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New assignment of the adenosine deaminase gene locus to chromosome 20q13·11 by study of a patient with interstitial deletion 20q

MICHAEL B PETERSEN*, LISBETH TRANEBJÆRG†,
NIELS TOMMERUP*, PER NYGAARD‡, AND HELLE EDWARDS†

*From *the Department of Medical Genetics, The John F Kennedy Institute, Glostrup; †Department of Paediatrics, Hvidovre Hospital, Hvidovre; and ‡University Institute of Biological Chemistry B, Copenhagen, Denmark.*

SUMMARY A karyotype 46,XY,del(20)(q11·23q13·11) was found in a three year old boy with mental and growth retardation, low set ears, broad nasal bridge, and macrostomia. Adenosine deaminase (ADA) activity was reduced by about 50%, assigning the gene locus to the deleted segment. A review of the previously reported regional assignments suggests that the ADA gene is in the region of band 20q13·11.

Long arm deletion of chromosome 20 (20q–) has been reported as a marker in certain haematological diseases like polycythaemia vera.[1] Constitutional 20q–, however, is extremely rare. Only one case of terminal deletion 20q has been published previously.[2]

The gene locus for the enzyme adenosine deaminase (ADA) has been assigned to chromosome 20,[3] and there are several reports on the regional localisation of the gene on 20q.[4–11] Purine nucleoside phosphorylase (PNP), another purine enzyme measured as a control, has been localised on chromosome 14.[12]

We present a case of interstitial deletion 20q where the ADA activity was investigated.

Methods

QFQ and GTG banding of chromosomes from peripheral lymphocyte and skin fibroblast cultures was performed in the usual way. High resolution banding was performed from RBA banded prometaphases using methotrexate synchronised peripheral lymphocyte cultures.[13]

ADA activity was determined by measuring the formation of inosine and hypoxanthine from adenosine by a radioisotopic method, as described previously.[11] ADA was measured in skin fibroblasts and in granulocytes, which were isolated from peripheral blood.[14] To ensure that enzyme activity had been conserved in the cells, PNP activity was determined by measuring the formation of hypoxanthine from inosine as described before.[11]

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Case report

The proband (fig 1) was the first child of non-consanguineous, healthy parents, the mother being 23 and the father 34 years of age at the time of birth. The father had a healthy daughter from a previous marriage. The pregnancy and delivery were uneventful, birth weight 2940 g, length 48 cm, and head circumference 32 cm. Moderate neonatal jaundice was treated by phototherapy.

Growth was markedly retarded, following the 3rd centile up to the age of one year, after which increasingly slow weight gain became obvious. At four years of age the weight was 13 kg and the height 90 cm, both well below the 3rd centile. Developmental milestones were markedly delayed. He sat at 15 months, walked alone at 19 months, and at three years four months he knew a few words, but had no speech.

Mild facial dysmorphism was noted including low set ears, broad nasal bridge, and macrostomia. A discrete heart murmur was heard, but ECG and chest *x* ray were normal and no symptoms of cardiac disease were present. At the age of three years a convergent strabismus was noted.

Febrile seizures occurred at three and a half years and since then prophylactic valproate therapy has

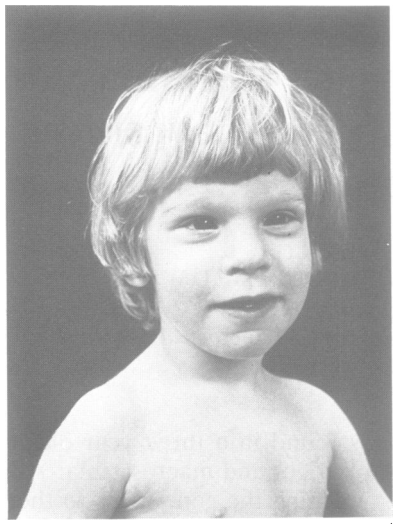

FIG 1 *The proband at three years of age.*

been given with no further seizures. EEG was normal. A cranial CT scan at the age of five and a half years was normal. No history of recurrent infections was recorded.

The bone age was retarded and was equivalent to three years when the biological age was five years two months. Clonidine test showed a normal concentration of growth hormone. The concentration of somatomedin C in serum was 5·4 nmol/l (normal 5·7 to 26·4 nmol/l) at five years two months.

The following laboratory tests were normal: TSH, T3, T4, creatinine, and urea. No excretion of amino acids or organic acids was demonstrated.

CYTOGENETIC FINDINGS

At the age of two years, conventional chromosome analysis of the proband was performed at another laboratory with the finding of a normal karyotype. At three years of age the patient was referred for chromosome analysis (fig 2) to our laboratory because of growth and mental retardation and slight facial dysmorphism. QFQ, GTG, and RBA banded chromosomes from peripheral lymphocyte cultures showed a deletion of the long arm of chromosome 20. A total of 27 cells was examined and in one cell a very small, metacentric, extra chromosome was found. A total of 19 skin fibroblasts was examined and no cells had an extra chromosome, but all cells showed the 20q deletion. With the three banding techniques the distal part of the deleted chromosome appeared normal with an intact band q13·2, but with the q12 band missing. High resolution banding revealed that bands q11·22 and q13·2 were probably intact. The karyotype was interpreted as 46,XY,del(20)(pter→q11·23:q13·11→qter).

The chromosomes of the mother were normal, but it was not possible to obtain the cooperation of the father. Thus, although the chromosomal findings in the proband were compatible with an interstitial deletion, we cannot totally exclude that the aberrant chromosome 20 was a derivative chromosome of paternal origin.

ENZYMATIC FINDINGS

Table 1 shows purine enzyme activities in skin fibroblasts from the proband (mean of three measurements), his mother (mean of two measurements), and controls ($\bar{x}\pm2$ SEM).

Table 2 shows purine enzyme activities in granulocytes from the proband, his mother, and controls ($\bar{x}\pm2$ SEM). This table also shows the activities from

TABLE 1 *Purine enzyme levels in skin fibroblasts.*

Origin	*ADA (nmol/min/mg/protein)*	*PNP*
Proband	6	35
Mother	14	60
Controls (n=15)	20±6	38±9

TABLE 2 *Purine enzyme levels in granulocytes.*

Origin	*ADA (nmol/min/mg/protein)*	*PNP*
Proband	4	46
Mother	8	45
Controls (n=14)	8±1	73±12
Mother of ADA patient	4	57
Father of ADA patient	3	53
Patient with ADA deficiency	<1	87

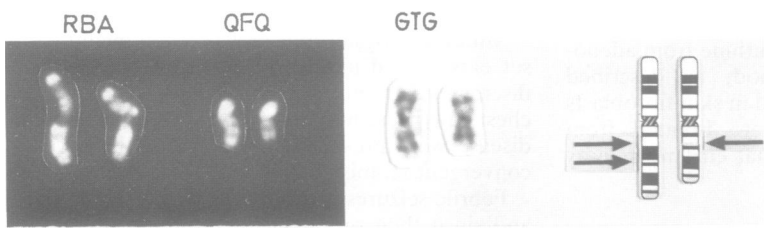

FIG 2 *Chromosomes 20 from the proband showing 20q deletion. Arrows in diagram show deletion breakpoints.*

a Danish family with a child suffering from severe combined immunodeficiency disease with ADA deficiency.

In both skin fibroblasts and granulocytes the ADA activity of the proband was about half the normal value, thus suggesting that he was hemizygous for the normal ADA gene. His PNP values were within the normal range.

Discussion

Anomalies involving chromosome 20 are rare and only 20p duplications have been reported several times.[15] A case of long arm deletion of an F group chromosome was reported before the banding era.[16] The patient was a 69 year old mentally retarded man, height 147 cm, with microcephaly, flat occiput, slanting eyes, epicanthic folds, and hypogonadism. Mosaicism 46,XX/45,XX,–20 was described in a phenotypically normal 37 year old woman with two normal children, two stillbirths, and a baby who died neonatally, all three grossly malformed.[17] Monosomy 20 was found in 25% of her lymphocytes.

Ring chromosome 20 with minor terminal deletions of both the short and long arms has been reported in a few cases.[15] These patients had various dysmorphic features, no severe malformations, normal growth, and rather late onset of epilepsy with mental retardation.

In reviewing published reports, we have found only one case of long arm deletion of chromosome 20 verified by banding techniques. This was reported by Fraisse *et al,*[2] who described a three month old boy with mental and growth retardation, epilepsy, upward slanting palpebral fissures, hypoplastic, nasal bridge, bulbous nose, long philtrum, microretrognathia, and aplasia of the middle phalanx of the fingers and toes. The deletion was de novo: 46,XY,del(20)(q13→qter).[2] We found, however, that the banding quality of the illustration does not allow further sublocalisation of the breakpoint to 20q13·2.[7] They found clinical manifestations similar to those characterising the ring 20 syndrome and thus concluded that the common symptoms were due to the partial terminal deletion of 20q. The only symptoms in common with our case are mental and growth retardation, but with different segments of 20q being deleted this is not surprising.

ADA is an enzyme that catalyses the deamination of adenosine to inosine. An isozyme polymorphism was demonstrated by Spencer *et al,*[18] who found three genetically determined phenotypes: ADA 1, ADA 2–1, and ADA–2, suggesting that the ADA phenotype is determined by two common alleles at an autosomal locus.

ADA deficiency is the cause of one form of severe combined immunodeficiency disease, first reported by Giblett *et al,*[19] which is inherited as an autosomal recessive disorder. Carrier detection is possible through measurement of ADA activity,[20] the carriers having activity decreased to about 50% of normal (table 2). We found ADA activity reduced by about 50% in our patient, but since we could not obtain cells for ADA activity analysis from the father, the decreased activity in the proband could in theory be due to an inherited silent allele. The gene frequency of such inactive alleles is unknown but must be very low. An estimate can be given by the fact that only one case of incompatibility of ADA phenotype was found among 4500 mothers and children investigated in a Danish series (J Dissing, 1986, personal communication). In that case the mother had phenotype ADA 2 and the child ADA 1, and both showed reduced enzyme activity. Therefore, the mother and her child appeared to be heterozygous for an inactive allele at the *ADA* locus. Thus, the gene frequency may be as low as 10^{-4}, suggesting that the decreased activity found in our patient was probably due to the visible deletion of 20q.

Tischfield *et al*[3] assigned *ADA* to chromosome 20 by demonstrating positive correlation between ADA expression and the presence of chromosome 20 using mouse-human hybrids. Aitken and Ferguson-Smith[4] found normal ADA levels in two patients with deletion and duplication, respectively, of the same region (pter→p11) of chromosome 20 and therefore assigned the *ADA* locus to 20p11→qter. Van der Weyden *et al*[5] described normal activities of ADA in cells of peripheral blood from three patients with polycythaemia rubra vera and deletions varying in size from one-third to two-thirds of the long arm of chromosome 20 in bone marrow preparations. They suggested that the ADA gene is not localised on distal 20q. However, deletion breakpoints were not described. Mohandas *et al*[6] assigned *ADA* to 20q, as a somatic cell hybrid containing a translocated chromosome X;20 (20pter→cen→Xqter) with no other chromosome 20 material did not express ADA. Fraisse *et al*[2 7] assayed the ADA activity in a patient with del(20)(q13→qter) and observed activity reduced by 50% in the patient compared to the parents and normal controls, thus assigning the *ADA* locus to the deleted fragment. Rudd *et al*[8] described an infant with an extra small chromosome, which was shown to be a number 20 with a deletion of the distal end of the long arm. The ADA activity of this patient was increased compared to controls, suggesting that she had three copies of the ADA gene. The breakpoint in the deleted chromosome was

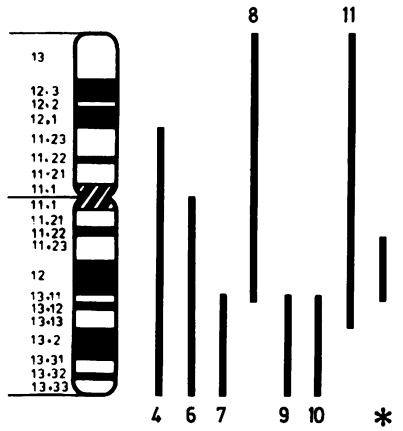

FIG 3 *Diagram showing reported regional assignments of the ADA locus to chromosome 20. Numbers refer to references. *Present case.*

at 20q11·3 according to the nomenclature of Francke,[21] which in high resolution banding nomenclature[22] corresponds to 20q13·11. This assigns *ADA* to 20pter→q13·11. Using somatic cell hybrids containing translocations involving human chromosome 20, *ADA* was localised to 20q13·1→qter by Mohandas *et al*[9] and to 20q13→qter by Honig *et al.*[10] Nielsen *et al*[11] described normal ADA activity in a patient trisomic for the distal part of 20q due to an unbalanced translocation t(3;20) and in this way excluded the *ADA* locus from 20q13·1→qter.

Our case of reduced ADA activity in a patient with del(20)(q11·23q13·11) assigns *ADA* to the deleted segment. When mapping the reported localisations of the *ADA* locus where band numbers were described,[4 6–11] the smallest region of overlap is found in band 20q13·11 (fig 3), suggesting that the *ADA* locus is in the region of this band, instead of the previous regional assignment to 20q13·2→qter.[23]

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Correspondence and requests for reprints to Dr Michael B Petersen, Department of Medical Genetics, The John F Kennedy Institute, Gl Landevej 7, DK–2600 Glostrup, Denmark.