ASSOCIATION OF FETAL BUT NOT MATERNAL P-GLYCOPROTEIN C3435T POLYMORPHISM WITH FETAL GROWTH AND BIRTH WEIGHT, A POSSIBLE RISK FACTOR FOR CARDIOVASCULAR DISEASES IN LATER LIFE

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SUMMARY

Background: The multidrug transporter P-glycoprotein (PGP) is expressed in the human placenta. In particular the C3435T ABCB1 polymorphism was associated with altered tissue expression of PGP in the human placenta. However, the potential functional impact of this polymorphism on the offspring is unknown so far.

Methods: We analyzed the impact of the ABCB1/C3435T polymorphism on fetal growth in 262 mother/child pairs. Fetal growth was assessed by differential ultrasound examination of the fetal body prior to birth and by measuring birth weight.

Results: The maternal ABCB1/C3435T polymorphism showed no trend for an association with birth weight or any ultrasound parameter describing late gestational fetal body shape. Genotyping the newborns, however, demonstrated that newborns carrying two copies of the T allele had a birth weight of 3176.39 g, whereas CT and CC newborns had a birth weight of 3345.04 g (p = 0.022). Adjusting for gestational age at delivery, child's gender, maternal BMI, maternal age and body weight at delivery confirmed this finding (p = 0.009). Considering gestational day of late ultrasound examination, gestational age at delivery, child's gender, maternal BMI, maternal age and maternal body weight at delivery, the fetal ABCB1/C3435T genotype revealed likewise a significant negative correlation with abdominal diameter and abdominal circumference (R² = 0.538, p = 0.010 and R² = 0.534, p = 0.005, respectively).

Conclusions: Low birth weight may be a risk factor for cardiovascular diseases in later life. The fetal ABCB1/C3435T gene polymorphism may contribute to this risk. Since PGP controls transport of various biological agents, we suggest that PGP is involved in the transport of biological agents to the fetus that are important for normal fetal growth.

INTRODUCTION

Cardiovascular diseases such as hypertension, coronary artery disease, and type 2 diabetes are inversely associated with birth weight [1]. Apart from maternal under- and also maternal over-nutrition, variation of fetal growth and thus final birth weight may likewise be caused by maternal genes [2] or fetal genes [3] controlling placenta function. P-glycoprotein (PGP), the product of the multidrug resistance gene (ABCB1), acts as an energy-dependent efflux pump that utilizes ATP to drive the efflux of its substrates out of cells and is therefore currently classified as an ABCB1 member of the ABC transporters superfamily. Apart from the multidrug transporter properties of PGP, normal physiolog-
calf functions of PGP have been reported as well [4]. PGP likely affects normal development and function of the placenta through apoptotic pathways [5-7]. Substantial variability in PGP expression has recently been observed among placentas in humans [8]. Although the variable expression may be caused by different environmental factors, genetic polymorphisms of the ABCB1 gene are believed to represent a major source of the PGP expression variability. Differences in placental PGP expression may lead to differences in the transport of biological agents to the fetus that may be essential for intrauterine growth. In the current study we investigated the effect of polymorphisms of the ABCB1 gene subunit (C3435T) on fetal growth.

MATERIALS AND METHODS

We invited a total of 402 Chinese women delivering at the Obstetric Department of the First Affiliated Hospital of Jinan University. Inclusion criteria were as follows: (1) the newborn was born without structural anomalies; (2) singleton pregnancy; (3) no HIV or syphilis; (4) no drug abuse; (5) no history of smoking or alcohol consumption during pregnancy; (6) pregnancies complicated by pregnancy-induced hypertension, diabetes mellitus, impaired glucose tolerance, and previous stillbirths were excluded from the study; (7) no use of steroid medication, anticancer drugs, immunosuppressants, cardiac glycosides, ß-adrenoceptor antagonists, nor antiepileptic drugs during pregnancy. After exclusion of cases that did not fulfill the inclusion criteria or were not willing to participate, we finally genotyped 262 mother and fetal cases. Differential ultrasound examination of the fetal body (head circumference, biparietal diameter, pectoral diameter, abdominal circumference, abdominal diameter and femur length) was done on average one week prior to delivery as described previously [9].

DNA was extracted from peripheral whole blood of each subject using a DNA extraction kit (Simgen, Hangzhou, China). A polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) was used for the detection of C3435T SNP. The PCR assay used sense primer: 5’-TGCTGGTCCTGAAGTGTGATC TGTGAAC-3’ and antisense primer: 5’-ACATTAGGC AGTGACTCGATGAAGGCA-3’. This was followed by digestion of a 248 bp PCR product with restriction enzyme Mbol (Takara Biotech, Dalian, China) for 24 hours at 37°C. Digested products were separated on a 3.0% agarose gel. All steps were done in duplicate. Only matching results were accepted. In order to ensure the quality of the RFLP method, we had selected 10% of PCR products, the PCR products that were also used for RFLP, see above, randomly from the original samples and sent them out for external sequencing (Sangon Biotech (Shanghai) Co., Ltd., www. sangoon.com). Our RFLP data sequences were confirmed in all cases.

Data were analyzed with SPSS version 17.0. The difference in allele or genotype frequency was determined using the chi-square test. Hardy-Weinberg genotype frequencies were assessed using the chi-square test. 95% confidence intervals were calculated for all observed allele frequencies. Results are presented as mean ± standard deviation (SD). Student’s unpaired t-test was used for comparison of continuous variables between groups. When examining the correlation between ABCB1/ C3435T polymorphism genotype and birth weight or ultrasound parameters, variables that are known to influence intrauterine growth and showed a significant bivariate correlation or association with birth weight or ultrasound parameters in our cohort, respectively, were used as co-variables. Pearson’s correlation coefficient and the regression coefficient B were used to estimate the strength of a correlation. A p-value of less than 0.05 was considered significant.

RESULTS

The descriptive data of the study population are shown in Table 1. The p values for the ABCB1/C3435T polymorphism genotype distribution and frequencies of the alleles is 0.06 indicating Hardy-Weinberg equilibrium. Newborns carrying two copies of the T allele had a birth weight of 3176.39 g, whereas CT and CC newborns had a birth weight of 3345.04 g (p = 0.022) (for details see Table 1 and Figure 1). The difference was statistically significant after adjustment for gestational age at delivery, maternal body weight at delivery, maternal BMI before pregnancy, maternal age, and the gender of the newborn (Table 2).

Considering gestational age at delivery, maternal body weight at delivery, maternal BMI before pregnancy, maternal age, and the gender of the newborn, the fetal ABCB1/C3435T genotype showed a significant negative correlation with abdominal circumference (R² = 0.538, p = 0.010) and abdominal diameter (R² = 0.534, p = 0.005) (for details see Table 2).

In the database, there were only 6 newborns delivered from non-Han mothers. When excluding the six non-Han birth cases from the multivariable analysis or adding maternal nation as another important confounding factor in the multivariable analysis, the results concerning birth weight and ultrasound data remained the same. Moreover, there were only 9 cases of newborns in our cohort with a birth weight below the 10th percentile for their gestational age (definition of low birth weight). When excluding these 9 cases from the analysis, the results concerning birth weight and ultrasound parameters remained the same. This indicates that our findings are not driven by low birth weight (data not shown).

Maternal ABCB1/ C3435T genotype was not significantly correlated with birth weight or any of the primary ultrasound measurements in any statistical models (data not shown).
# P-GLYCOPROTEIN C3435T POLYMORPHISM AS CARDIOVASCULAR RISK FACTOR

Table 1. Babies’ and maternal characteristics in different fetal ABCB1/C3435T genotype groups.

<table>
<thead>
<tr>
<th></th>
<th>CC+CT+</th>
<th>CC+CT</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth weight (g)</strong></td>
<td>3321.87 ± 412.36#</td>
<td>3345.04 ± 404.61#</td>
<td>3357.76 ± 432.04#</td>
<td>3337.38 ± 388.52#</td>
<td>3176.39 ± 436.30</td>
</tr>
<tr>
<td><strong>Gestational age at delivery (day)</strong></td>
<td>275.82 ± 7.40</td>
<td>276.03 ± 7.39</td>
<td>276.19 ± 7.09</td>
<td>275.94 ± 7.59</td>
<td>274.50 ± 7.44</td>
</tr>
<tr>
<td><strong>Gender (M/F)</strong></td>
<td>140/122</td>
<td>122/104</td>
<td>45/40</td>
<td>77/64</td>
<td>18/18</td>
</tr>
<tr>
<td><strong>APGAR 1 min after delivery</strong></td>
<td>8.91 ± 0.67</td>
<td>8.90 ± 0.72</td>
<td>8.84 ± 0.90</td>
<td>8.94 ± 0.59</td>
<td>8.97 ± 0.17</td>
</tr>
<tr>
<td><strong>APGAR 5 min after delivery</strong></td>
<td>9.95 ± 0.27</td>
<td>9.94 ± 0.28</td>
<td>9.94 ± 0.24</td>
<td>9.94 ± 0.31</td>
<td>9.97 ± 0.17</td>
</tr>
<tr>
<td><strong>APGAR 10 min after delivery</strong></td>
<td>10.00 ± 0.06</td>
<td>10.00 ± 0.07</td>
<td>9.99 ± 0.11</td>
<td>10.00 ± 0.00</td>
<td>10.00 ± 0.00</td>
</tr>
<tr>
<td><strong>Late ultrasound analysis time, day</strong></td>
<td>269.04 ± 12.46</td>
<td>269.02 ± 12.51</td>
<td>268.89 ± 13.30</td>
<td>269.10 ± 12.06</td>
<td>269.14 ± 12.31</td>
</tr>
<tr>
<td><strong>Late biparietal diameter, mm</strong></td>
<td>94.73 ± 4.59</td>
<td>94.75 ± 4.63</td>
<td>94.64 ± 5.19</td>
<td>94.82 ± 4.27</td>
<td>94.61 ± 4.40</td>
</tr>
<tr>
<td><strong>Late head circumference, mm</strong></td>
<td>331.36 ± 13.26</td>
<td>331.65 ± 13.29</td>
<td>332.11 ± 13.64</td>
<td>331.38 ± 13.12</td>
<td>329.56 ± 13.13</td>
</tr>
<tr>
<td><strong>Late pectoral diameter, mm</strong></td>
<td>90.36 ± 7.14</td>
<td>90.65 ± 7.15</td>
<td>91.03 ± 6.71</td>
<td>90.42 ± 7.42</td>
<td>88.59 ± 6.92</td>
</tr>
<tr>
<td><strong>Late abdominal diameter, mm</strong></td>
<td>102.99 ± 9.14</td>
<td>103.43 ± 9.12</td>
<td>103.02 ± 8.99</td>
<td>103.68 ± 9.22</td>
<td>100.28 ± 8.95</td>
</tr>
<tr>
<td><strong>Late abdominal circumference, mm</strong></td>
<td>339.60 ± 22.33</td>
<td>340.56 ± 22.30</td>
<td>339.55 ± 22.36</td>
<td>341.17 ± 22.32</td>
<td>333.61 ± 21.86</td>
</tr>
<tr>
<td><strong>Late femur length, mm</strong></td>
<td>70.93 ± 3.85</td>
<td>70.98 ± 3.77</td>
<td>70.73 ± 3.78</td>
<td>71.13 ± 3.78</td>
<td>70.67 ± 4.36</td>
</tr>
<tr>
<td><strong>Maternal age, years</strong></td>
<td>27.98 ± 3.47</td>
<td>28.01 ± 3.43</td>
<td>28.27 ± 3.30</td>
<td>27.85 ± 3.52</td>
<td>27.78 ± 3.74</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td>1.61 ± 1.05</td>
<td>1.61 ± 1.08</td>
<td>1.54 ± 0.89</td>
<td>1.65 ± 1.18</td>
<td>1.58 ± 0.87</td>
</tr>
<tr>
<td><strong>Maternal height, cm</strong></td>
<td>160.04 ± 4.31</td>
<td>159.91 ± 4.43</td>
<td>159.59 ± 4.17</td>
<td>160.10 ± 4.58</td>
<td>160.88 ± 3.45</td>
</tr>
<tr>
<td><strong>BMI before pregnancy, kg/m²</strong></td>
<td>19.95 ± 2.35</td>
<td>19.95 ± 2.33</td>
<td>19.69 ± 1.74</td>
<td>20.10 ± 2.63</td>
<td>19.94 ± 2.48</td>
</tr>
<tr>
<td><strong>Maternal weight before pregnancy, kg</strong></td>
<td>51.17 ± 7.00</td>
<td>51.09 ± 7.00</td>
<td>50.24 ± 5.65</td>
<td>51.61 ± 7.67</td>
<td>51.66 ± 7.07</td>
</tr>
<tr>
<td><strong>Maternal weight at delivery, kg</strong></td>
<td>67.45 ± 8.40</td>
<td>67.29 ± 8.41</td>
<td>66.53 ± 7.57</td>
<td>67.75 ± 8.88</td>
<td>68.46 ± 8.39</td>
</tr>
<tr>
<td><strong>Nation (Han/non-Han)</strong></td>
<td>256/6</td>
<td>222/4</td>
<td>85/0</td>
<td>137/4</td>
<td>34/2</td>
</tr>
<tr>
<td><strong>Method of delivery (Vaginal/C-section)</strong></td>
<td>93/169</td>
<td>77/149</td>
<td>29/56</td>
<td>48/93</td>
<td>16/20</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD, # p < 0.05 versus TT group.

Table 2. Multivariable regression analysis of the association between birth weight/late ultrasound measurements and fetal ABCB1/C3435T genotype: Overview.

<table>
<thead>
<tr>
<th></th>
<th>Birth weight</th>
<th>Biparietal diameter</th>
<th>Head circumference</th>
<th>Pectoral diameter</th>
<th>Abdominal diameter</th>
<th>Abdominal circumference</th>
<th>Femur length</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R²</strong></td>
<td>0.344</td>
<td>0.470</td>
<td>0.444</td>
<td>0.238</td>
<td>0.538</td>
<td>0.534</td>
<td>0.594</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>-79.85◆</td>
<td>-0.147</td>
<td>-1.092</td>
<td>-1.008</td>
<td>-1.684◆</td>
<td>-3.990◆</td>
<td>-0.242</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.009</td>
<td>0.631</td>
<td>0.224</td>
<td>0.087</td>
<td>0.010</td>
<td>0.005</td>
<td>0.281</td>
</tr>
<tr>
<td><strong>95% CI</strong></td>
<td>-139.96 - -19.74</td>
<td>-0.746 - -0.453</td>
<td>-2.859 - -0.674</td>
<td>-2.162 - -0.146</td>
<td>-2.961 - -0.408</td>
<td>-6.732 - -1.249</td>
<td>-0.684 - -0.199</td>
</tr>
</tbody>
</table>

For each variable, the multivariable regression analyses were performed considering gestational day of delivery/the ultrasound examination, the child’s sex, maternal BMI before pregnancy, maternal age and body weight at delivery, with the birth weight/ultrasound measurement being the dependent variables and the two groups of ABCB1/C3435T genotypes (CT+CC group vs. TT group) as the independent variables. Shown are the R², B, and p-values for the ABCB1/C3435T genotype. B: non-standardized regression coefficient. ◆: statistically significant.
DISCUSSION

We showed that the fetal ABCB1/C3435T TT genotype was associated with a lower birth weight. Moreover, we demonstrated an inverse relation between fetal ABCB1/C3435T genotype and fetal anthropometric parameters such as fetal abdominal circumference and abdominal diameter, parameters most likely correlated to fetal liver growth. PGP was recognized as a drug-exporting protein from cancer cells three decades ago. PGP is not only expressed in tumor cells, but also in cells of non-malignant origin: the biliary canalicular surface of hepatocytes and the apical surface of small biliary ductules; the apical surface of columnar epithelial cells of the small intestine and colon represent typical examples.

Expression of placental PGP has been confirmed at both gene and protein levels [8,10]. The function and role of placental PGP is unknown so far. Based on the placental expression, PGP was expected to play a role in the protection of the fetus against toxic xenobiotics and therapeutic compounds given to the mother [11]. Besides that, a physiological role as regulator of physiological molecule trafficking between the mother and the fetus of placental PGP was proposed [4]. Furthermore, it was suggested [12] that placental PGP augments the placental glucocorticoid barrier that protected the fetus from maternal glucocorticoids. In addition, PGP may affect normal development and function of the placenta related to fetal growth in utero. Hitzl et al. [14] demonstrated that carriers of the TT alleles have a lower PGP through apoptotic pathways. PGP may counteract apoptosis by regulating intracellular concentrations of intermediates in the apoptotic pathway, such as sphingomyelin [5]. Formation of the syncytiotrophoblast layer involves apoptotic processes, and increases in apoptosis are seen in complications during pregnancy, such as preeclampsia and intra-uterine growth restriction [6]. Interestingly, decreased expression of ABCB1 mRNA was observed in placenta complicated by idiopathic fetal growth restriction compared to controls [7].

So far, more than 100 SNPs of human ABCB1 gene have been identified in different populations. The C3435T, C2677T, and C1236T polymorphism were best-studied among them. They are in linkage disequilibrium, thus we decided to use the C3435T polymorphism for our study. The allelic frequencies of our cohort were similar to other experiment results in healthy Chinese population [13], suggesting that our findings are of general impact. Hitzl et al. demonstrated that placental PGP expression was significantly lower if both mother and infant were homozygous for the 3435T allele (TT/tt) compared to maternal and fetal homozygotes for the C allele. Moreover, placentas from mothers carrying polymorphisms for both 3435T and 2677T alleles also had a significantly lower PGP expression as compared to placentas of wild type individuals (CC/GG) [14]. Our study, for the first time, demonstrated that not maternal but fetal ABCB1/C3435T genotype was negatively reexpression that may lead to a lower transport capacity for PGP substrates. Based on our data, we suggest that a yet
unknown PGP substrate coming from the mother was transported to the fetus to a smaller extent in TT allele carriers and thus may result in reduced fetal growth. It is a study limitation that there was no replication cohort available. There are, to the best of our knowledge, no Asian birth cohorts available with DNA from mothers and newborns. We hope that our work will stimulate others to address similar questions. Given that birth weight is a multifactorial phenomenon involving factors such as non-genetic socioeconomic circumstances, food intake during pregnancy, offspring gender, paternal BMI as well as maternal and fetal gene alterations, the association of the 3435T allele is striking. The molecular pathways that mediate an impaired fetal growth associated with this allele need to be further clarified.

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Declaration of Interest:
The authors declared no conflicting interests.

References:

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