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Genetics

Localization of loci for hypoxanthine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase and biochemical evidence of nonrandom X chromosome expression from studies of a human X–autosome translocation

(allelic exclusion/X chromosome inactivation/DNA replication/somatic cell hybridization)

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ABSTRACT **We report a unique and complex karyotypic rearrangement involving chromosomes X, 3, 7, and 21. Blood cells and fibroblasts from the proband do not express the maternal allele for glucose-6-phosphate dehydrogenase (G6PD), providing biochemical evidence for nonrandom expression of X-linked genes in balanced X–autosome translocations. The break point on the X chromosome, at the junction of Xq27-Xq28, separates the loci for hypoxanthine phosphoribosyltransferase (HPRT) and G6PD. Studies of mouse–human hybrids derived from the proband's cells indicate that *G6PD*, at q28, is clearly distal to all other X loci now assigned. From these and previous studies, we can localize *HPRT* to that segment between Xq26 and Xq27. The studies also provide further evidence for the stability of the inactive X phenotype in hybrid cells.**

Among the loci most important to mammalian geneticists are the X-linked genes for glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) and hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8). The availability of spontaneous and induced variants at these loci in humans has contributed to their usefulness for study of basic genetic phenomena, specifically the regulation of X chromosome activity (1). It is likely that these genes will provide the means to obtain and identify X-chromosome-specific DNA. Although it is known that these loci are on the distal region of the long arm of the human X chromosome (2–4), their localization and order with respect to the centromere have not been unequivocally established. Studies of radiation-induced chromosome fragments in somatic cell hybrids suggest that the two loci are separable and that *G6PD* is distal to *HPRT* (5), but interpretation of these studies has been questioned (6).

Because more precise localization of these loci might facilitate isolation of these X-linked genes, we report studies utilizing X–autosome translocations. Our results indicate that the *HPRT* locus is between Xq26 and Xq27 and *G6PD* is more telomeric, being on band Xq28. Furthermore, studies of the human parental cells have provided biochemical evidence for nonrandom expression of X-linked genes in individuals with a balanced X–autosome translocation.

MATERIALS AND METHODS

Subjects. Skin fibroblast cultures were established from a 17-month-old black female with mental retardation and a complex karyotypic rearrangement (7). Cultures were also established from maternal skin fibroblasts as well as from leukocytes of the proband and both parents.

Medium. Cells were maintained in Eagle's minimal essential medium (GIBCO), enriched as described (8). Selection for hybrids was carried out in HOT medium—that is, hypoxanthine/amethopterin/thymidine (HAT) medium containing 1 μM ouabain (8). Medium containing 60 μM 6-thioguanine (6TG) was used to select for HPRT-deficient cells from the proband or from hybrid clones.

Cell Fusion. Fibroblasts from the proband in the second subculture were fused with A9 mouse fibroblasts lacking HPRT, using 50% (vol/vol) polyethyleneglycol 6000 (8). Petri dishes were plated with the equivalent of 9×10^5 mouse and 10^5 human cells. Hybrids were selected in HOT medium, isolated by using cloning cylinders, and maintained in the selective medium.

Selection of Hybrids Lacking HPRT. Several hybrid colonies selected in HOT medium were plated into nonselective medium and maintained in that medium for 1 week. These cells were replated into medium containing 6TG. Clones resistant to the 6TG were isolated by using cloning cylinders. Cultures established from these clones were maintained in 6TG and were assayed for their enzyme activity and karyotype.

Chromosome Studies. Lymphocyte cultures from the proband were studied with bromodeoxyuridine/acridine orange to determine the pattern of replication of X chromosomes as well as R banding (9). Hybrid clones were analyzed by sequential quinacrine and alkaline Giemsa staining to identify the human chromosomes in the hybrid (10). Karyotyping and enzyme analysis were carried out on replicate samples.

Biochemical Assays. For the enzyme assays, blood cells were prepared as described (11). Fibroblasts and hybrids were removed from petri dishes with a rubber policeman in 15 μl of NADP (100 μg/ml) and disrupted in a sonic cleaner. Electrophoresis of G6PD was performed on cellulose acetate gels as described, in Tris/glycine buffer (pH 9.2), 365 V for 25 min (11). Electrophoresis of phosphoglycerate kinase (PGK; EC 2.7.2.3) was performed at room temperature with Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX). The method was modified from that described by Meera Khan for Cellogel (12) as follows: The buffer was 40 mM Tris/1.18 mM citric acid/3.8 mM H_4EDTA/0.26 mM Na_2EDTA; the pH was adjusted to 8.0 with citric acid. The gel ran at a constant current of 12 mA for 25 min. Soluble superoxide dismutase-1

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Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; HPRT, hypoxanthine phosphoribosyltransferase; PGK, phosphoglycerate kinase; SOD, superoxide dismutase; 6TG, 6-thioguanine; HOT medium, hypoxanthine/amethopterin/thymidine medium containing 1 μM ouabain.

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(SOD-1; EC 1.15.1.1) was assayed by starch gel electrophoresis according to the method of Brewer (13). HPRT phenotype was detected by growth in selective media.

Selection for HPRT-Deficient Cells. Cells from the proband, as well as control and Lesch–Nyhan (HPRT-deficient) fibroblasts, were subjected to selection in medium containing 60 μM 6TG. Cells (10^3–10^5) were plated into a series of petri dishes containing growth or 6TG medium. Cells maintained in selective medium for 2 weeks were fixed *in situ*, stained, and analyzed for resistant clones.

RESULTS

Characteristics of human parental cells

Karyotype. Chromosome studies of lymphocytes and fibroblasts revealed that in all of them there was a complex *de novo* translocation with four chromosomes involved in rearrangements. The best interpretation of the karyotype is as follows: 46XX,t(X;3;7;21) (Xpter → q27::3p11 → pter; 3qter → q12::7p12 → q21::p12 → pter; 7qter → q21::3q12 → cen 3 or p11; 21qter → q21::p12 → q21::Xq28 → qter).

Two of the rearranged chromosomes contain X chromosome material. The derivative X (der X) has the major part of the X and most of the short arm of chromosome 3. The derivative 21 (der 21) has the major part of chromosome 21 with extra material at the end of its short arm. From R-banded karyotypes (Fig. 1) it seems that the dark band, q28 to qter, of the X, missing from the der X, has been translocated to the short arm of chromosome 21. In all of the 197 metaphases that were suitable for analysis, the normal X was always allocyclic, whereas the der X was early replicating (Fig. 2).

G6PD Phenotype. The enzyme from (*i*) erythrocytes, leukocytes, and fibroblasts of the proband was G6PD A; (*ii*) maternal erythrocytes, leukocytes, and fibroblasts was G6PD B, and (*iii*) paternal erythrocytes was G6PD A (Fig. 3). Thus the proband expressed only the paternal allele and excluded the maternal G6PD B allele. From this we infer that the maternal allele is located on the normal X chromosome, which is inactive in all of the proband's cells.

HPRT Phenotype. When fibroblasts from the proband were plated into medium containing 6TG, none were able to proliferate, indicating that all of these cells had HPRT activity.

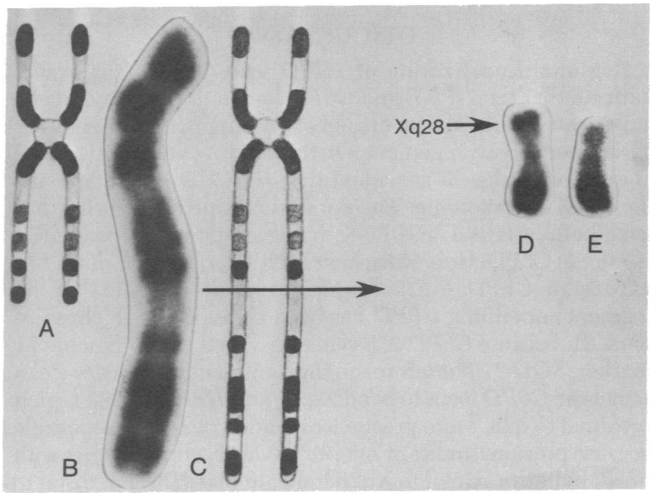

FIG. 1. X–autosome translocations in proband (diagram based on R bands). A, normal X; B and C, der X with break point at junction of Xq27-q28 and short arm of chromosome 3; D, der 21 with the segment Xq28-Xqter translocated to the short arm; E, normal 21.

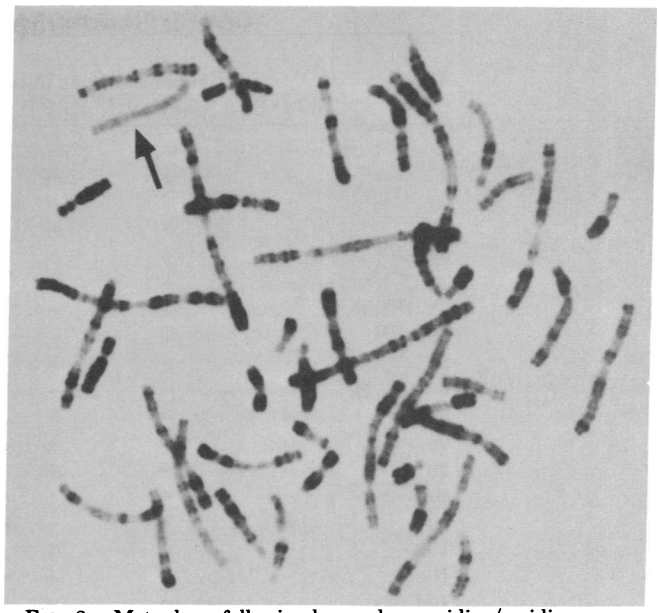

FIG. 2. Metaphase following bromodeoxyuridine/acridine orange treatment, showing that the normal X (arrow) is allocyclic.

Characteristics of somatic cell hybrids

The fusion of skin fibroblasts of the proband with HPRT-deficient mouse A9 fibroblasts resulted in 21 hybrid clones (Table 1). Because these cells were selected in HOT medium they all had human HPRT. Moreover, each expressed human PGK (Table 1, Fig. 4), an X-linked marker previously assigned to Xq13 (2). However, only 12 of the hybrid clones expressed human G6PD. In each case the human G6PD synthesized by the hybrid cells was G6PD A (Table 1, Fig. 4). Hybrids subjected to back selection in 6TG invariably lost their PGK activity, but four 6TG-resistant hybrids retained G6PD activity (Table 1).

Chromosome analysis of 11 hybrids selected and maintained in HOT medium indicated that all of them retained at least one copy of the der X translocation chromosome and many had two (Table 1, Fig. 5). The der X was not present in two hybrids that

FIG. 3. G6PD electrophoresis of extracts from fibroblasts from proband (lanes 2–4), erythrocytes from father (lanes 5–7) and mother (lanes 10–11), and fibroblasts (lanes 1 and 8) and erythrocytes (lane 9) from a G6PD AB control. The cathodal band in erythrocyte extracts is hemoglobin. Note proband does not express maternal *G6PD B* allele.

Table 1. Characteristics of human–mouse hybrid clones

Clone no.	Human enzyme phenotype					Number of human chromosomes		
	G6PD B	G6PD A	HPRT	PGK	SOD-1	Total	der X	G group
1	−	−	+	+	−	3–7	1–2	0–1
2	−	+	+	+				
3a	−	+	+	+	+			
3b	−	+	+	+	+	15–16	1	2
3b-1^{6TG}	−	+	−	−	+			
3b-2^{6TG}	−	+	−	−	+			
3b-3^{6TG}	−	+	−	−	+			
3b-4^{6TG}	−	+	−	−	+			
3c	−	+	+	+	+			
4	−	−	+	+	+	6–14	1	0–2
8	−	−	+	+		8	1	
9a	−	+	+	+				
9b	−	−	+	+				
9c	−	+	+		+		2	
10a	−	+	+	+	+			
10b	−	+	+	+	+	7–9	1–2	0–2
10c	−	+	+		+			
10d	−	+	+	+			1	
13	−	+	+	+	+			
14a	−	−	+	+		2–5	1	
14b	−	−	+	+	−			
14b^{6TG}	−	−	−	−		0–1	0	0
14c	−	−	+	+	−	4–7	1	0–1
14d	−	+	+	+	+	12–16	2	2–3
14d^{6TG}	−	−	−	−	−	0–2	0	0
14e	−	−	+	+		5–11	1–2	0–2
15	−	−	+	+				

were back selected in 6TG—an observation consistent with this chromosome having the *HPRT* locus.

The hybrids with human G6PD had a greater number of human chromosomes and more human small acrocentric chromosomes (G group) than those that had no G6PD (Table 1). Unfortunately, the resolution afforded by quinacrine banding did not permit a more precise identification. In any event, the results were compatible with the cytological evidence from the proband's cells that the X segment containing *G6PD* had been translocated to chromosome 21. Studies of hybrids with regard to SOD-1 phenotype supported this hypothesis because those clones that had G6PD A also expressed *SOD-1*, a marker assigned to the long arm of chromosome 21 (ref. 14, Table 1). However, the presence of SOD-1 activity did not completely correlate with G6PD activity: one clone, no. 4, had SOD-1, but no G6PD (Table 1). In this case, it is likely that the normal chromosome 21 was present in the hybrid cell.

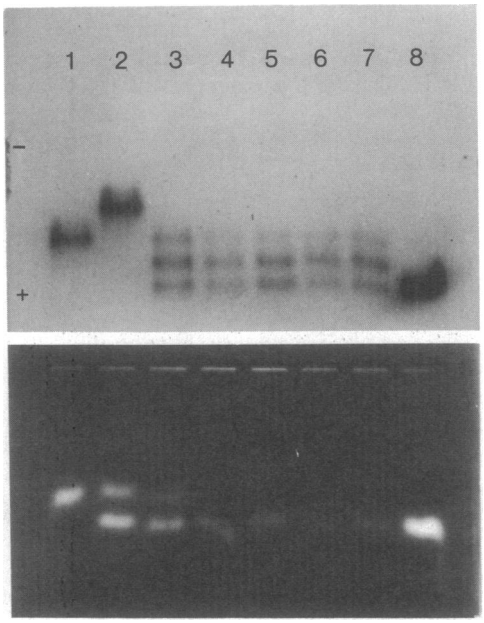

FIG. 4. Cellulose acetate gel electrophoresis of hybrid clones and controls showing independent segregation of G6PD from PGK. (*Upper*) G6PD. Lane 1, proband (G6PD A); lane 2, G6PD B control; lanes 3–7, hybrid clones 9a, 3b-1^{6TG}, 3b-2^{6TG}, 3b-3^{6TG}, and 3b-4^{6TG}, respectively; lane 8, mouse A9. (*Lower*) PGK. Same as *Upper* with exception of lane 2, which is a mixture of human and mouse extracts.

DISCUSSION

Regional Localization of *HPRT* and *G6PD*. Our results indicate that the *HPRT* and *G6PD* loci in the proband's cells have been separated by a break between them and subsequent chromosome rearrangement. On the basis of cytological as well as enzyme studies, it is evident that *HPRT* is associated with the der X chromosome. The der X is retained in hybrids that have human HPRT and PGK, but segregates independently of human G6PD. Hybrids lacking HPRT also lack the der X but may retain G6PD. Furthermore, our results indicate that the segment containing *G6PD* has been translocated to chromosome 21, because *G6PD A* is syntenic with the chromosome 21 marker, *SOD-1*. Therefore, on the basis of our studies, we can assign the *G6PD* locus to band Xq28, and *HPRT* to that region proximal to q28. More precise localization of *HPRT* is possible because previous studies of hybrids having X chromosomes with break points proximal to Xq28 indicate that *HPRT* is distal to Xq26 (2–4). Thus, *HPRT* is on segment Xq26-Xq27. The relationship of the two loci, with respect to the centromere, that we observed supports earlier observations of radiation-induced fragments (5).

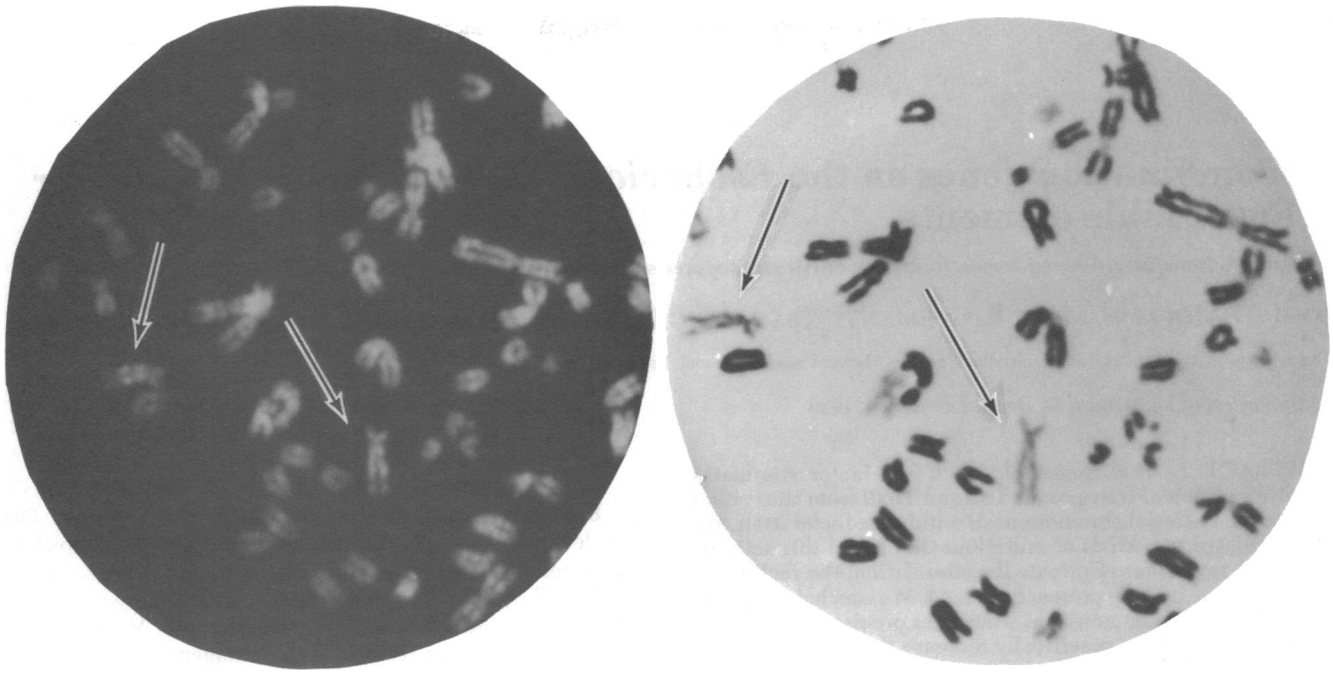

FIG. 5. Partial karyotype of hybrid clone 14d stained with quincrine (*Left*) and alkaline Giemsa (*Right*), showing the presence of two der X chromosomes.

Studies Relevant to X Inactivation. There is abundant evidence that the pattern of inactivation of the X chromosome in individuals with X-autosome translocation chromosomes is generally not random (reviewed in ref. 15). In those with unbalanced translocations that result in effective deletions of X chromatin, the normal X may be the active one in each cell. On the other hand, when the translocation is balanced, the normal X is usually inactive in all cells of the translocation carrier, as in our proband. In either case, the skewed pattern of X inactivation is attributable to selection for cells with the least genetic imbalance. All of the previous studies of inactivation in human X-autosome translocations have been based on observations of X chromosome replication. We have shown, using G6PD as the marker, that the maternal X is not expressed; our biochemical evidence, therefore, supports conclusions based on cytological observations that only one parental X is expressed in the great majority of cells with balanced X-autosome translocations. These studies demonstrate that the normal X not only replicates late but is genetically inactive as well.

In each case the human *G6PD* expressed in the hybrid cells was the one expressed in the proband, the paternal *G6PD A* allele. That none of the 27 hybrids analyzed for G6PD expressed the *G6PD B* allele on the inactive maternal X chromosome is further evidence that this locus on the inactive X chromosomes is not derepressed in interspecific hybrids (16).

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