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Paternal origin of the chromosomal deletion resulting in Wolf-Hirschhorn syndrome

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Abstract

DNA samples were obtained from children with Wolf-Hirschhorn syndrome and their parents to assist with gene mapping studies of 4p16.3 (the region known to contain the Huntington's disease gene). A panel of seven families was studied, using polymorphic DNA markers, to determine the parental origin of the chromosome abnormality resulting in Wolf-Hirschhorn syndrome. All seven cases were the result of de novo deletions or rearrangements of 4p and in each case the abnormality arose on the paternal chromosome. Analysis of the 3' hypervariable regions of the α globin and mucin loci indicated that non-paternity was unlikely to be an explanation for these results. A paternal age effect was not observed. The possibilities of an environmental influence or genetic imprinting require further consideration. This report extends information regarding the preponderance of the paternal origin of de novo structural deletion syndromes.

Wolf-Hirschhorn syndrome is a rare chromosomal deletion syndrome involving the distal short arm of chromosome 4 (4p). Affected children show prenatal growth retardation and profound postnatal growth and developmental delay. There is a characteristic facial phenotype that includes microcephaly, hypertelorism, colobomata, highly arched supraorbital ridge and eyebrows, prominent glabella, short philtrum, cleft lip and palate, low set, abnormal ears, ear pits, and scalp defects. Other abnormalities include seizures, cardiac and genital defects, overlapping toes, and decreased dermal ridges.[1] Depending on the number and severity of serious malformations, the syndrome is compatible with prolonged life.

Recently, the parental origin of de novo structural abnormalities has aroused much interest. Of particular note is the observation that Prader-Willi and Angelman's syndromes, two syndromes with non-overlapping clinical features, may be associated with similar deletions of the proximal long arm of chromosome 15. Deletions associated with Prader-Willi syndrome are almost always of paternal origin,[2-6] whereas those associated with Angelman's syndrome are of maternal origin.[7-13]

DNA samples were originally obtained from children with Wolf-Hirschhorn syndrome and their parents to assist with gene mapping studies of 4p16.3; this region is known to contain the Huntington's disease gene and has been the subject of intensive investigation since 1983.[14] A panel of seven families was studied using DNA polymorphisms from the relevant region in order to determine the parental origin of the chromosomal anomaly resulting in Wolf-Hirschhorn syndrome.

Patients and methods

Venous blood samples were obtained from children with Wolf-Hirschhorn syndrome and their parents. Local ethical committee approval had been obtained and each family was provided with a full explanation of the nature of the proposed research.

Chromosome preparations, suitable for high resolution studies, were obtained using deoxycytidine release of a thymidine block to induce synchronous cell division.[15] G banding was accomplished using a modification of the method of Seabright.[16] R banding was accomplished by a method adapted from that of Perry and Wolf[17] which included the addition of BrdU (20 μg/ml) for the last five hours of culture to release a methotrexate block, the exposure of the chromosome preparations to UV light, treatment with 2×SSC at 60°C for 10 minutes, and staining with 10% Giemsa.

DNA extracted from venous blood samples was digested to completion with the appropriate restriction enzyme according to the manufacturer's instructions. The particular combinations of DNA probe, restriction

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Table 1 Details of DNA probes used in this analysis.

Locus name	Probe name	Restriction enzyme	Allele sizes (kb)
D4S10	pk082	*Hin*dIII	17·5 and 15·0 4·9 and 3·7
	pk083	*Eco*RI	14·5 and 9·0
D4S125	pYNZ32	*Pst*I	3·0–2·0*
D4S95	p674	*Acc*I	4·1–4·5*
D4S115	p252.3	*Pst*I	2·5–2·3*
D4S111	p157.2	*Pst*I	2·2–1·8*

*Multiple alleles detected at these loci; therefore, allele sizes are approximate.

enzyme, and allele size used in this analysis are given in table 1. The fragments were separated by 0·7% agarose gel electrophoresis and transferred to nylon membrane filters (Hybond N, Amersham International). The filters were prehybridised, hybridised, washed, and exposed to x ray film (Konica) by standard methods.[18] Radiolabelled DNA probes were prepared by the random primer method.[19]

The loci detected by the DNA probes and their relative positions on chromosome 4p16.3 are illustrated in fig 1. Probes from the 3′ hypervariable regions of the α globin and mucin loci were used to check paternity (DNA profiling kit RPN 90, Amersham International). These probes detect variable number tandem repeats on chromosomes 16 and 1.

Results

All seven cases of Wolf–Hirschhorn syndrome were the result of de novo deletions or rearrangements which caused the loss of distal 4p material. The karyotypes of the affected children are listed in table 1. The most probable karyotypes were reported; however, in view of the known difficulty in distinguishing interstitial deletions, terminal deletions, and small de novo unbalanced translocations from one another, alternatives are possible.

Molecular studies indicated that the deletion occurred on the paternal chromosome in each of the seven cases. The probability of obtaining this result by chance is 1 in 128 (2^7). The loci at which absence of inheritance of the paternal allele was shown for each family are listed in table 2. The aim of the experiment was to determine the parental origin of the deletion; therefore, once the parental origin could be identified clearly for a particular patient no further analysis was undertaken.

Analysis of the 3′ hypervariable regions of the α globin and mucin loci indicated that non-paternity was not likely to be an alternative explanation for these results. Fig 2 provides an example of the molecular analysis for one of the families. In our population, the probability of excluding non-paternity using the 3′ hypervariable α globin probe is 83·69% (unpublished data), which is in close agreement with that published by Collaborative Research Incorporated (87·68%). Similarly, the probability of excluding non-paternity in our population, using the probe from the mucin locus, is 23·75%. Taken together, the probability of missing non-paternity in this study is less than 5%.

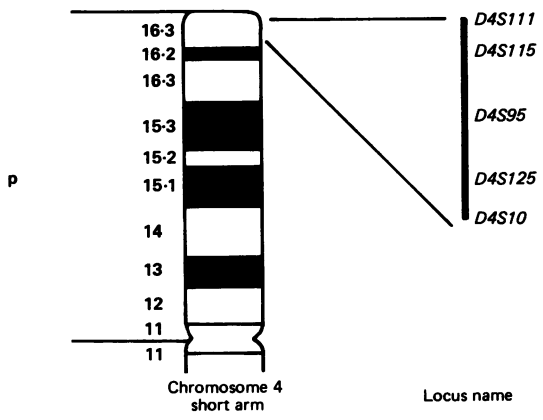

Figure 1 Ideogram of chromosome 4p which shows the order of loci detected by the DNA probes used in this study. In all cases probes detected loci from the most distal band, 4p16.3.

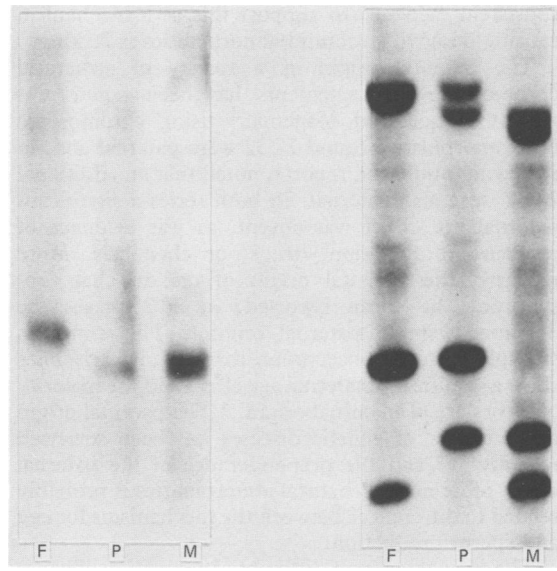

Figure 2 The left hand panel shows paternal allele loss from distal 4p in Wolf–Hirschhorn syndrome; in this case the locus D4S125 was detected by the probe pYNZ32. F=father, P=patient, M=mother. The right hand panel shows the same samples probed with the 3′ hypervariable regions of the α globin and mucin loci, confirming paternity as stated.

Table 2 Details of Wolf–Hirschhorn cases.

Case no	Paternal age (y)	Maternal age (y)	Loci which show paternal allele loss*	Karyotype
1	23	24	*D4S10, D4S95*	del(4)(p15.2)
2	18	17	*D4S125, D4S111*	t(4;?)(p15.2;?)
3	31	30	*D4S10, D4S95*	del(4)(p15.32)
4	37	31	*D4S125*	t(4;?)(p15.2;?)
5	30	29	*D4S115, D4S111*	del(4)(p15.32)
6	35	36	*D4S125*	del(4)(p16.1)
7	28	28	*D4S115*	del(4)(p15.31)
Average	28·8	27·8		

*Families were not informative with all probes.

Discussion

Our results indicate that de novo structural chromosomal abnormalities resulting in the Wolf–Hirschhorn phenotype occurred on the paternal chromosome in all seven cases studied. The parental origin of two other cases has been reported previously[20 21]; in each case the mutation occurred on the paternal chromosome. The probability that all nine mutations occurred on the paternal chromosome by chance is 1 in 512 (2^9). It is, therefore, likely that paternal chromosomes have a predisposition for this type of mutation. A paternal age effect was considered but is unlikely to be the explanation for this observation (table 2). The possibility of an environmental influence requires further consideration. There has been some evidence to suggest an increase in the occupational exposure to hydrocarbons among fathers of children with the Prader–Willi syndrome,[22] but consistent evidence to support this as a mechanism causing de novo structural abnormalities is lacking.

The parental origin of a variety of structural chromosome rearrangements has been noted previously. Olson and Magenis,[21] using chromosome heteromorphisms, found 27/32 were paternal and, in reviewing published reports, noted that an additional 28/39 were also paternal. In both series a significant paternal age effect was absent, as was evidence of exposure to radiation, drugs, or chemicals. More recently, the parental origin of cri du chat (5p) syndrome has been reported; in 20/25 cases the deletions were of paternal origin.[23] This contrasts strongly with the observation that de novo trisomies show a significant maternal age effect and the majority of errors are of maternal origin.[24] The parental origin of a number of genetic diseases has been reviewed recently[25 26] and the preponderance of the paternal origin of de novo structural abnormalities is probably related to differences between the mechanisms for egg and sperm production.

The phenomenon of differential expression of genes depending on their parental chromosome of origin, genetic imprinting, has received much attention recently.[27] Although much of the evidence favouring this as a mechanism during development comes from animal experiments, a clear example in humans is the hydatidiform mole, which results from a diploid paternal set of chromosomes. Imprinting is considered to be a possible explanation for the observation that children with Prader–Willi and Angelman's syndromes may have similar chromosomal deletions, but the particular phenotype depends on which parental set of genes is lost, usually paternal in the case of Prader-Willi syndrome and usually maternal in the case of Angelman's syndrome.[7 12 13]

It has been suggested that the distal part of chromosome 4p could be imprinted and thereby explain the phenomenon that patients with the severe juvenile form of Huntington's disease inherit the disease from their fathers.[28] It would be tempting to consider imprinting as a mechanism to explain the paternal origin of Wolf–Hirschhorn syndrome; this would imply that mutations of the maternal chromosome resulted in failure of embryonic development or fetal loss, a hypothesis which currently cannot be tested. Against imprinting being an explanation for the current observation is the fact that female carriers of balanced translocations involving 4p may produce chromosomally unbalanced offspring who have facial characteristics indistinguishable from de novo cases of Wolf–Hirschhorn syndrome.[29]

This report extends the documentation of the preponderance of paternal origin of particular chromosomal deletion syndromes. Additional studies of the parental origin of this and other deletion syndromes should provide valuable information on the mutational mechanisms underlying abnormalities of specific chromosomal regions.

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