Segregation of a t(3;20) translocation through three generations resulting in unbalanced karyotypes in six persons

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SUMMARY With the aid of high resolution chromosome banding, a t(3;20) translocation was discovered in a large family with six retarded members. The translocation involved very small terminal segments. The unbalanced products resulting in partial trisomy 20q and monosomy 3p were observed in the retarded subjects. Gene localisation studies of the ADA gene, with a presumed locus on the long arm of chromosome 20, were also carried out and seem to exclude this gene from the distal part of 20q (20q13.1→qter).

A number of cases of partial trisomy for the short arm of chromosome 20 have been reported,^{1,2} but very few cases have been described with trisomy involving the long arm. The first case of partial trisomy involving 20q was described by Pawlowitzki *et al*³ in a 9 month old girl with multiple anomalies.

We report here the clinical and cytogenetic findings in a family with a t(3;20) translocation segregating in three generations and resulting in partial trisomy 20q and partial monosomy 3p in six persons. Two further subjects with mental retardation and congenital malformations presumably also had unbalanced karyotypes, but had died by the time of the investigation.

This report illustrates the power of high resolution banding techniques in identifying very small chromosomal changes.

ADA (adenosine deaminase) activity in cultured skin fibroblasts and granulocytes from affected and normal members of the family were also investigated. Deficiency of ADA causes an autosomal recessive form of severe combined immunodeficiency. The catalytic activity of the enzyme resides in a single polypeptide encoded by a locus presumed to be on the long arm of chromosome 20.

Family history

The pedigree of the family is shown in fig 1. The

family was first studied by us in 1974, when III.7 asked for genetic counselling before pregnancy because of the family history of mental retardation. Her chromosomes, analysed with standard Q banding, showed a normal female karyotype. The karyotypes of the mentally retarded subjects were also found to be normal at the time. They were screened for possible metabolic diseases by analyses of urinary amino acids and acid mucopolysaccharides and lysosomal enzymes in leucocytes and serum, but no abnormalities were found. Accordingly, we had to advise the woman that although a hereditary disorder was suspected, we were unable to diagnose it. She later gave birth to IV.13, who was retarded. In 1981, the pregnant wife of III.3 asked for amniocentesis and prenatal diagnosis. Review of the family history suggested the possibility of transmission of a chromosomal translocation. The chromosomes of III.3, examined with high resolution banding techniques, revealed a translocation between chromosomes 3 and 20, involving very small chromosomal segments. The translocation could not be detected on the earlier Q banded preparations when these were re-examined.

After this finding, the chromosomes of all living members of the family were studied again with high resolution banding.

Cytogenetic investigations

A modification of the method of Yunis⁴ for high resolution banding was used. The chromosomes

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FIG 1 Pedigree of family.

were banded with GTG, or stained with acridine orange for RBA banding.

The mentally retarded subjects showed an unbalanced karyotype with an abnormal chromosome 3. The normal carriers additionally showed an abnormal chromosome 20. This was interpreted as a balanced reciprocal translocation: $46,XX$ or $XY,t(3;20)(3qter \rightarrow 3p25::20q13.1 \rightarrow 20qter; 20pter \rightarrow 20q13.1::3p25 \rightarrow 3pter)$.

The breakpoint in 3p was considered to be at the interface of 3p25 and 3p26. In GTG banded preparations, the altered banding pattern was clearly visible. The mentally retarded subjects were trisomic for the distal part of the long arm of chromosome 20 and monosomic for a small segment of the distal short arm of chromosome 3.

Amniotic fluid cells from the fetus IV.7 were cultured as usual and examined with QFQ and GTG banding. In GTG banded metaphases of good quality, it was apparent that the fetus had inherited the balanced translocation.

A partial karyotype from a carrier is shown in fig 2.

Enzyme determinations

Human fibroblasts were routinely maintained using standard tissue culture technique and grown in medium 199 with added pooled human serum (20%) and bovine embryo extract (4%). Approximately 1×10^6 cells from each person were trypsinised from near confluent cultures in T25 flasks (Nunc) and washed twice by centrifugation in saline buffer at 6000 g for five minutes. The cell pellets were resuspended in 100 μ l buffer containing 20 mmol/l

Tris-HCl, pH 8.0, 5 mmol/l magnesium acetate, 1 mmol/l dithiothreitol, 1 mmol/l EDTA, 25 mmol/l KCl, 10% glycerol, and 0.2% NP 40. The suspensions were rapidly frozen and thawed six times and centrifuged at 6000 g for five minutes. The supernatant was used for enzyme analysis. Granulocytes were isolated as previously described.⁵

FIG 2 Partial karyotype from carrier showing high resolution banding of chromosomes 3 and 20 (GTG banding). An ideogram shows breakpoints. (Abnormal chromosomes on the right.)

ADENOSINE DEAMINASE (ADA)

Enzyme activity was determined at 37°C by measuring the formation of inosine and hypoxanthine from adenosine. The assay mixture contained in a final volume of 50 µl: 150 µmol/l (8⁻¹⁴C) adenosine (5 µCi/µmol), 25 mmol/l Tris-succinate, pH=7.6, and cell extract 0.1 to 0.3 mg protein/ml. At two minute intervals, 5 µl samples were withdrawn, mixed with 5 µl (5 mmol/l adenosine, inosine, and hypoxanthine), and chromatographed on polyethylenimine impregnated cellulose plates. The chromatograms were developed in boric acid 10 g/l, LiCl 1.5 g/l, pH=7.0. This allowed the separation of hypoxanthine and inosine from adenosine. After drying, the spots were localised under UV light, cut out, and counted in a liquid scintillation spectrophotometer.

PURINE NUCLEOSIDE PHOSPHORYLASE (PNP)

Enzyme activity was determined by measuring the formation of hypoxanthine from inosine. The assay mixture contained 0.5 mmol/l (8⁻¹⁴C) inosine (5 µCi/µmol), sodium phosphate 0.1 mol/l, pH=7.1. Otherwise, the procedures used were as described above for ADA.

5' NUCLEOTIDASE (NUC)

Enzyme activity was determined by a previously described radiochemical method.⁶ The results are shown in tables 1 and 2. Table 1 shows enzyme activities in skin fibroblasts from controls and from one patient with partial trisomy 20q (IV.13) as well

as his carrier mother (III.7). Table 2 shows purine enzyme activities in granulocytes from the same patient, his carrier mother (III.7), and his chromosomally normal father.

In addition, we found it of interest, as an internal control of the method, to show ADA activities from the second Danish family with a child suffering from severe combined immunodeficiency. Heterozygote and homozygote levels in granulocytes are shown, as well as the presumed heterozygote level in amniotic fluid cells.

PNP and NUC were used as control enzymes to ensure that enzyme activity had been conserved in the cells.

Case reports

II.1 was a female born in 1914. Very little information was available. She was mentally retarded, lived in an institution, and died at 7 years of age from meningitis.

II.4 was a male born in 1923. He was the third of five children. Pregnancy and delivery were normal. His development was retarded: IQ was 31 (Binet) at the age of 13 years. He suffered from rachitis in childhood. Physical examination at that age showed small stature (below -2 SD), a small skull, and high arched palate. An umbilical hernia was noted. He lived at home until the age of 48 when he was institutionalised. At the age of 50 he had repeated urinary tract infections. Intravenous urography showed no abnormality. EEG was moderately abnormal. Examination at the age of 59 showed a severely mentally retarded male with a height of 175 cm and arm span of 166 cm. Slight facial dysmorphism was present in the form of a long narrow facies with a long nose and philtrum. The palate was high arched and the ears somewhat low set.

III.5 was a female born in 1947. She was the second of four children. Pregnancy and delivery were normal and birth weight was 3000 g. Development was retarded. Developmental quotient (Bühler-Hetzer) was 47 at 3 years of age. She was institutionalised at 5 years of age as she was difficult to manage at home. At the age of 18, she was noted to be slender with pronounced hypermetropia and was suspected of having Marfan's syndrome or homocystinuria. Analysis of urinary amino acids as well as acid mucopolysaccharides revealed nothing abnormal. When examined at 35 years she was 160 cm tall, weight 50 kg, arm span 145 cm. Her face was slightly dysmorphic with strabismus, deep set eyes, decreased interpupillary distance, narrow bridge of the nose, and protruding front teeth.

TABLE 1 *Purine enzyme levels in human fibroblasts.*

Origin of fibroblasts	ADA (nmol/min/mg protein)	PNP	NUC
<i>Skin</i>			
Control 1	13	19	67
Control 2	15	26	29
Control 3	13	30	32
Control 4	12	40	53
IV.13 (trisomy 20q)	16	28	82
III.7 (translocation carrier)	16	34	75
<i>Amniotic fluid</i>			
Heterozygote for ADA deficiency	9	47	38
Control A	33	40	44
Control B	35	46	58
Control C	22	38	40

TABLE 2 *Purine enzyme levels in granulocytes.*

Origin	ADA (nmol/min/mg protein)	PNP
Trisomy 20q (IV.13)	6.7	61
Father of IV.13	8.1	52
Mother of IV.13	12.2	86
Patient with ADA deficiency	0.6	87
Mother of ADA patient	4.3	57
Father of ADA patient	3.0	53
Controls (n=18)	7.6±1.2	68±20

III.10 was a male born in 1952. He was the third of six children. Birth weight was 4600 g. Anal atresia was immediately recognised and he was operated on when 1 day old. He failed to thrive and at 7 months he died during a further operation. Necropsy showed anal atresia and a hypertrophic left ventricle of the heart, but no other malformations were noted.

III.11 was a female born in 1954. She was the fourth of seven children. Pregnancy was complicated by first trimester bleeding, treated with bed rest. Delivery was normal, birth weight 4450 g, length 54 cm. Development was retarded. She walked at 21 months, spoke single words at 3 years, and sentences at 6 years. She was examined at 3 years, when she showed retarded development, slight facial dysmorphism with broad bridge of the nose, epicanthus, and irregular teeth, and hypermobile joints of the extremities. IQ (Binet) at the age of 10 was 44. She was institutionalised when 10 years old. Puberty and menstruation were normal. When examined at 31 years she had a height of 168 cm, slight facial dysmorphism with slight overbite, high arched palate, epicanthus, and hypermetropia. A dimple was present on the jaw. Neurological examination and hearing were normal.

IV.1 was a female born in 1968. She was the first of two children. Pregnancy was uncomplicated with delivery at term. Birth weight was 3450 g and length 52 cm. Moderate jaundice was present in the neonatal period. Thriving and growth were normal, with length corresponding to +2 SD for age. When examined at 1½ years old, she was noted to be retarded with slight facial dysmorphism. She had ample, coarse hair with a low hairline at the neck and forehead. The bridge of the nose was broad, the tongue large, the ears unremarkable, and the neck short. Slight universal hypotonia was noted. X-ray of the left hand showed normal bone age and x-ray of the skull and pelvis were normal. Psychomotor development was retarded. She walked at 2 years, spoke her first words at 2, and sentences at 5 to 6. At the age of 15, her height was 176 cm and head circumference 54 cm. Speech was well developed and she functioned in the moderately retarded range. Hearing was normal and eye examination revealed bilateral astigmatism.

IV.2 was a male born in 1971. He was the younger brother of *IV.1*. Pregnancy was uncomplicated, with delivery at term, birth weight 4000 g, length 54 cm. Severe jaundice occurred in the neonatal period due to ABO immunisation and he required exchange transfusions. Thriving was poor in the first months

with a tendency to vomiting. His growth curve showed length to be at +2 SD for age with weight at -2 SD for length. He was admitted to hospital when 5 months old for bronchitis and poor thriving and was noted to be developmentally retarded with universal hypotonia. Physical examination revealed normal head circumference and slight facial dysmorphism with ample hair with a low hairline, coarse facial features, large tongue, high arched palate, increased inner canthal distance, epicanthus, and convergent strabismus. The index fingers were noted to be broad distally. His bone age was normal for age. His psychomotor development was retarded: he walked at 22 months and spoke words at 4 to 5 years old. At the age of 11 years, height was 147 cm and head circumference 55.5 cm. He functioned in the severely retarded range with poor speech. Hearing was normal.

IV.13 was a male born in 1978 (fig 3). He was the second of two children. Pregnancy was uncomplicated and delivery normal, birth weight 3550 g, length 56 cm. He was noted to be tall and thin with long limbs. His development was somewhat retarded. He sat at 1 year and walked at 18 to 19 months. He spoke words at 3 years and sentences at 5 years. When examined at 5½ years he was 112 cm tall and weighed 18 kg with a head circumference of 49.3 cm. He was restless and could not concentrate, yet seemed cheerful and cooperative. Psychological

FIG 3 Case *IV.13* aged 5 years.

testing (Leiter) showed that he functioned in the mildly to moderately retarded range. His appearance was dysmorphic with a long thin trunk, long arms, and long thin fingers. The skull was small with ample coarse hair with a low hairline at the neck and forehead. The eyes were deep set and hypermetropia was present. There was a long philtrum, small low set ears, small triangular mouth, and high arched palate. An extra crease on the fifth finger was noted. One testis was undescended, but the external genitalia were otherwise normal. X-ray investigations of the skull, pelvis, and spine were normal. Bone age was slightly retarded. Amino acids in serum and urine and investigations of lysosomal enzymes in leucocytes and serum were normal.

Discussion

The unbalanced karyotypes in our patients were not associated with gross malformations and were compatible with a normal life span. However, two retarded subjects in the family died in childhood before the present investigations, and both probably had unbalanced karyotypes, although they might have had a different form of imbalance from the other retarded family members.

In addition to partial trisomy 20q, the retarded members of this family may be also monosomic for a small segment of 3p. Deletions of 3pter→p25 have often been described with strikingly similar clinical findings,⁷ including some features which were also present in our patients, such as low frontal and nuchal hairline, epicanthic folds, narrow nose, and long philtrum. Features not found in our patients were pre- and postnatal growth retardation and postaxial hexadactyly.

In the present family, where all living members have been karyotyped, six persons showed the same unbalanced karyotype, 13 persons were shown to be carriers, and one (I.2) must have been an obligate carrier. Eight members had a normal karyotype.

The unbalanced gamete, which led to the unbalanced karyotypes observed here, arose from a 2:2 disjunction and adjacent 1 segregation in a carrier. It was shown by Stene and Stengel-Rutkowski,⁸ who analysed reciprocal translocations involving 9p, 10p, and 12p, that the risk of unbalanced liveborn offspring was high for translocations leading to partial short arm trisomies through 2:2 disjunction and adjacent 1 segregation. The risk was of the order of 25 to 29%. They concluded that, in predicting the prospective risk for a reciprocal translocation, a high risk for unbalanced offspring is to be expected, if the resulting chromosomal imbalance is relatively small and can occur through a

common disjunction/segregation mechanism. This is the case in the present family who have shown a high risk for unbalanced offspring.

In our family, only one type of imbalance was found. The other gamete resulting from adjacent 1 segregation would have resulted in combined partial trisomy 3p and monosomy 20q. The genetic imbalance which might arise from adjacent 2 segregation or 3:1 disjunction is far greater and probably not compatible with survival beyond early fetal development. The ratio of balanced carriers to chromosomally normal subjects of 14:8 is not significantly ($p > 0.01$) in excess of the expected 1:1. The sex ratio among carriers was 1:1.

The enzyme adenosine deaminase (ADA) was assigned by Tischfeld *et al*⁹ to chromosome 20 by somatic cell hybrid studies. Further attempts have been made to map the intrachromosomal position of the ADA locus. Using an X;20 translocation and somatic cell hybrids, Mohandas *et al*¹⁰ showed the locus to be in the cen→qter region, and in an attempt to pinpoint the localisation further, Philip *et al*¹¹ studied the enzyme activity in a case of del(20)(q13-2→qter) and showed the activity to be in the heterozygous range, indicating the locus to be in the deleted fragment. The studies of Rudd *et al*¹² seem to disagree with this, as they found increased activity in a patient with a presumed trisomy for the short and proximal long arm (proximal to q11.3) of chromosome 20. They interpreted this as evidence for a gene dose effect, suggesting that the patient had three copies of the ADA gene and that the locus was proximal to q11.3.

The studies of Honig *et al*¹³ confirm the localisation of ADA to 20q13.2→qter.

Our investigations of heterozygotes from a family with ADA deficiency indicate that the assay is valid for gene dosage studies, and the results in a trisomic subject seem to exclude the ADA locus from the distal part of 20q (2q13.1→qter).

The apparent disagreement among several investigators might be explained by difficulties in exact localisations of breakpoints, or it could be due to the localisation of the ADA gene to band q12 which is between the previously suggested localisations.

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