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ELECTRONIC LETTER

Exclusion of maternal uniparental disomy of chromosome 14 in patients referred for Prader-Willi syndrome using a multiplex methylation polymerase chain reaction assay

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J Med Genet 2003;40:e46(<http://www.jmedgenet.com/cgi/content/full/40/4/e46>)

Uniparental disomy (UPD) is the inheritance of both homologues of a chromosome from one parent. For most of the autosomes, there is no definitive clinical consequence of this abnormal inheritance. However, UPDs of chromosomes 6, 7, 11, 14, and 15 are associated with abnormal phenotypes owing to overexpression or underexpression of imprinted genes on those chromosomes.^{1,2}

Maternal UPD(14) (matUPD(14)) has been described in over 20 cases and is primarily characterised by intrauterine growth retardation and precocious puberty. Additional features can include hypotonia at birth, feeding difficulties in early infancy, short stature, musculoskeletal findings including small hands and feet and scoliosis, mild developmental delay, and early childhood obesity. Most patients with matUPD(14) are described with minor facial dysmorphism including frontal bossing, short philtrum, and high arched palate. Paternal UPD(14) (patUPD(14)) is less common, more severe, and is characterised by polyhydramnios, facial and skeletal anomalies, and severe developmental delay.^{3,4} Recently, Wylie *et al*⁵ described reciprocally imprinted genes *DLK1* and *MEG3*, positioned ~90 kb apart at 14q32, which are candidate genes for the UPD(14) phenotypes. *DLK1*, a cell surface transmembrane protein, is paternally expressed, and *MEG3*, which lacks an open reading frame, is maternally expressed.⁵ *DLK1* knockout mice show features of matUPD(14), providing evidence that many of the phenotypic consequences of matUPD(14) may be attributed to a lack of *DLK1* expression in these patients.⁶

UPD(14) is usually ascertained through a combination of clinical features and a karyotype suggestive of UPD, such as confined placental mosaicism for trisomy 14, a non-homologous Robertsonian or reciprocal translocation involving chromosome 14, or an isochromosome 14. These karyotypes are consistent with, or predispose to, monosomy or trisomy rescue events, which are the most common mechanisms leading to UPD.² Recently, three patients with matUPD(14) were described who were originally referred for molecular analysis for Prader-Willi syndrome (PWS) based on clinical phenotypes suggestive of PWS. The authors suggested that there are enough phenotypic similarities between PWS and matUPD(14) such that some patients without PWS might have matUPD(14) syndrome.^{7,8}

We recently described a rapid multiplex methylation polymerase chain reaction (mPCR) assay to identify UPD for chromosome 14 based on parent of origin differential methylation associated with the promoter region of the *MEG3* gene, an imprinted gene on chromosome 14q32.⁹ In this communication, we report the analysis of 200 patients previously referred for PWS to determine if any were unrecognised as having matUPD(14) syndrome.

MATERIALS AND METHODS

Patient samples

Two hundred samples were selected from patients who were referred to our laboratory between 1995 and 2002 for DNA

Key points

- Maternal UPD for chromosome 14 (matUPD(14)) shows some phenotypic overlap with PWS, notably hypotonia, obesity, and hypogonadism in some patients. Recently, three patients with matUPD(14) were reported who were originally referred for a possible diagnosis of PWS. The identification of matUPD(14) in these patients suggested that there may be some use in testing for matUPD(14) in patients referred for PWS, who were not confirmed by molecular analysis.
- In this study we selected 200 patients initially referred for molecular diagnosis of PWS based on their clinical phenotype and who were normal by Southern blot or mPCR analysis of the *SNRPN* region. Patients were screened with a rapid bisulphite modification/multiplex mPCR method based upon the differential methylation associated with the imprinted *MEG3* gene on chromosome 14.
- All 200 samples from patients showed both the paternal and maternal specific PCR fragments, consistent with biparental inheritance of chromosome 14 and excluding matUPD(14).
- These data indicate that the incidence of matUPD(14) is likely to be low among patients referred for PWS.

testing for suspected PWS based on their clinical phenotype. All samples had normal chromosomes (46,XX or 46,XY) and had tested normal by Southern blot or mPCR analysis, excluding changes in methylation at the *SNRPN* locus associated with PWS.

Southern blot analysis

Genomic DNA was extracted from peripheral blood samples with a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Methylation analysis for PWS by Southern blotting with the PW71B (D15S63) probe was performed as described,¹⁰ except that the final posthybridisation wash was in 0.5 × SSC/1% SDS at 55°C for 20 minutes.

Methylation PCR analysis

Bisulphite modification of genomic DNA was performed as described.¹¹ For PWS analysis, methylation specific PCR

Abbreviations: CVS, chorionic villus sampling; matUPD(14), maternal UPD(14); mPCR, methylation polymerase chain reaction; patUPD(14), paternal UPD(14); PWS, Prader-Willi syndrome; UPD, uniparental disomy

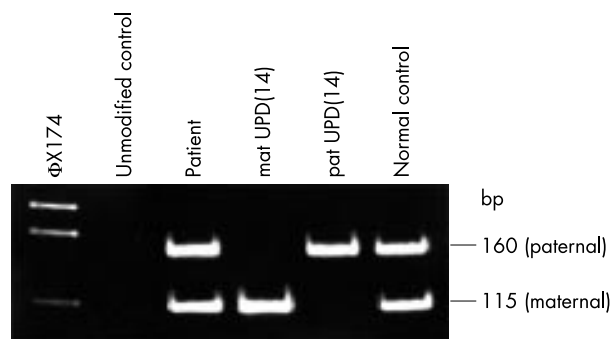


Figure 1 Methylation PCR of the differentially methylated region upstream of the *MEG3* promoter showed the presence of both maternal and paternal alleles in all 200 patients. The paternal and maternal UPD(14) controls showed only paternal or maternal alleles, respectively. Lane 1 was the molecular weight marker Φ X174 (Pharmacia, Piscataway, NJ); lane 2, control DNA without bisulphite modification; lane 3, representative patient sample; lane 4, matUPD(14) control; lane 5, patUPD(14) control; lane 6, normal control.

reactions with maternal and paternal oligonucleotide primers for the CpG island of the *SNRPN* gene were performed as described.¹²

Methylation specific PCR reactions with maternal and paternal oligonucleotide primers for the differentially methylated region 5' of the *MEG3* promoter on chromosome 14q32 were performed as described,⁹ except that the methylation specific forward oligonucleotide used was five nucleotides shorter at the 5' end: 5'-GGTAGTAATCGGGTTGTCCGC-3'. Annealing temperatures were five cycles at 65°C for 30 seconds, five cycles at 60°C for 30 seconds, and 31 cycles at 55°C for 30 seconds. Products of the PCR were separated on a 3% Nusieve agarose or a 6% non-denaturing acrylamide gel, stained with ethidium bromide, and visualised under UV illumination. Controls included normal samples, maternal UPD(14) (unpublished data), and paternal UPD(14).³

RESULTS

Samples from patients were selected from those referred to the laboratory for analysis for PWS, based on clinical phenotype. Two hundred patients were selected that had normal karyotypes (46,XX or 46,XY) and showed inheritance of both maternal and paternal alleles for chromosome 15 after methylation analysis by Southern blot or methylation PCR analysis. Methylation PCR analysis diagnostic for UPD(14) was used to determine if any of these patients were unrecognised matUPD(14). The matUPD(14) control sample showed only the 115 bp maternal PCR product, the patUPD(14) control showed only the 160 bp paternal PCR product, and the normal control showed both maternal and paternal specific PCR fragments as predicted (fig 1). All 200 patient samples showed both the 160 bp paternal and 115 bp maternal allele specific PCR fragments, consistent with biparental inheritance (fig 1).

DISCUSSION

Prader-Willi syndrome is a well recognised genetic disorder with a variable and evolving phenotype.¹³ Major criteria include hypotonia in infancy with associated feeding difficulties and failure to thrive. This is followed by rapid weight gain usually after 1 year of age resulting in notable obesity if uncontrolled. Other major criteria include hypogonadism and delay in motor and speech development. Characteristic facial features include bitemporal narrowing, almond shaped palpebral fissures, strabismus, narrow nasal bridge, and downturned corners of the mouth with a thin upper lip.

Musculoskeletal findings may include small hands and feet, which become more pronounced in mid-childhood, and scoliosis or kyphosis or both. Patients with PWS may have considerable behavioural issues which are quite characteristic to this syndrome, including tantrums, manipulative behaviour, and obsessive-compulsive tendencies. Interestingly, 17% of patients who tested positive by mPCR for PWS did not meet the diagnostic criteria, highlighting the phenotypic variability in this syndrome.¹³

Several aneusomies and Mendelian disorders can present with phenotypes that overlap with PWS. Patients with functional disomy for regions of the X chromosome, either from a duplication or supernumerary ring X chromosome, have phenotypic similarities to PWS, including polyphagia, neonatal growth retardation, and obesity in older children.¹⁴⁻¹⁶ Deletions of 6q also present with a PWS-like phenotype, including hypotonia, polyphagia, facial features, and obesity.^{17,18} Chudley *et al*¹⁹ described a family with an X linked disorder in whom the male patients presented with mental retardation, short stature, obesity, and hypogonadism, suggestive of PWS. Also, a group of patients with fragile X syndrome was reported with phenotypic overlap with PWS.²⁰

Recently, three patients were reported with matUPD(14) who were described as having a phenocopy of PWS, and who were originally referred for PWS testing on the basis of their clinical phenotype. The authors proposed that there was an overlap between the phenotypes of these two syndromes, and that some patients referred for PWS may be unrecognised as matUPD(14).^{7,8} We used a rapid multiplex mPCR assay for UPD(14)⁹ to screen 200 patients originally referred for PWS testing based on their clinical phenotype and found to be normal for PWS by molecular analysis. All 200 patients showed an mPCR profile consistent with biparental inheritance of chromosome 14 (fig 1), excluding UPD(14). Thus, the incidence of unrecognised matUPD(14) among PWS referrals is likely to be low.

None the less, the clinical findings for the two conditions have several similarities that merit further consideration. Many of the patients reported with matUPD(14) had phenotypic features overlapping with PWS to the extent that some were originally referred with a clinical diagnosis suggestive of PWS.^{7,8} A review of clinical data of 17 patients with matUPD(14) showed several features seen in PWS: hypotonia in 11/14, feeding difficulties in 9/10, childhood obesity in 6/15, motor delay in 12/15, and mental delay in 5/15.²¹ As noted by Sanlaville *et al*,²¹ the obesity in matUPD(14) was not as severe as in PWS and behavioural disorders were not as consistent in matUPD(14).

Conventional cytogenetic analysis is important in the diagnosis of UPD(14). Most patients with matUPD(14) reported to date have had rearrangements suggestive of UPD, that is, Robertsonian translocations or isochromosomes.²¹ Indeed, the two PWS-like patients with matUPD(14) described by Berends *et al*⁷ had a Robertsonian translocation and a chromosome 14 isochromosome, respectively, both karyotypes that would suggest a UPD(14) study in the context of phenotypic abnormalities. UPD(14) testing should be performed where cytogenetic analysis identifies a Robertsonian translocation involving chromosome 14 or isochromosome for chromosome 14,^{22,23} a supernumerary marker chromosome 14 (unpublished data), or in amniocytes secondary to identification of confined placental mosaicism for trisomy 14 in CVS.²⁴ Additional studies to test the hypothesis that there are unrecognised patients with matUPD(14) among referrals for PWS will ultimately determine the use of matUPD(14) testing in patients with PWS. The availability of a rapid multiplex mPCR test that does not require parental samples will facilitate these studies.

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