ELECTRONIC LETTER

Deletion in the *ABL* gene resulting from a meiotic recombination of a maternal (3;22;9)(q22;q12;q34.1) translocation

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Complex chromosome translocations involve changes between three or more chromosomes and are found very rarely in the general population.^{1,2} Abnormal phenotype, mental retardation, recurrent miscarriages, and infertility have been reported in carriers of apparently balanced complex chromosomal rearrangements.³ Phenotypic abnormalities in patients with apparently balanced chromosomal translocation could be the result of several mechanisms. A breakpoint in one derivative chromosome may have occurred within a gene leading to loss of gene function.⁴ In other cases, an abnormal phenotype could be the result of genomic imprinting, as observed in translocations involving chromosomes 11 or 15.^{5,6} Another explanation is the possibility of cryptic rearrangements such as deletion or duplication of chromosomal segments near the breakpoints.⁷ By using conventional cytogenetic techniques, these balanced chromosomal translocations have no apparent loss or gain of genetic material. However, the characterisation of subtle chromosome anomalies in chromosomal translocations is difficult because of the resolution limit of banding analysis.⁸ The use of fluorescence in situ hybridisation (FISH) has greatly improved the accuracy of cytogenetic diagnosis and uncovered a number of cryptic chromosomal anomalies.⁹

We report an inherited, apparently balanced complex translocation in a child with axial hypotonia and dysmorphism.

CASE REPORT

The proband was a male infant born after an uneventful term pregnancy. Apgar scores were 9 and 10 at one and five minutes, respectively. Birth weight, length, and head circumference were 3350 g, 48 cm, and 33 cm, respectively. He was the third child of healthy, non-consanguineous parents and his two older sisters were normal. Two miscarriages had occurred between the first and the second pregnancy and seven had occurred between the second and the third pregnancy; however, no cytogenetic studies had been performed. All the miscarriages had occurred between 6 and 8 weeks of gestation. The child was first admitted at the age of 8 months for gastroenteritis with severe dehydration (13% weight loss), which was rapidly cured. Physical examination showed moderate axial hypotonia (the child was not able to sit), hypertelorism and bilateral epicanthus, high arched palate with macroglossia, and downward slanting palpebral fissures. Laboratory data showed normal WBC (11 500 leucocytes/mm³ with 60% lymphocytes) and RBC counts (haemoglobin 13.5 g/dl, erythrocytes 4 500 000/mm³); platelet count was also normal (453 000/mm³). Blood coagulation tests were normal, as were serum electrolyte concentrations. There was no evidence of visceral malformation. Echocardiography and renal ultrasound examination were normal. At the age of 16 months, facial dysmorphic features were unchanged, but the child was able to stand alone for a few seconds even though mild axial hypotonia was still present. He was not able

Key points

- Small deletions near breakpoints may be an important cause of disease in apparently balanced chromosome rearrangements. We report the chromosomal findings in a boy with axial hypotonia and dysmorphism.
- Physical examination showed moderate axial hypotonia, hypertelorism and bilateral epicanthus, high arched palate, macroglossia, and downward slanting palpebral fissures. There was no evidence of visceral malformation. The mother has had nine miscarriages.
- By using conventional and molecular cytogenetic analyses, an apparently balanced translocation t(3;22;9) inherited from the mother was diagnosed. Fluorescence in situ hybridisation with different locus probes near the breakpoints showed a deletion of the *ABL* gene located at 9q34.1 in the patient. This deletion was not found in the mother or in the sister carrying the translocation.
- Deletion of *ABL* has been described once in a newborn with a complex cardiac anomaly. Molecular cytogenetic analysis of breakpoints in apparently balanced chromosomal rearrangements should be systematically carried out in patients with phenotypic abnormalities.

to walk alone. Weight, height, and head circumference were on the 50th centile. There was no evidence of any malformation and there were neither feeding nor sleeping disorders. The child was not available for photography.

MATERIALS AND METHODS

Cytogenetic investigations

Chromosome studies were performed on cultured lymphocytes using R banding techniques. The proband, the parents, and the two sisters were analysed.

Fluorescence in situ hybridisation

FISH studies using chromosome 3 whole paint probe, chromosome 9 whole paint probe, chromosome 22 whole paint probe (Vysis, Downers Grove, IL, USA), and probes specific for the 3q, 9q, and 22q chromosomal regions were performed on cultured lymphocytes. Single and double colour FISH experiments were performed on chromosome preparations. Probes were from bacterial artificial chromosomes (BAC) (Rocchi, Resources for Molecular Cytogenetics, Italy): bA778E2 located on 3qter, bA417A4 located on 9qter, bA145E17 located on 9q34.2, bA203J24 located on 9q34.13, bA342E24 located on 9q34.12, bA58C3 located on 9q33.1, and bk799F10 located on 22qter. Probes were nick translated with fluorescein or rhodamine 12-dUTP. Commercial probes containing the *ARSA* gene (Vysis, Downers Grove, IL, USA) located

Figure 1 RTG banded chromosomes 3, 22, and 9 from a metaphase of the proband showing a reciprocal translocation between chromosomes 3 and 22. Arrows indicate the derivative chromosomes 3 and 22. A similar banding pattern is observed for the two chromosomes 9.

on 22q13.3, the *BCR* gene (Vysis, Downers Grove, IL, USA) located on 22q11.2, N25 (Vysis, Downers Grove, IL, USA) located on 22q11.2, and the *ABL* gene (Vysis, ONCOR) located on 9q34.1 were also used. Three probes in the *ABL* region were used. The M-*BCR/ABL* probe (Oncor, Gaithersburg, MD) was used. This *ABL* probe is approximately 89 kb at the major breakpoint region of the *ABL* gene in chronic myeloid leukaemia. The LSI *BCR/ABL*-ES dual colour translocation DNA probe kit (Vysis, Downers Grove, IL, USA) was used. The spanning *ABL* probe labelled in SpectrumOrange is approximately 650 kb extending from an area centromeric of the arginosuccinate synthetase gene to well telomeric of the last *ABL* exon. The double fusion signal D-FISH *BCR/ABL* probe (Oncor, Gaithersburg, MD) was used. This *ABL* probe labelled with fluorescein isothiocyanate is approximately 600 kb, extending from an area well centromeric of the arginosuccinate synthetase gene to telomeric of the last *ABL* exon. This *ABL* probe is associated with the *BCR* probe, directly labelled with Texas Red, which is approximately 500 kb, beginning 5' of the first *BCR* exon and extending well beyond the last exon. FISH was performed according to standard protocols.

RESULTS

Conventional cytogenetic analysis

R banding of chromosomes derived from the proband's lymphocyte cultures showed a reciprocal translocation between chromosomes 3 and 22 (fig 1). Examination of peripheral blood lymphocytes obtained from the mother and the two sisters showed the translocation in the mother and one sister, and a normal karyotype in the other sister. The father showed a normal karyotype 46,XY. The translocation was first interpreted by conventional cytogenetic analysis as a t(3;22)(q22;q12) inherited from the mother.

FISH analysis

The use of chromosome 3 and 22 painting probes showed a translocation between chromosomes 3 and 22 and also hybridisation of the chromosome 22 probe on the q arm of one chromosome 9 identified using DAPI banding (fig 2A). Chromosome 9 painting probe showed a uniform hybridisation along the normal chromosome 9 and hybridisation along the derivative chromosome 9 except at the telomeric region of the q arm.

Results of FISH hybridisation in the proband's cells using probes specific for chromosomes 3, 9, and 22 are indicated in table 1. FISH using probes containing the *ABL* gene locus showed no deletion in the mother's cells (500 cells) (fig 2B) or

in the sister's cells carrying the translocation. A deletion with M-*BCR/ABL* probe and a diminution of the intensity of the hybridisation signal on 9q with *BCR/ABL*-ES and D-FISH *BCR/ABL* probes were observed in the proband's cells (fig 2C, D). Using the *ABL* gene probe, no mosaicism was observed in 500 of the proband's cells.

Molecular cytogenetic techniques showed that the chromosomal rearrangement was a complex translocation (3;22;9) with a deletion in the *ABL* gene in the proband (fig 3).

DISCUSSION

In the present study, molecular cytogenetic analyses were performed in an apparently inherited balanced complex translocation associated with phenotypic abnormalities. A cryptic deletion was found on the long arm of the chromosome 9 with a deletion in the *ABL* gene. FISH was first applied to define clearly the breakpoints of a reciprocal translocation between chromosomes 3 and 22 diagnosed by conventional cytogenetic techniques. This analysis showed that a chromosome 9 was also involved in the translocation. The size and the banding pattern in the region 9qter and 22qter did not allow the detection of the translocation between chromosomes 9 and 22 by conventional cytogenetic analysis. The original banded interpretation of this translocation between chromosomes 3 and 22 was shown by FISH to be a three way translocation between chromosomes 3, 22, and 9. As described in several reports, conventional cytogenetic techniques have limitations in the analysis of complex rearrangements.¹⁰⁻¹² In our case, the use of whole chromosome painting probes allowed us to identify the derivative chromosome 9 and specific locus probes near breakpoints showed the presence of a cryptic deletion of the chromosomal band 9q34.1. Wirth *et al*⁹ estimated that a significant proportion of cytogenetically balanced translocations (>10%) have submicroscopic deletions of several megabases that account for the associated clinical phenotype. To our knowledge, up to now cryptic deletions have been described only in de novo chromosomal translocations.^{2 7 9 11 13} In familial complex chromosomal translocations, abnormal phenotypes are often the result of adjacent 1 segregation.³ Our case illustrates that a submicroscopic deletion can occur near a breakpoint in an inherited translocation. This mechanism can explain normal and abnormal phenotypes associated with the same familial complex translocation.

Deletions of the 5' *ABL* region have been described recently in an apparently balanced translocation (9;22) in chronic myeloid leukaemia.^{14 15} Analysis of genomic *ABL* sequence has shown a high density of Alu elements which are distributed

Figure 2 (A) FISH with chromosomes 3 (green) and 22 (red) specific paint showing derivative chromosomes 3 and 22, and the derivative chromosome 9 with a segment of chromosome 22 translocated. (B) FISH with M-BCR/ABL probe in a metaphase from lymphocytes of the mother showing no deletion of the ABL locus. ABL is in green and BCR in red. (C) FISH with M-BCR/ABL probe in a metaphase from lymphocytes of the proband showing a deletion of the ABL locus (89 kb). ABL is in green and BCR in red. (D) FISH with D-FISH BCR/ABL probe in a metaphase and a nucleus from lymphocytes of the proband showing a diminution of the intensity of the hybridisation signal of the ABL region (600 kb). The ABL region is in green and the BCR region in red.

non-randomly.^{16, 17} The presence of this repetitive DNA in the vicinity of breakpoint regions may facilitate the generation of submicroscopic deletions, as described recently in deletions of the 5' ABL region in chronic myeloid leukaemia.¹⁸ In our case, the deletion has probably occurred in maternal meiosis; the deletion was observed in 100% of the child's cells and no deletion was observed in the mother's cells.

Interstitial deletions of the chromosome region 9q are rarely seen and concern large segments of 9q. Our report is the second case of an interstitial deletion 9q34.1 with a deletion in

the ABL gene. The first case described was a newborn with dysmorphism and a complex cardiac anomaly.¹⁹ The main phenotypic anomaly observed in the child was axial hypotonia. As emphasised by Kleyman *et al*,¹⁹ comparison of this cryptic deletion 9q34.1 with previous reports involving large deletions may be irrelevant. Hypotonia was also observed in a case of deletion 9q32q34 described by Turleau *et al*.²⁰ Recently, mutations in the gene encoding c-Abl-binding protein SH3BP2 were reported to cause cherubism.²¹ The proband exhibited no phenotypic abnormalities of cherubism. This

Table 1 Results of the FISH analysis using probes specific for chromosomes 3, 9, and 22 in the proband's cells

Probes	Location	Normal 3	der(3)	Normal 9	der(9)	Normal 22	der(22)
bA778E2	3qter	+	–	–	–	–	+
bA417A4	9qter	–	+	+	–	–	–
bA145E17	9q34.2	–	+	+	–	–	–
bA203J24	9q34.13	–	Dim	+	Dim	–	–
bA342E24	9q34.12	–	–	+	+	–	–
ABL*	9q34.1	–	–	+	–	–	–
ABL†	9q34.1	–	–	+	Dim	–	–
ABL‡	9q34.1	–	–	+	Dim	–	–
bA58C3	9q33.1	–	–	+	+	–	–
bK799F10	22qter	–	–	–	+	+	–
ARSA	22q13.3	–	–	–	+	+	–
BCR	22q11.2	–	–	–	–	+	+
N25	22q11.2	–	–	–	–	+	+

* , †, and ‡ correspond to the *ABL* region using a probe of 89 kb, 600 kb, and 650 kb, respectively.
 + and – correspond respectively to the presence and absence of the hybridisation signal on the chromosome studied. Dim corresponds to a diminution of the intensity of the hybridisation signal.

Figure 3 Idiogram of the chromosomal rearrangement (G banding). Arrows show breakpoints.

observation is in accordance with the hypothesis that mutations in the *SH3BP2* gene lead to a gain of function.

Mice homozygous for mutations that disrupt the *Abl* locus have multiple abnormalities including foreshortened crania, altered spleen and eye morphology, a susceptibility to respiratory and gastrointestinal infections, osteoporosis, defects in osteoblast maturation and in B cell maturation in bone marrow, abnormal peripheral lymphocyte function, reduced fertility, and increased perinatal mortality.^{22–25} Normal haematological parameters were observed in the child. As described in *c-abl* deficient mice, *abl* function is not essential for haematopoietic stem cells or myeloid and erythroid progenitors.²⁶

Although karyotypes of the abortuses were not available, the nine miscarriages of the mother were probably the result of the complex translocation.²⁷ The risk of spontaneous abortion for carriers of complex chromosomal rearrangements is estimated to be about 50%.^{3, 28}

FISH studies have allowed us to define correctly the complex translocation in this family and the deletion in the child. This is essential for appropriate reproductive risk assessment, genetic counselling, and further prenatal diagnosis. This report shows the possibility that several mechanisms can exist simultaneously in an apparently balanced translocation to explain an abnormal phenotype, such as the generation of an acting fusion gene or the disruption of a gene and the deletion of one or more genes adjacent to the translocation

breakpoints. This study emphasises that molecular cytogenetic analysis of breakpoints in apparently de novo or inherited balanced chromosomal translocations should be systematically carried out in patients with phenotypic abnormalities.

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