Association between G1733A (rs6152) polymorphism in androgen receptor gene and recurrent spontaneous abortions in Mexican population

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Abstract

Introduction Recurrent spontaneous abortion (RSA) is a multifactorial condition that occurs with a frequency of 0.2–5% in women of reproductive age. Among genetic factors, the single nucleotide polymorphism (SNP) G1733A in the androgen receptor (AR) gene has been associated with its presence in Greek and Iranian populations. Therefore, the aim of this study is to determine its possible association with RSA in this population.

Patients and methods A total of 156 Mexican RSA (with at least 2 consecutive abortions) unrelated patients and 152 unrelated healthy women were included, the presence of karyotype anomalies in the parents as well as uterine anomalies as well as antiphospholipid antibodies was excluded in patients; while all the controls presented at least two healthy pregnancies and no abortion. In all the included women, the presence

of the SNP G1733A was determined by restriction fragment length polymorphism (RFLP) technique.

Results No significant differences were observed in age between groups. The genotype GG, GA, and AA had a frequency of 0.70, 0.27, and 0.03 in controls and of 0.89, 0.10, and 0.01 in patients ($p < 0.001$); while the A allele frequency was of 0.06 and 0.16 in controls and patients, respectively ($p < 0.0001$). The difference in allele frequency increased 10–15% when patients with primary RSA (with no live births) and with at least three abortions were included.

Conclusions The SNP G1733A of the AR gene is significantly associated with RSA in Mexican patients. These results coincide with previous reports in other populations.

Keywords Recurrent spontaneous abortion · Androgen receptor gene · G1733A polymorphism

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Introduction

Recurrent spontaneous abortion (RSA) is a condition in women of reproductive age defined as three or more consecutive pregnancy losses before the 20th week of gestation and has been documented with a frequency of 0.5–2% [1]. However, some authors consider it two pregnancies losses [2], a condition that presents a frequency of 5% [3]. The RSA can be primary or secondary depending whether the couple has had a live birth previously (secondary) or not (primary) [3]. The presence of RSA remains unexplained in around 45–50% of patients [4, 5]. Regarding the main causes, it has been shown that the frequency of cytogenetic abnormalities in miscarriage tissues obtained from women with RSA is higher than in general population, although it is lower than in women with sporadic loss (25–50% versus 50–70%) [6, 7], and the frequency

of parental karyotyping abnormalities including balanced translocations is also higher in couples with history of RSA than in the general population (2–5 versus 0.2%) [8].

With respect to other causes, it has been proven that genetic, pathophysiologic, and environmental factors are associated with RSA; among the non-genetic factors are the uterine anatomic abnormalities, endocrine abnormalities, obesity, sperm quality, and parental age [8]. In addition, many mutations and polymorphisms in different genes have been associated with RSA, these have been reported in genes coding for cytokines, proteins related with blood coagulation (prothrombin and factor V Leiden), sex hormone receptors, and others (VEGF, MTHFR, p53) [9]; the X chromosome skewed inactivation (>90%) has also reported as a risk factor for RSA [10].

Among the sex hormone receptors, the androgen receptor (AR) gene (located between Xq11-q12), codes for a protein that functions as a nuclear transcription factor activated by a steroid hormone, and the single nucleotide polymorphism (SNP) (G1733A/rs6152) has been associated with RSA in Iranian and Greek populations [2, 11]; however, considering that this polymorphism has been studied in very few pathologies including RSA and no studies have been conducted in Mexican population, we decided to determine if its presence was associated with RSA in this population.

Patients and methods

Patients

A total of 156 unrelated women with primary and secondary RSA were included, the inclusion criteria were: Mexican women (from the West side) from 18 to 42 years of age with at least two consecutive pregnancy losses with the same partner before the 20th week of gestation, normal karyotype in the couple, normal hysterosalpingography, negative antiphospholipids antibodies, and without the diagnosis of polycystic ovary and diabetes.

The control group was formed of 152 unrelated healthy women from the West side of Mexico, with at least two live births, without history of abortions, and by interview, they denied the presence of uterine malformations, antiphospholipid syndrome, and polycystic ovary. All patients and controls signed an informed consent.

Methods

DNA was extracted from peripheral blood leukocytes by the salting-out method [12], and the DNA amplification was performed with the following primers: forward primer 5'-CTGGATGAGGAACAGCAACC-3' and reverse primer 5'-CGTTGTCAGAAATGGTCGAA-3' [11]; the polymerase

chain reaction (PCR) was performed with 35 cycles, each cycle containing denaturation at 94 °C for 25 s, annealing at 64 °C for 25 s, and extension at 72 °C for 90 s, with a final extension time of 4 min at 72 °C. The final product of 379 bp was posteriorly digested with the restriction enzyme *StuI* at 37 °C for 30 min and inactivated at 75 °C for 5 min. The mutated allele (A variant) produced a complete fragment of 379 bp, while the wild type allele (G variant) produced a fragment of 313 bp. The fragments were separated on 6% acrylamide gel stained with silver nitrate.

Statistical analysis

The descriptive statistics consisted in frequencies and percentages for the allele and genotype frequencies and of means and standard deviations for the quantitative variables (women age, number of abortions/pregnancies/deliveries). The comparison of the allele and genotype frequencies between the patients and controls was performed with chi-square and Fisher's exact tests and in the case of the allele frequencies, the odds ratio was also calculated. For the quantitative variables we used Student *T* test for independent samples, with a *p* value <0.05 considered as statistically significant for all probes. The statistical analyses were performed in SPSS version 10.0.

Results

The mean age of the patients was 32.16 ± 4.85 (18–43) years and of the controls was 28.88 ± 5.29 (18–43) years ($p > 0.05$); the number of pregnancies, normal deliveries, cesarean sections, and abortions in both groups are described in Table 1. The allele and genotype frequencies between patients and controls showed significant differences (Table 2), being the A allele (mutant) 10% more frequent in patients with RSA than in controls ($p < 0.0001$). The differences among the genotypes also showed statistical significance ($p < 0.001$), being the homozygous (AA) and the heterozygous (GA) more frequent in patients. If we analyze only the cases with three or more abortions ($N = 107$), slight changes are observed with an increase in the difference of the frequency of the A allele between patients and controls from 10 to 12% ($p < 0.0001$).

Table 1 Pregnancy history in patients and controls

	Patients (<i>N</i> = 156)	Controls (<i>N</i> = 152)	<i>P</i> value
Pregnancies	3.64 ± 1.16 (2–6)	2.41 ± 0.93 (1–4)	<0.0001
Normal deliveries	0.31 ± 0.65 (0–3)	1.68 ± 1.17 (0–4)	<0.0001
Cesarean sections	0.31 ± 0.577 (0–3)	0.74 ± 0.88 (0–3)	<0.0001
Abortions	2.96 ± 0.87 (2–6)	0 ± 0 (0–0)	<0.0001

Data is described in means $\pm$ SD (ranges), *P* value obtained with *T* test for independent samples

Table 2 Allele and genotype distribution between patients and controls

Genotype/allele N (%)	Patients N (%) (N = 156)	Controls N (%) (N = 152)	OR (95% CI)	P value
GG	110 (70.51)	135 (88.82)		<0.001
GA	42 (26.92)	16 (10.53)		
AA	4 (2.57)	1 (0.65)		
Allele			3.03 (1.67–5.55)	<0.0001
A	50 (16)	18 (6)		
G	262 (84)	286 (94)		

Data showing the number and percentages of allele and genotype frequencies in patients and controls. In the third column, it is described the OR with the interval coefficient of 95% for the comparison of allele frequencies

A similar change is observed when we only include patients without any live birth ($N = 80$) with a difference in the A allele frequency of 11.5% ($p < 0.0001$), and when we combine both characteristics (≥ 3 abortions and no live births) ($N = 54$), the difference increases to 15% ($p < 0.00001$). In contrast, this difference is reduced to 5%, being no significant) when we only analyze the patients with ≤ 2 abortions ($N = 49$, $p = 0.07$). The genotypic frequencies in patients and controls did not deviate from the Hardy-Weinberg equilibrium ($p > 0.05$).

Discussion

The prognosis of RSA has been reported favorable with a likelihood of live subsequent births around 50–80% [3]; however, each case is different and many factors contribute to the success in future pregnancies, like the cause of the RSA (if known), maternal age, the number of preceding losses and the presence of heart beat in 2–3 of the first three miscarriages presented [13].

In this study, we observed a significant association between the A allele of the G1733A SNP and RSA, results that coincide with the previous reports in Iranian and Greek populations [2, 11]; however in this report, the difference in the A allele frequency between patients and controls was lower than in the previous reports from Jahaninejad et al. and Karvela et al. (10% versus 37% and 14%, respectively). This difference can be attributed to the patient selection criteria, because in the previous reports only patients without a live birth were included, and in the case of the study of Karvela et al., patients with at least three abortions were included. When we used these criteria, a difference of 15% in the frequency of the A allele between patients and controls is observed, which is 1% higher than that found by Karvela et al. This differences suggest that the A allele can be more associated with primary RSA with more than three abortions. It is also important to consider that the allelic frequencies in controls were different in Mexican population, in which the A allele frequency was higher than in the previous reports (0.94 versus 0.84), which could also influence the associations observed in the different populations.

In this regard, taking into account that the G1733A SNP was also associated with RSA in a different population (Mexican) from the previously studied (Greek and Iranian), we consider that this SNP begins to be of interest for further research in other populations.

The G1733A polymorphism is a silent SNP that does not produce an amino acid change at the position 211 of the protein, which is the glutamic acid; however, it has been shown that some silent SNP's have a functional effect by modifying the co-translational folding and thus the tridimensional structure of the protein [14]. On the other hand, the G1733A SNP is located in exon 1 between 2 polymorphic trinucleotide repeats (CAG and GGC) that code for polyglutamine and polyglycine tracts [11], and in vitro studies have shown that these variants are related with AR gene activity [15, 16]; in addition, the codon 211 resides in the N-terminal domain of the AR gene, that harbors the major transcription activation functions [17]. Therefore, a possible link between G1733A SNP and functional and/or transcriptional effects can be related with linkage disequilibrium with other variants or can be driven by unknown mechanisms associated with its important position in the gene.

The lack and excess of circulating androgens in premature ovarian failure and polycystic ovary syndrome, respectively, are associated with increased risk to early fetal loss and late obstetric complications due to impaired placental function [18–20]. In addition, it has been shown that increased responsiveness to androgen signaling is associated with decidualization of human endometrial stromal cells through cytoskeletal organization and cell cycle regulation, functions that may be critical for trophoblast invasion and placental development [21]. Therefore, a change in AR activation and/or expression through genetic variants, including the G1733A SNP, could lead to modifications in the endometrium that increase the likelihood of miscarriage.

In conclusion, we observed a significant association between the A allele of the SNP G1733A and RSA in the allele and genotype frequencies, being higher when only patients with at least three abortions and without a live birth are included. These results coincide with the two previous reports that searched for this association, which suggests that this SNP

is a risk factor to develop RSA in different populations. However, considering that this SNP is silent, further experimental studies will determine its effects in the androgen receptor function and/or expression.

Compliance with ethical standards

Ethical approval The project was approved by the ethics committee of the Mexican Institute of Social Security with the number: R-2010-1305-3, and all patients signed an informed consent prior to be included in the study.

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