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* On leave from the Department of Immunology, Madurai University, Madurai, India.

17 May 1979

Imbalance in X-Chromosome Expression: Evidence for a Human X-Linked Gene Affecting Growth of Hemopoietic Cells

Abstract. *In each of six family members who were heterozygous at the X-linked locus for glucose-6-phosphate dehydrogenase, only one or the other of the two alleles at that locus was almost exclusively expressed. The data are consistent with evidence that X-chromosome inactivation is a random process that may be followed by selection for one of the two resulting cell types on the basis of an unknown gene, which is located on the X chromosome and which can affect the rate of proliferation of hemopoietic cells in humans.*

The structural gene for glucose-6-phosphate dehydrogenase (G6PD) is located on the X chromosome in humans (*1*). It is generally accepted that inactivation of the X chromosome occurs at random early in embryonic life (*2*). Only one allele is active in each somatic cell of women heterozygous at the *Gd* locus (*3*); this results in two populations of somatic cells. The factors determining the ratio between the two cell populations in various tissues are not yet fully understood (*4*). A marked deviation from a 1:1 ratio of the two cell types in adults is regarded as infrequent (*5*). This situation, in which one X chromosome is expressed to the almost complete exclusion of the other, will be referred to as an extremely unbalanced mosaic phenotype. There are two ways that this phenotype might be produced in a particular tissue: (i) by chance alone, if inactivation were to occur earlier than normal and were to affect the same chromosome (paternal or maternal X) in most cells of the whole embryo or of the primordial cell pool of that tissue, and (ii) by selection of one cell population over the other. An example of the latter mechanism was found to operate for the deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) in heterozygotes in whom erythroid cells consisted almost exclusively of the normal type, whereas in fibroblasts the expected two cell populations, HGPRT(+) and HGPRT(−), could be recognized (*6*).

We report that an extremely unbalanced mosaic phenotype can be demonstrated in heterozygotes for qualitative variants of G6PD. The data from one family suggest that at least one locus on the X chromosome (other than loci for G6PD or HGPRT) can affect the rate of proliferation of hemopoietic cells.

Hemolyzates were prepared and analyzed for G6PD by starch-gel (*7*) and cellogel (*8*) electrophoresis (*9*). In Nigeria, where the present study was carried out, the commonest type of heterozygote has the AB phenotype (*10, 11*). The relative proportions of the two G6PD types can be estimated semiquantitatively by visual inspection of gels; cases with a marked excess of either the A or B band have been reported (*12*). In addition, several G6PD variants can be easily resolved and more accurately quantitated by chromatography on DEAE-Sephadex columns (*13*), from which each enzyme has been found to elute at a characteristic and highly reproducible concentration of KCl (*14–16*). This technique shows that the relative amounts of two allelic G6PD types present in heterozygotes exhibit, in a number of cases, a large imbalance in favor of the mutant or of the normal enzyme type (Fig. 1).

To prove that at least some of such extreme phenotypes are caused by somatic cell selection, we studied a Nigerian family in detail (Fig. 2). The propositus, III-5, was a patient with homozygous sickle-cell anemia who had a slow-moving type of G6PD, the Ilesha variant (*17*). Upon testing 11 members in three generations of the family, one additional hemizygote and five heterozygotes for the Ilesha variant were found. In all five of them, the ratio between the two enzyme types was markedly different from unity. There was an approximately tenfold excess of G6PD Ilesha in four (I-2, II-4, III-2, and III-3) and a more than tenfold excess of G6PD B in the fifth (II-3) heterozygote.

In three members (II-4, III-2, and III-3), the white blood cells were also examined. Their G6PD phenotype was similar to the red cell phenotype. In member III-3, a repeat examination after 1 year showed that the ratio between G6PD B and G6PD Ilesha was stable, and chromosome studies, including G-banding, revealed a normal karyotype.

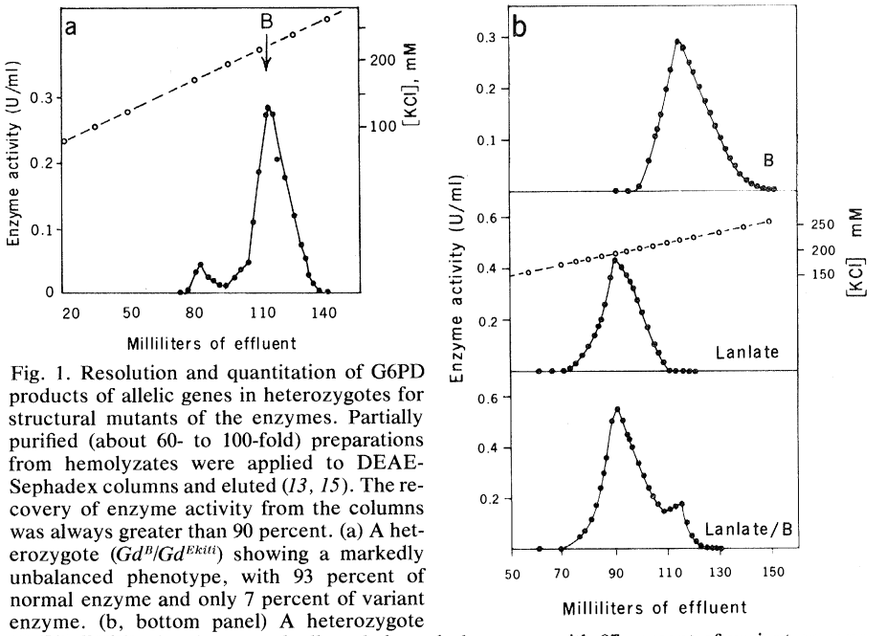

Fig. 1. Resolution and quantitation of G6PD products of allelic genes in heterozygotes for structural mutants of the enzymes. Partially purified (about 60- to 100-fold) preparations from hemolyzates were applied to DEAE-Sephadex columns and eluted (*13, 15*). The recovery of enzyme activity from the columns was always greater than 90 percent. (a) A heterozygote (Gd^B/Gd^{Ekiti}) showing a markedly unbalanced phenotype, with 93 percent of normal enzyme and only 7 percent of variant enzyme. (b, bottom panel) A heterozygote ($Gd^B/Gd^{Lanlate}$) showing a markedly unbalanced phenotype with 97 percent of variant enzyme and only 3 percent of normal enzyme. The upper two panels show control runs of G6PD type B and type Lanlate alone. The B band in the $Gd^B/Gd^{Lanlate}$ heterozygote could not be seen on starch gel. Characterization of these variants has been published (*15*).

0036-8075/79/0928-1418$00.50/0

The frequency of extremely imbalanced mosaic phenotypes was reported (*18*) to be about 5 percent in an unbiased sample of 77 Sardinian women heterozygous for G6PD deficiency. A comparable estimate for the Nigerian population is not yet available; however, among 42 heterozygous Nigerian girls tested, 7, or 17 percent (*19*), had G6PD activity levels in the normal or homozygous-deficient range. In families with sporadic G6PD variants (*15*), in whom we have been able to test heterozygotes by column chromatography, we found an extreme phenotype in two out of four members—excluding G6PD Ilesha. Although it is not possible to decide in any individual case whether such a phenotype results by chance or by selection, an analysis of the pedigree of family O.O (Fig. 2) appears to be compatible only with selection. Indeed, if we accept .05 as the a priori probability of an extremely imbalanced mosaic phenotype in any given heterozygote, the probability of six such phenotypes occurring in the same family is negligibly small ($.05^6$). Thus, we infer that the extremely unbalanced mosaic phenotypes in this family depend on some feature of the X chromosomes involved. We rule out the possibility that the expression of the Gd^{Ilesha} allele is favored by itself over the wild-type allele, since in subject II-3 the reverse was true.

The quantitative ratio between the two cell types that constitute a genetic mosaic in females who are heterozygous for any X-linked gene is, at least to some extent, under genetic control. The main piece of evidence so far has been the finding of a higher rate of concordance in monozygous twins compared to dizygous twins in the ratio between two G6PD types (*20*). The ratio in a particular tissue may be determined by a single X-linked gene. Indeed, with reference to the alternatives mentioned in the first paragraph, we are left with only two possible explanations of the findings in our pedigree (Fig. 2). First, there may be an inherited tendency for inactivation to take place earlier than normal. This would increase the likelihood of an extremely unbalanced mosaic phenotype, but balanced phenotypes would still be expected to occur in most cases. None was encountered in this family. Second, there may be specific selection in favor of cells with a particular X chromosome active (*21*). Since chromosomal abnormalities are well known to affect X-chromosome expression (*22*), a small deletion or a small (balanced) X-autosomal translocation (undetected by our chromosome studies) would cause preferential inactivation or selection of cells

Table 1. Quantitation of allelic G6PD types in heterozygotes. Quantitation was carried out by measuring areas under respective peaks in elution patterns such as that shown in Fig. 1.

Gd genotype	Subject	G6PD markedly underrepresented in heterozygote	
		Type	Percent
Gd^B/Gd^{Ekiti}	See Fig. 1a	Variant	11
$Gd^B/Gd^{Lanlate}$	See Fig. 1b (bottom panel)	Normal	9
Gd^B/Gd^{Ilesha}	I-2, Fig. 2	Variant	6
Gd^B/Gd^{Ilesha}	II-3, Fig. 2	Normal	10

in which either the normal or the abnormal X is active. However, we have no evidence for that possibility, which would be expected to cause some phenotypic abnormality in males in the case of deletion, or segregation of abnormal phenotypes in subjects in whom translocations were to become unbalanced. More likely, cell selection operates on the basis of an unknown gene that in one allelic form affects the growth rate of hemopoietic cells, as previously predicted (*23*). In the case of the subject family, the gene involved cannot be too closely linked to *Gd*, since recombination between the two loci has occurred in at least three out of five scorable cases (Fig. 2). We ignore whether the more common allele is one that is associated with faster or slower proliferation of hemopoietic cells. However, we can see that it was introduced in the pedigree at least twice. Indeed, if a "slow proliferation" gene is in coupling with Gd^B in subject I-2, it must have crossed over to the Gd^{Ilesha} chromosome in subject II-3. Since the Ilesha variant again prevails in subjects III-2 and III-3, a new, slow proliferation gene, in coupling with Gd^B, must have been supplied by subject II-2 (*24*). Subjects II-2 and II-3 came from different towns and are not related. Thus, the gene that affects hemopoietic cell proliferation may not be very rare

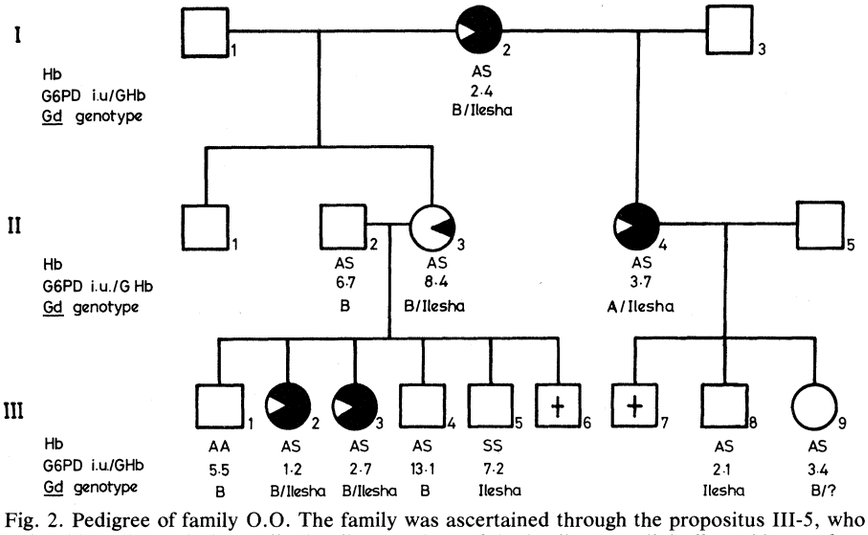

Fig. 2. Pedigree of family O.O. The family was ascertained through the propositus III-5, who had sickle-cell anemia (*17*). All other live members of the family were clinically and hematologically normal. Subjects III-6 and III-7 had died at the time of the study, the latter from severe neonatal jaundice. Under each symbol are given the hemoglobin electrophoretic pattern, the G6PD activity, and the *Gd* genotype—determined from quantitation and electrophoresis. The wedge-shaped shading in the heterozygotes who were fully investigated indicates the predominance of G6PD Ilesha (in black) or G6PD A or B (in white). The relative amounts of B and Ilesha were measured in subjects I-2 and II-3 by column chromatography (see Table 1). In the remaining cases, marked imbalance was seen on starch-gel patterns and estimated by visual comparison with artificial mixtures, in known proportions, of G6PD B and G6PD Ilesha samples run side by side with the unknowns. The symbols without entries under them represent subjects who could not be tested. For some of them, the *Gd* genotype can be inferred from the pedigree: I-1, Gd^B; I-3, Gd^A; II-5, Gd^B. The hemolyzate of subject III-9 on starch-gel electrophoresis showed only a single band of G6PD B. However, on the basis of her mother's genotype, she cannot be a Gd^B homozygote; rather, her genotype must be either Gd^B/Gd^A or Gd^B/Gd^{Ilesha}. Therefore, her phenotype also consists of an unbalanced mosaic in favor of the cells with Gd^B on the active X chromosome. The low values of G6PD activity in the hemizygous Gd^{Ilesha} subject, III-8, and in the phenotypically similar subjects I-2, II-4, III-2, and III-3 are explained by the fact that G6PD Ilesha is a mildly deficient variant (*15*). The normality of the enzyme activity value in the case of III-5, who is also a Gd^{Ilesha} hemizygote, is easily explained by the association of his younger red cell population with sickle-cell anemia (*17*).

and may account for a significant fraction of all heterozygous females with extreme phenotypes.

Our interpretation of extremely unbalanced mosaic phenotypes in terms of selection for an X-linked gene is supported by various lines of evidence, including the tissue specificity of the selection process recently discussed by Migeon (*25*). A similar explanation has been proposed to explain differences in the expression of parental X chromosomes in the mule, the interspecific hybrid between horse and donkey (*26*). In the subject family it was not possible to study somatic cells other than erythrocytes and leukocytes, but the possibility of growth advantage conferred by a single gene could be tested experimentally in mixed cell cultures (*27*) that mimic the somatic cell structure of female heterozygotes who are X-chromosome mosaics (*28*).

LUCIO LUZZATTO
International Institute of Genetics and Biophysics, 80125 Naples, Italy
ESSIEN A. USANGA
Department of Haematology, University College Hospital, Ibadan, Nigeria
ULRICH BIENZLE
Bernhard Nocht Institute for Tropical Diseases, Hamburg 4, Federal Republic of Germany
G. FOLAYAN J. ESAN
FELIX A. FASUAN
Department of Haematology, University College Hospital, Ibadan

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21. A third possibility [previously suggested by H. H. Ropers, T. F. Wienker, T. Grimm, K. Schroetter, K. Bender, *Am. J. Hum. Genet.* **29**, 361 (1977) to explain some unusual phenotypes in heterozygotes for α-galactosidase deficiency] is preferential inactivation of one X chromosome. From a formal point of view the result would be the same as in the second possibility; therefore we cannot completely rule out preferential inactivation, although there seems to be no direct evidence for this in humans.
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25 May 1979

Perinatal Exposure to Cannabinoids Alters Male Reproductive Function in Mice

Abstract. *Oral administration of Δ^9-tetrahydrocannabinol or cannabinol to female mice late in pregnancy and during early lactation alters body weight regulation and pituitary-gonadal function and suppresses adult copulatory activity in their male offspring. These findings suggest that both psychoactive and nonpsychoactive constituents of marihuana can affect the development of male reproductive functions in mice.*

Marihuana can affect the reproductive system and androgen-dependent behavior in adult males of several species (*1*). However, the effects of cannabinoids on male sexual differentiation in the fetus have not been examined. Cannabinoids cross the placental barrier and accumulate in a wide variety of fetal tissues, including the mitochondrial fraction of the brain (*2*). Moreover, newborn mammals may be exposed to cannabinoids through milk. Labeled Δ^9-tetrahydrocannabinol (THC) accumulates in the milk of the ewe, and radioactivity can be detected in suckling rat pups after treatment of the lactating female with ^{14}C-labeled THC (*2*). Thus, it is conceivable that fetal pituitary and testicular function could be affected by maternal exposure to cannabinoids during critical periods of development. In rodents, the testis is reported to produce increasing amounts of androgen during perinatal sexual differentiation. In the mouse, this increasing testosterone production is controlled by the fetal pituitary (*3*).

Manipulation of pituitary gonadotropins or gonadal steroids during certain critical perinatal periods of sexual differentiation can alter the development of reproductive structures and sex-typical behavioral responses, including copulatory behavior (*4*). In addition, it has been reported (*5*) that prenatal exposure to cannabinoids affects learning ability in adult rats. Hormonal status may moderate these effects on central nervous system function, since male, but not female, rats prenatally exposed to cannabinoids exhibited inferior performance in a maze learning task.

We have recently reported (*6*) that perinatal exposure to THC, the main psychoactive component of marihuana, or cannabinol (CBN), a nonpsychoactive component, affects reproductive functions and body weight regulation in male mice prior to sexual maturation. The present study determined the consequences of perinatal exposure to THC or CBN on body weight, pituitary-gonadal function, and sexual behavior in adult male mice.

Adult primiparous female mice were obtained from a colony of randomly bred mice at the Worcester Foundation (*7*). They were housed with a sexually experienced male and checked daily for the appearance of a copulatory plug. The day the plug appeared was designated day 1 of pregnancy. Approximately 24 hours prior to parturition (day 20), the female received an oral dose of 50 mg of THC or CBN (50 mg per kilogram of body weight) in sesame oil (20 μl) or a dose of sesame oil alone. This dosage of THC or CBN will alter testis function in adult male mice (*7*). The second dose of cannabinoids was administered on the day of parturition, and treatment

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