ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20230454

miR604A>G gene polymorphism is associated with recurrent pregnancy loss in Turkish women

Esra Tekcan[1*], Ulas Coban[2], Mesut Onal[2], Sengul Tural[3]

SUMMARY

OBJECTIVE: Recurrent pregnancy loss is considerably a reproductive health problem for couples. Genetic, epigenetic, and environmental factors play an important role in the development of recurrent pregnancy loss. While there are many causes, genetic and epigenetic factors are common. In this study, we aimed to examine the association between miR604 (rs2368393) A>G gene polymorphism and the risk of recurrent miscarriage in the Turkish population.

METHODS: The study included 250 participants (i.e., 150 patients and 100 controls). DNA samples were isolated from peripheral blood, and polymerase chain reactions and restriction fragment length polymorphism methodologies were applied.

RESULTS: The genotype distribution and allele frequencies of miR604A>G gene showed statistically significant differences between patients and control groups (p=0.002 and p<0.002, respectively).

CONCLUSION: As a result of the study, we found that the AA genotype and A allele of the miR604A>G gene were statistically significant for the risk of recurrent pregnancy loss in Turkish women.

KEYWORDS: Gene polymorphism. microRNA. Recurrent miscarriages.

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as two or more spontaneous pregnancy loss until the 20th gestational week. Generally, pregnancy losses occur in the first trimester. The risk of recurrence of pregnancy loss is higher in individuals whose first pregnancy resulted in spontaneous abortion. There are many causes of RPL, including genetic factors, anatomical disorders, hormonal disorders, placental structure, infection, and other reasons[1,2]. Genetic factors are of great importance among them. In recent years, epigenetic factors have become more popular. Especially microRNAs (miRNAs) have recently been studied intensively. miRNAs are short, 17-25-nucleotide-long, non-coding small RNA molecules that regulate gene expression. miRNAs have been reported to have critical regulatory roles in controlling genes associated with cellular and molecular activity and maintenance of pregnancy[3]. It has been revealed that most of the pregnancy-associated miRNAs are expressed in reproductive tissues[4,5]. miR604A>G showed a relation that it binds targets to related placenta retention. Based on these data, we aimed to investigate the relationship between miR604 (rs2368393) A>G gene polymorphism and the risk of RPL in the Turkish population.

METHODS

Study sample

The study was carried out with women who applied to the obstetrics and gynecology department between 2019 and 2021 with a history of idiopathic recurrent miscarriage and women in the control group who had a healthy pregnancy and did not have any miscarriages. A questionnaire was applied to the participants in which age, body mass index, number of miscarriages, number of pregnancies, smoking and alcohol use, occupation, and hormone values were questioned. The individuals participating in the study signed an informed consent form, and the study included 250 female volunteers (i.e., 150 patients and 100 controls). Women with a history of two or more pregnancy losses were defined as having a recurrent miscarriage. The control group women have a healthy pregnancy and no history of pregnancy loss. The mean age of the patients was 33.21±4.52 years, and the mean age of the control group was 33.61±5.38 years. The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine on May 31, 2023 (OMU KAEK 2023/178).

[1]Ondokuz Mayıs University, Faculty of Medicine, Central Laboratory – Samsun, Turkey.
[2] Ondokuz Mayıs University, Faculty of Medicine, Department of Obstetrics and Gynecology – Samsun, Turkey.
[3]Ondokuz Mayıs University, Faculty of Medicine, Department of Medical Biology – Samsun, Turkey.
*Corresponding author: esrabkr@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on May 24, 2023. Accepted on May 27, 2023.
This document has an erratum: https://doi.org/10.1590/1806-9282.20230454ERRATUM

DNA isolation and polymerase chain reaction-restriction fragment length polymorphism methods

A total of 2 mL of peripheral blood was collected from participants in EDTA tubes. DNA was isolated from the peripheral blood sample by kit methodology (Invitrogen). The obtained DNAs were stored in the freezer. Following DNA isolation, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methodology was performed. The miR604 (rs2368393) A>G polymorphism was detected using the forward and reverse primers, namely, 5′-CTT GGC TCA GTG GTC TGT TT-3′ and 5′-GTA CAG GGA CTG AAA GGT GAA G-3′, respectively. The 243 bp PCR product was digested with BssSI enzyme (New England BioLabs, Ipswich, MA, USA) to AA type (169 and 74 bp), AG type (243, 169, and 74 bp), and GG type (243 bp), under conditions of initial denaturation at 95°C for 15 min, 40 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 40 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Genotypes were visualized on 2% agarose gel with an image analysis system.

Statistical analysis

The SPSS.20 (Chicago, IL, USA) program and OpenEpi Info software package program were used for statistical analysis. Genotype distribution and allele frequencies are calculated and compared by chi-square (χ^2) analysis. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Statistical results reached the significant value of $p<0.05$ (two-tailed).

RESULTS

The clinical and demographic findings of the patient and control groups are shown in Table 1. Genotype distribution and allele frequencies of miR604A>G gene are shown in Table 2.

Table 1. Clinical and laboratory findings of the patient and control groups.

Characteristics	Patients (n=150) (mean±SD)	Median (min–max)	Controls (n=100)	Median (min–max)
BMI (kg/m²)	25.52±3.572	28.00 (12-45)	28.38±4.186	28 (26-42)
Age at menopause (years)	45.22±5.824	47.90 (35-60)	46.56±4.674	45 (33-58)
Age at menarche (years)	13.52±1.371	13.0 (9-18)	13.46±1.51	12 (10-17)
Number of birth (n)	3.56±1.828	3.0 (0-10)	3.0±1.468	3 (0-6)
Serum calcium (mg/dL)	9.63±0.540	9.75 (8.35-11.6)	9.57±0.481	9.55 (8.5-10)
Serum phosphorus (mg/dL)	3.73±2.55	3.52 (2.1-25.40)	3.78±0.876	4 (2.00-5.32)
Serum ALP (U/L)	187.03±61.83	187.355 (2.42-430)	177.65±45.146	186.6 (96-310)
Serum PTH (pg/mL)	77.03±53.95	77.23 (11.70-563)	75.32±27.563	72.7 (30-167)
Homocysteine (µmol/L)	7.28±1.95	8.05 (6.30-8.2)	NA	NA
Folate (mg/mL)	14.25±11.85	15.25(12.42-15.4)	NA	NA
PAI-1 (ng/mL)	10.43±5.81	11.15(9.95-15.0)	NA	NA

NA: not applicable.

Table 2. Genotype and allele frequencies of miR604A>G gene polymorphism.

Genotype/Allele	Patients (n=150)		Controls (n=100)		χ^2 (95%CI)	p-value	OR
	n	%	n	%			
Genotype							
AA	48	32.0	22	22.0	χ^2=12.325	p=0.002*	
AG	78	52.0	43	43.0			
GG	24	16.0	35	35.0			
Total	150		100				
Allele							
A	171	57	87	44	χ^2=9.486 (1.226-2.53)	p<0.002*	1.761
G	126	42	113	56			

*p<0.05 is statistically significant.

Patients' body mass index and mean standard deviation (SD) were 25.52±3.572 and 28.38±4.186 in control. The mean age±SD was 33.21±4.52 years in patients and 33.61±5.38 years in the control group, respectively. The mean homocysteine±SD was 7.28±1.95. The mean folate±SD was 14.25±11.85 and PAI-1±SD was 10.43±5.81 in patients. Table 2 represents the distribution of miR604A>G gene polymorphism genotypes in patients and control groups. miR604A>G gene AA, AG, and GG genotype frequencies of patients were 32, 52, and 16%, respectively. In the control group, AA, AG, and GG genotype frequencies were 22, 43, and 35%, respectively. AA genotype frequency was high in the patient group (p=0.002), and A allele frequency was also higher in the patient group (p<0.002), which reached a statistically significant value.

DISCUSSION

RPL is affecting 1-3% of pregnancies. Its exact etiology is poorly understood due to different definitions and limitations in access to abortion material. Few factors such as parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, anatomical anomalies of the uterus, and antiphospholipid antibody syndrome are important for pregnancy losses. Other possible etiological factors include endocrine disorders, hereditary and/or acquired thrombophilia, immunological abnormalities, infections, and environmental factors. However, the cause of almost half of RPLs is still unexplained. Many couples in recent days suffer from recurrent miscarriages that also considerably affect their quality of life. Diagnostic biomarkers, which are increasing day by day, are beneficial for patients in terms of determining the predisposition and taking the necessary precautions. SNPs in miRNA genes, genes encoding miRNA machinery proteins, or miRNAs that target genes involved in miRNA synthesis or function will affect processes regulated by miRNAs. They can adversely affect downstream gene expression[6,7]. Various studies showed that miRNAs play an important role in RPL. In Cho et al.'s study, a construct containing the 3'UTR MTHFR gene was performed using luciferase to measure the binding affinity of variant and major alleles of miR604 G>A to MTHFR[8]. These analyses showed that the binding affinity of miR604 was found to be stronger in cell lines transfected with the major A allele compared with those transfected with the minor G allele. The data therefore suggest that miR604A>G expression may affect the binding affinity of this miRNA to the 3'-UTR of MTHFR. It is also suggested that the liberation of MTHFR expression by the A to G change in miR604 (rs2368393) may have an effect on one-carbon metabolism. One-carbon metabolism is also associated with vascular and defects in blood coagulation factors, low, abnormal plasma urate, folate, and homocysteine levels, which are risk factors for RPL. The results of Cho et al. suggest that the miR604A>G polymorphism and its effect on MTHFR may contribute to RPL and therefore should be considered when evaluating RPL patients[8]. In our study, we aimed to examine whether the miR604A>G gene polymorphic region could be a potential biomarker for RPL (CHOO). Our results revealed that AA genotype and A allele of the miR604A>G gene were statistically significant for the risk of RPL. Several studies have provided evidence supporting a critical role for miRNAs in RPL[9,10]. According to the findings of Cho et al., the miR604A>G polymorphism is linked to RPL[8]. They reported that the miR604A>G polymorphism is associated with recurrent miscarriage, which is consistent with our findings. Few associated studies have been conducted in this field, and the results of this investigation do not definitively prove the significance of these polymorphisms in recurrent miscarriages[11,12]. Further research into this and other pre-miRNA polymorphisms in other ethnic populations, in combination with functional studies, will aid in understanding the role of miRNA polymorphisms in RPL and its susceptibility. In a recent study, Kim et al. investigated miR604A>G polymorphism and found that miR604A>G polymorphism was statistically significant for recurrent implantation failure[13]. To confirm our results, further studies are needed by novel and detailed analysis[14,15].

CONCLUSION

Only limited studies exist which investigated miR604A>G gene polymorphism and its relation with RPL, and as we know that this is the first study examining the miR604A>G gene polymorphism in relation with RPL in the Turkish population, our results would contribute to the literature.

ACKNOWLEDGMENTS

We would like to thank the Ondokuz Mayıs University, Karadeniz Advanced Technology Research and Application Center, where we carried out our laboratory studies.

AUTHORS' CONTRIBUTIONS

ET: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Writing - original draft. **UC:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision. **MO:** Funding acquisition, Resources, Software, Supervision, Visualization. **ST:** Data curation, Formal Analysis, Funding acquisition, Resources, Software, Validation.

REFERENCES

1. Coulam CB, Clark DA, Beer AE, Kutteh WH, Silver R, Kwak J, et al. Clinical guidelines recommendation committee for diagnosis and treatment of recurrent spontaneous abortion. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. Am J Reprod Immunol. 1997;38:57-74. https://doi.org/10.1111/j.1600-0897.1997.tb00277.x
2. Choi YS, Kwon H, Kim JH, Shin JE, Choi Y, Yoon TK, et al. Haplotype-based association of ACE I/D, AT1R 1166A>C, and AGT M235T polymorphisms in renin-angiotensin-aldosterone system genes in Korean women with idiopathic recurrent spontaneous abortions. Eur J Obstet Gynecol Reprod Biol. 2011;158:225-8. https://doi.org/10.1016/j.ejogrb.2011.04.028
3. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer. 2010;10:389-402. https://doi.org/10.1038/nrc2867
4. Kim YR, Ryu CS, Kim JO, An HJ, Cho SH, Ahn EH, et al. Association study of AGO1 and AGO2 genes polymorphisms with recurrent pregnancy loss. Sci Rep. 2019;9:15591. https://doi.org/10.1038/s41598-019-52073-0
5. Du E, Cao Y, Feng C, Lu J, Yang H, Zhang Y. The possible involvement of miR-371a-5p regulating XIAP in the pathogenesis of recurrent pregnancy loss. Reprod Sci. 2019;26:1468-75. https://doi.org/10.1177/1933719119828051
6. Baek KH. Aberrant gene expression associated with recurrent pregnancy loss. Mol Hum Reprod. 2004;10:291-7. https://doi.org/10.1093/molehr/gah049
7. Krieg SA, Fan X, Hong Y, Sang QX, Giaccia A, Westphal LM, et al. Global alteration in gene expression profiles of deciduas from women with idiopathic recurrent pregnancy loss. Mol Hum Reprod. 2012;18:442-50. https://doi.org/10.1093/molehr/gas017
8. Cho SH, Kim JH, An HJ, Kim YR, Ahn EH, Lee JR, et al. Genetic Polymorphisms in miR-604A>G, miR-938G>A, miR-1302-3C>T and the risk of idiopathic recurrent pregnancy loss. Int J Mol Sci. 2021;22:6127. https://doi.org/10.3390/ijms22116127
9. Dong F, Zhang Y, Xia F, Yang Y, Xiong S, Jin L, et al. Genome-wide miRNA profiling of villus and decidua of recurrent spontaneous abortion patients. Reproduction. 2014;148:33-41. https://doi.org/10.1530/REP-14-0095
10. Hu Y, Liu CM, Qi L, He TZ, Shi-Guo L, Hao CJ, et al. Two common SNPs in pri-miR-125a alter the mature miRNA expression and associate with recurrent pregnancy loss in a Han-Chinese population. RNA Biol. 2011;8:861-72. https://doi.org/10.4161/rna.8.5.16034
11. Amin-Beidokhti M, Mirfakhraie R, Zare-Karizi S, Karamoddin F. The role of parental microRNA alleles in recurrent pregnancy loss: an association study. Reprod Biomed Online. 2017;34:325-30. https://doi.org/10.1016/j.rbmo.2016.12.004
12. Cho SH, Chung KW, Kim JO, Jang H, Yoo JK, Choi Y, et al. Association of miR-146aC>G, miR-149C>T, miR-196a2T>C, and miR-499A>G polymorphisms with risk of recurrent implantation failure in Korean women. Eur J Obstet Gynecol Reprod Biol. 2016;202:14-9. https://doi.org/10.1016/j.ejogrb.2016.04.009
13. Kim JO, Ahn EH, Sakong JH, An HJ, Park HS, Kim YR. Association of miR-27aA>G, miR-423C>a, miR-449bA>G, and miR-604A>G Polymorphisms with risk of recurrent implantation failure. Reprod Sci. 2020;27:29-38. https://doi.org/10.1007/s43032-019-00031-6
14. Niyaz L, Tural S, Yucel OE, Can E, Ariturk N, Celik ZB, et al. Chromosomal microarray analysis of patients with Duane retraction syndrome. Int Ophthalmol. 2019;39:2057-67. https://doi.org/10.1007/s10792-018-1042-8
15. Tural S, Yuce MK, Polat A, Tekcan E, Celik BZ, Karabacak U, et al. Novel RET proto-oncogene variants identified in Turkish patients with thyroid carcinoma. Gene. 2020;746:144611. https://doi.org/10.1016/j.gene.2020.144611