In the present study, we demonstrated that the concentration of fetal DNA within maternal plasma is increased in cases of placenta previa, especially in patients with invasive placenta. We believe that invasion of trophoblasts into uterine muscle in these patients led to increased concentrations of cell-free fetal DNA within maternal plasma. Because the concentration of fetal DNA was high in the plasma of women with invasive placenta, antenatal diagnosis might be achieved through analysis of fetal DNA concentrations within maternal plasma. Placenta previa and a previous history of uterine surgery, including cesarean delivery, are considered risk factors for invasive placenta. In women who have these risk factors, fetal DNA quantification would be especially useful in the detection of invasive placenta.

Although we observed an increase in the plasma fetal DNA concentrations of women with placenta previa, the reason for this is unclear. However, the presence of thin and dysfunctional decidua at the lower segment of the uterus might be related to this increase.

In conclusion, we report here that fetal DNA is increased in the maternal plasma of patients with invasive placenta. We propose that the concentration of fetal DNA within maternal plasma might be a useful marker by which to arrive at an antepartum diagnosis of invasive placenta.

This work was supported in part by the High-Technology Research Center Project of the Ministry of Education, Science, Sport and Culture of Japan; the Ichiro Kanehara Foundation; the Kurozumi Foundation; and the Foundation of Boshi-Kenko-Kyokai.

References

Monitoring of Congenital Adrenal Hyperplasia by Microbore HPLC–Electrospray Ionization Tandem Mass Spectrometry of Dried Blood Spots, Chien-Chen Lai, Chung-Hai Tsai, Fua-Jen Tsai, Jer-Yuarn Wu, Wei-De Lin, and Cheng-Chun Lee (Department of Medical Genetics and Medical Research, China Medical College Hospital, Taichung, 404 Taiwan; * address correspondence to this author at: Department of Pediatrics, Medical Genetics and Medical Research, China Medical College Hospital, No. 2, Yuh-Der Road, Taichung, 404 Taiwan; fax 886-4-22033295, e-mail d0704@www.cmch.org.tw)

Congenital adrenal hyperplasia (CAH), a disorder caused by a deficiency of the 21-hydroxylase enzyme, is the most common inborn error of the adrenal steroid pathways. Early diagnosis of CAH can be lifesaving, and screening for CAH in newborns by measuring 17α-hydroxyprogesterone (17OHP) or other steroids has become a routine part of many programs (1, 2). These steroid hormones have been measured by fluorometry (3, 4), immunoassay (5–8), and HPLC (4, 9, 10). Most methods are affected by interferences or cross-reactivity with other steroids. Currently, neonatal screening and monitoring for CAH use immunoassays (3, 4). This approach, although practical, lacks specificity because cross-reacting congeners are inseparable from 17OHP in the direct assay (4, 11–13).

Electrospray ionization (ESI) has become an important method for the generation of gas-phase ions from biomolecules for mass spectrometric analysis, but the low proton affinity of natural steroids compromises their measurement by ESI. To improve sensitivity, we have derivatized steroids to form a covalent bond containing a permanent positively charged nitrogen atom. The carbonyl compound 17OHP was derivatized with a quaternary ammonium salt, Girard reagent P (GirP), to form water-soluble hydrazones with a permanently charged pyridine moiety. This derivative was selected for its introduction of a positive charge into the molecule of ketosteroid 17OHP and for the ease of its synthesis (14).

The purpose of this study was to evaluate the applicability of liquid chromatography-tandem mass spectrometry (LC-MS/MS) to clinical analysis of 17OHP in dried filter-paper blood samples from patients with CAH caused by 21-hydroxylase deficiency. Although others have proposed the detection of several corticosteroids by LC-MS/MS (15–18), large blood or urea sample volumes were needed, and published results of clinical LC-MS/MS analysis of steroids in whole blood are lacking.

Glacial acetic acid, 17OHP, 6α-methylprednisolone (6MP), GirP, and related compounds were purchased from Sigma. HPLC-grade methanol and acetonitrile were obtained from LAB-SCAN Analytical Science (Labscan Ltd.). Blank human whole-blood samples were obtained from China Medical College Hospital (Taichung, Taiwan).

Standardized filter-paper forms (Standardized SE&S 903 filter paper; Schleicher & Schuell) impregnated with whole capillary blood from CAH patients or 2- to 5-day-old infants were collected from the Department of Genetics, China Medical College Hospital (Taichung, Taiwan).
Patients with confirmed CAH were between 1 and 14 years of age (three girls and one boy). All patients or their parents gave informed consent. The National Taiwan University Hospital (Taipei, Taiwan) kindly provided five dried filter-paper blood samples from CAH infants. Samples were prepared from blood spots by simple solvent extraction. Four 3.175-mm (1/8-inch) circles from each blood spot (equivalent to 11.5 mL of whole blood) were excised from a 12.7-mm (1/2-inch) diameter dried-blood spot and placed in a flat-bottomed 96-well block automatically (individual 250-μL wells; Corning Incorporated) by a DELFIA DBS puncher (Wallac). A stock solution of extraction solvent (methanol) containing a known concentration of internal standard (50 g/L 6MP) was prepared and added to each well (200 μL). The wells were capped and shaken on a Vibromix 203E flatbed shaker (Tehtnica Co.) for 50 min. Subsequently, the extracts were transferred, using a multichannel pipette, into a clean V-bottomed 96-well microplate (individual 220-μL wells; Corning). Each 96-well microplate was placed in an evaporator [Techne (Cambridge) Ltd], and the solutions were evaporated to dryness under a gentle stream of dry nitrogen. The residue in each well was derivatized with 160 μL of GirP solution (10 g/L in ethanol, 1 mL/L of trichloroacetic acid as a catalyst), incubated at 65 °C for 50 min, and evaporated to dryness under a gentle stream of dry nitrogen. The GirP-derivatized 17OHP (GirP-17OHP) and 6MP (GirP-6MP) were reconstituted in 30 μL of 500 mL/L acetonitrile. The plate was covered with aluminum foil and placed on an autosampler tray for microbore HPLC-ESI-MS/MS analysis.

The HPLC system consisted of two Perkin-Elmer Series 200 micropumps (PE-Sciex). HPLC analysis was performed in a 5-μm C4 microbore (Vydac) column [50 × 1.0 mm (i.d.)] operated at ambient temperature. A guard column (C4 cartridge; Vydac) was used to prolong the life of the HPLC column. The mobile phase was water-acetonitrile (50:50 by volume), and the flow rate was 50 μL/min. The autosampler was a Perkin-Elmer Series 200 autosampler fitted with a 10-μL loop (PE-Sciex) and equipped with a 96-well sample plate stack.

We used an API 2000 bench-top triple quadrupole mass spectrometer (PE-Sciex) operated in ion evaporation mode with a TurbolonSpray ionization probe source (operated at 5 kV). The TurbolonSpray ionization probe source was operated with the turbo gas on (5 L/min; sensor temperature, 300 °C). The collision energy (Q0-RO2) was varied from 30 to 40 V. The orifice (OR) and ring (RNG) voltages were set at 50 and 360 V, respectively. Sample control (Ver. 1.4), TurboQuant (Ver. 1.0), and Microsoft Excel (Ver. 6.0) were used for data processing and statistical analysis. Background subtraction and a three-point smoothing algorithm were applied to all ion chromatograms and viewed using MultiView (Ver. 1.4) software.

Immunoassay of 17OHP was carried out with the IMMULITE analyzer (DPC), and the procedures for preparation, setup, dilutions, adjustment, assay, and quality control procedures as given in the IMMULITE operator’s manual (19).

For GirP-17OHP and GirP-6MP, the [M]+ (m/z 299 and

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>17OHP concentration, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn (n = 300)</td>
<td>2–5 days</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Children (n = 10)</td>
<td>1–14 years</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients with CAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
</tbody>
</table>

Values in parentheses are for dried blood spots obtained after therapy with hydrocortisone.
The study was funded by a grant from China Medical College Hospital (DMR-91-101). We thank National Taiwan University Hospital for providing five dried filter-paper blood samples from CAH infants.

References