

Association Study of the miR-196a2T>C and miR-499A>G Polymorphisms with the Incidence of Idiopathic Recurrent Spontaneous Abortions



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Abstract

MicroRNAs (miRNAs) are small, noncoding RNA molecules characterized as posttranscriptional negative regulators of gene expression and play a critical role in cell proliferation, cell development, cell differentiation, apoptosis, metabolism, and signal transduction. The miR-196a2T>C and miR-499A>G polymorphisms have been implicated as risk factors for recurrent spontaneous abortions (RSAs). Mature miR-196a-2 represses the expression of transcription factor HOXB8, a home box protein involved in implantation, and of annexin A1 (ANXA1) related to anti-inflammation inhibiting NF-κB pathway. Mature miR-499 preferentially inhibits the transcription of SOX6 mRNA, a transcription repressor of FGF-3 associated with cell proliferation and cell differentiation of developing fetal tissues.

Blood samples were collected from 135 patients with RSAs and 97 fertile women, from which genomic DNA was extracted, and PCR and Real-time-PCR were performed to identify the miR-196a2T>C and miR-499A>G genotypes. RSA patients exhibited higher frequency of miR-196a2CC and miR-499AA+GG genotypes, whereas miR-196a2TT+TC and miR-499AG genotypes have been showed higher frequency in the control group, but the frequency distribution is not significantly different between the comparing groups. Based on our study results, the presence of miR-196a2T>C and miR-499A>G polymorphisms is independent and not related to RSAs. The above results are very important, constituting a data base for doctors and researchers in order to know which miRNAs polymorphisms should be ruled out in the investigation of RSAs. Larger study group should be investigated in order to confirm our preliminary results.

Keywords: MicroRNA; Recurrent spontaneous Abortions; Polymorphism

Abbreviations: RSA: Recurrent Spontaneous Abortions; RSM: Recurrent Spontaneous Miscarriage; UTR: 3-Untranslated Region; SNPs : Single Nucleotide Polymorphisms

Introduction

Recurrent spontaneous miscarriage (RSM), defined as the occurrence of three or more consecutive abortions before the 20th week of pregnancy [1-3], is one of the greatest challenges of reproductive medicine due to its vast and sometimes poorly defined etiology. Approximately 1-3% of fertile couples will experience recurrent spontaneous miscarriage [4]. Endocrine dysfunction, chromosomal and anatomical anomalies, maternal infections and immune reactions have all been implicated as etiologic factors [5,6]. However, in about 50% of RSM cases no specific pathology is detected [7-10]. Posttranscriptional target genes regulation is

solid associated with microRNAs. In addition important regulatory functions are served by microRNAs such as maintenance of stem cell-ness and modulation of differentiation. MiRNAs are short, single stranded RNA molecules of approximately 19 to 23 nucleotides; they are also noncoding small regulatory RNAs locating into the RNA-induced silencing complex, recognizing the 3-untranslated region (UTR) of target mRNAs, and thereby regulating their expression by translational repression or mRNA degradation [11].

Endometriosis, infertility and preeclampsia may be associated with miRNAs based on recent studies [12, 13]. Recently, different

human cardiomyopathies have been associated with differential expression of miRNAs [11]. The single nucleotide polymorphisms (SNPs) of miR-196a2 gene, miR-196a2T>C (rs11614913) and of miR-499 gene, miR-499A>G (rs3746444) have been reported to be a possible genetic risk factor for recurrent spontaneous abortion (RSA) in a Korean population [12,13]. The aim of this study is to investigate the potential association of miR-196a2T>C and miR-499A>G polymorphisms in patients with recurrent spontaneous abortions (RSAs).

Materials and Methods

Study Participants

This was a retrospective case study performed in Alexandra Maternity Hospital in Athens, Greece. A total of 232 women were included in the study. The study group comprised of 135 patients with at least two miscarriages of unexplained etiology, before the 20th week of gestation who visited the recurrent miscarriage outpatient clinic of Alexandra Hospital (mean age \pm Standard Error of Mean [SEM] 33 \pm 0.64 years; BMI \pm SEM: 24.9 \pm 0.88). Women with a history of autoimmune disease, endocrine, chromosomal, infectious or thromboembolic disorders were excluded from the study. The control group consisted of 97 unrelated Caucasian multiparous women with no know past history of pregnancy loss (mean age \pm SEM 44 \pm 1.64 years; BMI \pm SEM: 25.4 \pm 0.47). The study protocol was approved by the Alexandra Hospital review board. Blood samples were collected by venipuncture in EDTA containing tubes for serum preparation and genomic DNA extraction using a commercially available kit (PureLink® Genomic DNA Mini Kit, Invitrogen™, Life Technologies, USA).

Genotyping

Polymorphisms miR-499A>G Was Detected Using PCR: Blood samples were collected from 135 patients with RSAs and 97 fertile women, from which genomic DNA was extracted. Subsequently, PCR and Real time PCR were performed to identify the miR-196a2T>C and miR-499A>G genotypes, using specific

primers and hybridization probes. The results from the two groups were evaluated for possible statistical correlations. The two single nucleotide polymorphisms (SNPs) studied was in complete Hardy-Weinberg equilibrium.

Results

In the control group, the percentages of women homozygous for the wild type allele, miR-196a2TT, women heterozygous, miR-196a2TC and women homozygous for the miR-196a2T>C polymorphism, miR-196a2CC were 45, 4%, 46, 4%, and 8, 2%, respectively. In the group of patients with recurrent spontaneous miscarriages, 59 women (43, 7%) were found to be homozygous for the wild type allele, miR-196a2TT, 61 women (45, 2%) were heterozygous, miR-196a2TC and 15 women (11, 1%) homozygous for the miR-196a2T>C polymorphism, miR-196a2CC. As far as the miR-499A>G polymorphism is concerned, in the control group, 42 (48, 3%) women homozygous for the wild type allele, miR-499AA, 38 (43, 7%) women heterozygous, miR-499AG, and 7 (8, 0%) women homozygous for the miR-499A>G polymorphism, miR-499GG, were observed.

In the group of patients with recurrent spontaneous miscarriages, 57 women (57, 6%) were homozygous for the wild type allele, miR-499AA, 33 women (33, 3%) were heterozygous, miR-499AG, while 9 women (9, 1%) were revealed homozygous for the miR-499A>G polymorphism, miR-499GG. The frequencies of miR-196a2CC (OR=1.398, $P_{\text{value}}=0.485$, 95% Confidence Interval [CI] =0.545-3.589) and miR-499AA+GG (OR=0.947, $P_{\text{value}}=0.921$, 95% CI =0.327-2.748) genotypes were higher in patients with at least two consecutive spontaneous miscarriages than control group. However the differences were not statistically significant. On the other hand, the miR-196a2TT+TC (OR=1.011, $P_{\text{value}}=0.969$, 95% CI =0.584-1.749) and miR-499AG (OR=0.640, $P_{\text{value}}=0.153$, 95% CI =0.346-1.182) genotypes have been showed higher frequency in the control group than RSA patients, but the frequency distribution is not significantly different between the comparing groups (Table 1).

Table 1: Genotype frequencies of miR-196a2T>C and miR-499A>G in RSA patients and control subjects.

Genotypes	Controls		RSA patients		p-value ¹	OR [95% CI] ²	p-values ³
	n=97	%	n=135	%			
miR-196a2T>C	n=97	%	n=135	%	p-value ¹	OR [95% CI] ²	p-values ³
TT	44	45,4%	59	43,7%	0,480	reference genotype	
TC	45	46,4%	61	45,2%		1,011 [0,584-1,749]	0,969
CC	8	8,2%	15	11,1%		1,398 [0,545-3,589]	0,485
Alleles							
T	133	68,6%	179	66,3%		reference allele	
C	61	31,4%	91	33,7%		1,108 [0,747-1,644]	0,609
miR-499A>G	n=87	%	n=99	%	p-value ¹	OR [95% CI] ²	p-values ³

AA	42	48,3%	57	57,6%	0,115	reference genotype	
AG	38	43,7%	33	33,3%		0,640 [0,346-1,182]	0,153
GG	7	8,0%	9	9,1%		0,947 [0,327-2,748]	0,921
Alleles							
A	122	70,1%	147	74,2%		reference allele	
G	52	29,9%	51	25,8%		0,814 [0,517-1,283]	0,375

Discussion

The association of variants of miRNAs with recurrent miscarriages is still not very clear. Implantation leading to a newborn infant is the result of a perfectly orchestrated association between a viable embryo and a receptive endometrial; a variety of key proteins and growth factors are implicated via playing a pivotal role to this process. Micro-RNAs have also been involved in this process. However their specific role in normal embryo formation, endometrial formation for pregnancy, and in early stages of gestation is still unclear [14]. miR-196a2T>C and miR-499A>G have been associated with a lot of cancer types maybe by affecting RSA through cell proliferation since the aberrant cell cycle regulation is the causing root of cancer [13]. Mature miR-196a-2 represses the expression of transcription factor HOXB8, a home box protein which is involved in implantation and myeloid differentiation.

The pivotal roles of HOX genes in implantation are clear based on various studies. In addition, miR-196a-2 has another important target, annexin A1 (ANXA1), a calcium- and phospholipid-binding protein related to anti-inflammation through inhibiting NF- κ B pathway. Increased miR-196a2 levels by the miR-196a2CC genotype could decrease ANXA1 transcripts, leading to excessive inflammation. Abortive process may be caused by dysregulated inflammation [13]. Mature miR-499 preferentially inhibits the transcription of SOX6 mRNA, a transcription repressor of fibroblast growth factor -3 (FGF-3) associated with cell proliferation and cell differentiation of developing fetal tissues [13]. In our study we showed the percentages revealing in a European- Caucasian population opposite to the Korean population as has been shown in recent published studies [12]. Based on our study results, the presence of miR-196a2T>C and miR-499A>G polymorphisms is independent and not related to RSAs. The above results are very important, constituting a data base for doctors and researchers in order to know which miRNAs polymorphisms should be ruled out in the investigation of RSAs.

Conclusion

In conclusion, our findings seem to be controversial. The small number of samples may play its role to this controversial result. A larger study group should be investigated in order to confirm the association between miR-196a2T>C and miR-499A>G polymorphisms and RSAs.

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References

1. Stirrat GM (1990) Recurrent miscarriage. *Lancet* 336(8716): 673-675.
2. Coulam CB (1991) Epidemiology of recurrent spontaneous abortion. *Am J Repro Immunol* 26(1): 23-27.
3. Branch DW, Gibson M, Silver RM (2010) Recurrent miscarriage. *New Engl J Med* 363: 1740-1747.
4. (2011) Royal College of Obstetricians and Gynaecologists (RCOG) (2011) The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. Green-top Guideline No. 17. Royal College of Obstetricians and Gynaecologists (RCOG).
5. Pandey MK, Rani R, Agrawal S (2005) An update in recurrent spontaneous abortion. *Arch Gynecol Obstet* 272(2): 95-108.
6. Pfeiffer KA, Fimmers R, Engels G, van der Ven H, van der Ven K (2001) The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. *Mol Hum Reprod* 7(4): 373-378.
7. Clifford K, Rai R, Watson H (1994) An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod* 9(7): 1328-1332.
8. Stephenson MD (1996) Frequency of factors associated with habitual abortion in 197 couples. *Fertil Steril* 66(1): 24-29.
9. Jaslow CR, Carney JL, Kutteh WH (2010) Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril* 93(4): 1234-1243.
10. Yue J, Tong Y, Zhou J, Liu Q, Yang J (2015) Genetic variant in interleukin-18 is associated with idiopathic recurrent miscarriage in Chinese Han population. *Int J Mol Sci* 16(2): 4180-4189.
11. Sluijter JP, Van Mil A, Van Vliet P, Metz CH, Liu J, et al. (2010) MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol* 30(4): 859-868.
12. Jeon Young Joo, Choi Yi Seul, Hyung Chul Rah, Su Yeoun Kim, Dong Hee Choi, et al. (2011) Association study of microRNA polymorphisms with Risk of idiopathic recurrent spontaneous abortion in Korean women. *Gene* 494(2): 168-173.
13. Jeon Young Joo, Kim Su Yeoun, Hyung Chul Rah, Dong Hee Choi, Sun Hee Cha, et al. (2012) Association of the miR-146aC>G, miR-149T>C, miR-196a2T>C, and miR-499A>G Polymorphisms with Risk of Spontaneously Aborted Fetuses. *American Journal of Reproductive Immunology* 68 (5): 408-417.
14. Galliano D, Pellicer A (2014) MicroRNA and implantation. *Fertil Steril* 101(6): 1531-1544.



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