Hereditary Sideroblastic Anemia and Glucose-6-Phosphate Dehydrogenase Deficiency in a Negro Family

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ABSTRACT Detailed clinical and genetic studies have been performed in a Negro family, which segregated for sex-linked sideroblastic anemia and glucose-6-phosphate dehydrogenase (G-6-DP) deficiency. This is the first such pedigree reported. Males affected with sideroblastic anemia had growth retardation, hypochromic microcytic anemia, elevated serum iron, decreased unsaturated iron-binding capacity, increased ^{59}Fe clearance, low ^{59}Fe incorporation into erythrocytes, normal erythrocyte survival (^{51}Cr), normal hemoglobin electrophoretic pattern, erythroblastic hyperplasia of marrow with increased iron, and marked increase in marrow sideroblasts, particularly ringed sideroblasts. Perinuclear deposition of ferric aggregates was demonstrated to be intramitochondrial by electron microscopy. Female carriers of the sideroblastic gene were normal but exhibited a dimorphic population of erythrocytes including normocytic and microcytic cells. The bone marrow studies in the female (mother) showed ringed marrow sideroblasts.

Studies of G-6-PD involved the methemoglobin elution test for G-6-PD activity of individual erythrocytes, quantitative G-6-PD assay, and electrophoresis. In the pedigree, linkage information was obtained from a doubly heterozygous woman, four of her sons, and five of her daughters. Three sons were doubly affected, and one was normal. One daughter appeared to be a recombinant. The genes appeared to be linked in the coupling phase in the mother. The maximum likelihood estimate of the recombination value was 0.14.

By means of Price-Jones curves, the microcytic red cells in peripheral blood were quantitated in female carriers. The sideroblast count in the bone marrow in the mother corresponded closely to the percentage of microcytic cells in peripheral blood. This is the second example in which the cellular expression of a sex-linked trait has been documented in the human red cells, the first one being G-6-PD deficiency. The coexistence of the two genes in doubly heterozygous females has made it possible to study correlations in cell counts; our studies showed a strong positive correlation except in the probable recombinant in which a reciprocal relation held which indicated that X-inactivation was at least regional, rather than locus by locus.

This paper was presented at the 10th Annual Meeting of the American Society of Hematology, Toronto, Canada, 3 December 1967.

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Received for publication 27 December 1967 and in revised form 19 February 1968.

INTRODUCTION

Several investigators (1–21) have described the occurrence of a hypochromic microcytic anemia,

not due to iron deficiency or thalassemia, which seemed to be familial. The mode of inheritance appeared to be sex-linked, but sufficient data to determine this with some certainty have been presented in only a few instances (1–5). In those cases in which bone marrow studies were performed, an excessive number of sideroblasts was seen and the entity has been called hereditary sex-linked sideroblastic anemia.

This paper presents studies of the first Negro family with sex-linked sideroblastic anemia. This family is also the first to be studied in which glucose-6-phosphate dehydrogenase (G-6-PD) deficiency coexists with sideroblastic anemia. This has provided us with a unique opportunity to study the linkage of the two genes and to study the relationships of the cellular expressions of the two genes in various genotypes, particularly double heterozygotes.

METHODS

Three doubly affected boys, seven heterozygous carriers of one or the other gene, three presumed normal, and three G-6-PD–deficient members of this family were studied in detail. Besides history, physical examination, and routine blood tests (fasting blood sugar, blood urea nitrogen, serum bilirubin, alkaline phosphatase, calcium phosphorus, and total lipids) complete hematological studies including bone marrow examination were carried out. The bone marrow smears were stained for iron (22) and the number of abnormal sideroblasts were counted (23). Normoblasts containing more than five coarse ferric aggregates or those containing aggregates arranged around the nucleus (ringed type) were considered abnormal (23). In addition, the bone marrow was fixed in buffered glutaraldehyde (24) and postfixed in 1% osmium tetroxide (25) for electron microscopic examination. Fetal hemoglobin and electrophoretic patterns were performed by standard methods (26). Serum iron and unsaturated iron-binding capacity were determined by the methods as outlined by Meites and Faulkner (27). The plasma disappearance rate of ^{59}Fe and its incorporation into red cells was also measured (28). Erythrocyte survival was measured by the use of ^{51}Cr-labeled red blood cells (29). Price-Jones curves were obtained by measuring two diameters and taking the average for each red blood cell on photographic prints of peripheral blood smears obtained at different times (30). A total of 2500 cells were measured in each subject. The percentages of normal and microcytic cells were calculated from the Price-Jones curves. Total serum protein was determined by the biuret method (31). Electrophoretic patterns of serum proteins were obtained on cellulose acetate. Fibrinogen in the plasma was quantitated by the method of Ware, Guest, and Seegers (32). Other blood coagulation studies included recalcification time, partial thromboplastin time, one stage prothrombin time, accelerator-globulin, antihemophilic globulin, antithrombin, thrombin time, and euglobulin lysis time (33).[1]

Studies of G-6-PD involved the methemoglobin elution test (MET) for G-6-PD activity of individual erythrocytes (34), quantitative G-6-PD assay (35), and starch-gel electrophoresis (36). The activities of aldolase, lactic, and malic dehydrogenases in the red blood cells were determined in several subjects with kits made available by Calbiochem, Los Angeles, Calif. Pyruvic kinase activity in the red cells was measured according to the method of Tanaka, Valentine, and Miwa (37). Glyceraldehyde-3-phosphate dehydrogenase activity of red cells was assayed by the method of Bergmeyer (38).

RESULTS

Pedigree and clinical observations in affected males

The pedigree is shown in Fig. 1. The affected[2] males (II-2, II-3, and II-16) were 12, 11, and 15 yr old, respectively. All three were essentially asymptomatic except for dyspnea on exertion. 5 yr before this study they were diagnosed elsewhere as having anemia of unknown eitology. One male sibling, II-14, died in 1961, also with a diagnosis of anemia of undetermined etiology. This patient, II-14, was said to have a bleeding diathesis. In this connection, it is of interest that one of the affected males, II-16, under investigation was admitted to the hospital during this study because of epistaxis.

All three boys were anemic and physically underdeveloped for their ages (below three percentiles of normal curves for height and weight). The spleen was barely palpable in two of the three cases and was not felt in the third. No evidence of color blindness was obtained in any of the family members.

Anemia was moderately severe in the affected boys (Table I, II-2, II-3, and II-16). Blood films of the peripheral blood stained with Wright's stain revealed the presence of markedly hypochromic microcytic cells, with extreme poikilocytosis. Hemoglobin was of the A type on electrophoresis; concentrations of fetal and A_2 hemoglobins were within normal limits. Serum iron was increased and unsaturated iron-binding capacity was decreased (Table I).

[1] We wish to thank Dr. E. Mammen and Dr. W. H. Seegers for coagulation studies.

[2] The term "affected" will be used to refer to males affected with sideroblastic anemia.

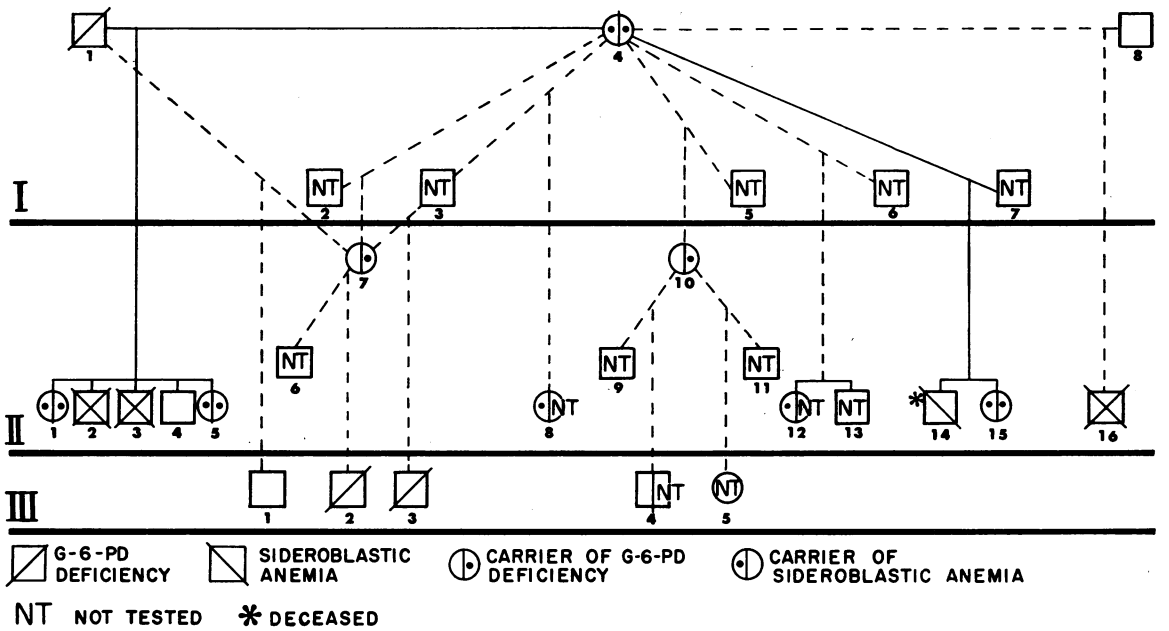

FIGURE 1 The pedigree of the family is shown in this figure.

Plasma ^{59}Fe disappearance rate was increased but ^{59}Fe utilization by red blood cells was markedly decreased (Table II). The red blood cell survival time as measured by ^{51}Cr-labeled red cells was within normal limits in the two patients in which this was determined (Table II). The bone marrow examination showed marked erythroblastic hyperplasia, increased stainable iron, and an increased number of sideroblasts. Most of the sideroblasts were of the ringed type. The electron microscopic examination of the bone marrow demonstrated the perinuclear deposition of the ferric aggregates to be intramitochondrial. The serum gamma globulin levels were normal but the plasma fibrinogen concentration ranged from 78 to 136 mg/100 ml in all three affected males (normal, 200–300 mg/100 ml). All other plasma-clotting factors were within normal limits. The activities in hemolysates of pyruvic kinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, lactic dehydrogenase, and malic dehydrogenase were within normal limits or in some cases slightly elevated in the affected males.

Heterozygous females. Studies in the female members of this family indicated that several were probable heterozygous carriers of the gene for sideroblastic anemia (I-4, II-1, II-5, II-8, II-12, and II-15). Although these women were hematologically normal, they exhibited dual populations of red blood cells in the peripheral blood: one normal and the other showing hypochromasia, microcytosis, and anisocytosis, not due to iron deficiency. The bone marrow studies in the heterozygous mother (I-4) revealed the presence of 9% abnormal sideroblasts (Table III).

Correlative studies of G-6-PD and sideroblastic anemia

Affected males. Table III shows the results of G-6-PD studies in various members of the family. The electrophoretic pattern of G-6-PD in the males affected with sideroblastic anemia was of the A type and the red cell G-6-PD activity was markedly reduced in all three subjects. In the MET, 67–76% of the cells failed to stain in the sideroblastic, G-6-PD– deficient males, which indicated little G-6-PD activity. This can be contrasted with the more usual value of approximately 80% (I-1, III-2, and III-3) in nonsideroblastic, G-6-PD– deficient males. Table III also shows the percentage of abnormal cells resulting from the sideroblastic gene (primarily microcytic cells) as detected by the Price-Jones technique, and the percentage of abnormal sideroblasts in the bone marrow in various subjects. The peripheral blood smear of the affected boys revealed an abnormal

TABLE I

Hematological Data in All Cases Studied

Pedigree No.	Hemoglobin	Hematocrit %	RBC in millions per mm³	Reticulocytes %	Hemoglobin types A	Hemoglobin types A_2	Hemoglobin types F	Serum iron	Unsaturated iron-binding capacity	Morphology
	g/100 ml					%		*μg/100 ml*	*μg/100 ml*	
I-1	14	40	4.6	—	A	1.5	<1	120	280	Normal
I-4	13.9	46	5.1	—	A	2.1	<1	117	248	Aniso and poikilocytosis, hypochromic cells, a few target cells and rare pencil cells, dimorphic pattern of red cells
I-8	13.7	43	4.7	1.4	A	4.7	<1	130	190	Normal
II-1	12.4	41	4.7	—	A	0.7	<1	112	240	Aniso and poikilocytosis, rare pencil cells, target cells, some hypochromasia, dimorphic pattern of red cells
II-2	7.5 10.0*	30.5	5.25	1.7	A	2	<1	275	0	Marked hypochromasia, moderate poikilocytosis, moderate anisocytosis, moderate microcytosis, occasional target cells, no siderocytes seen
II-3	6.3	27.5	5.12	2.1	A	1.8	<1	240	0	Marked hypochromasia, marked aniso and poikilocytosis, some pencil cells, some spherocytes, some target cells and leptocytes, some basophilic stippling, no siderocytes seen
II-4	12.3	41	4.8	—	A	2.0	<1	110	240	Normal
II-5	12.4	40	4.7	—				130	202	Hypochromasia, microcytosis, anisocytosis and rare target cells, dimorphic pattern of red cells
II-7	12.8	40	4.3	—	A	1.6	<1	100	250	Normal
II-8	12.6	41	5.0	—	—	—	—	83	250	Aniso and poikilocytosis, rare target cells, dimorphic pattern of red cells
II-10	12.1	41	4.5	—	A	1.8	<1	100	276	Normal
II-12	13	43	4.98	—	A	2	<1	108	230	Slight aniso and poikilocytosis, hypochromasia, dimorphic pattern of red cells
II-14†	6.2	22	3.82	1.3	A	1.5	<1	220	0	Marked hypochromasia, moderate aniso and poikilocytosis, microcytosis, occasional target cells, occasional basophilic stippling
II-15	12	40	4.4	—	A	1.9	<1	120	230	Moderate aniso and poikilocytosis, hypochromasia, dimorphic pattern of red cells
II-16	6.2 11.0*	27	4.7	0.3	A	1.6	<1	217	0	Marked hypochromasia, marked anisocytosis, moderate poikilocytosis, some target cells, some leptocytes, occasional spherocytes, no siderocytes seen
III-1	12.4	41	5.0	—	—	—	—	100	276	Normal
III-2	12.6	42	4.6	—	—	—	—	—	—	Normal
III-3	14	43	5.1	—	—	—	—	117	282	Normal
III-4	10.6	38	4.1	—	—	—	—	—	—	Slight anisocytosis
III-5	12.1	38	4.1	—	—	—	—	100	220	Slight anisocytosis
Normal values	16 ± 2‡	47 ± 5‡	5.4 ± 0.8‡	<2	A	2.46 ± 0.47	<2	87 ± 14	250 ± 50	

* After pyridoxine, 100 mg daily for 6 months.

† Deceased.

‡ These values are for males.

TABLE II
Summary of ^{59}Fe and ^{51}Cr Studies in Patients

Pedigree No.	Plasma ^{59}Fe clearance $t_{\frac{1}{2}}$	Red blood cells ^{59}Fe utilization in 8 days	Red cell survival time (^{51}Cr) $t_{\frac{1}{2}}$
	min	%	*days*
II-2	34	10	—
II-3	40	10	24
II-16	35	8.7	23
Normal values	60–60	>95	23–27

cell population consisting primarily of hypochromic microcytic cells and the Price-Jones technique showed a single curve which was shifted to the left (Fig. 2).

Heterozygous females. The Price-Jones technique was employed in an attempt to obtain a quantitative estimate of the percentage of abnormal cells in doubly heterozygous carriers, so that these counts could be correlated with the percentage of G-6-PD–deficient cells as measured by the MET (Table III). On the assumption that the two genes were linked in coupling (see linkage studies) in the mother (I-4), the two types of counts (Table III, columns 4 and 5) agree reasonably well in three of the four doubly heterozygous carriers (I-4, II-1, and II-15). On the other hand, the cell counts in II-5 are more compatible with the genes being present in repulsion, since the two counts are approximate reciprocals of each other. This observation suggests that II-5 may be a recombinant. In this connection, the presence of both G-6-PD electrophoretic type A and B in II-1 and II-5 when the putative father (I-1) should have transmitted the A type, and the mother's sideroblastic gene was linked to the A type, suggests either recombination or paternal exclusion. Blood types exclude I-1 as the father of II-1, but are completely compatible with I-1 being the actual father of II-5.

Thus, our interpretation in the case of II-1 is that she is not a recombinant, in view of the paternal exclusion and the agreement in cell counts. On the other hand, in the case of II-5, the lack of paternal exclusion, and the reciprocal relationship in cell counts, suggests that she may be a recombinant. Further evidence on this point was obtained by studies of the morphology of the cells on the MET slide. We have been able to show that in the case of heterozygotes I-4, II-1, and II-15, the nonstaining, G-6-PD–deficient cells are the morphologically abnormal ones (Fig. 3). On the other hand, in the case of II-5, the nonstaining G-6-PD–deficient cells appear to be normal morphologically while the cells which showed G-6-PD activity are

TABLE III
Correlative Studies of G-6-PD and Sideroblastic Anemia

Pedigree No.	G-6-PD electrophoretic type*	G-6-PD activity‡	MET, per cent non-staining cells	Microcytic cells in peripheral blood	Bone marrow abnormal sideroblasts
				%	%
I-1	A-	18	82	None	—
I-4	B	254	11	12	9
II-1	A-, B	100	13	12	—
II-2	A-	27	76	Majority	56
II-3	A-	24	67	Majority	62
II-4	B	266	0	None	—
II-5	A-, B	27	73	18	—
II-7	B	241	24	0	0
II-10	A	131	26	0	0
II-15	A	131	27	14	—
II-16	A-	31	69	Majority	66
III-1	B	245	0	None	—
III-2	A-	11	79	None	—
III-3	A-	19	80	None	—
Normal	B	200–350	> 95%	None	None

* The minus sign refers to a relative deficiency of enzyme activity detected on the starch gel.
‡ Expressed as μmoles of TPN reduced per gram of hemoglobin per hour at 23°C.

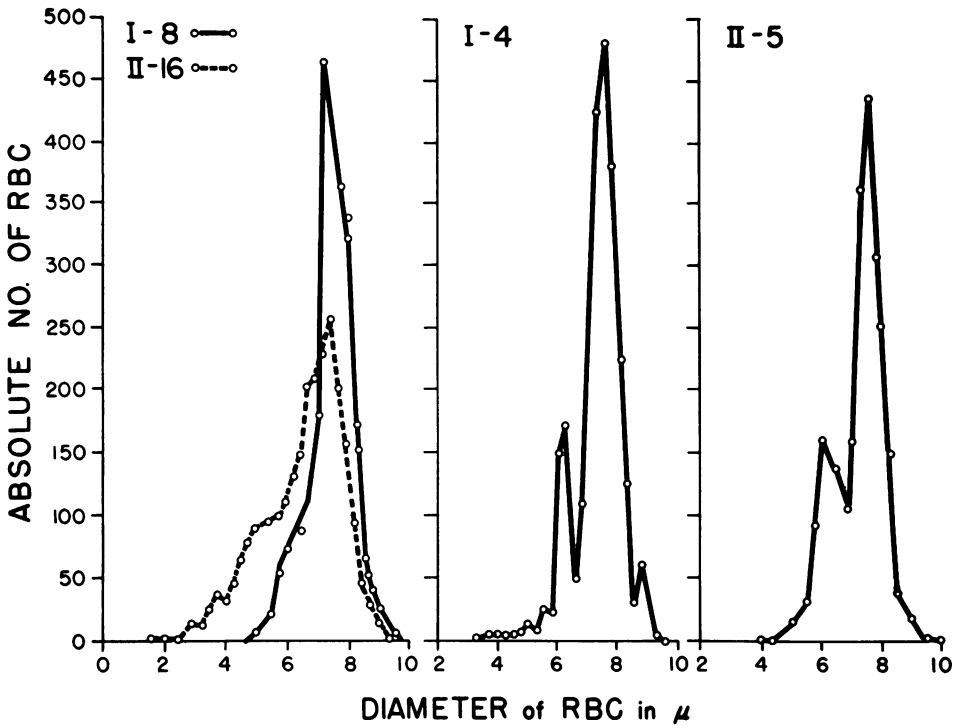

FIGURE 2 Price-Jones curves are shown here. I-8, normal father, II-16, affected male, I-4, doubly heterozygote mother, and II-5, recombinant female. In the case of the normal father a single normal curve was seen whereas in the case of the affected male a single abnormal curve was noted. The heterozygous females showed two cell populations, one normal and the other microcytic.

abnormal. Thus, we have a cellular expression of the two genes which allows genetic inferences to be made.

Another interesting point in the pedigree is the absence of expression of the G-6-PD A electrophoretic type in I-4 and II-7. The progeny of these two females reveal the presence of the A gene in both. The MET made the diagnosis of G-6-PD deficiency in I-4 (11% abnormal cells) but even the MET was within normal limits in II-7 (4% abnormal cells). Up to 5% abnormal cells may occasionally be found in normal individuals.

Linkage studies

For linkage studies, the simplest approach consists of computing a likelihood function. The most likely recombination value given this pedigree information was computed in the manner described by Smith (39). The recombination value, R_i, was taken to be the one which maximizes the conditional probability function, Prob (R_i| this pedigree). Assuming that the loci involved are both on the X chromosome and that the initial probabilities of the recombination values, Prob (R_i), are equal, it is easily shown that:

$$\text{Prob } (R_i \mid \text{Pedigree}) = \text{Prob } (\text{Pedigree} \mid R_i).$$

Therefore, the linkage analysis involved calculation of the probability of obtaining the observed phenotoypes for various recombination values. For those individuals with an incompletely specified genotype, the array of possible genotypes was considered. The probability of obtaining the pedigree becomes the weighted average of all possible combinations of genotypes which could occur. The weights were determined by the relative frequencies of occurrence in the general population of each of the combinations. The recombination value which maximizes the probability of obtaining this pedigree is 0.14 (Table IV). These results are based on the assumption that the mother (I-4) is a coupling heterozygote, II-1 has a random father, and the frequencies of the G-6-PD and sidero-

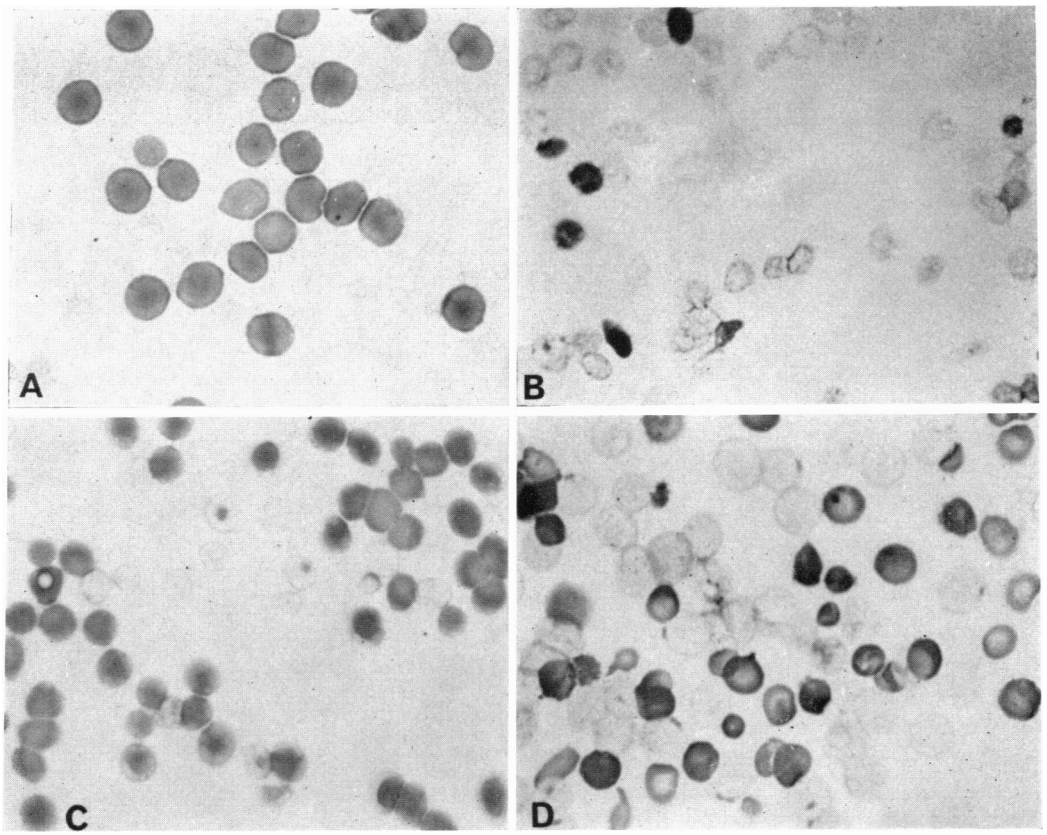

FIGURE 3 Methemoglobin elution test slides are shown here. A, normal male (II-4); B, affected male (II-16); C, doubly heterozygote female (I-4); and D, Recombinant female (II-5). The normal male showed normal G-6-PD activity in morphologically normal cells whereas in the affected male all cells were morphologically abnormal, an occasional young cell showing activity of this enzyme. In the mother (I-4), cells that showed microcytosis, anisocytosis, and poikilocytosis, were devoid of G-6-PD activity whereas in the recombinant female (II-5), morphologically normal cells showed no activity of this enzyme. × 800.

TABLE IV
Linkage Studies

R_i	Prob (Pedigree| R_i)
0.01	0.15194*
0.05	0.18060
0.10	0.20108
0.13	0.20526
0.14	0.20540
0.15	0.20495
0.20	0.19493
0.25	0.17485
0.30	0.14878
0.35	0.12039
0.40	0.09267
0.45	0.06771
0.50	0.04677

* Multiplied by 10 raised to the − 31 power.

blastic genes in the population are 0.125 and 0.001, respectively.

DISCUSSION

The term "sideroblastic anemia" has been used recently to refer to the anemias in which an abnormal increase of iron is detected in erythroblasts. As such it is found in various disorders, such as the Di Guglielmo Syndrome (40), acquired and congenital hemolytic anemias (41), some cases of aplastic anemias, leukemias, thalassemia, and lead poisoning. Our cases represent examples of "hereditary sideroblastic anemia" similar to those reported by others (1–5). On clinical grounds alone, the differential diagnosis between hereditary sideroblastic anemia and heterozygous thalassemia may present some difficulties. Normal levels of A_2 and

fetal hemoglobin, normal red cell life span, sex-linked mode of inheritance, completely saturated iron-binding capacity, and partial response to pyridoxine therapy provide evidences for the diagnosis of hereditary sideroblastic anemia.

Losowsky and Hall (5) and Ludin (13) have found a markedly increased incidence of this anemia in the male members of the affected families, although in their studies, a few females may also have been affected. One such female subject responded to pyridoxine therapy (5). Response to pyridoxine has been reported by several investigators in "hereditary" and "acquired" type of sideroblastic anemias (3, 9, 10, 14, 15, 42–45). Pyridoxine administration (100 mg daily) for 6 months resulted in an increase in the hemoglobin concentration of 2–4 g/100 ml in the two cases (II-2, II-16) indicating a partial response.

One affected male child (II-14) was reported to have died because of hemorrhage and another (II-16) gave evidence of epistaxis. This led us to investigate these patients for bleeding disorders. The coagulation studies were unremarkable except for hypofibrinogenemia which was noted in all three affected boys. The biochemical and genetic relationships of hypofibrinogenemia and the sideroblastic abnormality in these patients remain unknown at present.

In our studies, evidences for other six-linked traits such as color blindness, hypogammaglobulinemia, or hemophilia were not found except for the deficiency of G-6-PD in the red cells. Studies of the X_g^a blood group revealed no informative segregation.

The concomitant occurrence of the gene for G-6-PD deficiency and the gene for sideroblastic anemia in the same family offers several unique opportunities because it is possible to detect, in a fairly high proportion of cells, the expression of each of these two sex-linked genes at the individual cellular level. This is a result of X-chromosomal inactivation in females (46, 47) and the existence of mosaicism, that is, two cell types in females heterozygous for G-6-PD deficiency (48, 49). Of course, the Price-Jones curves take into account only one of the effects of the sideroblastic gene, namely, microcytosis. It does not utilize hypochromicity, nor for that matter, abnormalities of cell shape. In future studies it may be possible to further refine the methodology in terms of evaluating these additional cellular effects of the sideroblastic gene. Even now, however, we are able to show a single, somewhat microcytic cell population in males affected with sideroblastic anemia, and two cell populations, one microcytic and one normal, in heterozygotes.

Two approaches were employed to study the cellular relationship of the two genes in double heterozygotes. One approach was to make two independent cell counts, one of G-6-PD–deficient cells on the MET slide, and one of microcytic cells on Wright-stained differential slides. Using this approach, we obtained fairly good agreement in the counts in three of four double heterozygotes, in keeping with a probable coupling relationship of the genes. In the fourth double heterozygote, in whom there is reasonable additional grounds for suspecting a recombination, the two types of cell counts bore a reciprocal relationship to each other.

The second approach was to examine the cells on the MET slide concomitantly for G-6-PD deficiency and abnormal cellular morphology. This approach, which at this point has been done only qualitatively, indicated that in heterozygotes I-4, II-1, and II-15 the abnormalities coexisted in the same cells, while in heterozygote II-5 they existed in different cells.

The occurrence of the two abnormalities in the same cells in three double heterozygotes and in opposite cells in the fourth, rather than a random association in cells, indicates that the X chromosome of human females is inactivated at least regionally and not locus by locus. These are the first data on this point in the human.

The linkage estimate of $R_i = 0.14$ between these two sex-linked genes should be recognized as preliminary, because of the scantiness of the data. Hopefully, the linkage data can be increased as additional pedigrees are studied. It should be noted that demonstration of two cell populations, as well as the detection of abnormal sideroblasts in the bone marrow, should be useful tools in the diagnosis of the sideroblastic carrier state. This increased ability to diagnose heterozygosity should help in the analysis of future pedigrees, as well as in genetic counselling.

ACKNOWLEDGMENTS

We wish to thank Dr. Victor A. McKusick for his helpful suggestions concerning this study. We are grateful to

Dr. Robinson Abner of the Children's Hospital, Detroit, Mich., for providing information concerning some of the members of this family.

This paper was supported in part by U. S. Public Health Service grant AM-08142, a Michigan Cancer Society Grant, U. S. Public Health Service grant AM-09381, U. S. Public Health Service Career Development Award 5-K3-AM-7959, and AEC Contract AT(11-1)-1552.

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