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Association study of *interleukin-10* and *P53* polymorphisms and their influence on Iranian women with recurrent abortion

İnterlökin-10 ve P53 polimorfizmlerinin ilişki çalışması ve bunların tekrarlayan düşük yapan İranlı kadınlar üzerindeki etkileri

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Abstract

Objective: Recurrent spontaneous abortion (RSA), characterized by multiple miscarriages without a known cause, includes both genetic and non-genetic factors. In this research, we studied the association between two polymorphisms of the *interleukin (IL)-10* and *P53* genes and RSA for the first time in the southwest of Iran.

Materials and Methods: This was a case-control study involving 62 patients with a history of at least two RSA of unknown etiology, as well as 66 healthy individuals. Clinical factors were analyzed. Genomic DNA was extracted from whole blood. Genotyping was performed using amplification refractory mutation system-polymerase chain technique to investigate two single nucleotide polymorphisms (SNPs) of *P53* and *IL-10* genes. Gene-gene interactions were analyzed by logistic regression. Statistical analysis was performed using a significance level of $p<0.05$.

Results: Allelic and genotypic frequencies as well as dominant, recessive and over dominant models for two SNPs, rs1042522 and rs1800871, were investigated. No significant association with RSA ($p>0.05$) was found. The combination of the homozygote CC for the polymorphism rs1042522 in the P53 gene and the homozygote CC for the polymorphism rs1800871 in the homozygote CC for the polymorphism rs1800871 in the *IL-10* gene was associated with an increased risk of spontaneous abortion ($p=0.01$). Meanwhile, the phenotypic frequency of individuals with a history of consanguineous marriage was statistically significant between the case and control groups ($p=0.003$).

Conclusion: Limited studies have been conducted on the association between these two polymorphisms and RSA, and conflicting results have been obtained. Further investigation with a larger sample size may confirm results. Genetic research, such as this, helps understand genetic factors associated with the risk of RSA.

Keywords: Polymorphism, infertility, abortion, spontaneous

PRECIS: In this research, the association of *interleukin-10* and *P53* genes polymorphisms with recurrent spontaneous abortion was studied.

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Öz

Amaç: Bilinen bir nedeni olmayan birden fazla düşükle karakterize tekrarlayan spontan düşükler (RSA), hem genetik hem de genetik olmayan faktörler ile ilişkilidir. Bu araştırmada, ilk kez İran'ın güneybatısında *interlökin (IL)-10* ve *P53* genlerinin iki polimorfizmi ile RSA arasındaki ilişkiyi inceledik.

Gereç ve Yöntemler: Bu çalışma, en az iki bilinmeyen etiyolojiye sahip RSA öyküsü olan 62 hastayı ve 66 sağlıklı grubu içeren bir olgu kontrol çalışmasıydı. Klinik faktörler analiz edildi. Tam kandan genomik DNA çıkarıldı. *P53* ve *IL-10* genlerinin iki tek nükleotid polimorfizmini (SNP) araştırmak için amplifikasyona dirençli mutasyon sistemi-polimeraz zincir reaksiyonu tekniği kullanılarak genotipleme yapıldı. Gen-gen etkileşimleri lojistik regresyonla analiz edildi. İstatistiksel analiz p<0,05 dikkate alınarak yapıldı.

Bulgular: İki SNP olan rs1042522 ve rs1800871 için allelik ve genotipik frekanslar ile dominant, resesif ve aşırı dominant modeller araştırıldı. RSA ile anlamlı bir ilişki bulunamadı (p>0,05). Polimorfizm rs1042522 *P53* geni için homozigot CC ve polimorfizm rs1800871 *IL-10* geni için homozigot CC'ye sahip olanların genotip kombinasyonu spontan düşük riskini artırmıştır (p=0,01). Akraba evliliği öyküsü olan bireylerin fenotipik sıklığı olgu ve kontrol grupları arasında istatistiksel açıdan anlamlı olarak farklıydı (p=0,003).

Sonuç: Bu iki polimorfizm ile RSA arasındaki ilişki üzerine sınırlı sayıda çalışma yürütülmüş ve çelişkili sonuçlar elde edilmiştir. Daha büyük bir örneklem büyüklüğü ile yapılacak daha fazla araştırma bu çalışmanın sonuçlarını doğrulayabilir. Bu gibi genetik araştırmalar, RSA riskiyle ilişkili genetik faktörleri anlamaya yardımcı olmaktadır.

Anahtar Kelimeler: Polimorfizm, kısırlık, kürtaj, spontan

Introduction

Miscarriage, clinically termed spontaneous abortion, represents one of the prevailing complications in pregnancy, defined as the natural termination and expulsion of a fetus before it can independently sustain life. The term "miscarriage" commonly encompasses all forms of pregnancy loss occurring prior to the 20th week of gestation[1]. Recurrent spontaneous abortion (RSA) occurs when spontaneous abortion occurs more than twice during the initial 20 weeks of pregnancy, posing a significant challenge as it affects up to 20% of known pregnancies[2]. There are approximately one to two percent of women of childbearing suffering from RSA, which describes women who suffer from three or more continuous miscarriages in a row[2]. Predominantly observed between 8 and 12 weeks of gestation, with reduced prevalence in instances where etiology diverges, RSA substantially impacts pregnancy outcomes, warranting exploration into its underlying genetic determinants[3]. Furthermore, the prevalence of RSA approximates one in every 300 births, underscoring its clinical significance[3]. Notably, in individuals with a history of RSA, the likelihood of subsequent miscarriage increases progressively, with probabilities of 24%, 30%, and 40-50% after two, three, and four miscarriages, respectively[3]. RSA, from a medical standpoint, may also precipitate infertility owing to successive pregnancy losses[3].

The identifiable causes of RSA primarily include chromosomal abnormalities, anatomical irregularities, and hormonal imbalances (such as progesterone, estrogen, diabetes, thyroid disorders)[4]. However, over half of RSA cases (>50%) are attributed to deficiencies in blood coagulation proteins[5]. This deficiency in the blood clotting system is often associated with defects in coagulation inhibitors or proteins involved in the fibrinolytic pathway, resulting in an increased risk of blood clot formation[5]. Limited literature reports instances where deficiencies in clotting factors serve as the underlying cause of RSA. The most common deficiencies implicated include factor XIII, factor XII, and fibrinogen, which includes afibrinogenemia and dysfibrinogenemia[6].

RSA is a multifactorial disease, and different genes affect the occurrence of this disease. Genetic involvement in pregnancy complications is substantiated by candidate gene-based association studies, wherein specific polymorphic variants of genes are scrutinized for their potential roles in pregnancy[7]. In this way, several polymorphisms are associated with adverse pregnancy outcomes such as RSA[8,9].

Interleukin (IL)-10 orchestrates intricate interactions with various factors and cell types pivotal to pregnancy[10]. A successful gestation relies on maintaining equilibrium between immune responses mediated by Th1 and Th2 cells[11]. Notably, fetal survival correlates with a prevailing Th2 immune response, while a Th1-dominant response is linked to pregnancy failure[12]. IL-10, acting as a crucial regulator of Th2 immune responses, exerts potent anti-inflammatory effects by suppressing proinflammatory cytokine synthesis, thereby fostering a Th2 cytokine milieu that downregulates Th1 cytokine expression[13]. Enhanced maternal IL-10 production is associated with successful pregnancies, whereas diminished levels are implicated in recurrent fetal loss, potentially predisposing to immune system compromise and placental vascular insufficiency during pregnancy[13].

The regulation of IL-10 production is genetically determined and controlled at the transcriptional level, likely involving regulatory sequences within its promoter region[14]. Several studies suggested that certain IL-10 polymorphisms may heighten the risk of spontaneous abortion or confer protective effects against it[15]. Notably, several single nucleotide polymorphisms within the *IL-10* gene promoter region, such as -1082(A/G) (rs1800896), -819 (C/T) (rs1800871), and -592 (C/A) (rs1800872), have been implicated in increased rates of spontaneous abortions among some populations[16]. However, the precise role of *IL-10* gene polymorphisms in RSA remains a subject of debate.

On the other hand, the *P53* gene, known for its pleiotropic effects, plays a crucial role in vasculogenesis and cell apoptosis, essential processes for successful trophoblast cell invasion[17].

Recent investigations have revealed elevated apoptosis levels in chorionic villi and decidua, alongside heightened *P53* gene expression in placental villi among patients with recurrent unexplained spontaneous abortions[18]. These findings suggest that *P53* gene-induced apoptosis may contribute to RSA, underscoring its potential role as a pregnancy mediator with estrogen and progesterone activities. Various reports have linked several polymorphisms of the *P53* gene with RSA.

In a multivariate analysis, the P53 p.Pro72Arg (rs1042522) polymorphism was investigated, revealing an association with RSA and increased risk. The *P53* gene induces the expression of leukemia inhibitory factor (LIF); it is appropriate to consider the association of *P53* gene polymorphisms in patients who experience RSA and infertility, compared to fertile control groups[19]. A proline-rich domain at codon 72 is associated with reduced LIF expression, lower apoptosis rates, and G1 cell cycle arrest compared with arginine at codon 72[20]. These findings suggest that this polymorphism in the *P53* gene may serve as a risk factor for spontaneous abortion. In this study, for the first time, we aimed to investigate the potential association between genetic polymorphisms in the *IL-10* gene (rs1800871) and the *P53* gene (rs1042522) with RSA of unknown etiology in the population of Southwestern Iran.

Materials and Methods

This case-control study targeted 62 women with a primary diagnosis of RSA as the patient group and 66 women with no history of abortion as the control group. Firstly, informed consent forms were obtained from all patients and healthy individuals participating in the study. The study protocol was approved by the Ethics Committee of Islamic Azad University North Tehran Branch (approval no: IR.IAU.TNB.REC.1401.065, date: 13.12.2022). Blood samples were collected from infertility treatment clinics for women in Ahvaz city, referred by gynecologists. The samples were then transferred to the Genetics Department Laboratory of Jundishapur University of Medical Sciences, Ahvaz, following strict adherence to cold chain protocols.

Inclusion and Exclusion Criteria for Patients with RSA and A Healthy Control Group

In this study, women experiencing RSA, characterized by a history of at least two spontaneous abortions before the 20th week of pregnancy, were selected as the patient cohort. Medical records and examination results of all enrolled subjects were thoroughly reviewed for evaluation of chromosomal, anatomical, and pathological abnormalities, as well as common coagulation factors (c.C667T, c.A1298C), MTHFR, FACTOR II (c. G20210A), FACTOR V (c.G1691A), PAI 1 (4G/5G). This was done to ensure normalcy. Additionally, structured questionnaires were administered to gather pertinent information, including age, ethnicity, parity, history of abortion or infertility in the family, and consanguinity. Exclusion criteria encompassed the presence of chromosomal abnormalities in children and parents, anatomical uterine and ovarian disorders, antiphospholipid syndrome, and mutations in coagulation factors. A cohort of women free from a history of miscarriage, infertility, hormonal and anatomical abnormalities, and possessing at least two healthy children was chosen as the control group. Similar to the approach used for the patient cohort, a comprehensive questionnaire was administered to gather demographic and clinical data.

Blood DNA Extraction and Genotyping

Genomic DNA was extracted from anticoagulated peripheral blood samples using a DNA blood extraction kit (Sina Clon Company, Iran), and stored at -20 °C. All DNA extraction procedures were meticulously carried out in a Biosafety Level 2 laboratory. Genotyping of rs1800871 (c.-C819T) in IL-10 and rs1042522 (p.Arg72Pro) in P53 was performed using the amplification refractory mutation system-polymerase chain reaction (PCR) method. Designed primers were used for PCR amplification, with details provided in Table 1[21,22]. PCR reactions were conducted in a thermal cycler, and the resulting amplicons were analyzed via 1% agarose gel electrophoresis. Subsequently, cycles of denaturation, annealing, and extension were carried out. The reaction mixture for both polymorphisms underwent an initial denaturation step at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing and extension at 72 °C for 30 seconds for both single nucleotide polymorphisms (SNPs). Concerning the rs1800871 polymorphism, the annealing step was set at 60 °C for 30 seconds, and for the rs1042522 polymorphism, the annealing step was set at 64 °C for 30 seconds. Both protocols concluded with a final extension step: 72 °C for 2 minutes for both SNPs. Then, 10% of the samples were evaluated to genotype both polymorphisms by the Sanger sequencing method.

Statistical Analysis

In this research, the Statistical Package for the Social Sciences, version 26.0 (Chicago, Illinois, USA), and the χ^2 test were used for data analyses. Continuous variables were expressed in the form of mean ± standard deviation. The Kolmogorov-Smirnov test was applied to check whether the data were normally distributed. Normally distributed and abnormally distributed data between two groups were calculated by t-test and Mann-Whitney U test, respectively.

In order to verify any genotyping error, Hardy-Weinberg equilibrium (HWE) was calculated using the chi-squared test. The odds ratio (OR) and 95% confidence intervals (CIs) women with miscarriage compared to fertile women were calculated among four frequent genetic inheritance models, including the allelic model, dominant, recessive, and overdominant model. The p-value less than 0.05 was considered significant. At the end of the work to verify the specificity of the primers used and the accuracy of genotyping, Sanger sequencing was performed using the PCR primer and Big Dye Terminators (model 3130 Genetic Analyzer, Applied Biosystems Foster City CA USA).

Then, Sanger sequence analysis was conducted by Chromas 2.6.6 software.

Results

Demographic Characteristics of the Studied Subjects

Sampling for this study was conducted exclusively among case and control groups in Southwest Iran. The patient cohort with RSA, for whom the cause remained undiagnosed despite specialist consultation and genetic/pathological investigations, requires further study. The average age of the patient group was 30.69±4.76 years, with an average history of 3.18 miscarriages. On the other hand, the average age of the healthy control group was 32.73±5.31 years. Key demographic information, including average age, smoking habits, consanguinity, and history of abortions and infertility, is summarized in Table 2. A statistically significant disparity in the rate of consanguineous marriages among parents was observed between the patient and control groups (p=0.003), highlighting a potential contributing factor to RSA. Furthermore, no significant difference was noted in the average age between patients and healthy controls (p=0.1), and smoking habits were comparable across both groups (p=0.6). The t-test was employed for quantitative variables such as age, whereas the χ^2 test, was utilized to assess smoking habits as smoking habits are qualitative.

Association Study of rs1800871 and rs1042522 Polymorphisms with Recurrent Spontaneous Abortion

Statistical analysis of genotypic and allelic distributions for both rs1800871 and rs1042522 SNPs, is presented in Table 3. No significant associations were observed in genotypic frequencies between control subjects and patients in these two polymorphisms. Herein, we presented different models, including allelic, dominant, recessive, and over dominant models in Tables 4 and 5. Besides, we compared heterozygote with normal homozygote genotypes in the case and control groups for both SNPs. No significant differences between case and control groups, when using different models for both studied polymorphisms in *P53* and *IL-10* genes (Tables 4 and 5). Furthermore, OR calculations for the CT and TT genotypes using rs1800871 polymorphism, as well as the CG and GG genotypes for rs1042522 polymorphism did not demonstrate significant associations with RSA (p>0.05) (Tables 4 and 5). The HWE equilibrium analysis confirmed the balance of the rs1800871 and rs1042522 SNPs within the study population.

Combined Genotype Distribution for rs1800871 and rs1042522 Polymorphism

The combined genotype distribution of rs1800871 and rs1042522 SNPs was analyzed in both RSA cases and control groups. Among the comparisons that have been made, the CC + CC genotype combination was significantly more frequent in patients with RSA compared to the control group in this study (16.1% vs. 3.0%, OR: 0.163, 95% CI: 0.034-0.755, p=0.011). However, other genotype combinations, including GG + CC, CC + CT, CC + TT, CG + CC, CG + CT, CG + TT, GG + CT, and GG + TT, did not demonstrate significant differences between the two groups (Table 6). Overall, these findings suggest a potential association between specific genotype combinations of rs1800871 and rs1042522 SNPs and susceptibility to recurrent abortion, providing insights into the genetic factors underlying this condition.

Table 1. The primers used to survey rs1042522 and rs1800871 polymorphisms[22-41]

Gene name	Polymorphim	Primer type	Primer sequence (5' to 3')
IL-10	Rs1800871	R*	AGGATGTGTTCCAGGCTCCT
IL-10 N*	Rs1800871	F*	CCCTTGTACAGGTGATGATGTAAC
IL-10 M*	Rs1800871	F	ACCCTTGTACAGGTGATGTAAT
P53 N	Rs1042522	F	TCCCCCTTGCCGTCCCAA
P53 N	Rs1042522	R	CTGGTGCAGGGGCCACGC
P53 M	Rs1042522	F	GCCAGAGGCTGCTCCCCC
P53 M*	Rs1042522	R	CGTGCAAGTCACAGACTT

*R: Reverse, N: Normal, *F: Forward, M: Mutant, IL: Interleukin

Table 2. Clinical and demographic characteristics of the studied subjects

Characteristics	Patients (n=62)	Controls (n=66)	p-value
Age (mean)	30.69±4.76	32.73±5.31	0.104
Consanguineous marriage (Percentage %)	69.2% (n=27)	30.8% (n=12)	**0.003**
History of abortion and infertility (Percentage %)	25.92% (n=14)	-	-
Tobacco consumption (Percentage %)	3.7% (n=2)	5.6% (n=3)	0.647

n: Number

Table 3. Frequency distribution of rs1042522 and rs1800871 polymorphisms in all cases and controls

	Genotypes	Case	Control	Total	χ^2	p-value
P53	CC	35.5% (n=22)	27.3% (n=18)	31.3% (n=40)	1.499	0.473
	CG	54.8% (n=34)	57.6% (n=38)	56.3% (n=72)		
	GG	9.7% (n=6)	15.2% (n=10)	12.5% (n=16)		
	Total	100.0% (n=62)	100.0% (n=66)	100.0% (n=128)		
IL-10	CC	32.3% (n=20)	31.8% (n=21)	32.0% (n=41)	0.18	0.91
	CT	54.8% (n=34)	57.6% (n=38)	56.3% (n=72)		
	TT	12.9% (n=8)	10.6% (n=7)	11.7% (n=15)		
	Total	100.0% (n=62)	100.0% (n=66)	100.0% (n=128)		

IL: Interleukin

Table 4. Association study and different genetic models analysis of polymorphism rs1800871 between recurrent spontaneous abortion and control groups

Genotype/allele	Type	Patient	Control	Odds ratio	(95% confidence interval)		p-value
		n (%)	n (%)		Lower	Upper	
CC	Genotype	19 (35.2%)	19 (35.2%)	-	-	-	-
CT	Genotype	31 (57.4%)	30 (55.6%)	1.033	0.46	2.323	0.937
TT	Genotype	4 (7.4%)	5 (9.3%)	0.8	0.186	3.446	0.255
TT vs CC +CT recessive	Genetic model	50 (92.5%)	49 (90.7%)	0.874	0.199	3.093	0.727
CT +TT vs CC dominant	Genetic model	35 (64.8%)	35 (64.8%)	0.968	0.43	2.179	0.938
CT vs TT+CC over dominant	Genetic model	23 (42.5%)	24 (44.4%)	1.078	0.504	2.308	0.846
C	Allele	69 (63.9%)	68 (63.0%)	-	-	-	-
T	Allele	39 (36.1%)	40 (37.0%)	0.961	0.552	1.672	0.888

Discussion

To date, the simultaneous association of the SNPs rs1042522 and rs1800871 with RSA has not been investigated on a global scale. In this research, we studied the association of two polymorphisms in *P53* and *IL-10* genes with RSA, in the southwest of Iran for the first time. One of our main findings indicated that the genotypes frequency of P53 rs1042522 and IL-10 rs1800871 SNPs didn't show significant differences between RSA and control groups (p>0.05). Meanwhile, the comparison of alleles, recessive, dominant, and over dominant models did not demonstrate significant association between patients and healthy controls.

Previous research has analyzed the genotype and alleles of these polymorphisms individually, yielding results that either align with or contradict our findings. These findings demonstrated a complex association between *P53* and *IL-10* gene polymorphisms and RSA, which may vary across different populations. Concerning the rs1042522 polymorphism, similar results lacking an association between this polymorphism and RSA have been reported in previous studies. For instance, Yoon et al.[23] in 2015 in Korea studied 594 individuals (294 patients

Table 5. Association study and different genetic models analysis of polymorphism rs1042522 between recurrent spontaneous abortion and control groups

Polymorphism	Type	Patients	Control	(95% confidence interval)		Odds ratio	p-value
		n (%)	n (%)	Lower	Upper		
CC	Genotype	23 (42.6%)	15 (27.8%)	-	-	-	-
CG	Genotype	25 (46.3%)	31 (57.4%)	0.228	1.215	0.526	0.131
GG	Genotype	6 (11.1%)	8 (14.8%)	0.141	1.694	0.489	0.255
CG+GG vs CC dominant	Genetic model	31 (57.4%)	39 (72.2%)	0.232	1.158	0.518	0.107
GG vs CC+CG recessive	Genetic model	48 (88.9%)	46 (85.2%)	0.231	2.232	0.719	0.567
CG vs GG+CC over dominant	Genetic model	29 (53.7%)	23 (42.6%)	0.299	1.367	0.64	0.248
C	Allele	71 (65.7%)	61 (56.5%)	-	-	-	-
G	Allele	37 (34.3%)	47 (43.5%)	0.39	1.172	0.676	0.163

Table 6. Combined genotype distribution for rs1800871 and rs1042522 polymorphisms in recurrent spontaneous abortion and control group

p.Arg 72 pro + c.- C819T	Case (n=62)	Control (n=66)	Odds ratio	p-value
CC+CC	16.1% (n=10)	3.0% (n=2)	0.163 (0.034-0.755)	0.011
CC+CT	14.5% (n=9)	19.7% (n=13)	0.692 (0.273-1.757)	0.437
CC+TT	4.8% (n=3)	4.5% (n=3)	1.068 (0.207-5.500)	0.937
CG+CC	14.5% (n=9)	22.7% (n=15)	0.577 (0.232-1.4360)	0.234
CG+CT	32.3% (n=20)	30.3% (n=20)	1.095 (0.519-2.313)	0.812
CG+TT	8.1% (n=5)	4.5% (n=3)	1.842 (0.421-8.056)	0.411
GG+CC	1.6% (n=1)	6.1% (n=4)	0.254 (0.028-2.339)	0.194
GG+CT	8.1% (n=5)	7.6% (n=5)	1.070 (0.294-3.892)	0.918
GG+TT	0.0% (n=0)	1.5% (n=1)	1.015 (0.985-1.046)	0.331

and 300 healthy individuals) and revealed no significant association (p=0.3) between the rs1042522 polymorphism and RSA. On the other hand, Allafan et al.[25] in 2015 in Iran, Mashhad city (in the northeast of Iran), investigated 120 individuals (80 patients and 40 healthy individuals) and reported no association between the rs1042522 polymorphism and repeated in vitro fertilization (IVF) failure (p>0.05). In 2011, Wiwanitkit[26] studied a population in India a population including 302 patients, 302 patients, 57 controls, and 70 IVF failure cases. They reported no significant association between rs1042522 polymorphism and RSA or recurrent implantation failure (p>0.05).

Conversely, contrasting results have been reported in some studies for the rs1042522 polymorphism. For instance, in one of the cities in the center of Iran, Firouzabadi et al.[27] conducted a study in 2009, involving 62 individuals (41 patients and 21 healthy individuals), which revealed that the rs1042522 polymorphism may be a contributing factor in recurrent miscarriage and implantation failure (p=0.038). In 2013, a study in Spain by Lldeo et al.[28] demonstrated a significant association (p<0.05) between the rs1042522 polymorphism and RSA in a population, which included 98 patients and 83 healthy individuals. Similarly, a study in Spain in 2018 by Turienzo et al.[29] reported a significant association between the rs1042522 polymorphism and RSA in a non-selective population comprising 89 patients and 89 healthy individuals (p<0.05). Another study on RSA, conducted in Greece in 2022 by Dedousi et al.[30], involving 206 individuals (100 patients and 106 controls), confirmed a significant association between the studied polymorphism and RSA (p=0.002).

Concerning association studies of the rs1800871 polymorphism in IL-10 with RSA, various studies have demonstrated contradictory results. For instance, similarly to our results, in 2013, in India, Parveen et al.[31] found no significant association between RSA and rs1800871 polymorphism in a population of 298 individuals (134 patients and 164 healthy individuals) (p=0.3). Similarly, in 2014 in Bahrain, Qaddourah et al.[32] demonstrated no significant association between RSA and the rs1800871 polymorphism [-819(C/T)] in an Arab population comprising 296 patients and 305 healthy individuals (p=0.3).

Conversely, some studies have confirmed the association of the rs1800871 polymorphism with RSA. In Iran, in 2014, Bahadori et al.[33] investigated the association between rs1800871 polymorphism and RSA in a population of 196 individuals, comprising 191 patients and 95 healthy controls, in Tehran, and the results of their research were reported to be significant (p=0.006). In another study in 2014 in Ukraine, Zastavna et al.[34] demonstrated a significant association between rs1800871 polymorphism and RSA in a population of 100 patients and 73 healthy individuals (p<0.001). Similarly, in Romania in the same year, Bohiltea and Radoi[35] found a significant association between the rs1800871 polymorphism and RSA in a population of 69 patients and 64 healthy individuals (p=0.02). In 2015, Liu et al.[36] showed a significant association between the rs1800871 polymorphism and RSA in a Chinese Asian population comprising 284 patients and 284 healthy individuals. Additionally, in 2017, in the Indian Asian population, Vidyadhari et al.[23] showed a significant association between the rs1800871 polymorphism and RSA in a population of 180 individuals (100 healthy and 80 patients) (p<0.001).

Regarding all contradictory results in different studies, the non-significance observed in our study's findings may be attributed to ethnic differences and possibly the need for a larger sample size to investigate these polymorphisms further. Despite the high allelic frequency of these two studied polymorphisms, studying more samples across various countries and ethnicities is necessary to obtain a more accurate understanding of its association with RSA. Such studies are crucial for identifying factors involved for women with a history of RSA in different populations.

The second main result of this study was that individuals with the CC homozygous genotype for rs1042522 and rs1800871 polymorphisms were at an increased risk of spontaneous abortion (p=0.01) (Table 6). This is the first study that demonstrates the combined genotype distribution of these two polymorphisms, with RSA, which might provide novel insights into their combined influence on RSA. However, further studies need to be performed to confirm this effect.

The last main findings in our study were from investigating several phenotypic characteristics such as consanguineous marriage and the age of women in the target and control groups. According to the results obtained from this study, consanguineous marriage is likely to be associated with RSA (p=0.003), although so far most studies have been mainly cross-sectional and selected in specific regions and ethnic groups. The highest rate of consanguineous marriage can be observed in Middle Eastern countries. the prevalence of consanguineous marriage in Iran is about 38.6%, Due to socio-cultural factors. There is controversy as to whether there is any correlation between RSA and consanguinity[36]. Rad[38] determined that RSA occurs more frequently among related couples than those unrelated in India. An Iranian study conducted in 2010 found RSA to be more prevalent in the consanguineous group as compared to the non-consanguineous group. However, Saad and Jauniaux[39] found in 2002 that consanguinity did not correlate with RSA in Qatar. They concluded that this finding could be explained by the fact that autosomal recessive alleles are uncommon in Qatar. It could also be explained by the lack of association between consanguinity and RSA. In 2011, Gowri et al.[40] reported that consanguinity appears not to have any significant impact on the etiology of RSA and has no association with it in Oman. Further studies will be necessary to draw a conclusion.

Conclusion

As a result, research on rs1042522 and rs1800871 SNP and RSA is limited in Iran. Previous studies have *linked IL-10* gene rs1800871 and *P53* gene rs1042522 polymorphisms with RSA with different results. Research in this area is necessary to enhance understanding of the genetic factors of RSA and to identify possible risk factors for it.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Islamic Azad University North Tehran Branch (approval no: IR.IAU.TNB.REC.1401.065, date: 13.12.2022).
Informed Consent: Informed consent was obtained from the patients.

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Footnotes

Authorship Contributions

Concept: P.G., Design: P.G., Data Collection or Processing: N.R.B., K.S., F.S., Analysis or Interpretation: K.S., F.S., P.G., Literature Search: N.R.Z., P.K., Writing: N.R.Z.
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