Nephrogenic Diabetes Insipidus: Absence of
Close Linkage with Xg

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INTRODUCTION

Nephrogenic diabetes insipidus (NDI) is an inherited disorder which presents clinically with polyuria, polydipsia, and vasopressin-resistant hyposthenuria (Williams and Henry 1947). The hyposthenuria is associated with high levels of biologically active vasopressin, in both serum and urine (Holliday et al. 1963). These manifestations have led to the hypothesis that the basic defect is end-organ resistance.

Studies of pedigree patterns have established that the inheritance is X linked, and there is reason to believe that only one gene locus is involved (Bode and Crawford 1969). Affected males become symptomatic soon after birth, but polyuria is rarely recognized until late in the first year of life unless specifically sought. In many instances the disease leads to severe hypertonic dehydration with failure to thrive, mental retardation, and death. In heterozygous females the symptoms are milder and variable. Some heterozygotes are totally asymptomatic, but the vast majority of heterozygous females, whether symptomatic or not, have a relative inability to concentrate urine. The inability is more marked after puberty and is particularly evident during pregnancy; after menopause the hyposthenuria again becomes milder suggesting some kind of modulation by estrogen. The concentrating deficit in heterozygous females has been shown to be sufficient for a rather reliable determination of the carrier state (Carter and Simpkiss 1956). No female who is homozygous for the NDI gene has yet been reported.

The linkage relationships of NDI locus have not been studied previously. The present study was designed to explore its distance from the sites of Xg and color-blindness. The estimation of distance to deuteranopia, however, failed in that none of 43 males tested using Stilling's tables (Hertel 1939) was found colorblind.

MATERIALS AND METHODS

The study was based on the five families which were investigated earlier to establish the mode of inheritance (Bode and Crawford 1969). These families were partially

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reexamined for the present purpose. The propositus of each family was a former patient at the Massachusetts General Hospital. Although not clearly related, all five families share the same distant ancestry, being descendants of the "Ulster Scot" Presbyterians who settled in Colchester County, Nova Scotia, and in the Merrimac Valley of New Hampshire, in the eighteenth century.

Detection of the NDI gene. In males the presence of the NDI gene was established by the documentation of persistent hyposthenuria after water deprivation and administration of vasopressin.

Females were considered heterozygous when gene transmission could be documented or when—in the absence of renal disease, alcoholism, or diuretics—the excretion of hypotonic urine after 12 hr of water deprivation was observed on each of at least two occasions. That this test permits an adequate determination of carrier status was first suggested by Carter and Simpkiss (1956): none of the 14 heterozygous females in their series was able to concentrate urine above a specific gravity of 1.018 on three separate occasions. In a larger series, we found some overlap between genetically-determined heterozygotes and normal homozygotes when considering urine concentration in terms of specific gravity as well as osmolality (Bode and Crawford 1969). On the basis of these observations it was deemed reliable to consider a woman, not classifiable on the basis of gene transmission, to be a carrier if repeated urine samples obtained after water deprivation did not exceed a specific gravity of 1.017 and osmolality of 700 milliosmols/kilogram. If these values were at least 1.020 and 900 milliosmols/kilogram, respectively, the woman was classified as normal. If a woman fell into neither of these categories and there was no definite genetic evidence of heterozygosity, she was considered to be of unknown genotype.

Determination of Xg antigen. The presence or absence of the Xg antigen was determined on red cells obtained from clotted blood. These were incubated for one hour in the presence of Xg antiserum supplied by Ortho Diagnostic, Raritan, New Jersey; hemagglutination was taken as an index of a positive response.

The pedigrees and estimation of linkage. Of the five families in the earlier study, a total of 84 persons submitted to the withdrawal of a blood sample. This included 18 affected males and 29 female carriers of the NDI gene. Only four of the five families proved informative for the present linkage study. In the fifth family there was no indication of individuals heterozygous for Xg. The known and relevant aspects of these families are shown in figure 1.

The frequency of recombination between the NDI and Xg loci was estimated according to the likelihood ratio ("backward odds") method of Haldane and Smith (1947). The computer program of Renwick and Schulze (1961) was not conveniently available to us, and the likelihood function was therefore derived algebraically for each family. Morton's ascertainment correction (Morton 1955) was not employed in the interest of simplicity and because its effect would be negligible in the circumstances of the present study. The evaluation of the likelihood ratio function at various values of the recombination fraction was carried out by computer. The likelihood ratio functions involve the frequency of the Xg gene. For this the value 0.65 was used (Sanger et al. 1962).

To illustrate the method of deriving the likelihood ratio function, we shall consider in detail the analysis of family 4 in figure 1. The female I-2 is either homozygous or heterozygous for the Xg gene. If she were homozygous, then the parental genotypes would imply that the probability of II-1 becoming NDI negative and Xg positive is $\frac{1}{2} \times 1 = \frac{1}{2}$; the probability of II-2 becoming Xg positive is 1; and the probability of II-3 becoming both NDI and Xg positive is $\frac{1}{2} \times 1 = \frac{1}{2}$. Thus, given that I-2 is homozygous for Xg, the advance probability of the observed characteristics of generation II is $\frac{1}{2} \times 1 \times \frac{1}{2} = \frac{1}{4}$:

$$\Pr(\text{generation II} \mid \text{I-2 is homozygous for Xg}) = \tfrac{1}{4} . \tag{1}$$

If I-2 is heterozygous and in coupling, then II-1 represents a crossover and arises with probability $\frac{1}{2}\theta$, where θ is the recombination fraction; the probability that II-2
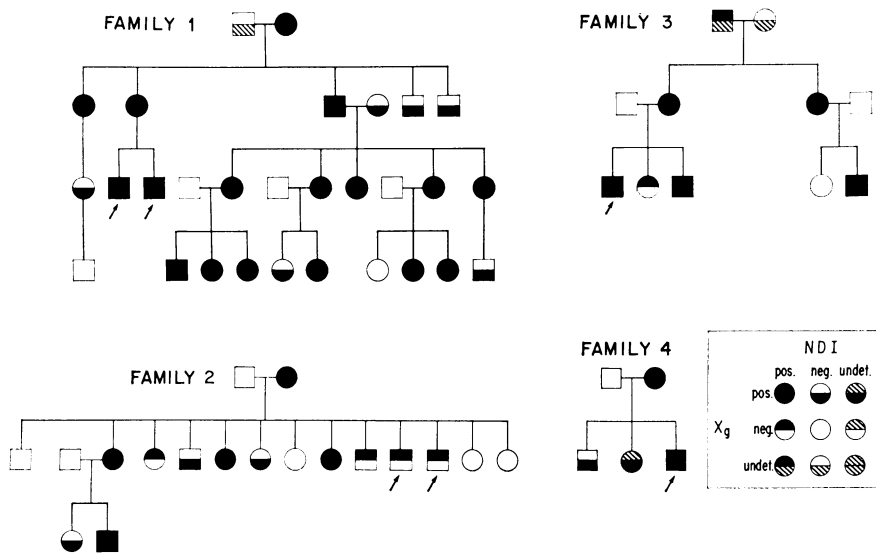

FIG. 1.—Four pedigrees in which linkage between nephrogenic diabetes insipidus and the red-cell antigen Xg was studied.

would become Xg positive is $\frac{1}{2}$; and the probability of II-3, a nonrecombinant, is $\frac{1}{2}(1 - \theta)$.
Thus,

$$\Pr(\text{generation II} \mid \text{I-2 is heterozygous in coupling}) =$$
$$\tfrac{1}{2}\theta \times \tfrac{1}{2} \times \tfrac{1}{2}(1 - \theta) = \theta(1 - \theta)/8 . \tag{2}$$

Similarly,

$$\Pr(\text{generation II} \mid \text{I-2 is heterozygous in repulsion}) =$$
$$\tfrac{1}{2}(1 - \theta) \times \tfrac{1}{2} \times \tfrac{1}{2}\theta = \theta(1 - \theta)/8 . \tag{3}$$

The relative probabilities of the conditions in equations (1), (2), and (3) may be represented by p^2, $2p(1 - p) \times \frac{1}{2} = p(1 - p)$, and $2p(1 - p) \times \frac{1}{2} = p(1 - p)$, re-

spectively, where p is the frequency of the Xg gene. Thus, the overall (unconditional) probability of observing the particular characteristics of generation II, given the phenotypes of generation I, is proportional to $p^2/4 + 2p(1 - p)\theta(1 - \theta)/8$ or to $p + (1 - p)\theta(1 - \theta)$. Therefore, the likelihood ratio function concerning the null hypothesis of independent recombination $(\theta = \frac{1}{2})$, for family 4, is $\lambda_4(\theta, \frac{1}{2}) = [p + (1 - p)\theta(1 - \theta)]/[p + (1 - p)/4] = 4[p + (1 - p)\theta - (1 - p)\theta^2]/(1 + 3p)$, with $p = .65$.

The derivation of the likelihood ratio function is more complex for family 3, and much more so for families 1 and 2. We shall forego the specifics in these families.

RESULTS

The values of the likelihood ratio functions for various values of the recombination fraction, θ, are given for each family in table 1. This table also gives the corresponding values of the overall likelihood ratio function $\lambda(\theta, \frac{1}{2})$ and of $\ln \lambda(\theta, \frac{1}{2})$.

TABLE 1

EVALUATION OF LIKELIHOOD RATIO $\lambda(\theta, \frac{1}{2})$

RECOMBINATION FRACTION θ	FAMILY				COMBINED	
	I $\lambda_1(\theta, \frac{1}{2})$	II $\lambda_2(\theta, \frac{1}{2})$	III $\lambda_3(\theta, \frac{1}{2})$	IV $\lambda_4(\theta, \frac{1}{2})$	$\lambda(\theta, \frac{1}{2})$	$\ln \lambda(\theta, \frac{1}{2})$
0.00	0.000	0.000	0.000	0.881	0.000	$-\infty$
0.05	0.354	0.000	0.587	0.904	0.000	-14.039
0.10	1.013	0.000	0.946	0.924	0.000	-7.823
0.15	1.607	0.006	1.135	0.942	0.010	-4.585
0.20	1.988	0.032	1.202	0.957	0.073	-2.608
0.25	2.132	0.106	1.191	0.970	0.261	-1.346
0.30	2.076	0.249	1.137	0.981	0.577	-0.552
0.35	1.880	0.459	1.071	0.989	0.914	-0.090
0.40	1.605	0.697	1.017	0.995	1.132	0.123
0.45	1.299	0.898	0.990	0.999	1.134	0.144
0.50	1.000	1.000	1.000	1.000	1.000	0.000

A graphical presentation of $\lambda(\theta, \frac{1}{2})$ is given in figure 2. The function has its maximum at $\hat{\theta} = 0.42$, and this, then, is the maximum likelihood estimate of the recombination fraction.

With a sufficiently large sample one may be $100(1 - \alpha)\%$ confident that the actual value θ satisfies the inequality $-2 \ln[\lambda(\theta, \frac{1}{2})]/\lambda(\hat{\theta}, \frac{1}{2}) < \chi_\alpha^2(1)$, where $\chi_\alpha^2(1)$ is the upper α point—the $100(1 - \alpha)$ percentile—of the χ^2 distribution with 1 df. This inequality may be recast in the form $\lambda(\theta, \frac{1}{2}) > \text{antilog}_e [\ln \lambda(\hat{\theta}, \frac{1}{2}) - (u_{1/2\alpha})^2/2]$, where $u_{1/2\alpha}$ is the upper $\frac{1}{2}\alpha$ point of the standard normal distribution. Thus, on the basis of the present data, one may be about 90% confident that θ satisfies $\lambda(\theta, \frac{1}{2}) = \text{antilog}_e [\ln(1.36) - 2.706/2] = 0.35$. From figure 2 it is seen that this bound $\lambda(\theta, \frac{1}{2}) = 0.35$ corresponds to $\theta = 0.26$. We thus conclude, with about 90% confidence, that $\theta \geq 0.26$. Therefore, while the data contain no evidence for linkage, they are, at the same time, insufficient to confine the possible values of θ to the vicinity of $\frac{1}{2}$, and only close linkage can be confidently excluded.

DISCUSSION

The estimation of the distance between the NDI and Xg loci assumes that only one locus is involved in each trait. This is well established for Xg. Strong supporting evidence for a single locus in NDI is provided by the common ancestry of the cases as well as the conformity of inheritance of the disorder in the pedigrees to X-linked transmission (Bode and Crawford 1969). This also provides a basis for pooling the information from the four families.

LIKELIHOOD RATIO FUNCTION
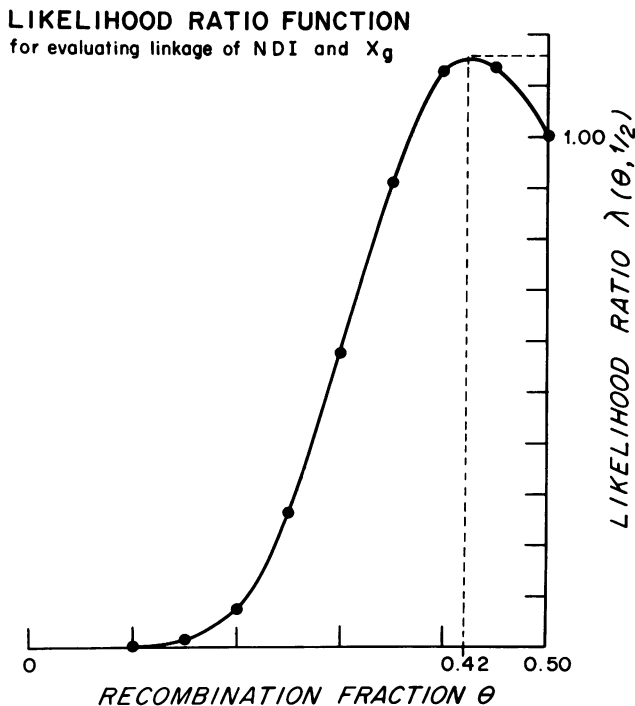for evaluating linkage of NDI and Xg

FIG. 2

The determination of the genotype for NDI in the male is not subject to error. The genetic and renal functional criteria used in determining the genotype in females seem sufficiently stringent: when the urine-concentration criteria were applied to the original cases of Bode and Crawford (1969), none of the 43 obligate carriers was classified as normal and two were not classifiable. Among their 28 normal homozygotes, none was classified as abnormal and six were not classifiable. Moreover, in the present study there was only one woman whose genotype remains indeterminate on the basis of the urine-concentration criteria.

There may be some question about the applicability of the simple asymptotic method of interval estimation. It seems, however, that the sample size is sufficient for a reasonable degree of accuracy. One alternative approach to this estimate would have been that suggested by Haldane and Smith (1947). This does not presuppose

large sample size but is definitely conservative. When applied to the present data, it permits one to conclude with a confidence of *at least* 90% that the recombination fraction is greater than 0.18, a figure in good agreement with that arrived at by the asymptotic method. The excessive conservatism of this method could have been avoided by using the iterative graphical procedure of Renwick and Schulze (1964).

The broad conclusion from the present study is that the linkage between the NDI and Xg loci is not a close one. Due to the failure to detect cases of colorblindness, the locus of which is not within a measurable distance from Xg (Siniscalco et al. 1966), it is not possible on the basis of the present data to determine the direction of the NDI locus relative to that of Xg. Inasmuch as the locus for NDI is remote from that for the Xg antigen, it could be close to that for colorblindness. To our knowledge the coincidence of these two defects has not been reported, and we have examined 43 males affected with NDI without encountering one with colorblindness. Since the gene frequency is 8% of the general population, its absence in this large group of males is remarkable. Granted that their common ancestry might somewhat reduce the frequency below the 8% affected when nonrelated males are examined, there were many opportunities for introduction of the gene into these families. Certainly colorblindness should be considered as a marker in the future studies of the linkage relationships of NDI.

SUMMARY

The linkage relationship of nephrogenic diabetes insipidus to the Xg blood antigen was studied. The material consisted of four families with common ancestry. There were 13 male cases and 22 female carriers. The data permitted the exclusion of close linkage, and it is concluded with about 90% confidence that the recombination fraction is greater than one-fourth, the maximum likelihood estimate obtained for this parameter being 0.42.

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