Linkage Between the X Chromosome Loci for Glucose-6-Phosphate Dehydrogenase Electrophoretic Variation and Hemophilia A

SAMUEL H. BOYER[1] AND JOHN B. GRAHAM[2]

[1]Division of Medical Genetics, Department of Medicine, Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland.
[2]Department of Pathology and Genetics Training Program, University of North Carolina Medical School, Chapel Hill, North Carolina.

THE A AND B ELECTROPHORETIC variants of glucose-6-phosphate dehydrogenase (G6PD) form an X-linked polymorphism among persons of West African ancestry (Boyer, Porter, and Weilbacher, 1962). For the purpose of linkage analysis in man, such variants provide a useful addition to the three previously employed polymorphic X-linked markers, viz., deutan colorblindness, Xg(a) erythrocyte type, and G6PD deficiency. It is our purpose to describe evidence for linkage between the structural G6PD locus and that for deficiency of antihemophilic factor (AHF) (hemophilia A). Such linkage has been inferred from other studies but heretofore has not been directly demonstrated.

SUBJECTS AND METHODS

Negro males with known AHF deficiency were sought through the use of records at the Johns Hopkins Hospital and at the University of North Carolina Medical Center. At Johns Hopkins the type of hemophilia was determined by Dr. Dudley P. Jackson and at North Carolina by one of us (J. B. G.). Probands and their relatives were examined for G6PD electrophoretic type by one or more methods (Boyer, Porter, and Weilbacher, 1962; Kirkman and Hendrickson, 1963; Porter *et al.*, 1964). The method described by Kirkman and Hendrickson (1963) is superior to other methods for typing erythrocytic enzyme since it permits detection of most Gd^A/Gd^B heterozygotes.* All electrophoretic methods allow detection of gross G6PD deficiency, i.e., phenotype Gd (A−), without resort to further assay. A total of six apparently unrelated sibships were examined. Two of these were detected at Johns Hopkins and the rest in North Carolina. Only three families (Fig. 1) contained doubly heterozygous mothers and were thus informative for linkage analysis. The remaining families contained only Gd (B) males and mothers. No hemophilic sibships with G6PD deficient subjects were encountered.

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*The nomenclature employed differs from that of earlier communications from one of us (S. H. B.) in that the gene for G6PD B+ is described as Gd^B and that for G6PD A+ as Gd^A.

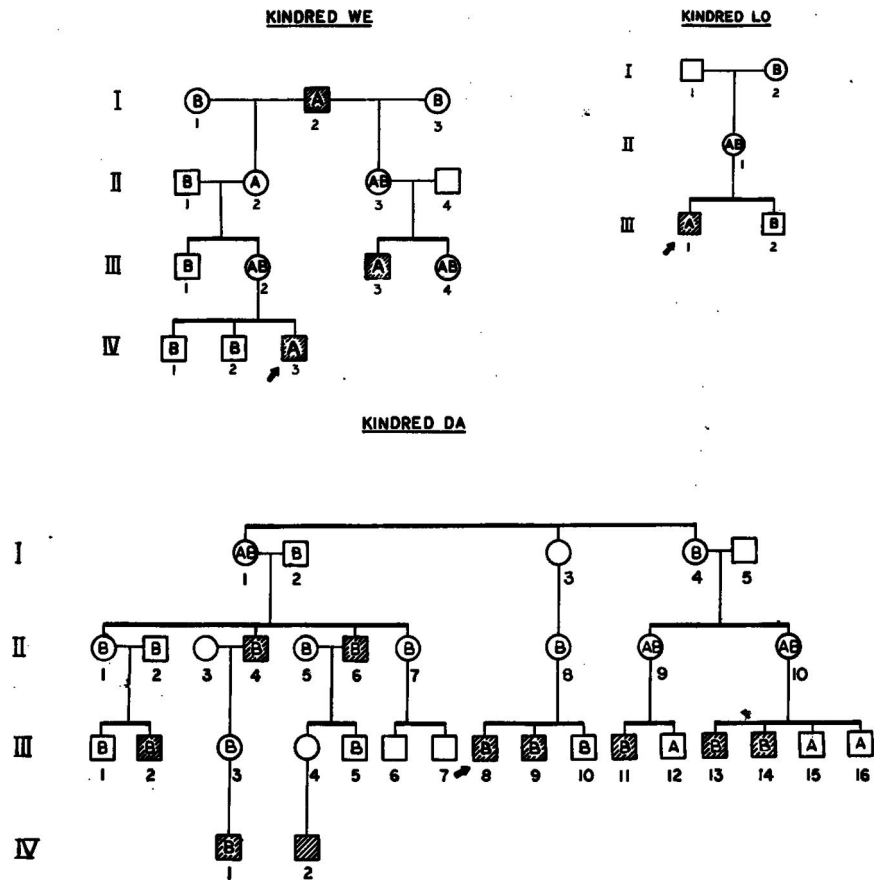

FIG. 1. Pedigrees of *We, Da* and *Lo* kindreds. Letters refer to electrophoretic type of G6PD in erythrocytes and leukocytes of kindred *We* and in erythrocytes of kindreds *Da* and *Lo*. Shading indicates hemophilia A and arrows denote probands. The G6PD type of *Da* III-4 and IV-2 was omitted from the pedigree. Both were B and thus uninformative.

RESULTS AND DISCUSSION

Kindred We (Johns Hopkins)

In kindred *We*, the women, II-2 and II-3, necessarily bear the genes for AHF deficiency and Gd (A+) in coupling. Both of these women are double heterozygotes, although the Gd (B) component is not detectable in either the leukocytes or erythrocytes of subject II-2. Opportunity for recombination between the *Gd* locus and that for AHF deficiency is manifest in individuals III-1, III-2, III-3, IV-1, IV-2, and IV-3. Among these six opportunities, it is unusual to be able to include a female, subject III-2; however, the antecedent and descendent pedigree as given clearly allows such inclusion. No recombinants are observed.

The discrepancy between G6PD genotype and phenotype in II-2 may be confusing and merits the following comment. Individual II-2 must have genotype Gd^A/Gd^B since she has a Gd (B) son whose filial relationship is

verified by blood group analysis. The discrepancy between genotype and phenotype in II-2 probably represents nothing more than the odd instance in which the phenomenon of X-chromosome inactivation (Lyon, 1962) has disproportionately involved the Gd^B gene to the point where its product is undetectable. Such discrepancies were observed three times among seventy families studied by Boyer, Porter, and Weilbacher (1962) and apparently involved, as in II-2 of kindred *We*, suppression of one particular X chromosome in *both* leukocytes and erythrocytes. The repeated detection of coincident inactivation of a particular X chromosome in two different types of cells would be unusual, unless these cells have a common primordial origin or there is a ubiquitous factor causing selective inactivation of a particular X chromosome. These alternatives could be tested by typing G6PD phenotype in some third tissue of appropriate subjects.

Kindred Da (North Carolina)

Evident opportunities for recombination are apparent among the offspring of I-1, II-9, and II-10. There are nine such offspring, viz., individuals II-1, II-4, II-6, III-11, III-12, III-13, III-14, III-15, and III-16. Unfortunately, the phase of linkage between the G6PD gene and that for hemophilia is not as certainly known as in kindred *We*. If subject I-4 is in fact homozygous for Gd^B rather than a cryptic Gd^A/Gd^B heterozygote, the genes for B and AHF deficiency are probably coupled in her daughters (II-9 and II-10). Accordingly, the six subjects, III-11 through III-16, are regarded as nonrecombinants. Individuals II-1, II-4, and I-6 represent either all recombinants or all nonrecombinants. The degree of linkage manifest in the preceding discussion distinctly favors nonrecombination.

Kindred Lo (North Carolina)

Kindred *Lo* represents a segment of a very large but generally uninformative family. Although the identity of individual I-1 is uncertain, he is probably the one of several men known to be hemophilic. Repeated analysis of blood from the woman, I-2, gives no evidence of a Gd^A gene. Consequently, it is presumed that the genes for both Gd^A and hemophilia were derived from I-1 and are coupled in subject II-1. Therefore, it is likely that both III-1 and III-2 represent nonrecombinants.

CONCLUSION

The absence of recombination among a total of 17 evident opportunities indicates close linkage between the loci for AHF deficiency and G6PD electrophoretic variation. In this situation, the maximum recombination fractions that could exist at the 95% and 99% confidence limits can be estimated by solution of the terminal term of the binomial expression. Such estimates are, respectively, 0.162 and 0.237. These values are compatible with a recombination fraction of 0.17 indirectly adduced from a recombination fraction of 0.12 between loci for AHF deficiency and deutan colorblindness (Whittaker, Copeland, and Graham, 1962; McKusick, 1964) and a recombination fraction

of 0.05 between deutan colorblindness and G6PD deficiency (Adam, 1961; Porter, Schulze, and McKusick, 1962), and from an assumption of the order of *Gd...deutan...AHF* (Adam *et al.*, 1963).

Our findings may also be examined by the likelihood expression. For mapping purposes, the median estimate may be preferable to the maximum likelihood estimate when the latter is zero, as it is in the present instance. A median estimate may be obtained by solving the likelihood expression to find that value of the recombination fraction which bisects the expression's integrated area. When no recombinants are observed among seventeen opportunities, the median recombination fraction is 0.038. This value suggests that the *Gd* structural locus may be considerably closer to that for AHF deficiency than previously supposed. If these two loci are in fact closely linked, then the order of X-chromosome loci may be *Xg...deutan...Gd...AHF* rather than *Xg...Gd...deutan...AHF*. Evidence for the latter arrangement has recently been summarized by McKusick (1964) and by Jackson, Symon, and Mann (1964). Such evidence largely derives from a recombination fraction of 0.27 between *Xg* and *Gd* (Adam *et al.*, 1962; Adam *et al.*, 1963), a recombination fraction of 0.42 between *Xg* and *deutan* (Jackson, Symon, and Mann, 1964), and a recombination fraction of 0.40 between *Xg* and *AHF* (Davies *et al.*, 1963). These, the most probable values, are inconsistent with an *Xg...deutan... Gd...AHF* arrangement. However such inconsistency may be more apparent than real, since in all *Xg* linkage analyses the 95% confidence limits (Renwick and Schulze, 1964) are sufficient to tolerate the latter arrangement. Another means of resolving possible inconsistency would lie in the existence of distinct loci for G6PD electrophoretic variation and G6PD deficiency. However, the genes for these characters apparently are closely linked if not identical (Boyer, Porter, and Weilbacher, 1962). Ethnic heterogeneity of the position of certain X-chromosome loci provides yet another means of resolving possible inconsistencies. In this connection, it is notable that the bulk of X-chromosome linkage studies have been conducted in non-Negro populations.

SUMMARY

Recombinants were not observed among seventeen evident opportunities for recombination between genes for glucose-6-phosphate dehydrogenase electrophoretic variation and hemophilia A. In a binomial distribution, this results in a maximum recombination fraction of 0.16 at the 95% confidence limit and 0.24 at the 99% confidence limit. The median estimate from the likelihood expression yields a recombination fraction of 0.038.

REFERENCES

ADAM, A. 1961. Linkage between deficiency of glucose-6-phosphate dehydrogenase and colour blindness. *Nature* 189: 686.

ADAM, A., SHEBA, C., RACE, R. R., SANGER, R., TIPPETT, P., HAMPER, J., AND GAVIN, J. 1962. Linkage relations of the X-borne genes responsible for glucose-6-phosphate dehydrogenase and for the Xg blood groups. *Lancet* i: 1188–1189.

ADAM, A., SHEBA, C., SANGER, R., RACE, R. R., TIPPETT, P., HAMPER, J., GAVIN, J., AND

FINNEY, D. J. 1963. Data for X-mapping calculations, Israeli families tested for Xg, g-6-pd and for colour vision. *Ann. Hum. Genet.* (Lond.) 26: 187–194.

BOYER, S. H., PORTER, I. H., AND WEILBACHER, R. G. 1962. Electrophoretic heterogeneity of glucose-6-phosphate dehydrogenase and its relationship to enzyme deficiency in man. *Proc. Nat. Acad. Sci.* (U. S.) 48: 1868–1876.

DAVIES, S. H., GAVIN, J., GOLDSMITH, K. L. G., GRAHAM, J. B., HAMPER, J., HARDISTY, R. M., HARRIS, J. B., HOLMAN, C. A., INGRAM, G. I. C., JONES, T. G., MCAFEE, L. A., MCKUSICK, V. A., O'BRIEN, J. R., RACE, R. R., SANGER, R., AND TIPPETT, P. 1963. The linkage relations of hemophilia A and hemophilia B (Christmas disease) to the Xg blood group system. *Amer. J. Hum. Genet.* 15: 481–492.

JACKSON, C. E., SYMON, W. E., AND MANN, J. D. 1964. X chromosome mapping of genes for red-green colorblindness and Xg. *Amer. J. Hum. Genet.* 16: 403–409.

KIRKMAN, H. N., AND HENDRICKSON, E. M. 1963. Sex-linked electrophoretic difference in glucose-6-phosphate dehydrogenase. *Amer. J. Hum. Genet.* 15: 241–258.

LYON, M. F. 1962. Sex chromatin and gene action in the mammalian X-chromosome. *Amer. J. Hum. Genet.* 14: 135–148.

MCKUSICK, V. A. 1964. *On the X Chromosome of Man.* Washington: American Institute of Biological Sciences.

PORTER, I. H., SCHULZE, J., AND MCKUSICK, V. A. 1962. Genetical linkage between the loci for glucose-6-phosphate dehydrogenase deficiency and colour blindness in American Negroes. *Ann. Hum. Genet.* (Lond.) 26: 107–122.

PORTER, I. H., BOYER, S. H., WATSON-WILLIAMS, E. J., ADAM, A., SZEINBERG, A., AND SINISCALCO, M. 1964. Variation of glucose-6-phosphate dehydrogenase in different populations. *Lancet* i: 895–899.

RENWICK, J. H., AND SCHULZE, J. 1964. An analysis of some data on the linkage between Xg and colorblindness in man. *Amer. J. Hum. Genet.* 16: 410–418.

WHITTAKER, D. L., COPELAND, D. L., AND GRAHAM, J. B. 1962. Linkage of color blindness to hemophilias A and B. *Amer. J. Hum. Genet.* 14: 149–158.