Genetic Linkage Analysis of Human Hemoglobin Variants

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The β-chain locus of human hemoglobin is known to be closely linked to the hemoglobin δ-chain locus (Mishu and Nance 1969) and to a locus which determined β-thalassemia (Pearson and Moore 1965), but despite the high prevalence of allelic variants in Negro and certain Asiatic populations, no additional linkages with other polymorphic genes have been established. The purpose of this report is to present linkage data for the *Hb* β-δ complex with genetic loci determining 17 other polymorphic systems.

MATERIALS AND METHODS

Hemoglobin typing was performed by starch gel electrophoresis with a tris-versene-borate buffer system (Nance 1968, p. 186–189). The parents of 1,053 Brazilian families of mixed racial origin were studied, along with 517 offspring in 117 families found to be segregating for genetic variants at the *Hb* β or *Hb* δ loci. The characteristics of the study population and the means of its selection have been described in detail elsewhere (Morton 1964). Blood typing was performed in duplicate, and hemoglobin, haptoglobin, Gm, and Inv typing were performed on frozen samples of glycerolized red blood cells and serum. The PTC typing was carried out with four concentrations of phenylthiocarbamide spanning the antimode of the taste-threshold distribution. Cholinesterase typing was accomplished by the technique of Harris and Robson (1963). Children acknowledged by the parents or proven by the blood typing results to be extramarital were omitted from the calculations. Linkage analysis was performed by the lod score method of Morton (1955) with a computer program written for an IBM 7040 computer. Maximum likelihood estimates of the recombination fraction θ were obtained by iteration of the lod scores. All hemoglobin variants used for the linkage studies were determined by codominant alleles. For test factors showing dominance, only "certain" families were scored, that is, those where the genotypes of both parents could be inferred from their phenotypes or from their children's phenotypes. Since the main factor (hemoglobin type) was ascertained by complete selection through the parents, no correction terms were required (Morton 1955).

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RESULTS

The results of hemoglobin typing are shown in table 1, and the overall population gene frequencies are given in table 2. Linkage analysis excluded close linkage ($\theta < 10\%$) at the 99% confidence level with 10 of the 17 loci, but was consistent with loose linkage between Hb β-δ and seven of the loci (table 3). The typing results for these seven loci were further analyzed to determine whether any significant difference could be demonstrated in the maximum likelihood estimates of the recombination fraction between segregating fathers and mothers. The results of these analyses are shown in table 4. Only in the case of the Duffy (Fy) locus did the evidence for heterogeneity between the estimates of the recombination frequency among males and females reach significance at the 2.5% confidence level.

DISCUSSION

The β-chain locus of human hemoglobin is known to be closely linked to the δ-chain locus, since no example of recombination has been observed among a total of

TABLE 1

DISTRIBUTION OF HEMOGLOBIN PHENOTYPES AMONG CHILDREN IN 1,053 BRAZILIAN FAMILIES

Mating Type		Number of Families	Offspring Phenotypes								Total Typed Offspring
Father	Mother		Hb A	Hb AS	Hb AC	Hb AF	Hb S	Hb C	Hb SC	Hb A_2A_2'	
Hb A	Hb A	936	.....	.....	.....	.....	.....	.....	.....	.....	.....
Hb AS	Hb A	35	69	79	.....	.....	.....	.....	.....	.....	148
Hb A	Hb AS	40	88	102	.....	.....	.....	.....	.....	1*	191
Hb AC	Hb A	16	39	.....	41	.....	.....	.....	.....	.....	80
Hb A	Hb AC	9	17	.....	19	.....	.....	.....	.....	.....	36
Hb AF	Hb A	3	6	.....	.....	3	.....	.....	.....	.....	9
Hb A	Hb AF	3	3	.....	.....	5	.....	.....	.....	.....	8
Hb AS	Hb AS	3	5	5	.....	.....	2	.....	.....	.....	12
Hb AC	Hb AC	1	3	.....	1	.....	.....	1	.....	.....	5
Hb AS	Hb AC	1	.....	3	.....	.....	.....	.....	3	.....	6
Hb AC	Hb AS	1	.....	1	2	.....	.....	.....	3	.....	6
Hb A_2'	Hb A	2	3	.....	.....	.....	.....	.....	.....	5	8
Hb A	Hb A_2'	3	7	.....	.....	.....	.....	.....	.....	1	8
Total.....		1,053	240	190	63	8	2	1	6	7	517

*Paternity exclusion.

TABLE 2

HEMOGLOBIN GENE FREQUENCIES IN A BRAZILIAN POPULATION

Allele	Frequency
$Hb^{\beta A}$	.9720 ± .0025
$Hb^{\beta S}$	.0197 ± .0021
$Hb^{\beta C}$	.0069 ± .0013
$Hb^{\beta F}$	.0014 ± .0006
$Hb^{\delta'}$	.0012 ± .0005

TABLE 3

ANALYSIS OF HEMOGLOBIN TYPING DATA FOR EVIDENCE OF LINKAGE WITH 17 OTHER LOCI

SYSTEM	FAMILIES	TYPED CHILDREN	z SCORES FOR RECOMBINATION VALUES (θ) RANGING FROM 5%–45%									MAXIMUM LIKELIHOOD ESTIMATE	
			5%	10%	15%	20%	25%	30%	35%	40%	45%	Odds	θ(%)
MNS	66	290	−45.96	−24.05	−13.17	− 6.89	−3.18	−1.12	−0.15	0.12	0.06	1.33	40.8
Gm	62	302	−47.37	−26.51	−15.77	− 9.28	−5.17	−2.61	−1.12	−0.36	−0.06	.....	.....
Hp	62	277	−56.45	−31.99	−19.40	−11.75	−6.85	−3.71	−1.77	−0.68	−0.15	.....	.....
Rh	56	256	−47.52	−26.46	−15.68	− 9.18	−5.10	−2.56	−1.10	−0.36	−0.07	.....	.....
ABO	51	253	−42.56	−23.36	−13.60	− 7.79	−4.20	−2.03	−0.81	−0.24	−0.04	.....	.....
Inv	39	226	−16.75	− 7.63	− 3.34	− 1.06	0.09	0.54	0.56	0.35	0.11	3.94	32.6
Sec	28	155	− 9.90	− 4.87	− 2.41	− 1.04	−0.28	0.09	0.20	0.15	0.05	1.58	35.6
Fy	27	131	−15.68	− 7.68	− 3.93	− 1.80	−0.59	0.04	0.26	0.22	0.08	1.86	36.5
Le	26	128	−10.76	− 5.64	− 3.07	− 1.60	−0.73	−0.26	−0.04	0.02	0.01	1.06	40.9
P	22	113	− 5.19	− 3.00	− 1.81	− 1.08	−0.61	−0.32	−0.14	−0.05	−0.01	.....	.....
PTC	22	112	− 9.43	− 5.03	− 2.75	− 1.40	−0.59	−0.15	0.04	0.07	0.03	1.19	38.7
E1	7	40	− 8.49	− 4.81	− 2.90	− 1.73	−0.97	−0.50	−0.21	−0.06	−0.01	.....	.....
Kidd	5	19	− 2.55	− 1.32	− 0.72	− 0.38	−0.19	−0.08	−0.03	−0.00	−0.00	.....	.....
Tf	4	24	− 0.91	0.16	0.58	0.72	0.70	0.59	0.41	0.22	0.06	5.36	21.8
Kell	4	22	− 5.98	− 3.56	− 2.28	− 1.48	−0.93	−0.55	−0.29	−0.12	−0.03	.....	.....
Ct	1	5	− 1.44	− 0.89	− 0.58	− 0.39	−0.25	−0.15	−0.08	−0.04	−0.01	.....	.....
Diego	1	2	− 0.72	− 0.44	− 0.29	− 0.19	−0.12	−0.07	−0.04	−0.01	−0.00	.....	.....

TABLE 4

COMPARISON OF HEMOGLOBIN LINKAGE ANALYSIS BETWEEN MALES AND FEMALES FOR SEVEN LOCI SHOWING POSITIVE z SCORES

System	Families	Typed Children	z Scores for Recombination Values (θ) Ranging from 5–45%									Maximum Likelihood Estimate		
			5%	10%	15%	20%	25%	30%	35%	40%	45%	Odds	θ(%)	χ^2
MNS:														
Father.....	33	142	−19.52	− 9.66	−4.87	−2.20	−0.72	0.02	0.27	0.22	0.08	1.89	36.3	0.70
Mother....	33	148	−26.44	−14.39	−8.29	−4.68	−2.46	−1.14	−0.42	−0.10	−0.01	1.01	45.5	
Inv:														
Father.....	20	75	− 6.85	− 2.68	−0.79	0.14	0.53	0.60	0.47	0.26	0.07	4.02	28.8	0.51
Mother....	19	71	− 9.90	− 4.96	−2.55	−1.20	−0.44	−0.05	0.09	0.09	0.03	1.27	37.2	
Le:														
Father.....	12	60	− 5.03	− 2.54	−1.32	−0.63	−0.24	−0.05	0.03	0.03	0.01	1.09	27.8	0.06
Mother....	14	68	− 5.73	− 3.10	−1.76	−0.97	−0.49	−0.21	−0.07	−0.01	0.00	1.00	45.5	
Sec:														
Father.....	11	55	− 2.36	− 0.98	−0.35	−0.05	0.08	0.11	0.09	0.05	0.01	1.30	29.9	0.61
Mother....	17	100	− 7.54	− 3.89	−2.06	−0.99	−0.36	−0.03	0.10	0.10	0.04	1.30	37.2	
Fy:														
Father.....	15	74	− 1.93	0.21	1.06	1.36	1.35	1.15	0.83	0.46	0.14	24.33	22.2	5.14*
Mother....	12	47	−13.35	− 7.89	−4.99	−3.16	−1.94	−1.11	−0.57	−0.23	−0.05	.....	.....	
PTC:														
Father.....	11	55	− 6.45	− 3.96	−2.56	−1.63	−1.00	−0.56	−0.27	−0.11	−0.02	.....	....	1.61
Mother....	11	57	− 2.99	− 1.07	−0.20	0.23	0.40	0.41	0.32	0.18	0.05	2.66	27.8	
Tf:														
Father.....	2	13	− 2.09	− 1.09	−0.60	−0.32	−0.16	−0.07	−0.02	−0.00	−0.00	.....	....	2.41
Mother....	2	11	1.18	1.25	1.18	1.04	0.86	0.66	0.44	0.22	0.06	17.95	9.1	

* $P < .025$.

61 opportunities (Mishu and Nance 1969). The locus for β-thalassemia is also closely linked, but, in this case, two crossovers have been reported among 29 relevant offspring (Pearson and Moore 1965). The predicted close linkage of the γ-chain locus to the β-δ complex (Nance 1963) has been confirmed in the mouse (Gilman and Smithies 1968), but similar data are lacking in man. The c locus for generalized oculocutaneous albinism is known to be closely linked to the Hb β locus in the rat (Brdicka 1966) and mouse (Popp 1962); it is interesting that the observed recombination frequency for c and Hb in mice was 2.49 ± 0.52 and $5.13 \pm 0.58\%$ in males and females, respectively (Popp and St. Amand 1960). The c mutation probably corresponds to the tyrosinase-negative form of albinism in man (Witkop et al. 1970), for which no conclusive linkage data are available. Massie and Hartmann (1957) reported a small pedigree where there was a suggestion of measurable linkage, but the type of albinism present in this family was not known. In a second small sibship of tyrosinase-positive albinism, no definite recombination was observed in three siblings (Nance et al. 1970); however, analysis of a much larger group of tyrosinase-positive families provided no evidence for measurable linkage (Witkop, personal communication, 1969).

In the present study, linkage analysis yielded positive z scores at high values of θ for seven of the 17 loci studied, but at no maximum likelihood value of θ did the pooled z score for males and females exceed 0.8. It now seems probable, however, that there may be a sex difference in the observed recombination frequency in man (Renwick 1968, 1969). Estimated map distances between markers in males range from about 33% (Rapley et al. 1968) to 83% (Mohr 1954) of those found between identical markers in females. For this reason, if any of the seven possible linkages are real, heterogeneity between the sexes in the observed frequency of recombination might be expected, with a shorter estimated map distance among segregating males. As shown in table 4, the evidence for linkage heterogeneity (Morton 1956) was significant at the 2.5% level only in the case of the Duffy locus. When the linkage data for the two sexes were analyzed separately, a maximum z score of 1.386 was observed at $\theta = 22.24$ in fathers, corresponding to a likelihood ratio of 24.33 to 1 favoring linkage. In females, on the other hand, no positive z scores were observed in the pooled data for values of θ ranging from 0 to 0.5.

Donahue et al. (1968) and Ying and Ives (1968) have shown that the Duffy locus is closely linked to a polymorphic secondary constriction found on one of the arms of chromosome no. 1, the largest metacentric, in about 0.5% of normal individuals. Therefore, if the suggestion of linkage in these data is confirmed by subsequent observations, it would indicate that Hb β is also located on chromosome no. 1.

The results for the MNS system are also of some interest. More informative families were observed for this locus than for any other studied. Positive z scores were obtained for large values of θ, and when the sexes were considered separately, increased evidence for linkage was found among segregating males. In 1947 (Snyder, Russell, and Graham) and 1949 (Snyder, Clarke, and Moore), Snyder and his coworkers reported data from nine families that suggested the existence of measurable linkage between the MNS and the sickle cell loci. Heterozygotes were not clearly distinguished from homozygotes in their studies, however, and subsequent observa-

tions failed to support the evidence for linkage (Neel et al. 1952; Waller et al. 1952). Most of the matings reported by these authors were intercrosses at the Hb locus, and among the backcrosses no indication of the sex of the affected parent was given, a fact which precluded reanalysis of the data to search for evidence of linkage heterogeneity between the sexes.

In contrast to the MNS and Duffy results, pooled data from the four families segregating for transferrin variants also yielded positive z scores, but when the sexes were considered separately, a smaller maximum likelihood estimate of θ was found in females than in males.

SUMMARY

Genetic linkage studies were performed on typing results from 117 two-generation families containing a total of 516 offspring, in which one or both parents were heterozygous for a genetic variant at the β or δ locus of human hemoglobin. No evidence for linkage to the Gm, Hp, Rh, ABO, P, E_1, Kidd, Kell, Catalase, or Diego loci was found, but positive z scores at large values of θ were obtained for the MNS, Inv, Sec, Le, Fy, PTC, and Tf loci. Of these, only the Duffy locus showed a significantly lower recombination frequency in males than females. It is suggested that a search for linkage heterogeneity between the sexes may prove to be a useful method for mapping distant autosomal loci in man.

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