Down's syndrome phenotype and autosomal gene inactivation in a child with presumed (X;21) de novo translocation

SUMMARY A 3½-year-old female with clinical features of Down's syndrome was found to have extra chromosome material on the long arm of one of the X chromosomes, 46,XXq+. The parental karyotypes were normal. In the light of the clinical features of the proband and the banding characteristics of the extra chromosome material, the patient was thought to have a de novo (X;21) translocation. The results of late replication studies with BUdR and enzyme superoxide dismutase (SOD) assays in the proband suggest that: (1) the presumed (X;21) translocation chromosome was the late replicating chromosome; (2) the spread of inactivation extended from the Xq segment of the translocation chromosome to the proximal part of the segment derived from chromosome 21, leading to the inactivation of the autosomal gene for enzyme SOD; (3) the remaining distal portion of the (X;21) translocation chromosome, a part of a segment presumably derived from chromosome 21, was spared from the spread of inactivation so that this part was still genetically active and responsible for the Down's phenotype; (4) therefore, the main determinants for a Down's phenotype may be located more distally (q22.2 or q22.3 or both) than the SOD gene (q22.1) on the long arm of chromosome 21.

In recent years several examples of balanced and unbalanced X:autosome translocations in man have been reported.1-3 These examples have provided important information concerning gene mapping of the X chromosome and X chromosome inactivation, and have been helpful in interpreting the phenotypic features in unbalanced subjects.

Recently we observed a child with the clinical features of Down's syndrome who had extra chromosome material on the distal end of one of the X chromosomes. Parental karyotypes were normal. After considering the clinical and cytogenetic findings, the extra chromosome material was thought to be the entire long arm of chromosome 21. We report here the clinical, cytogenetic, and biochemical findings in this patient who presumably has an (X;21) unbalanced de novo translocation.

Case report

The proband, a 3½-year-old white female, was referred for evaluation of developmental delay and dysmorphic features. She was the 1790 g, 39 week gestation product of a gravida 1, para 0, 15-year-old female. The pregnancy was complicated with recurrent urinary tract infections. The mother used alcohol and tobacco in small quantities during the pregnancy. The labour lasted ten hours and the delivery was vaginal with vertex presentation. The baby breathed and cried spontaneously. Her immediate neonatal course was uneventful, but her subsequent weight gain was poor. She had several admissions to hospital for repeated diarrhoea, otitis media, and pneumonia. She had two 'febrile' seizures for which she was placed on phenobarbital. Her development was markedly delayed. She smiled at 4 months, turned over at 7 months, walked at 18 months, and was not yet toilet trained. Her vocabulary was limited to ten simple words and she was not able to make sentences. The father, who was 20 years old, and the younger sister, 2 months old, were both in good health. There was no family history of mental retardation and birth defects and consanguinity between the parents was denied.

The physical examination revealed an underdeveloped, hyperactive child in no acute distress (fig 1). Her weight (9·1 kg) and height (88 cm) were below the 3rd centile and the head circumference (47 cm) was on the 25th centile for age. The box-shaped head had frontal prominence and the facial

FIG 1 Proband aged 3½ years.
profile was flat. The eyes had mild mongoloid slanting with bilateral epicanthic folds and hypertelorism (inner canthal distance 3 cm). There were several Brushfield spots on the irides. The nasal bridge was flat, the ears were low set, and the neck was short. She kept her mouth open most of the time. The cardiovascular examination revealed a 2/6 systolic murmur, best heard on the left sternal border. The liver was palpable 3 cm below the right costal margin. The hands were short and broad and the fingers were short with bilateral clinodactyly of the 5th fingers. There was a single flexion crease on the left 5th finger. The distance between the first and second toes was increased bilaterally. Dermatoglyphic examination revealed seven ulnar loops, three whorls, and normally placed axial triradii. Palmar creases were unremarkable, and hallucal patterns were wide distal loops bilaterally.

The normal laboratory studies included electrolytes, Ca, alkaline phosphatase, BUN, creatinine, serum proteins, and T4. The skull x-ray showed increased interorbital distance and the pelvic x-ray was normal. The EEG, CT scan, and EKG were also normal. The psychometric test results were in the mentally defective range (Cattell 46, Peabody 56, Vineland 53, and Verbal 42).

Methods

Chromosome studies were performed on peripheral lymphocyte and skin fibroblast cultures using Giemsa-trypsin banding. X inactivation studies were done on lymphocytes by the BUDR-acridine orange technique of Dutrillaux.

Superoxide dismutase (SOD) was measured by radioimmunoassay using a second antibody system. The sensitivity of the assay was 5 ng/ml SOD with an intra-assay coefficient of variation of 6·1. All specimens were assayed the same day. Heparinised blood was drawn and erythrocytes were separated by centrifugation. Red cells were washed three times in phosphate buffered saline (PBS) and stored frozen until assayed. Before assaying, red cells were lysed at 4°C in 10 volumes of lysing buffer (5 mM Tris-HCl, pH 7·4) for 10 minutes. The lysate was then centrifuged at 10 000 g for 20 minutes at 4°C. The supernatant was diluted to the appropriate dilution for assay. Protein determinations were done according to Bradford.

The fibroblast cell line was stored in the Human Genetic Mutant Cell Repository in Camden, New Jersey as GM 4108.

CYTOGENETIC STUDIES

The proband's modal chromosome number was 46. However, there was extra chromosome material on the long arm of one of the X chromosomes: 46,XXq+. The morphology and the banding characteristics of this extra chromosome material closely resembled the long arm of chromosome 21. The parental chromosomes were normal. Because of

![Fig 2 Giemsa-trypsin banded karyotype of proband showing presumed X;21 translocation (arrow).](image)
the clinical features of the proband and the cytogenetic features of the abnormal chromosome, we presumed that the patient had an X;21 translocation with breakpoints at Xq28 and 21q11 (figs 2, 3). The karyotype was: 46,XY,t(X;21)(q28;q11).

Late replication studies with BUdR revealed that the translocated X chromosome is late replicating and that the translocated material on its long arm is later replicating than the normal chromosomes 21 (fig 4).

**BIOCHEMICAL STUDIES**

Red blood cell SOD levels for the patient were compared with three other normal juveniles. The patient was found to have a slightly lower SOD level than the average for the other three controls. Red cell SOD levels for controls were 2.4, 4.0, and 2.9 µg SOD/mg protein, while that for the patient was 2.7 µg SOD/mg protein. As such, the patient was considered to have a normal SOD level.

**Discussion**

The patient presented here had several clinical features of Down's syndrome. Since neither of the parents carried a balanced X;21 translocation, positive identification of the extra chromosome material as 21 was not possible. Therefore, the interpretation of the extra chromosome material on the long arm of the X chromosome depended heavily on the clinical features of the patient. In order to
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substantiate our clinical impression of Down's syndrome, we tried to quantify the clinical and dermatoglyphic findings using previously described diagnostic indices.

The probability of the patient having Down's syndrome, in that she met 11 clinical criteria set by Jackson et al., was 84%. She fell on the overlapping area in the dermatoglyphic nomogram by Reed et al., with a total score of 75.8. When we used the diagnostic index of Preus, who used a combination of clinical and dermatoglyphic features, the patient scored +1.54 with 95% probability of having Down's syndrome. Based on these findings, in addition to our clinical impression and the banding characteristic of the abnormal X chromosome, it was concluded that the patient had an unbalanced X;21 translocation. In order to confirm this, serum and erythrocyte SOD-1 assays were performed. Since the gene for the enzyme SOD-1 was previously shown to be located on the long arm of chromosome 21, sub-band 22.1, we expected to find increased enzyme activity in the patient's cells. However, the results were normal. Meanwhile the result of the X late replication studies with BUdR revealed that the X;autosome translocation chromosome was late replicating. Furthermore, the extra chromosome material, translocated to the inactivated X chromosome, appeared to be more lightly stained than both free chromosomes 21, presumably indicating the spread of inactivation to the autosomal segment. How can we interpret these data?

Recent studies on the replication pattern of the X chromosome in subjects with X;autosome translocation indicated that, in general, the normal X chromosome in the balanced X;autosome carriers, and the abnormal X chromosome in the unbalanced subjects, are the late replicating X chromosomes. Our findings in BUdR studies support this observation. In addition, it has been shown that either complete or incomplete spreading of inactivation to include the autosomal segment in X;autosome translocations may occur. Phenotypic expression of spreading of inactivation to the autosomal segment in X;autosome translocations has been shown in several cases. Phenotypic expression in unbalanced monosomic or trisomic patients depends on the extent of the inactivation of the autosomal segment on the X;autosome translocation. Reduced phenotypic manifestations of trisomy 21 because of autosomal gene inactivation were recently reported by Couturier et al. in a family where an X;21 translocation was segregating. In this family, one of the children, who was trisomic for almost the entire long arm of chromosome 21, had only a few mild features of Down's syndrome, normal IQ, and moderately increased SOD activity. They explained the reduced Down's phenotype and only moderately increased SOD activity by the spreading of inactivation from the X chromosome to the autosomal segment in a percentage of cells.

The main determinants of the Down's phenotype have repeatedly been shown to be located in the distal euchromatic segment (q22) of the long arm of chromosome 21. Sinet et al. suggested that the precise location of the Down's determinants is on the sub-band 22.1, which is the same location as the SOD gene.

In the light of the available information, one can explain the clinical, cytogenetic, and biochemical findings of our patient in one of the following ways. (1) In the patient reported here, the spread of inactivation was incomplete and involved only the proximal segment of chromosome 21 material (21q22.1) on the X;21 translocation, leading to the inactivation of the SOD gene, but not the more distal sub-bands which were still genetically active, resulting in the Down's phenotype. If this is correct, then one could infer that the determinants for the Down's phenotype are located on sub-bands 21q22.2 or 22.3 or both. (2) The clinical effect or damage of the trisomic state may have been determined before the X inactivation at the blastocyst stage. (3) Finally, it is possible that the extra chromosome segment translocated to the X chromosome is not chromosome 21 material and the unidentified chromosome segment is producing a phenocopy of the Down's phenotype. This possibility seems unlikely because of the banding characteristics of the extra chromosome material, and because of the substantiation of the clinical impression by the diagnostic indices applied.

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A case of inverted insertion assessed by R and G banding

SUMMARY Cytogenetic studies in a 2-year-old boy referred to our laboratory for developmental delay showed an unusual karyotype with a three break rearrangement. R and G banding were both necessary to determine the exact nature of the rearrangement which is described as 46, XY,inv ins(16;3)(q22;p26p13). Several features coincide with the reported description of another patient where 3p26 was missing, and the coincidence is explained as a position effect.

Case report

The proband was the third of three sibs. His parents were healthy and were 26 years old at the time of his birth. His elder brother and sister were aged 3 and 1 at the time. They both have a history of febrile convulsions. Family history is otherwise unremarkable.

The patient was born by breech delivery after 35 weeks’ gestation. Birthweight was 1·39 kg, length 40·6 cm, and head circumference 30·5 cm. The patient spent 2 months in the Special Care Baby Unit. He had mild hyaline membrane disease and jaundice. A right-sided inguinal hernia was repaired at 3 months.

During his first year of life he had numerous admissions to hospital because of recurrent respiratory infections. He also had seborrhoic dermatitis and his development was retarded. X-ray showed only spina bifida of S1. Both little fingers were inwardly curved.

The patient was reassessed at 2 years of age when he showed psychomotor retardation of approximately 6 months. He was able to walk and run with a broad based gait and his vocabulary consisted of 6 to 10 words. His height (75 cm) and weight (10 kg) were less than the 3rd centile. His head circumference was 49·5 cm (50th to 75th centile).

Physical examination showed the following: visible veins over the skull, arched eyebrows, slight mongoloid slant to the eyes, epicanthic folds, broad nasal bridge, long philtrum, low set ears, and notched teeth. He also had divarication of the recti and partial syndactyly of toes 2 and 3. His seborrhoic dermatitis was still present. He had persistent lymphadenopathy in the left posterior cervical triangle for over 12 months. Neurological examination was normal. A cytogenetic investigation was undertaken at this stage.

Cytogenetic studies and discussion

Chromosomes were studied in peripheral leucocyte cultures from the three sibs and their parents. R and G banding were performed in all. Only the index patient showed abnormal chromosomes. All his cells showed insertion of nearly the whole short arm of chromosome 3 into the median region of the
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