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Fetal DNA Analyzed in Plasma from a Mother's Three Consecutive Pregnancies to Detect Paternally Inherited Aneuploidy, *Chih-Ping Chen,[1,2*] Schu-Rern Chern,[2] and Wayseen Wang[2]* (Departments of [1] Obstetrics and Gynecology, and [2] Medical Research, Mackay Memorial Hospital, Taipei, Taiwan; * address correspondence to this author at: Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Rd., Taipei, Taiwan; fax 886-2-25433642, e-mail cpc_mmh@yahoo.com)

The recent demonstration of fetal DNA in maternal plasma and serum at concentrations much higher than those present in the cellular fraction has introduced new possibilities for noninvasive prenatal diagnosis of paternally inherited dominant disorders *(1–3)*. To date, prenatal detection of fetal aneuploidy in maternal blood has focused on searching intact cells using fluorescence in situ hybridization. The use of fetal DNA in maternal plasma to determine fetal aneuploidy has rarely been described. We previously reported prenatal detection of a paternally inherited fetal aneuploidy from fetal DNA in maternal plasma *(4)*. Here we report the application of such a technique in an additional case involving a mother's three consecutive pregnancies.

We studied fetal DNA in maternal plasma from a pregnant woman whose fetuses possibly had paternally inherited aneuploidy. Her husband had a balanced reciprocal translocation between the long arm of chromosome 10 and the short arm of chromosome 22, 46,XY,t(10;22)(q24.1;p11.2). The woman's karyotype was normal. During her first pregnancy, genetic amniocentesis was performed at 19 weeks of gestation, and the maternal blood sample was collected at 22 gestational weeks before termination of the pregnancy. In contrast, during her second and third pregnancies, the maternal blood samples were collected at 14 and 18 gestational weeks, respectively, before amniocentesis. The amniocentesis of the first pregnancy revealed fetal distal 10q trisomy (10q24.1→qter), 46,XX,der(22)t(10;22)(q24.1;p11.2), resulting from paternal t(10;22) reciprocal translocation. The amniocentesis of the second and third pregnancies showed a balanced translocation the same as the paternal karyotype, 46,XY,t(10;22)(q24.1;p11.2).

We collected 5 mL of both paternal and maternal peripheral blood into EDTA-containing tubes. Blood samples were centrifuged at 3000*g*, and the plasma was carefully removed without disturbing the buffy coat. The maternal plasma sample was recentrifuged, and the supernatant was collected for processing. DNA was extracted from buffy coat and 600-μL plasma samples using a DNA extraction reagent set (QIAamp® DNA Blood Mini Kit). We used fluorescent PCR assays and polymorphic small tandem repeats (STRs) to analyze DNA in maternal plasma. Five pairs of highly polymorphic primers were used separately to amplify the following loci (www.gdb.org): *D10S541* (chromosome 10q22-q23; heterozygosity, 78%), *D10S574* (chromosome 10q; heterozygosity, 75%), *D10S534* (chromosome 10q23-q25; heterozygosity, 78%), *D10S187* (chromosome 10q; heterozygosity, 84%), and *D10S186* (chromosome 10q; heterozygosity, 81%). Each of the forward primers was labeled at the 5′ end with one of the following fluorescent dyes: 6-carboxyfluorescein (FAM); 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (HEX); or 4,7,2′,7′-tetrachloro-6-carboxyfluorescein (TET).

The PCR conditions followed the protocol recommended by the manufacturer. We used 60 ng of parental white blood cell (WBC) DNA (according to the measured absorbance) and a maternal-plasma DNA aliquot equivalent to 1/20 of the starting plasma as template. Normal controls were performed by amplifying maternal plasma and WBC DNA from women carrying fetuses not affected by chromosomal aneuploidies. After initial denaturation at 95 °C for 5 min, 10 cycles of PCR amplification were performed: denaturation for 15 s at 94 °C, annealing for

15 s at 55 °C, and extension for 30 s at 72 °C. Subsequently, 20 cycles of PCR amplification were performed: denaturation for 15 s at 89 °C, annealing for 15 s at 55 °C, and extension for 30 s at 72 °C. The final extension was at 72 °C for 10 min. The DNA fragments were diluted 10-fold (with HEX-labeled products) or 20-fold (with 6-FAM- or TET-labeled products) and were then mixed with formamide and GS-500 TAMRA size standard (Applied Biosystems). The DNA fragments were resolved on a DNA sequencer (ABI 377 model) by Genescan Analysis software (Ver. 2.1; Applied Biosystems). The sizes of the amplified alleles were estimated based on the peaks on the electrophoretograms. The specimen of maternal plasma in the first pregnancy showed disomy for paternal 10q by two informative markers, *D10S534* and *D10S186*, whereas the maternal plasma samples in the second and the third pregnancies showed monosomy for paternal 10q (Table 1 and Fig. 1). Therefore, results obtained by PCR assays were consistent with the cytogenetic results.

Our presentation demonstrates the application of polymorphic markers outside the Y chromosome in maternal plasma for noninvasive detection of possible fetal 10q

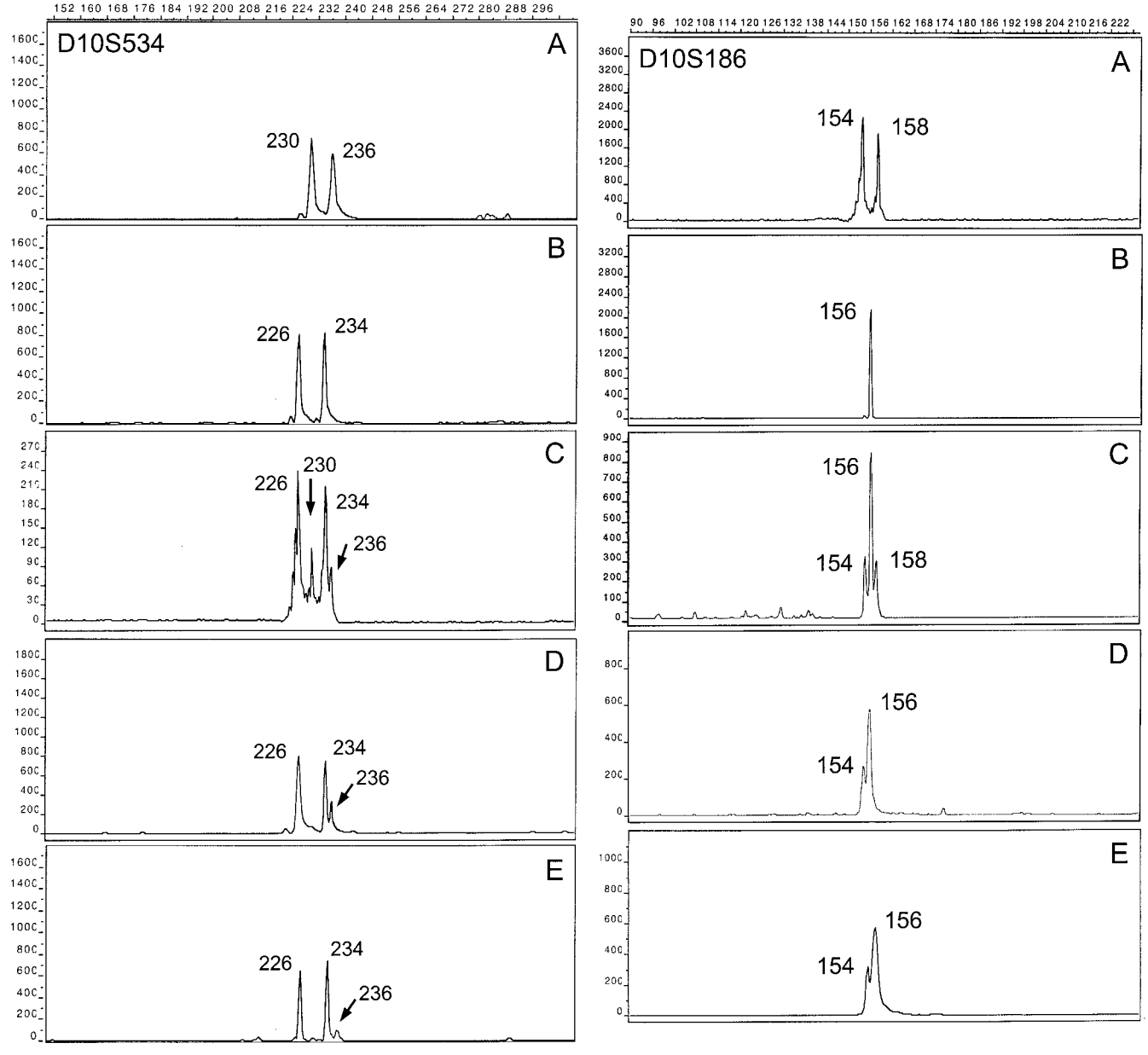

Fig. 1. Detection of fetal-derived paternally inherited 10q STR polymorphic markers in maternal plasma in three consecutive pregnancies.

STR markers *D10S534* (*left panels*) and *D10S186* (*right panels*) were informative. (*A*), paternal WBCs. (*B*), maternal WBCs. (*C*), two paternally inherited alleles were detected in the maternal plasma sample of the first pregnancy. (*D*), only one paternally inherited allele was detected in the maternal plasma sample of the second pregnancy. (*E*), only one paternally inherited allele was detected in the maternal plasma sample of the third pregnancy.

Table 1. Genotypic information of parental WBCs and maternal plasma, based on five STR markers specific for 10q, obtained by fluorescent PCR assays in three consecutive pregnancies.

Marker	Paternal WBCs (*A*)[a]	Maternal WBCs (*B*)	Maternal plasma (*C*)	Maternal plasma (*D*)	Maternal plasma (*E*)
D10S541	253, 259	255, 261	*253*,[b] 255, 261	255, *259*, 261	255, *259*, 261
D10S574	128, 132	128, 128	128, *132*	128	128
D10S534	230, 236	226, 234	226, *230*, 234, *236*	226, 234, *236*	226, 234, *236*
D10S187	94, 94	96, 102	*94*, 96, 102	*94*, 96, 102	*94*, 96, 102
D10S186	154, 158	156, 156	*154*, 156, *158*	*154*, 156	*154*, 156

[a] Karyotypes:
A, father: 46,XY,t(10;22)(q24.1;p11.2)
B, mother: 46,XX
C, fetus 1: 46,XX,der(22)t(10;22)(q24.1;p11.2)
D, fetus 2: 46,XY,t(10;22)(q24.1;p11.2)
E, fetus 3: 46,XY,t(10;22)(q24.1;p11.2)
[b] Italics indicate allele of paternal origin.

trisomy in three consecutive pregnancies in the presence of a fully known paternal balanced translocation. This demonstration is similar to our previous report of detection of fetal 3p trisomy resulting from a paternal t(3;7) translocation *(4)*. In our study, the earliest gestational age at which fetal DNA was detected was 14 weeks. This suggests that STR analysis of maternal plasma can be used for early second trimester, noninvasive prenatal diagnosis. However, because of the overriding presence of maternal DNA in the maternal plasma, our method should be applied with caution to fetal aneuploidy involving the inheritance of two copies of the paternal chromosomal material, and well-selected, informative STR markers should be used. Recently, Tang et al. *(5)* successfully detected fetal-derived paternally inherited X-chromosomal polymorphisms in maternal plasma and opened up new possibilities for noninvasive investigation of sex-linked genetic disorders and fetal DNA abnormalities in female fetuses.

In conclusion, we have shown that fetal aneuploidy can be detected by analyzing STR markers of fetal DNA in maternal plasma. With the development of polymorphic STR markers, we believe that molecular analysis of fetal DNA in maternal plasma can be used for the noninvasive detection of fetal-derived paternally inherited aneuploidy.

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Stability of Serum β-Crosslaps during Storage: Influence of pH and Storage Temperature, *Markus Herrmann, Gerd Pape, and Wolfgang Herrmann*[*] (Department of Laboratory Medicine, University of the Saarland, D-66424 Homburg/Saar, Germany; * author for correspondence: fax 49-6841-163109, e-mail kchwher@med-rz.uni-saarland.de)

Measurements of serum concentrations of β-crosslaps are used for longitudinal monitoring of the individual situation of bone metabolism in patients undergoing antiresorptive therapy *(1–5)*. It thus is important to guarantee comparability of measurements. To realize this, a precise test is needed as well as a correct sampling procedure and well-defined preanalytical handling of samples. Samples sent to an external laboratory may be in transit ≥24 h. We undertook the following study to estimate the influence of duration and temperature of storage as well as possible changes in sample pH on serum β-crosslaps concentrations.

Five untreated samples of human serum with increased β-crosslaps were treated according to the following scheme: Each serum sample was divided into six aliquots. One of these six aliquots served as control sample without any manipulation. The pH in the serum samples without manipulation was 8.7 on average. Each of the other five aliquots was adjusted to pH 6.5, 7.0, 7.3, 7.6, or 8.0. We performed an initial measurement of the β-crosslaps in the six aliquots. After this measurement, each aliquot was subdivided into three subsamples. Each of the three subsamples was stored at a different temperature (4, 21, or 37 °C) for the duration of the experiments. In the 37 °C samples, β-crosslaps were reanalyzed after 2 days, in the 21 °C samples repeated analyses were performed after 2 and 5 days, and in the 4 °C samples after 5 and 10 days. The measured β-crosslaps concentrations at the various check points of follow-up were compared with the values of the corresponding unmanipulated samples.

We measured β-crosslaps with the Elecsys®/β-crosslaps method for serum samples (Roche Diagnostics). We used a paired *t*-test with Bonferroni correction for multiple comparisons of paired samples.