A polymorphism in the CYP17 gene and intrauterine fetal growth restriction

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Intrauterine fetal growth restriction is a multifactorial disorder, and its aetiology includes both environmental and genetic components. We aimed to investigate whether maternal genetic polymorphisms of metabolic enzymes affects fetal growth and pregnancy duration. Genomic DNA was obtained from 134 women who experienced singleton deliveries beyond 24 weeks of gestation. Maternal age, birth weight, gestational age at birth and frequencies of fetal growth restriction, prematurity and pregnancy-induced hypertension were compared among genotypic subgroups of cytochrome P450 (CYP) and glutathione S-transferase (GST) genes. The polymorphisms of CYP1A1 (MspI), CYP17 (MspAI) and GSTP1 (BsmAI) genotypes, and the presence or absence of GSTM1 and GSTT1 genes were analysed by PCR-based methods. The frequency of fetal growth restriction (<10th percentile/<±1.5 SD; 22.7%/11.4%) in 44 women who were homozygous for the A1 allele (A1A1) of CYP17 was significantly higher than that (7.8%/2.2%) in 90 women who carried the A2 allele (A1A2/A2A2) of CYP17 (P < 0.05), with an odds ratio =3.41 (95% confidence interval = 1.18–9.84). The gestational age at birth (mean ± SD, 37.5 ± 3.1 weeks) in 67 women with GSTM1 null genotype was significantly lower than that (38.5 ± 2.4 weeks) in 67 women who carried GSTM1 (P < 0.05). The polymorphism of CYP17 that encodes the cytochrome P450c17α enzyme might be associated with the pathophysiology underlying fetal growth restriction.

Key words: CYP1A1/CYP17/genetic polymorphism/GST/fetal growth restriction

Introduction

The conditions of fetal growth are controlled mainly by genetics during the first trimester of pregnancy and are affected chiefly by the maternal environment in the second and third trimesters of pregnancy (Polani, 1973). Intrauterine fetal growth restriction is a primary cause of low birth weight and accounts for notable perinatal morbidity. When fetal growth is slowed sufficiently to result clinically in intrauterine fetal growth restriction, there is a significantly increased risk of cerebral palsy, short stature and sub-normal intellectual and psychological performance during later childhood (Barker, 1999).

The pathophysiological processes that occur at the cellular and molecular levels in fetal growth restriction are still largely unknown, though recent studies have demonstrated that many genetic factors significantly influence fetal weight. The maternal MspI polymorphism of cytochrome P450 1A1 gene (CYP1A1) and glutathione S-transferase T1 (GSTT1) null genotype were found to be associated with reduction in birth weight among women who smoked cigarettes, suggesting an interaction between metabolic genes and environmental factors (Wang et al., 2002). The association between maternal congenital thrombophilia and fetal growth restriction was investigated in several studies which have shown an increased incidence of thrombophilia in pregnancies complicated by fetal growth restriction; congenital thrombophilia included factor V Leiden mutation, the prothrombin mutation and protein S deficiency (Kuperminc et al., 1999, 2002; Martinelli et al., 2001). High frequencies of Thr235 allele in the angiotensinogen gene were also demonstrated in mothers with fetal growth restriction as well as in affected fetuses (Zhang et al., 2003). In the promoter polymorphism, the absence of the wild-type allele in the gene of insulin-like growth factor-I (IGF-I) in individuals was found to be associated with low birth weight (Vaessen et al., 2002). In addition, children carrying the 191-allele of IGF-I were found to have reductions in birth weight, length and head circumference, as well as persisting short stature (Arends et al., 2002).

Enzymes belonging to the GST and cytochrome P450 (CYP) families are involved in the two-stage detoxification process of a wide range of environmental toxins and carcinogens. The genes for these enzymes are part of the aryl hydrocarbon (Ah) gene battery and are under Ah receptor control (Nebert and Gonzalez, 1987). The Ah receptor binds a number of different classes of chemicals, including halogenated aromatics such as dioxin and polycyclic aromatic hydrocarbons, which induce transcription of the genes in this battery (Safe, 1995). Ah hydroxylase, encoded by the CYP1A1 gene, is a well studied phase 1 enzyme and is particularly relevant to the metabolism

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of chemicals in cigarette smoke. The phase 2 metabolism is a detoxification process in which these metabolic intermediates are detoxified by enzymes such as GSTs. The GSTs are a supergene family of enzymes that are believed to exert a critical role in cellular protection against toxic foreign chemicals and oxidative stress (Hayes and Strange, 2000). GSTM1 is involved in detoxification of carcinogens, including polycyclic aromatic hydrocarbons (Harris, 1991; Nebert, 1991). GSTT1 enzyme is important in protecting against certain forms of genotoxic damage, such as sister chromatid exchanges (Schroder et al., 1995) and the formation of haemoglobin adducts due to ethylene oxide (Fennell et al., 2000). GSTP1 is a major enzyme involved in the inactivation of carcinogens such as benzo[a]pyrene diol epoxide and toxic constituents such as acrolein (Haines and Pulford, 1995). The polymorphisms of these GST genes were found to be associated with susceptibility to malignant tumours (Harries et al., 1997; Strange and Fryer, 1999).

In this study, we aimed to investigate whether maternal genetic polymorphisms of metabolic enzymes affected fetal growth and pregnancy duration. Birth weight, gestational age at birth and the frequencies of fetal growth restriction, prematurity and pregnancy-induced hypertension (PIH) were compared among genotypic subgroups of CYP1A1, CYP17, GSTM1, GSTT1 and GSTP1.

Subjects and methods

Characteristics of subjects

This cohort study was performed in the city of Sapporo, Japan, and conducted with informed consent from all of the subjects, and was approved by the institutional ethical board for human gene and genome studies of Hokkaido University Graduate School of Medicine. Peripheral blood was obtained 1 month post-partum from 134 consecutive Japanese women aged 21–42 years who experienced singleton deliveries beyond 24 weeks of gestation (GW) in the obstetric ward of Hokkaido University Hospital and who gave us written informed consent. Women who had autoimmune disease, anti-phospholipid antibody syndrome or congenital thrombophilia, developed gestational diabetes mellitus, and women who delivered malformed infants in the index pregnancies were excluded from the study. Maternal and infantile medical records were reviewed to obtain clinical data including pregnancy complication such as PIH and birth outcomes such as birth weight, gestational age, presence or absence of growth restriction and prematurity, i.e. deliveries at less than 37 GW. Of 134 women, six developed PIH and 20 experienced premature deliveries. Seventeen infants were born with fetal growth restriction, which was defined as weighing less than the 10th percentile for gestational age from the standard growth curve, seven infants were found to have been born with fetal growth restriction, which was defined as weighing less than the 10th percentile for gestational age from the standard growth curve, and 26 infants were born with fetal growth restriction, which was defined as weighing less than the 10th percentile for gestational age from the standard growth curve. Values of maternal age, birth weight, gestational age at birth and the frequencies of fetal growth restriction, prematurity and PIH in all 134 women were compared among genotypic subgroups of CYP1A1, CYP17, GSTM1, GSTT1 and GSTP1.

Statistical analysis

The Fisher’s exact test (two-tail) was used to analyse the results. The t-test was used for the comparison of maternal age, birth weight and gestational week at birth. Values of P < 0.05 were considered statistically significant. We calculated the age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) associated with the CYP17 genotypes by logistic regression analysis, using SPSS (SPSS Inc., Chicago, IL). Hardy–Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies using a chi-square test.

Results

Maternal age, birth weight, gestational age at birth and the frequencies of fetal growth restriction, prematurity and PIH in all 134 women were compared among genotypic subgroups of CYP1A1, CYP17, GSTM1, GSTT1 and GSTP1. These data with genotype frequencies are shown in Table I.

We first performed Fisher’s exact test (two-tail) to detect any association between genotypes in the five different genes and fetal growth restriction, and found significant association only among the CYP17 genotypes. The frequency of fetal growth restriction (<10th percentile (<−1.5 SD; 22.7%/11.4%) in 44 women who were homozygous for the A1 allele (A1A1) of CYP17 was significantly higher than that (7.8%/2.2%) in 90 women who carried the A2 allele (A1A2/A2A2) of CYP17 (P = 0.025/P = 0.038). Similarly, the birth weight (mean ± SD, 2634 ± 610 g) of the babies born to women homozygous for the A1 allele of CYP17 was relatively lower than that (2851 ± 593 g) in the women who carried the A2 allele of CYP17 (P = 0.052). If correction for multiple comparisons (Bonferroni) among the five genes was performed, the results were no longer significant. Maternal age, gestational age at birth and the frequencies of prematurity and PIH were not statistically different among the genotypic subgroups of CYP17.
Additionally, the gestational age at birth (mean ± SD, 37.5 ± 3.1 weeks) in the offspring of 67 women with GSTM1 null genotype was significantly lower than that (38.5 ± 2.4 weeks) in the offspring of 67 women who carried GSTM1 (GSTM1 present) (P < 0.05). Maternal age, birth weight and the frequencies of fetal growth restriction, prematurity and PH were not statistically different between women with GSTM1 null and women with GSTM1 present.

Among genotypic subgroups of CYP1A1, GSTT1 and GSTP1, maternal age, birth weight, gestational age at birth and the frequencies of fetal growth restriction, prematurity and PH were not statistically different.

By logistic regression analysis, there was a significant difference in frequencies of the homozygous A1 allele of CYP17 (A1A1 versus A1A2/A2A2) between women with fetal growth restriction (<10th percentile) and women without. In the present cohort study, the risk of fetal growth restriction was higher in the women with the homozygous for the A1 allele (A1A1) than in carriers of the A2 allele (A1A2/ A2A2), with an OR = 3.41 (95% CI = 1.18–9.84). The ORs of fetal growth restriction among three groups were as follows: A1A1, 1.00 (reference); A1A2, 3.28 (95% CI = 2.48–4.37); and A2A2, 0.35 (0.25–0.49). No significant differences were found between the A1A2 and the A2A2 genotypes. Therefore, the A2 allele had dominant, but no additive effects. If an additional case–control study is planned, to detect an OR of 3.0 with 80% power at the 5% significance, a total of 62 cases in each group, each of with and without fetal growth restriction, will be needed (Armitage and Berry, 1994).

The distribution of each genotype of CYP1A1, CYP17 and GSTP1 followed Hardy–Weinberg equilibrium, which indicated that no selection occurred among genotypes.

### Discussion

Intrauterine fetal growth restriction is a multifactorial disorder, and its aetiology includes both environmental and genetic components (Johnstone and Inglis, 1974; Khoury et al., 1989; Magnus et al., 1997). Epidemiological studies have demonstrated a possible role of genetic and familial factors in determining birth weight (Bakketeig et al., 1979; Hackman et al., 1983; Klebanoff et al., 1984, 1997; Winkvist et al., 1998). Recently, the maternal CYP1A1 MspI polymorphism and GSTT1 null genotype were found to be associated with a reduced birth weight in women who smoked cigarettes, suggesting an interaction between metabolic genes and environmental factors (Wang et al., 2002). The authors demonstrated that women who smoked cigarettes and carried the m2 allele of CYP1A1 and GSTT1 null genotype had a greater risk of low birth weight, while among women who had never smoked neither genotype had an influence on birth weight. These gene polymorphisms have been associated with their encoded enzyme activity; it is suggested that CYP1A1 MspI variant genotypes increase enzyme activity (Landi et al., 1994), and GSTT1 null genotypes lead to an absence of enzyme activity (Pemble et al., 1994). We found that the mother’s genotype of CYP1A1, GSTT1 and GSTP1 had little affect on birth weight, pregnancy duration or the frequencies of fetal growth restriction and prematurity, although a possible confounding factor such as maternal smoking was not evaluated in the present study. It was found that women carrying the GSTM1 null genotype had a shorter pregnancy duration, ~1 week shorter than that in women carrying the GSTM1.

High frequencies of the GSTM1 null genotype have been found in patients with lung cancer, bladder cancer, cutaneous tumours, chronic bronchitis and endometriosis, which are regarded as environmentally induced (Baranova et al., 1997). We have also revealed that the GSTM1 null genotype increases the risk of recurrent pregnancy loss, and have suggested the involvement of this metabolic enzyme in the aetiology of reproductive failure (Sata et al., 2003). Thus, the GSTM1 null genotype may adversely affect the establishment of pregnancy and embryo development as well as the maintenance of pregnancy, probably through interaction with environmental factors that are yet undetermined.

The CYP17 gene, which is located on chromosome 10 and consists of eight exons, encodes the cytochrome P450c17α enzyme (Picado-Leonard and Miller, 1987). The cytochrome P450c17α enzyme 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 T-to-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. 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). It is suggested that the A2 allele may provide an additional promoter activity with an increased rate of transcription of CYP17 mRNA (Carey et al., 1994), leading to an increased production of precursor androgens and to subsequent conversion to estrogens. Serum levels of androgens, estrone and estradiol have been shown to be elevated in women who carry the A2 allele (Feigelson et al., 1998; Haiman et al., 1999). A recent study demonstrated that the age at menarche in women carrying the A2 allele was younger than in those who were homozygous for the A1 allele, suggesting that the estrogen-metabolizing CYP17 genotype influences age at menarche (Gorai et al., 2003). Women carrying the A2 allele of CYP17 were reported to have an increased risk of advanced breast cancer (Feigelson et al., 1997), early-onset breast cancer (Bergman-Jungestrom et al., 1999; Spurdle et al., 2000) and late-onset breast cancer (Miyoishi et al., 2000). Consistent positive correlations between pregnancy estrogens and fetal size have been demonstrated (Loriaux et al., 1972; Gerhard et al., 1986; Petridou et al., 1990; Gardner et al., 1997; Markestad et al., 1997; Peck et al., 2003); and a decrease in serum estradiol levels has been found in women with fetal growth restriction (Salas et al., 1993). Estrogens are generally acknowledged to stimulate cell proliferation and placenta growth, possibly through estrogen receptor alpha (Bukovsky et al., 2003) and are, thus, important determinants of fetal growth (Petridou et al., 1990). Trichopoulos et al. (Trichopoulos, 1990) has hypothesized that the risk of breast cancer in the adult offspring is related to high estrogen exposure in utero. This hypothesis has sparked a substantial amount of recent work on the association between pre- and perinatal estrogen exposures and breast cancer risk. A review of epidemiological studies suggested that a higher birth weight increased the risk of breast cancer compared with a normal birth weight (Potischman and Troisi, 1999). Compared with women with a birth weight of 2000 g or less, women with a birth weight of >3500 g had a 12-fold increase in breast cancer risk (Kajiser et al., 2001). In the present study, we demonstrated for the first time that women homozygous for the A1 allele of CYP17 had higher frequencies of fetal growth restriction; i.e. women carrying the A2 allele of CYP17 had lower risks of fetal growth restriction. Although serum estrogen levels during pregnancy were not evaluated in the present study, the CYP17 genotype was likely to affect fetal growth due to modification of maternal estrogen production. If this is the case, Trichopoulos’ hypothesis, in which high birth weights increase the risk of breast cancer in the adult offspring, might be merely reflected by a genetic linkage from mothers to fetuses in carriers with the A2 allele of CYP17, which was both factors of the breast cancer risk and the promotion of fetal growth due to elevated estrogen production. This point should be clarified by further genetic and epidemiological studies.

To the best of our knowledge, this is the first study to investigate the possible role of CYP17, which encodes the key enzyme for sex steroid hormone biosynthesis, in relation to intrauterine fetal growth. However, this study has several limitations. The number of subjects in this study population is rather small. Our results became no longer significant when multiple testing was taken into consideration (Bonferroni correction). However, this correction is a controversial matter since reduction of a type I error for null association increases the type II error for those that are not null ( Rothman, 1990). A power calculation suggests that for a future study, a total of 62 cases in each group with and without fetal growth restriction should be included to obtain completely unambiguous results.

As our subjects were community dwelling, our results may not apply to other populations. We cannot exclude the possibility of confounding effects resulting from uncontrolled or inadequately controlled risk factors such as nutritional status, alcohol consumption and cigarette use. These points should be further clarified in case-controlled and multivariate studies that consider the interaction between environmental factors and multigenetic factors including the fetal genotype.

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