Familial Wolf-Hirschhorn syndrome resulting from a cryptic translocation: a clinical and molecular study

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Abstract
We present three cousins who have normal karyotypes, despite having clinical features of Wolf-Hirschhorn syndrome. Fluorescence in situ hybridisation techniques confirmed that all three relatives were monosomic for the distal short arm of chromosome 4 and that a cryptic translocation involving chromosomes 4 and 11 was segregating within the family. Segregation analysis indicated that the risk of an affected child being born to a parent carrying the translocation was 15%. Molecular analysis showed that loci D4S111 and D4S115 were not deleted in the proband, thus excluding these loci from the "Wolf-Hirschhorn critical region". Surprisingly, DNA studies also suggested that the translocation breakpoint on chromosome 4 was within the region of a pre-existing paracentric inversion.

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Key words: Wolf-Hirschhorn syndrome; cryptic complex chromosomal rearrangement; critical region.

Wolf-Hirschhorn syndrome is caused by loss of chromosome material from the distal short arm of chromosome 4. Characteristic clinical features are severe growth retardation, mental handicap, microcephaly, ocular colobomata, cleft lip and palate, congenital cardiac defects, and genital abnormalities. In 90% of cases the chromosome 4 deletion arises as a de novo event, whereas in the remainder it is derived from a familial balanced translocation. Several cases have been described where Wolf-Hirschhorn syndrome was caused by a chromosome 4 abnormality detectable solely by molecular genetic techniques.

We describe a large family where the karyotypically normal proband had clinical features suggestive of Wolf-Hirschhorn syndrome. Two distant relatives were affected by multiple congenital abnormalities and we suspected that a translocation involving chromosome 4 was segregating within the family. Molecular genetic in situ hybridisation techniques were required to show the presence of a submicroscopic, reciprocal translocation between chromosomes 4 and 11. Unexpectedly, DNA studies suggested that the translocation breakpoint on chromosome 4 was within the region of a pre-existing paracentric inversion.

Molecular genetic studies have delineated a roughly 2 Mb long "Wolf-Hirschhorn critical region", the shortest area of overlap of deletions causing Wolf-Hirschhorn syndrome, between loci D4S43 and D4S90. DNA studies in our proband and her parents allowed us to shorten the critical region by excluding loci D4S111 and D4S115 from it.

Subjects and methods
CASE 1 (FIG 1, V-9 AND FIG 2): PROBAND
After an uncomplicated pregnancy, the proband was born at term by forceps delivery for fetal distress, weighing 3320 g. She was noted to have a cleft soft palate, a high nasal root, low set, posteriorly rotated ears, a well demarcated philtrum, cupid's bow mouth, and microcephaly. Unusual prominence of the glabella and nasal root led to a clinical suspicion of Wolf-Hirschhorn syndrome. Her early postnatal course was notable for failure to thrive and poor feeding. A ventricular septal defect and mild pulmonary valve stenosis were diagnosed in infancy. She developed epilepsy at approximately 6 months of age. Initially, this took the form of partial motor seizures, but later she developed generalised tonic-clonic seizures. She had bilateral secretory otitis media at 18 months. Subsequent development has been characterised by severe growth retardation (below the 10th centiles for height, weight, and head circumference) and intellectual disability.

CASES 2 (IV-8) AND 3 (IV-10) (FIG 3)
This brother and sister, aged 31 years and 25 years respectively, were second cousins once removed of case 1. They had normal birth weights at term and a similar pattern of abnormalities (severe cognitive disability, microcephaly, seizures, cleft palate, postnatal growth retardation) to the proband. Despite having normal birth weights, these sibs were thought to have an underlying diagnosis of Seckel syndrome.

CYTGENETIC ANALYSIS
Using standard cytogenetic procedures, metaphase chromosome spreads were obtained from the proband and her parents. Repeated conventional and high resolution study of Giemsa stained preparations showed normal karyotypes with no detectable abnormality of chromosome 4p.
IN SITU HYBRIDISATION
pK082, a 5.5 kb subclone of G8 in pBR328\(^8\) was used as a probe for region 4p16.3 and phins 311, an 8.6 kb DNA clone in pBR322, for 11p15.5.\(^5\) Both probes are available from the American Type Culture Collection. Telomere specific probes for 4p (in combination with a chromosome 4 centromere probe) and 11p were purchased from Oncor (Gaithersburg, MD).

The probes were used for either in situ hybridisation with enzymatic detection according to Garson \textit{et al.}\(^6\) (pK082 hybridisations to IV-11, IV-12, IV-16, IV-17, V-9) or for fluorescence in situ hybridisation (FISH), as described by Pinkel \textit{et al.}\(^7\) with modifications detailed by Carter \textit{et al.}\(^12\). Fluorescent images were captured and stored using the Smart-capture digital imaging system (Digital Scientific, Cambridge, UK). Pseudocoloured images were produced in monochrome for publication.

For both enzymatic and fluorescence detection procedures involving pK082 and phins 311, signals visualised on the posthybridisation metaphases were marked on the photographs of prehybridisation, banded metaphases. The distribution of hybridisation signals in chromosome spreads was analysed statistically using the $\chi^2$ test.

Metaphases were assessed for hybridisation of telomeric 11p and 4p probes to chromosomes 4 and 11 after identification of these chromosomes by DAPI (4,6 diamidino-2-phenylindole) band enhancement using Smart-capture software.

DNA ANALYSIS
DNA was extracted by standard techniques from the blood of the proband and her parents. Polymerase chain reaction amplifications were carried out as previously described for the variable number tandem repeat sequences at loci D4S111, D4S114, D4S115, and D4S43 and the trinucleotide repeat sequence within the Huntington's gene.\(^13-15\) Reaction product was electrophoresed on a 2% agarose gel or a 6% acrylamide gel, depending on its size.

Results
Segregation analysis was carried out according to the method described by Gardner and Sutherland.\(^6\) Excluding the proband and her direct antecedents, 13 offspring were born to carrier parents and two children were affected by Wolf-Hirschhorn syndrome. The risk of a carrier having an affected child is therefore 15\%, with a standard error of 10\%.

IN SITU HYBRIDISATION
The proband had a signal peak on only one chromosome 4 following hybridisation to pK082. This confirmed the clinical suspicion of Wolf-Hirschhorn syndrome. The proband's
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46,XX,−4,+der(4)t(4;11)(p16.3;p15.5)pat.

IV-8 and IV-10 (cases 2 and 3) had similar results to the proband following hybridisation to pK082 and phins 311. Examination of other family members showed four phenotypically normal subjects (III-6, IV-7, IV-11, IV-14) with signal peaks on chromosomes 4 and 11 following hybridisation to pK082, indicating that they were carriers of the balanced translocation. These findings were confirmed by the probe phins 311, which also showed that these four people had signal peaks on chromosomes 4 and 11. III-6 had signal on one chromosome 4 and one chromosome 11 with telomere specific probes for both chromosome 11p and 4p, confirming the reciprocal nature of the translocation involving the two chromosomes.

Three normal subjects (IV-12, IV-16, IV-17) had signal peaks on chromosome 4 only with probe pK082, indicating that they did not carry the translocation. A further five cases (III-10, III-13, IV-1, IV-4, IV-21) had only a chromosome 4 signal peak following hybridisation with pK082 and only a chromosome 11 signal peak following hybridisation with phins 311, again indicating that they did not carry the translocation. One carrier of the balanced translocation (IV-7) became pregnant during the course of our investigations. Amniocentesis was performed at 14 weeks of gestation and FISH with probes pK082 and phins 311 was performed on cultured amniocytes. The fetus had a normal female chromosome constitution (46, XX), without molecular cytogenetic evidence of a t(4;11) translocation. A phenotypically normal female infant weighing 3490 g was born at term.

Results of molecular genetic analysis on proband and parents

<table>
<thead>
<tr>
<th>Locus</th>
<th>Proband Allele</th>
<th>Father Allele</th>
<th>Mother Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>1/2</td>
<td>3/3</td>
<td>1/2</td>
</tr>
<tr>
<td>D4S43</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>D4S114</td>
<td>Uninformative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D4S115</td>
<td>1/3</td>
<td>1/2</td>
<td>3/4</td>
</tr>
<tr>
<td>D4S111</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
</tr>
</tbody>
</table>
DNA ANALYSIS
The results of the polymerase chain reaction amplifications are shown in the table. The proband was hemizygous for a region between the Huntington’s disease locus and D4S43, but heterozygous at the more telomeric loci D4S111 and D4S115 (fig 5).

Discussion
Normal cytogenetic results are an indication for molecular genetic investigation in patients who have phenotypic features strongly suggestive of an underlying chromosome imbalance. Submicroscopic deletions or translocations have been detected using DNA probes with Southern blot hybridisation, polymerase chain reaction based polymorphisms, and fluorescence in situ hybridisation in a variety of conditions, including sporadic and familial (sib or first cousin) cases of Wolf-Hirschhorn syndrome. In our family, it was initially thought that the proband had the same autosomal recessive disorder (Seckel syndrome) which had been diagnosed in distantly related male and female sibs. However, from the appearance of the proband and inspection of the family pedigree, we suspected that a translocation involving the short arm of chromosome 4 was segregating within the family. No abnormality of chromosome 4 could be visualised using high resolution chromosome banding but molecular genetic in situ hybridisation techniques successfully detected a submicroscopic translocation between chromosomes 4 and 11. The clinical diagnosis of Wolf-Hirschhorn syndrome was confirmed in the proband and her cousins by showing that they have the translocation in an unbalanced form and are monosomic for a segment of distal 4p. Four phenotypically normal subjects have the cryptic t(4;11) translocation in a balanced form and eight subjects were confirmed as having a normal karyotype. Segregation analysis in this extensive kindred showed that the risk that a carrier of the balanced translocation might have an affected liveborn child was 15%, with a standard error of 10%. This is in exact agreement with the results of segregation analysis carried out on a large family with Wolf-Hirschhorn syndrome resulting from a cytogenetically visible, reciprocal translocation.

We successfully carried out prenatal diagnosis in a carrier of the balanced t(4;11) translocation, using fluorescence in situ hybridisation on cultured amniocytes. In a family with Wolf-Hirschhorn syndrome caused by submicroscopic translocation, Alther et al used DNA probes with Southern blot hybridisation to show that DNA from a cultured chorionic villus sample was disomic for the short arm of chromosome 4. Goodship et al used the same techniques in combination with fluorescence in situ hybridisation for prenatal diagnosis in a similar family, again using cultured chorionic villus cells. They suggested that in families with a subtle translocation, fluorescence in situ hybridisation should be the technique of choice for prenatal diagnosis since it allows identification of all unbalanced products, it can distinguish between a normal karyotype and a balanced carrier, and its accuracy is not affected by recombination events. Pettenati et al detected an unbalanced chromosome abnormality of the type which causes cri du chat syndrome by using fluorescence in situ hybridisation on uncultured amniocytes. Our result adds to the emerging body of evidence that fluorescence in situ hybridisation is a reliable technique for prenatal detection of cryptic translocations.
An unexpected finding in our family was that a novel chromosomal rearrangement involving the translocated chromosome 4 has occurred. The PCR based molecular results in the proband show that she is hemizygous for a region between the Huntington locus and locus D4S43. However, she is heterozygous at two loci, D4S111 and D4S115, located telomeric to D4S43. The in situ hybridisation results on III-6 clearly indicate that the telomeric regions of the short arms of chromosomes 4 and 11 are reciprocally exchanged in carriers of the balanced translocation. The PCR based molecular data therefore suggest that the breakpoint on the translocated chromosome 4 was within the region of a pre-existing paracentric inversion (fig 6). Complex chromosomal rearrangements which are entirely submicroscopic have not previously been described, but study of light microscopically visible complex rearrangements with molecular techniques has shown unsuspected cryptic abnormalities. Light microscopically visible translocations are seldom intensively investigated by molecular techniques and it may be that cryptic complex chromosomal rearrangements are more common than currently suspected.

Analysis of DNA from patients with deletions of distal chromosome 4p has allowed the definition of a Wolf-Hirschhorn "critical region", the smallest area of overlap of all the deletions causing Wolf-Hirschhorn syndrome. Gandelman et al defined the critical region as being a 2-2 Mb long section in 4p16.3 between loci D4S43 and D4F26. Johnson et al shortened the region at its telomeric end by excluding locus D4F26. Our proband is hemizygous for the PCR based markers at Huntington locus and locus D4S43. She is heterozygous at loci D4S111 and D4S115, excluding these loci from the telomeric end of the critical region and suggesting that it lies between loci D4S43 and D4S115 (fig 5).

Our modification of the critical region relies on the proband having classical clinical features of Wolf-Hirschhorn syndrome, including growth and mental retardation, microcephaly, seizures, congenital heart disease, and cleft palate. Ocular colobamata and genital abnormalities were absent. Perhaps the single most useful diagnostic feature of Wolf-Hirschhorn syndrome is the facial "gestalt" and it was recognition of this in the proband which was the impetus for the molecular cytogenetic studies which confirmed the diagnosis. She had no specific features of trisomy 11p, which might have complicated the clinical picture.

A preliminary phenotypic map of chromosome 4p16 has been prepared recently. The clinical and molecular findings in our proband are compatible with this phenotypic map, with two exceptions: firstly, as discussed above, the definition of the extent of the Wolf-Hirschhorn critical region, and, secondly, our data suggest that D4S111 and D4S115 can be excluded from the region of deletions which cause a prominent glabella.

It is not yet known which genes must be deleted to cause the Wolf-Hirschhorn phenotype. It has been suggested that a zinc finger gene mapping to locus D4S90 might be a candidate gene. Both our results and the results of Johnson et al suggest that this locus is outside the Wolf-Hirschhorn critical region and tend to exclude the gene from playing a significant pathogenetic role.

Figure 6. Schematic diagram of the mechanisms by which the derivative chromosome 4 may have arisen. (A) Normal chromosomes 4 and 11, with a schema of the relative physical order of chromosome 4 marker loci shown left; (B) a small inversion occurs, reversing the order of the marker loci; (C) a reciprocal translocation involving chromosomes 4 and 11 occurs; the breakpoint on chromosome 4 is within the pre-existing inversion, between loci D4S43 and D4S115; (D) the translocation in the unbalanced form in the proband. The proband is heterozygous at loci D4S111 and D4S115, but hemizygous at D4S43 and the Huntington's disease locus (HD).
In summary, this family reinforces the fact that submicroscopic chromosomal abnormalities are an important, underdiagnosed cause of genetic disease. Clinical suspicion and simple inspection of the family tree are as crucial to their detection as sophisticated molecular cytogenetic techniques.

Probe pK082 was the generous gift of Dr James F Gusella. We thank Jean Hislop for illustrations, Professor Robin Winter for his suggestion, made in 1988, that the proband had features of Wolf-Hirschhorn syndrome, and many members of the extended family for their patience and cooperation.


