CONCISE REPORT

Genetic Evidence for Fetal Origin of Transcobalamin II in Human Cord Blood

By Henk J. Porck, Marijke Fräter-Schröder, Rune R. Frants, Lucja Kierat, and Aldur W. Eriksson

Phenotypes of transcobalamin II (TC2) were determined in 95 maternal–cord serum pairs in order to identify the origin of TC2 in human cord blood. Unsaturated (apo) TC2 in serum was labeled with radioactive (57Co) cobalamin (Cbl) and separated into isoproteins by polyacrylamide gel electrophoresis and autoradiography. Discordancy between the maternal and the cord serum type was observed in 45% of the pairs. The results demonstrated that, at the end of pregnancy, the fetus is capable of TC2 synthesis and that maternal TC2 is transferred and mediates Cbl uptake, and recycling of Cbl.2 Children with this disorder developed megaloblastic anemia within the first weeks or months of postnatal life. As clinical symptoms were absent at birth, several authors suggested that maternal TC2 is transferred and mediates Cbl utilization in the fetus.2'4 Maternofetal transfer is also proposed elsewhere.7,8

We investigated the origin of TC2 in fetal blood at the end of normal pregnancies by comparing the TC2 phenotypes in maternal–cord serum pairs, taking advantage of the genetic polymorphism of TC2.9,10 The analysis technique, autoradiography of electrophoretic patterns of 57-Co-Cbl-labeled serum, offers possibilities to detect both apo-TC2, and after exchange of endogenously bound Cbl with 57Co-Cbl, also holo-TC2. It is shown that at the end of pregnancy, the apo-TC2 content in maternal serum is higher than in cord serum and serum of nonpregnant women.11,12 Overall reduction in the apo-TC2 level in maternal serum during pregnancy is also reported.13 Our collection of maternal–cord serum pairs offered the opportunity to verify and extend these contradicting studies by determination of apo-TC2 and total (apo + holo) immunoreactive TC2.

MATERIALS AND METHODS

Maternal and cord serum. Blood samples were obtained from normal pregnancies at Dutch and Swiss hospitals. Cord blood was collected at the time of delivery, maternal blood within 24 hr before delivery. A control group of healthy male and female adults was used for comparison. Serum samples were stored at −20°C until analysis.

TC2 phenotyping. A modification of an established TC2 typing procedure14 was used. Serum was treated with neuraminidase (30 min at 37°C; 0.25 IU neuraminidase/ml serum; Behring-Werke, Mannheim, Germany) to prevent electrophoretic overlap of TC2 with cobalophilin. Apo-TC2 was labeled by incubation with 2,270 pg (50 nCi) 57Co-Cbl (Radiochemical Center, Amersham) per ml serum (15 min at 20°C). Samples, equivalent to 4 μl serum, were separated by vertical discontinuous polyacrylamide slab gel electrophoresis (electrode and gel buffers contained 1 mM EDTA). Autoradiographs of the dried gels were analyzed with a Quick Scan densitometer (Helena Laboratories). The detection limit was 55 pM TC2 Cbl equivalent (6.5% of the total TC2 serum content in the control group). An approximation of the expected discordancy of the TC2 phenotypes in the maternal–cord serum pairs was calculated from the frequencies of the most common genes in the population, TC2* M (p = 0.6) and TC2* X (q = 0.4): 2pq1 + 4pq2 + 2q = 0.48. A newly proposed nomenclature for TC2 variants is used in the text.14

From the Institute of Human Genetics, Free University, Amsterdam, The Netherlands, and the Department of Pediatrics, University of Zürich, Zürich, Switzerland.

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Address reprint requests to Dr. H. J. Porck, Institute of Human Genetics, Faculty of Medicine, Free University, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands.

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**Table 1. TC2 Phenotypes in 95 Maternal–Cord Serum Pairs**

<table>
<thead>
<tr>
<th>Concordant Pairs</th>
<th>Discordant Pairs: Mother TC2 Homozygous</th>
<th>Discordant Pairs: Mother TC2 Heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mother-Cord</td>
<td>n</td>
</tr>
<tr>
<td>17</td>
<td>MX-MX</td>
<td>9</td>
</tr>
<tr>
<td>26</td>
<td>M-M</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>X-X</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>23</td>
</tr>
</tbody>
</table>

**Labeling of serum holo-TC2.** The endogenously bound Cbl in holo-TC2 (±10% of the total TC2 serum content in the control group) was almost completely exchanged after incubation with 22,700 pg $^{57}$Co-Cbl/ml serum for 72 hr at 37°C. Details of this exchange procedure will be described elsewhere.

**Quantification of total and apo-TC2.** The serum content of total TC2 (pM Cbl equivalent) was determined by a radioimmunosorbent technique with immobilized rabbit anti-human TC2 antiserum and $^{57}$Co-Cbl–human TC2 complex as tracer. The serum content of apo-TC2 (pM Cbl equivalent) was determined after separation of TC2 from cobalophilin and free Cbl by cation-exchange chromatography, using two-step elution of CM-Sephadex C-50 (Pharmacia, Uppsala, Sweden) columns. A small sample t test for independent means was used for statistical evaluation of the results. Concentrations were expressed as means ± SD.

**RESULTS**

The results of TC2 phenotyping in 95 maternal–cord serum pairs are shown in Table 1. Concordant TC2 types were found in 55% (expected: 52%), discordant types in 45% (expected: 48%) of the pairs. In 23 discordant pairs, the maternal type was homoygous and the cord type heterozygous, indicating TC2 synthesis by the fetus. One of the cord sera was heterozygous for TC2*M and the rare allele TC2*DPAV-like. In 20 discordant pairs, the maternal type was heterozygous and the cord type homoygous, indicating lack of transplacental passage of maternal apo-TC2. In 9 of the latter informative pairs, the presence of maternal holo-TC2 in cord serum was examined by the $^{57}$Co-Cbl exchange procedure. Two representative pairs are shown in Fig. 1. Holo-TC2 labeling clearly resulted in intensification (up to 25%) of the TC2 pattern, but did not lead to the appearance of additional maternal bands in the cord serum phenotype (Fig. 1, C1*, C2*), suggesting that holo-TC2 in cord serum is only of fetal origin.

Immunologic quantification (Fig. 2) showed that the total TC2 content in 23 maternal serum samples (812 ± 175 pM Cbl equivalent) was significantly higher than in the corresponding cord sera (605 ± 148 pM; p < 0.001) and did not significantly differ from the value in the control group (841 ± 192 pM).

The apo-TC2 content in 12 maternal serum samples (760 ± 347 pM) was significantly higher than in the corresponding cord sera (501 ± 254 pM; p < 0.05) and did not significantly differ from the value in the control group (747 ± 137 pM). Apo-TC2 levels, determined by Sephadex G-150 gel filtration, in 5 additional maternal–cord serum pairs confirmed these results.

**DISCUSSION**

Genetic polymorphism offered the opportunity to differentiate between maternal and fetal contributions...
for several proteins in amniotic fluid\textsuperscript{19} and fetal circulation. Phenotyping in cord serum of orosomucoid,\textsuperscript{18} transferrin,\textsuperscript{19} haptoglobin,\textsuperscript{20,21} group-specific component\textsuperscript{21} the third component of complement,\textsuperscript{22} and $\alpha_1$-antitrypsin\textsuperscript{27} provided proof for fetal protein synthesis, and, except for transferrin and $\alpha_1$-antitrypsin, also indicated lack of transplacental passage. Using radiolabeled proteins, maternofetal transfer is demonstrated for orosomucoid, apparently below the detection limit of the applied phenotyping procedure, and for several other proteins.\textsuperscript{24-26}

In the present study, genetic evidence is obtained that at the end of pregnancy, the fetus is capable of TC2 synthesis, and that, within the detection limit, there is no transplacental passage of maternal apo-and holo-TC2 to the fetal circulation. As studies in man\textsuperscript{12} and rat\textsuperscript{27} indicate that only late in pregnancy is the bulk of Cbl is transferred from mother to fetus, absence of a substantial maternal contribution of TC2 in full-term cord serum favors the idea that there is no maternofetal transfer of TC2 throughout pregnancy and that the role of maternal TC2 in fetal development is restricted only to the transport of Cbl to the placenta, using the specific placental receptors for TC2.\textsuperscript{28-30}

We conclude that it is unlikely that in congenital TC2 deficiency maternal TC2 is responsible for Cbl transport and delivery in the fetus, as has been suggested.\textsuperscript{2,4} The explanation for normal in utero development in spite of this genetic defect thus remains unclear. Our finding that TC2 in human cord blood is of fetal rather than maternal origin suggests an essential role for fetal TC2 in Cbl utilization and offers the possibility for diagnosis, and starting adequate treatment of congenital TC2 deficiency immediately after birth.

The immunologic data show that at the end of pregnancy, the total TC2 content in maternal serum is significantly higher than in the corresponding cord sera and does not significantly differ from the value in a control group of healthy male and female adults. At the end of pregnancy, the apo-TC2 content in maternal serum is significantly higher than in the corresponding cord sera, which confirms earlier findings.\textsuperscript{5,11,12} The apo-TC2 level in maternal serum does not significantly differ from the value in the control group, which contradicts the increased, as well as the reduced values that have been reported for maternal apo-TC2 at the end of pregnancy.\textsuperscript{12,13} There is no apparent explanation for this discrepancy. Interpretation of the findings with regard to the regulation of Cbl transport during pregnancy will be premature until further data on both apo- and holo-transcobalamin levels become available.

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