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α_1 Antitrypsin Deficiency: A Study of the Relationship between the Pi System and Genetic Markers

M. G. SCHMITT, JR.,[1] R. B. PHILLIPS,[2] R. N. MATZEN,[3] AND G. RODEY[4]

INTRODUCTION

Alpha$_1$ antitrypsin, the principal protease inhibitor (Pi) in human serum, is determined by a series of codominant alleles (Pi alleles) [1]. Six different alleles [2], each completely expressed, and 21 phenotypes [3] have been reported for the Pi system. The phenotype Pi MM is most common, while phenotype Pi ZZ results in a severe serum deficiency of α_1 antitrypsin which is associated with pulmonary emphysema [4] and cirrhosis [5].

We have recently studied an adolescent female with cirrhosis and α_1 antitrypsin deficiency (Pi ZZ) and 23 family members representing 4 generations. Three α_1 antitrypsin alleles (Pi^M, Pi^I, and Pi^Z) and five phenotypes (MM, MZ, IM, IZ, and ZZ) were detected in family members. The quinacrine fluorescent banding technique was successfully utilized to reveal differential chromosomal markers in individual members of this family. In an attempt to identify the chromosomal location of the Pi locus, linkage of the Pi system in this family with these chromosomal markers was investigated. In addition, we have sought evidence for linkage of the Pi system with red cell and HL-A antigen systems.

MATERIALS AND METHODS

Venous blood samples were obtained from 24 members of the family. Serum samples were frozen at −30° C until Pi phenotyping was performed. Fresh whole blood (heparinized, phenol free) was used for HL-A antigen typing, red cell antigen typing, and for lymphocyte cultures necessary for chromosomal banding.

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[1] Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin 53226. Present address: Rockford Clinic, 2300 North Rockton Avenue, Rockford, Illinois 61101. Address reprint requests to M. Schmitt.

[2] Department of Zoology, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201.

[3] Caylor-Nickel Research Foundation, Bluffton, Indiana 46714.

[4] Milwaukee Blood Center, Milwaukee, Wisconsin 53233.

α_1 Antitrypsin Phenotyping

Phenotyping of serum α_1 antitrypsin was performed using a modification of the Fagerhol method [6, 7]. Horizontal acid starch gel electrophoresis of serum followed by electrophoresis at right angles into agarose gel containing human α_1 antitrypsin antibody (Behring Diagnostics, Woodbury, N.Y.) produced zones of α_1 antitrypsin which were classified according to Pi phenotypes previously described for this method.

Red Cell Antigens

Red cell antigens of eight alloantigen systems (ABO, MNS, P, Rh, Kell [K], Duffy [Fy], and Kidd [Jk]) were determined using standard blood bank agglutination and antiglobulin techniques.

HL-A Antigens

Standard microlymphocytotoxicity techniques [8] were employed for haplotype analysis of HL-A antigens. Each individual was screened for the following HL-A antigens: LA series—HL-A1, 2, 3, 9, 10, 11, W28, W29, W30, W32; 4 series—HL-A5, 7, 8, 12, 13, W5, W22, W27, W14, W15, W17, W18, W10.

Chromosomal Banding

Lymphocyte cultures were prepared by a modification of the standard Moorhead method [9]. Cultured lymphocytes were stained with 10% Giemsa for conventional karyotyping and with 0.5% quinacrine dihydrochloride (Atebrin) for fluorescence microscopy. Karyotypes were prepared according to a standard method [10].

RESULTS

Three α_1 antitrypsin alleles (Pi^M, Pi^I, and Pi^Z) and five phenotypes (MM, MZ, IM, IZ, and ZZ) were identified in 24 members of this family. The Pi phenotype of each family member is indicated on the family pedigree (fig. 1). Chromosomal banding patterns, HL-A antigens, and red cell antigens were determined in 22

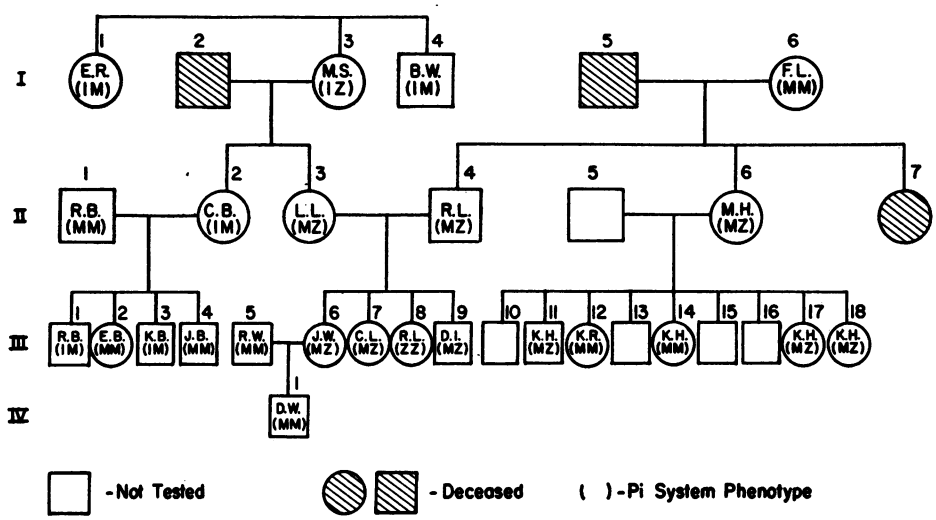

FIG. 1.—Family pedigree with Pi phenotypes indicated for 24 family members

TABLE 1

HL-A AND FOUR POLYMORPHIC RED CELL GENOTYPES IN 22 FAMILY MEMBERS

FAMILY MEMBER	HL-A ANTIGEN		RED CELL ANTIGEN			
			Rh	MNS	Fy	P_1
E. R., I-1	3/7	2/W27	R^1R^1	NSNs	a+b+	P_1—
M. S., I-3	3/7	9/...	rr	NSNs	a+b—	P_1—
B. W., I-4	2/W27	9/...	R^1r	MSNS	a+b—	P_zP_z
F. L., I-6	...	...	R^1r	NsNs	a+b+	P_1—
R. B., II-1	1/8	10/5	R^1R^1	MSMs	a+b+	P_zP_z
C. B., II-2	3/7	3/7	rr	MSNs	a+b+	P_1P_z
L. L., II-3	3/7	2/W10	rr	MSNs	a+b—	P_1—
R. L., II-4	2/7	3/W15	R^2r	MSNs	a+b+	P_1—
M. H., II-6	2/7	1/W17	R^1R^1	MSNs	a+b+	P_1—
R. B., III-1	3/7	1/8	R^1r	MSMs	a+b+	P_zP_z
E. B., III-2	3/7	1/8	R^1r	MsMs	a+b+	P_1P_z
K. B., III-3	3/7	10/...	R^1r	MsMs	a+b+	P_zP_z
J. B., III-4	3/7	10/5	R^1r	MsMs	a+b+	P_zP_z
R. W., III-5	3/7	2/12	R^1r	NsNs	a+b+	P_zP_z
J. W., III-6	3/7	3/W15	R^2r	NsNs	a+b—	P_1—
C. L., III-7	3/7	3/W15	rr	NsNs	a+b+	P_1—
D. L., III-9	3/7	3/W15	rr	MsNs	a+b—	P_1—
K. R., III-12	3/5	1/W17	R^1R^2	MSNs	a+b+	P_1—
K. H., III-14	9/...	1/W17	R^1r	MSNs	a+b+	P_1—
K. H., III-17	3/5	1/W17	R^1r	MsNs	a+b+	P_1—
K. H., III-18	2/7	9/...	R^1R^2	MSMS	a+b+	P_1—
D. W., IV-1	3/7	3/7	R^1r	NsNs	a+b+	P_1P_z

family members. The HL-A antigens and four red cell antigens (Rh, MNS, Fy, and P) were significantly polymorphic and informative in this pedigree (table 1). Fluorescent banding of chromosomes with quinacrine dihydrochloride demonstrated a total of 13 polymorphic Q bands, eight of which were informative for linkage determinations (table 2). The eight informative Q bands identified were as follows: (1) a bright centric region on chromosome 3; (2) a bright centric region with a large dull satellite on chromosome 13 (13a); (3) a bright centric region with bright satellite on chromosome 13 (13b); (4) a bright small satellite on chromosome 14 (14a); (5) a bright large satellite on chromosome 14 (14b); (6) a bright giant satellite on chromosome 21; (7) a large dull satellite on chromosome 22 (22a); and (8) a bright large satellite on chromosome 22 (22b) (fig. 2).

Evidence for linkage of the Pi locus with the chromosomal markers was obtained by computing lod scores for each combination according to the method of Morton [11, 12] (table 3). The negative scores exclude close linkage between the Pi locus and the markers on chromosomes 3, 13, 14, 21, and 22. In the case of linkage between Pi and MNS, lod scores were computed by combining our data with those

TABLE 2

CHROMOSOME MARKERS IN 22 FAMILY MEMBERS

FAMILY MEMBER	CHROMOSOME MARKER							
	3	13a	13b	14a	14b	21	22a	22b
E. R., I-1	−	−	−	−	−	−	−	−
M. S., I-4	+	+	−	−	−	−	+	−
B. W., I-4	+	+	−	−	−	−	+	−
F. L., I-6	−	−	−	−	−	−	−	−
R. B., II-1	+	−	−	−	+	+	−	−
C. B., II-2	−	+	−	+	−	−	+	+
L. L., II-3	−	+	−	−	−	−	+	−
R. L., II-4	+	−	+	+	−	+	−	−
M. H., II-6	+	−	−	+	−	+	−	−
R. B., III-1	−	+	−	+	+	+	−	+
E. B., III-2	−	+	−	+	+	−	+	−
K. B., III-3	+	+	−	+	−	+	−	+
J. B., III-4	+	+	−	−	+	−	−	+
R. W., III-5	−	−	−	−	−	−	−	−
J. W., III-6	+	−	+	−	−	−	+	−
C. L., III-7	+	−	+	−	−	−	+	−
D. L., III-9	+	+	−	−	−	+	+	−
K. R., III-12	+	−	−	−	−	+	−	−
K. H., III-14	−	−	−	−	−	+	−	−
K. H., III-17	−	−	−	−	−	−	−	−
K. H., III-18	+	−	−	+	−	−	−	−
D. W., IV-1	−	−	+	−	−	−	−	−

NOTE.— + = presence of chromosomal fluorescent marker.

of Kueppers and Bearn [13], who found four recombinants and four nonrecombinants in a 3-generation family. From the combined data, close linkage between the MNS locus and the Pi locus can be excluded. Since informative progeny were limited for determination of linkage of Pi to the other antigenic markers, no lod scores were computed for these combinations. However, both recombinant and nonrecombinant progeny were found for Pi and Rh, Pi and HL-A, and Pi and P.

DISCUSSION

The term Pi has been designated to describe a system of codominant alleles which are responsible for determining the presence of α_1 antitrypsin in human serum [1]. Several studies on genetic linkage of the Pi system with other genetic markers have been reported. Gedde-Dahl et al. [14] have presented evidence for linkage of the Pi system with the Gm system of immunoglobulin G. Kueppers and Bearn [13] studied a family of 11 subjects (3 generations) and did not find evidence of linkage of the Pi system with red cell antigens ABO, Rh, P, MNS, Fy, or Jk, nor with the Gc or haptoglobin serum groups.

The chromosome possessing the Pi locus is unknown. Staining techniques which produce differential banding of mitotic chromosomes have only recently been developed. These techniques have revolutionized the study of cytogenetics [15], but their application to linkage studies of genetic disease in man has not been

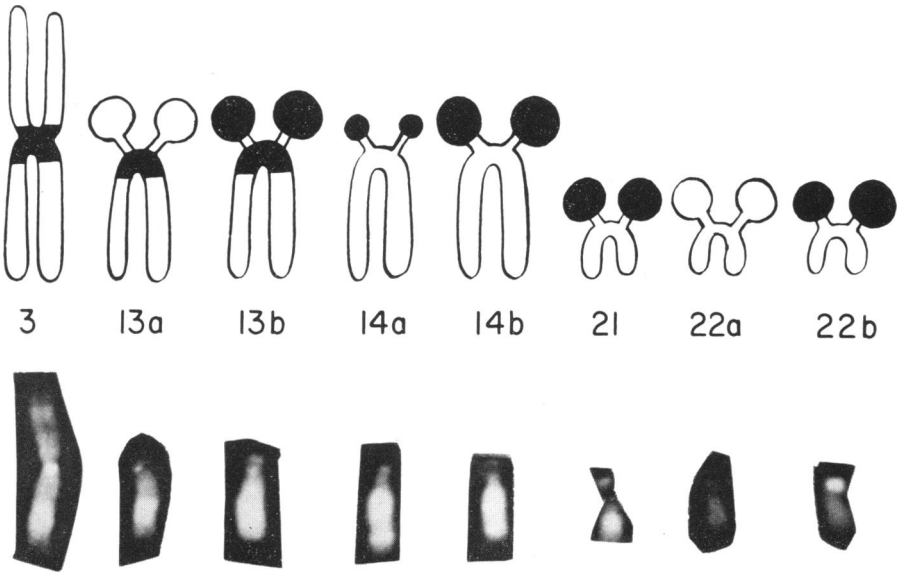

FIG. 2.—Schematic diagram illustrating eight chromosomal markers. The bright fluorescent region of the chromosome is shown in black.

reported. In this study we were successful in detecting differential banding of human chromosomes by the quinacrine dihydrochloride technique in members of a family with α_1 antitrypsin deficiency. Linkage of the Pi system to these chromosomal markers was sought in an attempt to identify the chromosome bearing the α_1 antitrypsin locus.

A total of 13 different chromosomal markers were detected in the family under study; however, only eight markers were significantly polymorphic to be informa-

TABLE 3

LOD SCORES FOR PI AND OTHER GENETIC MARKERS

MARKER	No. OFFSPRING	RECOMBINATION FRACTION .05	.1	.2	.3	.4
Chromosome 3	8	−1.905	−1.116	−0.448	−0.163	−0.036
Chromosome 13	8	−2.884	−1.775	−0.776	−0.303	−0.071
Chromosome 14	10	−2.626	−1.561	−0.642	−0.240	−0.055
Chromosome 21	6	−3.742	−2.581	−1.458	−0.824	−0.371
Chromosome 22	6	−3.442	−2.286	−1.184	−0.596	−0.230
MNS*	14	−4.069	−2.447	−1.029	−0.390	−0.089

NOTE.—For cases in which one parent carried a chromosomal marker and the other parent was not tested, the latter was assumed to be negative for the marker in question. In our experience, even when both parents have a marker on the same chromosome, they can be distinguished by careful analysis.

* Lod scores computed by combining our data with those of Kueppers and Bearn [13].

tive. These eight markers were located on the centromeric region of chromosome 3, the centromere and short arms of chromosome 13, and the short arms of chromosomes 14, 21, and 22. The absence of association between the Pi system and these chromosomal markers suggests that the Pi locus is not located on these chromosomes in or near the regions which exhibited fluorescence.

The genetic loci of the four red cell antigens which were polymorphic in this study have already been assigned to specific chromosomes, so that linkage between the fluorescent chromosomal markers in this family and the red cell antigens was not expected. The Rh and Fy loci are located on the long arm of chromosome 1 [16], the MNS locus is found near the centromere on the long arm of chromosome 2 [17], while P is located near the centromere on the long arm of chromosome 20 [16]. The inability to detect linkage of the Pi system with these four red cell antigens would be indirect evidence that the Pi locus is not located on chromosomes 1, 2, or 20 within measurable distance of these loci. More information is needed to define linkage relationships for Pi and the Rh, Fy, and P antigenic systems; however, our data combined with those of Kueppers and Bearn [13] exclude close linkage of Pi to the MNS locus which is found near the centromere on the long arm of chromosome 2.

On the basis of our study in this family, the genetic locus which determines the presence of α_1 antitrypsin in human serum does not appear to be located on the long arm of chromosome 2 near the centromere; on or near the centromeric region of chromosome 3; near the centromere or short arms of chromosome 13; or on the short arms of chromosomes 14, 21, or 22.

SUMMARY

Twenty-four members (4 generations) of a family with α_1 antitrypsin deficiency were studied in an attempt to determine the chromosomal location of the Pi system locus. Three α_1 antitrypsin alleles (Pi^M, Pi^I, and Pi^Z) and five phenotypes (MM, MZ, MI, IZ, and ZZ) were detected in family members. The quinacrine fluorescent banding technique was successfully utilized to reveal eight polymorphic chromosomal markers in family members. Eight red cell antigens and HL-A antigens were identified for each family member. No linkage between the Pi system and chromosomal markers, four polymorphic red cell antigens, and HL-A antigens was detected. On the basis of this family study, the Pi locus as defined by α_1 antitrypsin deficiency does not appear to be on chromosomes 2, 3, 13, 14, 21, or 22 within measurable distance of the markers used.

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