

Down syndrome: characterisation of a case with partial trisomy of chromosome 21 owing to a paternal balanced translocation (15;21)(q26;q22.1) by FISH

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Abstract

A patient with a typical Down syndrome (DS) phenotype and a normal karyotype was studied by FISH. Using painting probes, we found that the patient had partial trisomy of chromosome 21 owing to an unbalanced translocation t(15;21)(q26;q22.1) of paternal origin. To correlate genotype with phenotype as accurately as possible, we localised the breakpoint using a contig of YACs from the long arm of chromosome 21 as probes and performed FISH. We ended up with two YACs, the most telomeric giving signal on the der(15) in addition to signal on the normal chromosome 21 and the most centromeric giving signal only on both normal chromosomes 21. From these results we could conclude that the breakpoint must be located within the region encompassing YACs 280B1 and 814C1, most likely near one end of either YAC or between them, since neither YAC 814C1 nor 280B1 crossed the breakpoint (most likely between marker D21S304 and marker D21S302) on band 21q22.1. The same study was performed on the chromosomes of the father and of a sister and a brother of the patient; all three carried a balanced translocation between chromosomes 15 and 21 and had a normal phenotype. We also performed a prenatal study using FISH for the sister. The fetus was also a carrier of the balanced translocation.

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Down syndrome is the major cause of mental retardation and congenital heart disease in humans, affecting one in 700 newborns.¹ In 95% of cases DS is the result of full trisomy 21, 1% of cases are reported to be mosaics, and the remaining 4% are the result of unbalanced rearrangements resulting in total, or infrequently in partial, trisomy of chromosome 21.² In 1974 Niebuhr³ suggested the existence of a critical minimal region on band q22 of chromosome 21 which, when duplicated, is responsible for the DS phenotype. More recently, molecular studies provided evidence that the critical region for DS is located in the

region corresponding to marker D21S55,⁴ whereas other studies suggested that genes outside this region also contribute to the DS phenotype.⁵

The few published cases with DS resulting from a partial trisomy⁴⁻⁶ have been very important in establishing a correlation between genotype and phenotype, even though most of these cases have other chromosomal abnormalities, which might contribute to the clinical findings. To define or corroborate the phenotypic map of chromosome 21 further, it is necessary that new cases of partial trisomies are identified and that the chromosomal breakpoints are characterised.

Before the development of fluorescence in situ hybridisation (FISH) techniques in 1986,⁷ it was only possible to study chromosomal rearrangements by conventional cytogenetics or at best by high resolution cytogenetics. FISH studies now permit the identification of previously undetectable cryptic chromosomal rearrangements, which contribute greatly not only to the correct diagnosis and to adequate genetic counselling but to research studies and the characterisation of the genes interrupted by these rearrangements. With the recent development of genetic maps using highly polymorphic markers^{8,9} and the construction of contigs of ordered overlapping YACs and cosmid clones covering the entire long arm of chromosome 21,^{10,11} the use of mapped DNA clones in FISH analysis has been possible and easy. We present here the clinical, cytogenetic, and molecular characterisation of a subject with partial trisomy of chromosome 21 and DS, along with three other members of the family, all of whom carry a balanced translocation between chromosomes 15 and 21. We also performed a prenatal diagnosis for one of the members studied.

Patient and methods

The DS patient was ascertained through his sister, who came to our genetic clinic for counselling. The sister, 29 years old and pregnant, was married to a first cousin on the mother's side and was concerned about the risk to the fetus because of parental consanguinity. She had two brothers, the oldest of whom had been diagnosed with Down syndrome. Family history on the mother's side was unremarkable. No information could be obtained on the father.

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er's side as he was adopted as an infant. In order to assess the risk of the fetus having DS, a cytogenetic study was performed on a peripheral blood sample from the mentally retarded brother. Cytogenetic G banding showed an apparently normal karyotype. The patient was then assessed in our genetic clinic and the DS phenotype was verified.

CYTOGENETIC ANALYSIS AND FISH

Metaphase chromosome spreads were prepared from amniocyte and lymphocyte cultures according to standard methods. GTL banding techniques were performed in all cases.

ALU-PCR AMPLIFICATION, LABELLING, AND HYBRIDISATION OF THE PROBES

We generated painting probes from chromosomes 15 and 21 in order to detect the partial trisomy of chromosome 21 resulting from the unbalanced translocation (15;21). For chromosome 21 we used the hybrid (mouse-human) cell line WAV-17, which has chromosome 21 as its only human component.¹² We extracted the DNA and performed inter *Alu*-PCR using the following primers: A33: 5'-CGCGTGCACCACTGCACTCCAGCCTGGGCG-3'; A44: 5'-CGCGTGCACGGGATTACAGGCG-3'.^{10,13} For the chromosome 15 painting probe, we performed *Alu*-PCR using DNA from the Human Genetic Mutant Cell Repository (NIMGS panel)¹⁴ containing chromosome 15 as the only human chromosome. PCR products were separated on a 1.5% agarose gel. If suitable, the PCR products were ethanol precipitated and resuspended in 40 µl of distilled water before labelling. To define the partial trisomy of chromosome 21 further, YACs from the long arm of chromosome 21 were selected¹⁵ and amplified by inter *Alu*-PCR. Two micrograms of each probe were labelled with either dig-11dUTP or bio-16dUTP (Boehringer Mannheim) in a standard nick translation reaction,¹⁶ and purified by gel filtration. Then 400 ng of the product were precipitated with 1 µg of *Cot*I-DNA (GIBCO-

BRL) and 1 µg of salmon sperm DNA (Sigma) and the pellet resuspended in hybridisation mix containing 50% formamide and 50% (20% dextran sulphate in 3 × SSC); 10 µl of the hybridisation mix were applied to each slide (when hybridising two probes at the same time, 7 µl of each mix were used instead). After heat denaturation of the probes and the chromosome preparations, slides were incubated overnight in a humid chamber at 37°C. Post hybridisation washes were performed in three changes of 50% formamide/2 × SSC at 42°C, followed by three changes of 0.1 × SSC at 60°C. For detection of the signals slides were incubated at 37°C with sheep antidigoxigenin-FITC (Boehringer Mannheim), avidin-FITC, or avidin-TRITC (Vector Laboratories) for 20 minutes, and then washed in three changes of 4 × SSC/Tween 20 at 37°C. None of our probes needed further amplification of the signal. Slides were mounted with 40 µl of antifade solution (Vector Laboratories) containing 0.5 mg/ml of propidium iodide or 150 ng/ml of DAPI.¹⁷ Slides were viewed with an Olympus AH-3 (VANOX) fluorescence microscope equipped with the appropriate filter set. Images were analysed with the Cytovision system (Applied Imaging).

Results

CLINICAL FINDINGS

The patient was a 27 year old male, born after an uneventful term pregnancy, with a birth weight of 2550 g. During infancy he showed marked developmental delay and it was discovered at the age of 11 years that he suffered from severe sensorineural deafness. At the time of examination he showed absent speech and severe mental retardation. He had the distinct craniofacial appearance of DS including brachycephaly, flat occiput, flat face, short and upward slanting palpebral fissures, epicanthic folds, depressed nasal bridge, small nose with anteverted nostrils, protruding tongue, mid-facial hypoplasia, short and webbed neck, and small ears (table 1). Arms and legs showed rizo-melic and mesomelic shortening and a marked limitation of the pronosupination of the elbows was also present. The hands were broad and short with a bilateral single fifth finger crease. There was a single transverse palmar crease on one hand and a Sydney line on the other. Hypogonadism with bilateral cryptorchidism and a small penis was observed. In addition, his testosterone level was reduced. Cardiological examination was normal. Additional diagnostic procedures did not show other internal defects or endocrine dysfunction.

CHROMOSOMAL ANALYSIS, PARTIAL TRISOMY OF CHROMOSOME 21, AND PARTIAL MONOSOMY OF CHROMOSOME 15

Cytogenetic analysis of high resolution chromosomes from the patient by standard G banding showed a normal karyotype. Conventional cytogenetic studies on the chromosomes of the other members of the family were also normal. The clinical findings of the patient strongly

Table 1 Classical features of trisomy 21 in patients with duplication of the 21q22-qter region

Phenotypic features	Ref 4	Ref 5	Present case
Short stature	?	+/-	++
Mental retardation	?	+	+
Microcephaly	?	+	+
Brachycephaly	?	+	+
Oblique eye fissures	+	+	+
Epicanthic folds	+	+	+
Brushfield spots	+	-	-
Flat nasal bridge	+	-	+
Flat face	?	+	+
Open mouth	?	+	+
High arched palate	?	+	+
Furrowed tongue	?	-	-
Malpositioned ears	?	+	-
Small dysmorphic ears	+	+	+
Short neck	+	+	+
Short and broad hands	+	+	+
Clinodactyly 5th finger	+	+	+
Transverse palmar crease	-	-	+
Abnormal dermatoglyphics	+	?	+
Hypotonia	?	-	+
Congenital heart defect	+	+	-
Other visceral anomalies	-	-	-

+ indicates presence of the feature.

- indicates absence of the feature.



Figure 1 Fluorescence in situ hybridisation (FISH) analysis of chromosomes of a patient with Down syndrome and a partial trisomy owing to a $t(15;21)$ using painting probes of chromosomes 15 (green) and 21 (red). The arrow points to the translocation resulting in the partial trisomy of chromosome 21.

suggested a DS phenotype; therefore, we performed FISH with a chromosome painting probe from chromosome 21 and detected two normal chromosomes 21 and a translocated region of the same chromosome onto the distal region of an acrocentric chromosome from group D, presumably chromosome 15. To confirm these results, we performed a two colour painting probe hybridisation with chromosomes 15 and 21. The patient showed one chromosome 15 with a dual colour indicating that it was the translocated chromosome and that the patient was partially trisomic for chromosome 21 (fig 1). The same assay was performed on the other members of the family and showed that the sister and the brother were carriers of a reciprocal balanced translocation between chromosomes 15 and 21 inherited from their father, who is also a carrier of the balanced translocation.

Table 2 Summary of the YACs used in the FISH analysis of a patient with DS and partial chromosome 21 trisomy owing to a $t(15;21)$

YACs	Markers	der(15)
814C1	D21S82, D21S388, D21S226, D21S213, D21S300 D21S301, D21S302	-
280B1	D21S304, D21S305, D21S310, D21S306	+
876D4	IFNAR, D21S323, GART, D21S320, D21S316, D21S219, D21S319	+
667B10	D21S404, D21S262, D21S315, D21S316	+
72H9	D21S393, AML1, D21S65, D21S328 D21S332, D21S325	+
A222A12	D21S17, D21S211, D21S393	+
230E8	CBR, D21S333, D21S334	+
152F7	D21S270, D21S394, D21S336, D21S267	+
238B1	D21S337, D21S55	+
374B8	D21S259, D21S342, D21S339, D21S341 D21S233, ERG	+
767B3	D21S3, ACTFIB, ETS2, D21S259	+

+ indicates the presence of three signals in the metaphase.
- indicates the presence of two signals.

YAC MAPPING OF THE $t(15;21)$ BREAKPOINT

Before selection of the YAC clones used in the study, a set of 50 YAC clones from the long arm of chromosome 21 were tested by FISH for chimerism on control slides and were rejected if chimeric. Eight non-overlapping YACs were hybridised to the chromosomes of the patient (table 2). Hybridisations were performed and scored for double or triple signals (fig 2). The breakpoint was located on band 21q22.1 in the region encompassed by two neighbouring YACs. The more telomeric YAC (280B1) gave signal on both normal chromosomes 21 as well as on the der(15). The more centromeric YAC (814C1) gave signal only on both normal chromosomes 21 (table 2). To determine the extent of the monosomic region of chromosome 15, we performed a similar analysis using chromosome 15 YACs. The chromosome 15 breakpoint is located in band q26, distal to YAC 963C3 (containing markers D15S963, WI-6813, D15S158, STSG-10186, and WI-5507).

PRENATAL DIAGNOSIS

After detecting and characterising the translocation in this family, which results in partial trisomy of chromosome 21 and DS in one of the members, we performed a prenatal diagnosis. Given that the sister was a balanced carrier of the translocation, we performed the prenatal study on the metaphase chromosomes of a chorionic villus sample by FISH using the painting probes and YACs. The YACs used for this study were selected according to the previous results on the maternal chromosomes; we looked for double or triple signals. The fetus was a male carrying the balanced translocation $t(15;21)$.

Discussion

We have identified a patient with partial trisomy of chromosome 21 and a DS phenotype caused by a paternally inherited unbalanced translocation between chromosomes 15 and 21. A balanced (15;21) translocation was present in his two sibs as well as in the fetus carried by his sister. By using FISH with YAC probes from the long arm of chromosome 21, we have been able to identify the breakpoint of this translocation at 21q22.1, within the region covered by YACs 280B1 and 814C1. Clinical correlation analysis confirms that the majority of DS features map to 21q22-qter (from marker D21S304 to the telomere).

Comparison of the clinical features resulting from partial chromosome 21 duplications has provided the basis for construction of the DS phenotypic map. The coverage of chromosome 21 by a panel of YAC clones and the use of these YACs for FISH allows us to combine the phenotypic information from our DS patient with a fairly accurate molecular definition of the duplicated region. This approach is much faster and more precise than the dosage studies or polymorphic marker methods previously used.⁴⁵ Delabar *et al*⁴ has mapped 24 features of DS to different regions of chromosome 21, according to the clinical features of 10 patients

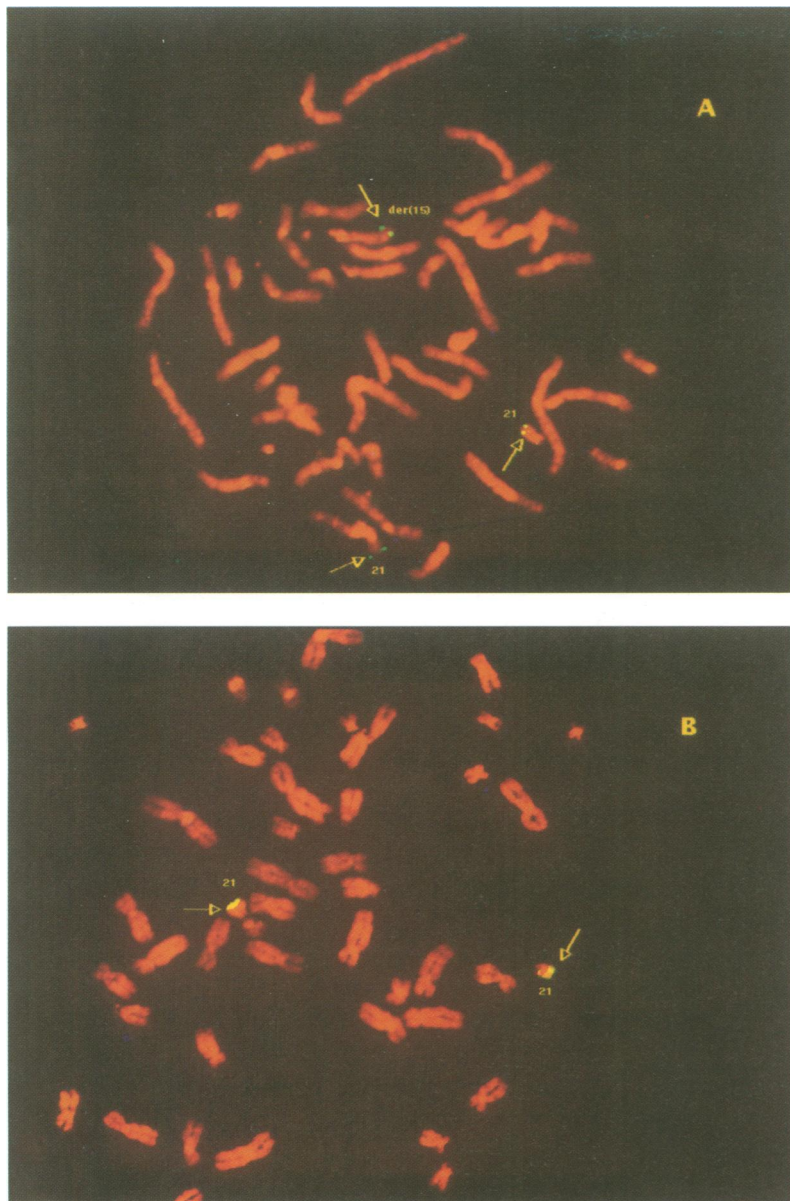


Figure 2 Fluorescence in situ hybridisation (FISH) analysis of chromosomes of a Down syndrome patient with partial trisomy of chromosome 21 owing to a $t(15;21)$ with two YAC clones from chromosome 21. (A) YAC 280B1 showed three signals, corresponding to the two normal chromosomes 21 and the translocated chromosome. (B) YAC 814C1 showed two signals on both normal chromosomes 21 and no signal on the translocated chromosome.

with partial trisomy 21. On the basis of the corresponding trisomic segments, they defined six chromosomal regions, each one including several clinical features, but defining the q22.2-q22.3 region as that contributing to most of the complex DS phenotype. Similarly, but with different conclusions, Korenberg *et al*⁵ constructed another phenotypic map of DS with 25 features assigned to regions spanning 2 to 20 Mb and concluded that DS is a contiguous gene syndrome, with duplication of regions distinct from distal 21q22 contributing to the main features of DS. The trisomic region of the DS patient reported here extends from marker D21S304 to the telomere, which covers about 17 Mb of chromosome 21, as judged from the physical map.¹⁸ Two other cases have been described⁴⁵ with a similar duplicated region. As shown in table 1, our case further

confirms that the majority of the phenotypic features of DS map to 21q21.3, as suggested by Korenberg *et al*,⁵ but also 21q22.

One of the difficulties in the construction of a phenotypic map, based on cases of partial trisomy, is that a large proportion of these cases have in addition other chromosomal abnormalities, which may also contribute to the clinical findings. Our patient presents severe mental retardation with absent speech and short stature. However, since these features are associated with a large number of chromosomal aberrations, it is difficult to evaluate the contribution of the chromosome 21 duplication to these. In fact, deletion of 15q26-qter, as in our patient, has been associated with both pre- and postnatal growth retardation, clinodactyly, microcephaly, developmental and speech delay, café au lait spots, and discrepancy in leg length.¹⁹ A recent study suggested that growth retardation may be the result of loss or disruption of the insulin-like growth factor receptor I (IGFRI) gene, assigned to 15q26. Although short stature in our patient may be partially caused by the partial trisomy 21, the presence of a marked shortening of the extremities, more evident in the arms, is probably the result of partial monosomy of chromosome 15. Application of available molecular cytogenetic tools to study additional cases with similar chromosomal arrangements will help to clarify this issue.

The study presented here highlights several important aspects of the diagnosis of cases of partial trisomy 21. The combination of chromosome painting and FISH using YAC clones as probes permits a rapid characterisation of the chromosomal breakpoint and its correlation with the clinical phenotype. As illustrated in this study, this has important implications for genetic counselling and also allows a fast and reliable prenatal diagnosis to be performed. In addition, this patient contributes to the definition of the phenotypic map of DS which, so far, is based upon a relatively small number of cases with partial trisomy of chromosome 21.

With the continued development of molecular cytogenetic tools and the availability of methods and probes for the accurate determination of the chromosomal rearrangements in each case, phenotype/genotype analysis can be performed with a high degree of accuracy and diagnosis can be performed at the molecular level with a high degree of certainty.

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