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

L G Dietz, A A Wylie, K A Rauen, S K Murphy, R L Jirtle, P D Cotter

Electronic Letter

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.

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. Recently, Wylie et al. 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.

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.

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. Recently, three patients with matUPD(14) were described who were originally referred for a possible diagnosis of PWS. The authors 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.

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.

**MATERIALS AND METHODS**

**Patient samples**

Two hundred samples were selected from patients who were referred to our laboratory between 1995 and 2002 for DNA 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, except that the final posthybridisation wash was in 0.5 x SSC/1% SDS at 55°C for 20 minutes.

**Methylation PCR analysis**

Bisulphite modification of genomic DNA was performed as described. 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
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.\textsuperscript{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.\textsuperscript{14} Deletions of 6q also present with a PWS-like phenotype, including hypotonia, polyphagia, facial features, and obesity.\textsuperscript{15} Chudley et al\textsuperscript{16} 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.\textsuperscript{17}

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).\textsuperscript{20} We used a rapid multiplex mPCR assay for UPD(14)\textsuperscript{21} 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 of 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.\textsuperscript{22} 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.\textsuperscript{23} As noted by Sanlaville et al,\textsuperscript{24} 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.\textsuperscript{25} Indeed, the two PWS-like patients with matUPD(14) described by Berends et al\textsuperscript{26} 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,\textsuperscript{17,26} a supernumerary marker chromosome 14 (unpublished data), or in amniocytes secondary to identification of confined placental mosaicism for trisomy 14 in CVS.\textsuperscript{27} 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.
Authors’ affiliations
L G Dietz, P D Cotter, Division of Medical Genetics and Department of Pathology, Children’s Hospital and Research Center at Oakland, 747 Fiftieth Street, Oakland, CA 94609, USA; pcotter@itsa.ucsf.edu
A A Wylie, S K Murphy, R L Jirile, Department of Radiation Oncology, Duke University Medical Center, Durham, NC 27710, USA
K A Rauen, P D Cotter, Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA 94143, USA
K A Rauen, Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA 94115, USA
P D Cotter, Division of Genetics, US Labs, 2601 Campus Drive, Irvine, CA 92612, USA

Correspondence to: Dr P D Cotter, Division of Genetics, US Labs, 2601 Campus Drive, Irvine, CA 92612, USA; pcotter@itsa.ucsf.edu

References