Familial complex chromosome rearrangement ascertained by in situ hybridisation

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Abstract

A complex familial chromosome translocation has been ascertained by combining classical cytogenetics and CISS (chromosomal in situ suppression). Cytogenetic analysis of a chorionic villus sample with G banding showed a 47,XX,-2,+der(2)t(2;22),+der(22)t(2;22) karyotype. Analysis of peripheral blood lymphocytes from the parents by G banding and CISS showed a more complex translocation in the father: 46,XY,-2,-11,-22,+der(2)t(2;11)(q13;q23),+der(11)t(11;22)(q23;q11.2),+der(22)t(2;22)(q13;q11.2). Definitive analysis of cultured amniotic fluid cells showed a double partial trisomy of chromosomes 11 and 22. The couple decided to continue the pregnancy. The fetal karyotype was confirmed at birth. Clinical abnormalities present in our patient were typical of an unbalanced 11;22 translocation. Our findings confirm that chromosome painting techniques allow a better characterisation of complex chromosome rearrangements which may be difficult to detect in G banded karyotypes.

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Keywords: prenatal diagnosis; complex chromosomal translocation; chromosomal in situ hybridisation.

Many chromosomal rearrangements may go unnoticed when using classical banding techniques, especially if they involve small fragments or fragments with similar or identical banding patterns. The characterisation of such anomalies is extremely important in prenatal diagnosis and in patients with congenital malformations. In some cases, a given chromosome anomaly may be difficult to characterise in amniocytes or in chorionic villus samples even if a balanced reorganisation is known to be present in one of the parents.¹⁻³ Often, these problems may be solved by using in situ hybridisation techniques that allow characterisation of subtle rearrangements⁴⁻⁶ and detection of some de novo structural anomalies.⁷⁻⁹

Here, we report a complex chromosome rearrangement not fully resolved by chromosome banding of chorionic villus cells. Chromosome painting disclosed an unbalanced translocation involving three chromosomes, inherited from the balanced, carrier father.

Case report

A 31 year old woman requested a prenatal diagnosis when she was in the 11th week of

pregnancy. Previously, she had had three spontaneous abortions and a phenotypically normal son. Cytogenetic analysis of chorionic villus cells after Wright's G banding showed an unbalanced translocation that was initially diagnosed as a 47,XX,-2,+der(2)t(2;22),+der(22)t(2;22).

Analysis of peripheral blood lymphocytes from the parents showed the presence in the father of the same derivative chromosomes 2 and 22. However, the higher degree of resolution obtained in G banded peripheral blood preparations suggested that the bands in der(2) did not correspond to chromosome 22, and indicated the possible presence of a more complex translocation.

Chromosomal in situ suppression (CISS) hybridisation using DNA libraries for chromosomes 2 and 22 (Cambio, Cambridge) labelled with biotin was performed on peripheral blood preparations from the father following instructions from the supplier. Detection of hybridisation was performed using the immunoperoxidase technique as described by Pérez-Losada *et al.*¹⁰ At least 10 metaphases per probe were analysed. The results showed that the region distal to 2q13 was translocated to 22q11.2, while the region distal to 22q11.2 was not translocated to chromosome 2, but to a chromosome from group C. The reassessment of G banded preparations suggested the possible involvement of chromosome 11. A CISS hybridisation was then performed with a DNA library for that chromosome pair, which implicated chromosome 11 in this complex translocation. The three derivative chromosomes resulting from the translocation were characterised as der(2)t(2;11)(q13;q23), der(11)t(11;22)(q23;q11.2), and der(22)t(2;22)(q13;q11.2) (fig1).

Subsequent classical cytogenetic and in situ hybridisation (CISS) analysis of amniocytes allowed us to reinterpret the karyotype of the fetus as 47,XX,-2,+der(2)t(2;11)(q13;q23),+der(22)t(2;22)(q13;q11.2). The presence of two normal chromosomes 11 and 22 in addition to the derivative chromosomes resulted in double partial trisomies of chromosomes 11 and 22 (figs 2 and 3). The couple decided to continue the pregnancy. Cytogenetic study of the neonate verified the fetal karyotype and no mosaicism was found in 73 metaphases analysed. She was born at term with a head circumference of 34 cm, length 51 cm, and weight 2840 g. The most important clinical features since the child was born have been retarded physical growth, obvious psychomotor retardation, muscular hypotonia,

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Figure 1 G banded partial karyotype from the father and G banded diagram of the chromosomes involved in the translocations: $der(2)t(2;11)(q13;q23)$, $der(11)t(11;22)(q23;q11.2)$, $der(22)t(2;22)(q13;q11.2)$. Arrowheads indicate the three derivative chromosomes.

talipes calcaneovarus, congenital heart disease (intra-auricular connection), cleft palate, broad nose resulting from a short septum, and preauricular pits. The child is now 14 months old and her parents refuse further investigation.

Chromosome analysis of the different members of the family showed that the complex balanced translocation was also present in the paternal grandmother (fig 4). Other members of this family were not available for cytogenetic study.

Figure 2 G banded partial karyotype from an amniocyte showing normal and derivative chromosomes

Figure 3 CISS hybridisation on amniocyte metaphases using libraries from chromosome 2 (A), chromosome 11 (B), and chromosome 22 (C). In (A) the arrowhead points to the normal chromosome 2 and the arrows to $der(2)$ and $der(22)$; in (B) arrowheads point to the two normal chromosomes 11 and the arrow to $der(2)$; in (C) arrowheads point to the two normal chromosomes 22 and the arrow to $der(22)$.

Discussion

Our results, as well as those of other authors,⁴⁻⁶ emphasise the need to combine different approaches in chromosome rearrangements not resolved by standard cytogenetic techniques. The most simple combination includes painting, to identify the chromosome frag-

Figure 4 Pedigree of the family.

ments involved in the reorganisation, and high resolution G banding to identify the breakpoints.^{11 12} In fact, the incidence of subtle and complex rearrangements is probably much higher than previously thought,¹³ as shown by the increasing number of cases that are re-evaluated using in situ hybridisation techniques to show previously unnoticed anomalies.^{1 14 15}

About one third of complex chromosome rearrangements are inherited^{16 17} and result in duplications and deficiencies responsible for abortions or congenital malformations in the offspring. Daniel *et al*¹⁸ observed that in most inherited cases the carrier of the balanced rearrangement was the mother, and suggested that in the male the presence of a balanced rearrangement could interfere with spermatogenesis. However, Farrell *et al*¹⁹ have recently described a complex familial rearrangement in which three men were inferred by pedigree analysis to have been carriers, indicating that in their case the rearrangement allowed for male fertility. In our case, the paternal transmission of the rearrangement supports the idea that male fertility would depend on the type of chromosome reorganisation and on the chromosomes involved.

The cytogenetic origin of complex reorganisations could be the result of three or more independent breaks that rejoined at random, resulting in the production of the derivative chromosomes. This hypothesis is in agreement with the fact that two thirds of complex reorganisations, which may affect as many as four¹⁴ or even seven chromosomes,²⁰ are de novo in origin. However, in our case the complex t(2;11) may have originated from a previous t(11;22), because the breakpoints in the derivative chromosomes 11 and 22 coincide with those of t(11;22)(q23;q11.2) which is the most frequent reciprocal translocation in humans.²¹

The only viable patients with unbalanced 11;22 translocations are trisomic for the centromeric segment of 22 (approximately 22pter-q11) and for the distal segment of 11 (approximately 11q23-qter).²² In our case the same chromosome (added to 2q) segments are implicated in the partial trisomy and the clinical abnormalities are coincidentally more characteristic of the unbalanced 11;22 translocation phenotype.^{21 23-26}

The present observation substantiates the effectiveness of the combined use of G banding and chromosome painting techniques in raising the quality of prenatal diagnosis and

disclosing rearrangements not resolved by standard cytogenetics.

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