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Relationship between Cytochrome P450 2E1 Gene Polymorphism and Susceptibility of Low Birth Weight Infants

Xiaoli Liu, Juan Du, Pei He, Xuemei Gao and Xiaoyan Deng#

Department of Obstetrics and Gynecology, Wuhan No.1 Hospital, Wuhan 430022, China

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ABSTRACT The purpose of this study is to investigate the relationship between cytochrome P450 2E1 (CYP2E1) RsaI/PstI and DraI single gene polymorphism (SNP) and low birth weight infants (LBW) of Chinese population. The researchers detected CYP2E1 RsaI/PstI and DraI SNPs by real-time PCR cycling and HRM analysis in 461 premature delivery and 461 healthy pregnant women. This paper found that RsaI/PstI C_2C_2 was statistically significantly correlation with increased risk of LBW (Odds ratios= 0.66,95% CI: 0.44–0.98, P = 0.04) and (Odds ratios = 0.75 , 95% CI: 0.56–0.99, P=0.04) for C_2C_2, compared with C_1C_1 and C_1C_1 + C_1C_2, respectively. When CYP2E1 RsaI/PstI and DraI SNPs combine, the risk of low birth weight infants was increased in individuals with both DraI TT and RsaI/PstI C_2C_2 genotypes (Odds ratios=0.59, 95% CI=0.36–0.97). The current study demonstrates CYP2E1 RsaI/PstI polymorphism is significantly related to LBW and the interaction between the RsaI/PstI and DraI polymorphisms has a significant impact on LBW.

INTRODUCTION

Cytochrome P450 (CYP450) is a type of multifunctional enzyme system, which mainly exists in the liver. CYP450 participates biotransformation in biological endogenous compounds including fatty acids, steroids and bile acids and exogenous compounds including drugs, procarcinogens and pro-toxicants (Rocejanasaro et al. 2014). In the human CYP450 super family, cytochrome P4502E1 (CYP2E1) accounts for seven percent of the total amount of CYP450 in the liver (Gonzalez 2007). CYP2E1 is mainly participate in the metabolism and activation of many small molecules for instance ethanol, nitrosamines, halogenated hydrocarbons and benzene (Wang et al. 1999). Furthermore, the physiological function of CYP2E1 may be mainly related to the glycogen genesis of acetone (Hu et al. 1997). It also involved in drug metabolism, and can catalyse the activation process of many pre-carcinogens and pro-toxins (Wu et al. 1997). As the SNPs in five flanking regions of the gene can cause obvious individual variations in its transcription activity, it can lead to obvious individual differences in enzyme activity (Howell et al. 2008). *Rsa*I (rs2031920) and *Pst*I (rs3813867) are SNPs in five flanking regions. The two SNPs were in complete linkage disequilibrium (LD), According to reports, this will alter the transcriptional activity of genes, which results in three different genotypes, that is, the homozygote of normal alleles (C_1C_1, Rsa I+ /Pst I-), the nucleotide heterozygote (C_1C_2) and homozygote (C_2C_2, Rsa I-/Pst I+) after nucleotide substitution (Han and Zhou 2000). Another important SNP is DraI (rs6413432) in intron 6, which is a mutation from T to A that is reported to alter the transcription of the CYP2E1 gene (Tang et al. 2000).

Low birth weight (LBW) refers to babies whose birth weight is less than 2500 grams (Cai 2002), which is an adverse pregnancy outcome. LBW can lead to higher mortality making babies susceptible to mental retardation and stunted growth.(Pinz et al. 2015; Doritsakou et al. 2016). The adverse pregnancy outcome of low birth weight in new-borns has many influencing factors including social, nutritional, psychological, environmental, infection, and genetic factors (Zhang et al. 2008; Grazuleviciene et al. 2015; Yu et al. 2012; Tiwari et al. 2015). Some researchers at home and abroad have studied the susceptibility of many related genes to LBW, but the results of the studies are inconsistent.

Objective

In order to clarify the possible impact of genes or the interaction between genes and

#*Address for correspondence:*
Xiaoyan Deng,
Wuhan No. 1 Hospital,
215 Zhongshan Avenue,
Wuhan City, Hubei Province, China
Telephone: 027-85332316,
E-mail: Xiaoyandengdr@yeah.net

genes on LBW, the researchers conducted an epidemiological investigation of the Department of Obstetrics and Gynaecology Wuhan No. 1 Hospital. The goal of this article is to observe observe the influence of the polymorphism at the 5 terminal Rsa I site of the gene CYP2E1 on the LBW

MATERIAL AND METHODS

Subjects

This case-control study involved 922 pregnancy cases undergoing delivery, the case group consisting 461 blood samples from mothers of preterm infants, and the control group consisting 461 maternal blood samples of normal delivery in the same period. They were all recruited from the Department of Obstetrics and Gyneecology Wuhan No. 1 Hospital from November 2017 to February 2021.

Mothers were included in the standard for no obstetric complications, such as eclampsia, diabetes and placenta previa.The standard of choosing LBW infants is gestational age >28 weeks, weight <2500 grams., should have been a ormal delivery, excluding premature infants due to accidents, and eliminated twins or more tyres with congenital fetus malformation. The criteria for choosing normal weight infants are: >28 weeks of gestational weeks of birth in the same hospital, >2500 grams of weight, or difference in birth time between <7 days, normal single birth babies, and not including pregnant women and newborns with complications and pregnancy complications. No statistical meaning exist between the two groups (P > 0.05). The study was approved by the Institutional Ethics Committee of Wuhan No. 1 Hospital, and all procedures follow agency guidelines.

DNA Extraction and Genotyping

According to reagent instructions, QIAamp DNA microkit (Qiagen) was used to extract DNA from whole blood samples.Through polymerase chain reaction high resolution melts (PCR-HRM) and LightCycler® 480 System (Roche Applied Science, Germany),the PCR products of the analyzed variants were analyzed by high-resolution melt (HRM) analysis. RsaI/PstI primers used included F 52 -GAGAGGTGCAGTGTFAGGTGC-32 , R 52 - CA-GACCCTCITCCACCITCTATG-3', and DraI: F52 -TCG TCA GTT CCT GAA AG C AGG-32 , R52 -GAG CTC TGA TGC AAG TAT CGC-32 .At 10 ¼l amplification in volume, mixed with wild type DNA in samples as standard. Distinguish wild-type and mutant homozygotes by PCR-labeled samples with known genotype sequences. Using unknown genome DNA (30 ng) as template, and another 3 ng of known wild-type DNA (confirmed by sequencing before). The PCR reaction conditions are as follows, that is, first pre-denaturation at 94 °C for 3 minutes of 1 cycle, then at 94 °C denaturation for 30 seconds, 57 °C refolding for 45 seconds, 68 °C extension for 45 seconds for 35 cycles, and finally at 68 °C for 7 minutes of 1 cycle. Results were analysed by the LightCycler® 480 Gene Scanning software v1.2 (Roche Diagnostics, Penzberg, Germany). As mentioned earlier, in 96 well plates, PCR cycles and HRM were applied to all experimental steps (Sun et al. 2015). Direct sequencing was used to verify the 50 random samples selected on the ABI7500 sequence detection system(Applied Biosystems, USA). The HRM genotyping results are consistent with the direct sequencing results.

Statistical Analysis

All analyses were carried out in the social science statistical package (SPSS) 20. Hardy-Weinberg equilibrium (HWE) of the RsaI/PstI alleles in the controls was assessed χ2-test. Odds ratios (ORs) and their 95 percent confidence intervals (CIs) calculateing by unconditional logistic regression models were performed to estimate the association between polymorphism and LBW risk. P<0.05 were considered statistical significant.

RESULTS

Association of RsaI/PstI and DraI Gene Polymorphism with LBW

The researchers performed PCR-HRM to analyse RsaI/PstI and DraI polymorphism. showed Three different graphs that can detect three different genotypes of RsaI/PstI and DraI polymorphism were shown in Figure 1. Table 1 showed the distributions of RsaI/PstI and DraI genotypes in cases and controls. The genotype

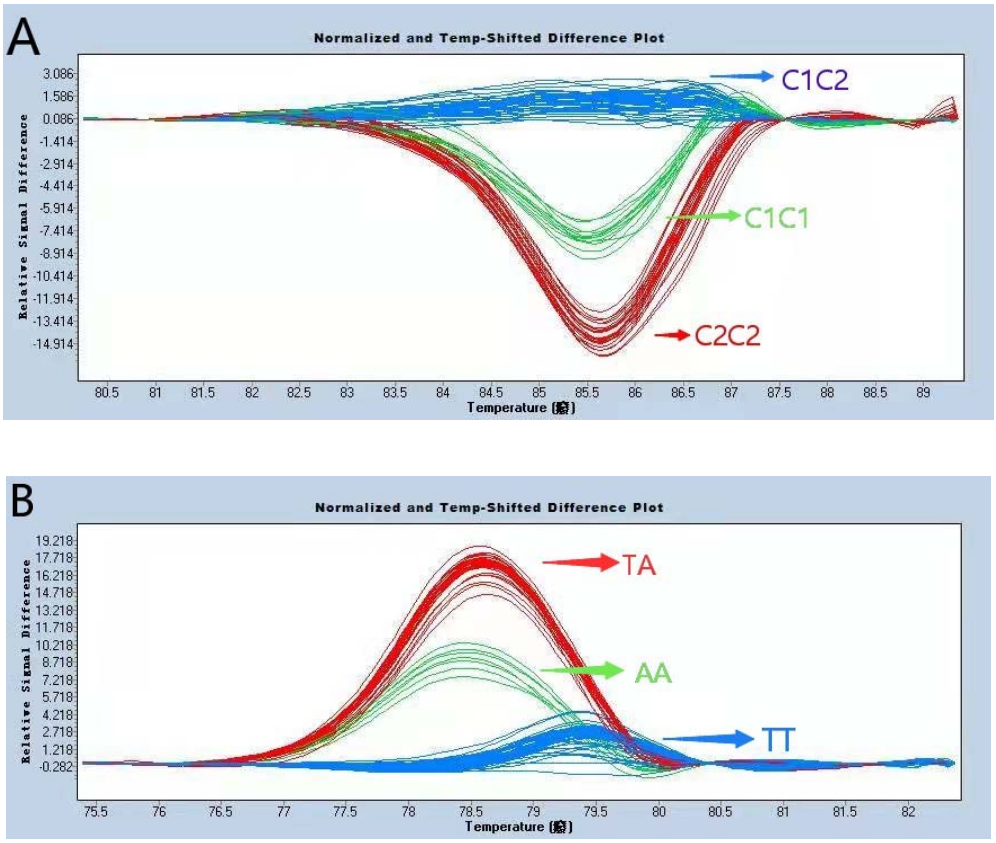

Fig.1. Melting curves of CYP2E1genotyping by high resolution melting analysis. There were three different genotypes for the RsaI/PstI (A) and DraI (B) polymorphisms in the study population

Table 1: Association of CYP2E1 RsaI/PstI and DraI gene polymorphism with LBW

Genotype	*Case (N=461), N (%)*	*(N=461), N (%)*	*OR (95% CI)*	*P-value*	*Control HWE*
RsaI/PstI					0.06
c1c1	84 (18.2)	68 (14.8)	1.00		
c1c2	255 (55.3)	243 (52.7)	0.85 (0.59-1.22)	0.38	
c2c2	122 (26.5)	150 (32.5)	0.66 (0.44-0.98)	0.04*	
Alleles					
c1	423 (45.9)	379 (41.1)	1.00		
c2	499 (54.1)	543 (58.9)	0.82 (0.68-0.99)	0.04*	
DraI					0.50
T T	291 (63.1)	285 (61.8)	1.00		
TA	153 (33.2)	158 (34.3)	0.95 (0.72-1.25)	0.71	
AA	17 (3.7)	18 (3.9)	0.93 (0.47-1.83)	0.82	
Alleles					
T	735 (79.7)	728 (79.0)	1.00		
A	187 (20.3)	194 (21.0)	0.96 (0.76-1.20)	0.69	

LBW = Low birth weight; n (%) = frequency; OR = odds ratio; CI = confidence interval.*P *-value* $\leq$ 0.05.

frequencies of RsaI/PstI and DraI in the control group were consistent with HWE (P = 0.06 for RsaI/PstI, and P = 0.50 for *Dra*I). The frequencies of RsaI/PstI C_1C_1, C_1C_2 and C_2C_2 genotypes were 18.2 percent, 55.3 percent and 26.5 percent respectively, while those in the control group were 14.8 percent, 52.7 percent and 32.5 percent respectively. There were significant differences in genotype distribution between case and control groups ccompared with C_1C_1 and $C_1C_1 + C_1C_2$, the ORs of C_1C_2 were 0.66 (95% CI: 0.44–0.98, P = 0.04) and 0.75 0.75 (95% CI: 0.56–0.99, P =0.04), respectively. The C_2C_2 genotype appeared in patients was with a higher frequency of statistical significance Furthermore, ccompared with subjects with C_1 alleles, subjects with C_2 alleles showed significant increases (OR = 0.82, 95% CI =0.68–0.99, P = 0.04). The results showed that the C allele of pregnant women may be a risk factor for LBW.

The frequencies of DraI TT, TA and AA in the cases were 63.1 percent, 33.2 percent and 3.7 percent respectively, while those in the control group were 61.2 percent, 34.3 percent and 3.9 percent respectively.TA and AA genotype had no significant effect on LBW, comparing with TT genotype, ORs was 0.95 (95% CI: 0.72 - 1.25) and 0.93 (95% CI: 0.47 - 1.83).

Interaction between CYP2E1 RsaI/PstI Polymorphism and Gene DraI Polymorphism on LBW

The combination of RsaI / PSII polymorphism and drai polymorphism were shown in Table 2. In DraI homozygote, the frequency of RsaI / PstI C_2C_2 genotype markedly lower than of controls (24.7% and 34.0%, respectively, P < 0.05). In addition, the presence of DraI TT and RsaI/PstI C_2C_2 genotypes were interrelated with an increased of LBW risk compared with individuals lacking both genotypes (or = 0.59, 95% CI = 0.36 – 0.97). These results indicate that DraI TT and RsaI/PstI C_2C_2 genotypes interact with each other in increasing LBW risk.

DISCUSSION

In this study, the researchers explored the relationship between the polymorphism at the RsaI/PstI site at the CYP2E152 -flank region and the polymorphism at the DraI site in intron 6. Through the analysis of 461 cases and 461 controls the researchers found that the homozygous gene type of RsaI/PstI c2c2 were statistically significantly associated with a increased risk of LBW risk. This indicates that mothers carrying CYP2E1 RsaI/PstI gene polymorphisms are risk factors for LBW, which will greatly increase the risk of LBW infants. However, the wild homozygous genotype of DraI compared with the mutant homozygous genotype/heterozygous genotype did not significantly increase the risk of LBW, which indicates that the gene CYP2E1 DraI site polymorphism has nothing to do with LBW. In addition, the correlation of 2 SNPs and LBW risk exhibit a gene-gene interaction, and the risk of LBW in subjects with DraI TT is not remarkable. Nevertheless, the existence of DraI TT and RsaI/PstI C2C2 genotypes are more sensitive to LBW.

CYP2E1 gene is mainly expressed in the liver, and its products can metabolise many drugs

Table 2: Risk of LBW associated with DraI genotypes by RsaI/PstI genotypes

Genotype		*Cases, n (%)*	*Controls, n (%)*	*OR (95% CI)*	*P-value*
DraI	*RsaI/PstI*				
T T	c1c1	57 (12.4)	44 (9.5)	1.00 (reference)	
	c1c2	162 (35.1)	147 (31.9)	0.85 (0.54-1.34)	0.48
	c2c2	72 (15.6)	94 (20.4)	0.59 (0.36-0.97)	0.04*
TA	c1c1	25 (5.4)	21 (4.6)	0.92 (0.46-1.85)	0.81
	c1c2	82 (17.8)	87 (18.9)	0.73 (0.44-1.19)	0.21
	c2c2	46 (10.0)	50 (10.8)	0.71 (0.41-1.25)	0.23
AA	c1c1	2 (0.4)	3 (0.7)	0.52 (0.08-3.21)	0.48
	c1c2	11 (2.4)	9 (1.9)	0.94 (0.36-2.48)	0.91
	c2c2	4 (0.9)	6 (1.3)	0.52 (0.14-1.94)	0.33

LBW = Low birth weight; n (%) = frequency; OR = odds ratio; CI = confidence interval. **P -value ≤ 0.05.

and exogenous substances to inactivate it. At the same time, it can also activate many exogenous substrates such as environmental pollutants benzene, herbicides, pesticides and food additives to form liver poisons or carcinogens, causing a variety of human diseases (Hu et al. 1997; Zhang et al. 2010; Balaji et al. 2011; Kato et al. 2011; Cai et al. 2005). The genetic polymorphisms of CYP2E1 are related to those diseases and physical conditions including diabetes, familial hypercholesterolemia, atherosclerosis, neonatal neural tube defects, and acute leukemia (Jiang et al. 2008). Vieira et al. discovered that CYP2E1 gene expression was very little in the foetal period of human development, but its expression level increased significantly after foetal birth (Vieira et al. 1996). Oesterheld reports that different CYP450 genes are activated and expressed at different stages of individual development, and among them, CYP2E1 gene begins to be expressed during the second trimester of foetal development, and it may be involved in the metabolism of foetal alcohol (Oesterheld 1998). Rasheed et al. found that pregnant women with alcohol drinking history at the end of pregnancy can activate CYP2E1, make it express in large quantities, produce active enzymes, and then decompose alcohol to form a large number of toxic substances, resulting in the reduction of foetal head after birth (Rasheed et al. 1997). In this study, the researchers analysed the relationship between the polymorphisms of CYP2E1 and LBW. From the results, the CYP2E1 RsaI/PstI c2c2 homozygous genotype was significantly related to LBW. After adjusting for environmental factors, there was still a significant correlation. However, its biological mechanism is still not very clear, and further research is needed.

The researchers collected clinical and epidemiological data from 922 pregnancy cases undergoing delivery. The large number of samples makes the results more reliable. In addition, the population surveyed is a low-risk group (best childbearing age, married women with early prenatal health care), which allows the researchers to eliminate the social environment factors and study the underlying causes of gene and LBW.

Some limitations should be considered in the study. First, the sources of the research subjects were different, and their age and ethnic composition are different. Second, the adverse pregnancy outcome of low birth weight in newborns is the result of the combined effect of genetic and environmental factors. However, the study is only conducted from the perspective of individual genes, which is not comprehensive enough. Therefore, when conducting research, in addition to increasing the sample size, it is also necessary to consider the gene environment interaction, and between genes and genes.

CONCLUSION

The current study demonstrates that CYP2E1 52 -flank region RsaI/PstI polymorphism is significantly related to LBW. However, the genetic DraI TT polymorphism was not significantly related to LBW, and the interaction between the two genetic polymorphisms has a significant impact on the occurrence of LBW.

RECOMMENDATIONS

More specific functions of CYP2E1 polymorphisms in the LBW are expected to be discovered in the near future. The biological mechanism in it is still not very clear and needs to be further clarified.

ABBREVIATIONS

- CYP450: Cytochrome P450
- CYP2E1: cytochrome P450 2E1
- LBW: low birth weight
- SNP: single gene polymorphism
- LDÿlinkage disequilibrium
- PCR-HRM: real-time polymerase chain reaction high-resolution melt
- HRM: high-resolution melt

STATISTICAL SYMBOL

- SPSS: Statistical Package for the Social Science
- HWE: Hardy-Weinberg equilibrium
- χ^2: Chi square test
- ORs: odds ratios
- CIs: confidence intervals

ETHICS APPROVAL

All the study participants gave a written informed consent. A formal consent was also is-

sued the Department of Obstetrics and Gynoecology Wuhan No. 1 Hospital (Approval number, 13.15.2021).

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