Diagnosis of maternal uniparental disomy of chromosome 7 with a methylation specific PCR assay

EDITOR—In approximately 10% of patients with Silver-Russell syndrome, pre- and postnatal growth retardation with relative macrocephaly, triangular facies, and asymmetry is associated with maternal uniparental disomy of chromosome 7 (UPD(7)mat). The purpose of this report is to present a novel assay to diagnose UPD(7)mat by analysing the methylation status of PEG1/MEST, the only known imprinted gene on chromosome 7, which encodes a protein with sequence homology to alpha/beta-hydrolase. Like its mouse homologue Peg1/Mest, the human PEG1/MEST gene is expressed from the paternal allele but not from the maternal allele. The promoter of the paternal allele is unmethylated whereas that of the maternal allele is methylated. An exception to this strict correlation between the expression and methylation of the promoter is observed in lymphocytes, in which both the paternal and the maternal alleles of PEG1/MEST are expressed. Recently, we have shown that an alternative isoform of PEG1/MEST is expressed concurrently with the original isoform in lymphocytes and that the original isoform is expressed only from the paternal allele while the alternative isoform is expressed from both the paternal and maternal alleles. We concluded that parent of origin specific loss of the isoform 1 expression is strictly correlated with the methylation of the promoter of isoform 1. Documentation of this tight correlation validates the use of methylation analysis of the promoter of isoform 1 as a diagnostic assay for UPD(7)mat.

In order to evaluate the methylation status of the PEG1/MEST gene, methylation specific PCR (MSP) was used. The principle of MSP is as follows. Methylated cytosines in the CpG dinucleotide are resistant to chemical modification by sodium bisulphite. In contrast, bisulphite treatment converts all unmethylated cytosines to uracil. Based on this differential effect, the bisulphite modified DNA sequence of a methylated allele can be distinguished from the unmethylated allele using two sets of allele specific primer pairs. Recently, Zeschnerg et al reported a MSP assay for the detection of hypermethylated alleles at the retinoblastoma loci.

A total of four UPD(7)mat and one UPD(7)pat cases were analysed by MSP. Genomic DNA from peripheral blood of three patients with UPD(7)mat (two from the University of British Columbia, Vancouver and one from Baylor College of Medicine, Houston) were evaluated. Molecular diagnosis of UPD(7)mat was confirmed by haplotype based analysis using multiple STR (short tandem repeats) on chromosome 7. Diagnosis of UPD(7)mat was evident when the child failed to inherit an allele from the father. The fourth UPD(7)mat sample cell line GM11496, a lymphoblastoid cell line established from an isodisomic UPD(7)mat, was obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, New Jersey, USA). A lymphoblastoid cell line from an isodisomic UPD(7)pat patient was also analysed. DNA samples from 50 normal, unrelated controls were derived from anonymous samples of persons presenting for clinical molecular diagnosis at Keio University. The samples were coded by laboratory number and no patient names or identifiers were retained for use in this study. The appropriate informed consent was obtained before clinical diagnostic tests performed on all patients evaluated in this study. Coded DNA samples from normal, UPD(7)mat, and UPD(7)pat were analysed in a blinded fashion.

From the published genomic sequence of the 5' flanking region of exon 1 of the PEG1/MEST gene (GenBank accession number Y10620), DNA sequences of the methylated and the unmethylated alleles after bisulphite modification were deduced. The sequence for the modified methylated strand was deduced by converting all cytosines to thymines. The sequence for the modified unmethylated strand was deduced by converting all thymines with the exception of those in the CpG dinucleotide.

A methylated allele specific primer pair (MET) (5'-tagttgcgtttcgtaaggtagtgc-3'; 5'-acacaatcctccgctcgccta-3') and an unmethylated allele specific primer pair (UNMET) (5'-gtagttgcgtttcgtaaggtagtgc-3'; 5'-cacacaatcctccgctcgccta-3') were designed. DNA amplification was accomplished by PCR using 40 cycles at 94°C for one minute, 58°C for one minute, and 72°C for one minute. PCR products were separated on a 2% agarose gel in 1× TAE buffer, stained with ethidium bromide, and directly visualised under UV illumination.

![Figure 1](http://jmg.bmj.com/37/9/e19)
The results of the test are presented in fig 1. Bisulphite modified DNA from four patients with UPD(7)mat amplified only with the MET primer pair, while bisulphite modified DNA from a patient with paternal uniparental disomy for chromosome 7 amplified only with the UNMET primer pair. Modified DNA from 50 normal, unrelated subjects amplified with both primer pairs. Nascent unmodified genomic DNA was not amplified either by the MET or UNMET primer pairs under the PCR condition described above (data not shown). We conclude that this MSP assay is a rapid, accurate, and robust method for diagnosing uniparental disomy of chromosome 7 and can be used to distinguish maternal disomy from paternal disomy 7.

The method presented has the following significant advantages over conventional haplotype analysis using STR markers: (1) MSP can be performed without the need for testing parental DNA. In this regard, MSP is not subject to the problem of non-paternity; (2) MSP requires only two sets of PCR based primers, whereas haplotype analysis with STR markers requires evaluation of multiple markers on chromosome 7 because informativeness of the markers is not known before testing.

When applying this assay, which is based on analysis of the PEG1/MEST promoter region alone, two points are to be considered. First, Silver-Russell syndrome, a genetically heterogeneous disorder, can occur through mechanisms distinct from UPD(7)mat. These patients may well have normal methylation at PEG1/MEST and will not be detected by MSP. Second, a recent report of negative mutation analysis of PEG1/MEST among 35 patients with Silver-Russell syndrome suggests that an imprinted gene on chromosome 7 other than PEG1/MEST may be involved in the pathogenesis of Silver-Russell syndrome.

If that is the case, segmental uniparental disomy including the putative gene could lead to the Silver-Russell syndrome phenotype, and hence MSP based on PEG1/MEST methylation could erroneously give a normal result. To rule out segmental uniparental disomy, haplotype analysis with multiple STR markers covering the entire chromosome 7 need to be evaluated. However, segmental UPD, although well established on chromosome 11p in rare cases of Beckwith-Wiedemann syndrome, to date has not been found to occur on chromosome 7. In any event, MSP would detect uniparental disomy of the whole chromosome 7 whether PEG1/MEST is directly involved in the pathogenesis of RSS or not.

In summary, MSP can be used for rapid screening for UPD(7)mat patients with a suspected diagnosis of Silver-Russell syndrome in a robust and efficient manner.

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