Electronic letter

J Med Genet 2000;37 (http://jmedgenet.com/cgi/content/full/37/9/e19)

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

EDITOR—In approximately 10% of patients with Silver-Russell syndrome,[1–3] pre- and postnatal growth retardation with relative macrocephaly, triangular facies, and asymmetry is associated with maternal uniparental disomy of chromosome 7 (UPD(7)mat).[4–7] 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,[8] which encodes a protein with sequence homology to alpha/beta-hydrolase.[9 10] Like its mouse homologue *Peg1/Mest*,[10 11] the human *PEG1/MEST* gene is expressed from the paternal allele but not from the maternal allele.[12] The promoter of the paternal allele is unmethylated whereas that of the maternal allele is methylated.[12 13] 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.[12 14] 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.[15] 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 *PEG1/MEST* gene in lymphocytes 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.[16–19] 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, Zeschnigk *et al*[20] 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.[4] 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,[21] 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[22] 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 unmethylated strand was deduced by converting all cytosines to thymines. The sequence for the modified methylated strand was deduced by converting all cytosines to thymines with the exception of those in the CpG dinucleotide. A methylated allele specific primer pair (MET) (5'-tagttgcgtttcgtaaggtagtgtc-3'; 5'-acacaatcctccgctcgccta-3') and an unmethylated allele specific primer pair (UNMET) (5'-gtggtagttgtgttttgtaagtgtagtgtt-3'; 5'-cacacaatcctccactcacctaca-3') were designed. Allele specific 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.

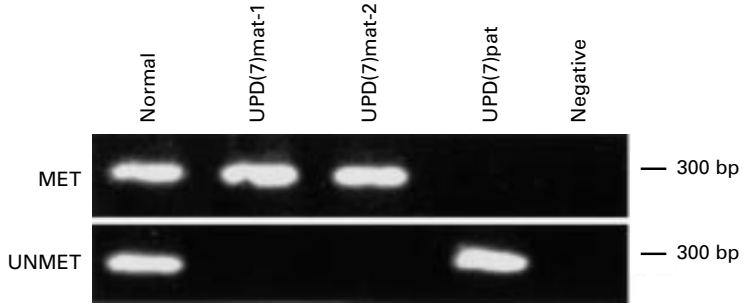

Figure 1 Diagnosis of uniparental disomy of chromosome 7 using methylation specific PCR. PCR products from a bisulphite modified DNA using the MET (top) and UNMET (bottom) primer pairs were electrophoresed on a 2% agarose gel. Note a normal subject (lane 1) had both alleles. Patients with maternal uniparental disomy of chromosome 7 (lanes 2 and 3) had only the methylated allele while the one with paternal uniparental disomy (lane 4) had only the unmethylated allele.

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[5]: (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.[23]

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,[24] to date has not been found to occur on chromosome 7.[4] 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.

We thank Mr Taichi Suzuki from Tokyo Technical College for excellent laboratory assistance. This work was supported in part by a grant to SS from the Novo-Nordisk.

KENJIRO KOSAKI*
RIKA KOSAKI†
WENDY P ROBINSON‡
WILLIAM J CRAIGEN§
LISA G SHAFFER§
SEIJI SATO*
NOBUTAKE MATSUO*

**Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan*

†*Health Center, Keio University, Tokyo, Japan*

‡*Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada*

§*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston Texas, USA*

Correspondence to: Dr K Kosaki, Division of Medical Genetics, Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan, kkosaki@med.keio.ac.jp

1 Russell A. A syndrome of intrauterine dwarfism recognizable at birth with craniofacial dysostosis, disproportionate short arms and other anomalies. *Proc R Soc Med* 1954;**47**:1040-4.
2 Silver H, Kiyasu W, George J, Deamer W. Syndrome of congenital hypertrophy, shortness of stature and elevated gonadotropins. *Pediatrics* 1953;**12**: 356-68.
3 Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet* 1999;**36**:837-42.
4 Bernard LE, Penaherrera MS, Van Allen MI, Wang MS, Yong SL, Gareis F, Langlois S, Robinson WP. Clinical and molecular findings in two patients with Russell-Silver syndrome and UPD7: comparison with non-UPD7 cases. *Am J Med Genet* 1999;**87**:230-6.
5 Kotzot D, Schmitt S, Bernasconi F, Robinson WP, Lurie IW, Ilyina H, Mehes K, Hamel BC, Otten BJ, Hergersberg M. Uniparental disomy 7 in Silver-Russell syndrome and primordial growth retardation. *Hum Mol Genet* 1995;**4**:583-7.
6 Preece MA, Price SM, Davies V, Clough L, Stanier P, Trembath RC, Moore GE. Maternal uniparental disomy 7 in Silver-Russell syndrome. *J Med Genet* 1997;**34**:6-9.
7 Eggermann T, Wollmann HA, Kuner R, Eggermann K, Enders H, Kaiser P, Ranke MB. Molecular studies in 37 Silver-Russell syndrome patients: frequency and etiology of uniparental disomy. *Hum Genet* 1997;**100**:415-19.
8 Kobayashi S, Kohda T, Miyoshi N, Kuroiwa Y, Aisaka K, Tsutsumi O, Kaneko-Ishino T, Ishino F. Human PEG1/MEST, an imprinted gene on chromosome 7. *Hum Mol Genet* 1997;**6**:781-6.
9 Sado T, Nakajima N, Tada M, Takagi N. A novel mesoderm-specific cDNA isolated from a mouse embryonal carcinoma cell line. *Dev Growth Differ* 1993;**35**:551-60.
10 Kaneko-Ishino T, Kuroiwa Y, Miyoshi N, Kohda T, Suzuki R, Yokoyama M, Viville S, Barton SC, Ishino F, Surani MA. Peg1/Mest imprinted gene on chromosome 6 identified by cDNA subtraction hybridization. *Nat Genet* 1995;**11**:52-9.
11 Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA. Abnormal maternal behaviour and growth retardation associated with loss of imprinted gene Mest. *Nat Genet* 1998;**20**:163-9.
12 Riesewijk AM, Hu L, Schulz U, Tariverdian G, Hoglund P, Kere J, Ropers HH, Kalscheuer VM. Monoallelic expression of human PEG1/MEST is paralleled by parent- specific methylation in fetuses. *Genomics* 1997;**42**: 236-44.
13 Lefebvre L, Viville S, Barton SC, Ishino F, Surani MA. Genomic structure and parent-of-origin-specific methylation of Peg1. *Hum Mol Genet* 1997;**6**: 1907-15.
14 Cuisset L, Le Stunff C, Dupont JM, Vasseur C, Cartigny M, Despert F, Delpech M, Bougnere P, Jeanpierre M. PEG1 expression in maternal uniparental disomy 7. *Ann Genet* 1997;**40**:211-15.
15 Kosaki K, Kosaki R, Craigen WJ, Matsuo N. Isoform-specific imprinting of the human PEG1/MEST gene. *Am J Hum Genet* 2000;**66**:309-12.
16 Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. *Eur J Hum Genet* 1997;**5**:94-8.
17 Kubota T, Das S, Christian SL, Baylin SB, Herman JG, Ledbetter DH. Methylation-specific PCR simplifies imprinting analysis. *Nat Genet* 1997;**16**:16-17.
18 Kosaki K, McGinniss MJ, Veraksa AN, McGinnis WJ, Jones KL. Prader-Willi syndrome and Angelman syndromes: diagnosis with a bisulfite treated-methylation specific PCR method. *Am J Med Genet* 1997;**73**:308-13.
19 Herman JG, Graff JR, Myohanen, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 1996;**93**:9821-6.
20 Zeschnigk M, Lohmann D, Horsthemke B. A PCR test for the detection of hypermethylated alleles at the retinoblastoma locus. *J Med Genet* 1999;**36**: 793-4.
21 Spence JE, Perciaccante RG, Greig GM, Willard HF, Ledbetter DH, Hejtmancik JF, Pollack MS, We OB, Beaudet AL. Uniparental disomy as a mechanism for human genetic disease. *Am J Hum Genet* 1988;**42**:217-26.
22 Pan Y, McCaskill CD, Thompson KH, Hicks J, Casey B, Shaffer LG, Craigen WJ. Paternal isodisomy of chromosome 7 associated with complete situs inversus and immotile cilia. *Am J Hum Genet* 1998;**62**:1551-5.
23 Riesewijk AM, Blagitko N, Schinzel AA, Hu L, Schulz U, Hamel BC, Ropers HH, Kalscheuer VM. Evidence against a major role of PEG1/MEST in Silver-Russell syndrome. *Eur J Hum Genet* 1998;**6**:114-20.
24 Catchpoole D, Lam WW, Valler D, Temple IK, Joyce JA, Reik W, Schofield PN, Maher ER. Epigenetic modification and uniparental inheritance of H19 in Beckwith-Wiedemann syndrome. *J Med Genet* 1997;**34**:353-9.