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ARTICLE

Unbalanced segregation of a complex four-break 5q23–31 insertion in the 5p13 band in a malformed child

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A rec(5)dup(5)(q23.2q31.3) inherited from a maternal ins(5)(p13.1q23.2q31.3) was detected in a 4-month-old male child who showed hypotonia, microcephaly, cardiac defects, pulmonary hypoplasia and stenosis, bilateral hydronephrosis, hydrocele, testicular hypoplasia and phimosis. Dysmorphisms were also observed. We compare the clinical characteristics of our patient with those of the previously reported dup5q cases in an attempt to define the phenotype–karyotype correlation. The maternal insertion responsible for the duplicated 5q23.2–31.3 region in the child was characterized in detail by FISH analysis, which identified a complex rearrangement involving four breakpoints (bkp's): a 5q segment excised following breakage at 5q23.2 and 5q31.3 became inverted and inserted at 5p13.1, probably coincidentally with an internal breakage at 5q23.3 causing a 180° rotation of the two subsegments. The mother's karyotype was consequently defined as 46,XX, ins(5)(pter→p13.1::q23.3→q23.2::q31.3→q23.3::p13.1→q23.2::q31.3→qter). There are clusters of Alu sequences in the genomic clones spanning all the four bkp's, suggesting their possible involvement in the rearrangement. No clinical phenotype was associated with this balanced rearrangement in the mother and a number of other carriers in the same family.
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Introduction

It has been estimated that chromosomal rearrangements involving three breaks, such as insertions, are relatively rare: 1/5000 live births.[1] A single crossover between any of the three breakpoints (bkp's) leads to unbalanced recombinants, with duplications or deletions of a chromosomal segment.

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Only a few descriptions of intrachromosomal insertions have so far been published (see review by Madan and Menko[2]).

We describe here a male child with a recombinant chromosome 5, resolved by FISH analyses to have a 5q23.2–31.3 duplication, inherited from his phenotypically normal mother who carries, in accordance with the term proposed by Madam and Menko,[2] a balanced 'pericentric' insertion of these bands in 5p13.1.

Attempts to establish the phenotype–karyotype correlation of partial trisomy 5q were hampered by the different lengths of the 5q duplications and their occurrence with chromosomal monosomies, as a result of malsegregation from a balanced translocation carrier.[3,4] There are only two

published cases with an apparently pure 5q duplication similar in size to the trisomic segment found in our patient.[5,6]

We report here the refined FISH characterization of the 5q duplication affecting our patient with the aim of defining a genotype–phenotype correlation. We also describe the detailed FISH analysis of the maternal rearrangement, which revealed an additional bkp within the inserted segment leading to further rearrangement within the insertion, and speculate on the possible genomic motifs priming this rearrangement.

Clinical report

The proband is a male child born by Cesarean section after 38 weeks of gestation with a birth weight of 2390 g and a length of 49 cm. His 1- and 5-min Apgar scores were 8 and 9, respectively. The pregnancy of his mother was complicated by oligohydramnios, cerebral vasodilatation and intrauterine growth retardation after eight gestational months.

A clinical examination performed when the child was 4 months old showed that he had a weight of 4500 g (<3rd centile), a height of 59.5 cm (<10th centile) and a head circumference of 36.5 cm (<5th centile). He exhibited hypotonia, microcephaly, hypertelorism, cranial asymmetry, a prominent occiput, low-set ears, retrognathia, a hypoplastic mandible, a club foot and partial syndactyly of the second and third toes of both feet. His cardiac defects included ventricular hypertrophy and hyperkinesias. Pulmonary hypoplasia and stenosis, bilateral hydronephrosis, hydrocele, testicular hypoplasia and phimosis were also observed.

Cytogenetic and FISH studies

A conventional karyotype performed on the proband's lymphocytes revealed a structurally abnormal chromosome 5, with additional material on 5p (not shown). The paternal karyotype was normal, but the mother carried an

Table 1 Representative YAC FISH results on ins(5) and rec(5) chromosomes

	YAC (y) code	*YAC name*	*Contigs*	*Localization*	*FISH signal*	
					ins(5)	*rec(5)*
	1	889B10	WC5.4	5p13.1	st	st
X	2	822F1	WC5.4	5p13.1	sp	sp
	3	806G10	WC5.4	5p13.1	sp	sp
	4	896E5	WC5.4	5p13.1	st	st
	5	965D11	WC5.9	5q23.1	st	st
A	6	933G11	WC5.9	5q23.1–23.2	sp	st at 5q and dim mv at 5p13
	7	844D4	WC5.9	5q23.2	sp	st at 5q and dim mv at 5p13
	8	772F7	WC5.9	5q23.2	mv	++
	9	905E9	WC5.9	5q23.2	mv	++
	10	764G6	WC5.9	5q23.2	mv	++
B	11	790E12	WC5.9	5q23.2	mv	++
	12	814A3	WC5.9	5q23.2–q23.3	mv	++
	13	852F2	WC5.9	5q23.3	mv	++
	14	779B12	WC5.9	5q23.3	mv	++
	15	808H6	WC5.9	5q23.3	mv	++
C	16	952D12	WC5.9	5q23.3	mvx2	++x2
	17	959A6	WC5.9	5q23.3	mvx2	++x2
	18	935H9	WC5.9	5q23.3	mvx2	++x2
D	19	796B12	WC5.10	5q31.1	mv	++
	20	752F7	WC5.10	5q31.1	mv	++
	21	745F8	WC5.10	5q31.1	mv	++
	22	737B9	WC5.10	5q31.1	mv	++
	23	857H10	WC5.10	5q31.2	mv	++
	24	802D5	WC5.10	5q31.2	mv	++
	25	941H2	WC5.11	5q31.3	mv	++
E	26	762F5	WC5.11	5q31.3	sp	st at 5q and dim mv at 5p13
	27	769G6	WC5.11	5q31.3	st	st
	28	844D6	WC5.11	5q31.3	st	st

st, stationary signal (signal remaining at native location); sp, split signal; dim, diminished signal intensity; mv, moved signal; ++, duplicated signal; x2, two signals seen. All the used YACs have insert sizes ranging from 0.5 to 1.5 Mb.

intrachromosomal insertion in one chromosome 5 (not shown). By means of conventional cytogenetics, the abnormal chromosome was interpreted as an ins(5)(p13q22q23) and, consequently, the proband's karyotype as 46,XY, rec(5)dup(5)(q22q23)ins(5)(p13q22q23)-mat.

FISH studies of the proband and mother using the chromosome 5-specific library (ONCOR) and the D5S23 5p15.2-specific probe (ONCOR) indicated that the 5p+ consists entirely of chromosome 5 sequences, and that the cri du chat region (recognized by the D5S23 probe) is not involved in the rearrangement (data not shown). In order to define the extent and bkp's of the insertion, FISH experiments were performed using YAC clones selected from the Genome Database (http://www.genome.wi.mit.edu) and anchored to the WC5.0, WC5.3, WC5.4, WC5.5, WC5.6 and WC5.9 contigs. The probes were labelled with biotin or digoxigenin (Roche, Basel, Switzerland) by means of nick translation. Significant results were obtained with the YACs belonging to the WC5.4 (numbered from 1 to 4), WC5.9 (numbered from 5 to 18), WC5.10 (numbered from 19 to 24) and WC5.11 (numbered from 25 to 28) contigs. Table 1 summarizes the results obtained on the mother's ins(5) and child's rec(5) chromosomes. As reported in Table 1 and illustrated in Figure 1a and d, the 5p bkp was mapped to p13.1 band, by using y2 and y3. YACs 6 and 7 allowed us to establish the proximal 5q bkp, q23.2, as shown in Figure 1b and e. The distal 5q bkp, q31.3, was mapped by FISH of y 26 (Table 1). All YACs included between 6 and 26 gave atypical 5p location signals on ins(5) and two signals (one at the native 5q location and the other at 5p) on rec(5) (Table 1). The only exception was for the overlapping YACs 16, 17 and 18, mapping to 5q23.3, which showed two distinguishable signals at the 5p13.1 insertion site (Table1 and Figures 1c and f). The fact that this hybridization pattern was not detected on the normal five homologues suggested breakage of the target region. So, we performed six additional dual color FISH experiments using different combinations of selected 5q YACs (letters A–E in Table 1) with the aim of establishing the orientation of the inserted chromosomal segment. As shown in Figure 2, we found that an additional break had occurred within the excised 5q segment in the 5q23.3 region spanned by the overlapping YACs 16, 17 and 18. This was followed by a rotation of each of the two resulting segments by 180°: the segment between YACs 6 and 16 and

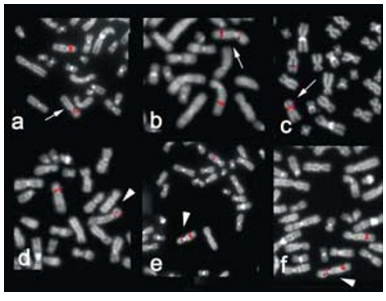

Figure 1 FISH YAC mapping of 5p and 5q bkp's in mother (top) and child (bottom). See Table 1 for YAC names and chromosomal location. The arrow points to ins(5) chromosome and the arrowhead indicates rec(5) chromosome. Mother's and child's abnormal chromosome 5 showed split signals with y2 (**a** and **b**). y6 revealed a split signal at 5q23 and 5p on ins(5) (**b**), but, together with the expected stationary signal at 5q33, a diminished signal at 5p on rec(5) (**e**). y16 showed two distinguishable signals at the 5p13.1 insertion site (**c** and **f**).

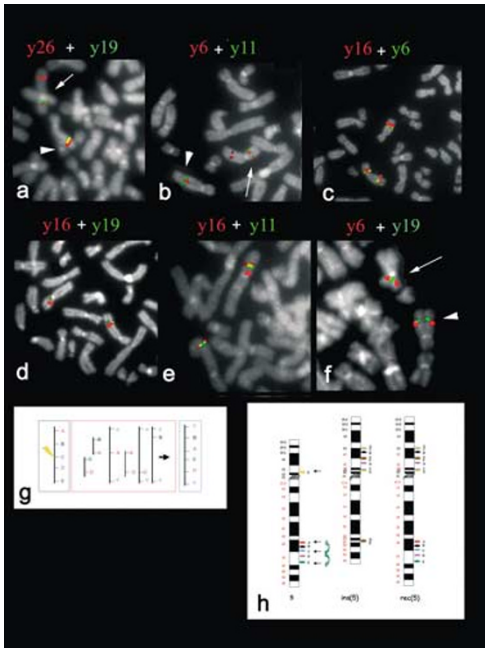

Figure 2 Dual colour FISH of selected 5q YACs. Maternal metaphases are shown in all cases with the exception of (**c**), which shows a metaphase of the proband. YAC codes are written with the color of the given signal at the top of each photograph. YACs show: an inverted signal location on the 5p inserted segment (arrowed) in comparison with the native 5q location (arrowhead) (**a** and **b**); red signals at the insertion end points with a green signal in the middle (**c**); red signals at both insertion end points and an interstitial green signal close to the centromeric (**d**) and the telomeric (**e**) insertion end point; signals ordered in 5p (arrowed) as on 5q (arrowhead) (**f**). The above six dual FISH experiments are also illustrated in the middle photograph of (**g**). The relative cen-tel location of the used YACs is shown in the left photograph, with the arrow pointing to the 5q bkp, and their order on ins(5) chromosome is shown in the right photograph. The ideograms of the ins(5) and rec(5) chromosomes as compared to that of normal 5 chromosome are shown in (**h**). Numbering of bands is in black for 5p and in red for 5q. The arrows point to 5p and 5q bkp's: the colored ovals indicate the crucial YACs spanning the bkp's and giving split signals (half ovals). For YAC designation by letters and YAC codes see Table 1.

Table 2 Clinical characteristics of reported patients with 5q22–q35 duplication

	NJ Martin et al[5]	*D Martin et al*[6]	*This report*
Age	4 months	2 days	4 months
Sex	M	M	M
Birth weight (g)	2290	2295	2390
Birth length (cm)	47.5	44	49
Microcephaly	+	–	+
Psychomotor retardation	+	Not evaluable	+
Growth delay	+	–	+
Eyes	Strabismus	Hypertelorism	Hypertelorism
Nose	Bulbous	Broad nasal root	–
Ears	Low-set ears	Normal	Low-set ears
Feet	Partial syndactyly of second and third toes of both feet. Rocker-bottom feet		Partial syndactyly of second and third toes of both feet. Club foot
Hypotonia	?	?	+
Heart defect	Nonstructural heart lesion	+ (B IAA*)	Ventricular hypertrophy and hyperkinesia
Duplicated region	q22–q33	q31.1–q35.1	q23.2–q31.3

*Type B interrupted aortic arch
? not mentioned.

26, and the segment between YACs 16 and 26. The diagram in Figure 2g recapitulates the results of the dual color FISH experiments with the native orientation of the genomic clones in the 5q segment, at extreme left, and that observed in the ins(5) chromosome, at extreme right. Figure 2h shows the ideograms of the chromosomes ins(5) and rec(5) as compared to that of normal chromosome 5, with the identified bkp's.

In brief, by means of molecular cytogenetics, the abnormal maternal chromosome was defined as an ins(5)(pter→p13.1::q23.3→q23.2::q31.3→q23.3::p13.1→q23.2::q31.3→qter) allowing the trisomic region responsible for the child's clinical characteristics to be delimited by YACs 6 and 26. According to the physical distance between the most centromeric STS of y6 (D5S1891) and the most telomeric STS of y26 (d5S2424), the size of the trisomic segment was estimated to be approximately 20 Mb (htpp//www.genome.ucsc). More than 250 genes are located in this region (http://www.ncbi.nlm.nih.gov).

The conventional cytogenetic study was then extended to the family, and revealed the presence of ins(5) in the karyotype of proband's maternal grandfather, two great-uncles, one great-aunt and three aunts. We detected seven carriers and seven noncarriers in the progeny of the heterozygotes. Between the progenies of two balanced carriers, there were one spontaneous abortion and two children deceased at 9 years and 9 months of age, respectively.

Discussion

The child's phenotype

The abnormalities in our patient are direct consequences of the pure 5q23.2–q31.3 duplication resulting from recombination between the maternal 5 chromosomes, one of which carrying a balanced intrachromosomal insertion. An accurate karyotype–phenotype correlation of partial 5q duplications is complicated because they are frequently associated with concurrent monosomies of other chromosomal regions due to the malsegregation of balanced translocations.[3,4] Furthermore, the previously reported cases concern patients with duplications of different lengths, and the boundary of the trisomic region has generally been defined using standard cytogenetic banding techniques.

The analysis of the reported partial 5q duplications revealed only two patients with a trisomic region overlapping that of our patient (Table 2).[5,6] The clinical features of our patient and that of NJ Martin[5] are quite similar, whereas the patient described by D Martin[6] has an interrupted aortic arch, a rare conotruncal heart defect that the authors hypothesized as being due to dose-sensitive genes within the 5q31q35 segment. The absence of this defect in our patient suggests that the putative genes affecting heart development may be located in the q32–q35 region.

Owing to the extension of the trisomic segment (20 Mb), and the great number of genes involved (>250), the role of specific gene/s in determining particular clinical features of the proband is hard to assess.

Origin of the abnormal chromosomes

The proband's mother is a balanced carrier of an intrachromosomal pericentric insertion, a chromosomal rearrangement normally involving three breaks which, in this case, underwent a fourth break causing its peculiar polarity with respect to the centromere. The absence of any phenotype anomaly in the mother indicates that no genes were structurally disrupted or deregulated by position

effects. The events leading to this complex rearrangement were the inverted insertion of 5q bands into the 5p segment and a simultaneous or superimposed break within the inserted region leading to 180° rotation of the two subsegments.

Repeated sequences located on the same chromosome may be responsible for bkp's at a distance of few megabases underlying inversions or more-complex rearrangements.[7] Clusters of Alu sequences within the genomic clones spanning all three 5q bkp's and the 5p bkp may have stimulated illegitimate recombination playing a role in generating the complex chromosomal rearrangement. This hypothesis is in line with the widespread literature on the influence of sequence-specific genomic motifs on non-recurrent chromosome rearrangements.[8] A single pachytene crossover between 5p13.3 and 5q23.2 during maternal meiosis would lead to a rec(5)dup(5q) chromosome such as that inherited by the child.

Reproductive outcome in the family

There is no evidence of a higher risk of spontaneous abortions among the insertion carriers in the family, which confirms the observations reported by Madan and Menko.[2] Of the two spontaneous abortions in the extended family, only one was among the progeny of a balanced carrier. No other family members were shown to carry the recombinant chromosome. Family records show that the progeny of two balanced carriers died at, respectively, 9 years and 9 months of age. We do not know the clinical history of these uninvestigated cases. The ratio of carriers to non-carriers in the normal progeny of heterozygous individuals seems to be 1:1 in the two generations. Our results do not confirm the trend toward more carriers revealed by pooling the previously reported data from sufficiently large families with intrachromosomal insertions.[2]

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