ELECTRONIC LETTER

Comprehensive microsatellite marker analysis contradicts previous report of segmental maternal heterodisomy of chromosome 14

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Uniparental disomy of chromosome 14 (UPD(14)) results in one of two distinct abnormal phenotypes depending upon the parent of origin.¹ The discordance between the maternal and paternal UPD(14) phenotypes may result from overexpression and/or underexpression of one or more imprinted genes located on chromosome 14q. A cluster of imprinted genes has been identified and localised to 14q32.²⁻⁵ However, it is unknown whether the altered expression of these genes results in any of the phenotypic features associated with maternal and paternal UPD(14). To refine the candidate imprinted region, it would be useful to identify cases of segmental UPD with clinical features associated with paternal or maternal UPD(14). We recently reported the first case of segmental paternal isodisomy of chromosome 14.⁶ In addition, there are two reported cases of segmental maternal UPD(14).^{7,8} We recently had the opportunity to re-evaluate the patient reported by Martin *et al.*⁷

CASE REPORT

The patient was admitted to the Texas Children's Hospital General Clinical Research Center after parental informed consent for participation in our Baylor College of Medicine Institutional Review Board approved research protocol. On physical examination, her weight and length were normal (50th centile). She showed relative macrocephaly, with head circumference at the 97th centile. Her only dysmorphic features were a high forehead and a pointed chin. She had normal hands and feet and did not have scoliosis, joint laxity, or any evidence of precocious puberty. Brain MRI, ophthalmological evaluation, skeletal survey, echocardiogram, cholesterol level, growth hormone stimulation testing, and FSH/LH stimulation testing were all normal. She scored 98, slightly below average but within the normal range, on the Griffith Developmental Scale (average = 100). She was mildly autistic on the Childhood Autism Rating Scale (score = 32). Therefore,

Key points

- We performed comprehensive microsatellite marker analysis on a previously reported case of segmental maternal uniparental disomy for chromosome 14.
- Of the 103 markers tested, only one suggested maternal uniparental disomy. Other informative markers within the same linkage group indicated biparental inheritance of chromosome 14.
- We therefore conclude that this most likely represents a mutation of a polymorphic marker, rather than uniparental disomy.

our clinical evaluation did not find any of the key features common in cases of holochromosomal maternal UPD(14), thus providing no clinical evidence to support maternal segmental heterodisomy for chromosome 14.

METHODS

Genomic DNA was extracted according to standard methods from cultured peripheral blood lymphoblasts from the patient and both parents. We constructed and analysed monochromosomal somatic cell hybrids containing either the patient's maternally derived ("maternal") or paternally derived ("paternal") chromosome 14, as previously described.^{6,9} We screened clonal hybrid cell lines using standard PCR amplification of markers D14S261 and D14S306 that are outside the region in question and identified two maternal (M1 and M2) and two paternal (P1 and P2) somatic cell hybrid cell lines (data not shown) used for further analysis. This allows for unequivocal construction of haplotypes for the maternal and paternal chromosomes. We then performed comprehensive

Table 1 Selected informative microsatellite marker results

Marker	Location	Het	Genomic DNA			Somatic cell hybrids				Interpretation
			M	C	F	Mat1	Mat2	Pat1	Pat2	
D14S289	14q23.3	0.78	23	24	14	2	2	4	4	Biparental
D14S277	14q23.3	0.78	13	13	23	1	1	3	3	Biparental by hybrids
D14S268	14q23.3	0.75	13	12	12	1	1	2	2	Biparental by hybrids
D14S1028	14q23.3	0.81	22	12	13	2	2	1	1	Biparental
D14S77	14q24.1	0.94	24	24	13	4	4	2	2	Paternal allele mutation
D14S1025	14q24.1	0.87	13	12	22	1	1	2	2	Biparental

Locations of markers were assigned by the NCBI database or assembled sequence contigs for chromosome 14 (<http://www.ncbi.nlm.nih.gov>). Het=heterozygosity coefficient of the marker, M=maternal sample, C=child's sample, F=paternal sample, Mat1 and Mat2 indicate two different hybrids that each contain the maternally derived chromosome 14 from the child, Pat1 and Pat2 indicate two different hybrids that each contain the paternally derived chromosome 14 from the child. Biparental by hybrids indicates those allele assignments that are based on analysis of the maternally and paternally inherited chromosomes 14 segregated into two different somatic cell hybrids. In making the final assignments we considered additional markers contained within the same linkage group.

microsatellite marker analysis of genomic DNA extracted from the mother, child, father, and each of the four hybrid cell lines using 103 highly polymorphic microsatellite markers spanning the long arm of chromosome 14. Direct labelled PCR reactions were carried out as previously described, followed by electrophoresis and autoradiography.¹⁰ Fifty-one of the 103 markers were informative (table 1). Martin *et al*⁷ reported results for 10 microsatellite markers, three of which they report as indicating maternal heterodisomy or UPD(14) (D14S277, D14S268, and D14S77).

RESULTS

Our results for marker D14S277 do not agree with those reported by Martin *et al*⁷ (table 1). The discrepancy can be

explained by examining the results from our monochromosomal hybrids. It is clear that the child inherited the upper allele from her mother (fig 1A). It is possible that Martin *et al*⁷ misinterpreted the lowest band as a common band present in all three subjects and therefore not reflecting a true allele, as this band was not presented in their figure. However, our monochromosomal hybrid analysis showed that this band is in fact a true allele (table 1, biparental by hybrids). This allele is present only in the paternal hybrids, which suggests that the child's lower allele was inherited from her father; however, because the lowest band is a shared allele, it could have originated from either parent. Therefore, this marker is uninformative with respect to the diagnosis of segmental maternal heterodisomy.

Figure 1 Selected STS marker results using genomic DNA from mother (M), child (C), father (F), maternally derived chromosome 14 hybrids (Mat1 and Mat2), and paternally derived chromosome 14 hybrids (Pat1 and Pat2). Panel A illustrates the partially informative marker D14S277. The child's upper allele is common only to the mother, whereas the lower allele is common to both parents. The maternal hybrids contain only the upper allele, whereas the paternal hybrids contain only the lower allele, indicating that the child inherited the upper allele from her mother and the lower allele from her father. Thus, the hybrid results show biparental inheritance for this marker ("biparental by hybrids", table 1). Panel B illustrates marker D14S268 for which the hybrids show biparental inheritance. In this case, the child inherited her upper allele from the mother and the lower allele from her father. Panel C illustrates results for D14S77. The child appears to have inherited two alleles common to her mother and no alleles common to her father. Hybrid analysis indicates that the child inherited the lower allele from her mother. The upper allele is present in the paternal hybrids, indicating that this allele is carried on the paternally inherited chromosome 14. See text for a detailed description of this locus. Panel D illustrates the fully informative marker D14S1028, located within the same linkage group as D14S77, showing biparental inheritance.

DISCUSSION

Martin *et al*⁷ did not present a figure reflecting their results for D14S268, which they reported as “heterozygous, parental origin undetermined (presumed hetero-UPD(14)mat)”. Our results indicate that the child inherited her lower allele from her father, and therefore does not have maternal heterodisomy for this marker (fig 1B, table 1). Our hybrid analysis shows that the child has biparental inheritance for this marker.

For marker D14S77, the child appears to have inherited two alleles common only to her mother. While this could be consistent with maternal heterodisomy, the markers immediately proximal (D14S1028) and distal (D14S1025) to D14S77 show biparental inheritance (table 1). Therefore, this scenario is improbable as it would require a somatic double recombination event within a very small genomic segment, once between markers D14S1028 and D14S77 and again between D14S77 and D14S1025, in order to generate segmental maternal heterodisomy. D14S77 and D14S1025 are separated by approximately 700 kb. Because there is currently a gap between the genomic contigs containing D14S1028 and D14S77, it is difficult to determine the exact physical distance between these markers. However, D14S1028, which shows biparental inheritance, belongs to the same linkage group as D14S77 (Marshfield Center for Medical Genetics, <http://research.marshfieldclinic.org>). The probability of a recombination event within such a small region is exceedingly low, as the recombination frequency between D14S1028 and D14S77 over 188 meioses is zero (Marshfield Center for Medical Genetics, <http://research.marshfieldclinic.org>).

Problematical, and probably leading to misinterpretation, is that for D14S77, the father's two alleles are not common to the mother or child (fig 1C). Our hybrid results indicate that the child inherited her lower allele on the maternally derived chromosome 14 and her upper allele on the paternally derived chromosome 14. The child's upper allele may reflect a mutation of her father's lower allele. Huang *et al*¹¹ recently reported a genome wide analysis of microsatellite mutations with an average microsatellite mutation rate of 1.94×10^{-4} /meiosis. Therefore, based on the genetic map of this region and relatively high likelihood for microsatellite mutation, we assert that this case does not have segmental maternal UPD(14). In summary, our clinical evaluation of this patient found none of the classical features associated with maternal UPD(14) and our molecular analysis showed a single, probably mutated allele within a region of otherwise complete biparental inheritance of chromosome 14. Therefore, we suggest that this case no longer be considered a report of segmental maternal heterodisomy of chromosome 14. Kotzot¹² recently published a comparative study of maternal UPD(14) case reports in an attempt to delineate potential imprinted regions. The author's analysis includes the Martin *et al*⁷ and Eggermann *et al*⁸ cases, and therefore should be interpreted with caution. The analysis of this case shows the utility of monochromosomal somatic cell hybrids in determining the

origin of alleles when markers are not fully informative. In addition, we suggest that comprehensive allele analysis at the highest possible density is warranted when evaluating for segmental UPD.

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