Am. J. Hum. Genet. 42:516–520, 1988

Glucose 6-Phosphate Dehydrogenase Deficiency and Incidence of Hematologic Malignancy

Anna Maria Ferraris,* Giorgio Broccia,† Tullio Meloni,‡ Gavino Forteleoni,‡ and Gian Franco Gaetani*

*Division of Hematology, University of Genova, Italy; †Division of Hematology, Ospedale A. Businco, Cagliari, Italy; and ‡Department of Pediatrics, University of Sassari, Italy

Summary

We have evaluated the hypothesis of a negative association between glucose 6-phosphate dehydrogenase (G6PD) deficiency and cancer in a cohort of 481 Sardinian males with hematological malignancies. The frequency of G6PD deficiency in the patients was not different from the incidence in a group of 16,219 controls. The same conclusion resulted from the comparison of the frequency of expression of the Gd^B gene in 23 heterozygous women having a clonal hematologic disease and a control group of 37 healthy heterozygotes. Therefore at present there is no evidence that G6PD deficiency has a protective effect against development of hematologic neoplasms.

Introduction

The identification of congenital factors predisposing to—or protecting from—development of cancer has raised considerable interest in recent years. Although a negative correlation between glucose 6-phosphate dehydrogenase (G6PD) deficiency and neoplasia has been suggested for both the Mediterranean (Sulis 1972) and the A^- variants (Naik and Anderson 1970, 1971), no convincing evidence to support this view has been so far reported. Since extensive epidemiological investigations on this topic were lacking, we evaluated the incidence of G6PD deficiency in a population of 481 Sardinian males with hematological neoplasms and compared it with the incidence in the general population, derived from the results of a screening program for G6PD deficiency in Sardinia.

If there is a negative association between G6PD deficiency and cancer, we ought to assume that cells carrying the active Gd^{Med} allele are less susceptible to the mutational event(s) leading to neoplasia. To test this hypothesis, we examined 23 Gd^B/Gd^{Med} women with a clonal hematological disease to determine whether, compared with the expected frequency of the Gd^B gene in the heterozygous population, there was an excess of leukemias expressing the B-type enzyme.

We then decided to reevaluate the theory of variability of the Gd^B/Gd^{Med} phenotype within normal heterozygotes, a variability that was suggested to result from events subsequent to X inactivation and to cause a selective advantage for the cells bearing the active Gd^B allele in some mature tissues (Rinaldi et al. 1976; Luzzatto et al. 1979). To this end, we determined Gd^B/Gd^{Med} mosaicism in hemopoietic and nonhemopoietic cells of 37 healthy heterozygous females. Ascertainment of mosaicism can be performed with various methods. For several years, we have been using the 2-deoxy glucose 6-phosphate (2dG6P) technique—a technique based on the different affinity for 2dG6P (an analogue of the normal substrate) of the B enzyme versus the Mediterranean variant—to determine, with a high degree of accuracy, G6PD mosaicism in hemopoietic and nonhemopoietic cells (Ferraris et al. 1981). With this technique we have been able to demonstrate clonality of hemopoietic cell populations in several subjects with

Received February 12, 1987; final revision received August 6, 1987.

Address for correspondence: Dr. G. F. Gaetani, University of Genova, ISMI, Viale Benedetto XV, 6 16132 Genova, Italy.

0002-9297/88/4203-0015$02.00

hematological malignancies (Gaetani et al. 1982; Ferraris et al. 1984, 1985).

The purpose of this study is therefore to provide adequate epidemiological and statistical data to (1) critically assess the hypothesis of a protective effect of G6PD deficiency against neoplasms of the hemopoietic system and (2) better define the role of events subsequent to X inactivation in determining the adult Gd^{B}/Gd^{Med} phenotype of healthy heterozygotes.

Material and Methods

Data on normal males were provided by the Regional Administration of Sardinia 1981 Campaign against Favism; G6PD analysis was performed with the fluorescent spot test.

The medical records of all male patients admitted at the Ospedale Oncologico, Cagliari, between January 1977 and June 1986 for hematological disorders were reviewed; erythrocyte G6PD had been determined in 481 cases by means of quantitative spectrophotometric assay.

In our laboratory, blood samples were obtained from 33 normal males, 20 G6PD Mediterranean men, and 37 heterozygotes, the latter being defined as women of Sardinian origin who had a G6PD-deficient father or son. Cell purification procedures were performed as described elsewhere for erythrocytes, granulocytes, platelets, lymphocytes, and monocytes (Gaetani et al. 1982). Scalp hair roots were examined in all heterozygotes and in ~50% of the normal and G6PD-deficient subjects.

Samples of skin, hair follicles, and peripheral blood were examined at diagnosis in 23 heterozygous women with various hematologic disorders. They were 23 consecutive patients in whom heterozygosity was demonstrated on the basis of skin or hair-follicle study with the 2dG6P technique. Results of seven cases have been published (Ferraris et al. 1983, 1984, 1985). Techniques used for skin fibroblast culture, hemopoietic cell separation, and preparation of cell extracts have been described elsewhere (Gaetani et al. 1982).

All purified cell preparations were tested for 2dG6P utilization according to the method of Ferraris et al. (1981).

Results

During the 1981 regional screening program for favism 16,219 healthy volunteer men were tested for G6PD activity in 345 Sardinian villages and towns. The overall incidence of G6PD deficiency was 15.1%; however, results showed significant variations between the four provinces, ranging from 8.1% in the province of Sassari to 26.3% in the province of Oristano. Results are shown in table 1 and are in good agreement with previous reports on the high variability of the frequency of the Gd^{Med} gene in different areas of Sardinia (Salvidio et al. 1969).

Table 1

G6PD Type of Patients and Controls, Stratified According to Province of Origin

	Controls		Patients	
Province	G6PD B	G6PD Med	G6PD B	G6PD Med
Cagliari	5,894	1,328	239	59
Nuoro	5,061	628	65	11
Oristano ...	896	319	47	19
Sassari	1,923	170	36	4
Total	13,774	2,445	387	93

In 538 male patients admitted between 1977 and 1986 with a diagnosis of hematologic malignancy, erythrocyte G6PD activity had been determined in 481 cases as part of routine laboratory investigation. All medical records were reviewed by two of us; we considered as neoplasms of the hemopoietic system the following disorders, in which a clonal development of hemopoietic lineages has been demonstrated with different approaches (Fialkow 1976, 1985; Arnold et al. 1983; Ferraris et al. 1983, 1984, 1985): chronic myelogenous leukemia (CML), essential thrombocythemia (ET), polycythemia vera (PV), agnogenic myeloid metaplasia (AMM), refractory anemia (RA), myelodysplastic syndromes (MDS), aplastic anemia (AA), acute lymphoblastic leukemia (ALL), acute nonlymphocytic leukemia (ANLL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and non-Hodgkin lymphoma (NHL). We also included all cases of hairy-cell leukemia (HCL) and Hodgkin disease (HD). Table 2 shows the distribution of normal and G6PD-deficient cases according to diagnosis. The overall incidence of G6PD deficiency was 19.3%; however, because of the high variability of the Gd^{Med} gene in different areas of Sardinia, 480 patients (no information on the place of birth of one subject was available) were subdivided into four groups according to the province of origin. Results of this analysis are also reported in table 1. We compared the observed frequency of G6PD defi-

Table 2

Distribution of 481 Male Patients with Hematological Malignancies, According to Disease and G6PD Type

Disease	G6PD B	G6PD Med	Total (% G6PD Med)
CML	32	8	40 (20.0)
PV	4	0	4 (0)
ET	3	0	3 (0)
AMM	5	2	7 (28.6)
MDS	13	8	21 (38.1)
RA	6	1	7 (14.3)
AA	3	1	4 (25.0)
ALL	39	5	44 (11.4)
ANLL	34	7	41 (17.1)
CLL	44	15	59 (25.4)
MM	30	9	39 (23.1)
HCL	5	1	6 (16.7)
NHL	106	25	131 (19.1)
HD	64	11	75 (14.7)

ciency in the four groups of patients with the frequency determined for normal subjects in each province included in the study described above. As represented in figure 1, for the provinces of Cagliari, Oristano, Nuoro, and Sassari, the incidence of G6PD deficiency in patients with hematological disorders was not different from the frequency observed in the normal population. Significance levels were evaluated with Fisher's exact test and with the χ^2-test for differences among proportions.

Percentages of 2dG6P utilization of Gd^B, Gd^{Med}, and Gd^B/Gd^{Med} healthy subjects are reported in table 3. The difference between the arithmetic means for the 2dG6P values of the three groups is statistically

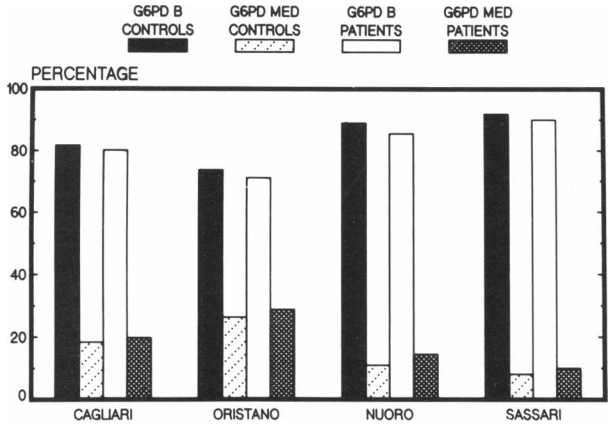

Figure 1 Percentage distribution of G6PD type in patients and controls, stratified according to the province of origin.

Table 3

Relative 2dG6P Utilization of Controls

Control Group (*N*)	Mean ± SD 2dG6P (%)
Gd B (33)	4.1 ± 0.9
Gd Med (20)	37.8 ± 7.3
Gd B/Gd Med (37)	13.2 ± 4.9

significant ($P < .0005$). Of the 37 healthy heterozygotes, various hemopoietic and nonhemopoietic tissues were examined with the 2dG6P method, and no variability in G6PD mosaicism between different cell populations within the same individual was observed (correlation coefficient [r] = .95; range .76–1.00).

In table 4 are summarized some clinical and laboratory data of the 23 heterozygous women having a clonal hematologic disease. Relative utilization of 2dG6P in nonhemopoietic tissues of heterozygote patients and controls are compared in figure 2. The distribution of 2dG6P values is skewed in favor of a

Table 4

Relative 2dG6P Utilization of 23 Gd^B/Gd^{Med} Women with Clonal Hematologic Disorders

Patient (Age), Disease	2dG6P Utilization (%)	
	Nonhemopoietic Cells	Neoplastic Clone
VN (73), ET	11.2	31.3
MM (16), ANLL	11.3	4.8
CM (2), ALL	11.4	29.7
PM (68), ET	10.0	41.7
RA (45), NHL	8.7	2.9
MA (60), CLL	19.1	48.6
GG (68), ANLL	8.7	32.7
CL (61), ET	11.7	3.6
ML (63), ANLL	10.9	5.4
IG (55), ALL	15.4	4.7
FC (9), ALL	11.5	3.0
CS (48), ANLL	7.4	5.0
DG (50), AMM	7.4	3.5
NL (25), CML	15.2	2.3
PM (31), CML	9.1	5.1
PA (38), ANLL	10.3	5.1
SG (40), CML	7.9	4.9
LP (40), ANLL	20.0	4.9
PS (51), PV	15.5	4.8
FL (36), RA	12.9	43.8
PP (11), ALL	17.8	26.9
VM (44), ANLL	12.3	5.3
PG (62), CML	9.8	36.5

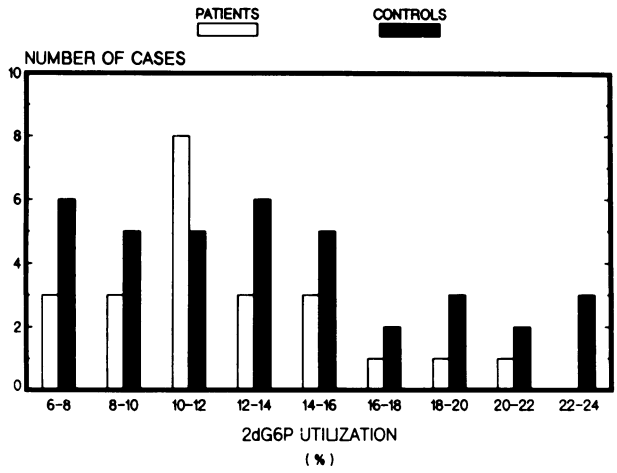

Figure 2 Degree of G6PD mosaicism, expressed as 2dG6P relative utilization of nonhemopoietic tissues, of heterozygous patients and controls.

prevalent expression of G6PD B, thus indicating a preferential inactivation of the Gd^{Med} allele in the heterozygous population. Therefore, the finding that the leukemic clone expressed G6PD Med in eight patients and G6PD B in 15 is not significantly different from the expected frequency ($P < .25$; χ^2 binomial test of proportions).

It must be pointed out, however, that although the deviation from the null hypothesis is not significant, the sample studied is too small to provide a definite confirmation of the null hypothesis on the basis of this evidence only.

Discussion

After a few mainly anecdotal reports suggesting an inverse relationship between G6PD deficiency and the incidence of cancer (Naik et al. 1970, 1971; Sulis 1972), no comprehensive studies have been published. In the present report the number of subjects studied and the application of appropriate statistical tests allow for the first time an adequate evaluation of the hypothesis of an inverse correlation between G6PD deficiency and cancer. The investigation of 481 patients with hematologic neoplasms clearly shows that the incidence of G6PD deficiency in leukemic subjects is not different from the incidence in the normal population. An important factor is represented by the variability of frequency of G6PD deficiency in different areas of Sardinia. We therefore thought it appropriate to compare separately patients and controls from each of the four provinces, and, on doing so, found the same percentage of G6PD deficiency for the four subgroups (fig. 1). There is, then, no evidence to suggest that G6PD-deficient subjects have a lower incidence of hematologic neoplasms. This conclusion is further strengthened by the possibility of a slight overestimation of the percentage of G6PD-deficient subjects in the normal population. Since the data that we used were collected through a regional campaign screening for favism, it is conceivable that subjects with a family or personal history of hemolytic crises may have been more eager to volunteer for testing. On the other hand, some Gd^{Med} patients with hematological disorders may have gone undetected: whenever the G6PD assay is performed in hemolysate from a G6PD-deficient leukemic subject with a high white cell count, contamination with nucleated cells that contain an appreciable amount of enzyme may easily occur, leading to an erroneously normal result for the test. In table 2 the frequency of G6PD deficiency in the various groups of diseases is detailed, and the overall incidence is not different from the expected incidence. However, the higher percentage of G6PD deficiency observed in the myelodysplastic-syndromes group and in the chronic-lymphoproliferative-disorders group is easily explained, since most of these patients came from the provinces of Cagliari and Oristano, where the incidence of G6PD deficiency is higher. Conversely, most of the cases with ALL were from the province of Sassari, and that accounts for the slightly lower incidence of G6PD deficiency in the acute-leukemias group (table 1).

That the presence of the mutated Gd^{Med} gene on the active X chromosome does not confer a diminished susceptibility to cancer development is further demonstrated by the study of the 23 heterozygous women with a clonal hematological disease, since the percentage of leukemias expressing the G6PD B phenotype did not exceed the expected frequency of the Gd gene in the heterozygous population.

As a final point, we believe that, as a general rule, events subsequent to X inactivation cannot play an important role in the determination of the heterozygous phenotype. The lack of variability in G6PD mosaicism between hemopoietic and nonhemopoietic cell populations of the 37 healthy heterozygous women studied strongly argues against the theory of a selective phenomenon acting in differentiated tissues (Rinaldi et al. 1976; Luzzatto et al. 1979; Beutler 1984). The finding of a preferential expression of the Gd^{B} allele in Gd^{B}/Gd^{Med} heterozygotes is more

compatible with the hypothesis that a selective event causes inactivation of the Gd^{Med}-bearing X chromosome at the first stages of embryogenesis. However, the undisputable role played by host defenses in the development of cancer deserves to be further explored.

Acknowledgments

We thank Dr. Pierluigi Cocco of the University of Cagliari for his cooperation. This work was supported by a grant from the Italian National Research Council, Special Project "Oncology," contract 86.00409.44, and by funds from the Associazione Italiana per la Ricerca sul Cancro.

References

Arnold, A., J. Cossman, A. Bakhshi, E. S. Jaffe, T. A. Waldmann, and S. J. Korsmeyer. 1983. Immunoglobulin gene rearrangements as unique clonal markers in human lymphoid neoplasms. N. Engl. J. Med. 309:1593–1599.

Beutler, E. 1984. "Clonal" remission in acute leukemia (letter). N. Engl. J. Med. 311:922.

Ferraris, A. M., G. Broccia, T. Meloni, L. Canepa, M. Sessarego, and G. F. Gaetani. 1984. Clonal origin of cells restricted to monocytic differentiation in acute nonlymphocytic leukemia. Blood 61:817–820.

Ferraris, A. M., L. Canepa, C. Mareni, G. Baule, T. Meloni, E. Salvidio, G. Forteleoni, and G. F. Gaetani. 1983. Reexpression of normal stem cells in erythroleukemia during remission. Blood 62:177–179.

Ferraris, A. M., L. Canepa, L. Massimo, G. Dini, G. Broccia, T. Meloni, G. Forteleoni, C. Melani, and G. F. Gaetani. 1985. Clonal development from a progenitor with restricted differentiative expression in acute lymphoblastic leukemia. Am. J. Hematol. 20:81–83.

Ferraris, A. M., P. Giuntini, S. Galiano, and G. F. Gaetani. 1981. 2-Deoxy glucose 6-phosphate utilization in the study of glucose 6-phosphate dehydrogenase mosaicism. Am. J. Hum. Genet. 33:307–313.

Fialkow, P. J. 1985. Clonal development, heterogeneity, and multiple pathogenesis of human leukemias. Pp. 491–503 in R. P. Gale and D. W. Golde, eds. Leukemia: recent advances in biology and treatment. Alan R. Liss, New York.

———. 1976. Clonal origin of human tumors. Biochim. Biophys. Acta. 458:283–321.

Gaetani, G. F., A. M. Ferraris, S. Galiano, P. Giuntini, L. Canepa, and M. D'Urso. 1982. Primary thrombocythemia: clonal origin of platelets, erythrocytes and granulocytes in a $Gd^{B}/Gd^{Mediterranean}$ subject. Blood 59: 76–79.

Luzzatto, L., E. A. Usanga, U. Bienzle, G. F. J. Esan, and F. A. Fasuan. 1979. Imbalance in X-chromosome expression: evidence for a human X-linked gene affecting growth of hemopoietic cells. Science 205:1418–1420.

Naik, S. N., and D. E. Anderson. 1970. G6PD deficiency and cancer. Lancet 1:1060–1061.

———. 1971. The association between glucose 6-phosphate dehydrogenase deficiency and cancer in American Negroes. Oncology 25:356–364.

Rinaldi, A., G. Filippi, and M. Siniscalco. 1976. 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.

Salvidio, E., I. Pannacciulli, A. Tizianello, G. Gaetani, and G. Paravidino. 1969. Glucose 6-phosphate dehydrogenase deficiency in Italy: a study of the distribution and severity of the enzymatic defect. Acta Haematol. 41: 331–348.

Sulis, E. 1972. G6PD deficiency and cancer (letter). Lancet 1:1185.