Duplication of 8p23.1: a cytogenetic anomaly with no established clinical significance

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Abstract

We present seven families with a cytogenetic duplication of the short arm of chromosome 8 at band 8p23.1. The duplication has been transmitted from parents to offspring in four of the seven families.

In three families, the source of the extra material and its euchromatic origin were established using FISH with a YAC which was mapped to 8p23.1 and a whole chromosome paint for chromosome 8. FISH signals from this YAC were significantly larger on the duplicated chromosome compared with the normal chromosome in all six family members tested. Comparative genomic hybridisation (CGH) on a representative subject was consistent with these results.

The families were ascertained for a variety of mostly incidental reasons including prenatal diagnosis for advanced maternal age. The transmission of this duplication by multiple phenotypically normal family members with no history of reproductive loss suggests the existence of a novel class of 8p23.1 duplications, which can be regarded as euchromatic variants or duplications with no phenotypic effect.
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Keywords: duplication; chromosome 8p23.1; normal phenotype; miscarriages

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In recent years the resolution of diagnostic cytogenetic analysis has increased and complementary molecular cytogenetic and molecular genetic techniques have been widely applied to characterise cytogenetic abnormalities. This has led to the ascertainment of an increasing number of families with euchromatic imbalances at the cytogenetic level, which are not consistently associated with any clinical or reproductive effects. Many of these deletions,[1–10] duplications,[11–14] and unbalanced translocations[15–20] are unique, but others occur in multiple families or unrelated subjects. Among these are the interstitial duplications of the proximal long arm of chromosome 15 which do not contain the Prader Willi/Angelman critical region (PWACR)[21 22] and the so-called euchromatic variants in which extra euchromatin is present within the long arm heterochromatin of chromosome 9[23] or adjacent to the short arm centromeric heterochromatin of chromosomes 9[24] or 16.[25]

In this paper we present seven examples of cytogenetically consistent duplications of the short arm of chromosome 8 which may represent another class of euchromatic variant or duplication without clinical significance.

Methods

Chromosomes were prepared by standard techniques after semi-synchronisation with FdU and release with thymidine.[26] Fluorescence in situ hybridisation (FISH) was carried out using modifications of the method of Pinkel *et al.*[27] Chromosomes were counterstained with DAPI and viewed through a Zeiss Axioskop microscope. Images were captured, enhanced, and analysed using a Photometrics cooled CCD camera and the Smart Capture Extensions for QUIPS software package (Vysis). A minimum of five cells was examined and findings corroborated by an independent observer.

Comparative genomic hybridisation (CGH) was performed using a protocol modified from that of Kallioniemi *et al.*[28] Genomic DNA was salt extracted[29] and directly labelled by nick translation with fluorescein-12-dUTP and Texas Red-5-UTP (Dupont) for test and reference DNA respectively. A total of 600 ng of labelled DNA from each of the test and reference sources was used for each of the hybridisation mixtures which were denatured at 72°C for eight minutes and applied to normal male target metaphase slides (Vysis) according to the manufacturer's instructions. Following cohybridisation for three days at 37°C, the slides were washed, counterstained, and inspected under a Zeiss Axioskop fluorescence microscope. Images were captured with the same cooled CCD camera used for conventional FISH and enhanced and analysed using Quips CGH software (Vysis). Each metaphase used in the analysis was karyotyped and green to red fluorescence intensity ratios along the length of each chromosome were calculated. The data from five to 10 metaphases were combined to give a mean ratio profile for each chromosome.

Results

VALIDATION OF YAC HTY3020

YAC HTY3020 was hybridised to a known complex rearrangement of chromosome 8 (inv dup del(8)(:p11.2→p23.1::p23.1→qter).[30 31] The abnormal chromosome has a deletion of the distal short arm of chromosome 8 (p23.1→pter) and a duplication of most of the medial short arm (p11.22→p23.1). This inverted duplication is consistent with those described by Floridia *et al*[32] in which the

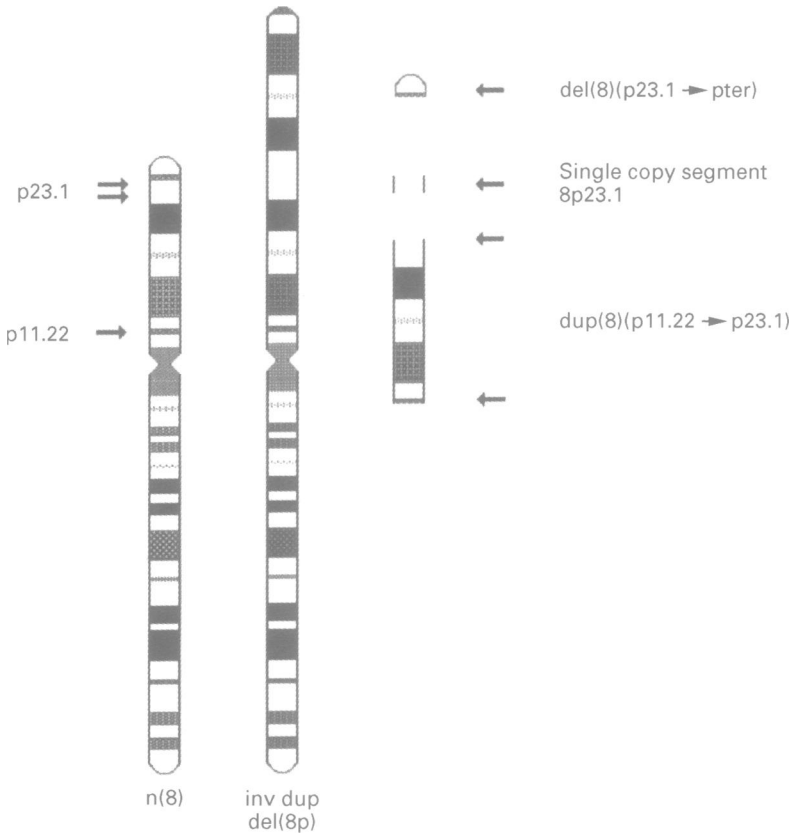

Figure 1 Idiogram of the complex inverted duplication and deletion of chromosome 8 used to map YAC HTY3020. Note the central single copy segment of 8p23.1.

duplicated segment is separated from its original location by a short single copy segment of band p23.1 (fig 1). YAC HTY3020 hybridised to the middle of the short arm of the duplicated chromosome with no evidence of more than one signal or of increased signal strength (fig 2). This excludes HTY3020 from the distal short arm (p23.1→pter) and strongly suggests that the YAC maps to the short single copy segment between the duplicated segments and, therefore, to band 8p23.1.

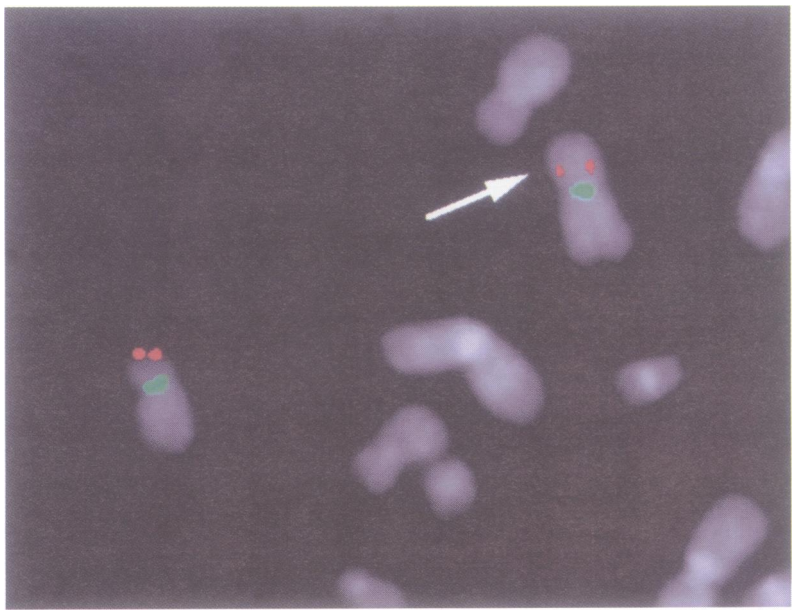

Figure 2 Hybridisation of YAC HTY3020 (red signals) to the central short arm of the inv dup del(8) chromosome (white arrow). Note the similar signal strength on both the rearranged and normal chromosomes 8.

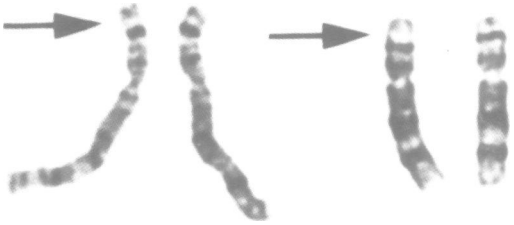

Figure 3 Partial karyotypes from the proband in family 1 (II.8). Note the consistent increase in G light material in the 8p23.1 region at different levels of resolution. The duplicated chromosome is on the left of each pair and the additional material is indicated by the arrows.

This YAC has not, to our knowledge, been mapped using other means and does not yet have a GDB accession number. It is not known whether it contains coding sequences.

Family reports

FAMILY 1

A cytogenetic duplication of chromosome 8 (dup(8)(p23.1p23.1)) (fig 3) was found in the 39 year old male partner (II.8, fig 4) of a couple referred because of four miscarriages (gestational ages not known). His wife (II.9) was aged 38 and they had also had two liveborn children. Four of his five available sibs (II.1, 3, 7,11) had the same duplication. One of the four had no partner, but the other three had six children and one miscarriage between them. Their mother (I.2) was also a duplication carrier (data not shown). She had no history of miscarriages. In metaphases from the proband the additional G light material was C band negative and a whole chromosome paint (Cambio) hybridised along the length of the duplicated chromosome (data not shown). In metaphases from the proband and his oldest brother, the YAC HTY3020 showed a significant contrast in signal strength between anomalous and normal chromosomes 8 consistent with duplication or amplification of the sequences detected (fig 5). In metaphases from the mother, the duplication could be identified with certainty in only 5/30 G banded cells from a suboptimal preparation. FISH with HTY3020, however, showed a consistent contrast in signal intensity between the chromosomes 8 in each of the 24 metaphases available and we concluded that she was a non-mosaic carrier. CGH using DNA from the proband (II.8) showed an excess of green signal in the 8p23.1 region resulting in a peak (fig 6) not seen in a normal control preparation consisting of cohybridised DNA from two normal subjects. This indicates a gain of material from this region. It is not known whether the duplicated 8 or some derivative of it was present in any of the miscarried fetuses. A cell line from the proband (II.8) is available from this laboratory under reference number 9602986.

FAMILY 2

Amniocentesis of both twins of a dichorionic diamniotic pregnancy was carried out because the mother was 40 years of age (I.2, fig 4). Twin 1 had an apparently normal female karyotype. In twin 2, 6/35 cells from one of three cultures had two independent balanced translocations

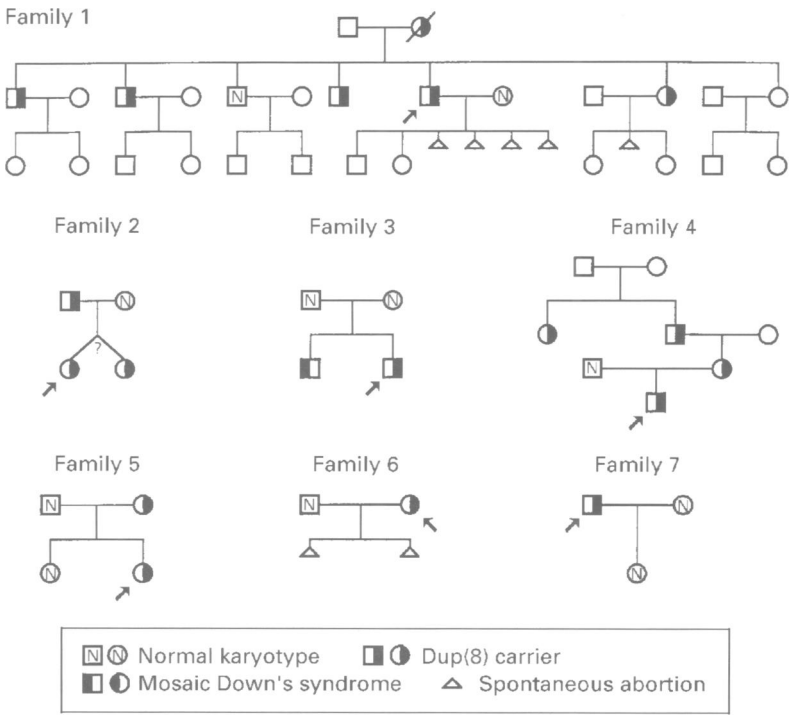

Figure 4 Pedigrees of the seven families numbered in the same order as in the text. Duplication 8 carriers have symbols shaded on the right, an N denotes subjects with normal chromosomes, and those with empty symbols have not been karyotyped. The symbol shaded on the left for II.1 in family 3 indicates mosaic trisomy 21.

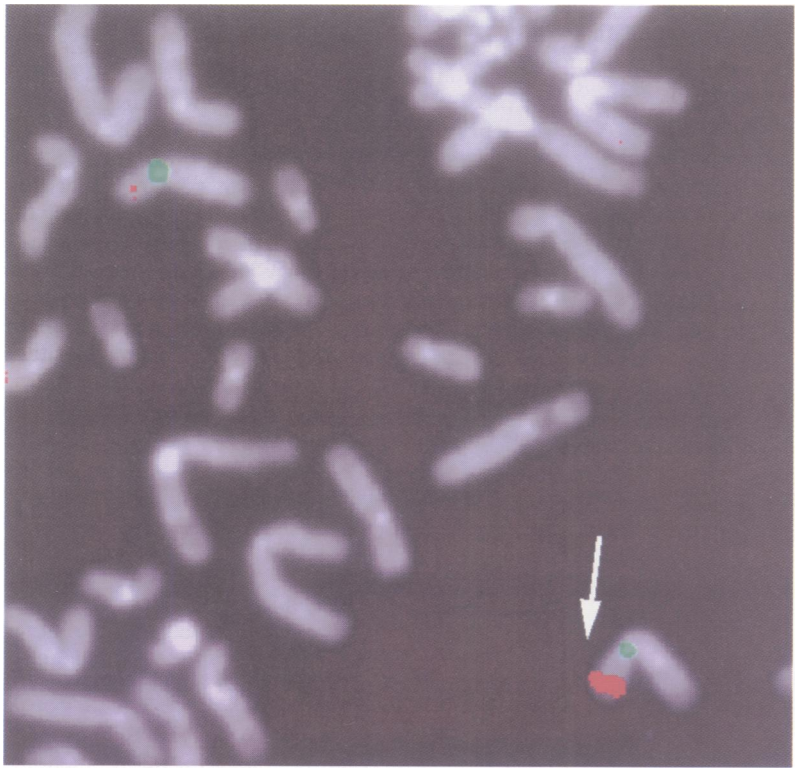

Figure 5 Hybridisation of YAC HTY3020 (red signals) to chromosomes from II.8 in family 1. Note the increased signal strength on the chromosome indicated by the white arrow. D8Z2 was used to highlight the chromosome 8 centromere (green signals).

(t(1;4) and t(5;18)), but the remaining 29 cells were apparently normal female, as were 30 cells from each of two further cultures. At delivery, a cord blood sample from twin 2 showed no evidence of the translocations seen at amniocentesis but a duplication of 8p23.1 was observed. Twin 1 and her father had the same 8p23.1 duplication, while the mother had a normal karyotype. Both twins are developmentally

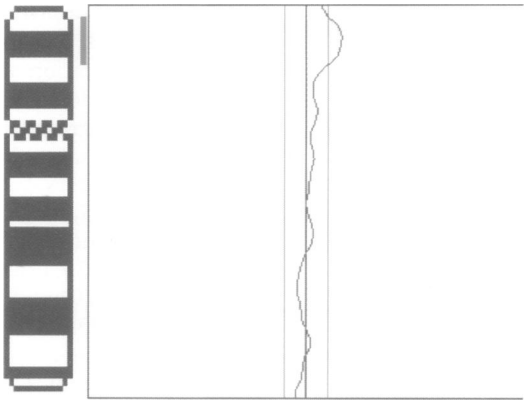

Figure 6 CGH showing the average ratio profile for chromosome 8 in patient II.8 from family 1. The vertical midline represents a green:red ratio of 1:1, the red line to the left a ratio of 0.9:1, and the green line to the right a ratio of 1:1.1. Deviation of the profile beyond the green line is consistent with a gain of material from this region in the test DNA.

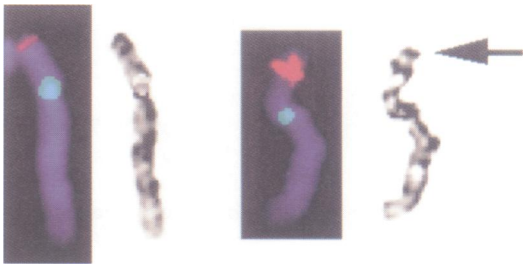

Figure 7 Partial karyotype of the father (I.1) from family 2 showing hybridisation of YAC HTY3020 (red signals) and D8Z2 (green signals) to the normal (left hand pair) and duplicated chromosomes 8 (right hand pair). The right hand chromosome of each pair shows the computer enhanced G banding derived from the DAPI counterstain of the same chromosomes used for FISH. Note the increased signal strength on the right hand FISH image corresponding to the right hand chromosome with additional G light material.

normal at 6 months of age. FISH with the HTY3020 on metaphases from the father (fig 7) and one of the twins was consistent with the results in family 1. The father had dyslexia but was developmentally normal and university educated. There is no history of learning difficulties or pregnancy loss in this family. Further family screening was not pursued.

FAMILY 3

A 31 year old, gravida 2, para 1 woman had an amniocentesis because of a previous liveborn child with mosaic Down's syndrome (II.1, fig 4). A duplication of band 8p23.1 was detected (fig 8). FISH with a whole chromosome 8 paint and hybridisation with HTY3020 were consistent with the results in families 1 and 2 (fig 9). Parental karyotypes were normal and molecular analysis using a β actin polymorphism on chromosome 6 showed no discrepant paternity (data not shown). The couple decided to continue the pregnancy which is ongoing. Detailed ultrasound scan at 19 weeks' gestation did not show any abnormality.

FAMILIES 4–7

All four of these families were ascertained before families 1 to 3 and before appropriate FISH techniques were available.

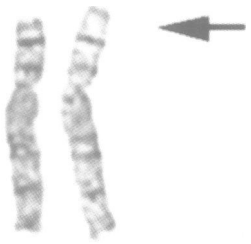

Figure 8 Partial karyotype of chromosomes 8 from the proband in family 3. The arrow indicates the extra material in the 8p23.1 region of the right hand chromosome.

In family 4, a primagravida (III.2, fig 4) had amniocentesis for a maternal age of 38. An 8p23.1 duplication was detected (fig 10) and subsequently identified in the mother, grandfather, and great aunt. No phenotypic or reproductive anomalies have been recorded in either the carriers or the neonate at term.

In family 5, a girl of 9 (II.2, fig 4) was referred because her height was on the 3rd centile. An 8p23.1 duplication was found in the proband (fig 10) and her mother, whose height was on the 25th centile, but not in her elder daughter whose height was under the 3rd centile at the age of 14. The father had normal chromosomes and was of average height (50th centile). No dysmorphic features were found in any family members. A family history of premature puberty or menarche did not cosegregate with the duplication.

In family 6, an 8p23.1 duplication was found in a female of 29 (I.2, fig 4) referred following two spontaneous 10 week miscarriages (fig 10). The duplicated region was C band and NOR band negative, Q band light, and not methyl-green/DAPI bright (data not shown). Her partner was 28 years of age and had a normal karyotype.

In family 7, a pregnant 26 year old female (I.2, fig 4) was referred because her cousin had Down's syndrome. Her karyotype was normal but a duplication of 8p23.1 was found in her partner (fig 10). The couple opted for amniocentesis because of the duplication and a normal female karyotype was found in the fetus. Delivery was premature and no abnormalities were recorded.

Recall of all families 4-7 for further tests has not yet been successful and no cell lines are available.

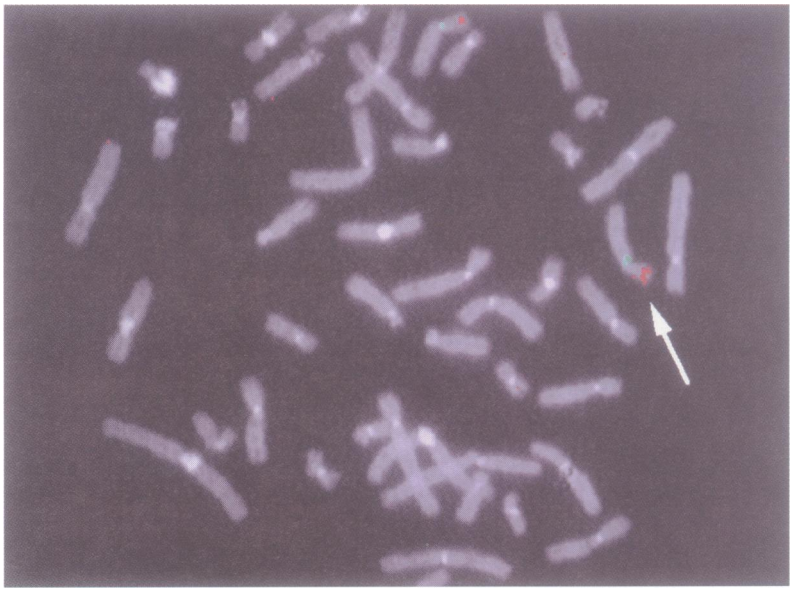

Figure 9 Hybridisation of YAC HTY 3020 and D8Z2 to chromosomes from the proband of family 3. Note the increased signal strength on the chromosome indicated by the white arrow.

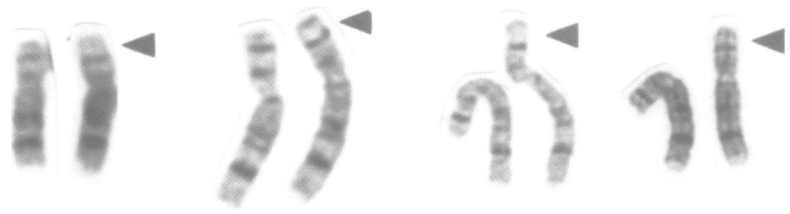

Figure 10 G banded partial karyotypes of members of family 4 (left hand pair), family 5 (first pair to the right), family 6 (second pair to the right), and family 7 (right hand pair).

Discussion

We have provided details of seven families in which extra G light material detectable at the cytogenetic level was found within the short arm of chromosome 8 at band p23.1. C banding, whole chromosome painting, and FISH with a YAC mapped to this band are consistent with a euchromatic duplication of band p23.1 itself. A fine G dark band is seen at the centre of the enlarged 8p23.1 band, which resembles the fine band seen within 8p23.1 in normal high resolution chromosomes 8, but differs in that the contrast between the duplicated and normal homologues is consistently found in both high and low resolution cells (fig 3). This band is not recognised by ISCN[33] but is described at the 1250 band level as 8p23.12.[34] Its location at the centre of the enlarged 8p23.1 (fig 11) implies that the duplication is either a direct duplication of 8p23.11 or an inverted duplication of 8p23.11 to 8p23.13. The striking increase in signal strength of YAC HTY3020 on each anomalous chromosome 8 suggests that the extra material could also be a limited amplification of a smaller part of band 8p23.1 for which this YAC is specific.

In an attempt to substantiate the possibility of an amplification, CGH was used on one representative subject and showed a clear gain of material from distal 8p. However, if band 8p23.1 represents 0.22% of haploid autosomal genome length,[35] then the maximum size of the duplication can be crudely estimated at 6.6 Mb, which is close to published estimates of the limits of resolution of the CGH technique.[28] For this reason, the extent of the gain detected could have resulted from either a duplication of the whole 8p23.1 band or an amplification of a smaller segment which would not normally be detected by CGH in the absence of multiple extra copies. This result was, nevertheless, important as the method involves the simultaneous cohybridisation of equimolar amounts of patient and control DNA to normal metaphase chromosomes. This gain of material is, therefore, likely to represent additional euchromatic DNA extracted from the region of interest on chromosome 8 rather than a heritable alteration of chromosome conformation.

As far as we are aware, all previous duplications confined to 8p23.1, including the present families, have been reported in abstract form only.[31 36-38] The available details of these 12 families are listed in table 1. In 10 of these 12 families and 25 out of 27 duplication carriers, no phenotypic abnormality has been recorded although the phenotype of the eight patients ascertained at prenatal diagnosis has only been reported as normal at six months (two cases), at term (three cases), or sonographically normal in utero (three cases). In one of the other two families, a de novo duplication was found in a boy of 18 months referred for developmental

Table 1 Families and subjects with duplications of 8p23.1

Reason for referral	*Sex of proband*	*Age*	*Inheritance*	*No of carriers*	*Reference*
Prenatal diagnosis	—	—	Paternal	2	Krasikov *et al*[37]
Prenatal diagnosis	—	—	Paternal	2	Krasikov *et al*[37]
Prenatal diagnosis	—	—	Maternal	2	Krasikov *et al*[37]
Prenatal diagnosis (increased serum screened risk of Down's syndrome)	—	—	Paternal	2	Williams *et al*,[38] case 1
Developmental delay	Male	18/12	De novo	1	Williams *et al*,[38] case 2
Spontaneous miscarriages × 4	Male	38	Maternal × 4	6	Present family 1
Follow up of prenatal diagnosis	Female	6/12	Paternal	3	Present family 2
Prenatal diagnosis (previous child with mosaic Down's syndrome)	Male	—	De novo	1	Present family 3
Prenatal diagnosis (advanced maternal age)	—	—	Maternal and grandpaternal	4	Present family 4
Short stature	Female	9	Maternal	2	Present family 5
Spontaneous miscarriages × 2	Female	29	Not determined	1	Present family 6
Family history of Down's syndrome in partner	Male	27	Not determined	1	Present family 7

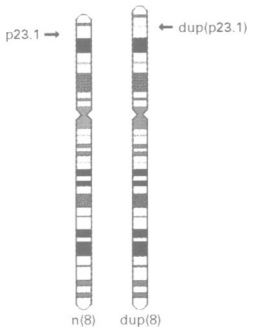

Figure 11 Idiogram of the duplication of 8p found in the subjects of this report. Note the fine G dark band interpreted as 8p23.12 at the centre of an enlarged G light 8p23.1 band.

delay which was thought to be "spontaneously resolving" by the age of 2 years.[38] The relationship between the duplication and his developmental delay remains speculative. In the second of the other two families (present family 5), a 9 year old girl referred for short stature had inherited the 8p23.1 duplication from her mother whose height was on the 25th centile. Her 14 year old sister was, however, just as short for her age and had not inherited the duplication. This suggests that the short stature and the duplication are likely to be coincidental findings in this family.

Two of the 10 families without phenotypic abnormalities were referred for spontaneous miscarriages. In the present family 1, a 39 year old woman and her 38 year old carrier partner had four miscarriages and two liveborn children and, in family 6, a 29 year old female had two 10 week miscarriages and no liveborns. At the same time, however, four of the male partner's five tested sibs in family 1 were also duplication carriers and three of these four had six children and only one miscarriage between them. It seems unlikely that duplications of this kind are the direct cause of spontaneous miscarriages when most liveborn carriers have no detectable phenotypic abnormality and the two exceptional patients have only transient developmental delay or short stature. In addition, the duplicated material is likely to form an unpaired duplication loop at meiosis in which recombination will not occur and recombinant products are unlikely to be formed.[39] None of the spontaneously miscarried fetuses have, however, been examined cytogenetically.

The duplication has been stably transmitted in eight of these families, with paternal transmission in four, maternal transmission in three, and transmission from carriers of both sex in the present family 4. This makes it unlikely that parent specific imprinting accounts for the absence of phenotypic effect in normal carriers.[13] The duplication has arisen de novo in two cases (Williams *et al*[38] and the present family 3) and the parents of duplication carriers have not been examined in two families (present families 6 and 7).

In the present series, the combination of G banding and FISH provides evidence of a consistent anomaly in each family and subject tested. Provided future cases can be clearly differentiated from other imbalances of 8p, it should be possible to treat them as clinically innocuous rearrangements which do not warrant family follow up or the offer of prenatal cytogenetic analysis to carriers. In the meantime, if an 8p23.1 duplication is detected for the first time during prenatal chromosome analysis, it remains prudent to request chromosomes from both parents and sibs so that the familial pattern of inheritance from normal carriers can be established as quickly as possible. While it is not yet possible to assume that an apparently de novo duplication would be equally free of phenotypic consequences,[40] the existence of other families in which a cytogenetically identical rearrangement has no phenotypic effect suggests that any increased risk is small.

In conclusion, the families reported here suggest that duplication of 8p23.1 is a cytogenetic anomaly of no established significance. It seems likely that the miscarriages and mild phenotypic effects associated with the duplication in a minority of subjects represent bias of ascertainment. It is, however, important that further examples are published in order to substantiate this suggestion and to provide evidence with which to reassure future families ascertained with the same anomaly.

Note added in proof

A physical examination at 11 weeks of age confirmed a normal outcome of pregnancy in family 3.

We are particularly grateful to all the members of family 1 who contributed samples and to C E Browne, T M Hutchings, K J Moore, and N D Wyre for karyotyping additional family members. We thank Dr A Jauch for supplying and Dr H Donis-Keller for generating YAC HTY3020. We acknowledge Dr J Whittaker for the molecular analysis of the β actin locus in family 3 and are grateful to Dr P G Johnston for providing additional details on family 5. JCN is supported by the Wessex Cancer Trust and the Parthenon Trust. The Digital Scientific (VYSIS) Image Enhancement equipment was provided by the Wellcome Foundation. We are grateful to Judy Gladding for preparation of the manuscript.

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