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Clinical and cytogenetic characterisation of a patient with Down syndrome resulting from a 21q22.1→qter duplication

EDITOR—Trisomy of human chromosome 21 is one of the most frequent aneuploidies in humans and results in Down syndrome (DS), affecting approximately 1 in 700 live births.¹ DS is a major cause of mental retardation and congenital heart disease in humans, but is also associated with other major features, such as characteristic facies, skeletal abnormalities, and an increased risk of leukaemia and Alzheimer's disease. In most cases (95%), the trisomy of chromosome 21 involves the whole chromosome through maternal (most frequent) or paternal non-disjunction, whereas in some cases (4%) Down syndrome is the result of an unbalanced translocation. Among the latter, a very small proportion of cases are the result of partial trisomy of chromosome 21 arising either from non-Robertsonian translocations or from intrachromosomal duplications.² The DS phenotype is also found in cases (1%) of mosaicism of trisomy 21.³

During the last decade, considerable progress has been made towards discovering the gene content of chromosome 21, but the functions of most of these genes and their specific contributions to the final DS phenotype still remain unknown. The identification and characterisation of cases of partial trisomy of chromosome 21 has allowed a phenotypic map of this chromosome and DS to be constructed. Clinical and molecular studies of these cases

have suggested that most of the phenotypic features of DS are the result of the triple dose of the genes contained in the region around marker D21S55. This region is called the Down syndrome critical region (DSCR).⁴⁻⁶ However, recent evidence suggests that genes outside this region may also contribute to the DS phenotype.⁷ To evaluate DS patients clinically, a checklist of 25 clinical traits was first reported by Jackson *et al*⁸ and later revised by Epstein *et al*.⁹ The accurate and exhaustive clinical evaluation of the patients, along with the characterisation of the extent of each trisomy, should help to establish genotype/phenotype correlations. Comparison of the clinical findings in DS patients with similar partial trisomies is now possible through the database created by J Delabar (<http://www.infobiogen.fr/services/aneu21>), which includes the

Figure 1 Patient with a duplication 21q22-qter at the age of 2 years 11 months. Note the flat face, brachycephaly, pale periorbital fissures, epicanthic folds, and malformed and low set ears.

Figure 2 Schematic representation of human chromosome 21, indicating the region involved in the partial trisomy of the patient with dup(21q22.1→qter). A panel of 18 YACs covering the proximal region involved in the duplication is shown. Circled YACs were used in FISH experiments. The markers near the duplication breakpoint (D21S304, D21S306, D21S310, and D21S305) are indicated in bold. Other markers outside the duplication breakpoint are shown as reference loci.

molecular characterisation and clinical findings of patients with aneuploidy of chromosome 21. FISH analysis has proven very useful for characterising the extent of partial trisomies of chromosome 21, since a contig of YACs covering the whole long arm of the chromosome is available^{10 11} and the sequenced clones are constantly released to the public databases.

We report here the clinical findings of a patient with a partial trisomy of chromosome 21 resulting from a dup(21q22.1-qter). The patient has some clear signs of DS

but also lacks some of the characteristic features of the disorder. The extent of the duplication was characterised by FISH. It extends from marker D21S304 to the telomere and the centromeric breakpoint is localised in the chromosomal region encompassed by YAC 280b1, centromeric to the *SOD1* gene.

The patient is a boy from São Paulo, Brazil. The family history is unknown and the patient is currently in a Shelter House of the Brazilian Government waiting for adoption. The patient was first seen at the age of 1 year 10 months.

Table 1 Clinical features of Down syndrome in patients with partial trisomy of chromosome 21q22-qter

Phenotypic features	Cases			Present case
	DL*	SOL†	t(15;21)‡	
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	-	-	-	-

+, presence of the feature.

-, absence of the feature.

?, unknown.

*Delabar *et al.*,⁶ †Korenberg *et al.*,⁷ ‡Nadal *et al.*,¹⁴

The first examination showed some clinical features suggestive of DS, such as oblique eye fissures, low set ears, high and narrow palate, mild hypotonia, and protruding tongue, as well as delayed psychomotor milestones. A karyotype was performed then and showed 46,XY,21q+. This cytogenetic result prompted a further clinical examination of the patient according to the protocols described by Epstein *et al.*⁹ At this second examination, the patient was 2 years 11 months old (fig 1).

Chromosome analyses were performed on standard phytohaemagglutinin (PHA) stimulated lymphocyte cultures from peripheral blood. Some chromosome spreads were G banded and 5-bromo-2'-deoxyuridine (BrdU) was added to two cultures to perform R banding. We generated painting probes from chromosome 21 in order to elucidate the origin of the extra material. We used the hybrid (mouse-human) cell line WAV-17 that has chromosome 21 as its only human component.¹² We extracted the DNA and performed inter *Alu*-PCR.¹³ To define the partial trisomy further, YACs from the long arm of chromosome 21 were selected¹⁰ and also amplified by inter *Alu*-PCR. A total of 14 YACs were hybridised (fig 2). Probes were labelled and hybridised as described elsewhere.¹⁴ Twenty metaphases and 50 interphase nuclei were studied for each probe.

The clinical evaluation of the patient at the age of 2 years 11 months showed short stature, brachycephaly, flat facies, oblique palpebral fissures, epicanthic folds, flat nasal bridge, high palate, malformed and low set ears, short and broad hands with clinodactyly of the fifth finger, and moderate mental retardation. The patient also had delayed psychomotor development; he sat at the age of 8 months and walked at 16 months (table 1, fig 1). Dermatoglyphics were normal and an electrocardiogram showed that the patient had an incomplete block of the right branch (with no charge), but no major congenital heart defect. Abdominal scan and audiometry were normal. The cytogenetic study performed with both G and R banding showed that out of 45 metaphases analysed, 37 had extra material on chromosome 21q. A more detailed study of the case by FISH using a painting probe that covers the whole long arm of chromosome 21 showed that all the extra material attached to one chromosome 21 was derived from the same chromosome. FISH performed with YACs along the q arm

Figure 3 FISH analysis on metaphase spreads of a patient with Down syndrome phenotype and a dup(21q22.1→qter). (A) Metaphase of the patient showing a duplication in one of the chromosome 21 homologues when hybridised with YAC280b1. In the left corner, R banding image of dup(21) and its homologue. (B) Metaphase of the same patient hybridised with YAC 814c1 showing only one signal on both chromosomes 21, indicating that the partial trisomy does not include the markers contained in this YAC.

of chromosome 21, showed that the patient had a duplication 21q22.1→qter. The contig of YACs used showed that the breakpoint was located just proximal to *SOD1*, within YAC 280b1, which contains markers D21S213 to D21S306 (fig 2). All distances between the double signals on the dup(21) were identical for all YACs studied. All the metaphases analysed by FISH showed the duplication, discounting the possibility of mosaicism observed using banding techniques (fig 3).

We have identified a case of partial trisomy of chromosome 21 and a DS phenotype caused by duplication of 21q22.1→qter. Using FISH with YAC probes from the long arm of chromosome 21, we have identified the extent of this duplication as well as its orientation. The trisomic region spans marker D21S213 to the telomere, which covers about 13 Mb of the chromosome, as judged from the physical map.¹⁵ Comparison of the clinical features resulting from partial trisomy of chromosome 21 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 in FISH analyses 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.^{6,7} The phenotypic map of DS constructed by Korenberg *et al.*⁷ assigned 25 features to regions spanning 2 to 20 Mb and they concluded that DS is a contiguous gene syndrome with duplications distinct from distal 21q22 contributing to the main features of DS. Given that the partial trisomy of chromosome 21 in the patient does not involve any other chromosome, it further supports the hypothesis that the genes contained in the region from 21q22 to the telomere are responsible for the majority of the features of DS, as previously reported by Korenberg *et al.*⁷ Three other cases have been reported with a similar duplicated region.^{6,7,14} As shown in table 1, the case reported here further confirms that the majority of the phenotypic features of DS are contained in the region triplicated in the four cases. However, the penetrance of the majority of the clinical features of DS is not complete, so to establish the correlation only the presence (not the absence) of a given trait should be taken into account. When the three published cases of DS with a similar partial trisomy and the present case are compared, we can see that almost all the physical traits typical of DS are present, perhaps with the exception of the furrowed tongue which has an overall frequency of 55% in the DS population.¹⁶ One of the difficulties in the construction of a phenotypic map of DS, based on cases of partial trisomy, is that a large proportion of these cases have in addition other chromosomal abnormalities which may contribute to the clinical findings. Our patient presents no other chromosomal abnormality, so all his clinical traits can be assumed to be associated with the duplication of chromosome 21. However, we have established comparisons with other cases with a very small partial monosomy of another chromosome, which may have a very small contribution to the final phenotype of the patients.

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 correlations can be obtained 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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Stable non-Robertsonian dicentric chromosomes: four new cases and a review

EDITOR—Dicentric autosomes are rarely encountered as stable constitutional chromosomes in humans, with the exception of Robertsonian translocations. The presence of two alpha satellite sequences on the same chromosome leads to a high risk of attachment of the same chromatid to the mitotic spindle from opposite poles and to the forma-

tion of anaphase bridge during cell division. Therefore, breakage of the dicentric can occur with subsequent cell death.¹ Stability can be achieved when the centromeres are very close together and form only one heterochromatin block, or when one of them is inactivated. One report by Vianna-Morgante and Rosenberg² shows a rarer mechanism of stabilisation, the deletion of one centromere. Several mechanisms can lead to dicentric chromosomes: meiotic recombination within a paracentric inversion loop, isochromatid break with U shaped rejoining, mitotic crossing over, Robertsonian translocation, and non-homologous non-Robertsonian translocation. Non-homologous dicentric autosomes are expected to be formed by the latter. We