G1733A Polymorphism of the Androgen Receptor Gene and Recurrent Spontaneous Abortion in Saudi’s Women

Afrah F. Alkhuriji¹, Suliman Alomar¹, Zeneb A. Babay², Mahmoud I. M. Khalil³

Abstract

Various gene polymorphisms have been reported to influence the risk of recurrent spontaneous abortion (RSA). In females, androgen receptor (AR) is expressed in reproductive tissues; however, the causal association of androgens receptors (AR: G1733A) in RSA has not been investigated in Saudi’s women with RSA. The aim of the present study was to investigate the potential effect of the G1733A polymorphism of the AR gene in the occurrence of RSA in Saudi women. The study group comprised of 65 Saudi women with unexplained RSA. The reference population consisted of 65 Saudi women who had at least 3 children, and were without known pregnancy losses or any known medical illnesses. Peripheral venous puncture, DNA extraction, PCR, and sequencing were employed to genotype women for the presence of a polymorphism at position G1733A of androgen receptor gene. For the G1733A polymorphism, the frequency of the A allele in the Saudi healthy females is 16.2%. The frequencies of homozygous AA were 6.2% and 0% in the control and RSA group, respectively, but the difference was not significant (p>0.05). In addition the frequency of heterozygous GA was 20% for the control group and 26.2% for the patient group but the difference was not significant (P>0.05). Regarding the G1729A polymorphism, the frequency of the A allele in the Saudi healthy females was 3.1%. The frequencies of GA+AA were 6.2% and 1.5% in the control and patient groups, respectively, and the difference was not significant (p>0.05). These findings confirmed that there were no causal association between the occurrence of the A allele of the G1733A and G1729A polymorphisms of the AR gene and increased risk of RSA in Saudi’s women.

Keywords: Recurrent spontaneous abortion, Androgen receptor, Gene polymorphism, Risk factor.

INTRODUCTION

Recurrent spontaneous abortion (RSA) is a clinical problem and occurs in women of reproductive age with a frequency of 1%–3% (Regan, 1998; Pandey et al., 2005; Shin et al., 2010). According to Royal College of Obstetrician and Gynaecologists, recurrent abortion is defined as 3 or more consecutive fetal loss before 20 weeks of gestation (green top guideline 17) (Li et al., 2002; Jahaninejad et al., 2013; Regan et al., 2010). The pathogenesis of RSA is very complicated (Xiao et al., 2014). The risk of miscarriage in a subsequent pregnancy is about 40 % to 50 % in women with a history of recurrent miscarriage (Jahaninejad et al., 2013). It is enhanced by a variety of factors including advanced maternal age, anatomic abnormality, chromosome abnormalities, hormonal disorders, hereditary thrombophilia, immune-logic factors, infections, and nutritional and environmental
Androgens are lipophilic hormones with several physiologic effects in both sexes (Quigley et al., 1995). In females, androgens production is very important for decidualization, a process that controls embryo implantation and placentation (Guay et al., 2004). Androgen receptor (AR) is commonly expressed in female reproductive tissue such as endometrium (Apparao et al., 2002). AR gene encodes a ligand-activated nuclear transcription factor that is activated by steroid hormones such as testosterone or dihydrotestosterone (Roy et al., 1999; Gelmann, 2002). Activated AR is transferred into the nucleus, where it regulates the transcription of androgen-responsive genes (Burger, 2002).

The AR gene is highly conserved, located on the chromosome Xq11-12 locus and is composed of eight exons (Brown et al., 1989). In humans, AR is a 110 kD protein composed of 919 amino acids (Trapman et al., 1998). The AR contains four domains: the amino terminal activation domain (NTD); the DNA-binding domain (DBD); the hinge region (HR); and the carboxyl ligand-binding domain (LBD) (Chang et al., 1995; Yong et al., 1998). AR exon 1 encodes the entire N-terminal domain (NTD) (a.a. 1-556) which comprises the bulk of the AR and is the least conserved of the four domains. This variability allows AR to differentially recruit co-regulators conferring androgen specific transactivation (Eisermann et al., 2013). Exon 1 of the gene harbours two microsatellites, two polymorphic trinucleotide repeats (CAG and GGC). Between these two repeats, a single nucleotide polymorphism (SNP) determined as a G to A substitution (G1733A; rs6152) in the third nucleotide position of the 211 codon has been previously described (Esteban et al., 2005). This codon resides in the N-terminal domain of the AR, that harbors the major transcription activation functions (Grad et al., 2001), raising the possibility that any variation in this codon may affect AR transactivational activity (5). G1733A polymorphism is a synonymous change as the glutamic acid remains unchanged (35), and it occurs in 13% to 20% of Caucasian populations (Lu and Danielsen, 1996; Ellis et al., 2001).

It was reported that there is a statistically significant association between the A allele of the G1733A polymorphism and the increased risk of prostate cancer (Medeiros et al., 2003; Kucerova et al., 2014). Other studies showed that women with pre-eclampsia have significantly higher androgen levels when compared to controls (Carlsen et al., 2005; Saarela et al., 2005). Furthermore, it has been shown that androgens play a significant role in the development of hypertension (Bachmann et al., 1991; Mantzoros et al., 1995) and obesity (Gustafson et al., 2003). Around 1,000 mutations are reported in the AR (Gottlieb et al., 2012). While the number of mutations reported continues to rise, the relevance of these mutations in RSA requires more studies. Few studies suggested that G1733A polymorphism is associated with a higher risk for RSA in some populations (Jahaninejad et al., 2013; Karvela et al., 2010). However the causal relationship between the risk of RSA and G1733A polymorphism has not been poorly understood.

In view of the review presented above. The aim of our study was to determine whether the AR G1733A polymorphism is associated with an increased risk for RSA in Saudi women.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board (IRB), College of Medicine, King Saud University.

The patients participating in this study were referred to the Recurrent Abortion Clinic, King Khaled University Hospital, Riyadh, Kingdom of Saudi Arabia from January 2010 to January 2011. There were no ethnic differences between cases and controls. 60% of the invited cases and controls declined to participate. The study group comprised of 65 women (mean age: 34.1 ± 6.2 years, range: 15-45 years) with unexplained RSA, consecutively referred. The reference population (controls) consisted of 65 women who had at least 3 children, and were without known pregnancy losses or any known medical illnesses. Routine analysis at the hospital laboratory were performed to exclude known causes of abortion: parental karyotypes; hormone levels; toxoplasmosis; cytomegalovirus; rubella; antiphospholipid antibodies; protein C; protein S; glucose level; hysteroscopy; hysterosalpingography; and serial ultrasound when needed. The criteria for inclusion were: females presenting with unexplained RSA after all the tests mentioned above were normal. The protocol of this investigation was approved by the Medical Ethics Committee of King Khalid University Hospital and the Ethical Committee of King Saud University, Riyadh, Saudi Arabia. All women were required to sign the informed consent form to participate.
Table 1. Demographic and clinical characteristics of unexplained recurrent spontaneous abortion patients in comparison with the control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>34.1 ±0.77</td>
<td>34.6 ±0.97</td>
<td>0.6</td>
</tr>
<tr>
<td>Height, m</td>
<td>156.97 ±0.75</td>
<td>158.56 ±0.68</td>
<td>0.414</td>
</tr>
<tr>
<td>Weight, Kg</td>
<td>75.15 ±2.45</td>
<td>71.34 ±1.64</td>
<td>0.126</td>
</tr>
<tr>
<td>BMI, Kg/m²</td>
<td>30.66 ±1.01</td>
<td>28.28 ±0.62</td>
<td>0.047*</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>6.5 ±0.38</td>
<td>3.9 ±0.22</td>
<td>0.0001*</td>
</tr>
<tr>
<td>No. of children</td>
<td>2.1 ±0.27</td>
<td>3.8 ±0.22</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

*statistically significant

Table 2. Genotype and allele frequencies of G1733A polymorphism of AR gene among patients with unexplained recurrent spontaneous abortion and controls.

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Patients (n= 65)</th>
<th>Controls (n= 65)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>48 (73.8)</td>
<td>48 (73.8)</td>
<td>1.00 (0.46-2.19)</td>
<td>1.00</td>
</tr>
<tr>
<td>GA</td>
<td>17 (26.2)</td>
<td>13 (20.0)</td>
<td>1.42 (0.62-3.22)</td>
<td>0.53</td>
</tr>
<tr>
<td>AA</td>
<td>0 (0)</td>
<td>4 (6.2)</td>
<td>0.11 (0.01-1.98)</td>
<td>0.12</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>113 (86.9)</td>
<td>109 (83.8)</td>
<td>0.78 (0.39-1.56)</td>
<td>0.60*</td>
</tr>
<tr>
<td>A</td>
<td>17 (13.1)</td>
<td>21 (16.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test.

Genotyping

Ten ml blood samples were extracted by venipuncture in ethylenediaminetetraacetic acid tubes from each case and control. Genomic DNA was extracted using the DNA Pure gene purification kit (Qiagen, Venlo, Netherlands). DNA was stored at 4°C until analysis. The purified DNA was amplified by PCR using sequence specific primers designed using the Primer3 program. The PCR amplification primers were as follows: forward primer: 5'-GAGGATGGTTCTCCCATCAAG-3'; reverse primer: 5'-GGACTCAATGTCTCCAACG-3'. PCR amplification was carried out in 25 µl volume containing 5U/µl of hot star Taq-polymerase (Qiagen, Venlo, Netherlands), 2.5 mMdNTPs (Amersham Pharmacia Biotech, NJ, USA), 10 µM/µl of each primer (Invitrogen, CA, USA), and ~50 ng DNA. PCR conditions comprised an initial denaturing step at 95°C for 15 min, followed by 36 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 2 min, and a final extension at 72°C for 10 min. After PCR, The generated 500 bp amplicons were subjected to sequencing using Applied Biosystems Integrated (ABI) 3130xl Genetic Analyzer following the kit manufacture instructions.

Statistical analysis

All statistical analyses were carried out using the Statistical Package for Social Sciences version 12 for Microsoft Window (SPSS Inc, Chicago, IL, USA) and the genotype and allele frequencies of the patient were compared with control using X² and Fisher exact test analysis and the difference was considered statistically significant if p<0.05.

RESULTS

The demographic and clinical characteristics of the patient and control groups are shown in Table 1. There were no significant differences between patients and controls concerning average age (p = 0.6). The average BMI (P= 0.047) and number of pregnancies (P=0.0001) of patients are significantly higher compared to the control women. The control women have significantly more live born children on average compared to patients (P=0.04).

This study was done to investigate G1733A polymorphism of the AR gene for 65 patients and 65 controls, the genotype and allele frequencies of G1733A polymorphism were shown in Table 2. In the present study, 48 patients had the GG genotype, and the other 17 patients had the GA genotype. 48 control subjects had the GG genotype, 13 had the GA genotype, and the other 4 had the AA genotype. No mutant homozygous (AA) carriers were identified in the patient group. Figure 1 is showing the sequence diagrams of G1733A locus in AR gene. The frequencies of the GG and GA genotypes of the G1733A polymorphism were 73.8%, and 26.2%,
Figure 1. Sequence diagrams of G1733A locus in AR gene. (1) Represented G allele, (2) represented A allele (homozygous), (3) represented genotype of GA (heterozygote).

Table 3. Genotype and allele frequencies of G1729A polymorphism of AR gene among patients with unexplained recurrent spontaneous abortion and controls

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Patients (n= 65)</th>
<th>Controls (n= 65)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>64 (98.5)</td>
<td>61 (93.8)</td>
<td>4.20 (0.46-38.63)</td>
<td>0.36</td>
</tr>
<tr>
<td>GA + AA</td>
<td>1 + 0 (1.5)</td>
<td>4 + 0 (6.2)</td>
<td>0.23 (0.03-2.19)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>129 (99.2)</td>
<td>126 (96.9)</td>
<td>0.24 (0.03-2.22)</td>
<td>0.37*</td>
</tr>
<tr>
<td>A</td>
<td>1 (0.8)</td>
<td>4 (3.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test.

respectively, for the patient group and 73.8%, 20%, and 6.2%, respectively, for the control group. Allele frequencies of the G1733A polymorphism among patients and controls were 86.9% and 83.8%, respectively, for the most frequent allele (G) (wild type) and 13.1% and 16.2%, respectively, for the A allele (mutant). Statistical analysis of the genotype frequencies showed that there are no significant differences between the two groups. Furthermore, as shown in Table 2, the analysis of allele frequencies indicated insignificant differences (P = 0.60; odds ratio 0.78) between women with RSA and controls. The χ² values of Hardy-Weinberg equilibrium test of G1733A locus for the patient group were 1.29 (P = 0.53), while χ² values of the control group were 4.43 (P = 0.11). These results suggested that the 2 groups in our study were in Hardy-Weinberg equilibrium and were demographically representative.

In addition, we identified, unexpectedly, a rare variation (G1729A) upstream the G1733A locus. The genotype and allele frequencies of G1729A variation of AR gene among the same patients with RSA and same controls are shown in Table 3. 64 patients had the GG genotype, and the other patient had the GA genotype. 61 control subjects had the GG genotype, and the other 4 had the GA genotype. No mutant homozygous (AA) carriers were identified in either the patient or control group. Figure 2 is showing the sequence diagrams of G1729A locus in AR gene. The frequencies of the GG and (GA+AA) genotypes of the G1729A variation were 98.5% and 1.5%, respectively, for the patient group and 93.8% and 6.2%, respectively, for the control group. Allele frequencies of the G1729A variation among patients and controls were 99.2% and 96.9%, respectively, for the most frequent allele (G) (wild type) and 0.8% and 3.1%, respectively, for the A allele (mutant). Statistical analysis of the genotype frequencies showed that there are no
Furthermore, as shown in Table 3, the analysis of allele frequencies indicated insignificant differences ($P = 0.37$; odds ratio 0.24) between women with unexplained recurrent spontaneous abortion and controls.

**DISCUSSION**

The progression of RSA is complicated and may be controlled by different hereditary pathways. It was reported that RSA is associated with proteins, encoded from different genes, involved in distinct biologic pathways (Buchholz et al., 2004; Tempfer et al., 2001). A number of studies were performed to explore whether polymorphisms of distinctive genes could contribute to RSA development. Polymorphisms in the genes coding for endothelial nitric oxide synthase (15, 43, 44), cytokine genes (16, 45), vascular endothelial growth factor (16), factor V (17), the angiotensinogen gene (14), Glutathione S-transferase A1 gene (46), prothrombin mutation and methylenetetrahydrofolate reductase genes (Lino et al., 2014), and folic acid metabolism-related genes (48) have been studied for the risk of RSA. Notwithstanding, the outcomes from diverse studies are frequently contentious, prompting indeterminate decisions about the role of these polymorphisms in RSA pathogenesis.

Steroid hormones and their receptors are involved as initiators or promoters in diverse ailments. AR CAG repeat length have been reported in several studies to be associated with prostate carcinogenesis (Medeiros et al., 2003; Montgomery et al., 2001; Kim et al., 2008), benign prostatic hyperplasia (Giovannucci et al., 1999; Roberts et al., 2004), and infertility among men (Ochsenkuhn and De Kretser, 2003; Davis-Dao et al., 2007; Giagulli et al., 2014), in addition to ovarian (Terry et al., 2005) and endometrial cancer risk (McGrath et al., 2006), polycystic ovary syndrome (Kim et al., 2008), autoimmunity in women with lupus (Olsen et al., 2014), and osteoporosis (Langdahl et al., 2003) in females. Several reported mechanisms pointed out the involvement of androgens in the development of breast cancer in females (Chen et al., 2014). One suggested mechanism included the conversions of androgens to E2 or their coupling to the estrogen receptor and/or AR (Elhaji et al., 2001; Giguere et al., 2001; Cox et al., 2006). Besides, it was shown by two laboratories the important role of AR in normal female reproduction (64-65). They provided the evidence that female AR$^-$ mice are subfertile. Accordingly, it is possible that polymorphisms that may affect AR function could be associated with RSA.

In the present study we attempted to investigate the potential effect of the G1733A polymorphism in the 211 codon of the AR gene in the occurrence of RSA. In addition, we identified, unexpectedly, a rare variation (G1729A) in the second nucleotide position of the 210 codon that had not been previously reported. G1729A polymorphism is a non-synonymous change as the arginine is changed to lysine. Beside two other reports (5, 18), This is the third report concerning the association of G1733A polymorphism to RSA. Previous data indicated that the allele frequencies of the G1733A polymorphism vary among different populations. In Mediterranean populations, the A allele frequency increases from 8% in
coastal Sardinians to 22%in Spanish Basques (Esteban et al., 2005), whereas it has been found to be13% in white North Americans (Lu and Danielsen, 1996), 2% in Brazilians (Ribeiro et al., 2002), and approximately 27% in North African populations (Esteban et al., 2005).

For the G1733A polymorphism, our present results indicated that the frequency of the A allele in the Saudi females is 16.2%. The frequency of homozygous AA is 6.2% and 0% in the control and patient groups, respectively, whereas the frequency of heterozygous GA is 20% for the control group and 26.2% for the patient group. For the G1729A polymorphism, our present results indicated that the frequency of the A allele in the Saudi females is 3.1%. The frequency of GA+AA is 6.2% and 1.5% in the control and patient groups, respectively. Statistical analysis indicated that there are no significant differences between the two groups and the two polymorphisms investigated in terms of both genotype distribution and allele frequencies. The presence of the A polymorphisms investigated in terms of both genotype and 1.5% in the control and patient groups, respectively.

CONCLUSIONS

Based on the findings of the present study, it can be concluded that there is no association between G1733A Polymorphism of androgen receptor and recurrent spontaneous abortion (RSA) in Saudi women. In addition, there was no association between AR G1729A polymorphism and RSA in Saudi’s women.

ACKNOWLEDGEMENTS

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Conflict of Interests

The authors declare that they have no conflict of interests.

REFERENCES


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