervical [8], and breast [9] cancer. MEG3 can inhibit the occurrence of tumor through various mechanisms. Firstly, MEG3 can inhibit the proliferation of tumor cells and consequently induce apoptosis, which has been confirmed by in vitro experiments and animal models [10]. Secondly, MEG3 plays a role in epigenetic regulation and can alter the function of cancer cells by affecting DNA methylation and regulating the functions of snoRNA and miRNA [11, 12]. Moreover, MEG3 is involved in the regulation of many tumor-related signaling pathways, including the classic p53, MDM2, and pRb pathway [13].

The genetic variants may influence the expression or function of MEG3 and consequently alter cancer susceptibility. However, there are no investigation to explore the relationship between MEG3 polymorphisms and breast cancer. In this study, we genotyped three polymorphisms (rs3087918, rs11160608 rs7158663) in MEG3 gene based on 434 BC patients and 700 healthy controls, to explore their relationship with breast cancer.

Materials And Methods

Study subjects

In total, 1134 females were recruited for this population-based case-control study. Among these, 434 breast cancers were enrolled in the Department of Oncology, the Second Affiliated Hospital, Xi'an Jiaotong University, from 2013 to 2015. 700 healthy females were randomly recruited from medical center of the same hospital during the same period. All BC patients were diagnosed by pathology and detailed immunohistochemical analysis. BC patients who had a history of other malignant diseases or receiving chemotherapy or radiotherapy were excluded. The controls were matched to cases by age (± 2 years) and had no history of malignant tumors, no history of chemoradiotherapy, no obvious abnormality in blood routine examination. The protocol of this study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi’an Jiaotong University Shaanxi Province (Xi’an, China). All patients gave written informed consent prior to participation in the study.

SNP selection and Genotyping

SNPs were selected according to NCBI database and literature. First, the minor allele frequency (MAF) was no less than 0.05 among Asian population. Secondly, the detection rate is less than 95%. Thirdly, The genotype distribution of the three polymorphisms in control groups accorded with Hardy–Weinberg Equilibrium (HWE). Peripheral blood samples were collected in EDTA-coated tubes and conserved at -80°C. Genome DNA were extracted from whole blood samples using ComWin BloodGen Mini Kit (QIAGEN, China, Beijing). Ultraviolet spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA) was

utilized to measure the purity and concentration of extracted DNA. We designed multiplexed SNP MassEXTEND assay using Sequenom MassARRAY Assay Design 3.0 software. DNA samples were genotyped by Sequenom MassARRAY RS1000 according to the standard protocol. The primers applied for the three SNPs were shown in supplemental table S1.

Statistical analysis

The HWE of the three SNPs were calculated using Fisher's exact test in controls group. Student's t test was adopted to evaluated the difference of age distribution and body mass index (BMI) between BC patients and healthy controls. Two-sided Pearson's chi-square tests were applied to access the differences in the categorical variables between cases and controls, such as age (< = 49 and > 49), BMI, menstrual-status, and allelic frequencies. $P < 0.05$ was considered statistically significant. We also calculated odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression analysis. Haplotype analysis were conducted by Haploview 4.2. Other statistical analyses were performed using the version R 3.5.2 software.

Function prediction based on databases

We used RNAfold (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi) and LncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP/) database to predict the effect of SNP on MEG3. RNAfold is a classic database to predict RNAs structure. Free energy represents the amount of energy that needs to be injected to change the structure. The smaller the corresponding value is, the more stable the structure will be. LncRNASNP2 is a novel database containing 7260238 SNPs on 141353 human lncRNA transcripts and 3921448 SNPs on 117405 mouse lncRNA transcripts [14]. We used this database to predict the potential function of the MEG3 polymorphisms.

Results

Demographical and clinical information of study population

This study contained 434 BC cases and 700 healthy control. All the subjects were Han Chinses women from northwest China. There were no statistically significant differences in age distribution, BMI and menopausal status between the patients and the control group. The detail demographical and clinical information was display in Table 1. BMI was a statistical index to estimate the body fat in people of any age. In this study, BMI was divided into four levels (underweight, normal weight, overweight, and obese) based on Chinese reference standard.

Table 1
Demographic information.

Characteristics		Cases (%)	Control (%)	P value
Number		434	700	
Age (mean ± SD)		51.95 ± 10.35	51.83 ± 17.28	0.879[a]
≦ 49		180(41.5)	298(42.6)	
49		254(58.5)	402(57.4)	0.716
BMI, kg/m2 (mean ± SD)		22.38 ± 2.61	22.71 ± 4.00	0.084[a]
Menopausal status				
Premenopausal		157(36.2)	188(41.8)	
Postmenopausal		277(63.8)	262(58.2)	0.506
TNM Stage				
		114(26.3)	-	-
		192(44.2)	-	-
		89(20.5)	-	-
		39(9)	-	-
Immunohistochemistry results				
ER	–	142(32.7)	-	-
	+	292(67.3)	-	-
PR	–	189(43.5)	-	-
	+	245(56.5)	-	-
Her-2	–	250(57.6)	-	-
	+	184(42.4)	-	-

[a] Student's t-test

BMI: body mass index; ER: estrogen receptor; PR: progesterone receptor; Her-2: human epidermal growth factor receptor-2.

The associations between MEG3 SNPs and BC risk

Three SNP locus of MEG3 (rs3087918, rs11160608 rs7158663) were genotyped in all recruited subjects, and their respond rate were 99.1%, 99.2% and 99.4%, respectively. The genotype distribution of the three polymorphisms in control groups accorded with HWE (rs11160608: P_{HWE} = 0.844; rs3087918: P_{HWE} = 0.968; rs7158663: P_{HWE} = 0.334. We didn't find statistical significance for rs11160608, rs7158663 and breast cancer (P > 0.05 in all genetic models). Pooled analysis indicated that rs3087918 was related to a decreased risk of breast cancer [GG vs. TT: OR (95%CI) = 0.67(0.45–0.99), P = 0.042; GG vs. TT + TG: OR (95% CI) = 0.69(0.48 0.99), P = 0.046]. The detail results were showed in Table 2.

Table 2
Association between MEG3 gene polymorphisms and risk of breast cancer (rs11160608, rs3087918, rs7158663)

SNPs	Genotype	Case(%)	Control(%)	OR (95%CI)	P value
genetic model		N = 434	N = 700		
rs11160608					
Co-dominate	AA	126(29.7)	227(32.4)	reference	
	AC	218(51.4)	341(48.7)	1.15(0.87–1.52)	0.316
	CC	80(18.9)	132(18.9)	1.09(0.77–1.55)	0.625
Dominate	AA	126(29.7)	227(32.4)	reference	
	AC + CC	298(70.3)	473(67.6)	1.14(0.87–1.48)	0.342
Recessive	AA + AC	344(81.1)	568(81.1)	reference	
	CC	80(18.9)	132(18.9)	1.00(0.74–1.36)	0.996
Allele	A	470(55.4)	795(56.8)	reference	
	C	378(44.6)	605(43.2)	1.06(0.89–1.26)	0.528
rs3087918					
Co-dominate	TT	171(40.2)	259(37.0)	reference	
	TG	207(48.7)	334(47.7)	0.94(0.72–1.22)	0.633
	GG	47(11.1)	107(15.3)	0.67(0.45–0.99)	0.042*
Dominate	TT	171(40.2)	259(37.0)	reference	
	TG + GG	254(59.8)	441(63.0)	0.87(0.68–1.12)	0.279
Recessive	TT + TG	378(88.9)	593(84.7)	reference	
	GG	47(11.1)	107(15.3)	0.69(0.48–0.99)	0.046*
Allele	T	549(64.6)	852(60.9)	reference	
	G	301(35.4)	548(39.1)	0.85(0.71–1.02)	0.077
rs7158663					
Co-dominate	GG	224(52.5)	403(0.6)	reference	

*The P Value < 0.05.

OR: odds ratio; CI: confidence interval.

SNPs	Genotype	Case(%)	Control(%)	OR (95%CI)	P value
genetic model		N = 434	N = 700		
	GA	170(39.8)	250(0.4)	1.22(0.95–1.58)	0.12
	AA	33(7.7)	47(0.1)	1.26(0.79–2.03)	0.333
Dominate	GG	224(52.5)	403(0.6)	reference	
	GA + AA	203(47.5)	297(0.4)	1.23(0.97–1.57)	0.094
Recessive	GG + GA	394(92.3)	653(0.9)	reference	
	AA	33(7.7)	47(0.1)	1.16(0.73–1.85)	0.52
Allele	G	618(72.4)	1056(75.4)	reference	
	A	236(27.6)	344(24.6)	1.17(0.97–1.42)	0.107
*The P Value < 0.05.					
OR: odds ratio; CI: confidence interval.					

Stratified Analysis by age, BMI and menopausal status

Then, we conducted stratified analysis based on age, BMI and menopausal status to further explore their effect on relationship between BC susceptibility and the three SNPs in MEG3. BMI was divided into two levels (BMI < 24 kg/m^2 and BMI > = 24 kg/m^2). No association was found between rs11160608, rs7158663 and breast cancer when stratified by age, BMI and menopausal status (Supplemental Table S2). Rs3087918 was related to a reduced susceptibility for women aged 49 and above [GG vs. TT: OR(95%CI) = 0.40(0.22–0.73), P = 0.02] (Table 3).

Table 3
Stratified Analysis of rs3087918 by age, BMI and menopausal status.

Group	rs3087918 (Case/Control)			
	TT	TG	GG	TG + GG
Age				
<=49	69/93	87/141	19/64	106/205
OR(95%CI)	1.00 (reference)	0.83(0.55–1.25)	0.40(0.22–0.73)	0.70(0.47–1.03)
P-value		0.378	0.002*	0.069
> 49	102/166	120/193	28/43	148/236
OR(95%CI)	1.00 (reference)	1.01(0.72–1.42)	1.06(0.62–1.81)	1.02(0.74–1.41)
P-value		0.945	0.832	0.901
BMI(kg/m2)				
< 24	134/206	147/254	35/74	182/328
OR(95%CI)	1.00 (reference)	0.89(0.66–1.20)	0.73(0.46–1.15)	0.85(0.64–1.13)
P-value		0.441	0.171	0.271
>=24	37/53	60/80	12/33	72/113
OR(95%CI)	1.00 (reference)	1.07(0.63–1.84)	0.52(0.24–1.14)	0.91(0.55–1.53)
P-value		0.794	0.100	0.727
Menstrual-status				
postmenopausal	114/167	128/201	29/65	157/266
OR(95%CI)	1.00 (reference)	0.93(0.67–1.29)	0.65(0.40–1.08)	0.87(0.64–1.18)
P-value		0.675	0.093	0.356
menstruating	57/92	79/133	18/42	97/175
OR(95%CI)	1.00 (reference)	0.96(0.62–1.48)	0.69(0.36–1.32)	0.90(0.59–1.35)
P-value		0.848	0.260	0.597
*The P Value < 0.05.				
BMI: body mass index; OR: odds ratio; CI: confidence interval.				

Relationship between MEG3 rs3087918 and clinical characteristics of BC

To further explore the effect of rs3087918 loci and clinicopathological information on BC susceptibility, correlation analysis was conducted in the cases group defined by age, BMI, menopausal status, tumor size, metastasis, clinical stage, ER/PR status and Her-2. As showed in Table 4, GG and TG + GG genotypes had an increased trend when Her-2 expressed positive [GG vs. TT: OR(95%CI) = 2.37(1.24–4.63), P = 0.010; TG + GG vs. TT: OR(95%CI) = 1.50(1.01–2.24), P = 0.045]. We further divided the cases into luminal, Her-2 and triple negative breast cancer (TNBC) groups according to molecular classification. However, we found no association between three SNPs of MEG3 and the different molecular typing states of BC (Table S3).

Table 4

Relationship between MEG3 rs3087918 and clinical characteristics of cases.

rs3087918	TT	TG	GG	TG + GG
Age				
> 49/<=49	102/69	120/87	28/19	148/106
OR(95%CI)	1.00 (reference)	0.93(0.62–1.408)	1.00(0.52–1.95)	0.94(0.64–1.40)
P-value		0.742	0.993	0.777
BMI(kg/m^2)				
>=24/<24	37/134	60/147	12/35	72/182
OR(95%CI)	1.00 (reference)	1.48(0.92–2.37)	1.24(0.59–2.63)	1.43(0.91–2.26)
P-value		0.104	0.571	0.12
Menstrual status				
yes/no	114/57	128/79	29/18	157/97
OR(95%CI)	1.00 (reference)	0.81(0.53–1.24)	0.81(0.42–1.59)	0.81(0.54–1.21)
P-value		0.33	0.526	0.307
Tumor size(cm)				
> 2/<=2	85/86	107/100	31/16	138/116
OR(95%CI)	1.00 (reference)	1.08(0.72–1.62)	1.96(1.01–3.92)	1.20(0.82–1.73)
P-value		0.701	0.05	0.35
Metastasis				
Positive/negtive	93/78	104/103	24/23	128/126
OR(95%CI)	1.00 (reference)	0.85(0.56–1.27)	0.88(0.46–1.68)	0.85(0.58–1.26)
P-value		0.422	0.686	0.419
Clinical Stage				
III-IV/I-II	51/120	59/148	16/31	75/179
OR(95%CI)	1.00 (reference)	0.94(0.60–1.47)	1.21(0.60–2.39)	0.99(0.65–1.51)

*The P Value < 0.05.

BMI: body mass index; ER: estrogen receptor; PR: progesterone receptor; Her-2: human epidermal growth factor receptor-2; OR: odds ratio; CI: confidence interval.

rs3087918	TT	TG	GG	TG + GG
P-value		0.778	0.579	0.948
ER				
Positive/negtive	115/56	138/69	33/14	171/83
OR(95%CI)	1.00 (reference)	0.97(0.63–1.50)	1.15(0.58–2.37)	1.00(0.66–1.51)
P-value		0.904	0.7	0.988
PR				
Positive/negtive	94/77	112/95	33/14	145/109
OR(95%CI)	1.00 (reference)	0.97(0.64–1.45)	1.93(0.98–3.97)	1.09(0.74–1.61)
P-value		0.867	0.063	0.666
Her-2				
Positive/negtive	62/109	90/117	27/20	117/137
OR(95%CI)	1.00 (reference)	1.35(0.89–2.05)	2.37(1.24–4.63)	1.50(1.01–2.24)
P-value		0.155	0.01*	0.045*
*The P Value < 0.05.				
BMI: body mass index; ER: estrogen receptor; PR: progesterone receptor; Her-2: human epidermal growth factor receptor-2; OR: odds ratio; CI: confidence interval.				

Haplotype analysis of MEG3 SNPs and associations with the risk of BC

To explore the combined effect the three SNPs in MEG3, we performed haplotype analysis by Haploview. The results of the haploid analysis indicated that TCG haplotype may increase the risk of breast cancer compared with the wild haplotype TAG [OR (95%CI) = 2.97(1.66–5.31), P < 0.001]. Other haplotypes showed no association with BC (Table 5). The order of the three SNPs was rs3087918, rs11160608 rs7158663.

Table 5
Haplotype analysis of MEG3 rs3087918.

Haplotypes	Control (%)	Case (%)	OR (95%)	P
TAG	293(41.89)	155(37.44)	reference	-
GCG	206(29.89)	105(25.36)	0.96(0.71–1.31)	0.811
TAA	94(13.89)	67(16.18)	1.35(0.93–1.95)	0.113
GCA	57(8.89)	33(7.97)	1.09(0.68–1.75)	0.707
TCG	21(3.89)	33(7.97)	2.97 (1.66–5.31)	< 0.001*
*The P Value < 0.05.				
The order of the three SNPs was rs3087918, rs11160608 rs7158663. Haplotypes with frequency less than 0.03 were excluded. OR: odds ratio; CI: confidence interval.				

The function prediction of the rs3087918 in MEG3

We used RNAfold (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi) and LncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP/) database to predict the potential function of rs3078918. The centroid secondary structure of rs3087918 was shown in Fig. 1, we learned that mutant allele “G” would significantly change the centroid secondary structure of MEG3. Moreover, its minimum free energy was change from − 28.87 kcal to -26. 90 kcal/mol, which mean rs3087918 may increase the structural stability of MEG3. The results of LncRNASNP2 indicated that rs3087918 may gain the targets of hsa-miR-1203 to MEG3 (lncRNA ID: NONHSAT039760.2), while loss the target of hsa-miR-139-3p and hsa-miR-5091 to MEG3 (See supplemental Table S3 and Figure S1).

Discussion

The occurrence of breast cancer is a result of a long-term complex interaction between individual genetic background and environmental exposure factors. As the most common type of genetic mutation, SNP is of great significance for breast cancer risk, diagnosis, individualized treatment and prognosis prediction. This study is aimed to investigate the association between MEG3 polymorphisms (rs3087918, rs11160608 rs7158663) and breast cancer. Our study recruited 1134 subjects containing 434 breast cancer patients and 700 healthy controls. The results indicated that the mutant homozygous GG of rs3087918 may associated with a decreased risk of BC, especially in females age < = 49. Comparison between case groups showed genotype GG and TG/GG of rs3087918 were correlated with her-2 receptor expression. The results of haplotype analysis for MEG3 showed that compared with wild haploid TAG, TCG haplotype may increase the risk of breast cancer, while other haplotypes were not significantly correlated with breast cancer risk. Furthermore, we found rs3087918 may influence the secondary structure of MEG3 and affect the bind of MEG3 to some miRNAs.

Previous evidences showed that MEG3 was highly expressed in normal tissues such as brain, pituitary, placenta and adrenal gland, and its transcripts can be detected in several human organs including ovary, testes, spleen, pancreas, liver, and mammary gland [4]. However, the expression of MEG3 was lower in various human tumors compared with that in normal human tissues, including breast cancer [15]. MEG3 was recognized as a tumor suppressor deponed on recent researches. In vitro experiments showed that restoring the expression of MEG3 could inhibit cancer cells proliferation and induce their apoptosis [16], and a similar tumor inhibition effect was found in nude mice [13]. MEG3 can also participant in epigenetic regulation of transcripts in the MEG3 region, such as DNA methylation [17, 18], snoRNA/microRNA regulation [19–22]. We used a database named LncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP/) to predict the potential function of rs3087918 on MEG3 gene. The results indicated that rs3087918 may influence MEG3 binding to miRNAs. In detail, rs3087918 may gain the targets of hsa-miR-1203 to MEG3, while loss the target of hsa-miR-139-3p and hsa-miR-5091 to MEG3. A study performed by Tomoyuki Okumura et al. found has-miR-1203 significantly associated with tumor recurrence [23]. Downregulation of has-miR-139-3p could induce cancer cell migration and invasion [24–26], and a pooled analysis proved that high has-miR-139-3p expression was related to a better prognosis for hepatocellular carcinoma [27]. Thus, has-miR-139-3p was attributed as a tumor suppressor. Hsa-miR-5091 was also reported as a biomarker with better prognosis for pancreatic ductal adenocarcinoma [28]. These were coincident with our results that rs3087918 was related to a decreased risk of breast cancer.

To be best of our knowledge, this is the first study to explore the association between MEG3 SNPs (rs3087918, rs11160608 rs7158663) and breast cancer risk. We used scientific methods and designs and finally obtained credible results. However, there are still some potential limitation should to be clarify. First, we failed to consider the potential influence of environmental, lifestyle and other unknow risk factors on our study. Secondly, this is a one center case-control study with a small samples scale, we should not ignore the selective bias. In the future, more complete and larger sample scale study need to accomplish.

Conclusion

The wild-type homozygous GG of MEG3 rs3087918 was associated with a decreased risk of breast cancer. MEG3 haplotype TCG (SNP sequence: rs3087918, rs11160608, rs7158663) may increase the risk of breast cancer. And the protest effect of rs3087918 on breast cancer may owe to its effect on the structure and function for MEG3.

Supplemental Attachments

Figure S1. The prediction results of s3087918 affect the bind of MEG3 to miRNAs. (A) rs3087918 caused has-miR1203 target gain; (B) rs3087918 caused has-miR-139-3p target loss; (C) rs3087918 caused has-miR-5091 target loss.

Table S1. Primers used for this study.

Table S2. Stratified Analysis of rs11160608 and rs7158663 by age, BMI and menopausal status.

Table S3. Association analysis between three SNPs inMEG3 and Molecular typing of breast cancer.

Table S4. Rs3087918 influence MEG3 binding to miRNAs based on LncRNASNP2 database.

List Of Abbreviations

BC: Breast cancer; MEG3: Maternally expressed gene 3; lncRNA: long non-coding RNA; MAF: minor allele frequency; HWE: Hardy–Weinberg Equilibrium; BMI: body mass index; ORs: odds ratios; CIs: 95% confidence intervals;

Declarations

Ethics approval and consent to participate

The protocol of this study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University Shaanxi Province (Xi'an, China). All patients gave written informed consent prior to participation in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declare no conflicts of interest for this study.

Funding

Not applicable.

Authors' contributions

Kang Liu, Yuyao Zhu, Zhen Zhai, and Ying Wu collected the samples. Shuqian Wang, Peng Xu, Yujiao Deng, Shuai Lin and Na Li detected the SNPs. Zhijun Dai, Gaixia Zhu, and Yi Zheng also provided patients. Zhijun Dai and Gaixia Zhu guided experiments. Yi Zheng and Meng Wang analyzed and

interpreted the data. Yi Zheng was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Figures

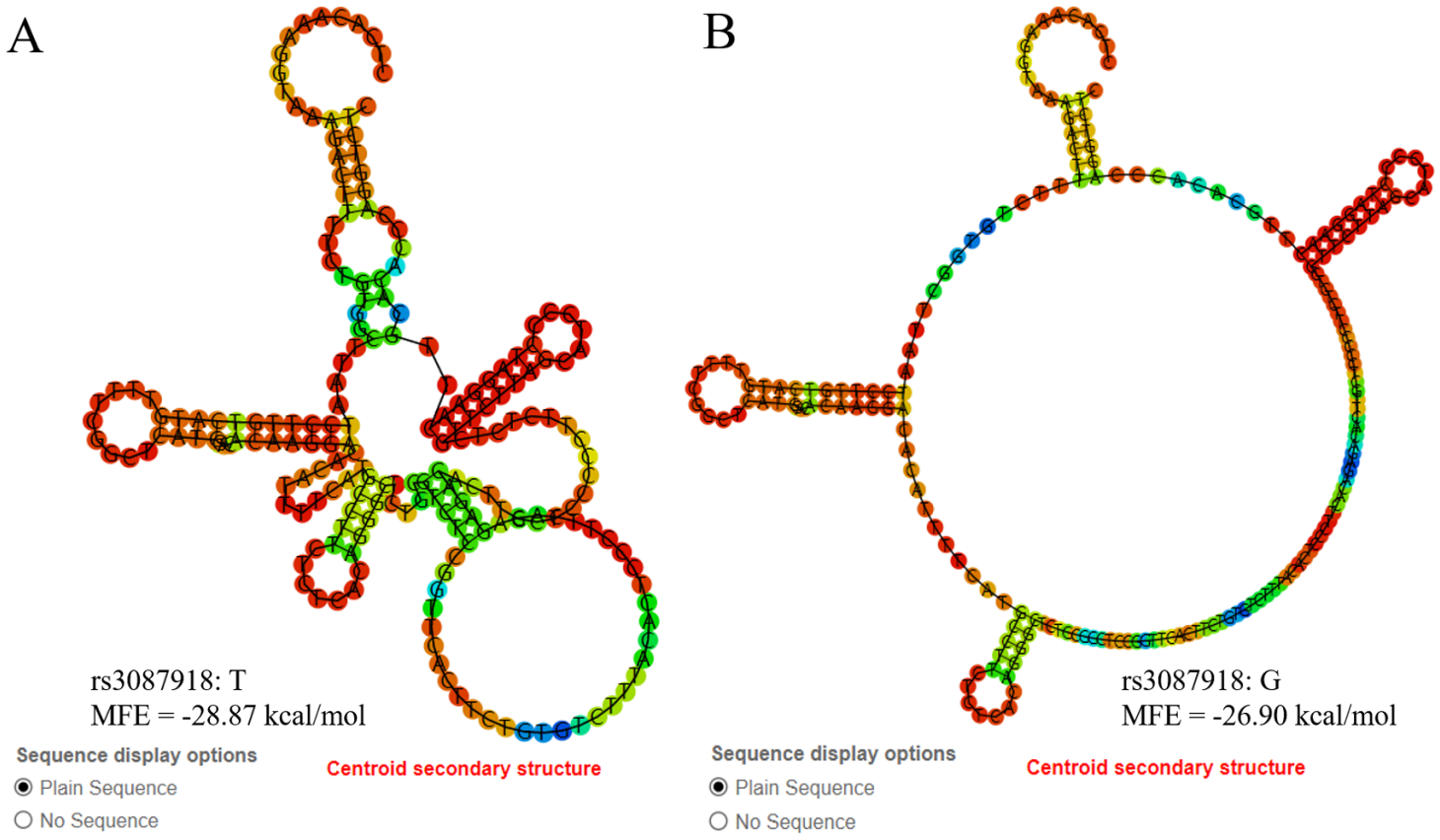

Figure 1

he RNAfold algorithm in silico predicting the impact of rs3087918. MFE: minimum free energy.

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