Genetic Linkage between a Locus for 6-PGD and the Rh Locus: Evaluation of Possible Heterogeneity in the Recombination Fraction between Sexes and among Families

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In a survey of the linkage relations between a locus for 6-phosphogluconate dehydrogenase (6-PGD) and 16 other loci, Weitkamp et al.[1] noted some evidence favoring linkage with the Rhesus (Rh) blood group locus. We now present a summary and analysis of data from 35 families informative with respect to linkage between the Rh and 6-PGD loci. Part of the material in this paper has been previously published in abstract form [2, 3].

MATERIALS AND METHODS

Individuals heterozygous for 6-PGD were ascertained by screening discarded blood samples from the hematology and clinical chemistry laboratories of Strong Memorial Hospital and cord blood samples obtained from the Department of Obstetrics and Gynecology, University of Rochester. Twenty families segregating at the 6-PGD locus cooperated in the project. Of these, only the 13 families informative with respect to the linkage relations of the loci for 6-PGD and Rh are included in this report.

Hemolysates were prepared by shaking washed red cells for 30 minutes with an equal volume of distilled water and two volumes of toluene, the toluene layer being discarded. The specimens were then centrifuged at 30,000 *g* for 30 minutes to remove the remaining stroma. The procedure for typing 6-PGD is detailed in Weitkamp et al. [4]. The samples were typed for ABO, MN, Fy^a and K as well as Rh.

The linkage analysis for each family follows the pattern described by Haldane and Smith [5], Morton [6], and Smith [7]. That is, specifying θ as the recombination fraction and $(1 - \theta)$ as the nonrecombination fraction, the likelihood or algebraic probability expression for the occurrence of the segregation pattern in the pedigree was computed by summing the probabilities of occurrence for each of the possible genotypically different pedigrees consistent with the observed or assigned phenotypes in the pedigree. Following the method used by Morton [6], we inferred Rh gene complexes from the genotypes of informative offspring. (On the basis of the phenotypes of their parents and offspring, individuals H011-29 and H011-61 are CD^ue/cDE and H011-88 is CDe/cDE. The genotypes of H011-28 and H011-95 are uncertain.) Genotypes for untyped individuals were assigned either when the part of the Rh genotype relevant to linkage analysis could be inferred from the types of the offspring (both parents of H008-7, one of the untested

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6-PGD heterozygous parents of H009-5, and the untested parents of H011-72, H011-54, H021-13, and H021-34), or when 6-PGD heterozygosity could be similarly inferred (one of the untested Rh heterozygous parents of H008-7, both parents of H009-5, and the untested parents of H011-54, H021-13, and H021-34).

In order to minimize the complexity of the calculations, no genotypes were assigned a probability based on population gene frequencies and the postulate of random mating. Three individuals were assumed to be 6-PGD A homozygotes (one of the parents of H008-7 and the untested parents of H011-72 and H029-5), since the chance of heterozygosity in each case is no more than 0.05 (see [8, p. 492]). The resultant probability expressions are thus in some instances approximations of the complete pedigree probabilities and may not extract all the information from the data. However, since most individuals have been directly typed, the additional accuracy obtainable from complete pedigree probabilities is unlikely to modify the results in any important way. The relative probability (standardized probability ratio) for any specific value of the recombination fraction, θ, was obtained by dividing the solution of the probability expression using the specific value of θ by the solution obtained when $\theta = 0.5$ (no linkage).

RESULTS AND DISCUSSION

The analysis is based on 35 kindreds informative for the segregation of alleles at the 6-PGD locus, including 21 two-generation families previously reported [1].The pedigrees of 13 new kindreds (H001, H003, H008, H009, H010, H011, H015, H016, H017, H020, H021, H026, and H029) and an expanded version of Pedigree 10854 [9], containing 220 tested individuals, are included in Appendix I.* Genotyping data at six loci (6-PGD, Rh, ABO, MN, Fy^a, and K), as well as sex and age for each individual, are listed in Appendix II. One individual (H010-5) found to have a phenotype incompatible with the expected segregation of parental genes was excluded from the study. The expressions used in the calculation of the probability ratios for the 14 new pedigrees are given in Appendix III, and the probability ratios for each of the 35 pedigrees at 11 different values of the recombination fraction are shown in Appendix IV.

Demonstration of the Presence of Linkage

It has been customary to assume a uniform prior probability distribution over all values of θ between 0 and 0.5 based on evidence for an average autosomal length of 1 morgan [10]. On this premise the average probability ratio or likelihood, Λ, is 69,199 when estimated by the trapezoidal rule and 67,632 when estimated by Simpson's rule. Using Smith's [7] approximate a priori expectation of finding linkage in man of one in 22, the probability of linkage between the Rh and 6-PGD loci, $\Lambda/(\Lambda + 21)$, is 0.9997.

Renwick [11] has recently proposed more conservative prior odds for linkage in man, citing in part evidence favoring a total autosomal map length of 33 morgans. In his method, the value for the odds on linkage, Λ, is the inverse of the ratio of the area under the graph of the prior distribution of probability to the area under the graph of the data-modified distribution of probability. In each

* Appendixes I-VI have been deposited as NAPS document number 01490 with the ASIS National Auxiliary Publications Service, c/o CCM Information Corporation, 909 Third Ave., New York, N.Y. 10022, and are available on request.

case, the probability is plotted against map interval, w, which is obtained from θ by the transformation $w = 1/4 \tanh^{-1} 2\theta + \tan^{-1} 2\theta$ [12]. The values used here for the prior probability, $P_{(w)}$, for various values of w are given in table 1.

TABLE 1

LINKAGE OF LOCI FOR Rh AND 6-PGD: PRIOR AND POSTERIOR PROBABILITIES FOR VARIOUS VALUES OF THE MAP INTERVAL, w

w (morgans)	θ	Probability Ratio	Prior Probability ($P_{(w)}$)	Posterior Probability ($P_{(w)} \times$ Probability Ratio)
0	0	0	1.11	0
0.05	.05	0	1.07	0
0.10	.10	4	1.04	4
0.15	.15	9,714	1.00	9,714
0.20	.20	216,470	0.96	207,811
0.25	.25	349,350	0.92	321,402
0.31	.30	105,492	0.88	92,833
0.37	.35	10,412	0.83	8,642
0.44	.40	527	0.78	411
0.55	.45	21	0.70	15
3.04	~.50	1	0	0

These values were derived from Renwick [11, table 2], assuming the scaling factor, r, is equal to 0.555 [13]. The data-modified or relative posterior probability distribution for the Rh/6-PGD linkage (table 1) is plotted against w

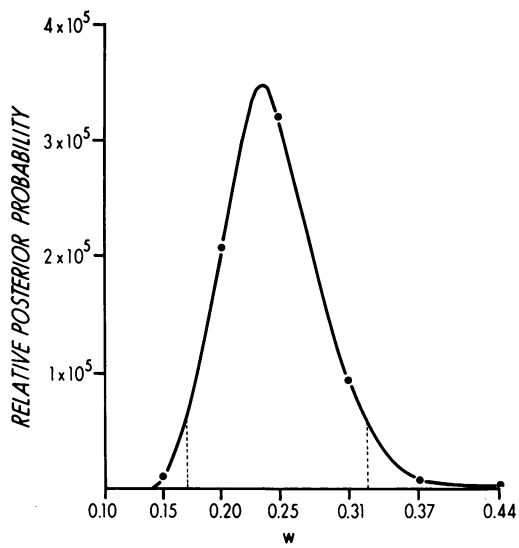

FIG. 1.—Relative posterior probability of linkage between Rh and 6-PGD loci for various values of the map interval, w (in morgans). The probability ratios from 35 pedigrees have been modified by the prior probability distribution (table 1).

in figure 1. Setting the area under the graph of the prior probability distribution in figure 7 of Browne et al. [13] as measured by a planimeter to 1, Λ' is about 34,000. If the prior odds of finding two loci on the same autosome are 1:17.5 [11], the posterior odds are Λ':17.5 or 34,000:17.5 (i.e., about 2,000:1). The corresponding probability (0.9995) is sufficiently large to accept the hypothesis that loci for Rh and 6-PGD lie on the same autosome. Figure 1 shows that the maximum probability value for the linkage distance is approximately at $w = 0.24$ morgans. The narrowest 95% probability limits (see [14]) estimated by planimetry are at $w = 0.17$ morgans and 0.325 morgans.

Possible Difference in the Recombination Fraction in Males and Females

Four previously described human autosomal linkage pairs have had a recombination fraction high enough to permit an analysis of the influence of sex on the estimate of θ. Renwick [15] defines the ratio of the female estimate of map length to the male one as the susceptibility ratio, s; he points out that s may not have a constant value for the whole chromosome complement since there is an apparent inconsistency from region to region in the mouse [16]. In man, estimates of s have varied from 1.55 to 1.74; the ratio of $\hat{\theta}_f$ to $\hat{\theta}_m$ is 0.146:0.084 for the nail-patella (NP)/ABO linkage [17], 0.163:0.105 for the Lutheran/Secretor linkage [15, 18], 0.19:0.11 for the transferrin/cholinesterase E_1 linkage [19], and 0.17:0.10 for the adenylate kinase (AK)/ABO linkage. (The values for $\hat{\theta}_f$ and $\hat{\theta}_m$ in the AK/ABO linkage were derived from the data of Rapley et al. [20], Renwick [15], and Weitkamp et al. [4]. Lod scores for various pairs of values of the recombination fractions, θ_f and θ_m, using the combined data are given in Appendix V. Since the AK and NP loci are closely linked [21], the estimates for θ_f and θ_m in the AK/ABO and NP/ABO linkage pairs involve similar portions of the chromosomal complement.

The standardized probability ratios (relative likelihood) for various pairs of male and female recombination fractions for the 6-PGD/Rh linkage are presented in table 2. The probability ratios for the male and female recombination fractions

TABLE 2

LIKELIHOODS FOR SELECTED PAIRS OF RECOMBINATION FRACTIONS θ_m FOR MALES AND θ_f FOR FEMALES

	θ_m						
	.10	.15	.20	.25	.30	.35	.40
θ_f:							
.15	0	**4**	8	4	1	0	0
.20	6	68	**117**	67	19	3	0
.25	20	226	389	**224**	62	11	1
.30	25	283	488	281	**78**	13	2
.35	16	186	320	184	51	**9**	1
.40	7	82	141	81	23	4	**0**
.45	3	31	53	30	8	1	0
.50	1	12	20	12	3	1	0

NOTE.—The values for which $\theta_m = \theta_f$ are in bold face. If all values are multiplied by 10^3, then the value at $\theta_m = \theta_f = 0.5$ will be 1.

considered separately for each pedigree are indicated in Appendix IV for the 21 two-generation families reported previously, and can be calculated for the remainder of the families from the probability expressions in Appendix III. The values of the probability ratios for the homogeneity situation, $\theta_m = \theta_f$, are lower than those listed in table 1 because the information was omitted for the few cases where sex of the informative parent was unknown. Inspection of table 2 indicates that the maximum height of the surface (maximum probability ratio) is approximately 500,000 at a point corresponding to $\theta_m = 0.20$ and $\theta_f = 0.29$. The same values of θ for the joint maximum probability estimates are obtained more formally by using the method of Smith [17, appendix 2]. At present, the data are insufficient to warrant the effort of transformation into map intervals: the estimate of s is approximately 1.5. Indeed, this estimate may require revision if heterogeneity in the recombination fraction among families proves to be present (see below).

Possible Heterogeneity in the Recombination Fraction among Families

The variable distribution of values for $\hat{\theta}$ for different families (see Appendix IV, especially families H003, H0011, H015, H021, and 10854) suggested the possibility of heterogeneity in the recombination fraction among families. Four hypotheses which might account for the distribution of values for θ among families were considered (see [17]): one true value of θ with linkage (H_L), one true value of θ without linkage (H_U), two true values of θ with linkage in one instance only (H_{LU}), or two true values of θ with linkage in both instances (H_{LL}). The expressions (6) and (7) of Smith [22] were calculated for a range of values of θ_{I} and α and the whole calculation repeated with various values from 0 to 0.5 for the value $\theta_{\mathrm{II}} = 0.5$.

In this manner, a series of tables of likelihood values (probability ratios) for combinations of two recombination fractions, θ_{I} and θ_{II}, each representing a likelihood surface for a specific value of α, the proportion of pedigrees with θ_{I}, were generated. The likelihood values for the situation in which $\alpha = 0.5$ is presented in table 4 as an example. Tables I–IV in Appendix VI list the likelihood values for combinations of θ_{I} and θ_{II} for $\alpha = 0.1$–0.4. The values for $\alpha = 0$ or 1 are the same as for $\theta_{\mathrm{I}} = \theta_{\mathrm{II}}$, and the value for $\alpha = 0.6$–0.9 can be obtained by substituting θ_{I} for θ_{II} in tables I–IV, Appendix VI, for $\alpha = 0.4$–0.1, respectively.

It was assumed in the case of linkage that the initial probability distributions of θ and α are uniform between 0 and 0.5 [10] and between 0 and 1 [22], respectively, although Renwick [11] has suggested a different distribution in the former case and a cosine distribution may be preferable in the latter instance [17]. The assumptions chosen do permit an easy calculation of the average likelihood, Λ, for each value of α (averaged over values for θ_{I} and θ_{II} using the trapezoidal rule in each direction). The values for Λ_{LU} and Λ_{LL}, corresponding to the hypotheses H_{LU} and H_{LL}, are given in table 4. The value of Λ_U is 1 by design and $\Lambda_L = \Lambda_{LL}$ when $\alpha = 0$ or 1. The average likelihood, $\overline{\Lambda}$, for each hypothesis was obtained by averaging with the trapezoidal rule over all values of α: $\overline{\Lambda}_{LU} = 55 \times 10^3$ and $\overline{\Lambda}_{LL} = 434 \times 10^3$. Since $\overline{\Lambda}_L = 70 \times 10^3$, the average likelihood for H_{LL} is about six times that for H_L. Assuming there are two true

recombination fractions among families, we find the most likely values to be roughly at $\theta = 0.05$ and 0.35, each in approximately equal frequency (from inspection of tables 3 and 4; see also Appendix VI).

TABLE 3

LIKELIHOODS FOR COMBINATIONS OF TWO RECOMBINATION FRACTIONS, θ_I AND θ_{II}, AT $\alpha^* = 0.5$.

	θ_{II}										
	0	.05	.10	.15	.20	.25	.30	.35	.40	.45	.50
θ_I:											
0 ...	**0**	0	0	0	23	499	2,324	3,637	2,576	1,039	283
.05 ...	0	**0**	0	1	81	1,203	4,575	6,701	4,880	2,100	588
.10 ...	0	0	**0**	2	101	987	2,850	3,484	2,271	921	253
.15 ...	0	1	2	**10**	116	607	1,235	1,211	694	267	76
.20 ...	23	81	101	116	**216**	428	527	389	193	74	26
.25 ...	499	1,203	987	607	428	**349**	244	128	53	21	9
.30 ...	2,324	4,575	2,850	1,235	527	244	**105**	39	13	5	2
.35 ...	3,637	6,701	3,484	1,211	389	128	39	**10**	3	1	0
.40 ...	2,576	4,880	2,271	694	193	53	13	3	**1**	0	0
.45 ...	1,039	2,100	921	267	74	21	5	1	0	**0**	0
.50 ...	283	588	253	76	26	9	2	0	0	0	**0**

NOTE.—The values in boldface type are the likelihoods when $\theta_I = \theta_{II}$ (or $\alpha = 0$). All values are to be multiplied by 10^3.

* α = Proportion of pedigrees with θ_I.

The preceding values, of course, assume a uniform recombination fraction with respect to sex. A determination of the joint maximum likelihood estimate for θ_{If}, θ_{Im}, θ_{IIf}, and θ_{IIm} for different values of α is beyond the scope of this paper. However, in a preliminary way, the extent to which heterogeneity in the recombination fraction between males and females is reflected in the likelihood values for heterogeneity among families can be tested by computing the values of Λ for the male and female informative families separately. The results are given in table 4; in each case the probability distribution has substantially the same shape as that obtained for the complete pedigrees. The overall ratio, $\overline{\Lambda}_{LL}:\overline{\Lambda}_L$, is about 7.5:1 after thus allowing for sex differences. Since the number of informative offspring from male and female double heterozygotes in this study is approximately the same, the relative smallness of the likelihood values for females compared with males simply reflects the fact that the higher the recombination fraction, the lower the likelihood obtained from a given number of informative offspring [10].

The confidence which may be placed in the assessment of heterogeneity in the recombination fraction among families depends not only on one's opinion of the validity of the approximations made in the calculations of the likelihoods but also on one's prior expectation for finding such heterogeneity. Renwick and Schulze [17] have presented a tentative mathematical estimation of prior odds for the situation where there are two mimic loci for a phenotype; but as Smith [22] points out, there is little evidence to indicate how often a variable recombination fraction may be expected. The prior subjective odds for heterogeneity in the recombination fraction in this linkage will be influenced by two special considera-

TABLE 4

AVERAGE LIKELIHOODS, Λ_{LU} AND Λ_{LL}, FOR VARIOUS VALUES OF α^* IN THE COMPLETE PEDIGREES AND CONSIDERING MALE AND FEMALE RECOMBINATION FRACTIONS SEPARATELY

LIKELIHOOD	α^*											
	0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	$\bar{\Lambda}$
Complete pedigree:†												
Λ_{LU}	0	0	8	37	82	110	101	73	54	55	70	55
Λ_{LL}	70	131	288	535	760	847	760	535	288	131	70	434
Male:‡												
Λ_{LU}	0	0	5	21	58	115	164	176	141	87	49	79
Λ_{LL}	49	82	137	201	253	273	253	201	137	82	49	167
Female:												
Λ_{LU}	1.0	3.0	7.0	11.9	15.4	16.4	15.3	13.4	11.4	9.5	7.5	10.8
Λ_{LL}	7.5	11.1	15.4	19.4	22.0	22.9	22.0	19.4	15.4	11.1	7.5	16.6

NOTE.—The corresponding average likelihood, $\bar{\Lambda}$, is taken over all values of α. At $\alpha = 0$ or $\alpha = 1$, $\Lambda_{LL} = \Lambda_L$.

* α = Proportion of families with θ_I.

† All values to be multiplied by 10^3.

‡ All values to be multiplied by 10^2.

tions. First, the only previous evidence of heterogeneity in the autosomal recombination fraction among human families is for the Rh/elliptocytosis linkage [6, 22–24]. This has been attributed to the existence of two loci for elliptocytosis [6], one being closely linked to the Rh locus ($\theta = 0.05$) and the other being unlinked. However, another possibility would be some region-specific effect of the chromosome, such that other linkages with Rh (rather than elliptocytosis) demonstrate heterogeneity in the recombination fraction. Second, although there is no evidence to indicate heterogeneity in the electrophoretic phenotype of 6-PGD AB heterozygotes among the families in this study, and indeed thus far no direct evidence to indicate more than one human 6-PGD locus, Tuchinda et al. [25] and Ritter et al. [26] have presumed on the basis of the number of 6-PGD electrophoretic bands that there are at least two 6-PGD loci. It is conceivable that variant alleles at different 6-PGD loci may be phenotypically indistinguishable, providing another possible mechanism for variability in the recombination fraction among families.

In view of the subjective nature of the prior expectations of heterogeneity in the recombination fractions among families, the fact that likelihoods are improved by increasing the number of parameters, and the small difference in the value of the likelihoods for H_L and H_{LL}, no calculation of the final odds on heterogeneity has been attempted. However, we do feel that the data warrant further extensive collection of large pedigrees to examine the linkage relations of 6-PGD, Rh, and elliptocytosis in order to investigate the possibilities outlined above.

SUMMARY

A linkage analysis of 35 pedigrees in which variants of 6-PGD are segregating is presented. The data considered as a whole are highly indicative of linkage betweeen loci for 6-PGD and Rh (posterior probability >0.999). The maximum likelihood value for the recombination fraction in males is equal to 0.20, and in females, 0.29, a sex difference consistent with that in four previously described human linkage pairs. The data are not inconsistent with two different recombination fractions among families, a possibility to be explored further.

The 6-PGD/Rh linkage is notable in that if it has one true value for the recombination fraction, it is the loosest linkage yet described in man. If it has truly two values for the recombination fraction, this linkage will have special interest in view of the previously reported variability in the recombination fraction for the Rh/elliptocytosis linkage.

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