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Genetics

Assignment of the AK_1:*Np*:*ABO* linkage group to human chromosome 9

(somatic cell hybrids/enzyme markers/gene localization)

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ABSTRACT **In man–Chinese hamster somatic cell hybrids the segregation patterns of the loci for 25 human enzyme markers and human chromosomes were studied. The results provide evidence for the localization of the gene for adenylate kinase-1 (AK_1) on chromosome 9. Since the loci for the ABO blood group (ABO), nail-patella syndrome (Np), and AK_1 are known to be linked in man, the *ABO*:*Np*:AK_1 linkage group may be assigned to chromosome 9.**

In man several electrophoretically separable isoenzymes for adenylate kinase (AK; EC 2.7.4.3) have been described (1). On zymograms Van Cong *et al.* (2) have identified four isozymes of AK in various human tissues, designated as I, II, III, and IV. The isozyme III represents the only form present in the red blood cells. This isozyme is polymorphic in most of the human populations and is known as AK_1. AK_1 polymorphism is determined by a pair of alleles (AK_1^1 and AK_1^2) occurring at an autosomal locus (3). The occurrence of rare alleles (AK_1^3, AK_1^4, and AK_1^5) was also reported in different populations (4–6).

AK_1 was found to be linked to the *ABO* blood group locus and to the locus for the nail-patella syndrome (Np) in man (7–9). Thus an assignment of one of these loci to a specific chromosome will assign this linkage group to a specific chromosome.

The isozyme II described by Van Cong *et al.* (2) was shown to be determined by an independent gene segregating from the gene for AK_1 in man–rodent hybrids. This locus, which is called AK_2, is assigned to chromosome 1 (10). Until now the AK_1 locus was not assigned to a specific chromosome.

This report deals with the evidence for the assignment of AK_1 to chromosome 9. The segregation patterns of 25 human enzyme markers including AK_1 were related to the presence or absence of human chromosomes in man–Chinese hamster cells.

MATERIALS AND METHODS

Hybrid cell lines were obtained by fusion of Chinese hamster mutant cells with human fibroblasts or leukocytes. The isolation and growth characteristics of the Chinese hamster cell lines a3 and a23, both thymidine kinase deficient, and wg3-h, hypoxanthine guanine phosphoribosyl transferase (HPRT) deficient, were described earlier (11). The human fusion partners involved were normal fibroblasts, HPRT-deficient fibroblasts derived from a patient with the Lesch-Nyhan syndrome, or human leukocytes. The human leukocytes were obtained from peripheral blood of male and female donors. The details on production, isolation, and propagation of these hybrids have been published elsewhere (11).

In the hybrid and parental cell populations the following enzymes were analyzed by means of Cellogel electrophoresis: glucose-6-phosphate dehydrogenase (G6PD); phosphoglycerate kinase (PGK); α-galactosidase-A (α-Gal A); lactate dehydrogenases (LDH-A, LDH-B); 6-phosphogluconate dehydrogenase (6PGD); phosphoglucomutases (PGM_1, PGM_3); superoxide dismutases (SOD-1, SOD-2); NAD-dependent malate dehydrogenase, supernatant form (MDH-1); NADP-dependent isocitrate dehydrogenase, supernatant form (IDH-1); cytoplasmic malic enzyme (ME_1); glucose phosphate isomerase (GPI); adenylate kinase-1 (AK_1); glutamate oxaloacetate transaminase (GOT); adenosine deaminase (ADA); peptidases (Pep-B, Pep-C); hexosaminidases (Hex-A, Hex-B); purine nucleoside phosphorylase (NP); mannose phosphate isomerase (MPI); pyruvate kinase (PK-3); and aconitase (ACO). The methods used for the characterization of these enzymes as well as for the preparation of cell lysates have been described elsewhere (12, 13). Chromosomes were analyzed as described before (14, 15).

For the recognition of specific human chromosomes the preparations were stained with the Giemsa-11 technique (16). The staining time for an optimal differentiation between Chinese hamster and human chromosomes is between 15 and 20 min. The air-dried preparations were stored for seven days at room temperature before staining.

RESULTS

To define adenylate kinase isoenzymes expressed in different cell types electrophoresis was carried out with lysates from cultured human fibroblasts, man–Chinese hamster cell hybrids, and red and white blood cells (Fig. 1). Red blood cells from a heterozygote of AK_1 with the phenotype 2-1 (channel 2) show an electrophoretic pattern similar to that of white blood cells and fibroblasts obtained from the same individual (channels 4 and 6). The homozygote whose red cell AK phenotype was known to be 1 exhibits one band for the three different cell types (channels 1, 3, and 5). The electrophoretic pattern of AK in the hybrids is similar to that of a mixture of human and Chinese hamster cell lysates, indicating that no heteropolymeric molecules of AK are produced in the hybrid cell.

The segregation pattern of AK_1 together with the other markers mentioned in *Materials and Methods* is shown in Table 1. No concordant segregation for AK_1 and the other enzyme markers studied was found. Thus the expression of human AK_1 is not related to the presence of any of the other markers investigated. The table also shows that AK_1 is absent more often than any other marker tested in these hybrids.

Abbreviations: AK_1, adenylate kinase-1 or red cell adenylate kinase; ABO, ABO blood group; Np, nail-patella syndrome.

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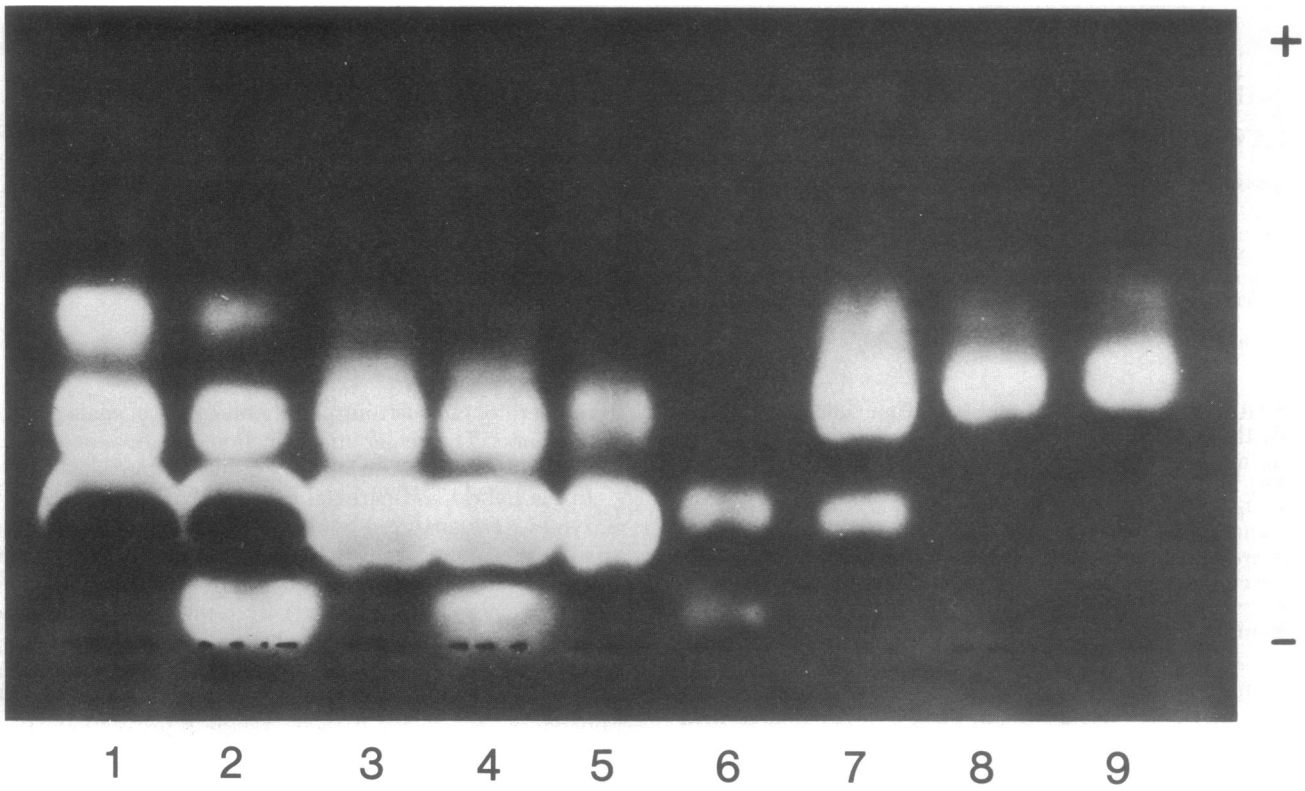

FIG. 1. Electrophoretic patterns of adenylate kinase in the erythrocytes (channels 1 and 2), leukocytes (channels 3 and 4), and fibroblasts (channels 5 and 6) of man, man–Chinese hamster cell hybrids (channels 7 and 8), and Chinese hamster fibroblasts (channel 9) on Cellogel. The red cells, white cells, and fibroblasts, respectively, of channels 1, 3, and 5 are from donors whose red cell AK_1 phenotype was already known to be 1, while those of channels 2, 4, and 6 were obtained from an individual whose red cell AK_1 was originally found to be 2-1. The hybrid in channel 7 is positive and that in 8 is negative for AK_1. Electrophoresis was performed according to van Someren *et al.* (13). In this procedure the zones of AK activity are seen as white bands against blue background.

The chromosome data of Table 2 suggest that the presence or absence of AK_1 does not correlate with the presence or absence of any human chromosome except no. 9. The data from primary clones included in Table 2 are separated and shown in Table 3. Of the 42 primary clones 24 had retained AK_1 and chromosome 9, 17 clones had lost AK_1 and chromosome 9, and one clone was found which did express human AK_1 but had no recognizable chromosome 9. No clone was found which had an intact chromosome 9 and lost human AK_1.

Table 1. Synteny relationships between AK_1 and 14 enzyme markers

Other marker	AK_1/Marker, number of clones			
	+ +	+ −	− +	− −
G6PD	23	22	101	138
LDH-A	29	14	91	123
LDH-B	29	14	84	129
6PGD	22	20	60	106
PGM_3	15	30	19	144
SOD-1	36	12	116	111
MDH-1	24	23	27	152
GPI	33	16	105	122
GOT	8	30	25	179
ADA	33	16	75	151
Hex-A	16	28	60	104
Hex-B	20	24	53	113
NP	29	9	53	97
ACO	8	26	20	38

For well-established synteny groups data of only one marker are presented. ++ means both markers are present; + − means AK_1 present, but the other absent; − + means AK_1 absent, whereas the other marker is present; − − both markers are absent. Data were obtained from primary as well as secondary hybrid clones.

To check the concordant segregation between AK_1 and chromosome 9, 22 randomly selected clones, and the two clones which were positive for human AK_1 but did not contain a human chromosome 9 as shown by analyzing quinacrine or trypsin-Giemsa stained preparations (Table 2), were investigated with the Giemsa-11 (G-11) technique. With this technique (16) the human chromosome 9 can be identified by the bright red secondary constriction. In the present study this technique was found to be useful in identifying human chromosomes in man–Chinese hamster hybrids (Fig. 2). Chinese hamster chromosomes are stained violet or magenta, whereas the human chromosomes are blue and the G-11 positive material at sites mentioned by Bobrow (16) is red. The human chromosomes which have no stained red dots by this technique can be differentiated from the Chinese hamster chromosomes by their lighter color. Also with the G-11 method the AK_1 chromosome 9 concordance was observed (Table 4). Thirteen clones had retained chromosome 9 and expressed AK_1; 9 clones had lost both the marker and chromosome 9. The two exceptional clones of Table 2 were found to be negative for an intact chromosome 9 also with the G-11 technique. However, with this technique it was shown that these two hybrid lines did contain interspecific man–Chinese hamster translocation chromosomes. The human pieces of these interspecific chromosomes could not be identified.

Table 2. Relationships between presence and absence of human AK_1 and human chromosomes

Chromosome	Chromosome/AK_1, number of clones			
	+ +	+ −	− +	− −
1	5	15	12	36
2	4	0	12	46
3	5	19	12	28
4	2	3	13	42
5	8	10	8	35
6	5	31	11	16
7	4	6	12	41
8	8	27	8	19
9	34	0	2	57
10	2	5	13	42
11	11	30	6	21
12	12	19	23	27
13	2	2	14	44
14	8	6	9	41
15	5	15	11	32
16	6	19	10	25
17	12	24	3	22
18	8	10	8	24
19	10	21	6	5
20	9	20	7	9
21	12	45	5	33
22	6	38	1	9
X	2	28	10	33

Data were obtained from primary as well as secondary hybrid clones. ++ means both AK_1 and chromosome are present; + −, chromosome present, AK_1 absent; − +, chromosome absent, AK_1 present; − −, AK_1 and chromosome absent.

DISCUSSION

The electrophoretic patterns of adenylate kinase in red cells, white cells, and fibroblasts derived from an individual heterozygous for red cell *AK* (AK_1) were found to be identical (see Fig. 1). It is very unlikely that an individual heterozygous for AK_1 is heterozygous also at another *AK* locus which is expressed in fibroblasts and white blood cells and exhibits the same electrophoretic behavior. Therefore, the most cathodal band seen in the fibroblasts appears to be coded by the same locus as that for the red cell *AK*, or AK_1.

The loci for the enzymes presented in Table 1 are either firmly or tentatively assigned to particular chromosomes. From the table it can be concluded that the AK_1 is not syntenic with any of the loci for the tested enzymes. Therefore, the localization of AK_1 to one of the chromosomes of the following assignments: X (*G6PD*); 1 (PGM_1); 2 (*MDH-1*); 2 or 3 (*ACO*); 5 (*Hex-B*); 6 (PGM_3); 10 (*GOT*); 11 (*LDH-A*); 12 (*LDH-B*); 14 (*NP*); 15 (*Hex-A*); 19 (*GPI*); 20 (*ADA*); and 21 (*SOD-1*); can be excluded. For an extensive review see *Rotterdam Conference 1974* (17). The list of exclusions is supported and extended by the chromosome data presented in Table 2. Only chromosome 9 segregated concordantly with AK_1 and, therefore, we conclude that AK_1 is most probably located on chromosome 9. The two clones presented in Table 2 in which human AK_1 was present in the absence of a detectable human chromosome 9 can be explained in terms of chromosomal breakage, a phenomenon occasionally occurring in hybrid cells (14). As a consequence of this breakage chromosomal material, though present, will not be recognized cytologically, whereas the locus concerned may be retained and expressed. One of the reasons that this human chromosome piece will not be recognized may be that it is translocated to Chinese hamster chromosomal material. The Giemsa-11 technique is of great importance for the recognition of translocations between human and Chinese hamster chromosomes in man–Chinese hamster hybrid cells (18). We were not able to analyze the human chromosomal material of the two clones having the interspecific translocations. Therefore the presence of chromosome 9 material in these clones could not be established.

Table 3. Linkage relationships between human AK_1 and human chromosome 9 in primary man–Chinese hamster cell hybrids

		Chromosome 9	
		+	−
AK_1	+	24	1
	−	0	17

The data have been broken down to 2 × 2 format and tabulated in ++, + −, − +, and − − categories. The results are given in absolute numbers of clones.

Table 4. Linkage relationships between AK_1 and human chromosome 9 in clones tested by the G-11 method

		Chromosome 9	
		+	−
AK_1	+	13	2
	−	0	9

The data have been broken down to 2 × 2 format and tabulated in ++, + −, − +, and − − categories. The results are given in absolute numbers. The data of these clones are included also in Table 2.

Although the mouse AK can be distinguished from human AK_1, the locus for AK_1 could not be assigned as yet to a particular chromosome by using man–mouse hybrids. In these hybrids AK_1 was always found to be lost (2, 19). Also in man–Chinese hamster hybrids (Tables 1 and 2) chromosome 9 appears to be lost more frequently than most of the other chromosomes.

A large body of family data collected and analyzed by Rapley *et al.* (5) suggested linkage between the locus for red cell adenylate kinase and the ABO blood group locus. This linkage was confirmed and the most likely value for the recombination fraction (θ) was found to be 0.15 with a lod score more than 8.0 (8). Earlier studies have indicated that the loci for ABO blood group and nail-patella syndrome (Np) are within measurable distance (7). Schleuterman *et al.* (9) observed no instance of recombination between *Np* and AK_1 among 53 opportunities. A strong evidence for linkage between loci for xeroderma pigmentosum (Xp) and ABO with a lod score higher than 5 at $\theta = 0.179$ was also reported (20). The initial suggestion obtained for linkage between the locus for Waardenburg syndrome-type I (WS_1) and that for ABO blood group (21) was supported by data obtained elsewhere (22). The combined lod score was reported to be + 1.634 at $\theta = 0.20$. The direct chromosome assignment of

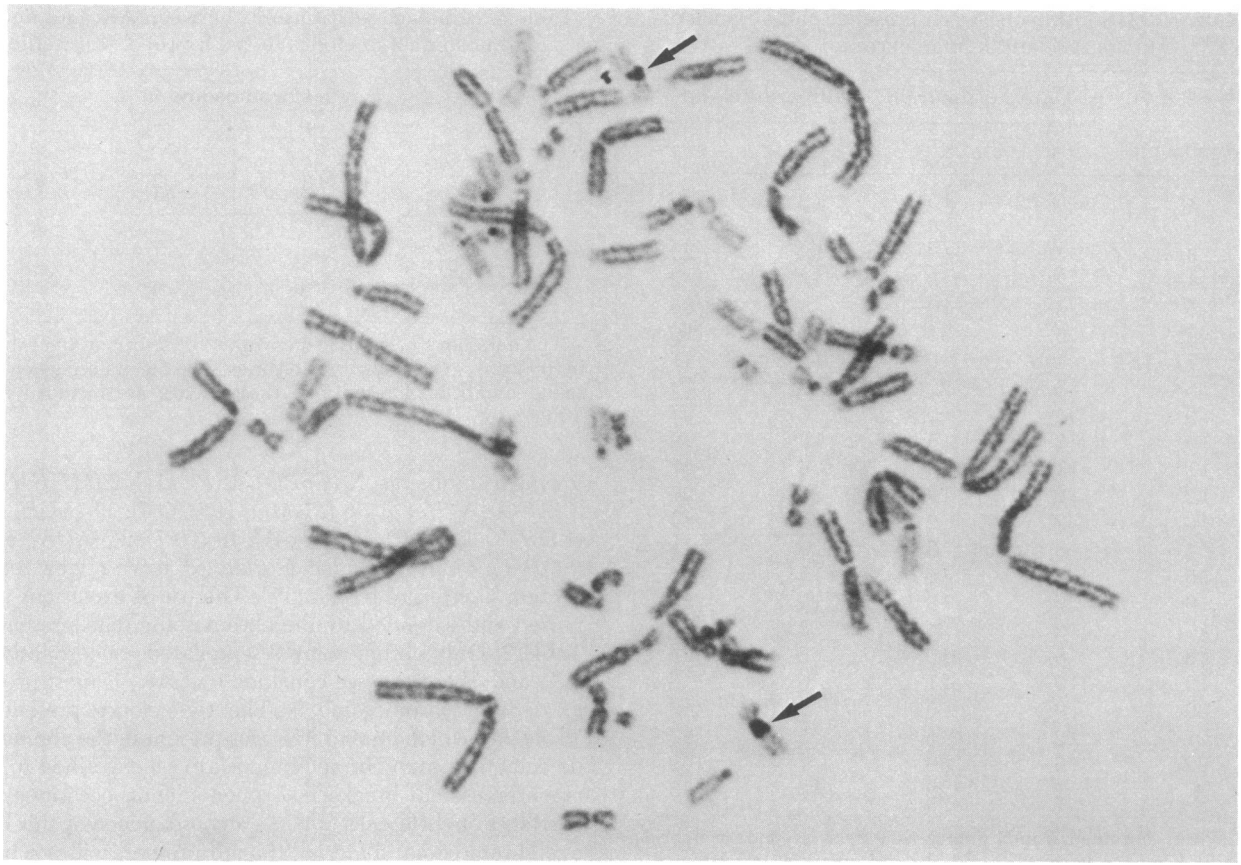

FIG. 2. Chromosomes of a man–Chinese hamster hybrid cell stained by Giemsa-11 technique. Human chromosome 9 is indicated by an arrow.

AK_1 thus assigns the *ABO* linkage group comprising *ABO*, *Np*, AK_1, *Xp*, and probably WS_1 to chromosome 9.

There are reports which are in apparent conflict with the present assignment. Yoder *et al.* (23) have studied a large kindred with a p^+ variant of chromosome 15 and obtained a lod score of 1.428 at $\theta = 0.32$ between the p^+ region of 15 and the *ABO* locus. Analyzing a large series of families segregating for centromeric autosomal polymorphisms and marker genes, Ferguson-Smith *et al.* (24) have obtained a lod score of 3.0 at a recombination fraction of 0.1 for *ABO* and $1qh^+$ and consequently they have considered the possibility of assigning the *ABO* to chromosome 1. The fact that none of the markers assigned to chromosome 1 was found to be syntenic with AK_1 either in man–mouse (2, 19) or in man–Chinese hamster cell hybrids (present report) does not favor the assignment of AK_1 to chromosome 1. Similarly, in our data no member of the linkage group *MPI*:PK_3:*Hex-A*, which was assigned to chromosome 15 (25), was found to be syntenic with AK_1. Therefore, the present data assign the *ABO* linkage group to chromosome 9 through AK_1.

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