Two further cases of WHS with unbalanced de novo translocation t(4;8) characterised by CGH and FISH

Holger Tönnies, Markus Stumm, Luitgard Neumann, Marianne Volleth, Uta Grumpelt, Jörg Müsebeck, Gisela Annuss, Heidemarie Neitzel

EDITOR—In the October 2000 issue of the journal, five new cases of unbalanced translocations with partial monosomy 4p and partial trisomy 8p were described by Wieczorek et al and the authors concluded that de novo translocations causing Wolf-Hirschhorn syndrome (WHS) are more frequent than previously estimated. In particular, unbalanced de novo translocations involving the short arms of chromosomes 4 and 8 seem to be frequent in WHS patients. Furthermore, because of the limited resolution of cytogenetic techniques, some cryptic translocations can be missed by routinely performed cytogenetic differential diagnosis. Therefore, the authors emphasised the necessity of investigating all patients with WHS and visible chromosomal imbalances in chromosome 4p by fluorescence in situ hybridisation (FISH), using a chromosome 4 specific painting probe (whole chromosome painting, wcp) to detect possible translocations. Here we report on two further cases with unbalanced de novo translocations t(4;8). The unbalanced translocation was not detectable by conventional cytogenetics alone and was also not detectable just by painting with a chromosome 4 specific library. We have chosen a different strategy using the comparative genomic hybridisation (CGH) technique as a straightforward molecular cytogenetic assay to unravel unbalanced chromosomal translocations. Subsequently, the CGH results were confirmed by FISH.

Case reports
Patient 1 was the first child born to a 25 year old mother and a 30 year old father. The boy was born spontaneously in the 42nd week of gestation after an uncomplicated pregnancy. Birth measurements were in the lower range, weight 3320 g (10th-25th centile), length 52 cm (25th-50th centile), and occipitofrontal circumference (OFC) 34 cm (3rd-10th centile). Apgar scores were 9/9/8. In addition to the clinical manifestations listed in table 1, the child presented with microphthalmia on the right side and incomplete anophthalmia on the left side, with bluish swellings of the lower lids. Bilateral orbital cysts were histopathologically proven as glioependymal. They were excised at the age of 8 months and orbital prostheses were implanted. Ophthalmological examination further showed atrophy of the optic nerves and the child has no light vision. At the age of 18 months he showed growth delay (height 74 cm, <3rd centile; weight 9900 g, 10th-15th centile; microcephaly, OFC 45.5 cm, <3rd centile), and general muscular hypotonia with poor head control.

Table 1 Clinical findings of both patients

<table>
<thead>
<tr>
<th>Birth</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational weeks</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3320</td>
<td>2070</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>52</td>
<td>44</td>
</tr>
<tr>
<td>OFC (cm)</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Apgar score</td>
<td>9/9/8</td>
<td>1/1/5</td>
</tr>
<tr>
<td>Age at examination (mth)</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

| Hypertelorism | + | + |
| Strabismus | - | - |
| Downturned corners of mouth | + | + |
| Micrognathia | + | + |
| Cleft lip/palate | + | + |
| Facial defects | + | + |
| Hypospadias | Hypospadias |
| Seizures | + | + |

**Figure 1** (A) GTG banded chromosomes 4 of patients 1 (left) and 2 (right). The derivative chromosomes 4 (arrows) show additional unknown material at the distal end of the short arm. (B) A FISH probe of the WHS deletion region 4p16.3 shows the deletion of the proximal part of the short arm in the derivative chromosome 4 in patient 1.
One week after birth, peripheral blood from patient 2 was referred for cytogenetic differential diagnosis of WHS because of intrauterine growth retardation, hypertelorism, hypospadias, and iris coloboma. Patient 2 was the first child of a 29 year old mother and a 26 year old father. Growth retardation was reported in the second half of the pregnancy. The boy was born by caesarean section in the 42nd week of gestation. The amniotic fluid was viscous and green coloured. Birth measures were all below the 3rd centile (weight 2070 g, length 44 cm, and OFC 33 cm). The Apgar scores were 1/1/5 and artificial respiration of the newborn was necessary. The clinical findings of both patients are summarised in table 1.

Blood samples from the patients and their parents were drawn after informed consent. High resolution chromosome analyses by GTG banding were performed using standard techniques. FISH for WHS differential diagnosis was performed using the commercially available probes localised in the WHS region of chromosome 4p (Vysis, Oncor). For further characterisation of the derivative chromosomes 4, genomic DNA from the patients was investigated by our CGH protocol. In brief, patient and control DNA were differently labelled by nick

![Figure 2](image-url)
Electronic letter

We read the letter by Tönnies et al describing two further cases of WHS with unbalanced de novo translocations t(4;8) with great interest. This observation emphasises our findings that particularly translocations t(4;8) are quite frequent in patients with WHS.

Tönnies et al successfully used comparative genomic hybridisation (CGH) as their cases were not detectable by conventional cytogenetics and painting with a chromosome 4 specific library alone. We agree that CGH is a very sensitive method of chromosome painting, as shown in our cases. More reliable methods for the detection of small translocations are available. CGH as a genome wide screening assay allows the identification and localisation of chromosomal imbalances in just one experiment. Alternative screening methods are multi-colour FISH techniques, which can just identify the chromosomal translocation partner but not the exact localisation of the additional chromosomal material. Therefore, our investigations clearly illustrate the potential of CGH for the precise characterisation of unbalanced chromosomal aberrations. We recommend the characterisation of de novo derivative chromosomes by CGH analysis followed by chromosome and/or locus specific probe hybridisations as reported in previous cases.

The authors thanks Anja Gerlach and Britta Teubner for excellent technical assistance in the molecular cytogenetic experiments.


This letter was shown to Drs Wiczerzek and Gillessen-Kaebach, who reply as follows.

We read the letter by Tönnies et al describing two further cases of WHS with unbalanced de novo translocations t(4;8) with great interest. This observation emphasises our findings that particularly translocations t(4;8) are quite frequent in patients with WHS.

Tönnies et al successfully used comparative genomic hybridisation (CGH) as their cases were not detectable by conventional cytogenetics and painting with a chromosome 4 specific library alone. We agree that CGH is a very sensitive method of chromosome painting, as shown in our cases. More reliable methods for the detection of small translocations are available. CGH as a genome wide screening assay allows the identification and localisation of chromosomal imbalances in just one experiment. Alternative screening methods are multi-colour FISH techniques, which can just identify the chromosomal translocation partner but not the exact localisation of the additional chromosomal material. Therefore, our investigations clearly illustrate the potential of CGH for the precise characterisation of unbalanced chromosomal aberrations. We recommend the characterisation of de novo derivative chromosomes by CGH analysis followed by chromosome and/or locus specific probe hybridisations as reported in previous cases.

The authors thanks Anja Gerlach and Britta Teubner for excellent technical assistance in the molecular cytogenetic experiments.


This letter was shown to Drs Wiczerzek and Gillessen-Kaebach, who reply as follows.

We read the letter by Tönnies et al describing two further cases of WHS with unbalanced de novo translocations t(4;8) with great interest. This observation emphasises our findings that particularly translocations t(4;8) are quite frequent in patients with WHS.

Tönnies et al successfully used comparative genomic hybridisation (CGH) as their cases were not detectable by conventional cytogenetics and painting with a chromosome 4 specific library alone. We agree that CGH is a very sensitive method of chromosome painting, as shown in our cases. More reliable methods for the detection of small translocations are available. CGH as a genome wide screening assay allows the identification and localisation of chromosomal imbalances in just one experiment. Alternative screening methods are multi-colour FISH techniques, which can just identify the chromosomal translocation partner but not the exact localisation of the additional chromosomal material. Therefore, our investigations clearly illustrate the potential of CGH for the precise characterisation of unbalanced chromosomal aberrations. We recommend the characterisation of de novo derivative chromosomes by CGH analysis followed by chromosome and/or locus specific probe hybridisations as reported in previous cases.

The authors thanks Anja Gerlach and Britta Teubner for excellent technical assistance in the molecular cytogenetic experiments.


This letter was shown to Drs Wiczerzek and Gillessen-Kaebach, who reply as follows.

We read the letter by Tönnies et al describing two further cases of WHS with unbalanced de novo translocations t(4;8) with great interest. This observation emphasises our findings that particularly translocations t(4;8) are quite frequent in patients with WHS.

Tönnies et al successfully used comparative genomic hybridisation (CGH) as their cases were not detectable by conventional cytogenetics and painting with a chromosome 4 specific library alone. We agree that CGH is a very sensitive method of chromosome painting, as shown in our cases. More reliable methods for the detection of small translocations are available. CGH as a genome wide screening assay allows the identification and localisation of chromosomal imbalances in just one experiment. Alternative screening methods are multi-colour FISH techniques, which can just identify the chromosomal translocation partner but not the exact localisation of the additional chromosomal material. Therefore, our investigations clearly illustrate the potential of CGH for the precise characterisation of unbalanced chromosomal aberrations. We recommend the characterisation of de novo derivative chromosomes by CGH analysis followed by chromosome and/or locus specific probe hybridisations as reported in previous cases.

The authors thanks Anja Gerlach and Britta Teubner for excellent technical assistance in the molecular cytogenetic experiments.

elegant method to identify unbalanced translocations. However, this method is restricted to the expertise of only a few laboratories. For routine analysis, chromosome painting should be followed by FISH with single copy probes from the distal part of the short arms of chromosome 8, for example, 8p telomere probe, which is commercially available.

In addition, patient 1 has an interesting phenotype. He shows some clinical findings, like normal birth weight and lack of postnatal growth retardation, which are unusual in WHS.

In summary, the description of two further cases of WHS with de novo unbalanced translocations t(4;8) by Tönnies et al supports our hypothesis that de novo unbalanced translocations are more frequent in WHS than suspected. In addition, they also confirm our observation that the clinical features of WHS patients with unbalanced translocations t(4;8) are by no means specific enough to distinguish between the phenotypes of simple monosomy 4p and WHS resulting from unbalanced translocation t(4;8).