An analysis of common isodisomic regions in five mUPD 16 probands


Abstract

Intrauterine growth retardation (IUGR) with or without additional abnormalities is recognised as a common feature of maternal uniparental disomy for chromosome 16 (mUPD 16) and is usually associated with confined placental mosaicism (CPM). Although it is likely that the CPM largely contributes to the IUGR, postnatal growth retardation and other common abnormalities may also be attributed to the mUPD. Five cases with mUPD 16 and CPM were analysed for common regions of isodisomy using polymorphic markers distributed along the length of the chromosome. In each case the aberration was consistent with a maternal meiosis I error. Complete isodisomy was not detected in any of the patients although two patients were found to be mixed with both iso- and heterodisomy. Interestingly, the patient with the greater region of isodisomy was the most severely affected. The fact that there were no common regions of isodisomy in any of the patients supports the hypothesis that imprinted genes, rather than recessive mutations, may play a role in the shared phenotypes.

Keywords: IUGR; mUPD; heterodisomy; isodisomy

Intrauterine growth retardation (IUGR) is defined as birth weight below the 10th centile for gestational age in infants born in the same community. It is one of three major causes of perinatal and childhood morbidity, after prematurity and major abnormalities. IUGR can result from maternal factors, such as pre-eclampsia, fetal karyotypic chromosomal abnormalities, and placental insufficiencies, but by far the largest group is idopathic.

In 1994, Kalousek documented 73 pregnancies with IUGR or intrauterine fetal death associated with confined placental mosaicism (CPM) (that is, the presence of two or more karyotypes in placental tissue only). IUGR can result from maternal factors, such as pre-eclampsia, fetal karyotypic chromosomal abnormalities, and placental insufficiencies. Two out of 35 were found to have CPM for chromosome 16 and also had imperforate anus, and were subsequently shown to be heterodisomic for maternal UPD for chromosome 16 (mUPD 16).

Several mechanisms have been postulated for the origins of UPD. These include (1) gametic complementation, for example, fertilisation of a disomic egg with a nullisomic sperm; (2) monosomic rescue, where a haploid zygote attains diploid status following mitosis; (3) somatic recombination which produces mosaicism with a normal cell line coexisting with a line with partial isodisomy; and (4) trisomic rescue, where fertilisation of a disomic egg by a haploid sperm produces a trisomic zygote. Following subsequent mitosis, loss of one of the homologous chromosomes present in triplicate generates a mosaic diploid/trisomy. Usually the trisomic cell line is confined to the placenta and the fetus is diploid. In one third of cases, the paternal chromosome is lost, leaving two copies of the maternal chromosome.

The effects of uniparental disomy become apparent when there is either exposure of a recessive gene(s) or an imprinted gene(s) on the chromosome. The first case of mUPD for chromosome 7 documented is that of a girl with cystic fibrosis (CF) and short stature, where only the mother was heterozygous for the CF mutation. Since the girl showed isodisomy for this chromosome, that is, had inherited both copies of chromosome 7 with the mutated CF allele from her mother, the recessive phenotype was exposed. In the case of imprinted genes, the unequal contribution of the maternal and paternal genome to the embryo (maternal genome contributing to the fetus, paternal genome contributing to extra-embryonic tissue) means that absence of the maternal or paternal genome in pUPD or mUPD respectively may lead to growth and developmental abnormalities.

The relative contributions of CPM and mUPD 16 to fetal growth retardation and particularly congenital abnormalities has yet to be determined. CPM is associated with structural placental abnormalities which contribute to poor fetal nutrition and blood supply, which in turn leads to growth retardation. Reports of CPM for chromosome 16 without associated fetal mUPD for chromosome 16 indicate that the presence of high levels of trisomic cells in placenta alone can produce fetal growth retardation and are associated with fetal loss. However, none of these cases had associated malformations.

Kalousek and Barrett reported two cases of fetal UPD for chromosome 16 with normal outcome. However, in a case report of a 4 year old patient with mUPD 16 described continuing short stature as a feature, without any additional major cognitive
This continued growth retardation could be the result of either a long term effect of a compromised placenta or the continued over- or underexpression of an imprinted gene(s).

The five cases described here were ascertained through IUGR being diagnosed in utero or subsequent detection of trisomy 16 confined to the placenta or both. Examination of CVS or term placental tissue in all five cases showed trisomy for chromosome 16 and mUPD of chromosome 16 in the baby. Three of the cases have previously been described. In this investigation, detailed molecular characterisation was undertaken on the five mUPD 16 probands, using additional polymorphic VNTR probes and PCR markers to those described previously, in order to identify heterodisomic or isodisomic regions along chromosome 16 for each case. The data obtained will be useful in postulating either the possibility of an imprinted gene(s) on chromosome 16 involved in growth or pockets of common isodisomy which could locate important recessive genes associated with imperforate anus or heart defects.

Materials and methods

SUBJECTS

Table 1 details the medical history of the five probands investigated. Ethical approval for this project was given by the Hammersmith Hospital Ethics Committee (94/4290) and all subjects gave informed consent. Cases 1 and 2 have previously been reported by our group as having 71% and 28% trisomic placentae respectively and fetal mUPD for chromosome 16.

Karyotyping of fetal brain, kidney, liver, lung, and ovary (50 cells each) in case 1 and informative polymorphic PCRs on fetal brain, kidney, liver, and lung in case 2 (unpublished data) ruled out fetal mosaicism in these tissues and cases. Case 3 was identified at birth as IUGR and term placental tissue showed 100% trisomy for chromosome 16; a postnatal blood specimen from the baby showed a normal karyotype.

Case 4 was brought to our attention from a CVS showing evidence of 100% placental trisomy for chromosome 16. The later amniocentesis showed a normal fetal karyotype. Polymorphic markers subsequently identified mUPD 16 for both cases. Cases 3 and 4 are alive so no further analysis of mosaicism has been possible. Case 5, showing 53% trisomic CVS, has also previously been reported. Case 5 died after 156 days and extensive postmortem tests were refused by the parents, although fibroblast cultures from two separate skin biopsies were diploid (15+30 cells) and peripheral blood lymphocyte culture was also diploid (30 cells). Paternity in all cases was confirmed using three polymorphic tetranucleotide PCR markers from chromosome 7, D7S2846, D7S2212, and D7S1826 (Research Genetics).

DNA EXTRACTION FROM BLOOD AND PLACENTA

DNA was extracted from parental blood, fetal blood, and placenta as in Abu-Amero et al.

SOUTHERN HYBRIDISATION AND RADIOLABELLED PCR

Southern hybridisation and radiolabelled PCR were carried out as described by Abu-Amero et al. Probes (HGMPRC) and polymorphic di- and tetranucleotide PCR markers (HGMPRC and Research Genetics) were used; their cytogenetic and physical locations and informativity are shown in fig 1. Polymorphic PCR markers were selected for high levels of heterozygosity and an even distribution along chromosome 16.

Table 1 Medical history of five mUPD 16 cases investigated

<table>
<thead>
<tr>
<th>Case</th>
<th>Weight at birth (g)</th>
<th>Age at birth (wk)</th>
<th>No of cells counted at amniocentesis</th>
<th>No of trisomic cells observed/total</th>
<th>Features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>350</td>
<td>24</td>
<td>ND</td>
<td>5/5</td>
<td>34/48 (71%)</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
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<td>13</td>
</tr>
<tr>
<td>3†</td>
<td>1690</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1650</td>
<td>35</td>
<td>50</td>
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<td>14</td>
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<tr>
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<td>1390</td>
<td>34</td>
<td>ND</td>
<td>9/9</td>
<td>28%</td>
<td>13</td>
</tr>
</tbody>
</table>

Each case was <3rd weight centile for gestational ages according to the 1988 Gardner-Pearson growth and development chart.

*All amniocenteses showed 100% diploidy.

†This case was diagnosed as IUGR at birth and placental tissue was taken at term and postnatal blood specimen from the baby followed. Cytogenetic discrepancy in the two different tissues led to the request for mUPD 16 analysis.

ND = not done.
16q22-24) was isodisomic for case 2, but all other informative markers (11/18) were heterodisomic. Case 5 showed mixed heterodisomy and isodisomy (8/18 and 5/18 markers respectively) along the length of chromosome 16.

Discussion
Intrauterine growth retardation is a commonly associated feature of confined placental mosaicism for many of the autosomes, irrespective of whether the fetus is UPD or not.17 Except for chromosome 7, the severity of IUGR correlates with percentage trisomy in the term placenta.10 17 Despite the level of trisomy being variable in our five cases, they all showed severe IUGR. However, the severity of IUGR is compounded in CPM with UPD; for example, out of six normal cases with CPM involving chromosome 16, only one had IUGR compared to 8/12 with mUPD 16.17 Whether the increased reduction in growth is a consequence of uncovering recessive genes or disturbance of the normal expression of imprinted genes remains to be determined.

A total of 18 informative markers were used in this study of five mUPD 16 cases to determine common regions of isodisomy, which would point to exposure of a recessive gene(s) being responsible for the IUGR and other malformations seen here. Cases 1, 3, and 4 showed only heterodisomy and isodisomy (8/18 and 5/18 markers respectively) along the length of chromosome 16.

Figure 1 Summary of genotypes in the five IUGR cases with mUPD 16. (A) Location of markers in centimorgans (cM). (B) Genotype of VNTR or polymorphic PCR marker(s) for the region for subjects 1, 2, 3, 4, and 5. (C) Ideogram of chromosome 16. The data show no common region of isodisomy along chromosome 16 in these five cases. H=heterodisomy, I=isodisomy, NI=not informative or not done (as no DNA left) or mUPD, but not informative as to hetero- or isodisomy. Genetic locations were not available for all of the markers in a single database, therefore the locations given are amalgated from three different sources: the Genetic Location Database (ftp://cedar.genetic.soton.ac.uk) (average of male and female chromosome 16 given), Research Genetics Inc, and Comparative Human Linkage Cooperation (http://www.chlc.org).
effectively normal except for the IUGR (table 1). Heart defects were also seen in case 4, although no regions of isodisomy were found to suggest exposure of a recessive gene(s). Case 5 had numerous, severe malformations in addition to IUGR. It is possible that some of the features in case 5 may be the result of uncovering of recessive mutations, as large regions of isodisomy were found.

Certain phenotypes, for example heart defects, inguinal hernia, etc., observed in our five cases have been described in mosaic trisomy conceptuses, with or without UPD in the disomic cell line (table 1). However, not all phenotypes described here, such as imperforate anus, have been found in such cases. It is not possible completely to rule out fetal mosaicism in any case, although multiple tissues from cases 1 and 2 and skin from two sites in case 5 were clearly diploid.

A number of markers (D16S403, D16S769, D16S261, D16S285, D16S288, and D16S299) were selected for their location around the centromere to determine whether non-disjunction occurred at maternal meiosis I or II. Non-disjunction at maternal meiosis I will result in heterodisomy at the centromere, whereas non-disjunction at maternal meiosis II results in isodisomy at the centromere despite recombination. All informative markers at the centromere were heterodisomic in cases 1-4, pointing to maternal meiosis I error.

No common regions of isodisomy were found in our five cases. Although this does not prove that there is an imprinted gene(s) on chromosome 16, the lack of common isodisomy among our five cases also suggests that exposure of a recessive gene(s) is less likely. Several other lines of evidence suggest that there is an imprinted gene(s) on chromosome 16 involved in growth and, from these five cases, possibly also in anal and heart development. Imprinted genes are generally associated with early development of the embryo and placenta. If the normal imprinting pattern of genes is altered by deletions, duplications, mutations, or UPD, specific growth disorders are observed. Since mUPD 16 has usually been reported with IUGR, it cannot be ruled out that there is an imprinted gene(s) important for growth on this chromosome. Of great interest, a region homologous to human 16q11-12 is on the imprinted part of mouse chromosome 7, suggesting that the region 16q11-12 may also be imprinted in humans.

The small selection of markers for case 5 in the original report stresses the importance of using as many polymorphic markers as possible to establish heterodisomy versus isodisomy, as too few markers may not show the true chromosomal constitution.

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