were the same as described for deoxycytidine (Table 3). However, products could be detected when hydralazine ($1.0 \times 10^{-1}M$) and [5-^{3}H]deoxycytidine ($1.0 \times 10^{-6}M$) were incubated for 4 days (Table 3). Thin-layer chromatography of the reaction mixture demonstrated at least four radioactive spots with R_F values of 0.66, 0.81, 0.85, and 0.92. The slowest moving spot was deoxycytidine. This reaction of deoxycytidine with hydralazine proceeded at a considerably slower rate than that of hydralazine with thymidine (Table 1). The reaction with deoxycytidine was similar to the reaction with thymidine in that the rate was increased by light irradiation (Table 3).

We have shown that hydralazine alters the pyrimidine bases of DNA. Modified DNA can be highly immunogenic (*18*). Thus, it is suggested that the interaction of SLE-inducing drugs with DNA can lead to a marked enhancement of the immunogenicity of DNA. An increase in the immunogenicity of DNA could explain drug-induced antibodies to nucleic acids and perhaps even to DNA. It is also possible that these drugs modify DNA of B cells or suppressor cells, thus altering the antibody response.

Studies on drug-induced SLE may provide clues to understanding idiopathic SLE. It is possible that some cases of idiopathic SLE may be induced by the unsuspected and undetected ingestion of drugs or exposure to chemical agents in the environment. For example, hydrazines are found in tobacco, tobacco smoke (*19*), and mushrooms (*20*); synthetic hydrazines are used in industry, agriculture, and medicine. Synthetic and naturally occurring compounds containing hydrazine that are present in the environment may induce disease in people with a proper genetic predisposition.

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Allele Increasing Susceptibility to Human Breast Cancer May Be Linked to the Glutamate-Pyruvate Transaminase Locus

Abstract. *The patterns of the occurrence of breast cancer in 11 high-risk families were evaluated by segregation and linkage analysis. These patterns were consistent with the hypothesis that increased susceptibility to breast cancer was inherited as an autosomal dominant allele with high penetrance in women. The postulated susceptibility allele in these families may be chromosomally linked to the glutamate-pyruvate transaminase (E.C. 2.6.1.2, alanine aminotransferase) locus. Confirmation of this linkage in other families would establish the existence of a gene increasing susceptibility to breast cancer. Since there is no association in the general population between a woman's glutamate-pyruvate transaminase genotype and her cancer risk, the glutamate-pyruvate transaminase linkage cannot be used as a screening test for breast cancer.*

Probably the single factor most dramatically increasing the risk of breast cancer is the presence of the disease in the immediate family, particularly if more than one relative has had breast cancer, or if the relative was affected bilaterally or at a young age (*1*). However, this increased risk may be due to social, dietary, or environmental factors that predispose the family to breast cancer, or to inherited factors that increase susceptibility to breast cancer. Whether the pattern of breast cancer in some families is consistent with Mendelian segregation of a "breast cancer susceptibility" gene, and whether this gene is physically (chromosomally) linked to a known, clinically innocuous, genetic marker locus have not previously been determined.

We have surveyed a registry of families that were recruited, or that had volunteered, for studies in cancer genetics because breast cancer was frequent in each. We have assumed that in these families the incidence of breast cancer was high and have tested the consistency of this elevated incidence with the pattern expected for a genetically influenced disease (*2*). Four models for transmission of susceptibility to breast cancer were tested to determine which best fit the observed patterns of breast cancer. These models postulated Mendelian inheritance of increased susceptibility to breast cancer through an autosomal dominant allele, an autosomal recessive allele, an X-linked dominant allele, or higher risk of breast cancer for all women in a family due solely to shared environmental factors. For each model, we assumed that men could not be affected with breast cancer, although they could carry and transmit susceptibility (*3*).

The autosomal dominant model for inheritance of breast cancer susceptibility best fit the observed distribution of breast cancer in each of the 11 families ($P > .05$ for rejection by the likelihood ratio test). The environmental model was clearly rejected ($P < .03$ for each family). The autosomal dominant model was up to 1500 times more likely than the recessive model and up to 7000 times more likely than the X-linked model. However, since neither the autosomal recessive nor the X-linked dominant models could be consistently rejected, all three genetic models were used in subsequent linkage analysis. The good fit of the autosomal dominant model corresponds to several observations that clinicians had made about these 11 families: occurrence of breast cancer in several generations, apparent transmission of susceptibility by (unaffected) fathers to their daughters, little family history of breast cancer among most individuals marrying into the pedigree, virtual absence of unaffected mothers with affected daughters, and appearance of breast cancer in about 50 percent of the daughters of breast cancer patients (*4*).

The consistency of a genetic model with the observed pattern of breast cancer in a family has to be interpreted cautiously, since a number of nongenetic

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characteristics are consistent with models of genetic transmission. For example, money is inherited, but not because it is encoded by DNA. The principal motivation for the use of linkage analysis in the study of breast cancer is to resolve this question of cultural mimicry of genetic transmission. Using linkage analysis, it is sometimes possible to confirm the existence of an allele increasing susceptibility to disease by locating it, even if its function remains unknown (*5*).

For linkage analysis, we obtained 20 ml of whole blood from each of 426 members of the 11 families. Genotypes at the following marker loci were determined by established immunologic and electrophoretic methods: ABO, acid phosphatase, adenosine deaminase, adenylate kinase, amylase 2, Duffy A and B, esterase D, glutamate-pyruvate transaminase, glyoxalase I, Gm immunoglobulins, haptoglobin, HLA A and B, Kell, Lewis A and B, MNSs, phosphoglucomutase 1, properdin factor B, Rh antigens C, c, D, E, and e, transcobalamin II, and vitamin D-binding globulin. Each marker was tested for linkage to hypothetical dominant and recessive susceptibility alleles.

Linkage results for glutamate-pyruvate transaminase (GPT) indicate that this locus may be close to a dominant allele increasing susceptibility to breast cancer (Table 1). No one family alone yielded very much information, but the cumulative lod scores are suggestive. Six of the families provide evidence in favor of close linkage. Five families provide very weak evidence against linkage; for each of these families, independent assortment of the GPT and susceptibility loci was slightly more likely than linkage. For the 11 families as a group, the strongest evidence in favor of linkage is at zero recombination ($\theta = 0$). However, the individual lod score for family B103 is greatest at $\theta = .36$. The true recombination fraction θ for GPT and the susceptibility allele is probably between zero and .36 and can more accurately be estimated when additional families are analyzed. No other markers appear to be closely linked to any susceptibility allele in these families: lod scores for the other markers ranged from $-\infty$ to $+0.18$ for the families as a group. Nor is there evidence for linkage of GPT to a recessive susceptibility allele: the maximum lod score for GPT under a recessive model is $+0.09$ at recombination fraction $\theta = .2$.

The interpretation of the GPT linkage result depends in part on assumptions of the homogeneity of breast cancer in this sample of families. One alternative is to assume that, if an allele for breast cancer susceptibility exists, then it must have the same chromosomal location in all 11 families. This is the appropriate assumption for investigating linkage between known genetic markers: the ABO locus, for example, is located on chromosome 9 in all 11 families. Furthermore, because ages of breast cancer diagnosis appear homogeneous in these families, they were combined for segregation analysis. Under this assumption, the lod score for GPT in all 11 families is 1.43, corresponding to 27 to 1 odds in favor of linkage. For this test alone, $P = .01$; but $P = .15$ after allowing for multiple comparisons.

An alternative approach is to recognize that breast cancer may be influenced by different autosomal dominant mechanisms in the 11 families. In this case, we would look for clusters of families, within a sample consistent with a genetic model, which appear to show similar linkage relationships. According to this interpretation, the strongest evidence for linkage is provided by the six families with a combined lod score of 1.84, corresponding to 80 to 1 odds in favor of linkage, and $P = .04$ after adjusting for multiple comparisons. Under this assumption, we would conclude that breast cancer susceptibility in families such as B108 with negative lod scores is probably influenced by a mechanism other than a dominant allele linked to GPT. However, the 11 lod scores reported in Table 1 are not significantly heterogeneous, so such an interpretation is not warranted at present. Since suggested linkages supported by lod scores of less than about 1.5 have frequently proved incorrect (*6*), it is essential to test our hypothesis in other families in which breast cancer incidence is consistent with a genetic model (*7*).

Table 1. Genetic analysis of breast cancer susceptibility in 11 families. M ± S.E., estimated mean ± standard error of ages at breast or ovarian cancer diagnosis; Lod (GPT), lod score for linkage of GPT to a dominant susceptibility allele at zero recombination.

Family	Family size (number sampled)	Breast cancer cases	Ovarian cancer cases	M ± S.E.	Lod (GPT)
B103	270 (81)	19	0	43.1 ± 3.0	+.65
B110	244 (34)	10	1	48.4 ± 4.1	+.05
B95	233 (66)	8	0	43.0 ± 4.0	+.26
B102	187 (30)	7	2	52.8 ± 4.6	+.12
B126	50 (22)	6	1	48.1 ± 8.1	+.35
B73	26 (14)	3	0	49.3 ± 8.7	+.41
B93	208 (116)	5	0	54.6 ± 4.0	−.003
B85	206 (11)	3	5	47.1 ± 1.7	−.003
B21	129 (29)	9	2	48.3 ± 3.5	−.08
B108	76 (11)	3	0	60.0 ± 9.7	−.01
B113	66 (12)	5	0	49.8 ± 3.9	−.21
Families indicating linkage				46.9 ± 0.5	+1.84 ($P = .003$)
All families				48.2 ± 0.5	+1.43 ($P = .01$)

The GPT locus has been provisionally mapped on chromosome 10, but no other markers polymorphic in Caucasian populations have been located on that chromosome (*8*). As polymorphic markers linked to GPT are discovered, they can be tested for linkage to the hypothesized susceptibility allele in the present families. If the linkage of GPT to a dominant allele increasing susceptibility to breast cancer can be confirmed, medical counseling for both susceptible and not susceptible women in such families will become a real possibility.

However, GPT cannot be used as a screening test for breast cancer. The GPT gene simply marks the chromosomal region where a susceptibility gene of still unknown function may be located. In the general population, there is no association between a woman's GPT genotype and her risk of breast cancer. Our results can only be used to counsel women in families in which genetic linkage between the GPT locus and a susceptibility gene can be confirmed.

Not all families with high incidence of breast cancer follow this pattern. In analyses of breast cancer in families other than the 11 reported here, we have found some pedigrees in which susceptibility is not consistent with a single-gene hypothesis, and still others in which susceptibility to breast or endometrial cancer late in life appears to be inherited as an autosomal dominant allele not linked to GPT.

Confirmation of the linkage of GPT and a breast cancer susceptibility gene may offer an opportunity to elucidate breast cancer etiology. It may be possible to determine the way in which susceptibility genes influence breast cancer risk by comparing immunological and hormonal profiles of genetically susceptible but unaffected young women with

those of young women who are not genetically susceptible. If we can determine how susceptibility genes for breast cancer are expressed, we can begin to investigate how to alter this expression to modify preclinical developments in the natural history of breast cancer.

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2. All families from the registry with at least three cases of breast cancer in mothers and daughters or sisters, for whom cancer diagnosis could be confirmed by pathology review, and with relatives from at least three generations available for sampling, were included in the investigation. Prior to genetic analysis, the 21 large families who met these requirements were classified into three groups on the basis of two epidemiologic criteria: ages at diagnosis of breast cancer patients and sites of other cancers present at higher than expected frequencies. Group 1: 11 families reported herein with breast and ovarian cancer or only breast cancer in excess (average age at diagnosis, 48 years); group 2: eight families with frequent postmenopausal breast cancer and endometrial cancer (average age at breast cancer diagnosis, 66 years); group 3: two families with breast cancer in very young women (average age, 34 years) and high frequencies of adrenal-cortical carcinoma, leukemia, brain tumors, and sarcomas. The occurrence of ovarian cancer among close relatives of breast cancer patients in four of the group 1 families led us to postulate a common susceptibility to breast and ovarian cancer in those families. More than 80 percent of breast cancers and all ovarian cancers were verified by pathology review. [H. T. Lynch, R. E. Harris, H. A. Guirgis, K. Maloney, L. L. Carmody, J. F. Lynch, *Cancer* **41**, 1543 (1978).]
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9. We thank J. Lynch, K. Maloney, and L. Rankin for family interviews and sampling; G. Rodgers, D. Lattanzio, M. E. Dupuy, and H. Williams for laboratory analysis; and E. B. Kaplan, R. Leung, and R. Harris for programming and statistical assistance. Supported by NIH grants and contracts CA 13556, CB 44003, and CB 84274.

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Survival of Mice Receiving Melanoma Transplants Is Promoted by Hydroquinone

Abstract. *In BALB/c female mice with melanoma transplants, the incidence of "takes" is decreased and survival is increased by hydroquinone, a melanocytolytic agent. The mechanism of drug action is suggested to be via DNA. The significant and high degree of positive response to hydroquinone treatment in vivo is encouraging for the clinical management of melanoma with melanocytolytic agents.*

Melanoma occurs in all known human populations, in both sexes and in all age groups; the incidence is highest in the Caucasoid population (*1*). Among the most malignant of human tumors, melanoma composes 1 to 2 percent of the total cancer incidence and about 20 percent of all skin cancer. Generally, the prognosis is poor; the best available clinical procedures, individually or in combination, offer only a limited degree of temporary relief (*2*). However, a number of agents have been found to selectively destroy normal integumental melanocytes in vivo (*3–7*). These melanocytolytic (depigmentary) agents may detrimentally affect abnormal melanocytes (melanoma cells); encouraging findings have been reported for some of these agents (*8, 9*). Here we report the effects in vivo of one such melanocytolytic agent, hydroquinone, on mice receiving melanoma transplants.

The NIH Harding Passey melanoma was grown in BALB/c female mice as follows. On day zero, 0.1 ml of melanoma brei (12 g of melanoma plus 10 ml of 0.9 percent saline) was injected subcutaneously in the right axilla to form the transplants. Following an NIH screening procedure (*10*), we injected subcutaneously either hydroquinone or 0.9 percent saline vehicle once daily on days 1 through 9. The animals were injected at 15 to 17:30 hours daily to avoid possible diel variation. The control and experimental groups are indicated in Table 1. After the initial 9 days of chemotherapy the mice received no further treatment. They were examined daily for the 140-day experimental period, after which time the surviving mice were killed. Autopsies were performed on all mice, and the melanomas were weighed. For statistical analysis of survival we used the log-rank method of life table analysis (*11*) which has been applied previously to clinical trials in cancer chemotherapy (*12*). Other data were analyzed by Student's t-test.

Comparison of the median survival times (Table 1) reveals that all of the control groups not implanted with melanoma have median survival times exceeding 140 days (the length of the study). Further, only one of the melanoma-bearing groups (injected with 80 mg of hydroquinone per kilogram of body weight) has a median survival exceeding 140 days. Median survival for the other melanoma-bearing groups ranges from 94 to 96 days.

Comparison of the survival curves of the melanoma-bearing groups treated with 16 or 80 mg of hydroquinone per kilogram revealed a statistically significant difference in survival rates ($\chi^2 = 12.12$, d.f. = 1, $P < .0005$). The estimated probability of survival to 140 days was .725 for the group that received the high dose and .350 for the group that received the low dose.

The two melanoma-bearing control groups not receiving chemotherapy (no treatment; 0.9 percent saline) were not significantly different in survival ($\chi^2 = 0.02$, d.f. = 11, not significant). Data for these two control groups, therefore, were combined for subsequent analyses. A comparison of each of the two hy-

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