Uniparental disomy 7 in Silver–Russell syndrome and primordial growth retardation

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Maternal uniparental disomy for the entire chromosome 7 has so far been reported in three patients with intrauterine and postnatal growth retardation. Two were detected because they were homozygous for a cystic fibrosis mutation for which only the mother was heterozygous, and one because he was homozygous for a rare COL1A2 mutation. We investigated 35 patients with either the Silver-Russell syndrome or primordial growth retardation and their parents with PCR markers to search for uniparental disomy 7. Four of 35 patients were found to have maternal disomy, including three with isodisomy and one with heterodisomy. The data confirm the hypothetical localization of a maternally imprinted gene (or more than one such gene) on chromosome 7. It is suggested to search for UPD 7 in families with an offspring with sporadic Silver-Russell syndrome or primordial growth retardation.

INTRODUCTION

Uniparental disomy (UPD), the inheritance of both homologous chromosomes from only one parent, has been found in some individuals with: (i) balanced interhomologous rearrangements (1); (ii) autosomal recessive disorders (2–7); (iii) fetuses or newborns after the determination of confined placental mosaicism (8–11); and (iv) in a proportion of patients with the Prader–Willi syndrome and the Angelman syndrome (12–15). In these two syndromes, the loss of the active homologue of one or several imprinted genes is considered to be responsible for the developmental anomalies. Segmental or mosaic paternal UPD 11 was found in a minority of cases with the Wiedemann–Beckwith syndrome (4,16–18). Excess Igf2 expression caused by two active Igf2 gene copies is suggested to be responsible for the latter syndrome. In mice, as in humans, the Igf2 gene is maternally and the H19 gene paternally imprinted (19,20). Both genes map to distal murine chromosome 7 which is partially homologous to the human chromosome 11. It has been demonstrated that murine paternal disomy 7 and hence overexpression of the Igf2 gene and lack of expression of the H19 gene leads to fetal overgrowth while maternal disomy causes lack of expression of the Igf2 gene and thus results in intrauterine growth retardation (review in reference 21).

In humans, primordial growth retardation (PGR) is characterized by prenatal as well as postnatal growth retardation. If no associated findings are present, it is considered to be PGR sensu stricto. PGR, however, can be a feature of a number of syndromes of known or unknown etiology; most of them are associated with mental deficiency. Examples include the fetal alcohol syndrome (FAS), the autosomal recessively inherited Bloom and Dubowitz syndromes and the Silver–Russell syndrome (SRS; 22–24). The latter syndrome predominantly occurs sporadically, although some instances of direct transmission from a parent to one child or several children (25) and of siblings born to unaffected parents (26) have been reported. In addition to PGR, the SRS is characterized by a disproportionately large head with a broad and prominent forehead and a small and narrow lower portion of the face giving it a triangular appearance, delayed closure of the anterior fontanelle, downturned corners of the mouth, hemihypotrophy of the limbs, clinodactyly and brachymesophalangy of little fingers, partial cutaneous syndactyly between second and third toes, areas of hypo- or hyperpigmentation of the skin, male genital hypoplasia, diminished subcutaneous tissue, delayed bone maturation and excessive sweating, especially over the forehead. None of these features is obligatory. Motor and mental development are normal in the majority of cases.

Three instances of maternal (2,3,27), one instance of maternal long arm and paternal short arm (28) and one instance of paternal UPD 7 (7) have recently been reported. All four

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maternal UPD cases were growth-retarded whereas the one with paternal isodisomy was not growth-retarded and was detected upon molecular investigation of a family with a proband with congenital chloride diarrhea who did not reveal growth retardation. Two isodisomic patients were discovered at molecular investigation of families with probands with cystic fibrosis (CF). Loci around the CF locus were found on chromosome 7q. Molecular investigation revealed paternal imprinted prenatal growth retardation gene on 7p in which case one of these would be a candidate. In order to determine whether maternal UPD 7 might be the cause of a significant proportion of patients with PGR and SRS, we investigated two series of patients with their parents with PCR markers for uniparental disomy 7; (i) 25 cases with sporadic SRS; (ii) 10 patients with PGR. We found maternal UPD 7 in four patients of the 35 patients investigated including three of 25 with SRS and one of 10 with PGR.

RESULTS

The study population

The study population consisted of 35 families with an offspring with either the SRS (n = 25) or PGR (n = 10), both of sporadic occurrence. Proband with chromosome aberrations and other, genetic and non-genetic causes for intrauterine growth retardation were excluded from the study population. In three families it was not possible to obtain blood from both parents. Ages of the probands ranged between 1 year 2 months and 16 years 9 months at examination.

Markers on chromosome 7

In four families, marker analysis revealed maternal UPD 7 (Table 1 and Fig. 1a,b). Patients 1, 3 and 4 were homozygous for all informative markers (7, 14 and 11, respectively) while patient 2 showed heterodisomy, inheritance of two different maternal alleles, for D7S504 and D7S640. At another informative locus (D7S489b), mother and child were homozygous for the b allele while the father was homozygous for the a allele. In addition, patient 2 had inherited maternal heterozygosity at 15 markers; there was no marker in whom the proband differed from the mother in terms of reduction to homogygosity. In patients 1, 3 and 4 the numbers of loci showing reduction of maternal heterozygosity to homogygosity were 6, 12 and 10, respectively, and the numbers of loci showing lack of inheritance of a paternal allele in the proband, but being uninformative for maternal homo- versus heterozygosity were 1, 2 and 1, respectively. Analysis of additional microsatellite markers on chromosomes 6, 11 and 15 revealed no evidence for non-paternity in any of the four patients.

The other 32 patients showed normal biparental inheritance for at least one informative marker on chromosome 7.

The four uniparental disomy 7 patients

Patients 1–3 were clinically classified as having the SRS before the investigations were performed while patient 4 was
considered to have PGR, but not the full SRS picture. Patients 1, 3 and 4 (all with isodisomy) were born to parents below 35 years of age while parental ages of patient 2 (with heterodisomy) were 39 years for the mother and 35 years for the father. Birth weight and length were below the third percentile for gestational age in all patients. Ages at examination ranged from 19 months (patient 1) to 8 years 6 months (patient 3). Length/height at last examination ranged between 3 and 5.5 SD below the mean. Psychomotor development was considered normal in all four patients. Patients 1–3 displayed the full spectrum of the SRS including the characteristic facies; however, patient 4, referred with the diagnosis of primordial growth retardation, also displayed the following SRS features: a triangular face with broad forehead and prominent philtrum and lips; short fifth fingers with clinodactyly; excessive sweating over the forehead; mild limb asymmetry; and a grossly retarded bone age.

**DISCUSSION**

Maternal uniparental disomy 7 was found in four of 35 cases with PGR (including 25 diagnosed with the SRS phenotype). This increases the number of published cases with full maternal UPD 7 to seven, and that of maternal UPD for the long arm of chromosome 7 to eight. Of these cases, the molecular results were compatible with complete maternal isodisomy in five (2,3; patients 1, 3 and 4 of the present study). These cases are most likely the result of a postzygotic mitotic nondisjunction of the maternal chromosome 7 and loss at any stage (pre-meiotically, meiotically or postmeiotically) of the paternal homolog. The results in one case (patient 2 of the present study) disclosed complete heterodisomy which would indicate meiosis 1 nondisjunction of chromosome 7 in an ‘older’ mother, possibly with lack of pairing and recombination. Long arm and proximal short arm homozygosity, but distal short arm heterozygosity of chromosome 7 markers would indicate meiosis 2 nondisjunction following recombination at 7p at meiosis 1 in the patient of Spotila et al. (27). The mechanism in the case of Eggerding et al. (28) with two isochromosomes, one, paternally derived, for 7p and the other, maternally derived, for 7q, is again different and complete homozygosity indicates postmeiotic origin of a structural rearrangement between the two chromosomes 7.

The present findings confirm that maternal UPD 7 is a cause of PGR, especially of the SRS-type, in a significant number of cases. Out of the four ‘positive’ cases of the present study, three had been classified as having the SRS while one (patient 4) was considered to have ‘simple’ PGR. However, the classification of SRS in patients with PGR is subjective, and as patient 4 exhibited some features beyond PGR which are also found in SRS (triangular facies, broad forehead, clinodactyly), it is possible that other clinicians would have classified her as a case of SRS. Clinically, the four UPD 7-positive patients do not differ in any way from the 31 other cases in whom UPD 7 was excluded.

In the 14 year old patient of Spence et al. (2) with maternal isodisomy 7 and cystic fibrosis, asymmetry of the legs was noticed, and the diagnosis of SRS was discussed, although she did not show the characteristic facies. Abnormal findings beyond growth retardation were not mentioned by Voss et al. (3). The patient of Spotila et al. (1992) was a 30 year old male who was mentioned to have had a pointed face at age 1 year. This might indicate an SRS-like facies. As the facies of SRS patients tend to become more normal with age and especially in adulthood, a (partial) SRS-like phenotype can probably not be excluded. Finally, Eggerding et al. (28) described SRS-features such as a triangular face, bilateral clinodactyly of little fingers and slight limb asymmetry, in a 27 month old girl without mentioning SRS. Thus, the patients so far observed with maternal UPD 7 seem to have PGR with a mild or incomplete SRS phenotype. However, it cannot be excluded that maternal UPD 7 may cause ‘pure’ PGR without additional abnormalities in some instances. It seems very unlikely that undetected mosaicism for trisomy 7 in the probands could cause growth retardation without mental retardation and variable other features, and it is excluded in the case with two isochromosomes (28) who could not have arisen through an initial trisomy.

The results of our investigation lend further support to the hypothesis already expressed by Voss et al. (3) and Spotila et al. (27) that there is at least one maternally imprinted gene on chromosome 7 which controls intrauterine and postnatal growth. Patients with maternal UPD 7 obviously have PGR with additional varying features of SRS. Although the number of investigated patients with SRS/PGR is still small, we judge that approximately 10% of sporadic patients with isolated PGR ± SRS features and no evidence for another etiology might result from maternal UPD 7. Thus, it is probably justified to investigate families with a sporadic offspring with PGR or SRS for UPD 7. Further indication for this etiology would be increased maternal age as in patient 2, the only case so far observed due to maternal meiosis 1 nondisjunction. This observation, in addition to the lack of any non-growth-retarded cases with maternal UPD 7, is a strong argument against reduction to homozygosity of a non-imprinted recessive gene as the cause of PGR in these probands. A review of genes mapping to human chromosome 7 reveals that the epidermal

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Order of alleles: proband/mother/father. Informative markers are in bold.
growth factor receptor gene (EGFR) maps to the short arm of chromosome 7 (29,30; Genome Database 1994). Its imprinting status in humans seemingly has not yet been studied. Thus, EGFR could be a candidate gene to cause growth retardation in maternal UPD 7. Furthermore, insulin-like growth factor binding proteins 1 and 3 (IGFBP-1 and IGFBP-3) both map to the short arm of chromosome 7 and have been suggested to regulate cell growth (31)

It is very likely that SRS is genetically heterogeneous. If, as our data suggest, loss or mutations of a maternally imprinted gene or genes account for a proportion of cases with this phenotype, one would then expect that mutations or microdeletions of that gene(s) or of the imprinting region could be responsible for an additional proportion of cases with SRS. As SRS patients are not necessarily infertile, this would imply that microdeletions or mutations could be transmitted in a dominant fashion from a father to one or two of his offspring. If the father would have inherited the mutation from his father, he would also show the SRS phenotype while he would be normal, and thus the pedigree be suggestive of recessive inheritance, if he had received the mutation from his mother. The same would be true for mutations which would falsely activate the imprinting process on the paternal allele. A linkage study of such families with chromosome 7 markers could help to narrow down the localization of such a gene or genes to one or several smaller regions of that chromosome. However, if, as it seems to be in the Prader-Willi syndrome, there is a series of genes involved in the phenotype, it would be less likely to find such pedigrees.

To summarize, using PCR marker analysis, we found four patients with maternal UPD 7 among 35 patients with PGR or SRS. This high incidence justifies that an investigation of UPD 7 should be performed in patients with sporadic PGR or SRS without further features or another etiology.

MATERIALS AND METHODS

Cyto genetic examinations were performed in the probands on blood lymphocyte cultures. GTG banded preparations were investigated, on the average 10 metaphases per case on the level of 400 bands.

DNA was extracted from blood samples as previously described (32), and microsatellite loci in the probands and their parents were analyzed using the polymerase chain reaction. In three families only one parent was available. A total of 27 microsatellite loci and one RFLP marker from chromosome 7 were studied. In addition, microsatellite loci from chromosomes 6, 11 and 15 were studied in order to exclude non-paternity. A complete description of the allele frequencies and primers or probes used to detect the loci is available from the Genome Data Base.

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ABBREVIATIONS

CF, cystic fibrosis; IGF, insulin-like growth factor; PCR, polymerase chain reaction; PGR, primordial growth retardation; SRS, Silver-Russell syndrome; SD, standard deviation; UPD, uniparental disomy.

REFERENCES