Detection of Fetal-derived Paternally Inherited X-Chromosome Polymorphisms in Maternal Plasma, Nelson L.S. Tang, Tse N. Leung, Jun Zhang, Tze K. Lau, and Y.M. Dennis Lo (Departments of Chemical Pathology and Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR; address correspondence to this author at: Department of Chemical Pathology, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Bldg., 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong Special Administrative Region; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

The recent demonstration of the presence of cell-free fetal DNA in the plasma and serum of pregnant women opens up new possibilities for noninvasive prenatal diagnosis (1-5). However, many published reports have used Y-chromosomal sequences that are present in a male fetus as a marker (1, 6, 7), an approach that is not applicable to the 50% of pregnancies that involve a female fetus. A marker allowing the positive identification of DNA from a female fetus in maternal plasma would be useful for the investigation of sex-linked genetic disorders and for the extension of studies on pathologies involving fetal DNA abnormalities (7, 8) to pregnancies involving female fetuses. We reason that because the female fetus would have inherited a paternally derived copy of the X-chromosome, short tandem repeat (STR) polymorphisms on this chromosome could potentially be used as a fetus-specific marker for female fetal DNA. In this study, we tested this hypothesis using a panel of STRs on the X-chromosome.

Blood samples from pregnant women were collected into tubes containing EDTA. Plasma was harvested as described previously (9). We studied samples from 25 women with female fetuses as ascertained at delivery. Informed consent was obtained, and the study was approved by the Clinical Research Ethics Committee. In 10 cases, samples were collected during the second trimester (mean gestational age, 18 weeks; range, 16–19 weeks) just before amniocentesis. For these cases, 2 mL of amniotic fluid was also collected at the time of amniocentesis for elucidation of the fetal genotype. The 15 third trimester cases had a mean gestational age of 36 weeks (range, 28–41 weeks). Following delivery of these third trimester cases, 5 mL of cord blood sample was also collected for fetal genotype ascertainment.

DNA was extracted from 0.5–0.8 mL of amniotic fluid and maternal plasma using a QIAamp blood kit (Qiagen, USA). DNA was extracted from cord blood using either a QIAamp blood kit or a Nucleon BACC kit (Amersham Life Science). Five pairs of Cys-5-labeled primers flanking STRs mapped to the X-chromosome were studied, including GATA72E05, GATA165B12, GATA31E08, GATA124B04, and GATA172D05 (Research Genetics). Primer sequences and amplification conditions were obtained from the website of the Cooperative Human Linkage Center (http://www.ncbi.nlm.nih.gov/CHLC/). One-fifth of the extracted DNA from maternal plasma was used in each PCR reaction. For fetal DNA extracted from amniotic fluid and cord blood, 2 ng was used for each amplification. PCR was performed in a 25-μL reaction volume containing 2.4 pmol of each primer, 200 μmol of dNTP, and 1 U of Taq enzyme (MBI Fermentas). PCR was performed for 35 cycles with the recommended temperature profiles. PCR product (3–5 μL) was loaded into a 5% Long Ranger denaturing polyacrylamide gel (FMC) for allele-typing in an ALF Express sequencer (Amersham Pharmacia). Allele size determination was performed with the AlleleLinks software. A DNA internal size standard (2.5 fmol) was also loaded together with each sample for quantification of STR peak size. PCR product fragment lengths were determined by alignment of the internal and an external 50-base ladder size standard.

To determine the sensitivity of the system, mixtures of DNA containing various proportions of DNA from two individuals carrying different STR alleles were prepared, with the minority allele present at 1%, 3%, 5%, 7%, and 10% of the total DNA. The detection limit for the minority allele, when 0.5 ng of total DNA was used for amplification, was between 3% and 5%.

A STR marker was defined as informative if its allele size in the paternally inherited X-chromosome was different from the two maternal alleles (Fig. 1A). The presence of the paternally derived allele in the maternal circulation was scored when the following conditions were fulfilled: (a) the STR marker was informative; and (b) a peak was present in the maternal plasma that corresponded to the paternal allele and had a fragment length equal to the paternal allele (as determined by analysis of amniotic fluid and cord blood samples), with a fragment size within a difference of 0.5 bp and with a peak height greater than the internal size marker (i.e., ≥2.5 fmol in amount; Fig. 1).

Among the 25 pairs of samples from women carrying a
Fig. 1. Detection of fetal-derived paternally inherited X-chromosome STR polymorphisms in maternal plasma.

(A), STR GATA124B04 was noninformative in this pair of samples because the fetus shared the same two alleles with the mother. (B), paternally inherited allele (arrow) was detected in a second trimester maternal plasma sample in addition to the maternal alleles (GATA172D05). (C), paternally inherited allele (arrow) was detected in a third trimester maternal plasma sample in addition to the maternal alleles (GATA165B12). •, internal size standards; □, position of detected allele.
female fetus, 19 (76%) were informative with at least one STR marker. Fourteen of these 19 pairs (74%) showed the presence of X-chromosomal DNA of paternal origin in maternal plasma (Table 1). Among the 14 third trimester sample pairs with informative STRs, the paternal allele could be detected in 10 (71%). A similar percentage was found for maternal plasma collected during the second trimester (80%; four of five). The earliest gestational age at which fetal DNA was detected was 16 weeks. These results suggested that STR analysis of maternal plasma could potentially be used for second trimester noninvasive prenatal diagnosis.

Because the STR PCR systems used in this study amplified both the majority maternal alleles and the minority fetal alleles at the same time (10), the sensitivity of detecting the minority fetal allele was lower than in previous reports that utilized fetus-specific amplification schemes (2, 4, 9). The use of other types of polymorphisms, e.g., single nucleotide polymorphisms (11), which are amenable to allele-specific amplification strategies (12), should allow more sensitive systems to be developed.

The use of five STR polymorphisms increased the chance that a fetomaternal pair would be informative. On average, 1.6 informative STR markers were found for each fetomaternal pair. If additional STRs are used, the chance of detecting the fetal alleles may be further increased. The use of multiple fluorescent labels and markers of easily distinguishable PCR product sizes may make it possible to perform this assay in a multiplex format, thus further increasing the speed and informativeness of the approach.

Although the objective of this study was to demonstrate the detection of fetal-derived paternally inherited X-chromosomal polymorphisms in maternal plasma, the approach could easily be applied to autosomal polymorphisms. This development would potentially allow maternal plasma to be used for the noninvasive prenatal diagnosis of a wide variety of genetic disorders.

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Table 1. Number of maternal plasma samples containing X-chromosome STRs of paternal origin.

<table>
<thead>
<tr>
<th>Gestational period</th>
<th>No. of informative cases</th>
<th>Informative cases with detectable paternal X-chromosomal polymorphisms</th>
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<tbody>
<tr>
<td>Second trimester</td>
<td>5/10 (50%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>14/15 (93%)</td>
<td>10/14 (71%)</td>
</tr>
<tr>
<td>All samples</td>
<td>19/25 (76%)</td>
<td>14/19 (74%)</td>
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Rapid, Cost-effective Gene Mutation Screening for Carnitine Palmitoyltransferase II Deficiency Using Whole Blood on Filter Paper, David Smail, Leah Gambino, Christopher Boles, and Georgirene D. Vladutiu* (State University of New York at Buffalo, 936 Delaware Ave., Buffalo, NY 14209; * author for correspondence: fax 716-878-7980, e-mail gdv@acsu.buffalo.edu)

Carnitine palmitoyltransferase II (CPT II; EC 2.3.1.21), an enzyme associated with the inner mitochondrial membrane, is important in the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation (1). CPT II deficiency presents as three distinct clinical phenotypes: adult myopathic (MIM 255110), lethal neonatal (MIM 600649), and a severe infantile phenotype (2). The adult form is the most common lipid myopathy in humans and is characterized by muscle pain, stiffness, and myoglobinuria triggered by exercise, fasting, anesthesia, or other metabolic stressors (3). CPT II is a homotetramer (4) encoded by a gene (MIM 600650) on chromosome 1p32 (5) that spans 20 kb and contains five exons ranging in length from 81 to 1305 bp (6). At least 15 mutations in CPT2 are associated with the adult and infantile disorders (7–9). CPT II deficiency is an autosomal recessive disorder (3, 9); however, recent biochemical and molecular evidence suggests the existence of manifesting carriers, predicting that the prevalence of the disease may be even higher than previously believed (9).

Screening for mutations in CPT2 has been performed using DNA isolated from biopsied muscle tissue (9),