Am J Hum Genet 30:339–345, 1978

Triplo-X Constitution of Mother Explains Apparent Occurrence of Two Recombinants in Sibship Segregating at Two Closely X-Linked Loci (G6PD and Deutan)

ANTONIETTINA RINALDI,[1,2] MARIA VELIVASAKIS,[3] BACHISIO LATTE,[4] GIORGIO FILIPPI,[5] AND MARCELLO SINISCALCO[3]

Several independent studies have established that the X-linked structural gene for human glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49) is closely linked to the genes for both deutan and protan color blindness [1–6]. To date, only one recombinant has been directly observed between G6PD and protan out of 51 scorable sibs and five between G6PD and deutan out of 240 scorable sibs (table 1). Four of the latter were found by our group in Sardinia, and, surprisingly enough, two of them were in the same sibship [7]. This puzzling finding called for additional studies which were delayed for several years owing to the reluctance of critical family members to undergo further investigations. The pedigree has now been reexamined and expanded (fig. 1). On the basis of their phenotypes (table 2) and the derived genotype of their father (fig. 1), individuals II-1 and II-3 in this pedigree appear to be double heterozygotes in coupling at the loci for G6PD and deutan color-vision ($d^{+}Gd^{B}/d^{-}Gd^{Mediterranean}$). The two mutant phenotypes (G6PD deficiency of Mediterranean type and severe deuteranopia) or the two corresponding normal phenotypes (G6PD B type and normal color-vision) segregate together without recombination among the 12 scorable descendants of II-1. However, only one of the three G6PD deficient sons of II-3 is deuteranope, while the others have normal color-vision, suggesting the occurrence of two recombination events during maternal oogenesis.

Among the alternative hypotheses to explain this finding, we considered the possibility that (1) II-3 carries two different G6PD mutant alleles: the $Gd^{Mediterranean}$ mutant, in coupling with the gene for deuteranopic color blindness (d^{-}) and another mutant, probably milder in its manifestation at the red cell level, associated with the

Received October 14, 1977; revised February 21, 1978.

The field and laboratory studies were supported by grant CA 17085 and CA 08048 from the National Cancer Institute and by the Italian National Research Council.

[1] Centro di Genetica Evoluzionistica del CNR, University of Rome, Italy,

[2] Istituto di Genetica, Facolta di Scienze, Universita di Cagliari, Italy.

[3] Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021. (Address for reprints)

[4] Ospedale Civile, Nuoro, Italy.

[5] Istituto di Genetica Medica, Universita di Trieste, Italy.

TABLE 1

STUDIES ESTIMATING DEGREE OF LINKAGE BETWEEN G6PD AND COLOR-VISION LOCI

	Segregating Loci	No. Informative Sibships or Pedigrees	No. Recombinants Reported	Total Scorable Sibs
Sardinian series [1, 7, 8]	Deutan-G6PD	57	4	183
	Protan-G6PD	10	1	27
Israeli series [2, 5]	Deutan-G6PD	5	0	25
	Protan-G6PD	2	0	7
	Color blindness Unspecified	12	0	49
Maryland series [3, 4]	Deutan-G6PD	6	1	30
	Protan-G6PD	2	0	8
Greek series [6]	Deutan-G6PD	1	0	2
	Protan-G6PD	2	0	9
Summary of data	Deutan-G6PD	69	5	240
	Protan-G6PD	16	1	51

NOTE.—The four G6PD recombinants of the Sardinian series [7] include III-9 and III-10 recombinants of figure 1. To correct for ascertainment, only one of them was included in the linkage data summarized in reference [8]. Removing them from the computation reduces the frequency of recombination between G6PD and deutan to 3/238 = .013 and between G6PD and protan to 1/51 = .020.

gene for normal color-vision (d^+); and (2) II-3 or the alleged recombinant sons (III-9 and III-10) bear an abnormal X-chromosomal constitution. The latter hypothesis proved to be the correct one, since the mother (II-3), though entirely normal both in her phenotype and mental capacity, has a 47,XXX chromosome complement as shown by the presence of two sex chromatin bodies in 50% of her buccal mucosa cells and by cytogenetic analysis of cultured peripheral blood lymphocytes and fibroblasts.

Since the incidence of triplo-X among phenotypically normal newborn females is of the order of 1×10^{-3} [17] and the chance of two recombinant sibs for the loci under consideration is at least as low, the possibility of a fortuitous association of both events in the same sibship can be safely disregarded. Thus we conclude that the two alleged recombinants (III-9 and III-10) and their double affected brother (III-13) are most likely segregants from a mother of genetic constitution $d^+Gd^{\text{Mediterranean}}/d^-Gd^{\text{Mediterranean}}/d^+Gd^{\text{B}}$. Additional findings which corroborate this conclusion are: (1) All cytogenically tested male and female children (III-9, III-10, III-12, and III-13) of the triplo-X mother have a normal chromosomal constitution, and both daughters have only one sex-chromatin body in their buccal mucosa cells.* (2) All three G6PD deficient sons and daughter III-12 have the G6PD red cell phenotype typical of the hemizygous and

* The screening for sex chromatin bodies was performed [18] on 200 buccal cells. The sex chromosome complement was determined by direct microscopy and by karyotype analysis of 10 to 20 metaphases with Giemsa banding according to Seabright [19], except for III-10 and III-13, whose karyotypes were established with normal Giemsa staining before the era of G-banding. Fibroblast cultures from II-3 and III-9 (GM 1973) and (GM 1917) are stored in the Human Genetic Mutant Cell Repository (Camden, N.J.).

TABLE 2

DATA ON G6PD PHENOTYPE OF CRITICAL MEMBERS OF PEDIGREE REPRODUCED IN FIGURE 1

	G6PD PHENOTYPE IN RED CELLS		PERCENT G6PD(+) CELLS		
	Assay*	Dye Test† (min)	Erythrocytes‡	Lymphocytes§	Fibroblasts§
II-1	2.09	35	n.t.	n.t.	n.t.
II-3 (triplo-X)	1.70	40	54	18	16
III-9	0.05	> 240	0.1	0.9	1.0
III-10	0.08	> 240	n.t.	n.t.	n.t.
III-11	2.21	35	99	100	n.t.
III-12	1.58	45	60	54	43
III-13	0.06	> 240	0.3	1	0.6
Averages:‖					
Hemizygotes for Gd^{B}	1.92 ± 0.66 (no. = 37)	41 ± 0.5 (no. = 115)	99.9 ± 0.04 (no. = 115)	90 ± 0.5 (no. = 20)	95 ± 0.7 (no. = 10)
Hemizygotes for $Gd^{Mediterranean}$	0.03 ± 0.01 (no. = 30)	> 240 (no. = 45)	0.4 ± 0.05 (no. = 45)	0.0 (no. = 20)	0.0 (no. = 10)
Homozygotes for Gd^{B}	2.14 ± 0.60 (no. = 20)	44 ± 0.7 (no. = 52)	99.6 ± 0.08 (no. = 52)	89 ± 0.4 (no. = 20)	92 ± 0.8 (no. = 10)
Heterozygotes for $Gd^{B}/Gd^{Mediterranean}$	1.51 ± 0.90 (no. = 15)	56 ± 3.6 (no. = 77)	58.1 ± 2.59 (no. = 77)	39 ± 1.0 (no. = 20)	43 ± 1.3 (no. = 10)
Homozygotes for $Gd^{Mediterranean}$	0.09 ± 0.07 (no. = 10)	> 240 (no. = 8)	0.8 ± 0.31 (no. = 8)	0.0 (no. = 20)	0.0 (no. = 10)

NOTE.—n.t. = not tested.

* G6PD activity, expressed as μM of NADP converted per min/10^{12} red cells, was assayed according to Motulsky et al. [9]. When required, the G6PD electrophoresis was carried out according to Rattazzi et al. [10]. Averages are those published for the same population by Piomelli and Siniscalco [11].

† Averages for the dye decoloration test of Motulsky and Campbell-Kraut [12] were reported for the Sardinian population by Rinaldi et al. [13].

‡ The percent of G6PD(+) erythrocytes was estimated on 1,000 cells by the method of Gall et al. [14] on freshly collected EDTA samples of peripheral bloods. The average values are those of Rinaldi et al. [13].

§ G6PD activity in lymphocytes and cultured fibroblasts were determined by histochemical staining of enzyme activity according to DeMars [15] and Chasin et al. [16] after exposure of the cells at 45°C for 10 min. Average values of G6PD(+) cells were obtained for individuals of various genotypes.

‖ With standard errors and no. observations.

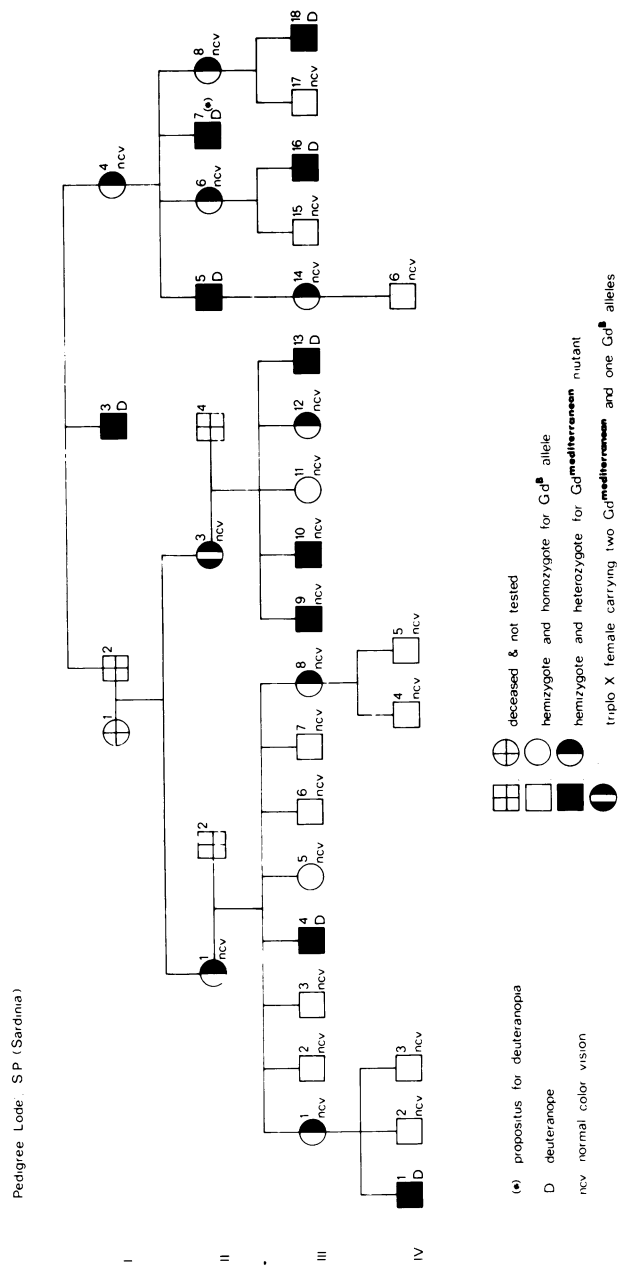

FIG. 1.—The branch of the pedigree including individuals I-3, I-4 and her descendants is the same as the pedigree reported in reference [1] under the code S.A.-Lode. See legend to table 2 for information on G6PD phenotype determination. Color-vision phenotypes were established with the Ishihara and HRR tables and the 15 hue Farnsworth test. The pedigree in question segregates for severe deuteranopia. The classification of the color-vision phenotype was confirmed with the anomaloscope of Nagel for individuals II-3, III-4, III-9, III-10, III-13, III-16, and IV-1.

heterozygous carriers of the $Gd^{Mediterranean}$ mutant (table 2). (3) The presence of a Gd^{B} allele in the mother's genotype is inferred from daughter III-11, who is classified as homozygote Gd^{B}/Gd^{B} on the basis of finding a homogeneous population of G6PD(+) cells in her peripheral blood erythrocytes and lymphocytes (table 2). (4) About 80% of the peripheral blood lymphocytes and cultured fibroblasts of the triplo-X mother express the mutant G6PD phenotype (table 2) detected at the individual cell level by histochemical staining of enzyme activity after heat denaturation at 45°C for 10 min [15, 16]. These findings are as expected, if two of her X chromosomes carry the $Gd^{Mediterranean}$ mutant and one the normal allele. The slight excess of G6PD(+) erythrocytes in her peripheral blood is consistent with one previous report that heterozygotes for this mutant have a distribution of red cell G6PD phenotypes skewed in favor of the normal G6PD(+) cells [13].

The findings described urged us to examine the chromosomes of the double heterozygous mothers of the other three reported Sardinian families in which a simple recombinant between G6PD and deutan or G6PD and protan had been scored [7, 8]. They were found to have a normal 46,XX chromosomal complement.

These results indicate that either the frequency of triplo-X among normal Sardinian adult women is appreciably higher than 1/1,112 found at birth in northern European populations, or the true frequency of meiotic recombination between the loci for G6PD and deutan is appreciably lower than the figure of .05 reported by Porter et al. [3, 4] and perhaps even of .013 (3/238) as derived from the data in table 2 after reclassifying III-9 and III-10 as segregants. From table 2, it appears also that the frequency of recombination between G6PD and protan is of the same order (1/51 = .020). These estimates have wide fiducial limits so that nothing can be said about a possible difference in the degree of linkage between the two sets of loci on the basis of these direct measurements of meiotic recombination. However, population data on linkage disequilibrium, recently reported by our group [20], suggest that the G6PD locus may be nearer to the protan than to the deutan locus.

This report is intended to alert students of human X-linkage to the fact that apparently rare recombinational events between closely X-linked loci may be explained by numerical aberrations of the X chromosome. Families such as those described by Vanderdonk and Verriest [21] and Arias and Rodriguez [22] with two recombinant sibs between deutan and protan loci should be checked for the X-chromosomal constitution of the double heterozygous mothers. Sibships with one or more apparent recombinants between closely linked autosomal loci such as those reported for histocompatibility antigens [23, 24] also warrant chromosomal study. A triple dose for an autosomal chromosome, however, is unlikely to be the explanation since grossly abnormal phenotypes have been associated with the autosomal trisomies thus far described.

SUMMARY

Two male sibs believed to be examples of meiotic recombinants between the closely linked loci for G6PD deficiency of Mediterranean type and severe deutan color blindness proved to be simple segregants of a triplo-X mother of genotype $d^{-}Gd^{Mediterranean}/d^{+}Gd^{Mediterranean}/d^{+}Gd^{B}$. This finding suggests that in Sardinia the linkage between the two loci under consideration may be tighter than previously assumed.

ACKNOWLEDGMENTS

We wish to express our deep gratitude to all members of the families investigated for their unlimited patience and to acknowledge the critical role of Dr. Costantino Marcello, medical officer at Lode, Sardinia, for the successful completion of our work.

REFERENCES

1. SINISCALCO M, MOTULSKY AG, LATTE B, BERNINI L: Indagini genetiche sulla predisposizione al favismo. II. Dati familiari. Associazione genica con il daltonismo. *Rend Accad Lincei Cl Sci* [*Ser* VIII] 28:903–909, 1960
2. ADAM A: Linkage between deficiency of glucose-6-phosphate dehydrogenase and colour-blindness. *Nature* 189:686, 1961
3. PORTER IH, SCHULZE J, MCKUSICK VA: Linkage between glucose-6-phosphate dehydrogenase deficiency and colour blindness. *Nature* 193:506, 1962
4. PORTER IH, SCHULZE J, MCKUSICK VA: Genetical linkage between the loci for glucose-6-phosphate dehydrogenase deficiency and color-blindness in American Negroes. *Ann Hum Genet* 26:107–122, 1962
5. ADAM A, SHEBA C, SANGER R, RACE RR, TIPPETT P, HAMPER J, GAVIN J: Data for X-mapping calculations, Israeli families tested for Xg, g-6-pd and for colour vision. *Ann Hum Genet* 26:187–194, 1963
6. FRASER GR, DEFARANAS B, KATTAMIS CA, RACE RR, SANGER R, STAMATOYANNOPOULOS G: Glucose-6-phosphate dehydrogenase, colourvision and Xg blood groups in Greece: linkage and population data. *Ann Hum Genet* 27:395–403, 1964
7. SINISCALCO M: Localization of genes on human chromosomes, in *Genetics Today,* Proceedings 11th International Congress of Genetics, The Hague, Netherlands, September 1963, New York, Pergamon Press, 1964, pp 851–870
8. SINISCALCO M, FILIPPI G, LATTE B: Recombination between protan and deutan genes; data on their relative position in respect of the g-6-pd locus. *Nature* 204:1062–1064, 1964
9. MOTULSKY AG, VANDEPITTE J, FRASER GR: Population genetic studies in the Congo. I. Glucose-6-phosphate dehydrogenase deficiency, hemoglobin S and malaria. *Am J Hum Genet* 18:514–537, 1966
10. RATTAZZI MC, BERNINI LF, FIORELLI G, MANNUCCI PM: Electrophoresis of glucose-6-phosphate dehydrogenase: a new technique. *Nature* 213:79–80, 1967
11. PIOMELLI S, SINISCALCO M: The hematological effect of glucose-6-phosphate dehydrogenase deficiency and thalassemia trait: interaction between the two genes at the phenotype level. *Br J Haematol* 16:537–549, 1969
12. MOTULSKY AG, CAMPBELL-KRAUT JM: Population genetics of glucose-6-phosphate dehydrogenase deficency of the red cell. *Proceedings of the Conference on Genetic Polymorphisms and Geographic Variations in Disease,* edited by BLUMBERG BS, New York, Grune & Stratton, 1961, pp 159–191
13. RINALDI A, FILIPPI G, SINISCALCO M: Variability of red cell phenotypes between and within individuals in an unbiased sample of 77 heterozygotes for G6PD deficiency in Sardinia. *Am J Hum Genet* 28:496–505, 1976
14. GALL JG, BREWER GJ, DERN RJ: Studies of glucose-6-phosphate dehydrogenase activity of individual erythrocytes: the methemoglobin elution test for identification of females heterozygous for G6PD deficiency. *Am J Hum Genet* 17:359–368, 1965
15. DEMARS R: A temperature-sensitive glucose-6-phosphate dehydrogenase in mutant cultured human cells. *Proc Natl Acad Sci USA* 61:562–569, 1968
16. CHASIN LA, ROSENSTRAUS M, URLAUB G: The induction of linked mutations in cultured mammalian cells. *Excerpta Medica Int Congr Series* 1(349):52–55, 1974
17. HAMERTON JL, RAY M, ABBOTT J, WILLIAMSON C, DUCASSE GC: Chromosome studies in a neonatal population. *Can Med Assoc J* 106:776–779, 1972

18. MOORE KL, BARR ML: Smears from the oral mucosa in the detection of chromosomal sex. *Lancet* 2:57, 1955
19. SEABRIGHT M: The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. *Chromosoma* 36:204–210, 1972
20. FILIPPI G, RINALDI A, PALMARINO R, SERAVALLI E, SINISCALCO M: Linkage disequilibrium for two X-linked genes in Sardina and its bearing on the statistical mapping of the human X-chromosome. *Genetics* 86:199–212, 1977
21. VANDERDONK R, VERRIEST G: Femme protanomale et hetérozygote mixte (genes de la protanomalie et de la deutéranopie en position de répulsion) ayant deux fils deutéranopes, un fils protanomal et deux fils normaux. *Biotypologie* 21:110–120, 1960
22. ARIAS S, RODRIGUEZ A: New families, one with two recombinants for estimation of recombination between the deutan and protan loci. *Humangenetik* 14:264–268, 1972
23. SUCIU-FOCA N, RUBINSTEIN P: Intra-HLA recombinations in juvenile diabetes mellitus. *Lancet* 1:371, 1976
24. SHAW JF, KANSAL PC, GATTI RA: Diabetes mellitus, chromosomal aberration, and malignancy. *Lancet* 2:315, 1976

New Editor

Beginning July 1, 1978, all manuscripts and correspondence concerning editorial matters should be directed to the new editor, Dr. David E. Comings, Department of Medical Genetics, City of Hope Medical Center, 1500 E. Duarte Road, Duarte, California 91010.