Paternal origin of the de novo deleted chromosome 4 in Wolf-Hirschhorn syndrome

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Abstract

The parental origin of the de novo deleted chromosome 4 was studied in five cases of Wolf-Hirschhorn syndrome using polymorphic probes mapping in the 4p16.3 region. In all the patients the deleted chromosome was found to be of paternal origin and these results, together with similar ones obtained by another group, make the preferential paternal origin of the de novo chromosome 4 deletion highly significant.

The parental origin of numerical and structural chromosome anomalies has been difficult to assess for years. Acrocentric chromosome polymorphisms, heterochromatic variants, and specific genetic markers are only occasionally informative.

Current DNA techniques provide a more precise means for studying the origin of chromosomal anomalies in man and recently several studies have indicated the possible existence of genomic imprinting, a phenomenon first discovered in the mouse (see Hall¹ for a review).

The Wolf-Hirschhorn syndrome (WHS), characterised by low birth weight, developmental delay, microcephaly, hypertelorism, downward slanting palpebral fissures, prominent glabella, short philtrum, cleft lip and palate, low set, abnormal ears, and other possible abnormalities such as seizures and genital defects, results from monosomy of the distal short arm of chromosome 4.² Most cases are sporadic.

We studied the parental origin of the deleted chromosome 4 in five patients with the WHS using probes mapping in the 4p16.3 region.

Materials and methods

Sex, age, and clinical data of the five patients are shown in the table. All five patients had severely delayed growth, profound mental retardation, Greek warrior helmet face, and recurrent convulsive attacks.

Chromosome preparations were made from cultures of blood lymphocytes and high resolution banding was obtained according to Dutrillaux and Viegas-Pequignot.³

DNA was extracted directly from blood lymphocytes. For RFLP detection, 2 µg of each DNA sample was digested to completion with the appropriate restriction enzyme (*AccI*, *PstI*, *PvuII*) as recommended by the supplier (Boehringer, Mannheim). The resultant DNA fragments were resolved according to size by agarose gel electrophoresis in TAE buffer. The DNA fragments in the gel were denatured in situ with 0.2 mol/l NaOH, 0.6 mol/l NaCl. The denatured DNA fragments were transferred to a nylon filter (Hybond N-Amersham) by Southern blotting and fixed by UV.

DNA probes (pBS674E-D, 252-3, and D5 corresponding to *D4S95*, *D4S115*, and *D4S90*, respectively) were labelled with (³²P)-dCTP (Amersham) by random oligonucleotide priming⁴ and then hybridised to the filter for 20 hours at 44°C in 50% formamide, 5 × SSC, 1 × Denhardt's solution, 20 mmol/l NaH₂PO₄, 10% Na dextran sulphate, 0.1% SDS, and 100 µg/ml of salmon sperm DNA. The filters

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Table Clinical findings.

	Patients				
	1	2	3	4	5
Sex	F	F	F	F	M
Date of birth	17.7.88	9.9.82	20.1.84	27.9.83	26.5.88
Paternal age at birth	26	41	41	36	29
Maternal age at birth	22	32	39	32	28
Birth weight (g)	2450	1800	1720	2050	2520
Length (cm)	49	45	45	46	49
Age at last examination	2.2/12	8.4/12	4.9/12	7.2/12	2.2/12
Weight (kg)	6.8		7.3	13.6	7.9
Height (cm)	78	102	85	108	76
Prominent glabella	+	+	+	+	+
Hypertelorism	+	+	+	+	+
Malformed ears	+	+	+	+	+
Stabismus	+	+	+	+	+
Iris defect	+	+	+	+	+
Broad nose	+	+	+	+	+
Short philtrum	+	+	+	+	+
Carp mouth	+	+	+	+	+
Cleft lip or palate	+	+	+	+	+
Sacral sinus or dimple	+	+	+	+	+
Heart defect	+	+	+	+	+
Renal malformations	+	+	+	+	+
Hypotonia	+	+	+	+	+
Seizures	+	+	+	+	+
Mental retardation	Profound	Profound	Profound	Profound	Profound

were washed twice in $2 \times \text{SSC}$ at 44°C for 15 minutes, and once in $2 \times \text{SSC}$ at 60°C for 15 minutes before autoradiography.

Results

In patients 1, 2, 3, and 4 chromosome analysis showed a deletion of the region $4p15.3 \rightarrow 4\text{pter}$. In patient 5 the abnormal chromosome 4 was derived from a translocation with a pericentromeric region of an acrocentric chromosome as indicated by the presence of the nucleolar organiser region. DA-DAPI staining, specific for chromosome 15, was negative. The monosomy of patient 5 included the region $4p16.1 \rightarrow \text{pter}$. The parents of the patients all had normal karyotypes.

Molecular studies were performed using probes pBS674E-D, 252-3, and D5 which are localised in $4p16.3$ distal to *D4S10*.⁵

RFLP analysis of pBS674E-D (*D4S95*), hybridised with a filter of genomic DNA digested by *AccI*, showed the paternal origin of the deleted chromosome 4 in patients 2 and 5 (fig 1).

RFLP analysis of 252-3 (*D4S115*), hybridised with a filter of genomic DNA

digested by *PstI*, showed the paternal origin of the deleted chromosome 4 in patients 1, 2, 3, and 4 (fig 2).

The paternal origin of the deleted chromosome 4 in patients 3 and 4 was confirmed by the RFLP analysis of D5 (*D4S90*), hybridised with genomic DNA digested by *PvuII* (fig 3).

Discussion

All our patients showed a de novo terminal deletion of the short arm of chromosome 4 including the region $4p15.3 \rightarrow \text{pter}$. In patient 5 monosomy $4p16.1 \rightarrow \text{pter}$ was the result of a de novo unbalanced translocation with an unidentified acrocentric chromosome. Our results are in agreement with previous reports suggesting that monosomy $4p16$ is sufficient for the full expression of the Wolf-Hirschhorn syndrome,⁶ whose locus should be distal to *D4S10*.⁷ The paternal origin of the abnormal chromosome has been shown for all of our five patients using probes mapping in the deleted region.

Quarrell *et al*⁸ reported seven WHS patients with de novo deletions or rearrangements of the short arm of chromosome 4 showing paternal origin of the abnormal chromosome. Pooling our data and those of Quarrell *et al*,⁸ the preferential paternal origin of the de novo chromosome anomaly is significant ($0.02 < p < 0.01$).

A chromosome imprinting effect may account for these results. The best known imprinting effect in man is related to the deletion of band $q11-q13$ of chromosome 15. The same deletion causes Angelman's syndrome if the deleted chromosome is of maternal origin

Figure 1 RFLP analysis of the pBS674E-D probe (*D4S95*), hybridised with a filter of genomic DNA digested by *AccI*, of patient 5 (DF), his father (F), and mother (M). The probe recognises two groups of polymorphic alleles of 7.0 and 1.5 kb and of 4.4, 4.3, 4.2, and 4.1 kb, respectively. In the proband only the maternal 1.5 kb allele is present while the paternal 7.0 kb allele is absent.

Figure 2 RFLP analysis of the 252-3 probe (*D4S115*), hybridised with a filter of genomic DNA digested by *PstI*, of patients 1 (CG), 2 (SSa), 3 (SSe), 4 (MP), and their parents (F=father, M=mother). The 252-3 probe recognises more than six alleles between 2.2 and 2.6 kb. In all four patients only the maternal allele is present.

Figure 3 RFLP analysis of probe D5 (*D4S90*), hybridised with genomic DNA digested by *PvuII*, of patients 3 (SSe) and 4 (MP) and their parents (F=father, M=mother). The probe recognises two alleles of 5.0 and 5.6 kb. Only the maternal 5.6 kb allele is present in the two patients.

and the Prader-Willi syndrome if the deleted chromosome is of paternal origin.⁹

The deletion of the paternal chromosome 4 and maternal hemizyosity for the same region would be responsible for the expression of the WHS. We can postulate that the maternal origin of the chromosomal anomaly remains undetected, being either lethal in the embryo or without phenotypic effect.

However, the observation that patients with derivative 4p monosomy owing to familial translocation always show the WHS, irrespective of the parental origin of the anomaly, is against this hypothesis. In a review of published reports, Narahara *et al*⁶ found that 13% of the 4p monosomies associated with WHS were the result of familial translocation. Among the 15 cases reported, five were of paternal and 10 of maternal origin.

It seems, therefore, that among these cases there was no selection against the deletions of the short arm of chromosome 4 of maternal origin and that the paternal chromosome 4 could be more prone to mutational events than the maternal one.

A similar discrepancy in the parental origin between familial and sporadic cases has been found in Beckwith-Wiedemann syndrome with an excess of maternal transmission in the former and paternal transmission in sporadic cases with chromosome 11p15.1 duplication.¹⁰

An imprinting phenomenon also related to chromosome 4 has been postulated to play some role in the expression of Huntington's disease (HD). The paternal transmission of the HD gene is correlated with an earlier onset and a more severe expression of the disease.¹¹ Thus, it could be suggested that the distal region of the short arm of chromosome 4 in man is likely to undergo genomic imprinting. In the mouse, the region corresponding to the

distal portion of 4p in man does not appear to be subject to imprinting.¹¹

Finally, it must be noted that the ratio between male and female WHS patients is 1:2. A distorted sex ratio has been observed in several chromosomal anomalies and is usually interpreted as resulting from differential survival in one sex. It is conceivable that the imprinting phenomenon may be responsible for the sex ratio distortion.

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