A Study of Various Blood Group Systems in Leprosy Patients and Controls in Cebu, Philippines [1, 2]

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A number of studies have been made on blood groups in relation to leprosy. Some are difficult to interpret because they deal with few persons, and lack controls, or use an outdated classification of the blood groups, while the results of other more recent studies are contradictory (Table 1).

This report presents the results of a controlled study of the distribution of blood groups in leprosy patients, in Cebu, Philippines. The study reported was part of a broader investigation undertaken under the auspices of the Leonard Wood Memorial with the aim of testing the hypothesis of a genetically-determined susceptibility to leprosy or to specific clinical types of this disease.

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Typing for the following blood groups was performed at the Johns Hopkins University Immunogenetics Laboratory located at Baltimore City Hospitals: ABO, the Rh groups (C, c, D, E, e), MNSs, Kidd (Jk^a), Kell (K), Cellano (k), Duffy (Fy^a), Lutheran (Lu^a), and P_1.

MATERIALS AND METHODS

Sampling. The sample consisted of 546 cases of leprosy and 435 controls, all residents of the island of Cebu. Among the patients 250 were classified as lepromatous, 223 as tuberculoid, 50 as borderline, and four as belonging to the indeterminate group; 19 were unclassified. Of the lepromatous patients, 140 had records of lepra reaction during the last four years; 83 had no such records, and in 27 the previous history of lepra reaction could not be ascertained. The leprosy group consisted of 291 patients hospitalized at the Eversley Childs Sanitarium (75.9% of them lepromatous), 76 outpatients treated by the Cebu Traveling Skin Clinics (only one lepromatous), and 179 patients followed by the Cebu Stationary Skin Clinics and the Leonard Wood Memorial Epidemiologic Unit in Cebu City (15.6% lepromatous).

Sampling methods differed among the various agencies cooperating in the study. At the Eversley Childs Sanitarium, a preliminary sampling of the patients was made from the files. A study group was selected, based on diagnosis, place of birth, ancestry, and age. Only those cases with a clear-cut description of lepromatous or tuberculoid disease were included. Lepromatous cases

were limited to patients born in Cebu Province. All females meeting these conditions were included, but since more males were available only a sample based on the initials of their names were selected. Because of the small number of tuberculoid cases, no birthplace restrictions were specified. Individuals with Chinese ancestry up to the third generation, determined by the patronyms and through interviews, were excluded. With a few exceptions, only persons between 14 years and 60 years of age were studied.

As a source of outpatients, eight of 53 health centers of the Traveling Skin Clinics were selected on the basis of the distance to be traveled from Cebu City. In these eight clinics, all of the registered leprosy patients were summoned. Blood was drawn, however, only from those of suitable age, without Chinese patronyms, and with definite lepromatous or tuberculoid lesions.

A similar procedure was adopted for the cases supervised by the Leonard Wood Memorial Epidemiologic Unit in Cebu City, except that only patients scheduled for follow-up examinations during the course of the study were eligible.

A total of 435 controls were drawn from the population as follows: 134 medical students at Cebu Institute of Technology, and hospital employees of the Chang-Hua Hospital, Cebu City; 15 healthy sibs of leprosy outpatients; 286 persons consulting at the Cebu Skin Clinic for dermatologic conditions. The medical students and hospital employees in the study were volunteers. The controls from the Skin Clinic were drawn in a sequential manner from patients being seen for dermatologic conditions over a period of two months.

To examine possible bias deriving from a lack of homogeneity within the control group, the subgroups were compared with regard to their ABO and MN distribution. Since no statistically significant differences were observed, all the controls were pooled in a single group.

Diagnostic criteria. Ascertainment of leprosy and classification of type of disease was carried out jointly by two, and at times three, of the authors, who utilized clinical

TABLE 1. *Review of studies on blood groups in leprosy.*

Author	Ref.	Country	No. of patients	No. of controls	Blood group typed	Remarks and results
Ali	1	Egypt	100	None	ABO	A more susceptible to leprosy
Beiguelman	2	Brazil	2,127	Blood donors	ABO	Excess O in tuberculoid as compared with lepromatous pts.
					Rh	
Cerri	4		100	181	ABO	No difference with respect to leprosy. Differences related to type of leprosy. Blood group classification outdated.
Cesarino Netto	5	Brazil	240 L	Relatives	ABO	No difference.
			91 L	Pop. data	Rh	No difference.
Gupta	9	Madhya Pradesh	300	4,720	ABO	AB group higher in leprosy.
Hasegawa	10, 11	Japan	1,400	Pop. data	ABO	No relationship with disease, type, sex, or age.
Herivaux	12	Madagascar	78	112	ABO	Excess O, B in leprosy.
Hsuen	13	S. India	526	1,000	ABO	Excess O and deficit B in leprosy. No age adjustment.
Kolpakov	15	U.S.S.R.	130		ABO	Many variables.

TABLE 1. *Continued.*

Lowe	17	India	200 L 200 N	1,538	ABO	No difference related to disease or type of disease.
Malinin & Strukov	19	U.S.S.R.	171	206	ABO	Descriptive
Malaihollo	18	Java	46 + 91	79 + 33	ABO	No relationship with leprosy or type of leprosy.
Marti	20	Spain	269		Presumably ABO	No relationship.
Omichi	23	Japan	89		ABO	Excess B.
Pacheco	24	Goa	Indians & Christians		ABO	O more frequent.
Paldrock	25	Lithuania	200		ABO	Analysis by age and type; no definite conclusion.
Paldrock	26	Estonia		Pop. data	ABO	Excess A and O in leprosy.
Pinetti	27	Sardinia	31	Pop. data Cagliari	ABO	Excess A in leprosy; no relationship with type of leprosy.
Povey	28	S. India	1,085	1,755 Blood donors	ABO	No relationship.
Puente	29	Argentina	233		ABO	Natives from Argentina and Italy; descriptive.
Rode	31	Fr. W. Africa	81		ABO	Relationship with age, sex, ethnic group, type.
Rudchenko	32	U.S.S.R.	186		ABO	Interpretation difficult (AB less resistant); association with course of the disease.
Sato *et al.*	35	Japan	469 406	Relatives	ABO	No relationship.
Valle	36	Pondichery	72	Hosp. persons 38	ABO	No conclusion; slight excess of O in leprosy patients.
Vermia & Dongre	37	India	594	1,000 Blood donors	ABO	No relationship.
Weidemann	38, 39	Latvia	206		ABO	No relationship.
Yankah	40	Ghana	400	400	ABO Rh	No relationship.

examination, bacteriology at various sites of the body, and, in 346 patients, a lepromin test, as a basis for their decision.

Since treatment for leprosy had been under way in Cebu Island for several years, many of the cases presented only residual lesions. Because of the requirements of the sample size, some of these inactive patients were included in the study: 70 cases of the lepromatous study group (28%) were inactive, and 118 (52.9%) of the tuberculoid group. Criteria for inactivity were (1) residual characteristics of the skin lesions; (2) stabilization or regression of nerve involvement; (3) bacteriologic negativity. For classification of type of disease in the inactive patients, past records were screened.

The average duration of observation was 10.2 years (range 1 to 38 years) for lepromatous cases and 5.6 years (range 1 to 23 years) for tuberculoid patients. The average number of bacteriologic examinations performed since diagnosis and registration in the presently inactive cases was 15.4 for the lepromatous and 5.0 for the tuberculoid.

The ascertainment of lepra reaction was limited to the lepromatous patients who had been hospitalized in the Eversley Childs Sanitarium. These patients, who constitute 88.4 per cent of the lepromatous study group, were followed continuously by one of the authors (J.G.T.) during the course of the last four years. However, for patients admitted to the leprosarium less than four years before sampling, and without any episode of lepra reaction following their admission, the presence of this condition prior to admission cannot be excluded.

Leprosy in medical students and hospital employees chosen as controls was excluded on the basis of questioning of the individuals, examination of any suspicious lesions, and referral to the Skin Clinics whenever it was indicated. Exclusion of leprosy in dermatologic patients resulted from the differential diagnosis, based on physical and dermatologic examination, with bacteriologic or mycologic examinations and skin tests whenever necessary.

Age, sex and birthplace characteristics. The age and sex distributions of the study groups are given in Table 2. Cases and controls showed slight differences in age distribution. The proportion of individuals under 40 was 67.9 per cent for the cases (mean age: 34.9 years) and 76.5 per cent for the controls (mean age: 32.8 years). No age differences were observed between the lepromatous and tuberculoid groups. The proportion of persons under 40 was 67.6 per cent for the lepromatous cases (mean age: 35.4 years) and 66.4 per cent for the tuberculoid cases (mean age: 35.7 years).

No marked differences in sex distribution of the various groups were observed.

In general, lepromatous cases were affected for a longer period than tuberculoid cases. Duration of disease was computed in two ways, viz., duration from time of registration, and duration from time of

TABLE 2. *Proportion of persons under 40 and over 40 years of age, and distribution by sex in the subgroups under study.*

	Age					Sex				
	<40			>40			Male		Female	
Subgroups	Total	No.	%	No.	%	Total	No.	%	No.	%
Controls	426	326	76.5	100	23.5	435	277	63.7	158	36.3
Cases	546	371	67.9	175	32.1	546	335	61.4	211	38.6
Lepromatous	250	169	67.6	81	32.4	250	155	62.0	95	38.0
Tuberculoid	223	148	66.4	75	33.6	223	132	59.2	91	40.8

onset as stated by the patients. The first method is an underestimate, since the time lapse between onset and diagnosis is not taken into account. The second method is likely to be an overestimate as suggested from the observation of cases in which the time of onset was known. The duration of disease following registration was 0 to three years in 42.4 per cent of the lepromatous, as compared with 55.4 per cent of the tuberculoid cases, and 10 years or more in 20.8 per cent of the lepromatous and 9.5 per cent of the tuberculoid cases. When the duration of the disease was computed from onset as stated by the patient at the time of registration, 13.6 per cent of the lepromatous and 37.4 per cent of the tuberculoid patients only were affected for three years or less, while up to 47.2 per cent of the lepromatous and 23.9 per cent of the tuberculoid stated the onset as 10 years ago or more.

With regard to birthplace, the proportions of persons born in the Vizayas Islands, including Cebu, Bohol, Iloilo, Masbate, Samar, Leyte, Romblon and Negros, were 67.8 per cent for the controls and 87.5 per cent for the cases, and 83.6 per cent for the lepromatous and 92.4 per cent for the tuberculoid patients.

Birthplace was not reported for eight cases (1.5%) and 115 controls (26.4%), all of them medical students or hospital employees in Cebu. Since there are many universities in the Philippines, students attending the university at Cebu may be assumed to have come from Cebu or from the neighboring islands. Hospital employees too could be assumed to be local natives. When the distributions of the ABO and MN blood groups in these controls with unknown birthplace were compared with the distribution of the other controls born in Cebu Island, no differences were found, and therefore these controls were not excluded from the sample.

Technics. All the specimens were collected by the authors or under their direct supervision. About 5 ml. of blood were collected from each individual in a 5 ml. vacutainer containing 5 mgm. of the sodium salt of ethylene-diamine tetra-acetic acid (EDTA). After centrifugation at 3,000 rpm, the plasma was removed with a Pasteur pipette and an equal volume of glycerol-citrate solution was then added to the red blood cells according to the technic of Mollison ([21]).

The specimens were stored at −16°C until shipment from Cebu City to Baltimore. They were packed in dry ice for shipping and then stored at −20°C until typing was done, for a period ranging from one week to four months. The samples were labeled with code numbers and the laboratory technicians had no means of distinguishing cases from controls.

At least 98 per cent of the specimens in each category were examined for ABO, MNSs, and Rh (DCcEe). Only part of the sample was studied for some blood groups, either because of the scarcity of certain antisera or because of the small amount of red cells in those specimens. The results of all typing are presented in Table 3.

Epidemiologic and clinical information, along with the laboratory results, was coded for computer-processing.

The results were tested for differences according to a number of parameters: leprosy, type of leprosy, lepra reaction, age (under 40 and 40 and over), sex, duration of disease (3 years duration or less since reported onset, and 10 years duration or more), and province of origin (Vizayas Islands and Mindanao).

Two tests of statistical significance were performed: (1) the chi-square test on the distribution of phenotypes, and (2) Z tests on the differences in proportions of the phenotypes. In addition, Z tests were performed on the differences in gene frequencies between those groups whose phenotypic proportions varied significantly. Differences were disregarded unless the statistical probability of their occurrence was 0.05 or less. Statistical tests were not valid in a number of comparisons because the subdivision into many groups resulted in cells with only a few individuals.

RESULTS

Table 3 records the observed blood group distribution of controls and total cases as well as cases subclassified by type of leprosy, i.e., tuberculoid or lepromatous,

TABLE 3. *Blood group distributions in the various study groups.*

	Cases		Controls		Lepromatous		Tuberculoid		With lepra reaction		Without lepra reaction	
	No.		No.		No.		No.		No.		No.	
Blood group	546	%	435	%	250	%	223	%	140	%	83	%
A	131	24.0	120	28.0	57	22.8	59	26.5	28	20.0	23	27.7
B	136	25.0	85	19.8	73	29.2	48	21.5	39	27.9	24	28.9
AB	40	7.3	25	5.8	15	6.0	20	9.0	11	7.9	2	2.4
O	238	43.7	199	46.4	105	42.0	96	43.0	62	44.3	34	41.0
CC	381	70.0	292	67.9	180	72.0	150	67.3	100	71.4	60	72.3
Cc	151	27.8	117	27.2	65	26.0	67	30.0	38	27.1	21	25.3
cc	12	2.2	21	4.9	5	2.0	6	2.7	2	1.4	2	2.4
DD or Dd	537	98.5	432	99.3	245	98.0	221	99.1	137	97.9	82	98.8
dd	8	1.5	3	0.7	5	2.0	2	0.9	3	2.1	1	1.2
EE	5	0.9	12	2.8	2	0.8	2	0.9	1	0.7	1	1.2
Ee	112	20.6	92	21.4	51	20.5	49	22.3	29	20.7	18	22.0
ee	426	78.4	326	75.8	196	78.7	169	76.8	110	78.6	63	76.8
MM	184	33.8	129	29.9	82	33.2	76	34.2	55	39.6	23	27.7
MN	274	50.3	238	55.2	124	49.8	111	50.0	64	46.0	42	50.6
NN	87	16.0	64	14.8	43	17.2	35	15.8	20	14.4	18	21.7
Ss	63	11.6	52	12.1	30	12.0	26	11.8	17	12.1	11	13.3
ss	481	88.4	377	87.9	220	88.0	195	88.2	123	87.9	72	86.7

Kk	1	0.2	0	0.0	1	0.4	0	0.0	0	0.0	0	0.0	0.0
kk	537	99.8	411	100.0	243	99.6	220	100.0	138	100.0	80	100.0	100.0
Kp^a/Kp^b	5	0.9	0	0.0	3	1.2	2	0.9	0	0.0	1	1.3	
Kp^b/Kp^b	527	99.1	316	100.0	238	98.8	217	99.1	136	100.0	76	98.7	
Fy^a+	528	98.5	419	98.8	242	96.8	217	99.1	137	99.3	78	98.7	
JK^a+	292	68.9	273	65.6	118	67.4	133	70.7	59	66.3	40	66.7	
Lu^a-	188	95.4	31	96.9	96	94.1	71	95.9	63	92.6	28	96.6	
P_1+	120	24.2	87	20.3	58	28.3	45	20.3	27	25.7	19	25.7	

and lepromatous cases by whether or not they showed the lepra reaction. Gene frequencies for the same categories are shown in Table 4.

ABO. No significant differences were observed in the total distribution of the ABO phenotypes between leprosy cases and controls or between lepromatous and tuberculoid patients. However, there is a suggestion of a few deviant patterns, such as a higher proportion of the B phenotype in the lepromatous group as compared with the control group ($Z = 2.71$; $P<0.01$) and a lower proportion of A in the lepromatous with a history of lepra reaction in comparison with the controls.

When duration of disease from reported onset was taken into account (Table 5), the following were observed: (1) a higher proportion of the O phenotype in the cases affected for 10 years or more than in the cases with a duration of three years or less ($Z = 3.07$; $P<0.01$); (2) a significant deficiency in the A phenotype among lepromatous patients with disease of long duration in comparison with controls ($Z = 2.23$; $P<0.05$); and (3) a relatively high proportion of the B phenotype among lepromatous patients with recent onset of the disease in comparison with controls, tuberculoid patients, or lepromatous patients with disease of long duration.

Gene frequencies, computed by the formulae given by Race and Sanger ([30]), yielded similar results.

No differences in the ABO distribution were observed when the data were subclassified by age. The only apparent sex difference was found in the tuberculoid cases; the tuberculoid females had a considerable excess of the O phenotype (0.527) as compared with the males (0.364) ($Z = 2.44$; $P<0.01$).

Rhesus. In the Rh blood group system the combination of five antisera led to the recognition of 18 phenotypes, corresponding to the 36 possible genotypes resulting from the various combinations of the alleles C, c, D, d, E, e. The distributions are presented in Table 7.

Typing for special alleles revealed only one C^u phenotype, identified in a dermatologic clinic control suffering with eczema.

TABLE 4. *Gene frequencies in the various study groups.*

Gene	Controls	Leprosy patients	Lepromatous cases	Tuberculoid cases	With lepra reaction	Without lepra reaction
A	0.181	0.162	0.157	0.178	0.136	0.189
B	0.133	0.167	0.196	0.147	0.184	0.196
O	0.681	0.660	0.648	0.656	0.665	0.640
M	0.575	0.585	0.580	0.590	0.626	0.530
N	0.425	0.415	0.420	0.410	0.374	0.470
S	0.062	0.058	0.062	0.061	0.064	0.069
s	0.938	0.942	0.938	0.939	0.936	0.931
C	0.815	0.839	0.850	0.823	0.850	0.849
c	0.185	0.161	0.150	0.177	0.150	0.151
D	0.917	0.878	0.859	0.905	0.855	0.890
d	0.083	0.122	0.141	0.095	0.145	0.110
E	0.135	0.112	0.110	0.120	0.111	0.122
e	0.865	0.888	0.890	0.880	0.889	0.878
Fy^a	0.891	0.878	0.911	0.905	0.916	0.886
JK^a	0.413	0.442	0.429	0.459	0.419	0.423

TABLE 5. *ABO phenotypes in controls and in various groups of patients according to duration of the disease since reported onset.*

Groups	Duration (yrs.)	A		B		AB		O	
		No.	%	No.	%	No.	%	No.	%
Controls		120	28.0	85	19.8	25	5.8	199	46.4
Cases		37	26.2	39	27.7	15	10.6	50	35.5
Lepromatous	3	9	26.5	15	44.1	3	8.8	7	20.6
Tuberculoid	3	24	28.9	18	21.7	10	12.0	31	37.3
Other cases	3	4	16.7	6	25.0	2	8.3	12	50.0
Cases		39	20.5	38	20.0	14	7.4	99	52.1
Lepromatous	10	22	18.6	29	24.6	7	5.9	60	50.8
Tuberculoid	10	15	28.3	6	11.3	6	11.3	26	49.1
Other cases	10	2	10.5	3	15.8	1	5.3	13	68.4

TABLE 6. *Distribution of MNSs genotypes.*

Genotype	Cases		Controls		Lepromatous		Tuberculoid		With lepra reaction		Without lepra reaction	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
MS/MS	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
MS/Ms	27	5.0	23	5.4	15	6.0	9	4.1	8	5.7	6	7.2
Ms/Ms	157	28.9	105	24.5	68	27.2	67	30.3	48	34.3	17	20.5
MS/NS	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
MS/NS + MS/Ns	27	5.0	24	5.6	11	4.4	14	6.3	8	5.7	2	2.4
Ms/Ns	246	45.2	213	49.6	113	45.2	96	43.4	56	40.0	40	48.2
NS/NS	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
MS/Ns	9	1.7	5	1.2	4	1.6	3	1.4	1	0.7	3	3.6
Ns/Ns	78	14.3	59	13.8	39	15.6	32	14.5	19	13.6	15	18.1
Total	544	100.0	429	100.0	250	100.0	221	100.0	140	100.0	83	100.0

The subject was negative for both C and c by the direct agglutination test. D^u was identified in four cases and three controls. An additional analysis with anti-f (anti-ce) serum of the blood specimens with Cc and Ee phenotypes was performed in 65 patients and 13 controls. Of the 65 patients, 32 (49.2%) and of the 13 controls, only two (15.4%) were anti-f-negative.

No differences were observed in the major reaction complexes, such as R_1 (CDe), R_2 (cDE), etc. However, there was a suggestion of a higher frequency of controls homozygous for "c" than among total leprosy cases ($Z = 2.09$; $P<0.05$) or than among the lepromatous patients only ($Z = 2.03$; $P<0.05$). Similarly, there appeared to be a higher frequency of controls homozygous for "E" (0.027) as compared to the total cases (0.009; $Z = 2.09$) or to lepromatous patients only (0.008; $Z = 2.04$).

MNSs. The MN reactions are given in Table 6. A significant excess of MM homozygotes in lepromatous patients with a history of lepra reaction (0.396) as compared with controls was noted, as well as a few other suggested deviations when duration of disease and age were taken into account. Nevertheless, all of the MN findings must be considered with caution in view of the instability of these reactions with aging recently reported ([3]).

With respect to the Ss blood groups, the tuberculoid cases aged 40 or over showed a significantly higher proportion of Ss heterozygotes (0.946) than younger patients (0.849; $Z = 2.90$; $P < 0.01$), whereas lepromatous patients 40 or over showed a deficiency of the Ss phenotypes (0.852) as compared with tuberculoid patients of this age (0.946), an observation which was of borderline significance ($Z = 1.96$).

The S^u reaction was tested in 365 specimens from leprosy cases (215 lepromatous and 150 tuberculoid, 224 males and 141 females), and found to be positive in all.

Kell. The distribution of the possible genotype combinations of K, k, Kp^a, and Kp^b was observed. All the controls were kKp^b/kKp^b, whereas among 529 cases, 523 were of this genotype, 5 were kKp^a/kKp^b (3 lepromatous and 2 tuberculoid), and 1 (lepromatous) was KKp^b/kKP^b.

TABLE 7. *Distribution of Rh phenotypes.*

Genotype	Cases		Controls		Lepromatous		Tuberculoid		With lepra reaction		Without lepra reaction	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
rr	2	0.4	1	0.2	0	0.0	2	0.9	0	0.0	0	0.0
R_oR_o	0	0.0	5	1.2	0	0.0	0	0.0	0	0.0	0	0.0
R″r	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R″R″	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R_2R_2, R″	5	0.9	11	2.6	2	0.8	2	0.9	1	0.7	1	1.2
R_2R_o, R_2r, R_oR''	5	0.9	4	0.9	3	1.2	2	0.9	1	0.7	1	1.2
R′r	3	0.6	1	0.2	3	1.2	0	0.0	2	1.4	1	1.2
R_1R_o, R_1r, R_oR'	55	10.1	43	10.0	22	8.8	21	9.5	12	8.6	7	8.5
R″R′, R_{yr}	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R_yR''	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R_1R_2, R_2R', R_1R'', R_oR_z, R_{zr}, R_yR_o	93	17.2	71	16.6	39	15.7	43	19.5	24	17.1	13	15.9
R_2R_z, $R''R_z$, R_yR_2	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
R′R′	3	0.6	1	0.2	2	0.8	0	0.0	1	0.7	0	0.0
R_1R_1, R_1R'	362	66.8	291	67.8	169	67.9	146	66.4	95	67.9	55	67.1
R_1R_z, $R'R_z$, R_yR_1	14	2.6	0	0.0	9	3.6	4	1.8	4	2.9	4	4.9
R_zR_z, R_yR_z	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R_yR'	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
R_yR_y	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	542	100.1	429	99.9	249	100.0	220	99.9	140	100.0	82	100.0

Other systems. No statistically significant differences were observed in the frequencies of Jk^a or Fy^a reactions with respect to leprosy, type of leprosy, lepra reaction, age or duration of disease. Similarly, tests with anti-Lu^a yielded no suggestion of a difference.

While the P system yielded one significant difference (lepromatous patients showed an excess of P^1 + as compared with controls), this finding will require further testing in fresh samples, since P_1 typing is recognized to be unreliable with frozen samples.

DISCUSSION

There are certain inherent problems in attempting to detect associations between genetic markers and disease by the case-control method. First, it is necessary to have cases and controls with similar exposure to the disease. Yet, the retrospective ascertainment of risk is particularly difficult in leprosy since no immunologic methods are available to determine past exposure to the disease. Second, the evaluation of risk based on a retrospective study of environmental factors, especially socio-economic conditions, is hazardous, both because the role of environment in the transmission of leprosy is not clearly delineated and because information on incidence of the disease in population groups of various socio-economic levels is lacking. Doull *et al.* ([8]) showed that in Cebu the transmission of leprosy can often be traced to the household. The risk of contracting the disease is as much as eight times higher for individuals in contact with lepromatous patients and twice as great for individuals in contact with tuberculoid patients as for individuals not known to have had household contacts with leprosy patients. Thus if controls were less exposed to the disease than cases, a possible association between leprosy and blood groups might be masked or distorted.

Age is another factor for consideration, since the probability of manifesting recognizable symptoms of leprosy increases with age. Nevertheless, the fact that cases in this study are on the average two years older than controls is not likely to cause the case and control groups to be unlike with regard to their probability of having developed leprosy symptoms. In a sample of leprosy patients from Cebu, Doull *et al.* ([7]) found that 84.9 per cent of the patients had manifestations of the disease before the age of 20. Only 45 out of 429 controls included in the present study for whom age was recorded, were under 20 years of age. Moreover, the controls were on the average older than the cases were at the time the latter showed the first signs of leprosy. Consequently, controls, on the basis of their age, had as ample an opportunity to develop apparent signs of leprosy as did the cases.

If the inherent pitfalls of variable exposure and age are disregarded, the first because it cannot be resolved, and the second because it probably presents no difficulty in this study, the findings of this investigation nevertheless still remain difficult to interpret. No truly striking differences were observed. Moreover, while it is necessary to examine the data by utilizing the various subcategories in numerous combinations to detect clues as to possible patterns of susceptibility and manifestation, the multiplication of comparisons increases the probability of obtaining significant associations. Which associations, then, are real, and which are spurious?

On the basis of the observation that lepromatous cases with leprosy for three years or less seemed to show a lower frequency of O phenotype and a higher frequency of B phenotype than either controls or lepromatous patients with leprosy for 10 years or more, is it possible to assume that there is a lower susceptibility to lepromatous leprosy in persons of blood group O and/or a greater susceptibility in persons of blood group B? Differential survival in cases with O and B phenotypes could account for the observed deviations, if the death rate is higher in lepromatous patients of blood group B and lower in patients of blood group O. Or an association like that observed might result from different recovery rates in lepromatous patients with O and B phenotypes; e.g., individuals of blood group B, although possibly more prone to develop lepromatous leprosy,

might recover more rapidly and be discharged sooner from the leprosarium than individuals of group O. Unfortunately, the data available thus far can neither confirm nor reject either alternative explanation, nor support unequivocally the observations for which these explanations are designed. Thus the associations in the ABO system, and in the other blood group systems as well, still remain to be resolved.

On the other hand, this investigation through analysis of the data by type of leprosy, duration of disease, and lepra reaction, as well as age and sex of the patients, has thereby been able to furnish provocative hypotheses for further study in leprosy, a field in which there has been a remarkable paucity of information on susceptibility factors despite a voluminous literature.

To elucidate the possible relationships between blood groups and leprosy, several types of studies should prove invaluable. Clearly, further cross sectional studies in other populations would be desirable. Since leprosy does not exist or has a low prevalence in populations where the B phenotype is absent or rare, as among the American Indians or the Berberes of North Africa, extensive and detailed geographic and historical surveys of leprosy and blood groups in various populations could eventually yield meaningful information.

Unquestionably what is needed most of all are prospective studies of high risk populations blood-typed before disease onset and followed longitudinally to determine whether there are differences in incidence of disease, mode of manifestation, course of disease, recovery, and/or mortality from leprosy associated with blood groups. Where such large scale population studies are not feasible, the intensive longitudinal follow-up of patients blood-typed at the time of diagnosis of disease could also provide an insight into possible differential disease prognosis associated with blood groups. When such studies are carried out on sizable, reliable samples, then, hopefully, the role of blood groups in leprosy will be clarified.

SUMMARY

As part of a study of genetic polymorphism and leprosy conducted under the auspices of the Leonard Wood Memorial, the distributions of eight blood group systems have been investigated in leprosy patients and controls in Cebu, Philippines.

The sample consisted of 546 patients, including 250 with lepromatous leprosy, 223 with tuberculoid leprosy, and 73 with other types of leprosy or unclassified, and 435 controls without signs of leprosy. The blood groups investigated included ABO, the Rh groups (C,c, D,E,e), MNSs, Kidd (JK^a), Kell (K), Cellano (k), Duffy (Fy^a) Lutheran (Lu^a), and P_1. The phenotypic distributions and the gene frequencies have been studied in relation to leprosy, the type of leprosy, the acute episode of lepromatous leprosy known as lepra reaction, age, sex, duration of disease, and birthplace.

Lepromatous patients affected with the disease for three years or less appeared to have a lower frequency of O phenotype and a higher frequency of B phenotype than controls without leprosy or lepromatous patients with a disease of long duration, i.e., 10 years or more. Although the different phenotypic distribution in lepromatous patients with recent onset and controls is suggestive of an association between ABO blood groups and differential susceptibility to lepromatous leprosy, such an association is difficult to explain. Possible hyptheses are discussed, such as differential death or recovery rates in lepromatous patients. However, to resolve this problem, similar cross-sectional studies in the population and longitudinal studies in patients and high risk populations classified according to ABO blood groups should be conducted. If confirmed, these findings might help to reconcile the conflicting data on blood groups and leprosy published to date, where neither the type nor the duration of disease had been considered.

RESUMEN

Como parte de un estudio sobre polimorfismo genético y lepra, realizado bajo el patrocinio del Leonard Wood Memorial, se investigó la distribución de ocho sistemas de grupos sanguíneos en enfermos de lepra y en controles, en Cebú, Filipinas.

La muestra estuvo formada por 546 enfermos, la que incluyó 250 casos de lepra lepromatosa, 223 de lepra tuberculoide y 73 casos de otras formas de lepra o lepra sin clasificar; el control lo constituyeron 435 personas sin manifestaciones de lepra. Los grupos sanguíneos estudiados incluyeron ABO, el grupo Rh (C,c,D,E,e), MNSs, Kidd (Jk^a), Kell (K), Cellano (k), Duffy (Fy^a), Lutheran (Lu^a), y P_1. La distribución de los fenotipos y la frecuencia de los genes se estudiaron en relación con lepra, tipo de lepra, episodios agudos en lepra lepromatosa conocidos como reacción leprosa, edad, sexo, duración de la enfermedad y lugar de nacimiento.

Enfermos con lepra lepromatosa, cuya enfermedad tenia tres o menos años de duración, parecian tener una frecuencia menor del fenotipo O y una frecuencia mas alta del fenotipo B que los controles sin lepra o enfermos lepromatosos con una enfermedad de larga duración, p. ej. 10 años o mas. Si bien la distribución diferente de los fenotipos en los enfermos lepromatosos de reciente comienzo y controles sugiere una asociación entre grupos sanguíneos ABO y suceptibilidad diferencial a la lepra lepromatosa, tal asociación es dificil de explicar. Se discuten posibles hipótesis, tales como tasas diferenciales de muerte y recuperación en enfermos lepromatosos. Sin embargo, para resolver este problema, deberán efectuarse estudios transversales similares en la población y estudios longitudinales en enfermos y poblaciones expuestas a un alto riesgo, clasificadas de acuerdo a los grupos sanguíneos ABO. Si estos hallazgos se confirmaran, ayudarian a resolver el desacuerdo que existe en la información sobre grupos sanguíneos y lepra publicada hasta hoy, en donde ni el tipo ni la duración de la enfermedad se ha tenido en cuenta.

RÉSUMÉ

Dans le cadre d'une étude du polymorphisme génétique et de la lèpre, menée sous les auspices du Leonard Wood Memorial, on a étudié les distributions de huit systèmes de groupes sanguins chez des malades de la lèpre et chez des témoins à Cebu, Philippines.

L'échantillon comprenait 546 malades, parmi lesquels 250 atteints de lèpre lépromateuse, 223 atteints de lèpre tuberculoïde, et 73 qui prèsentaient d'autres types de lèpre ou bien dont le type clinique n'était pas précisé. Les témoins sans manifestations de la maladie étaient au nombre de 435. Les groupes sanguins étudiées étaient les suivants: ABO, Rhesus (C, c, D, E, e), MNSs, Kidd (Jk^a), Kell (K), Cellano (k), Duffy (Fy^a), Lutheran (Lu^a) et P_1. Les distributions de phénotypes et les fréquences des divers gènes ont été étudiées en fonction de la lèpre du type de lèpre, de la réaction lépreuse, de l'âge, du sexe, de la durée de la maladie et du lieu de naissance.

Les malades lépromateux atteints par la maladie depuis trois ans ou moins présentaient une fréquence du phénotype O moins élevée, ainsi qu'une fréquence du phénotype B plus élevée, que les témoins indemnes de lèpre ou que les malades lépromateux avec maladie de longue durée, dix ans ou plus. Les différences notées dans les distributions phénotypiques chez les sujets lépromateux avec maladie d'apparition récente et chez les témoins, suggèrent qu'il existe une association entre les groupes sanguins ABO et la susceptibilité à la lèpre lépromateuse. Une telle association est toutefois difficile à expliquer. Différentes hypothèses sont discutées telles que des taux de mortalité ou des taux de guérison différents chez les malades lépromateux de divers phénotypes. Pour résoudre cette question, il serait nécessaire d'entreprendre des études semblable dans d'autres populations, ainsi que des études prospectives chez des malades et dans des populations fortement exposées à lèpre, classés selon le groupe sanguin. Si ces observations étaient confirmées, elles pourraient contribuer à expliquer les contradictions rencontrées dans les études publiées jusqu'à ce jour sur les groupes sanguins dans la lèpre, lorsqui' on ne tient pas compte du type de la maladie et de sa durée.

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REFERENCES

1. Ali, L. Blood groups among lepers. J. Egyptian Med. Assoc. **14** (1931) 119-122.
2. Beiguelman, B. Grupos sanguineos e lepra. Rev. brasileira Leprol. **31** (1963) 34-44.
3. Bias, W. B. and Cohen, B. H. Changes in reactivity of M and N blood group antigens as a function of sample age. Presented at Third International Congress of Human Genetics, Chicago, Ill., 5-10 September 1966. *In* abstracts of contributed papers, page 8.
4. Cerri, B. Sui gruppi sanguigni nella lebbra come eventuale elemento di resistenza. Gior. Italiano Dermat. & Sifil. **79** (1938) 791-809.
5. Cesarino Netto, J. B. Grupos sanguineos na lepra. Arq. mineiros Leprol. **12** (1952) 53-55.
6. Dobson, A. M. and Ikin, E. W. The ABO blood groups in the United Kingdom; frequencies based on a very large sample. J. Path. & Bact. **58** (1946) 221-227.
7. Doull, J. A., Rodriguez, J. N., Guinto, R. S. and Plantilla, F. C. A field study of leprosy in Cebu. Internat. J. Leprosy **4** (1936) 141-170.
8. Doull, J. A., Guinto, R. S., Rodriguez, J. N. and Bancroft, H. The incidence of leprosy in Cordova and Talisay, Cebu, P.I. Internat. J. Leprosy **10** (1942) 107-131.
9. Gupta, M. C. and Gupta, S. R. Blood groups in relation to pulmonary tuberculosis and leprosy. Indian J. Med. Sci. **20** (1966) 353-356.
10. Hasegawa, K. Über die Blutgruppen bei leprakranken in Japan. La Lepro **8** (1937) 59-61. (German abstract)
11. Hasegawa, K. Über die Blutgruppen bei Leprakranken in Japan. La Lepro **9** (1938) 600. (German abstract)
12. Herivaux, A. Les groupes sanguins dans la lèpre. Bull. Soc. Path. exot. **24** (1931) 618-619.
13. Hsuen, J., Thomas, E. and Jesudian, G. A.B.O. blood groups and leprosy. Leprosy Rev. **34** (1963) 143-147.
14. Jovellanos, A. C. The organization of the Eversley Childs Sanitarium, Mandawe, Cebu. Dept. of Health, Republic of the Philippines, 1961. (mimeographed)
15. Kolpakov, T. and Audrusso, V. Lepra und Blutgruppen. Zentralbl. f. Haut. u. Geschlechtskr. **35** (1931) 471. (abstract)
16. Lessa, A. and Ruffie, J. Sero antropologis dos Ilhas de Cabo Verde. Mesa Redonda sobre a Homen Cabo-Verdiano. Estudos Ensaios e Documentos. Lisbon, Junta de Investigacoes de Ultramar, Vol. 23, 1960.
17. Lowe, J. Leprosy and blood groups. Leprosy in India **14** (1942) 23-25.
18. Malaihollo, J. F. Lepra in de Desa Wates (Res. Batavia) en het resultaat van een bloed groepenonderzoek onder leprozen en gezonden in Wates en Flora. Geneesk. Tijdschr. Nederl.-India **80** (1940) 2296-2312.
19. Malinin, I. and Strukov, S. Die isoagglutinatorischen Eigenschaften des Blutes bei Aussatzigen. Zentralbl. f. Haut. u. Geschlechtskr. **35** (1931) 138.
20. Marti, R. R. Grupos sanguineos y lepra. Rev. Leprol. (Fontilles) **7** (1947) 609-616.
21. Mollison, P. L. Blood transfusion in clinical medicine. Oxford, Blackwell Scientific Publications, 1956.
22. Mourant, A. E., Kopec, A. C. and Domaniewska-Sobczak, K. The ABO blood groups; comprehensive maps and tables of world distribution. Springfield, Ill., Charles C Thomas, 1958.
23. Omichi, N. On isoagglutination in leprosy. Lep. Summary **16** (1929) 269. (abstract)
24. Pacheco de Figueredo. Quoted by Valle (36).
25. Paldrock, A. Die Blutgruppen der Leprosen in Estland. Arch. f. Schiffs. u. Tropen. Hyg. **33** (1929) 440-446.
26. Paldrock, A. Die Blutgruppen der Leprosen in Estland. 2. Mitteilung. Festschr.

Bernard Nocht. Hamburg, J. J. Augustin, 1937, pp. 440-445. (*Abstract in* Internat. J. Leprosy **7** (1939) 296)

27. Pinetti, P. Lo studio del sangue nella lepra. Gruppi sanguigni. Formula emoleucocitaria. Gior. Italiano Dermat. i Sifil. **72** (1931) 1319-1335.
28. Povey, M. S. and Horton, R. J. Leprosy and blood groups. Leprosy Rev. **37** (1966) 147-150.
29. Puente, J. J. Los grupos sanguineos en la lepra. Rev. Argentina Dermat. **12** (1927-1928) 125-137.
30. Race, R. R. and Sanger, R. Blood Groups in Man. London, Blackwell, 1962.
31. Rode, P. Le phenomène d'agglutination dans les recherches de groupes sanguins chez les lépreux. Comp. Rend. Sci. Soc. Biol. **122** (1936) 301-302.
32. Rudchenko, S. N. Blood groups in leprosy. Trop. Med. i Vet. **8** (1930) 34-38. (*Abstract in* Biol. Abstr. **7** (1933) 638)
33. Salzano, F. M. and Ferlauto, M. Grupos sanguineos e lepra. Cienc. e Cult. **14** (1962) 164.
34. Salzano, F. M., Sune, M. V. and Ferlauto, M. Novos dados sobre a relação entre grupos sanguineos e lepra. Cien. e Cult. **17** (1965) 201-202.
35. Sato, S., Mayama, A., Suzuki, T. and Matsumoto, S. ABO type and S type of lepra patients, especially relationship among relatives. La Lepro **18** (1949) 65-68. (*Abstract in* Internat. J. Leprosy **19** (1951) 390)
36. Valle. Détermination des groupes sanguins chez les lépreux de l'Hospice Prophylactique. Rev. Med. et Hyg. Trop. **29** (1937) 125. (abstract)
37. Verma, B. S. and Dongre, A. V. Leprosy and A.B.O. blood groups. Leprosy Rev. **36** (1965) 211-213.
38. Weidemann, M. Zur Verteilung der Blutgruppen bei den Leprösen in Lettsland. Zentralbl. f. Haut. u. Geschlechtskr. **36** (1931) 227-228. (abstract)
39. Weidemann, M. and Kaktin, A. Über die Verteilung der Blutgruppen bei Leprosen in Lettland. Zentralbl. f. Haut. u. Geschlechtskr. **32** (1930) 195.
40. Yankah, J. A. K. Observation on the frequency of A.B.O. and Rh blood groups in leprosy and non-leprosy people in Ghana. Leprosy Rev. **36** (1965) 73-74.