

Identification of interstitial maternal uniparental disomy (UPD) (14) and complete maternal UPD(20) in a cohort of growth retarded patients

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Abstract

The association of uniparental disomy (UPD) and short stature has been reported for different chromosomes and in several conditions. Therefore, we investigated a cohort of 21 patients referred because of intrauterine and postnatal growth retardation for UPD of chromosomes 2, 7, 9, 14, 16, and 20. Typing of short tandem repeats showed maternal UPD(14) and maternal UPD(20) in two cases. In the first case, an interstitial UPD(14) was detected and the growth retarded newborn showed some additional clinical signs in common with the putative “maternal UPD(14) syndrome”. The maternal UPD(20) patient showed minor features. However, since it is only the second maternal UPD(20) case it is too early to delineate a specific syndrome and the role of this constitution in growth remains to be investigated. Our data suggest that searching for UPD in growth retarded patients is a helpful approach to getting more information on the role of UPD in growth retardation. Based on our results, general considerations and indications for UPD testing are discussed.

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Uniparental disomy (UPD), the abnormal inheritance of both homologous chromosomes from only one parent, has been described for nearly every human chromosome. The phenotype ranges from normal in UPD(13), (21), and (22) to severely affected, for example in carriers of maternal and paternal UPD(15) in Prader-Willi and Angelman syndromes, respectively, or of paternal UPD(14).¹ For maternal UPD(14), a broad spectrum of features including intrauterine and postnatal growth retardation (IUGR, PGR) has been described.² In UPD of chromosomes 2, 9, and 16, IUGR and/or PGR have been reported as the dominant features.³ This association was also detected in the only published case of

maternal UPD(20).⁴ Maternal UPD(7) is involved in the aetiology of Silver-Russell syndrome (SRS), a disease characterised by IUGR, PGR, and other dysmorphic features, and is observed in nearly 10% of SRS cases.³ In rare cases, maternal UPD(7) has been described in growth retarded patients with no or minor features of SRS. IUGR may also occur in pregnancies in which confined placental mosaicism (CPM) for trisomies is present.⁵ Here, a trisomic rescue resulting in UPD has been shown for chromosomes 2, 7, 9, 15, and 16. Whether the IUGR is the result of the CPM, as suggested for trisomy 16, or of the UPD remains to be elucidated.

In order to ascertain the role of UPD of chromosomes 2, 7, 9, 14, 16, and 20 in short stature, we investigated 21 patients with intrauterine and postnatal growth retardation.

Material and methods

PATIENTS

Our study population consisted of 21 families with an offspring with IUGR and PGR (<3rd centile) of sporadic occurrence. These included 10 patients with isolated IUGR and PGR, four patients with additional single SRS-like features, and seven with different craniofacial dysmorphisms. Cytogenetic analyses of peripheral lymphocytes showed normal karyotypes in all patients. Additionally, we screened 30 SRS patients for UPD(20); in this group, UPD of chromosomes 2, 7, 9, 14, and 16 had been excluded previously.⁶ The study was approved by the appropriate ethics board of the University of Tübingen.

In two of these patients, interstitial maternal UPD(14) (case 1) and maternal UPD(20) (case 2) was identified.

Case 1 with maternal interstitial UPD(14)

This boy was born at 36 weeks of gestation to a 32 year old woman after an uneventful pregnancy. His birth weight was 1710 g (<3rd centile), length was 42 cm (<3rd centile), and head circumference 33.0 cm (<50th centile). He was relatively macrocephalic with a large fontanelle, a snub nose, and low set, malrotated ears. The mandible was hypoplastic and retracted. Clinodactyly was present. The eyes,

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mouth, and feet were normal and he had normal male genitalia with descended testes. On x ray, the long bones appeared shortened without a specific diagnosis. No further malformations (ultrasound of the brain and abdomen, MRI of the skull) or hydrocephalus were observed. Echocardiography and electroencephalography were normal for age. Neurological evaluation showed generalised muscle hypotonia and poor head control. There was slight developmental delay of 1 to 2 months at the age of 6 months. An incarcerated inguinal hernia was operated on and 5 cm of small bowel resected at the age of 3 months. The patient was a lazy feeder without hypoglycaemia and in spite of a high caloric intake he failed to thrive. The boy died at the age of 6 months from aspiration pneumonia in a peripheral hospital. He was referred for UPD testing because of some features of SRS.

Case 2 with maternal UPD(20)

This boy presented for clinical examination at the age of 17 months because of growth retardation. He is the first child of healthy, unrelated parents. Maternal age at his birth was 39 years. During pregnancy insufficiency of the placenta had been observed, but the pregnancy ended at term. Birth weight was 2150 g (<3rd centile) and length was 49 cm (<10th centile). At the age of 17 months he showed macrocephaly, strabismus, and clinodactyly of both hands. IGF-I and IGFBP3 levels were decreased (11 and 1481 ng/ml, respectively).

Table 1 Results of chromosome 14 STR typing in the maternal UPD(14) family. Data from markers other than chromosome 14 STRs are not shown

STR	Coordinate (cM)*	Location	Father	Mother	Proband	Informativity
D14S72	3	14q11.1-11.2	1-2	2-2	1-2	Paternal allele
D14S283†	7	14q11.1-11.2	3-3	1-1	1-2	Mutated allele
D14S80	19	14q12	1-3	2-2	2-3	Biparental
D14S285	47	14q13-23	1-1	2-2	2-2	Maternal UPD
D14S290	56	14q21-22	2-2	1-2	2-2	—
D14S258	64	14q23-24.3	2-3	1-1	1-1	Maternal UPD
D14S289	68	14q23-24.3	1-1	1-1	1-1	—
D14S277	69	14q23-24.3	1-2	3-4	4-4	Maternal UPD
D14S61	77	14q24.3-31	1-2	2-2	2-2	—
D14S68	86	14q24.3-31	1-1	2-2	2-2	Maternal UPD
D14S67	86	14q24.3-31	1-3	2-4	2-4	Maternal UPD
D14S81	100	14q31	1-1	1-1	1-1	—
D14S267	114	14q32	2-3	1-3	2-3	Paternal allele

*The genetic distances and order of markers correspond to the map published by Gyapay *et al.*¹⁹

†A mutation seemed to have occurred in the marker D14S283; the proband showed a maternal allele but also an allele that was larger than the paternal ones. We therefore confirmed paternity by typing 14 different STR markers on nine chromosomes other than 14, all of which showed allelic patterns consistent with paternal inheritance (data not shown). Assuming a mutational event, a dinucleotide repeat expansion would have affected the paternal allele. Such length variations may be caused by strand slippage during replication and have been described before.²⁰

Table 2 Results of STR typing in the maternal UPD(20) family. Data from markers other than chromosome 20 STRs are not shown

STR	Coordinate (cM)*	Father	Mother	Patient	Informativity
D20S117	2	1-1	1-1	1-1	—
D20S199	5	1-2	1-1	1-1	—
D20S95	16	1-3	2-3	2-3	—
D20S194	18	3-3	1-2	1-2	Maternal UPD
D20S170	74	1-4	2-3	2-3	Maternal UPD
D20S96	76	1-1	1-2	1-2	—
D20S178	84	1-3	2-4	2-4	Maternal UPD
D20S109	91	1-1	1-1	1-1	—
D20S120	99	2-2	1-3	1-3	Maternal UPD

*The genetic distances and the order of markers correspond to the map published by Gyapay *et al.*¹⁹

DNA STUDIES

DNA was isolated from peripheral lymphocytes from the patients and their parents. Biparental disomy or UPD for chromosomes 2, 7, 9, 14, 16, and 20 was determined by STR typing. In the case of chromosome 14, all families were typed with STRs localised in the region 14q23-q24 for which an interstitial UPD was published by Martin *et al.*⁷

For exclusion of UPD at least two informative markers on each chromosome were typed according to Lindor *et al.*⁶ and in the case of UPD a panel consisting of at least nine STRs on the specific chromosome was analysed. Paternity was confirmed by typing at least five informative STRs on chromosomes other than the UPD chromosome. Markers, primers, and PCR conditions are available from the authors on request.

Results

Molecular analysis of the 21 patients with IUGR and PGR showed biparental inheritance of chromosomes 2, 7, 9, and 16. Typing of STRs on chromosomes 14 and 20 showed UPD in two out of the 21 IUGR/PGR cases. No UPD(20) was detected among the SRS patients. In case 1, typing of the STRs D14S285, D14S258, D14S277, D14S68, and D14S67 showed a maternal UPD while analysis of proximal and distal markers on 14q (D14S72, D14S80, and D14S267) showed biparental inheritance (table 1). Thus, the UPD was restricted to an interstitial segment in 14q. The UPD interval may be as small as 39 cM (the distance between D14S285 and D14S67) or as large as 95 cM (the distance between the flanking markers D14S80 and D14S267, showing biparental inheritance). This region spans the cytogenetic bands 14q13-q31.

Typing of chromosome 20 specific STRs in case 2 showed maternal UPD(20) in four out of nine markers analysed (table 2). In four markers, maintenance of maternal heterozygosity was observed.

Discussion

Searching for UPD in a cohort of growth retarded patients showed two cases with maternal interstitial UPD(14) and complete UPD(20), respectively.

The interstitial UPD region in case 1 includes the segment 14q23-q24 for which a maternal interstitial UPD has been described by Martin *et al.*⁷ In this case, the UPD interval ranged between 2 and 21 cM and therefore gives strong evidence for an imprinting region in 14q23-q24. The mechanism of formation of this rare finding appears to be the same as that suggested by Martin *et al.*⁷ A mitotic double exchange occurred between the paternal and one of the maternal chromosomes in a trisomy 14 zygote, followed by the loss of the rearranged paternal chromosome. Since our case is the second with an interstitial UPD(14), we hypothesise that this chromosome might be prone to mitotic crossing over which may possibly be mediated by the architecture of the

Table 3 Clinical features in our maternal UPD(14) patient and comparison with published cases, based on the review by Fokstuen et al²

Clinical findings	Previously reported cases (n=13) ²	Present case
Low birth weight	9/11	+
Hypotonia	10/13	+
Hydrocephalus	4/13	(Relative macrocephaly)
Short stature	11/12	Failure to thrive
Mild developmental delay	9/13	+
Normal intelligence	8/10	Too young
Small hands	10/11	-
Hyperextensible joints	5/9	-
Scoliosis	4/10	Too young
Early onset of puberty	8/8	Too young
Recurrent otitis media	5/9	Too young

chromosome. In general, segmental UPD may be underestimated owing to the chance nature of its finding.

The patient described here was referred for clinical examination because of signs indicating Silver-Russell syndrome, for example, IUGR and facial dysmorphism including a triangular face. Asymmetry was not noted. Interestingly, the maternal UPD(14) case of Martin *et al*⁷ also showed a slightly triangular face. Comparing this case with previously published maternal UPD(14) probands, our patient was too young for ascertainment of the most characteristic maternal UPD(14) features, such as developmental delay, precocious puberty, and PGR (table 3). A further sign, hypotonia, has often been described in SRS as well as in maternal UPD(14) cases. The asphyxia, leading to early death, in our maternal UPD(14) patient, has not been described in maternal UPD(14) patients before and might be a coincidental finding.

Our patient and those cases previously published show the considerable phenotypic variation of a putative maternal UPD(14) syndrome, ranging from apparently normal as described by Papenhausen *et al*⁹ to a Prader-Willi-like phenotype.¹⁰ Of course, in the two interstitial UPD(14) cases the phenotypic variability might be caused by the extent of the UPD segment. Many of the symptoms of maternal UPD(14) cannot be ascertained in newborns (table 3). Thus, diagnosis of maternal UPD(14) in newborns is difficult, as shown here, owing to the non-specificity of the features observable at this age, such as a triangular face in our case.

In the second case with complete maternal uniparental heterodisomy 20, we concluded that the UPD originated from a maternal meiosis error followed by a trisomic rescue. Trisomy 20 itself is not viable, but mosaic trisomy 20 is detected in about 6% of amniotic fluid cell cultures. Children born thereafter are almost always normal and the trisomy 20 cell clone is thought to originate from epithelia of the urogenital tract. The non-disjunction in maternal meiosis resulting in trisomy 20, combined with subsequent loss of the paternal chromosome 20, is compatible with the advanced maternal age in our case. So far, only two cases with UPD(20) have been published, a phenotypically severely affected child with paternal UPD(20)¹¹ and the one with maternal UPD(20).⁴ The maternal UPD(20) patient

showed growth retardation, slight dysmorphism, and hyperactivity. The UPD(20) patient presented here only shares growth retardation as a common finding. It is therefore too soon to delineate a maternal UPD(20) phenotype.

Among others, human chromosome 20 is syntenic with mouse chromosome 2H3-H4 where a cluster of imprinted transcripts including the *Gnas* locus has been mapped.¹² The human *GNAS* gene (20q13.2) shows bidirectional imprinting¹³ and mutations in *GNAS* are associated with pseudohypoparathyroidism type IA and McCune-Albright syndrome. Features characteristic of these syndromes were not detected in our patient. Nevertheless, mutations in other genes located in a putative imprinting cluster might be responsible for growth retardation and further phenotypic effects.

No case of UPD(7) was detected among the growth retarded patients, which is consistent with previous studies.¹⁴⁻¹⁵ Very few non-SRS patients with maternal UPD(7) have been described. However, when looking at these patients' phenotypes, three out of five show at least some SRS-like features.¹⁶⁻¹⁸ We therefore conclude that maternal UPD(7) resulting in "pure" IUGR and PGR is a rare event.

As far as chromosomes 2, 9, and 16 are concerned, the majority of UPDs reported have been detected because of cytogenetic aberrations such as CPM. In the case of chromosome 16, the outcome is often normal and the clinical anomalies which have been reported in rare cases seemed to have occurred coincidentally. In maternal UPD(16), the prenatal growth retardation is more likely the result of placental insufficiency.

After exclusion of UPD in our patients, there remains the possibility that an undetected mosaicism in tissues other than blood might cause growth retardation and variable other features, as suggested by Kotzot.³ UPDs restricted to segments as described for UPD(14) cannot be ruled out either.

In summary, searching for UPD in pre- and postnatal growth retarded patients is a promising approach for shedding more light on this frequent clinical finding. Based on our results of UPD screening, we suggest that testing for UPD should be carried out in the following situations: (1) after cytogenetic analysis resulting in indications for chromosomal missegregations (these include CPMs and apparently balanced Robertsonian translocations and in these cases the respective chromosome(s) should be analysed); (2) in the case of unexpected homozygosity for a recessive allele; (3) in patients showing IUGR and PGR, hypotonia, and early onset of puberty when screening for UPD(14) should be performed (other features like small hands, hyperextensible joints, and developmental delay might support UPD(14) testing); (4) our data and other published reports indicate that searching for UPD(7) can be restricted to patients with SRS and SRS-like features and screening of SRS patients for UPDs other than UPD(7) need only be advised in rare cases with aberrant chromo-

somal constitutions. (5) Whether maternal UPD(20) is a frequent finding and associated with characteristic features leading to a “maternal UPD(20) syndrome” remains to be elucidated. Here, further cases with isolated IUGR should be screened.

Nevertheless, the phenotypic transitions in UPD patients may be fluid, therefore making consideration of different UPDs difficult, particularly among newborns with IUGR and non-specific dysmorphism. Generally, the number of cases with UPDs other than UPD(15) should be studied further to give more details concerning phenotypic consequences. Follow up studies are required to enable a reliable prognosis.

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