Double partial trisomy 9q34.1→qter and 21pter→q22.11: FISH and clinical findings

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Abstract

We describe a patient with double trisomy 9q34.1→qter and 21pter→q22.1 resulting from 3:1 segregation of a maternal balanced translocation. The patient shows a clinical syndrome similar to that observed in patients with duplication of the chromosome 9q distal region, while no signs of trisomy 21 were observed. The use of high resolution banding and FISH were of fundamental importance for the cytogenetic diagnosis and for definition of the break-points on both chromosomes 9 and 21.

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There are only a few reports of partial terminal trisomy of 9q.¹⁻⁸ We describe an unusual case of double trisomy 9q34.1→qter and 21pter→q22.1 owing to 3:1 segregation of a maternal balanced translocation. The patient shows a clinical syndrome similar to that observed in patients with duplication of the chromosome 9q distal regions, while no signs of partial trisomy 21 are present. Cytogenetic analysis with high resolution GTG banding and FISH were necessary for the identification of the chromosome imbalance.

Case report

The proband was referred at birth to our cytogenetics unit because of multiple congenital abnormalities (MCA). The patient was born after a term pregnancy; the parents are unrelated and apparently healthy and the father was 35 and the mother 33 years old at the time of the baby's birth.

On the maternal side of the family there was a malformed female first cousin, who had been referred to this cytogenetics unit because of MCA, but whose karyotype was normal.

The pregnancy was complicated by polyhydramnios. Delivery was by vacuum extraction. At birth the patient weighed 3020 g and suffered from asphyxia. Apgar scores were 4 and 6 at one and five minutes, respectively. Cyanotic spells were observed in the neonatal period.

Clinical examination showed a dysmorphic face with caput quadratum, a high and prominent forehead, narrow palpebral fissures, deep set eyes, divergent strabismus, horizontal nystagmus, narrow and prominent nasal bridge, small, beaked nose, short columella, short philtrum, small mouth with thin lips and downturned corners, high arched palate, low set, cup shaped, anteverted ears, receding chin, and a supernumerary nipple on the left. She had long limbs and arachnodactyly of the hands and feet (fig 1). The first metatarsal bones were short with hammer toes and the heels were prominent. There was hypertrophy of the clitoris. A cardiac murmur was heard and cardiac ultrasound scan showed a VSD. EKG showed an incomplete right branch block. Ultrasound scan of the genitourinary tract showed a hypoplastic left kidney. Skeletal x ray showed 14 thoracic vertebrae with 14 sets of ribs. Ophthalmological examination showed pale hypoplastic papillae. Brain MRI showed a hypoplastic corpus callosum, wide subarachnoid spaces, and dilatation of the ventricular system. EEG showed diffuse immaturity and slight reduction of the white matter. At present

Figure 1 Phenotype of the patient. (A) Facial appearance. (B) Arachnodactyly.

Figure 2 Cytogenetic findings. (A) Ideogram and high resolution G banding showing the abnormal (left) and normal (right) chromosomes 9 and 21. Arrows indicate the breakpoints. (B) QFQ banding: partial metaphase from the proband showing three chromosomes 21 (arrows). (C) FISH: partial metaphase from the mother with the double dots showing the D21S65 probe signal on the normal chromosome 21 and on the translocated chromosome 9 (arrows). (D) FISH: partial metaphase from the patient. Classical satellite probe D9Z1 was used to detect chromosomes 9, and *abl* probe signal mapping on chromosomes 9 and on the abnormal chromosome 21 (arrows).

the patient is 1 year old. She has profound mental retardation, marked hypotonia, and hypotrophy of the muscles. She is unable to hold up her head, she has aimless movements of the hands and legs, and she does not speak. Her weight is 6350 g (<<3rd centile), length is 74.5 cm (75th centile), and head circumference is 45.1 cm (50th centile). The patient has a weak cry and laryngeal stridor and suffers from frequent cyanotic spells during sleep. She has had frequent episodes of bronchopneumonia.

CYTOGENETIC FINDINGS

Karyotype analysis performed with standard GTG banding was first interpreted as 47,XX,+21. This result was not convincing as there was a remarkable discordance between phenotype and karyotype. We suspected that (1) the chromosome abnormality in our proband might be more complicated than a simple trisomy 21, and (2) there might be some connection between the cause of the clinical abnormalities in our proband and those in her malformed maternal cousin. Chromosome analysis was performed on the proband's parents, with special attention paid to the maternal karyotype, but at first the parental karyotypes were interpreted as normal. Cytogenetic analysis was then repeated on the proband and her parents with high resolution of chromosome banding and in situ hybridisation.

FLUORESCENT IN SITU HYBRIDISATION

As we suspected that the additional chromosome 21 might be rearranged, we selected a panel of probes mapping on chromosome 21 to analyse the three chromosomes 21 of the proband in detail. Three biotinylated probes were used in this study. One alpha satellite probe (D13Z1/D21Z1, from Oncor Inc, Gaithersburg, MD) was used to detect chromosome 21 centromeres. Cosmid probes were used to detect unique sequences in the long arm of chromosomes 21. The specific cosmid probes selected were SOD, which was used to detect band 21q22.11, and D21S65 (Oncor) to detect band 21q22.22. Cosmid DNA was labelled by nick translation with biotin-16-dUTP (Boehringer-Mannheim) and precipitated with ethanol. Labelled probe DNA was resuspended in 50% formamide/2 × SSC/10% dextran sulphate in a final concentration of 10 ng/μl.

FISH was performed according to the procedure of Lichter and Cremer.⁹ The hybridisation was detected by avidin-FITC (Sigma) with one cycle of amplification (biotinylated goat anti-avidin, Vector). Chromosomes were counterstained with DAPI (200 ng/ml 2 × SSC) and propidium iodide (0.5 μg/ml antifade) and analysed with a fluorescence microscope (Leitz Ortoplan); they were then photographed using a Kodak Ektachrome 400 ASA film.

Figure 3 Family pedigree.

In the proband only two of the three chromosomes 21 showed hybridisation spots with all the probes that were used for chromosome 21: D13Z1/D21Z1 alpha satellite probe, SOD specific cosmid probe, and D21S65 specific cosmid probe. The third chromosome 21 showed positive signals only with D13Z1/D21Z1 alpha satellite probe and SOD specific cosmid, but not with D21S65 specific cosmid probe. These results indicated that besides two normal chromosomes 21 there was an additional rearranged chromosome 21 where the region below band q22.1 was missing and had been replaced by material derived from some other chromosome. To identify the origin of this unknown chromosome material, as we suspected that the mother might be a carrier of a reciprocal balanced translocation, we performed FISH analysis with the D21S65 specific cosmid probe on the maternal metaphases. In the maternal preparation a hybridisation signal with D21S65 probe was seen in one chromosome 21 only, while the other hybridisation spot was seen on the distal region of the long arm of a chromosome 9 (fig 2C). These results indicated that the mother was a carrier of a reciprocal translocation involving chromosomes 21q22.1 and 9q. To define the breakpoint on chromosome 9, after the results of high resolution chromosome banding, we selected the specific cosmid probe human *abl* (Oncor), which was used to detect band 9q34.1, while a classical satellite probe, D9Z1 (Oncor), was used for the identification of chromosome 9. In the mother the hybridisation signal with the human *abl* probe was missing from the abnormal chromosome 9 and it could be seen on the derivative chromosome 21. Thus, according to our observations, the breakpoint on chromosome 21 is between SOD and D21S65 while the breakpoint on chromosome 9 is proximal to the *abl* locus. The karyotype of the proband's mother has been interpreted as 46,XX,t(9;21)(q34.1;q22.1).

In our proband the signal with the human *abl* probe was present on both chromosomes 9 (which were normal) and on the additional, rearranged chromosome 21 (fig 2D); her karyotype was then 47,XX,+der(21),t(9;21)(34.1;q22.1)mat (fig 2).

The paternal karyotype was normal. The karyotype of the maternal uncle and that of his malformed child were restudied using high

resolution chromosome banding and in situ hybridisation and were normal.

Discussion

Our patient is a carrier of a double partial trisomy 21pter→q22.1 and 9q34.1→qter owing to 3:1 segregation of a balanced maternal translocation.

Although in our patient trisomy 9q was suspected on the basis of the clinical findings, the chromosome imbalance had been misdiagnosed with standard karyotyping only and it was recognised only after high resolution banding and FISH. The use of single copy probes also allowed a clear definition of the breakpoints on both chromosomes 9 and 21. The family history had pointed our attention to the maternal side of the family; however, although the mother was in fact a carrier of a balanced translocation, thorough chromosome analysis in the maternal uncle and his malformed child failed to show any connection with the abnormality observed in our proband and her mother (fig 3).

There have only been a few reports of pure trisomy of distal portions of chromosome 9q. Allderdice *et al*¹ reported a family in which seven patients showed duplication of band 9q34. Other reports of cases of trisomy of 9q34.1→qter were the result of unbalanced segregation of reciprocal translocations,²⁻⁷ and therefore associated with some other chromosome imbalance. In the cases reported by Krauss *et al*³ and by Narahara *et al*⁴ a consistent contribution to the phenotype was coexisting deletion of 4p and 7q respectively, while in the cases reported by Houdou *et al*² and Spinner *et al*⁶ little if any phenotypic abnormality seemed to be the result of the coexisting deletions of chromosomes 3 and 12, respectively. In the cases reported by Wellesley *et al*,⁷ trisomy 9q34.13→qter was accompanied by deletion of 5p15.1→pter, but no signs of the well known cri du chat syndrome were present, except for laryngeal abnormalities observed in one patient, which, as our patient shows, might be related to the trisomy 9q itself.

In our case partial trisomy 9q is accompanied by trisomy 21pter→q22.1, but, as in many other reports,¹⁰ there are none of the dysmorphic features observed in patients with pure trisomy for this region, such as epicanthic folds, flat nasal bridge, large, open mouth, and short, broad hands and feet.^{11 12} By comparing the clinical findings in the patients reported by Allderdice *et al*,¹ who have pure trisomy 9q34, in those reported by Houdou *et al*,² Spinner *et al*,^{5 6} Wellesley *et al*,⁷ and in our case, a severe clinical picture with a consistent phenotype can be recognised. The main findings are dysmorphic face with dolichocephaly (observed in 9/12 patients), prominent forehead (4/12), deep set eyes (5/12) with narrow palpebral fissures (4/12), prominent nasal bridge or beaked nose (9/12), abnormally shaped, posteriorly rotated, or low set ears (10/12), high arched palate, small mouth with downturned corners (8/12), and receding chin (7/12). Patients have a narrow habitus with long limbs and striking arachnodactyly of the hands and feet (12/12)

and camptodactyly/large joint contractures (7/12). There may be a congenital heart defect (3/12). In males there may be abnormalities of the genitalia (small penis, cryptorchidism) (3/5). Patients have severe psychomotor (12/12) and speech retardation, hypotonia (9/12), and failure to thrive. Laryngeal abnormalities have been reported in two patients. A less severe phenotype was observed in a child with trisomy 9q34.3→qter, who lacked most of the cardinal dysmorphic features of the syndrome.⁸

An additional finding in our patient is the presence of 14 thoracic vertebrae and 14 ribs bilaterally. This finding is unusual in patients with imbalances of chromosome 9 and it has only rarely been observed in patients with mosaic and non-mosaic full trisomy 9¹³ and in patients with dup 9q32→qter.¹⁴⁻¹⁵ The phenotype described above is very similar to that already reported in patients having larger terminal 9q duplications.¹⁵⁻¹⁸ The present report allows the critical region for the full expression of the syndrome to be reduced to band 9q34.1→qter. However, patients with trisomy 9q34→qter apparently share a number of dysmorphic features, with patients having trisomy for more proximal, non-overlapping regions of chromosome 9q.¹⁶⁻¹⁸ In fact in previous reports it had been suggested that duplication of band 9q32 was critical for the manifestation of the clinical syndrome.¹⁵⁻¹⁷

Further observations with high resolution chromosome banding and precise identification of the breakpoints using FISH may prove useful in detecting minimal regions of overlap and to help in mapping the phenotypic abnormalities in relation to the cytogenetic imbalance.

Conclusions

This unusual case allows the following comments to be made. It stresses once more the importance of completing cytogenetic study with microcytogenetics and FISH, particularly when the phenotype/karyotype relationship is

unusual, and it provides further information with regard to the clinical abnormalities associated with distal trisomy 9q.

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