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.

Keywords: duplication, chromosome 8p23.1, normal phenotype, miscarriages

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 translocations15-18 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)22 and the so-called euchromatic variants in which extra euchromatin is present within the long arm heterochromatin of chromosome 923 or adjacent to the short arm centromeric heterochromatin of chromosomes 924 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 Kallioniem et al.28 Genomic DNA was salt extracted29 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→qter) 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 al32 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.

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 reports

Family 1

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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...
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Figure 1. Figure D8Z2 arrow.

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 chromosome 8 from 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).

Figure 6. CGH showing the average ratio profile for chromosome 8 in patient II.8 from family 1. The vertical midpoint 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. 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 (1.1) 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.
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 methylgreen/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.

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 but is described at the 1250 band level as 8p23.12.

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, 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. 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. 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
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Table 1  Families and subjects with duplication of 8p23.1

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

delay which was thought to be "spontaneously resolving" by the age of 2 years. The relationship between the duplication and its 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 recombiant products are unlikely to be formed. 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. The duplication has arisen de novo in two cases (Williams et al. 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, 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.

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