Association of the G2014G Genotype in Estrogen Receptor 1 Gene with Failure of the Mifepristone-Induced Termination of Early Pregnancy

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Mifepristone is a synthetic steroid compound that is used as a progesterone receptor antagonist. Dramatic disparities have been observed in the reactions to mifepristone. According to a study conducted in China, one to three percent of the subjects are very sensitive to mifepristone, achieving complete abortion before misoprostol is used (Sang et al. 1999). On the other hand, one to three percent of the subjects fail to achieve complete abortion even after receiving a combination oral treatment of mifepristone and misoprostol. Over 85% of the subjects achieve complete abortion after a combination of mifepristone and misoprostol (Sang et al. 1999). Success rates are different across countries because of variable regimens and/or route of administration, with a mean complete abortion rate of 94 to 96% (Kahn et al. 2000). Known etiologic factors of insensitivity to mifepristone are endocrine disorder, immunologic factors, infections, and environmental factors. In addition, there is evidence that a point mutation of genetic codon 722 in progesterone receptor gene (PGR) is often associated with mifepristone insensitivity in women (Gao et al. 1998). However, genetic etiology of mifepristone insensitivity remained largely unexplored.

It is a common phenomenon that reactions and side effects to the same drug differ greatly among different populations. This genetic diversity is reflected in three phases of drug-related gene polymorphisms: (1) some genes, such as cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4), ATP-binding cassette, sub-family B (MDR/TAP), member (ABCB1) and other related gene polymorphisms, take effect before drugs bind to corresponding receptors. (2) Some genes such as the PGR and ESR1 take effect while interacting with drugs. (3) Some genes such as matrix metalloproteinase (MMP) and prostaglandin E synthase 3 (PTGES3) take effect after drugs bind to their receptors.

SNPs are single-base variations that are widely distributed in the human genome, representing genetic diversity
among individuals (Kruglyak 1997). Inherited genetic disparities between individuals appear to account for each patient's response to medicine. Investigation of the relationship between genetic polymorphisms, particularly SNPs, and susceptibility or tolerance to a certain medicine in a population is likely to help clarify the genetic factors that influence the effectiveness of a particular drug (Shastry 2002).

Estrogen, the most important regulator in the female reproductive system, functions by binding with the estrogen receptor (ER). ER plays a central role in the estrogen/progesterone synergism and gene expression regulation. It is coded by the ESR1. PGR is one of the genes regulated by ESR1. There are 14 polymorphic sites in the ESR1 gene locus. All of the polymorphic sites are silent, and they do not alter the amino acid sequence. In this study, we focused on the three SNPs in the ESR1 gene, T-397C (rs2234693), C325G (rs1801132), and G2014A (rs2228480), which have been reported to be associated with dry eye syndrome, suicidal behavior, vertigo, heart disease, breast cancer, and stroke (Lee et al. 2007; Wang et al. 2007; Bos et al. 2008; Giegling et al. 2008; Imbert et al. 2008; Klos et al. 2008).

In this study, we examined the association between each of the three SNPs in the ESR1 and the sensitivity to mifepristone.

Materials and Methods

Subjects

This study was conducted from May 2002 to April 2003. Institutional review board (IRB) approval was obtained from the Academic Committee of the National Research Institute for Family Planning. A total of 90 pregnant women in Liaoning Province, China participated in this study. Informed consent was obtained from all of the subjects. They voluntarily chose mifepristone medical abortion to terminate early pregnancy (≤ 49 days of gestational age). The relevant information was collected such as basic information (e.g., age, weight, and height), health history, menstrual history, pregnancy history, delivery history, hereditary diseases, chronic diseases, current pregnancy (e.g., time of amenorrhea and diameter of the gestational sac), and reactions after oral administration of mifepristone.

All the subjects were requested to take 50 mg of mifepristone in the morning and 25 mg 12 hours later for two days. On the morning of the third study day, all the subjects took 600 µg of misoprostol and then stayed in hospital for six hours. The subjects’ reactions (e.g., frequency of vomiting, diarrhea, vaginal bleeding and gestational sac expulsion) were recorded by the nursing staff. The subjects then went home when their clinical condition was stable. Each was given a diary card to record the duration and amount of vaginal bleeding in comparison with their usual menstrual periods and any side effects of the drug. The subjects returned to hospital on the eighth study day, the 15th study day and the 30th study day after gestational sac expulsion.

Based on their symptoms of abortion, the 90 subjects were divided into two groups:

Failure group (n = 30): The subjects failed to achieve a complete abortion or had an ongoing pregnancy, and had to be treated with a surgical evacuation.

Success/control group (n = 60): The whole gestational sac was expelled after the combined use of mifepristone and misoprostol. The subjects experienced bleeding for less than two weeks. The urinary pregnancy test returned negative within two weeks.

Gene polymorphisms analysis

Genomic DNA isolation and restriction enzyme digestion followed a standard procedure (Iwase et al. 1996; Jin et al. 2004; Colson et al. 2006). See Table 1 for primers, annealing temperatures and the restriction enzymes used in the PCR or enzyme digestion systems.

Statistical analysis

Gene counting method was used to analyze the distributions of genotypic frequencies and allelic frequencies of polymorphisms in the ESR1. A Chi-square test was used to compare the genotypic frequencies and the allelic frequencies between the two groups. SPSS13.0 was used to examine the association. A p value of < 0.05 was considered statistically significant.

Results

The data for analysis were from 90 subjects (30 with failure in medical abortion and 60 success controls). Table 2 describes the characteristics for the two groups. Similarities existed between the two groups in terms of age and occupation. A gene counting method was employed to investigate the polymorphic region of the ESR1 between the success and the failure groups. Three SNPs were identified in the ESR1: T-397C in intron 1 (NCBI, dbSNP number, rs2234693), C325G in exon 4 (NCBI, dbSNP number, rs1801132; codon 325), and G2014A in exon 8 (NCBI, dbSNP number, rs2228480; codon 594). The distance between the T-397C and the G2014A is 256,714 bp.

When comparing the genotypic frequency and the allelic frequency of the T-397C between the two groups, we found no significant differences (p = 0.148 and p = 0.289, respectively).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer sets</th>
<th>Annealing temperatures</th>
<th>Restriction enzyme</th>
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<tbody>
<tr>
<td>T-397C</td>
<td>5'TGACACATGTCTGTGTGTTCA3' 5'CCAGAATATGTGTACCTAAAA3'</td>
<td>50°C</td>
<td>Pvu II</td>
</tr>
<tr>
<td>C325G</td>
<td>5'TCAGGATACGAAAAAGACC3' 5'GCCCATGTGACTGTTGAACC3'</td>
<td>59°C</td>
<td>Hinf I</td>
</tr>
<tr>
<td>G2014A</td>
<td>5'GAGGAGACGGACCAACAACCCAC3' 5'GCCATTGGTGTGGATGCATGC3'</td>
<td>67°C</td>
<td>Btg I</td>
</tr>
</tbody>
</table>

Table 1. The pairs of primers for SNPs in the ESR1.
respectively, in Table 3). Likewise, the genotypic and allelic frequencies of C325G showed no significant differences between the two groups ($p^a = 0.327$ and $p^b = 0.831$, respectively, in Table 4). Thus, there was no association between each of the two SNPs, T-397C and C325G, and the risk of failure in medical abortion.

When the frequency of the AA, AG, and GG genotypes of the G2014A polymorphism was compared between the two groups, a significant difference was observed ($p^a = 0.030$ in Table 5). In the failure group, the frequency of the GG homozygote was the highest (86.7%), while that of AA was the lowest (6.7%). With regard to the heterozygote distribution, the frequency of the AG genotype was higher in the success group (28.3%) than that in the failure group (6.7%). Moreover, the frequency of the A allele was higher in the success group (25.8%) and lower (10.0%) in the failure group; conversely, the frequency of the G allele was higher in the failure group ($p^b = 0.013$ in Table 5). These results indicate that the GG genotype of the G2014A polymorphism is associated with the risk of failure in the mifepristone-induced abortion.

**Discussion**

Combined treatment of mifepristone and misoprostol is a routine practice for terminating early gestation. One to three percent of subjects are very sensitive to this regimen, while one to three percent of subjects fail to react at all (Sang et al. 1999). Reactions to mifepristone are complex, caused by multiple genes and environmental factors. It remains unknown why the reactions vary so dramatically among individuals. Pharmacogenomic or pharmacogenetic methods are now applied to identify the genetic mechanism of different reactions to mifepristone. In this study, we analyzed the association between ESR1 polymorphisms and

<table>
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<th>Table 2. Characteristics of the subjects.</th>
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<tr>
<td><strong>Age</strong></td>
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<tr>
<td>20-25</td>
</tr>
<tr>
<td>Success (n = 60)</td>
</tr>
<tr>
<td>Failure (n = 30)</td>
</tr>
<tr>
<td>χ²a = 3.308</td>
</tr>
<tr>
<td>χ²b = 4.372</td>
</tr>
</tbody>
</table>

^a Comparison of the four age groups between success and failure groups using χ²-test with 4 × 2 contingency table.

^b Comparison of three kinds of occupations between success and failure groups using χ²-test with 3 × 2 contingency table.

Note: The subjects of the two groups are similar in terms of age and occupation.

<table>
<thead>
<tr>
<th>Table 3. Genotypic and allelic frequencies of the ESR1 T-397C.</th>
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<tbody>
<tr>
<td><strong>Genotypic distribution</strong></td>
</tr>
<tr>
<td>CC (n (%))</td>
</tr>
<tr>
<td>Success (n = 60)</td>
</tr>
<tr>
<td>Failure (n = 30)</td>
</tr>
<tr>
<td>χ²a = 3.827</td>
</tr>
<tr>
<td>χ²b = 1.125</td>
</tr>
</tbody>
</table>

^a Comparison of CC, CT and TT genotypic distribution between success and failure groups using χ²-test with 3 × 2 contingency table.

^b Comparison of C and T allelic distribution between success and failure groups using χ²-test with 2 × 2 contingency table.

Note: The subjects of the two groups are similar in the genotypic and allelic distribution.

<table>
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<th>Table 4. Genotypic and allelic frequencies of the ESR1 C325G.</th>
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<tr>
<td><strong>Genotypic distribution</strong></td>
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<tr>
<td>CC (n (%))</td>
</tr>
<tr>
<td>Success (n = 60)</td>
</tr>
<tr>
<td>Failure (n = 30)</td>
</tr>
<tr>
<td>χ²a = 2.233, p^a = 0.327</td>
</tr>
<tr>
<td>χ²b = 2.45, p^b = 0.451</td>
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</tbody>
</table>

^a Comparison of CC, CG and GG genotypic distribution between success and failure groups using χ²-test with 3 × 2 contingency table.

^b Comparison of C and G allelic distribution between success and failure groups using χ²-test with 2 × 2 contingency table.

Note: The subjects of the two groups are similar in the genotypic and allelic distribution.
individual reactions to mifepristone medical abortion in order to comprehend the way mifepristone affect target genes. We performed PCR-RFLP on regions where variants in the ESR1 were known to occur and found the GG genotype frequency in the G2014A polymorphism was significantly higher in the failure group than in the success group. Thus, the GG genotype of the G2014A polymorphism is associated with the risk of failure in the mifepristone-induced abortion.

In the subsequent sections, we discuss three questions regarding the G2014A polymorphism: How is it associated with the failure medical abortion? How does it cause the failure medical abortion? And what role does it play in estrogen-related disorders in a broad sense?

Mifepristone is a strong antagonist of the progesterone, and the binding force between mifepristone and the PR is five times higher than that of progesterone and the progesterone receptor (PR). Mifepristone concentration increases in the serum of pregnant women after mifepristone is taken. When mifepristone binds with the PR, the structure of the PR changes or PGR transcription is inhibited, causing the PR to decrease in cytoplasm and leading to abortion (Jiang et al. 2002). However, this study observed the association between the ESR1 polymorphisms and reactions to mifepristone-induced abortion, which can be explained by the crosstalk between the ER and the PR in the body at three levels: hormonal, transcriptional and protein synthesis.

At the hormonal level, higher concentration of mifepristone induces a decrease of PR in the decidua of pregnant women. Endometrial cells display degeneration and necrosis while estrogen increases in the meantime. ER is positively regulated by estrogen and negatively regulated by progesterone. Progesterone negative regulation is blocked by mifepristone, resulting in increase in the ER and decrease in the PR. Therefore, the increase of ER, complicated by upregulation of estrogen, changes the ratio of ER and PR. As a result, the development of endometrial is delayed and implantation of gestation sac becomes difficult (Jiang et al. 2002). At the transcriptional level, the PGR and ESR1 act independently as transcriptional regulators. PGR transcription is regulated by the ER (Patel et al. 2008). Estrogen responsive elements (ERE) on PGR promoters are activated by ER and with co-activator proteins such as AIB1 and CBP/P300 (Ciocca et al. 2006). ESR1 is mainly expressed in human endometrium (Lecce et al. 2001). ESR1 promotes transcription after combining with estrogen, followed by the activation of proliferation of endometrium is active. Human estrogen response element is located in the upstream of oxytocin receptor (OXTR) promoter (Vasudevan et al. 2001). Expression of OXTR mRNA is up-regulated by the combination between ER and estrogen. Decreased ESR1 results in the weakness of uterine contraction and the difficulty of endometrium discharge, prolonging uterine bleeding (Zhu et al. 2003). At the protein level, PR synthesis is dependent on the pathway of ER synthesis (Kimura et al. 1991). The mobility of PR is hastened by the coexistence of ER, but the mobility of ER is not changed by the existence of PR (Matsuda et al. 2008). Zhu et al. (2003) reported that the decreased ERα levels in the human endometrium could be related to prolonged uterine bleeding after medical abortion induced with mifepristone and misoprostol. It might be possible that the A-to-G transition at 2014 may cause the decrease in the ESR1 transcriptional efficiency and ER synthesis is decreased in the endometrial cells.

It should be noted that G2014A is a silent polymorphism that does not alter the amino acid sequence, which might be explained by the linkage disequilibrium between G2014A with and regulatory sequences in the 3'UTR of the ESR1 gene (Ongphiphadhanakul et al. 2001, 2003, 2005). Specifically, both 5' and 3'UTR have been reported to play an important role in the regulation of gene expression. The ESR1 gene, similar to other genes coding for steroid hormone receptors, has an extremely long 3'UTR. There is an AU(AT)-rich element playing a controlling role in the stability of mRNA in the 3'UTR region, and polymorphism in the 3'UTR in the vicinity of the ATTTA motifs has been
demonstrated to affect gene transcription as well as certain clinical features (Keaveney et al. 1993). Moreover, the 3’ UTR also contains sequences regulating poly adenylation which that affect mRNA processing and mediate rapid mRNA turnover (Kenealy et al. 2000). This response induces alterations in estrogen levels that interfere with pregnancy maintenance (Sundarraj et al. 1999).

This study revealed the effect of the G2014A polymorphism on mifepristone reactions. The subjects with the A allele were sensitive to mifepristone and successfully managed in early pregnancy termination. Conversely, the sensitivity to mifepristone decreased in the subjects with the G allele, as they failed in medical abortion. The G2014A polymorphism is associated with post-menopausal osteoporosis, bone mineral density in postmenopausal and breast cancer, all of which are hormone-dependent diseases (Curran et al. 2001; Ongphiphadhanakul et al. 2001, 2003). The frequency of the A allele is higher than that of the G allele in osteoporosis (Ongphiphadhanakul et al. 2001, 2003). The frequency of the G allele is higher than that of the A allele in breast cancer population; a subject has approximately a three-fold increase in the risk of developing breast cancer, if she does not express the AA genotype (Curran et al. 2001). All these studies suggest the important role of the G2014A polymorphism in the estrogen-associated diseases and call for further verification of its functions and application.

In conclusion, the G2014G genotype is significantly associated with the insensitivity to oral mifepristone plus misoprostol among Chinese women. In this sense, our finding regarding a potential association of \textit{ESR1} SNP and mifepristone insensitivity in women may provide guidance for individualized medication as well as for drug development in the future. However, multiple genes and environmental factors determine various reactions to mifepristone during termination of early pregnancy, and the effect of a single gene is limited. The study with a large number of isolated samples and high throughput screening is necessary to fully understand the genetic basis of the failure in medical abortion.

References


