ASSIGNMENT OF ABO LOCUS TO 9q31.3→qter BY STUDY OF A FAMILY IN WHICH AN INTRACHROMOSOMAL SHIFT INVOLVING CHROMOSOME 9 IS SEGREGATING

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Summary A family in which an intrachromosomal shift, *dir ins*(9)(q22.1q31.3;q34.3), was segregating is described. A meiotic crossing over in the noninsertional loop of the carrier mother appeared to have resulted in the proband with a recombinant chromosome 9 duplicating the interstitial segment 9q22.1→q31.3. The study of ABO and AK₁ phenotypes in the family showed that the proband was also a recombinant for the ABO locus. These results allowed us to assign the ABO locus to 9q31.3→qter on the cytogenetic basis.

INTRODUCTION

The determination of the locus for ABO blood group (ABO) on 9q34 has been founded on the linkage studies in families (Robson *et al.*, 1977; Cook *et al.*, 1978). The lack of the deletion mapping for ABO is due to the unavailability of a patient with monosomy for the distal portion of the long arm of chromosome 9 with an informative genotype of ABO. In Human Gene Mapping 8 (1985), cytogenetic evidence for the assignment of ABO to 9q34 has been presented by Allderdice *et al.* (1985), who have analyzed ABO, AK₁ (adenylate kinase 1) and ORM (orosomucoid) in kindreds in whom a specific rearrangement (an inverted insertional translocation of 9q34.1→q34.3 into 9q22.1) was segregating. Their data suggest that ABO maps to 9q22.1→q34.1, AK₁ to 9q34.1→q34.3 and ORM 9q34.3→qter. Similarly, we saw a family in which the proband was found to have a recombinant chromosome deriving from a maternal intrachromosomal shift, *dir ins*(9)(q22.1q31.3;q34.3). The segregation study of ABO and AK₁ in the family indicated that ABO can be assigned to 9q31.3→qter.

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MATERIALS AND METHODS

Case report. The proband, a 5 year 5 month-old girl, was referred to us for evaluation of short stature. Family history (Fig. 1) showed early trimester spontaneous abortions in the mother (II-3) and maternal grandmother (I-2), and low birth weight and short stature in her cousin (III-2). The parents and sibling were healthy. The patient was born after uneventful 40 weeks' gestation, when the mother was 22 years old and the father 28. Her birth weight was 2,430 g, and her length 46.0 cm. At two months of age, her dislocation of the right hip joint was conservatively treated. Although her psychomotor development was normal, the physical growth was markedly retarded. At the age of 5 years and 5 months, she measured only 85.5 cm, with the body weight of 10.2 kg and the head circumference of 44.3 cm (all figures far less than the 3rd percentile). Her short stature was proportionate. She was found to have hypertelorism with epicanthic folds, but otherwise appeared normal. Her IQ was estimated to be 92 (Tanaka-Binet Intelligence Scores). The bone age (2 years and 2 months) was much delayed for the chronological age, but endocrinological studies yielded normal results. Dermatoglyphic study showed digital patterns consisting of U.U.W.W.U on the left hand and U.U.W.W.U on the right, a low total finger ridge count (82), and bilateral simian creases. The main line formulae were 9.0.5".1-t"-A^u.O.O.O.O on the left palm and 9.7.5".1-t"-A^u.O.O.O.L^d on the right.

Cytogenetic study. Chromosome analyses were performed on cultured peripheral blood lymphocytes from the patient, sibling, parents and maternal grandparents. Cells in prophase and prometaphase were collected according to the method of Ikeuchi and Sasaki (1979). Chromosome preparations were processed by G-, Q-, R- and C-banding techniques.

Fig. 1. The family pedigree.

Gene marker studies. Blood group typing of freshly collected erythrocytes was carried out using the standard agglutination test. Erythrocyte activities of galactose-1-phosphate uridyl transferase (GALT) and adenylate kinase (AK) were assayed by the method of Beutler (1975). Starch gel electrophoresis was used to determine an AK phenotype, as described by Harris and Hopkinson (1976).

RESULTS

Cytogenetic study

The karyotype of the patient showed an extra amount of chromosome material on the terminal portion of the long arm of a chromosome 9 (Fig. 2, a). The mother was found to have an intrachromosomal shift of the interstitial segment of the long arm of chromosome 9 into its own distal end (Fig. 2, b). From the G- and R-banding patterns, the most pertinent interpretation of the rearrangement is a direct insertional translocation of the segment 9q22.1→q31.3 into the long arm at band 9q34.3. The abnormal chromosome of the patient seemed to be a recombinant resulting from a crossing over during meiosis in the mother. The karyotype of the patient was therefore 46,XX,rec(9),dup(q22.1→q31.3), dir ins(9)(pter→q22.1::q31.3

Fig. 2. The partial G- and R-banding karyotypes of the patient (a) and the mother (b). As depicted in the diagram of the inserted chromosome 9 of the mother [ins(9)], the interstitial segment 9q22.1→q31.3 (bracket) was transposed into 9q34.3 (arrow).

$\rightarrow q34.3::q22.1 \rightarrow q31.3::q34.3 \rightarrow qter$)mat. The father and sibling had normal chromosomes.

The sequential Q- and C-banding analysis showed that the inserted chromosome 9 of the mother contained a partially inverted C-heterochromatic region of 9q (9qh), while the normal homologue had a common type of 9qh heteromorphism (Fig. 3). The father and sibling had a common type of 9qh heteromorphism in both chromosomes 9. The 9qh heteromorphism in the recombinant chromosome 9 of the patient was identical to that in the normal chromosome 9 of the mother.

As shown in Fig. 1, the maternal grandfather (I-1) was also found to possess the insertional translocation. Although chromosome study was unavailable for the uncle (II-1) and the cousin (III-2), the phenotypic similarity between the patient and the cousin suggested that the uncle was a probable carrier of the insertional translocation and the cousin the recombinant chromosome.

Gene marker studies

Results of blood group typing in the available family members were given in Table 1. The affirmative probability of parentage on the patient was calculated to be 0.921. The family was informative for the segregation of ABO blood group. The father was homozygous (genotype OO), while the mother heterozygous (genotype AO). Since the maternal grandfather (a carrier of the shift) had a phenotype

Fig. 3. The partial C-banding karyotypes of the father (a), mother (b), sibling (c) and patient (d). Sequentially stained chromosomes 9 of the mother were shown in the hatched circle, and another pair of chromosomes 9 right to this circle.

Table 1. Results of blood group typing.

		Blood groups			
Maternal grandfather	O				
Maternal grandmother	A				
Father	O	MNss	P1	Le(a-b+) CcDEe kk Kp(a-b+) Fy(a+b-) JK(a+b+) Di(a-b+) Xg(a+) Jr(a+)	
Mother	A	MMss	P2	Le(a-b-) CcDEe kk Kp(a-b+) Fy(a+b+) JK(a+b-) Di(a-b+) Xg(a+) Jr(a+)	
Patient	O	MMss	P2	Le(a-b+) CcDEe kk Kp(a-b+) Fy(a+b+) JK(a+b+) Di(a-b+) Xg(a+) Jr(a+)	
Sibling	A	MMss	P2	Le(a-b+) CcDEe kk Kp(a-b+) Fy(a+b+) JK(a+b+) Di(a-b+) Xg(a+) Jr(a+)	

O and the grandmother A, the inserted chromosome 9 and the normal homologue of the mother must contain gene O and A, respectively. The blood type of the two children (the patient O and the sibling A) suggested that the patient was a recombinant as regards the ABO locus, too. It was reported that the uncle had a phenotype O.

The GALT and AK activities of erythrocytes from the patient were within the normal ranges (GALT: 20.2 U/g Hb, normal 20.1 ± 2.4 U/g Hb, AK: 258.2 U/g Hb, normal 232.6 ± 42.1 U/g Hb). Electrophoresis of hemolysates for AK isozyme showed that the patient and parents had an AK₁ phenotype 1.

DISCUSSION

The present study has provided another cytogenetic evidence for the physical assignment of the ABO locus to the distal 9q. As depicted in Fig. 4, the chromosomes 9 of the carrier mother are predisposed to the meiotic crossing over in the noninsertional loop, which is much longer than the insertional loop, and consequently appears to result in the recombinant chromosome seen in the patient. The preservation of the same 9qh heteromorphism in the recombinant chromosome of the patient as in the normal chromosome 9 of the mother suggests that there is no (or less likely an even number of) crossing over in the segment from the 9qh to the origin of the insertional loop (q22.1). The mean chiasma frequency on the long arm of chromosome 9 is reported to be 1.28 (Laurie *et al.*, 1982). This, together with the observation that the patient was a recombinant for the ABO locus, leads

Fig. 4. Schematic representation of the meiotic pairing and crossing over in the mother which produce the cytogenetic and gene marker's phenomena seen in the patient. The most reasonable explanation is that a single crossing over has occurred proximally to the ABO locus within the noninsertional loop.

us to assume that the ABO locus must be distal to the site of the meiotic crossing over.

The assignment of the ABO locus to the distal 9q was first established by the linkage study of AK₁ locus (Robson *et al.*, 1977), which was mapped to 9q34 by studies of somatic cell hybridization (Westerveld *et al.*, 1976) and gene dosage effects (Ferguson-Smith and Aitken, 1978). Cook *et al.* (1978), studying the segregation of ABO and AK in families with various abnormalities of chromosome 9, demonstrated that these loci have to be near the end of the long arm of chromosome 9. Two other loci, ALDA (aminolevulinate dehydratase) and ORM, have been found to be linked with ABO or AK₁ (Eiberg *et al.*, 1981; Eiberg *et al.*, 1983), and on the basis of the lod scores the gene order, ABO-AK₁-ALDA-ORM is suggested. The proposition of Allderdice *et al.* (1985) that ABO maps to 9q22.1→q34.1, AK₁ to 9q34.1→q34.3 and ORM to 9q34.3→qter is in good agreement with this gene order. In consideration of the proposition of Allderdice *et al.* (1985), we would like to conclude on the cytogenetic basis that the ABO locus lies within the region 9q31.3→q34.1. This physical assignment is not contradictory to the result of the genetic assignment that the distance between ABO and ORM is 27 centimorgans (cM) in males (Eiberg *et al.*, 1983), whose genetic length of the whole 9q is presumed to be 65 cM (Norton *et al.*, 1982).

Finally, individuals with a recombinant resulting from an intrachromosomal shift will show 'pure' deletion or duplication of the inserted segment. As far as we are aware, the present case is the first instance of pure trisomy for 9q22.1→q31.3. We believe that triplication of this chromosome segment produces few phenotypic abnormalities.

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