Metachromatic leucodystrophy (MLD) in a patient with a constitutional ring chromosome 22

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Abstract
Metachromatic leucodystrophy (MLD) is an autosomal recessive lysosomal storage disease resulting from a severe deficiency of arylsulphatase A. The arylsulphatase A gene is located on chromosome 22 at q13.3. An MLD patient is described who carries a common splicing mutation ("I" allele) and a de novo ring 22 deleted for the arylsulphatase A gene. The father was determined to be a heterozygous carrier of the "I" allele and the mother a heterozygous carrier of the arylsulphatase A pseudodeficiency allele. The ring 22 was shown by Southern blotting to be deleted in one copy of the arylsulphatase A gene. Minisatellite analysis showed the extent of the deletion and confirmed the biparental inheritance of chromosome 22 sequences. The carrier status of the parents and the patient’s 46,XX,r(22) karyotype complicated the initial diagnosis in this family. However, the causal relationship of the ring 22 and MLD have implications for the recurrence risk in this family.

Materials and methods
Case report
This female child was born to non-consanguineous parents. She showed delayed development in her first year and was investigated for deafness at 14 months. Unusual physical features including large ears, high forehead, upward slanting eyes, micrognathia, large extremities, and broad hands with short phalanges were noted at that time. She also had spina bifida occulta with a sacral dimple. Karyotype investigation showed a possible ring chromosome 22 in all cells examined. This was later confirmed in two subsequent karyotype studies. A partial spread of metaphase chromosomes from cultured fibroblasts is shown in fig 1. Neither parent showed the ring 22 formation. Because the child’s condition was worsening more rapidly than would be expected from the ring chromosome alone, she was investigated for the possibility of metabolic errors. Enzymatic assay of arylsulphatase A in fibroblast extracts gave a diagnosis of MLD when the child was 2 years 3 months. She deteriorated rapidly becoming decerebrate by the age of 2 years 4 months and died at 2 years 10 months.

Materials
Tag polymerase was purchased from Promega. ID Pol, a heat stable DNA polymerase from *Thermus flavus*, was obtained from ID Labs Inc (London, Ontario). Restriction enzymes were
from Pharmacia Biotech (Baie d’Urfe, Quebec), New England Biolabs (Mississauga, Ontario), or Boehringer Mannheim Canada (Laval, Quebec). \([^{32}P]dCTP\) (specific radioactivity 3000 Ci/mm mol) was from Amersham Canada. MapPairs™ for minisatellite analysis were purchased from Research Genetics (Huntsville, Alabama). A USB Random Primed DNA Labelling kit was purchased from Amersham Canada.

Determination of ASA Activity in Fibroblast Extracts

Fibroblast cultures were grown under standard conditions and harvested at about 75% confluence for assay. Cells were homogenised and cell free supernatants were used in assays with a p-nitroctochelate substrate in an assay specific for ASA (J Rip and B A Gordon, manuscript in preparation) modified from Baum et al.\(^{12}\)

Polymerase Chain Reaction (PCR) Amplification

The following oligonucleotide primers are from a series designed for screening for common mutations in the ASA gene and were used in the PCR amplifications described below and in table 1:

\[
\begin{align*}
(3) \; & 5'\; GCGGAAATTCTTTGATGCGAGGATGAGTCCGAC' \\
(4) \; & 5'\; GGCCTCAGGAAGGCTCTGCAAAAGGCTGCTGG' \\
(5) \; & 5'\; CATGGCCATCTTGACAGGGCAG' \\
(10) \; & 5'\; CACAGATTCAGGAGCTTGATGACTTGTCGAC' \\
(11) \; & 5'\; TGTCAGAAGCTGAGTCTCCATGTCGTA3' \\
(12) \; & 5'\; GGGCTAAGGTCGCAAAGCCATGGCAGGCTC'3'
\end{align*}
\]

Restriction enzyme recognition sites were incorporated into primers 3, 4, 6, and 10 and at the 5’ ends to facilitate cloning. Mismatched bases introduced in primers 11 and 12 to generate new restriction sites in the PCR product are underlined. All primers were synthesised using phosphoramidite chemistry on an automated DNA synthesiser.

PCR amplifications were performed using published procedures.\(^{13}\) Primers were added to a final concentration of 1 \(\mu\)mol/l each. Substrate DNA was added at 1 \(\mu\)g/100 \(\mu\)l. Taq and ID Pol polymerases were used interchangeably at 2-5 U/100 \(\mu\)l and 0-75 to 1-25/100 \(\mu\)l respectively. \(\text{MgCl}_2\) concentration was at 1·5 mmol/l unless otherwise stated. Cycling parameters were: denaturation - 94°, two minutes; annealing temperature as specified in table 1, 1·5 minutes; extension - 72°, two minutes; 30 cycles followed by a seven minute extension at 72°. All reaction mixtures were overlaid with mineral oil and amplification initiated by addition of polymerase to a hot (94°) reaction mixture (“hot start”). RT-PCR was performed using fibroblast mRNA MMuv reverse transcriptase as previously described.\(^{14}\)

Amplification for minisatellite analysis was performed as above with incubation times shortened to 45 seconds for denaturation and 30 seconds for annealing and extension and using MapPairs™ oligonucleotides\(^{15}\) specific for chromosome 22. An annealing temperature of 60° was used for all primer pairs except TO-PIP2 where 50° was used. Products were labelled during the amplification with \([^{32}P]dCTP\) (approximately 0·5 \(\mu\)Ci per 10 \(\mu\)l reaction volume), separated in a sequencing type polyacrylamide gel at 1500 V, and detected by autoradiography.

Southern Blot Analysis

DNA probes were excised from plasmids and labelled with \([^{32}P]dCTP\) by random primed labelling. Genomic DNA samples (5 \(\mu\)g each) were digested with \(\text{HindIII}\), and subjected to electrophoresis in 0·8% agarose, capillary blotting in neutral buffer, and hybridisation with probe by standard methods.\(^{17}\)

Results

ASA Activity in Fibroblast Extracts

Fibroblast extracts from the patient, her parents, and controls were analysed for ASA ac-

<table>
<thead>
<tr>
<th>Site</th>
<th>Primers</th>
<th>PCR product size (bp)</th>
<th>Annealing temperature</th>
<th>Restriction enzyme</th>
<th>Restriction site in normal sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;I-I&quot;</td>
<td>15/16</td>
<td>220</td>
<td>60°</td>
<td>MvaI</td>
<td>+</td>
</tr>
<tr>
<td>&quot;I-1,2-2&quot;†</td>
<td>10/4</td>
<td>738</td>
<td>65°</td>
<td>MvaI</td>
<td>+/+</td>
</tr>
<tr>
<td>&quot;(3)&quot;glycosylation†</td>
<td>3/19</td>
<td>860</td>
<td>68°</td>
<td>Brl</td>
<td>+/-</td>
</tr>
<tr>
<td>Poly A</td>
<td>17/11</td>
<td>147</td>
<td>65°</td>
<td>Rul</td>
<td>-</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>3/12</td>
<td>157</td>
<td>60°</td>
<td>MapIII</td>
<td>-</td>
</tr>
</tbody>
</table>

*Normal sequence as per accession number X52150 (EMBL).
†Simultaneous detection of the two sites.
Further support for this comes from PCR based analyses of the “I-2” and “I-3” polymorphisms and the pseudodeficiency mutations as outlined in table 1. The “I-1” and “I-2”, and the “I-3” and glycosylation mutations can be detected simultaneously (data not shown). The glycosylation mutation can also be identified independently of the “I-3”. Results for the poly A signal mutations are shown in fig 2B. The samples from the father and daughter were not cut by RsaI indicating that they were both homozygous negative for the mutation. The mother’s sample was partly cut by RsaI indicating that she is a carrier of the mutation. In summary, the father is heterozygous for all three “I” mutations and homozygous negative for the two pseudodeficiency mutations (glycosylation and poly A). The mother is homozygous negative for all three “I” mutations and a heterozygous carrier for the two pseudodeficiency mutations. The patient carries the three “I” mutations but not the two pseudodeficiency mutations. The finding of a pseudodeficiency allele in the mother accounts for her decreased level of ASA enzyme activity. Clearly none of the mother’s ASA markers, neither her pseudodeficiency mutations, nor her normal sequence at the sites of the “I” mutations, have been passed to her daughter, consistent with a complete deletion of the gene.

SOUTHERN BLOT ANALYSIS
DNA from family members was cut with restriction enzymes and subjected to agarose gel electrophoresis and Southern blot analysis. Results of HindIII digestion and hybridisation with a probe for the ASA cDNA are shown in fig 3. Visually the patient’s sample appears to be about half the density of that of the parents,

Table 2. ASA enzyme activity in fibroblast extracts*

<table>
<thead>
<tr>
<th>Sample</th>
<th>ASA activity (umol/mg protein)</th>
<th>% control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>17-01</td>
<td>44-9</td>
</tr>
<tr>
<td>Father</td>
<td>10-14</td>
<td>28-8</td>
</tr>
<tr>
<td>Patient</td>
<td>0-09</td>
<td>0-02</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>37-9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(22-3-49-6)</td>
<td></td>
</tr>
</tbody>
</table>

*Activity measured with p-nitrocatechol sulphate as described in Methods.

PCR/RESTRICTION ENZYME ANALYSIS
DNA from family members was tested for the “I-1” mutation using primers 15 and 16 and the restriction enzyme MvaI as outlined in table 1. Results shown in fig 2A clearly indicated that the father’s PCR product was partly cut by MvaI and thus he is a heterozygous carrier of this mutation. The patient’s sample appears to be homozygous for the “I-1” mutation; it was totally uncut. However, the mother’s sample was completely cut and therefore she does not carry the “I-1” mutation. This suggests that her daughter is probably deleted in one ASA allele and is actually hemizygous for the “I-1” mutation.

Figure 2. PCR/Restriction enzyme analysis. Genomic DNA was amplified by PCR and subjected to MvaI digestion. (A) “I-1” allele: lane 1, father (F); lane 2, patient (P); lane 3, mother (M). A 220 bp standard is indicated to the left. The 220 bp abnormal product is not cut by MvaI. The 175 bp product results from cutting of a normal 220 bp PCR product by MvaI. (B) Poly A pseudodeficiency mutations: samples are shown ± additions of RsaI. Sample 1, pseudodeficiency homozygote control (PD); sample 2, father (F); sample 3, patient (P); sample 4, mother (M). The 147 bp normal product is not cut by RsaI. The 117 bp product results from cutting of an abnormal 147 bp product by RsaI. A 154 bp standard is indicated on the left.

Figure 3. Hybridisation with an ASA cDNA probe. Lane 1, father (F); lane 2, patient (P); lane 3, mother (M); lane 4, control (C). Sizes of the restriction fragments are given in kb.
suggestive of deletion of one allele. Analysis of the same blot with a probe for the proximally located D22S55 site, pH9118 (data not shown), gave a similar result with a single band of 5.5 kb, again consistent with a deletion and suggesting that the loss of material from C22q extends proximal to the ASA gene at least as far as D22S55. The approximate location of D22S55 relative to the ASA gene is shown in fig 4.

MINISATELLITE ANALYSIS
Minisatellite analysis was performed on this family and the results are shown in fig 5. Approximate locations of all markers used are shown in fig 4. Amplification with the primers for the markers D22S315 and TOPIP2 clearly indicated inheritance of unique alleles from both parents in the child. The markers D22S283 and CYP2D also showed probable inheritance from both parents; however a common allele is shared by all three family members. Thus, these markers are proximal to the breakpoints involved in the ring formation. These data, in conjunction with the "I" allele inheritance pattern, confirm that there is biparental inheritance of chromosome 22 sequences and that the ring with the deletion is of maternal origin.

ASA SPECIFIC mRNA TRANSCRIPTION
The production of ASA specific mRNA in the patient was tested using RT-PCR as described in Methods. Virtually no product was detectable in the child’s sample although appropriate sized products were detected when primers for an unrelated gene (aspartyl-glucosaminidase) on another chromosome were used (data not shown). These results are consistent with the expectation of essentially no functional ASA specific mRNA where one allele is deleted and the other carries a major splicing mutation.

Discussion
We have reported here a case of a female patient with MLD who has inherited a paternal chromosome 22 carrying the infantile onset "I" allele and has a de novo constitutional ring chromosome 22 of maternal origin, deleted for the ASA gene. The deleterious "I-I" mutation is a splicing defect and is not expected to produce any active enzyme of functional mRNA. The deletion of the other ASA allele would also preclude production of functional mRNA. This was indeed the case, as no RT-PCR products of appropriate size were detectable in this child although the mRNA was able to support transcription of an unrelated cDNA and PCR product.

A typical clinical picture of a patient with a constitutional ring 22 includes significant mental retardation, microcephaly, growth failure, hypotonia, and perhaps a predisposition to meningioma formation. In this particular case the child was referred for cytogenetic analysis because of dysmorphic features, several of which have been noted in other cases of ring 22. De novo ring chromosomes affecting chromosome 22 are relatively rare, comprising approximately 9 to 10% of all constitutional autosomal rings. Autosomal rings occur in about 1/50 000 live births although they have also been detected as isolated findings in men.

![Figure 4](image_url)

**Figure 4** An ideogram of chromosome 22 showing the approximate locations of the markers described.

![Figure 5](image_url)

**Figure 5** Minisatellite analysis: 2 μl aliquots of 10 μl reactions were loaded onto the gel. The samples are from the father (F), patient (P), and mother (M) respectively in each set of three. Alleles are designated a, b, c, and d.
Metachromatic leukodystrophy (MLD) in a patient with a constitutional ring chromosome 22

In the paper, the authors discuss the clinical features and molecular genetics of metachromatic leukodystrophy (MLD), a neurodegenerative disorder characterized by the accumulation of sulfatides in the brain. The paper highlights the importance of constitutional ring chromosome 22, where the patient's condition is associated with a pseudodeficiency allele of the arylsulfatase A gene. This allele is known to cause a partial deficiency of arylsulfatase A, leading to the accumulation of sulfatide in the brain.

Key points from the paper:

1. Metachromatic leukodystrophy is a neurodegenerative disorder characterized by the accumulation of sulfatides in the brain.
2. Constitutional ring chromosome 22 is associated with a pseudodeficiency allele of the arylsulfatase A gene, leading to partial deficiency of arylsulfatase A.
3. This condition is often manifested in infancy or early childhood with symptoms such as seizures, developmental delay, and progressive spastic quadriplegia.
4. Molecular genetic analysis can be used to diagnose MLD, particularly in cases with constitutional ring chromosome 22.

The paper also discusses the importance of prenatal diagnosis and the potential for early intervention in the management of MLD. It emphasizes the need for further research to better understand the pathogenesis of this disorder and to develop effective therapeutic strategies.