Received: 25 August 2021 | Revised: 1 November 2021 | Accepted: 23 November 2021

DOI: 10.1002/jcla.24167

RESEARCH ARTICLE

WILEY

Association between the rs3802201 polymorphism of the lncRNA MIR2052HG gene and the risk of recurrent miscarriage in a Southern Chinese population

Hanran Mai[1,2] | Canhong Cai[3] | Kun Lin[1] | Linyuan Zhang[1] | Yishuai Wang[1,4] | Menghua He[5] | Yanxia Qu[6] | Yufen Xu[1] | Lanyan Fu[1] | Lei Pi[1] | Huazhong Zhou[1] | Dingke Zeng[7] | Di Che[1] | Liandong Zuo[2]

[1]Department of Clinical Biological Resource Bank, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

[2]Department of Andrology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

[3]Department of Clinical Lab, Chaozhou People's Hospital, Chaozhou, China

[4]School of Medicine, South China University of Technology, Guangzhou, China

[5]School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou, China

[6]Department of Gynecology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

[7]Department of Surgery, Guangzhou Chest Hospital, Guangzhou, China

Correspondence
Liandong Zuo, Department of Andrology, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, Guangdong, China.
Email: zuold@163.com

Funding information
This study was funded by the Guangdong Provincial Science and Technology Plan (China, 2017A030223003), Guangzhou Medical and Health Technology Projects (China, 20191A011021 and 20191A011033), the Guangdong Natural Science Foundation (China, 2019A1515012061), the Guangzhou Institute of Pediatrics/Guangzhou Women and Children's Medical Center Fund (China, YIP-2019-050), Guangzhou Science and Technology Program Key Projects (China, 201904010486), and the Guangzhou Health Commission (China, 20211A011034).

Abstract

Background: Plenty of studies have indicated that some genetic polymorphisms of the breast cancer which associated with its susceptibility may also be related to the susceptibility of abortion. MIR2052HG plays an important role in the onset and progression of breast cancer by maintaining the level of ERα, but to the best of our knowledge, the correlation between risk of recurrent abortion and MIR2052HG rs3802201 C>G polymorphism is still unclear. Therefore, we conducted this case-control study to investigate whether MIR2052HG rs3802201 C>G polymorphism is associated with susceptibility of recurrent miscarriage (RM).

Methods: We recruited 392 healthy controls and 248 patients with RM to process this research, the participants were all from southern China, and genotyping was performed by TaqMan method.

Results: Our results showed that there was no evidence indicates the MIR2052HG rs3802201 C>G is related to RM (CG and CC: adjusted OR = 0.970, 95% CI = 0.694–1.355, $p = 0.8577$; GG and CC: adjusted OR = 0.743, 95% CI = 0.416–1.330, $p = 0.3174$; dominant model: adjusted OR = 0.925, 95% CI = 0.672–1.272, $p = 0.6298$; recessive model: adjusted OR = 0.751, 95% CI = 0.430–1.321, $p = 0.3233$).

Hanran Mai and Canhong Cai are contributed equally to this study.

Conclusion: We verified that the MIR2052HG rs3802201 C>G allele might be uncorrelated to the RM risk, but these findings require further validation in multicenter studies with larger sample size and different ethnicities.

KEYWORDS
MIR2052HG, recurrent miscarriage, single nucleotide polymorphism

1 | INTRODUCTION

Recurrent miscarriage (RM) is defined as women had three or more consecutive loss of pregnancies before the 20th weeks of gestation with the same sexual partner.[1,2] About 1% of couples are affected by RM, and no less than 50% of those have no obvious pathogenesis to be identified.[3-5] RM is associated with multiple factors, such as genetics, uterine abnormalities, thrombotic tendency, hormone or metabolic disorders, infection, autoimmunity, age, and lifestyle problems.[6]

Number of studies had showed that genetic susceptibility may play an important role in RM.[7-9] In recent years, as the researches on RM gradually increased, the correlation between genetic polymorphisms and recurrent abortion is becoming more pronounced. Shi et al. recently published a large meta-analysis in which 53 polymorphisms of 37 genes were shown to be associated with RM.[8] Our laboratory had also conducted relevant researches by investigating the association between TOX3(rs3803662) and RM, but there was no significant correlation discoverd.[10] Inspiringly, other researches have found that some polymorphisms related to susceptibility of breast cancer are also participated in occurrence of RM.[11-13] Soon after, another results we conducted[14] have noticed the polymorphism of LncRNA HULC was associated with susceptibility of recurrent spontaneous abortion, which is also associated with the promotion of breast cancer.[15] On the other hand, we have also found that the polymorphism of LncRNA MALAT1 can decrease the susceptibility of RM.[16] Interestingly, LncRNA MALAT1 is also considered as a factor that helps suppress breast cancer metastasis.[17] Such interesting discoveries reveal the essential connection between LncRNA and RM. Therefore, exploring the relationship between LncRNA and the gene which connected with breast cancer pathogenesis can provide an essential reference for understanding the causes of RM.

MIR2052 host gene (MIR2052HG) is a long noncoding RNA(LncRNA) which is 32.4 kb in length and located in 8q21.11–q21.13.[18] LncRNA MIR2052HG participates in the development of breast cancer, and it has been reported to be expressed in multiple type of breast cancer, including the most common type-Erα+ breast cancer.[19] According to previous studies, MIR2052HG sustained estrogen receptor α (ERα) levels both by promoting protein kinase B/forkhead box O3 (Akt/FOXO3)-mediated ESR1 transcription and by limiting ubiquitin-mediated, proteasome-dependent degradation of ERα.[19,20] Thus, the depletion of MIR2052HG is considered to be associated with reduced estrogen level and decreased cellular proliferation as well as tumor metastasis. Some single nucleotide polymorphisms (SNPs) in or near MIR2052HG have been reported to help curing breast cancer.[19] Depending on previous reports, we believe that there may be correlation between SNP in MIR2052HG and RM risk. However, there are no previous studies on the association between MIR0252HG polymorphism rs3802201 C>G and RM. Thus, the main aim of the current study was to determine the relationship between rs3802201 and RM.

2 | MATERIALS AND METHODS

2.1 | Study population

Same as another article we had published, we recruited 248 women which were diagnosed with RM at Guangzhou Women and Children's Medical Center between June 2017 and July 2018 to conduct our research. The standard of diagnosis was defined as two or more consecutive pregnancy losses before 20 weeks of gestation.[21] Besides, this study also included 392 age-matched healthy controls with at least two normal pregnancies and had no history of miscarriage.

2.2 | Genotyping and DNA extraction

Clinical Biological Resource Bank of the Guangzhou Women's and Children's Medical Center would supply all the blood samples we need for the research. We extracted total genomic DNA from blood by using a TIANamp Blood DNA Kit (TianGen Biotech Co., Ltd.). We conducted the genotype of SNP rs3802201 by the TaqMan real-time polymerase chain reaction protocol on an ABI Q6 (QuantStudio™ 6 Flex Real-Time PCR System, Applied Biosystems) and performed in 384-well plates. Besides, about 10% of samples were randomly selected for sequencing for quality control purposes and validation of genotyping results. The results were 100% concordant.

2.3 | Statistical analysis

Goodness-of-fit chi-square test was conducted to calculate the Hardy-Weinberg equilibrium (HWE) of the control subjects. We compared the demographic and genotypic differences between cases and controls of RM by the chi-square test. Unconditional univariate and multivariate logistic regression analyses were performed. To assess the strength of the association between rs3802201 and

susceptibility to recurrent spontaneous abortion, we applied age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs). We also performed the stratified analysis of number of abortions and age. The *p*-value less than 0.05 was considered as statistically significant, and all statistical tests were bilateral and calculated using SAS software (version 9.1; SAS Institute).

A plausible value of important biological effect (an OR of 1.5 for risk effect and 0.67 for protective effect) combined with the number and *p* value of observation in the table (Tables 1 and 2) were using for calculated statistical power.[22-24]

2.4 | Ethics statement

The study was approved by the Ethics Review Committee of the Guangzhou Women and Children's Medical Center. Written informed consent was obtained from each participant.

3 | RESULT

3.1 | Association between MIR2052HG polymorphisms and RM susceptibility

The demographic characteristics of recurrent spontaneous abortion group and control group are listed in Table S1.

Table 1 has demonstrated the genotype distribution of the MIR2052HG rs3802201 C>G polymorphism in RM group and controls group. The HWE of control group was shown for MIR2052HG rs3802201 C>G genotypes (HWE = 0.636). However, we observed no significant association between MIR2052HG rs3802201 polymorphism and susceptibility to RM (CG and CC: adjusted OR = 0.970, 95% CI = 0.694–1.355, $p = 0.8577$; GG and CC: adjusted OR = 0.743, 95% CI = 0.416–1.330, $p = 0.3174$; dominant model: adjusted OR = 0.925, 95% CI = 0.672–1.272, $p = 0.6298$; recessive model: adjusted OR = 0.754, 95% CI = 0.430–1.321, $p = 0.3233$).

3.2 | Stratification analysis

We stratified the subjects by age and the number of abortions to further assess the effects of the MIR2042HG rs3802201 C>G polymorphism in RM group and control group (Table 2). Our stratification analysis results suggested that the MIR2052HG rs3802201 C>G polymorphism was not significantly associated with RM susceptibility in different age groups or the number of abortions.

4 | DISCUSSION

The occurrence and development of RM may be affected by genetic susceptibility.[25] However, studies on the pathogenesis of RM were mainly focused on the protein coding gene, such as NKG2D, HRG, and PDE8B. Few researches have explored the relationship between long noncoding RNA polymorphisms and RM.[26-28] In the present study, we investigate the association between LncRNA MIR2052HG gene polymorphism (rs3802201 C>G) and RM susceptibility to this condition for the first time.

As the functions of MIR2052HG were not yet fully explored, according to limited information, MIR2052HG regulates Lemur tyrosine kinase 3 (LMTK3) through interacting with the early growth response 1 (EGR1) protein and initiate movement of the MIR2052HG-EGR1 complex toward the LMTK3 locus to regulate the transcribe of LMTK3.[29] The low expression of LMTK3 will lead to the upregulation of aromatase, kinase B/forkhead box O3(Akt/FOXO3), resulting in the stability of ERα and ESR1. In short, depletion of MIR2052HG will lead to low level of aromatase, estrogen, and reduced cellular proliferation and tumor metastasis. But due to

TABLE 1 Genotype and allele frequencies of MIR2052HG in RM patients and controls

Genotype/allele	RM (*N* = 248)	Controls (*N* = 392)	*p*-value	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value[d]
mir/rs3802201 C>G (HWE =0.5725)							
CC	123 (49.60)	187 (47.70)		1.00	/	1.00	/
CG	105 (42.34)	164 (41.84)	/	0.973 (0.697–1.360)	0.8743	0.970 (0.694–1.355)	0.8577
GG	20 (8.06)	41 (10.46)	/	0.742 (0.415–1.326)	0.3133	0.743 (0.416–1.330)	0.3174
Additive			0.59	0.904 (0.708–1.155)	0.4196	0.904 (0.707–1.154)	0.4167
Dominant	125 (50.40)	205 (52.30)	0.6407	0.927 (0.674–1.274)	0.6406	0.925 (0.672–1.272)	0.6298
Recessive	228 (91.94)	351 (89.54)	0.3101	0.751 (0.429–1.315)	0.3162	0.754 (0.430–1.321)	0.3233

Note: Statistical power was calculated using an odds ratio of 0.67 (for protective effect) and the number and *p* value of observation in the table.
[d]P-value after adjustment.

TABLE 2 Stratification analysis for associations between MIR2052HG polymorphism and RM risk in an south Chinese population

	rs3802201 (cases/controls)						
Variable	**CGGG**	**CC**	***p***	**OR (95% CI)**	***p***	**Adjust OR (95% CI)**	***p***
Age							
<35	95/159	92/129	0.347	0.838 (0.579–1.212)	0.3471	/	/
35–40	28/43	24/49	0.4124	1.329 (0.672–2.629)	0.4131	/	/
>40	2/3	7/9	0.8822	0.857 (0.111–6.617)	0.8825	/	/
No. of abortion/%							
2–3	81/187	88/205	0.961	0.991 (0.691–1.422)	0.961	0.985 (0.686–1.416)	0.9364
4≥	42/187	37/205	0.3757	0.804 (0.495–1.304)	0.3762	0.808 (0.498–1.313)	0.3896

Note: Statistical power was calculated using an odds ratio of 0.67 (for protective effect)/1.5 (for risk effect) and the number and *p* value of observation in the table.

lack of consensus on the mechanisms of how exactly MIR2052HG takes part in the development of breast cancer, as well as insufficient studies on MIR2052HG, more researches are required for further understanding of MIR2052HG.

Although the function of most LncRNAs is still unclear, many studies have revealed that some LncRNAs involve in the onset and development of abortion.[29-31] We had also carry on a study about relationship between LncRNA TINCR (rs2288947) polymorphisms and susceptibility of RM, but the result showed there was no significant correlation between rs2288947 and RM.[32] Positive result had arisen in our following researches, which indicated that some LncRNA polymorphisms related to breast cancer were also associated with the development of RM. Thus, there is reason to believe that more LncRNAs polymorphisms which might be association with RM are waiting to be found.[14,16] Our laboratory had also found that the rs6983267 polymorphism of CCAT2, a LncRNA that takes part in the progression of intrahepatic cholangiocarcinoma, small cell cancer of lung and breast cancer, has association with recurrent spontaneous abortion. Similar to CCAT2, MIR2052HG is also associated with breast cancer susceptibility and it is possible that the polymorphisms of MIR2052HG are also related to recurrent abortion susceptibility.[33] But in our present case-control study, we did not find any significant association between the rs3802201 and RM susceptibility. This may due to the different role of the rs3803662 C>T polymorphism in different diseases, and larger sample sizes and multicenter cohorts are needed for confirm.

There were several limitations to our current findings. First, the population of our study is limited to Southern Chinese population, and no cases or controls from other ethnic groups were assess to this study, according to dbSNP database (https://www.ncbi.nlm.nih.gov/snp), and rs3802201 C>G frequencies are different between populations (East Asian, $G = 0.36$; Latin American, $G = 0.226$; African, $G = 0.1083$; European, $G = 0.34047$). Such differences may indicate the disease susceptibility of different ethnic groups is variable. As the incidence of abortion also varies greatly among different ethnic groups, more studies are need to be conducted to explore the function and distribution of rs3802201 C>G in different populations. Secondly, because of the absence of data, we did not consider other important factors such as smoking, drinking, and lifestyle habits in the stratified analysis, which may affect our result. Thirdly, our sample size in this study is relatively small and a larger sample size is needed to confirm our previous results or other polymorphisms of MIR2052HG.

In summary, in this case-control study, we recruit 248 RM patients and 392 healthy controls from Southern Chinese population, but no significant correlation between the MIR2052HG gene rs3802201C>G polymorphism and recurrent abortion was observed. In the stratified analysis, the association between LncRNA MIR2052HG rs3802201 C>G polymorphism and times of abortions or age groups was also not significant. These results may indicate that the LncRNA MIR2052HG rs3802201 C>G polymorphism is not a appropriate biomarker for the diagnosis or prognosis of RM. However, we need larger sample size to confirm these results.

ACKNOWLEDGMENTS

We would like to thank the Clinical Biological Resource Bank of Guangzhou Women and Children's Medical Center for providing all the clinical samples.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

ORCID

Hanran Mai https://orcid.org/0000-0002-6064-7872

REFERENCES

1. Yazdani N, Shekari Khaniani M, Bastami M, et al. HLA-G regulatory variants and haplotypes with susceptibility to recurrent pregnancy loss. *Int J Immunogenet*. 2018;45(4):181-189.
2. Diejomaoh MF. Recurrent spontaneous miscarriage is still a challenging diagnostic and therapeutic quagmire. *Med Princ Pract*. 2015;24(Suppl. 1):38-55.
3. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril*. 2010;93(4):1234-1243.

4. Kutteh WH. Novel strategies for the management of recurrent pregnancy loss. *Semin Reprod Med*. 2015;33(3):161-168.
5. Stirrat GM. Recurrent miscarriage. *Lancet*. 1990;336(8716):673-675.
6. Shahine L, Lathi R. Recurrent pregnancy loss: evaluation and treatment. *Obstet Gynecol Clin North Am*. 2015;42(1):117-134.
7. Arias-Sosa LA, Acosta ID, Lucena-Quevedo E, et al. Genetic and epigenetic variations associated with idiopathic recurrent pregnancy loss. *J Assist Reprod Genet*. 2018;35(3):355-366.
8. Shi X, Xie X, Jia Y, et al. Maternal genetic polymorphisms and unexplained recurrent miscarriage: a systematic review and meta-analysis. *Clin Genet*. 2017;91(2):265-284.
9. Pereza N, Ostojić S, Kapović M, et al. Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. *Fertil Steril*. 2017;107(1):150-159 e152.
10. Huang W, Zhou H, Li Q, et al. Association between the TOX3 rs3803662 C>T polymorphism and recurrent miscarriage in a southern Chinese population. *J Clin Lab Anal*. 2019;33(9):e22992.
11. Gallegos-Arreola MP, Marquez-Rosales MG, Sanchez-Corona J, et al. Association of the Del1518 promoter (rs3730485) polymorphism in the MDM2 gene with breast cancer in a Mexican population. *Ann Clin Lab Sci*. 2017;47(3):291-297.
12. Nejati-Azar A, Alivand MR. miRNA 196a2(rs11614913) & 146a(rs2910164) polymorphisms & breast cancer risk for women in an Iranian population. *Per Med*. 2018;15(4):279-289.
13. Parveen F, Agrawal S. Recurrent miscarriage and micro-RNA among north Indian women. *Reprod Sci*. 2015;22(4):410-415.
14. Fang Z, Yang Y, Xu Y, et al. LncRNA HULC polymorphism is associated with recurrent spontaneous abortion susceptibility in the southern Chinese population. *Front Genet*. 2019;10:918.
15. Wang N, Zhong C, Fu M, et al. Long non-coding RNA HULC promotes the development of breast cancer through regulating LYPD1 expression by sponging miR-6754-5p. *Onco Targets Ther*. 2019;12:10671-10679.
16. Che DI, Yang Y, Xu Y, et al. The lncRNA MALAT1 rs619586 G variant confers decreased susceptibility to recurrent miscarriage. *Front Physiol*. 2019;10:385.
17. Kim J, Piao H-L, Kim B-J, et al. Long noncoding RNA MALAT1 suppresses breast cancer metastasis. *Nat Genet*. 2018;50(12):1705-1715.
18. Melzer D, Perry JRB, Hernandez D, et al. A genome-wide association study identifies protein quantitative trait loci (pQTLs). *PLoS Genet*. 2008;4(5):e1000072.
19. Ingle JN, Xie F, Ellis MJ, et al. Genetic polymorphisms in the long noncoding RNA MIR2052HG offer a pharmacogenomic basis for the response of breast cancer patients to aromatase inhibitor therapy. *Cancer Res*. 2016;76(23):7012-7023.
20. Giamas G, Filipović A, Jacob J, et al. Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. *Nat Med*. 2011;17(6):715-719.
21. Rai R, Regan L. Recurrent miscarriage. *Lancet*. 2006;368(9535):601-611.
22. Wacholder S, Chanock S, Garcia-Closas M, et al. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst*. 2004;96(6):434-442.
23. Engel LS, Taioli E, Pfeiffer R, et al. Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review. *Am J Epidemiol*. 2002;156(2):95-109.
24. Marcus PM, Vineis P, Rothman N. NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. *Pharmacogenetics*. 2000;10(2):115-122.
25. Daher S, Mattar R, Gueuvoghlanian-Silva BY, et al. Genetic polymorphisms and recurrent spontaneous abortions: an overview of current knowledge. *Am J Reprod Immunol*. 2012;67(4):341-347.
26. Elenis E, Lindgren KE, Karypidis H, et al. The histidine-rich glycoprotein A1042G polymorphism and recurrent miscarriage: a pilot study. *Reprod Biol Endocrinol*. 2014;12:70.
27. Granfors M, Karypidis H, Hosseini F, et al. Phosphodiesterase 8B gene polymorphism in women with recurrent miscarriage: a retrospective case control study. *BMC Med Genet*. 2012;13:121.
28. Hizem S, Mtiraoui N, Massaoudi S, et al. Polymorphisms in genes coding for the NK-cell receptor NKG2D and its ligand MICA in recurrent miscarriage. *Am J Reprod Immunol*. 2014;72(6):577-585.
29. Barwal TS, Sharma U, Bazala S, et al. MicroRNAs and long noncoding RNAs as novel therapeutic targets in estrogen receptor-positive breast and ovarian cancers. *Int J Mol Sci*. 2021;22(8).
30. Wang H, Cao Q, Ge J, et al. LncRNA-regulated infection and inflammation pathways associated with pregnancy loss: genome wide differential expression of lncRNAs in early spontaneous abortion. *Am J Reprod Immunol*. 2014;72(4):359-375.
31. Wang L, Tang H, Xiong Y, et al. Differential expression profile of long noncoding RNAs in human chorionic villi of early recurrent miscarriage. *Clin Chim Acta*. 2017;464:17-23.
32. Huang W, Zhou H, Pi L, et al. Association between the rs2288947 polymorphism of the lncRNA TINCR gene and the risk of recurrent miscarriage in a Southern Chinese population. *J Clin Lab Anal*. 2019;33(6):e22919.
33. Che D, Huang W, Fang Z, et al. The lncRNA CCAT2 rs6983267 G allele is associated with decreased susceptibility to recurrent miscarriage. *J Cell Physiol*. 2019;234(11):20577-20583.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mai H, Cai C, Lin K, et al. Association between the rs3802201 polymorphism of the lncRNA MIR2052HG gene and the risk of recurrent miscarriage in a Southern Chinese population. *J Clin Lab Anal*. 2022;36:e24167. doi:10.1002/jcla.24167