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.

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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 idiopathic.

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, but by far the largest group is idiopathic.

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 also reported two cases of fetal UPD for chromosome 16 with normal outcome. However, a case report of a 4 year old patient with mUPD 16 described continuing short stature as a feature, without any additional major cognitive
effects.\textsuperscript{12} 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.\textsuperscript{13,14} 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 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 placenta respectively and fetal mUPD for chromosome 16.\textsuperscript{13} Karyotyping of fetal brain, kidney, liver, lung, and ovary (50 cells each) in case 1 and informative polymorphic PCRs on fetal kidney, brain, liver, and lung in case 2 (unpublished data) ruled out fetal mosaicism in these tissues and cases.\textsuperscript{13} 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% placentomal 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.\textsuperscript{14} 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).\textsuperscript{14} 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.\textsuperscript{15}

#### SOUTHERN HYBRIDISATION AND RADIOLABELLED PCR

Southern hybridisation and radiolabelled PCR were carried out as described by Abu-Amero et al.\textsuperscript{15} 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.

### Results

#### SOUTHERN HYBRIDISATION AND POLYMORPHIC PCR MARKERS

Alleles for parents and probands were scored according to gel mobility, and informativity for each probe and PCR marker is indicated in fig 1.

Cases 1, 3, and 4 were heterodisomic for all markers that were informative (15/18, 11/18, and 11/18 respectively). One marker (D16S7 -

### 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>10/10</td>
<td>34/48 (71%)</td>
</tr>
<tr>
<td>2</td>
<td>520</td>
<td>28</td>
<td>50</td>
<td>ND</td>
<td>ND</td>
<td>8/29 (28%)</td>
</tr>
<tr>
<td>3†</td>
<td>1690</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20/20 (100%)</td>
</tr>
<tr>
<td>4</td>
<td>1650</td>
<td>35</td>
<td>50</td>
<td>15/15</td>
<td>15/15</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>1390</td>
<td>34</td>
<td>ND</td>
<td>9/9</td>
<td>15/15</td>
<td>283/527 (53%)</td>
</tr>
</tbody>
</table>

Each case was <3rd weight centile for gestational ages according to the 1988 Gardner-Pearson\textsuperscript{16} 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. Except for chromosome 7, the severity of IUGR correlates with percentage trisomy in the term placenta. 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. 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 for all informative markers. Case 2 was mostly heterodisomic and isodisomic only for the q terminal region. Case 5 presented with mixed regions of heterodisomy and isodisomy and also had the most isodisomic regions. Indeed, four recombination events are necessary to explain the distribution of heterodisomy and isodisomy in this case.

Cases 1 and 2 both had imperforate anus. Case 3 had only giant cell hepatitis and was
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 five cases have been described in mosaic defects, inguinal hernia, etc, observed in our isodisomy were found.

Features in case 5 may be the result of uncovering to IUGR. It is possible that some of the had numerous, severe malformations in addition to IUGR, it cannot be ruled out mutations, or UPD, specific growth disorders genes is altered by deletions, duplications, centa. If the normal imprinting pattern of 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. A completely heterodisomic map lends support for imprinted gene(s) being responsible for the observed phenotype, whereas isodisomic pockets would suggest exposure of recessive gene(s) in addition to any imprinting effect.