International Journal of Research in Medical Sciences
Sharif FA et al. Int J Res Med Sci. 2015 Nov;3(11):3123-3125
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20151148

Research Article

The single nucleotide polymorphism rs2305957 G/A is not associated with recurrent pregnancy loss

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Received: 01 September 2015
Accepted: 06 October 2015

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ABSTRACT

Background: This study was conducted in order to investigate the association between the single nucleotide polymorphism (SNP) rs2305957 G/A and recurrent pregnancy loss (RPL) in a group of Palestinian women residing in Gaza strip.
Methods: A retrospective case-control study was carried out during the period (May to August, 2015). A total of 380 females, 190 RPL patients and 190 control women without previous history of RPL, aged 20–35 years were included in the study. The SNP was analyzed by tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR).
Results: No statistically significant difference existed between RPL cases and controls in terms of the allelic and genotypic distribution of rs2305957 G/A.
Conclusions: SNP rs2305957 G/A does not represent a risk factor for RPL in the investigated population.

Keywords: RSA, rs2305957, SNP, Gaza strip, Palestine

INTRODUCTION

Recurrent pregnancy loss (RPL), which is currently, defined as two or more consecutive pregnancy losses before the 20th week of gestation, affects as many as one in 20 couples seeking parenthood. Although many known causes of RPL including uterine anatomic (15%), infectious (1%–2%), hormonal (20%), immunological (20%), and genetic (2%–5%) have been identified, a significant number of cases (approximately 40%–50%) do not have known causes.[1]

Studies on mosaic embryos have shown that the occurrence of mitotic aneuploidies is common in human preimplantation embryos, and that, at least, some of those aneuploidies can lead to embryo loss.[2,3]

Recently, McCoy et al. (2015) have reported on the identification of a genetic variant, SNP rs2305957 G/A on maternal chromosome 4 that increases the aneuploidy risk in the embryos of women of European and East Asian ancestries.[4] Several genes are located in the vicinity of that SNP, but the authors attributed the association to PLK4 gene.[4]

Therefore, this study was designed in order to test whether this polymorphism is associated with RPL in Palestinian women.

METHODS

Study sample: The study was conducted on 190 Palestinian women, 18–35 years old, who had at least two RPLs ≤20 weeks of gestation. Age and ethnicity matched 190 women with at least two live births and without a previous history of abortion or pregnancy-associated complications served as the control group.

Ethical considerations: Informed consent was obtained from all participants.

DNA extraction and rs2305957 G/A genotyping: The DNA was isolated from whole blood samples using Wizard DNA extraction kit (Promega, USA) as described by the manufacturer. The rs2305957 G/A polymorphism were analyzed by T-ARMS-PCR. The primers used are presented in Table 1 and were designed using primer1 software (http://primer1.soton.ac.uk/ primer1.html). PCR cycling was performed at 95 °C for 4 min followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 45 s, extension at 72 °C for 45 s and a final extension at 72 °C for 8 min. The PCR products were separated by running the PCR products on ethidium bromide-stained 2% agarose gels and analyzed on a gel documentation system.

Statistical analysis: The genotype, allele frequency in RPL patients and the controls were analyzed by standard Chi-square test and odds ratio (OR) for risk of RPL at 95% confidence intervals (CI). Hardy-Weinberg equilibrium (HWE) was tested using a freely available software: (http: //www. oege. org/software/hwe-mr-calc.shtml).

RESULTS

Genotypic and allelic distribution of rs2305957 polymorphism in RPL subjects and controls: Genotype and allele frequencies of the tested polymorphism were not significantly different between RPL patients and controls (Table 2). Moreover, statistical analyses of the genotypes under recessive and dominant models (data no shown) indicated no significant difference between the two study groups.

Hardy-Weinberg equilibrium: Analysis of the observed and the calculated expected genotype frequencies of SNP rs2305957 G/A polymorphism in the control group showed that the distribution of genotypes are in Hardy-Weinberg equilibrium.

Table 1: Sequence of primers used and product size for SNP rs2305957 T-ARMS PCR.

Primer	Sequence 5`-3`	Product Size
Forward inner (A allele)	TTTGATTTTCTGCTGTGGGAATCTTTA	Control: 378 bp For A allele: 174 bp For G allele: 260 bp
Reverse inner (G allele)	TCCTTAATGCTTTTATCAAAAGACTTGAC	
Forward outer	CGCATCAGTAATTGAGAAGCAAAATT	
Reverse outer	AAGAACTTAGGAAAGAATTCCAGGTTCA	

Table 2: Genotype and allele frequencies of rs2305957 among RPL patients and controls.

Genotype/ allele	Patient, n= 190	Controls, n=190	Odds Ratio (95% CI)	P-value
GG	114 (60.0%)	118 (62.10%)	1.09 (0.72 to 1.65)	0.674
GA	68 (35.80%)	59 (31.05%)	1.24 (0.807 to 1.897)	0.328
AA	8 (4.20%)	13 (6.85%)	0.598 (0.242 to 1.479)	0.266
Allele G	296 (77.90%)	295 (77.60%)	0.985 (0.699 to 1.386)	0.930
Allele A	84 (22.10%)	85 (22.40%)		

DISCUSSION

Recurrent pregnancy loss (RPL) is considered as a multifactorial complication of pregnancy, and despite extensive research worldwide; causes of nearly half of the RPL cases remain elusive.

Genetic testing of miscarriage tissues offers the promise to identify pregnancy loss due to chromosome anomalies that may be responsible for lethal embryonic developmental defects.

RPL due to chromosomal aneuploidies may simply be a consequence of the dramatic increase of aneuploidy in ova associated with advanced maternal age. However, all the patients included in this study were of young age (≤35 yrs.).

It is possible that some embryos are at increased risk of mitotic chromosome aneuploidies as a result of genetic variations in the parental genomes. In their investigation on human embryos, McCoy et al. have shown that the maternal minor "A" allele of the SNP rs2305957 G/A is strongly associated with occurrence of post-zygotic mitotic aneuploidy.[4]

Testing this polymorphism in our RPL patients and controls did not reveal any association between the rs2305957 polymorphism and RPL. The frequency of the minor "A" allele was very similar in both groups (Table 2). This lack of association could be due to ethnic genetic variation unrelated to the investigated SNP or most probably due to linkage disequilibrium to other sequence variants in the vicinity of rs2305957.

In conclusion, results of the present work showed that the rs2305957 polymorphism does not contribute to the risk of RPL in Palestinian women.

Funding: Supported by the Scientific Research Council [Scientific Research Grant 2014-2015], Ministry of Education and Higher Education, State of Palestine
Conflict of interest: None declared
Ethical approval: Approved

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Cite this article as: Sharif FA, Ashour M. The single nucleotide polymorphism rs2305957 G/A is not associated with recurrent pregnancy loss. Int J Res Med Sci 2015;3:3123-5.