Genetic Characteristics and Cirrhosis: a Controlled Study of 200 Patients*

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A genetic basis for cirrhosis of the liver (Maddrey and Iber, 1964) was suggested by Cruz-Coke (1965) and Cruz-Coke and Varela (1966) on the strength of an apparent association between this disease and colour-blindness, an X-linked trait. Fialkow, Thuline, and Fenster (1966), on the other hand, concluded that the majority of colour-vision defects in their series of 46 alcoholic patients with cirrhosis were secondary either to the cirrhosis or to a reversible nutritional disease.

The data of both the Chilean and the Seattle investigators were presented as the incidence of colour-blindness in cirrhotic patients. A suitable matched control population was not utilized.

The present study was undertaken to further investigate the possible association of colour-blindness and cirrhosis and to determine whether an association exists between any of the blood group systems and cirrhosis. For this purpose the 100 cirrhotic patients were matched with non-cirrhotic subjects with respect to race and age (± 5 years).

Method

Control subjects were either hospital employees or non-cirrhotic patients attending the medical clinics of the hospitals in which the investigation was carried out.

The cirrhotic patients were attending the liver clinics of either the Baltimore City Hospitals or the Johns Hopkins Hospital. Hospitalized cirrhotic patients were included if they had been in the hospital for over four weeks or had been transferred to the clinical research unit direct from the liver clinic. This avoided most cases of alcoholic amblyopia and patients too obtunded as a result of the acuteness of their illness to comprehend the requirements of the tests. When

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inpatients were tested they were matched with non-cirrhotic inpatients.

Where possible the diagnosis of cirrhosis was confirmed by biopsy. When not confirmed by biopsy there was unequivocal evidence of cirrhosis on historical, clinical, and laboratory data. Of the cirrhotic patients, 71% were proved by biopsy and 29% were diagnosed on clinical evidence. Alcoholism was the causative factor in 84% of the cirrhotic patients. In the remaining 16% the cause of the cirrhosis was post-necrotic scarring (7%), chronic active hepatitis (2%), primary biliary cirrhosis (1%), probable post-necrotic scarring (3%), and unknown (3%).

In each group there were 61 Caucasians and 39 Negroes. The average age of control subjects was 48 years, and of the cirrhotic patients 50.2 years. All subjects were categorized according to the following physical characteristics: hair colour (red, blond, brown, or black); eye colour (blue, green, brown, or black); skin colour (very fair, normal, or swarthy in Caucasians, and very light, normal, or very black in Negroes); body type (ectomorphic, mesomorphic, or endomorphic).

Each patient was tested for ability to taste filter paper impregnated with phenylthiocarbamide at a concentration of 0.65 g./l. Dvorine pseudoisochromatic plates were used for the assessment of colour-blindness. Patients were allowed up to five seconds to view each of 14 plates. If they failed more than two of these they were considered to be colour-blind. If they were unable to recognize numbers they were tested with seven plates of similar type following a serpentine trail of contrasting colour. Failure to interpret correctly more than one of these seven plates also constituted colour-blindness.

In the first year of study all patients were tested with monochromatic plates developed by Professor Louise Sloan. These plates consisted of numbers presented in a single colour (grey or purple) similar to the Dvorine or Ishihara plates and permitted testing of both the co-operation of the patient and

FIG. 1.—Incidence of different hair colours in controls (a) and cirrhotics (b). Negro: $\chi^2=0.35$, $\chi^2_{0.05}=3.84$. Caucasian: $\chi^2=8.14$, $\chi^2_{0.05}=7.81$.
FIG. 2.—Distribution of skin colours in controls (a) and cirrhotics (b). Negro: $\chi^2=2.20$, $\chi^2_{0.05}=5.99$. Caucasian: $\chi^2=7.51$, $\chi^2_{0.05}=5.99$.
FIG. 3.—Distribution of eye colours in controls (a) and cirrhotics (b). Negro: $\chi^2=2.89$, $\chi^2_{0.05}=3.84$. Caucasian: $\chi^2=1.69$, $\chi^2_{0.05}=7.81$.

the testing for amblyopia accounting for apparent colour-blindness. From this experience the study of "acute" in-patients with cirrhosis was discontinued and only those who were withdrawn from their alcohol ingestion and nutritionally rehabilitated for one or more months were studied.

Venous blood was collected in acid-citrate-dextrose solution for blood group determinations. Aliquots of each sample were stored at -20°C . in 40% buffered glycerol (Mollison, 1961). The blood groups investigated were the following: ABO, rhesus (C, c, D, E, e), Duffy (Fy^a , Fy^b), Kidd (Jk^a), MN, Ss, Kell (K, k), and P_1 . Secretor status was also determined. The χ^2 test was used to determine statistical significance.

Results

Figs. 1 to 3 show the physical characteristics of control and cirrhotic subjects. A difference reaching statistical significance was found in both hair colour (Fig. 1) and skin colour (Fig. 2) in the Caucasian population. Thus Caucasian subjects with cirrhosis had an excess of brown hair and of very fair skin compared with controls. Eye colour (Fig. 3) was not significantly different. The control subjects and the patients had the same distribution of body types. Of the 61 Caucasian control subjects 35 tasted phenylthiocarbamide; of the 61 Caucasian cirrhotic subjects 36 tasted phenylthiocarbamide. Of 39 Negro control subjects 25 were tasters, and of 39 Negro cirrhotic subjects 30 were tasters. There was no difference between control subjects and patients with cirrhosis.

Colour-blindness was found in 17 patients (7.5% of the series). Four of these were in the control group and 13 in the cirrhotic. Although there are more colour-blind cirrhotics than colour-blind controls the difference is not statistically significant (Table I). The trend appears to be toward an increase of colour-blindness in male Negro cirrhotics. This trend is not apparent in male Caucasian patients or in females of either race.

TABLE I.—Distribution of Colour-blindness According to Race and Sex

	Male Subjects				Female Subjects			
	Negroes		Caucasians		Negroes		Caucasians	
	Colour-Blind	Normal	Colour-Blind	Normal	Colour-Blind	Normal	Colour-Blind	Normal
Control	1	20	2	32	1	17	0	27
Cirrhotic	5	17	4	32	4	13	0	25
$\chi^2 = 2.75$		$\chi^2 = 0.59$		$\chi^2 = 2.40$		$\chi^2 = 3.84$		
$\chi^2_{0.95} = 3.84$				$\chi^2_{0.95} = 3.84$				

TABLE IV.—Distribution of Other Genetic Markers in Control and Cirrhotic Subjects

			P ₁				Secretor				MN						Ss					
			Negro		Caucasian		Negro		Caucasian		Negro			Caucasian			Negro			Caucasian		
			+	-	+	-	+	-	+	-	MM	MN	NN	MM	MN	NN	SS	Ss	ss	SS	Ss	ss
			+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Control	..	..	34	2	42	13	26	13	37	18	12	21	6	9	33	19	2	10	27	4	25	32
Cirrhotic	..	..	36	3	52	9	20	12	42	11	6	22	11	8	41	12	4	9	26	6	30	25
			$\chi^2 = 0.26$		$\chi^2 = 1.42$		$\chi^2 = 0.12$		$\chi^2 = 1.92$		$\chi^2 = 3.49$			$\chi^2 = 2.50$			$\chi^2 = 0.74$			$\chi^2 = 1.71$		
			$\chi^2_{0.95} = 3.84$				$\chi^2_{0.95} = 3.84$				$\chi^2_{0.95} = 5.99$						$\chi^2_{0.95} = 5.99$					

Jk				Kk						Fy											
Negro		Caucasian		Negro			Caucasian			Negro				Caucasian							
+	-	+	-	KK	Kk	kk	KK	Kk	kk	a+b+	a+b-	a-b+	a-b-	a+b+	a+b-	a-b+	a-b-				
38	1	50	10	0	1	38	0	7	54	2	3	11	20	31	12	15	0				
37	2	42	19	1	5	33	1	6	54	11	5	15	8	36	12	7	0				
$\chi^2 = 0.35$		$\chi^2 = 2.83$		$\chi^2 = 4.02$			$\chi^2 = 1.08$			$\chi^2 = 12.39$				$\chi^2 = 3.19$							
$\chi^2_{0.95} = 3.84$				$\chi^2_{0.95} = 5.99$						$\chi^2_{0.95} = 7.81$								$\chi^2_{0.95} = 5.99$			

Blood group and secretor typing results are shown in Tables II, III, and IV. There are no significant differences between cirrhotics and controls for any system except for the Duffy blood group system in Negroes. The $\text{Fy}(\text{a}-\text{b}-)$ phenotype is

TABLE II.—ABO Distribution in Control and Cirrhotic Subjects

		Negro				Caucasian			
		O	A	B	AB	O	A	B	AB
Control	..	17	12	9	1	23	29	6	3
Cirrhotic	..	15	12	10	2	29	24	6	2
		$\chi^2 = 0.05$				$\chi^2 = 1.36$			
		$\chi^2_{0.95} = 7.81$							

TABLE III.—Reactions with Rh Antisera

D	C	c	E	e	Negro		Caucasians	
					Control	Cirrhosis	Control	Cirrhosis
0	0	+	0	+	4	0	12	7
+	+	+	0	+	8	11	18	19
+	+	0	0	+	1	1	7	11
+	+	+	+	+	1	4	10	12
+	0	+	0	+	15	18	4	3
+	0	+	+	+	6	4	5	7
+	0	+	+	0	3	0	4	2
+	+	0	+	+	1	0	1	0
Total					39	38	61	61
					$\chi^2 = 10.95$		$\chi^2 = 4.56$	
$\chi^2_{0.95} = 14.07$								

found in 20 Negro control subjects but in only eight cirrhotic patients. By contrast there are 11 $\text{Fy}(\text{a}+\text{b}+)$ heterozygotes in the Negro cirrhotic group but only two among the Negro controls. The χ^2 for this blood group is 12.39 with 3 degrees of freedom.

Discussion

In our investigation of 100 patients with cirrhosis matched for age and race with 100 non-cirrhotic subjects there was a significant increase among the 61 Caucasian cirrhotics in the incidence of brown hair and very light skin compared with the Caucasian controls. Observation of the physical characteristics was the only subjective assessment of the investigation and involved five separate observers. Interestingly, the results are opposite to those of Lea (1953), who found a greater than expected incidence of dark hair, dark skin, and brown eyes among British prisoners of war developing cirrhosis while interned in Asia during the second world war. Though our Caucasian cirrhotics had a higher incidence of blue eyes (Fig. 3), this was not significant at the 5% level.

In our study there was no significant difference in the incidence of colour-blindness between disease and control groups. The largest percentage (30) of colour-blind subjects in the series was found in the Negro male cirrhotic group. The finding of colour-blindness in four female subjects with cirrhosis was unexpected, but was confirmed by a second examination in each. We are unable to support the findings of increased colour-blindness among cirrhotics as reported by Cruz-Coke and Varela (1966).

The highly significant difference in the Duffy blood group systems between Negro cirrhotics and controls seems important. The finding of no significant difference with respect to this blood group in Caucasians suggests that the association may be an indirect one rather than linkage of a "gene" for cirrhosis with Duffy locus.

The Duffy system is perhaps the most distinctive of all the blood-group systems in showing racial differences. There are at least three alleles in this system Fy^a , Fy^b and Fy . (An allele for which no antibody has been found has no identifying superscript). Approximately 70% of Negroes are of the Fy ($a-b-$) phenotype, or $FyFy$ genotype. This phenotype is virtually non-existent in Caucasians. Race and Sanger (1962) estimated the Fy gene frequency in Caucasians to be approximately 0.03. In Negroes the frequency of this gene is approximately 0.82.

A possible explanation for the deficiency of Fy ($a-b-$) and the corresponding increase of Fy ($a+b+$) subjects among Negro cirrhotics is that this group has a greater White admixture than the control group.

The most objective measure of White admixture in Negroes is the deviation in the test population of gene frequencies of those markers which show the greatest differences between Negroes and Caucasians. In addition to Duffy, these would include colour-blindness and glucose-6-phosphate dehydrogenase deficiency. Studies of G-6-PD in cirrhotic and control subjects have been started.

The frequency for male colour-blindness in the Baltimore Negro population is approximately 0.03. For Caucasians it is about 0.08. Though the difference in incidence of this trait between Negro cirrhotics and matched controls is, as previously stated, not significant, the excess of colour-blindness in Negro cirrhotics, which was not observed in the Caucasian group, lends support to the hypothesis.

Since the frequency of the Fy gene in Negroes is 27 times greater than its frequency in Caucasians, this marker is obviously a better indicator of racial admixture. A degree of admixture which would not be significant on the basis of deviation in gene frequency for colour-blindness could be highly significant when the Duffy frequency deviations are observed.

This explanation for the significant difference in the Duffy blood groups between Negro cirrhotics and controls is supported by an observation of Cruz-Coke and Varela (1966) of a "highly significant correlation . . . between increasing prevalence of alcoholism, colour defectiveness, and Caucasian admixture" in their investigation of a primitive population of Chile.

If the incidence of cirrhosis is measurably higher in the Caucasian population than among non-Whites one would expect to find an increase in the frequency of the disease in those Negroes with the greatest White admixture. Unfortunately, reliable incidence figures for this disease are not

available. Recent studies, however, indicate that the death rates from cirrhosis are almost identical for Caucasians and Negroes in America when they are age-corrected (Dunn and Mendeloff, 1968) (Table V).

TABLE V.—*Ratios of Death Rates for Cirrhosis of Liver by Age, Colour, and Sex: United States, 1959-61 (Dunn and Mendeloff, 1968)*

Age	Ratio of Non-Whites to Whites	
	Males	Females
All groups		
Age adjusted	1.05	1.41
< 1	2.29	1.57
1-4	3.00	2.00
5-14	2.00	1.00
15-24	3.00	3.00
25-34	4.35	5.17
35-44	1.82	2.27
45-54	1.04	1.27
55-64	0.82	0.99
65-74	0.53	0.61
75-84	0.47	0.51

Mortality rates are not equivalent to incidence, however. The data in Table V suggest that Negroes succumb to cirrhosis at an earlier age than Caucasians. Because of this difference in age distribution and the possible difference in incidence between Negroes and Caucasians it is impossible to conclude decisively regarding the importance of White admixture.

Our study failed to support an excess of blood group A among cirrhotics as observed in Sydney by Billington (1956) and in Vienna by both Speiser (1958) and Wewalka (1960). Studies from Iowa (Buckwalter *et al.*, 1964) and from Lima (Muñoz Baratta, 1961) have failed to demonstrate an excess of group A compared with controls.

Summary

One hundred patients with cirrhosis were compared with control subjects in inherited physical characteristics, colour-blindness, and blood group substances. There was a significant increase among Caucasian cirrhotic subjects with brown hair and very light skin colour compared with controls. Among Negroes an increase in the Duffy blood group was observed in the cirrhotics compared with controls. No increase in colour-blindness was observed in cirrhotic subjects. These studies suggest a broad multifactorial base for susceptibility to cirrhosis and an increased frequency of these factors in Whites compared with non-Whites.

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