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Hemizygous Expression of Glucose-6-Phosphate Dehydrogenase in Erythrocytes of Heterozygotes for the Lesch-Nyhan Syndrome*

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Abstract. In women heterozygous for hypoxanthine guanine phosphoribosyl transferase deficiency, the activity of this enzyme in the erythrocyte is usually normal. In a key kindred two such obligate heterozygotes were also heterozygous for glucose-6-phosphate dehydrogenase types *A* and *B*. The *AB* genotype was confirmed in one by assay of skin fibroblasts. Erythrocytes were exclusively of type *B*. These observations suggest the clonal origin of the hematopoietic system in these women from a primordial cell line with a single active *X* chromosome.

Patients with complete deficiency of the activity of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) (E.C.2.4.2.8) present clinically with the Lesch-Nyhan syndrome, in which mental retardation, choreoathetosis, spastic cerebral palsy, and self-destructive biting occur along with hyperuricemia.[1, 2] The disorder is transmitted as an *X*-linked recessive trait.[3] In accordance with the Lyon hypothesis, the female carrier of the gene should be a mosaic, in which there are two populations of cells, one in which the activity of HGPRT is normal and the other in which the activity of HGPRT is completely absent. This has been shown to be the case in clones of fibroblasts grown in cell culture after biopsy of the skin of mothers of boys with the disease.[4, 5] If the inactivation of one of the *X* chromosomes in the female were a random process, the mean activity of an enzyme determined by the *X* chromosome in a series of mothers of deficient males should approximate 50 per cent of normal, unless there were some form of regulation after inactivation. However, assay of HGPRT activity in the erythrocytes of a group of obligate heterozygotes has indicated that activity is normal in virtually all of them.[6] Similarly, in autoradiographic studies of hypoxanthine incorporation in lymphocytes of heterozygous females, Dancis and colleagues[7] found no evidence of mosaicism. Observations on a family in which there were two types of glucose-6-phosphate dehydrogenase (G6PD) (E.C.1.1.1.49) as well as two types of HGPRT indicate that in the hematopoietic system there is selection for the cell with normal activity of HGPRT.

Materials and Methods. Fibroblast cultures were established following punch biopsy of the skin and carried in Eagle's minimal essential medium with 20% fetal calf serum.

Venous blood was drawn into Acid Citric Dextrose solution. HGPRT was assayed in erythrocytes[6] using a method in which labeled inosinic acid is isolated on an AG-50 column following incubation with ^{14}C-labeled hypoxanthine. This enzyme was also assayed using a method in which the nucleotide product is precipitated with lanthanun.[8] The isoenzymes of G6PD were separated using disk gel electrophoresis.[9] With this method, the mobilities of G6PD *A* and *B* are clearly different, and the two isozymes can be readily separated in an artificial mixture or material from a heterozygote.

Results. A partial pedigree of the kindred under study is illustrated in Figure 1. There have been four involved boys with hyperuricemia, cerebral

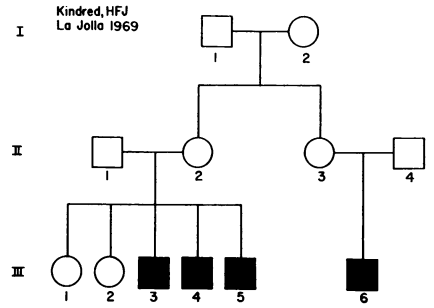

FIG. 1.—Pedigree of the HFJ kindred. The closed squares indicate males with complete deficiency of HGPRT. Patients III 3, 4, and 5 have all the clinical manifestations of the Lesch-Nyhan syndrome. III 6 is less than 6 months of age.

dysfunction, and self-mutilation in generation III. Patient III 5 has been reported by Marks and colleagues.[10] HGPRT activity has been measured in the erythrocytes of III 3, 4, 5, and 6 by two different methods[6, 8] and found to be completely deficient. In contrast, HGPRT activity has been found to be normal in the female siblings, III 1 and 2, and in the two obligate heterozygotes II 2 and 3, as well as in their mother I 2. Erythrocyte HGPRT activities in II 2 and 3 were 38.3 and 39.7 $m\mu M/hr/\mu l$ red blood cells, while the mean of 17 control individuals was 39.1 and the value for I 2 was also 39.1.

Glucose-6-phosphate dehydrogenase activity was measured quantitatively in the involved males and their mothers and found to be normal. Electrophoretic analysis of this kindred revealed that both G6PD types *A* and *B* were present (Fig. 2). The grandfather I 1 was type *B* and the grandmother I 2 type *A*. Therefore, their daughters II 2 and II 3 would be expected to be heterozygous *AB*. Furthermore, the husband of II 2, II 1, was type *A*, and their daughters III 1 and 2 were *A* and *AB*, which would also require II 2 to be *AB*. Her sons were types *B*, *B*, and *A*, which would also require her to be *AB*. However, her electrophoretic pattern was clearly only *B*. This finding was confirmed on two separate blood samples. One of them was also analyzed by Dr. H. N. Kirkman using starch gel electrophoresis of both young and old red cells and also found to contain only G6PD *B*. Leukocytes were assayed in our laboratory and by Dr. Kirkman and found also to contain only G6PD *B*. A similar discrepancy between genotype and phenotype was observed in II 3 whose erythrocytes contained only G6PD type *B*. Her son's hemolysate (III 6) can be seen to contain fetal hemoglobin as well as the faster-moving hemoglobin *A*. His G6PD type was *A*. It was concluded from these observations that the two mothers, II 2 and 3, who were obligate heterozygotes for HGPRT deficiency, were of genotype

AB for G6PD, but their red and white cells exhibited only the *B* phenotype.

This discrepancy was studied further in II 2 in extracts of cultured fibroblasts obtained following biopsy of the skin. Disk gel electrophoresis of the G6PD isozymes of her fibroblasts are shown in Figure 3. The gels illustrated were those of extracts of fibroblasts of I 2 whose G6PD type *A* was consistent with the type found in her erythrocytes and of a control individual contained G6PD type *B* as well as of II 2. In this analysis II 2 was shown clearly to be heterozygous, her fibroblasts containing both G6PD types *A* and *B*. The phenotype of these somatic cells, unlike that of cells of the hematopoietic system, was consistent with the *AB* genotype. Identical data were obtained on II 2's sister II 3.

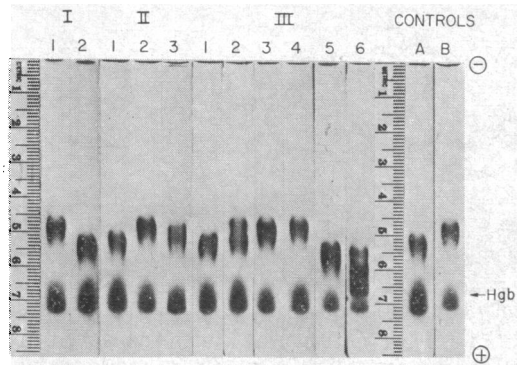

FIG. 2.—Disk gel electrophoresis of G6PD isozymes in erythrocyte hemolysates. Hemolysates of control individuals representing types *A* and *B* are shown at the right. From the left are shown the results obtained with hemolysates from the HFJ kindred, designated as in Figure 1.

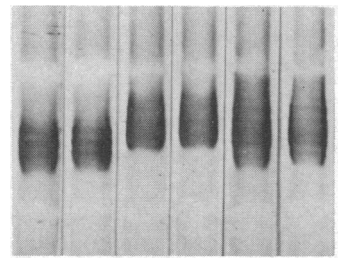

FIG. 3.—Disk gel electrophoresis of G6PD isozymes in extracts of cultured fibroblasts. From the left are illustrated 2 gels each of I 2, type *A;* control D.B., type *B*, and II 2, type *AB*.

Discussion. The data from the fibroblasts indicate that the discrepancy between genotype and phenotype observed with erythrocytes and leukocytes is not methodological. Of course, the analysis of the erythrocytes of II 2's daughter, III 2, indicated that the $G6PD^A/G6PD^B$ heterozygote is readily detected using this methodology. A faint A^- band could be missed in an $A^- B$ heterozygote, but then the type *A* sons would be A^-, while these sons were type *A* and not G6PD deficient. Similarly, the heterozygous females in whom the discordance was observed were not ill and were receiving no drugs. It appears likely, therefore, that the findings are due to a genetic event involving the marrow or a stem cell.

A number of genetic mechanisms could be responsible, including somatic mutation, recombination, or deletion. In the case of the *X* chromosome it seems more likely that the mechanism involves that of inactivation of the *X*. It appears, at least in the case of the marrow, that either the inactivation of the *X* chromosome in the heterozygous female is not random, or that following random inactivation there is selection against the cell with HGPRT deficiency. In either case we postulate a clonal origin of the HGPRT containing cells found in the peripheral blood of the heterozygous female.

These findings are most unusual. In a study of G6PD in over 100 mother-son pairs, Kirkman and Hendrickson[11] found no instances of discrepancy between the phenotype of the erythrocytes of mother and son. It seems likely that it is HGPRT deficiency that is selected against and that in these heterozygotes the HGPRT deficient gene is on the same X chromosome as that for G6PD A, while the other X chromosome specifies the normal form of HGRPT and G6PD B. This hypothesis can be assessed by cloning the fibroblasts of the double heterozygotes, II 2 and II 3, and examining for HGPRT and the isozymes of G6PD. Clonal analysis of this sort in II 2's girls, III 1 and 2, could provide important information on the hypothesis.

Data reported by Gandini and colleagues[12] could be interpreted as indicating that the hematopoietic system in some heterozygotes may stem from a single pool of primordial cells. In a study of 950 women in Sardinia, they found 4 among an estimated 300 $G6PD^{+/-}$ heterozygotes in whom the levels of activity of G6PD were indistinguishable from those of $G6PD^{-}$ hemizygotes, while study of their families, in which the activity of G6PD in a father or a son was normal, indicated them to be heterozygotes. G6PD activity was assayed in skin homogenates and found to be in the heterozygous range. The authors interpreted their data as indicating an embryonic cell population at the time of inactivation of eight cells or less. Their data appear, therefore, to be explainable by a different mechanism than those of selective inactivation or selection after inactivation that appear to be operating in the family we have studied.

The inactivation of elements in the genome is a fundamental problem in biology. The turning on and off of genes in sequence during development is basic to the question of differentiation. Among the autosomes that determine myeloma proteins there appears to be selective activation of parts of the genome such that there is optimal complementation of light and heavy chains.[13] The closeness of the fit is thought to result in a signal for the clonal selection of those cells with the appropriate part of the genome turned on. The X chromosome is unique in that the entire chromosome is inactivated. Nonrandom inactivation of the X chromosome has been observed by Lyon and colleagues[14] in the mouse in the special case of X-autosome translocation. In this situation, the heterozygous female has three chromosomes carrying X-chromosomal material, one intact X and two autosomes each carrying part of the other X. Simultaneous inactivation of the two translocated parts appears to exceed the capacity of this mechanism of dosage compensation, for it is always the intact X chromosome that is inactivated.

It is not possible to assess accurately the degree of linkage of the genes for G6PD and HGPRT on the X chromosome from the study of a single family. However, patients III 3 and 4 were of G6PD type B, while patients III 5 and 6 were of G6PD type A. Therefore, in view of at least two crossovers, it appears that the two genes are not close.

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Abbreviations: HGPRT, hypoxanthine guanine phosphoribosyl transferase; G6PD, glucose-6-phosphate; ACD, acid citric dextrose; Rbc, red blood cell; HFJ, the initials of the surnames of members of the kindred.

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