A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss

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The CYP17 gene encodes the enzyme cytochrome P450c17α, which mediates both 17α-hydroxylase and 17,20-lyase activity in the steroid biosynthesis pathway. A T→C polymorphism in the 5' promoter region of CYP17 has been described. To examine the association between recurrent pregnancy loss (RPL) and a polymorphism in CYP17, a case–control study of 117 cases with RPL and 164 controls was conducted. This polymorphism was investigated by PCR/restriction fragment length polymorphism using DNA from peripheral lymphocytes. The T→C transition in the variant allele (A2) creates a new recognition site for the restriction enzyme MspA1, which permits designation of the wildtype allele (A1) and A2. Women with the A2 allele of CYP17 had an increased risk of RPL [A1/A1 genotype (reference); A1/A2 genotype: odds ratio (OR), 1.68; 95% confidence interval (CI), 0.94–3.01; A2/A2 genotype: OR, 2.37; 95% CI, 1.16–4.83; P trend, 0.016]. Additionally, there was a similar tendency for the increased risk of primary RPL [A1/A1 genotype (reference); A1/A2 genotype: OR, 2.14; 95% CI, 1.14–4.01; A2/A2 genotype: OR, 2.50; 95% CI, 1.16–5.41; P trend, 0.015]. These results suggest that possession of the A2 variant of CYP17 may predispose to an increased risk of RPL with a gene dosage effect.

Key words: CYP17/estrogen/genetic polymorphism/molecular epidemiology/recurrent pregnancy loss

Introduction

About 10–14% of clinically recognized pregnancies end in pregnancy loss in the Japanese population as well as in Caucasians. The aetiology of recurrent pregnancy loss (RPL) remains largely unclear (Stirrat, 1990; Parazzini et al., 1991; Cramer and Wise, 2000; Yamada et al., 2001). Epidemiological studies have suggested that the condition may be multifactorial with a possible genetic predisposition and involvement of environmental factors in its pathogenesis (Parazzini et al., 1991; Fenster et al., 1991; Cramer and Wise, 2000). Recently, a number of genetic studies have revealed an association between RPL and genetic polymorphisms related to metabolic enzymes (Zusterzeel et al., 2000; Sata et al., 2003), cytokines (Reid et al., 2001; Unfried et al., 2001; Wang et al., 2002; Saijo et al., 2003), coagulation factors (Wrambsy et al., 2000; Pilhusch et al., 2001), methylenetetrahydrofolate reductase (MTHFR) (Lissak et al., 1999; Wrambsy et al., 2000; Pilhusch et al., 2001) and histocompatibility antigens (Aldrich et al., 2001).

The CYP17 gene, located on chromosome 10, consists of eight exons and encodes the cytochrome P450c17α enzyme (Picado-Leonard and Miller, 1987). Cytochrome P450c17α mediates both steroid 17α-hydroxylase and 17,20-lyase activities and functions at key steps in the genesis of human sex steroid hormones. The 5'-untranslated promoter region of CYP17 contains a single base pair (bp) T→C polymorphism that may create a new Sp-1 site (CCACC box) at 34 bp upstream from the initiation of translation and 27 bp downstream from the transcription start site (Carey et al., 1994). This polymorphism in CYP17 introduces a restriction site for MspA1, giving two alleles: A1 which does not contain the restriction site and A2 which contains the restriction site (Carey et al., 1994). Serum levels of androgens and estrogens have been shown to be elevated in women who carry the A2 allele (Feigelson et al., 1998; Haiman et al., 1999). These findings suggest that the A2 allele may provide an additional promoter activity with an increased rate of transcription of CYP17 mRNA (Carey et al., 1994). This may lead to increased production of precursor androgens and to subsequent conversion to estrogens. On the other hand, a more recent in-vitro study has shown no binding of the transcription factor Sp-1 to the variant sequence (Kristensen et al., 1999).

The 5'-untranslated promoter polymorphism of CYP17 has been investigated with respect to hormonally related cancers, especially breast cancer. Women carrying the A2 allele of CYP17 were reported to have an increased risk of some types of breast cancer (Feigelson et al., 1997; Bergman-Jungestrom et al., 1999; Miyoshi et al., 2000; Spurde et al., 2000), while other studies failed to demonstrate this association (Weston et al., 1998; Helzlsouer et al., 1998; Dunning et al., 1998; Kristensen et al., 1999; Hamajima et al., 2000). Meta-analyses have revealed that there were overall no significant associations between the promoter polymorphism of CYP17 and breast cancer (Dunning et al., 1999; Ye et al., 2002).

Although the A2 polymorphism of CYP17 has been a topic of a number of studies investigating hormonally related cancers, there has been no study concerning the CYP17 polymorphism in a field of infertility. As RPL might be a multifactorial disease with a possible genetic predisposition, we hypothesized that the A2 polymorphism of the CYP17 gene might play a crucial role as a modifier of risk factors of RPL because the A2 allele would be a susceptible marker for hormone-related diseases such as breast cancer, ovarian cancer...
In the present study, we set out to determine whether the A2 polymorphism of CYP17 is associated with any risk of RPL in a case-control study.

### Materials and methods

This case–control study was performed in the city of Sapporo, Japan, during the years 1999–2002. We studied 117 patients aged 20–43 years with a history of RPL and controls resident in Sapporo and the surrounding areas in Japan; all the patients and the controls were native Japanese. In recent times the geographical region has had little population immigration by different ethnic groups. The characteristics of the study groups are shown in Table I. RPL was defined as having a history of two or more spontaneous consecutive miscarriages or stillbirths. The primary RPL group comprised 102 women with a history of two or more pregnancy losses but no live birth. The 15 secondary RPL women experienced two or more pregnancy losses after at least one live birth. A total of 108 RPL women experienced all their miscarriages in the first trimester. Sixty-four of 117 RPL women experienced all their miscarriages at <9 weeks of gestation, and 44 women experienced at least one miscarriage between 9 and 13 weeks of gestation, but 44 women experienced all their miscarriages at <9 weeks of gestation. All the patients with RPL and controls were resident in Sapporo and the University Graduate School of Medicine. All the patients with RPL and controls were resident in Sapporo and the University Graduate School of Medicine.

### Results

The frequencies of the CYP17 genotypes were compared between 117 patients with RPL and 164 controls in a Japanese population (Table II). The distribution of genotypes in each group was in Hardy–Weinberg equilibrium. Sixty-four (54.7%) cases were heterozygous, and 29 (24.8%) were homozygous for the A2 variant of CYP17, compared with 84 (51.2%) and 27 (16.5%) controls. The adjusted OR for RPL associated with heterozygosity for the A2 variant was 1.68 (95% CI, 0.94–3.01), and the OR for homozygosity of the A2 variant was 2.37 (95% CI, 1.16–4.83).

We evaluated the risk of pregnancy loss in the subgroups of patients according to the type of RPL (primary or secondary), the number of pregnancy losses, and gestational ages of previous miscarriages. We found a similar tendency for the increased risk of primary RPL (Table III). Sixty-one (59.8%) cases were heterozygous, and 23 (22.6%) were homozygous for the A2 variant of CYP17 in the women with primary RPL. The adjusted OR for primary RPL associated with heterozygosity for the A2 variant was 2.14 (95% CI, 1.14–4.01), and the OR for homozygosity of the A2 variant was 2.50 (95% CI, 1.16–5.41). On the other hand, the number of pregnancy losses or gestational ages of previous miscarriages were not related to the risk of RPL (data not shown).
We also found a statistical significance in a logistic regression model for the risk of total RPL and primary RPL in correlation with an increasing number of the A2 allele (total RPL versus control, $P = 0.016$; OR, 1.55; 95% CI, 1.09–2.20; primary RPL versus control, $P = 0.015$; OR, 1.60; 95% CI, 1.09–2.33). The results indicate that the presence of the A2 allele may increase the risk of RPL and primary RPL with a gene dosage effect.

**Discussion**

It has been suggested that the CYP17 genotype is an important biomarker for the onset of ovulation in adolescents and the initiation of regular ovulatory cycles (Feigelson et al., 1997). RPL occurs in women of reproductive age whose estrogen levels fluctuate to a great extent during the menstrual cycle (Kado et al., 2002). Endocrinological abnormalities such as elevation of the free androgen index and low progesterone levels at the mid-luteal phase have been found in approximately a quarter of women with RPL (Li et al., 2000).

In the present study, we found that the possession of the A2 variant of CYP17 might have an increased risk of RPL with a gene dosage effect. There was a greater tendency for an increased risk of primary RPL in women who carried the A2 allele. The A2 allele of CYP17 is likely to have impacts on women of reproductive age by causing hormonal imbalance in their reproductive organs. To the best of our knowledge, this was the first study to investigate the possible role of CYP17, which encodes the key enzyme for sex steroid hormone biosynthesis, in relation to the aetiology of RPL. It is necessary to examine not only serum levels but also local levels of these hormones at the maternal-fetal interface such as the decidua, in order to elucidate the functional role of the A2 allele during the reproductive process.

The presence of the A2 allele of CYP17 has previously been described as an independent risk factor for some types of breast cancer (Feigelson et al., 1997; Bergman-Jungestroem et al., 1999; Young et al., 1999), even though conflicting results have been reported (Techatraisak et al., 1997; Dunning et al., 1998; Helzlsoer et al., 1998; Weston et al., 1998; Kristensen et al., 1999). From an epidemiological point of view, the A2/A2 genotype is infrequent, which leads to a small sample size of the compared groups and low statistical power (Kristensen et al., 1999). Elevated estrogen levels throughout the pre-menopausal period was suggested explanation of the observed association between the A2 allele and a higher risk of advanced breast cancer in elderly patients (Feigelson et al., 1997). There remains the possibility that elevated estrogen levels could confer a high-risk phenotype earlier in life such as RPL, especially primary RPL. When the precise mechanism of CYP17 genotype in sex steroid synthesis pathways is clarified in future studies, women with RPL carrying the A2 allele may be appropriate targets for medical interventions such as hormonal therapies.

The enzyme cytochrome P450c17a has a bifunctional active site: one catalytic centre performs the 17α-hydroxylation of pregnenolone and progesterone and another, the 17,20-lyase activity, is responsible for the conversions of 17α-hydroxypregneno- lone to dehydroepiandrosterone and 17α-hydroxyprogesterone to androstenedione, precursors of testosterone and estrogens (Helzlsoer et al., 1998; Weston et al., 1998). In the adrenal glands the bulk of 17α-hydroxyprogesterone is used in glucocorticoid production with minor fractions ending as dehydroepiandrosterone and androstenedione (Nakajin et al., 1984). The adrenal CYP17 expresses considerable 17α-hydroxylase activity, but little 17,20 lyase activity, suggesting tissue-specific regulation of gene expression (Voutilainen and Miller, 1986). On the other hand, ovarian theca cells express high levels of 17,20-lyase activity during the reproductive period, suggesting tissue-specific regulation of CYP17 (Kristensen et al., 1999). It would be of interest to search for interactions of the polymorphism in the 5'-untranslated promoter region of the CYP17 gene with tissue-specific transcription factors other than the relatively ubiquitous Sp-1, which is present in ovarian theca cells and uterine endometrial cells, but absent in the adrenal.

This study provides evidence that a genetic factor related to sex steroid hormone synthesis and metabolism may affect the risk of RPL. Additional molecular and epidemiological studies need to be performed to clearly elucidate the role of the variant form of CYP17 as well as other steroid-metabolizing genes.

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**References**


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*CYP17 and the risk of recurrent pregnancy loss*

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