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Association of rs7260002 of chorionic gonadotrophin β5 with idiopathic recurrent spontaneous abortion in Chinese population

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Abstract
Purpose Low levels of serum hCG during the first trimester is regarded as a predictor of miscarriage. This study was designed to examine whether variance in chorionic gonadotrophin β5 (*Cgb5*) gene confers risk to recurrent spontaneous abortions (RSA) in Chinese women.
Methods We recruited a total of 454 RSA subjects and 460 controls from our medical center between the years 2010 to 2013. rs7260002, which resides in the promoter region of *Cgb5*, was genotyped through direct sequencing.
Results The carriers with the minor allele of rs7260002 had reduced risk of RSA (P=0.018; P adjusted=0.032; OR: 0.76; 95 % CI: 0.61–0.96). Genotype frequency was further analyzed under additive, recessive, and dominant models. Significant differences between the RSA subjects and controls were detected under additive and recessive models (P additive= 0.040; P recessive=0.010).
Conclusions The current study identified a protective allele of the *Cgb5* gene against RSA. Functional studies are required to elucidate the effect of the identified SNP on CGB expression and HCG hormone activity.

Keywords Recurrent spontaneous abortion · Chorionic gonadotrophin β5 · Genotype · Genetic association study

Yong Sun and Xuan contributed equally to this work.

Capsule The *Cgb5* polymorphism is associated with the development of RSA in Chinese population.

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Introduction

Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses before 22 gestational weeks or the spontaneous abortion of an embryo/fetus weighing less than 500 g. RSA affects around 1–3 % of couples trying to conceive [1]. Although complicated tests, such as karyotype analysis, thrombosis-related tests, and endocrine or immunological tests, are employed to establish the specific causes of RSA, the underlying causes of nearly half of the RSA patients remain unknown [2]. Previous studies suggested the existence of a familial aggregation of RSA, indicating that genetic factors may contribute to the etiology of RSA [3].

An increasing number of genetic association studies are conducted to determine the genetic background of idiopathic RSA [4]. In these candidate-gene association studies, genes were evaluated based on their biological function and importance in pregnancy. Because cytokines, hormones, and angiogenic mediators have their important roles in implantation and gestation, the polymorphisms in these genes have been intensively studied [4]. A resequencing study using samples from Estonia and Finland indicated a possible role of the chorionic gonadotropin β-subunit gene (CGBs) polymorphisms in RSA [5]. *CGB* genes encode the receptor-binding and hormone-specific subunit of human chorionic gonadotrophin (hCG), which is an indispensable placental hormone in human pregnancy [6]. Low levels of hCG during the first trimester of pregnancy are related to miscarriage and ectopic pregnancy [7–9]. At the same time, high concentration of hCG, especially its beta subunit, is a diagnostic marker for gestational trophoblastic diseases [10]. There are six consecutive *CGB* genes (*CGB*, *CGB1*, *CGB2*, *CGB5*, *CGB7*, and *CGB8*), which originated from the nearby luteinizing hormone beta (LHB)

gene on chromosome 19q13.3. *CGB5* is one of the most actively transcribed genes among the six *CGB* genes [11]. CGB5 is regarded as the major contributor of hCG function during pregnancy [6, 12].

Existing studies have sequenced the coding regions of *CGB5*. The present study using a large data base examines the relationship study with larger sample size to examine the relation between single-nucleic polymorphisms (SNPs) of *CGB5* and RSA in Chinese women. Previous studies in the Caucasian population have indicated that two SNPs (rs59079581 and rs377204493) in the promoter region of CGB5, except their minor allele frequency (MAF), was too low in the Asian population [6]. Thus, rs7260002, which is also located in the promoter region of CGB5 with appropriate MAF, was selected. Rs7260002 was also suggested to be associated with elevated risk of breast cancer by determining levels of hCG hormone by changing the transcription activity and, hence, the pregnancy-related protection from breast cancer risk [13]. As a preliminary association study of CGB5 in the Eastern Asian population, rs7260002 in the promoter region was chosen because SNPs in the promoter are more likely to lead to altered transcription level of CBG5.

Materials and methods

Study population

As *CGB* genes are produced by trophoblast, maternally and paternally derived gene variants have equal contribution to the fetal genotype [14]. The participants of our study included the RSA women and their husbands who had experienced recurrent pregnancy losses. We recruited a total of 454 RSA subjects (179 couples and 96 single female patients with a history of three or more spontaneous abortions during the first trimester of pregnancy) and 460 controls (230 couples of proven fertility with normal menstrual cycles and ovary morphology, without the history of subfertility treatment.) from our medical center between the years 2010 to 2013. Our study was approved by the ethics committee of our medical center and informed consent, and informed consent was obtained from all the participants. All the subjects lived in the Shandong Province and are from the Chinese Han ethnical group. All cases were excluded from the study when routine clinical assessments identified all the possible causes for RSA, including anatomic, hormonal, chromosomal, infectious, autoimmune, or thrombotic ones.

Genotyping of rs7260002

Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The SNP genotyped in the current study, rs7260002 (−1595 A/C), resides in the promoter region of *CGB5*. The region containing rs7260002 was amplified using the polymerase chain reaction (PCR). The primers and reaction conditions were briefly stated as below. The primer sequences were as follows: forward, 5′ GTGAGCAATACT TCACTGGCG 3′; reverse, 5′ TTTCTGCCTGACCAGTTC CTG 3′. PCR conditions were 94 °C for 5 min, followed by 24 cycles at 94 °C for 30 s, 57 °C for 45 s, 72 °C for 45 s, and a final step at 72 °C for 10 min.

The PCR products were first analyzed using agarose gel electrophoresis and were then sequenced on an ABI3730XL capillary sequencer (Applied Biosystems, Forster City, CA) for automatic sequencing.

Statistics

Continuous variables were summarized with descriptive statistics (n, mean, and standard deviation) and analyzed using independent-samples *T* test. Categorical variables were described with counts and percentages and compared using chi-squared test. PLINK (v.1.07, http://pngu.mgh.harvard.edu/purcell/plink) was applied to test the Hardy-Weinberg equilibrium. Unconditional logistic regression analysis was used to minimize the impact of age. Genetic models were divided into the following: additive (+/+vs. +/−vs. −/−), dominant (+/+plus +/−vs. −/−), and recessive (+/+vs. +/−plus −/−). Logistic regression for disease trait was conducted to exclude the potential confounding effect of age. Statistical analyses were performed using the SPSS statistical software system (version 17.0; SPSS Inc., Chicago, IL). $P<0.05$ was considered statistically significant.

Results

All the RSA subjects ($n=454$) and controls ($n=460$) were of the Chinese Han ethnicity, and age of the RSA subjects at enrollment was more advanced than the controls (mean ± SD) (29.0±5.5 vs. 28.1±5.0), respectively. The RSA group and the control group have similar body mass index (BMI) (21.3± 2.3 vs. 21.6±2.6).

The allele and genotype distributions of rs7260002 among the RSA subjects and controls are shown in Table 1. The allele frequency in RSA subjects and controls showed no deviation from Hardy–Weinberg equilibrium. The minor allele frequency (MAF) was significantly different between cases and controls ($P=0.018$; odds ratio [OR], 0.76; 95 % confidence interval [CI], 0.61–0.96). Because the difference of age between RSA subjects and controls was significant, we adjusted the P value by age using the regression analysis. According to the result, the difference of allele distributions between RSA

Table 1 Frequency distribution of CGB5 alleles and genotypes in RSA and control group

	Allele		Genotype		
	C (freq)	A (freq)	C/C (freq)	A/C (freq)	C/C (freq)
Control (n=460)	218 (0.237)	702 (0.763)	55 (0.12)	108 (0.235)	297 (0.646)
RSA (n=454)	174 (0.192)	734 (0.808)	28 (0.062)	118 (0.26)	308 (0.678)
P value		0.018			0.016 *

* P value calculated under the recessive model [homozygotes of risk allele vs. (heterozygotes + homozygotes of non-risk allele)]

patients and controls remained significant after adjustment ($P_{adjusted}$=0.032). Using the chi-squared test, genotype frequency was further analyzed under additive, recessive, and dominant models. We detected significant differences between the RSA patients and controls under additive and recessive models ($P_{additive}$=0.040; $P_{recessive}$=0.010).

Discussion

Previous studies have confirmed that carriage of certain variances in the promoter region of *CGB5* seems to protect against RSA [5]. According to the results of meta-analysis across data from three populations (Estonians, Finns, and Danes), a modest but significant effect of the *CGB5* promoter variants rs59079581 (−155 G/C, OR= 0.64; 95 % CI, 0.44–0.94) and rs377204493 (−142 T/A, OR=0.66; 95 % CI, 0.45–0.94) in reducing the risk for RM has been established in Northern European population [15]. Considering the importance of physiological function of *CGB5* and ethical discrepancy, we decided to initiate the current study by focusing on the relation between SNPs of *CGB5* and RSA among Han Chinese population. However, the MAF of the two identified SNPs in the Northern European population (rs59079581 and rs377204493) are inappropriately low in East Asian population according to the 1,000 Genomes Database (http://browser.1000genomes.org/index.html). Another SNP, rs7260002, which was also located in the promoter region of CGB5 was chosen in the current study [13]. Taking advantage of our relatively large sample size, our study identified the association between rs7260002 and the susceptibility to RSA in Han Chinese population. Significant differences of genotype frequency between the RSA subjects and controls also were found under additive and recessive models.

HCG is essential for the successful progression of pregnancy. It is assumed that some miscarriages are due to a primary failure of the trophoblast to produce hCG. Low levels of hCG in the first trimester of pregnancy has long been considered as a predictor of miscarriage, ectopic pregnancy, and failure of IVF procedure [7–9]. Intensive studies have revealed that its function includes maintaining the production of steroid hormones and other growth factors in the corpus luteum, blastocyst implantation, uterine vascularization and angiogenesis, uterine quiescence, and immunological adaptation during pregnancy [16–19]. HCG, a glycoprotein hormone, is composed of identical alpha subunit similar to other glycoprotein hormones (LH, FSH, and TSH) in addition to a hormone-specific beta catalytic subunit [20]. *CGB5* genes encode the hormone-specific beta subunit and are thus indispensable for successful pregnancy maintenance. It has been found that in most normal placentas, *CGB5* was the most highly transcribed gene [11]. Another study has found a high level of expression of CGB5 and CGB8 in the placenta throughout pregnancy with a minor decrease during the second trimester, and the expression was found to be moderately correlated with hCG level in the maternal serum. Certain polymorphisms in the promoter region gene may enhance CGB5 transcription and thus increase the hCG level. According to F-SNP (http://compbio.cs.queensu.ca/F-SNP/), an online functional SNPs predicting software, rs7260002 is conserved across multiple species, which suggests rs7260002 might be functional. The protective allele found with lower prevalence in RSA, compared with control couples, suggests that some miscarriages in RM couples may be caused by polymorphisms in the CGB genes. Couples lacking such protective polymorphisms may benefit from hCG supplementation, but this hypothesis must be tested in prospective trials in the future.

Some potential limitations should be considered when interpreting the results of the present study. First, although our sample size was relatively large, our patients mainly came from the Shandong province of northern China. Considering the possible genetic discrepancy among the Han Chinese population from the different areas of China [21], our findings may not be generalizable until the association is successfully replicated in subjects from other areas of China. Furthermore, other *CGB5* variances located in other genetic components (exons, introns, and 3′ untranslated area) should be tested, which could provide more insight into the relation between *CGB5* variances and RSA. Lastly, functional study should be conducted to reveal the relation between rs7260002 and *CGB5* transcript.

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