excretion and that loss of glomerular anionic content may be associated with increased urinary GAG excretion.

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Prenatal Diagnosis of Myotonic Dystrophy Using Fetal DNA Obtained from Maternal Plasma, *Paola Amicucci,[1,2] Massimo Gennarelli,[3] Giuseppe Novelli,[1,2*] and Bruno Dallapiccola[1,2]* ([1] Department of Biopathology and Diagnostic Imaging, Tor Vergata University of Rome, Via Di Tor Vergata 135, 00133 Rome, Italy; [2] CSS-Mendel, Piazza Galeno 3, 00161 Rome, Italy; [3] Istituto di Ricovero e Cura a Carattere Scientifico, Fatebenefratelli, Via Pilastroni 4, 25125 Brescia, Italy; * author for correspondence: fax 39-06-20427313, e-mail novelli@med.uniroma2.it)

Myotonic dystrophy (DM; MIM 160900) is an autosomal dominant disorder associated with expansion of an unstable CTG trinucleotide repeat in the 3′ untranslated region of the DM kinase gene (*DMPK*) on chromosome 19q13 *(1)*. Patients are heterozygous for expanded alleles in the range of 50–4000 repeats *(1)*. The molecular diagnosis of DM routinely is performed by analyzing the CTG number on genomic DNA extracted from various biological sources, including trophoblast cells sampled at 10–11 weeks of amenorrhea during the first trimester of pregnancy *(2, 3)*. We evaluated the possibility of using maternal plasma for prenatal diagnosis of DM, by monitoring the pregnancy of an unaffected woman whose husband was affected by DM (70 CTG repeats).

All participants gave oral and written informed consent.

A blood sample (~10 mL) was collected at 10 weeks of gestation before chorionic villus sampling (CVS) and was centrifuged at 3000*g* for 10 min. Plasma was carefully removed from EDTA-containing tube and centrifuged again at 3000*g* for 10 min. DNA was then extracted from 2 mL of the centrifuged plasma with a QIAamp Blood Kit (Qiagen). The elution volume of the final step was 300 μL. Genomic DNA was also extracted from chorionic villi and peripheral blood lymphocytes of both parents.

To check for the presence of fetal DNA in maternal plasma, we performed microsatellite DNA analysis (CSF1PO) and Y-specific PCR (amelogenin) amplification after having ascertained that the fetus was a male (Fig. 1, A and B). *DMPK* CTG repeat amplification was carried out as reported previously *(2)* with a slight modification. A first round of PCR consisting of 15 cycles (30 s at 94 °C, 1 min at 62 °C, 5 min at 68 °C, and a final elongation of 5 min at 68 °C), was performed in 30 μL of reaction mixture, using 25 pmol each of forward and reverse primers DMK9003 (5′-CACAGGCTGAAGTGGCAGTTCCA-3′) and DMK11111 (5′-TGTCGGGGTCTCAGTGCATCCA-3′) *(2)*, and 5–10 μL of the extracted DNA. We reamplified 1 μL of this first-round reaction, using 25 pmol each of forward and reverse primers MDY-1D (5′-GCTCGAAGGGTCCTTGTAGCCG-3′) and MDY-Z2A (5′-TTCCCGAGTAAGCAGGCAGA-3′) *(3)* for 40 additional cycles, using the same cycling and reaction conditions. Amplicons were separated by 1% agarose gel electrophoresis and blotted onto a nylon membrane. Filters were hybridized with $(CTG)_5$ ^{32}P-labeled oligonucleotide as described *(3)*. The same protocol was used for genomic

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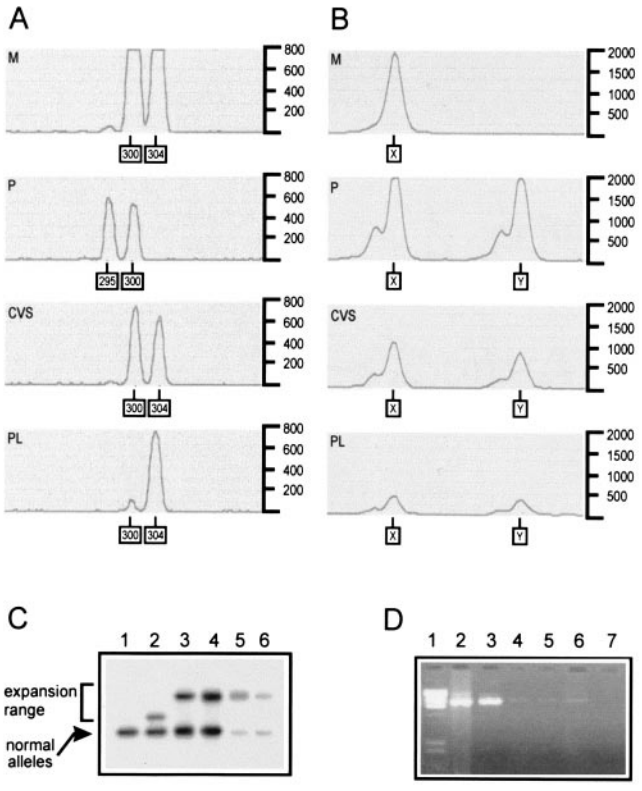

Fig. 1. Electropherograms of alleles at the *CFSPO* locus (*A*) and X-Y amelogenin PCR products (*B*), autoradiograph of CTG expansion at the *DMPK* locus (*C*), and gel showing amplification of *BPY2* (*D*).

(*A* and *B*), electropherograms of maternal genomic DNA (*M*), paternal genomic DNA (*P*), fetal genomic DNA (*CVS*), and maternal plasma DNA (*PL*). (*C*), *lane 1*, maternal genomic DNA; *lane 2*, paternal genomic DNA; *lanes 3* and *4*, fetal genomic DNA at two different concentrations; *lanes 5* and *6*, maternal plasma DNA at two different volumes (3 and 5 μL) of the QIAamp elution. (*D*), *lane 1*, DNA size marker; *lane 2*, paternal DNA; *lane 3*, fetal genomic DNA; *lanes 4–6*, maternal plasma DNA at different volumes (3 and 5 μL) of the QIAamp elution; *lane 7*, negative PCR control (water).

DNA extracted from CVS and peripheral blood lymphocytes.

CTG-expanded alleles were detected in paternal DNA (70 CTG repeats), maternal plasma DNA (150 CTG repeats), and trophoblast DNA (150 CTG repeats). A single wild-type allele of approximately five CTG repeats was found in the maternal genomic DNA (Fig. 1C).

To demonstrate that large, CTG-expanded *DMPK* alleles (up to 2000 CTGs) can be detected in maternal plasma, we performed a long-PCR to amplify an 8-kilobase DNA fragment of the basic protein Y2 (*BPY2*) gene mapping to the Y-chromosome (Fig. 1D). PCR consisting of 35 cycles (2 min at 94 °C, 30 s at 65 °C, 6 min at 68 °C, and a final elongation of 5 min at 68 °C) was performed in 30 μL of reaction mixture using 25 pmol each of forward and reverse primers 7R (5′-GGTATCTGAAGCTGGGTATATGAC-3′) and 7F (5′-AGATAACATCCATCGTGGCTCTG-3′; A. Pizzuti, unpublished data), and 5–10 μL of plasma extracted DNA.

These results support the possibility of performing prenatal diagnosis of DM with maternal plasma. At present, this test seems appropriate only for monitoring paternally inherited expanded alleles. Noninvasive DM prenatal diagnosis was reported previously by our group on trophoblast cells retrieved from the lower part of the uterine cavity *(4)*. However, the amount of fetal DNA recovered with that procedure is low compared with the amount of fetal DNA recovered from maternal plasma *(4–6)*. We conclude that this noninvasive method, which allows first-trimester DM prenatal diagnosis using maternal plasma, has the potential to become an alternative procedure in selected cases.

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