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Increased Prevalence of the *HFE C282Y* Hemochromatosis Allele in Women with Breast Cancer

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Abstract

Individuals with the major hemochromatosis (*HFE*) allele *C282Y* and iron overload develop hepatocellular and some extrahepatic malignancies at increased rates. No association has been previously reported between the *C282Y* allele and breast cancer. We hypothesized that due to the pro-oxidant properties of iron, altered iron metabolism in *C282Y* carriers may promote breast carcinogenesis. Because 1 in 10 Caucasians of Northern European ancestry carries this allele, any impact it may have on breast cancer burden is potentially great. We determined *C282Y* genotypes in 168 patients who underwent high-dose chemotherapy and blood cell transplantation for cancer: 41 with breast cancer and 127 with predominantly hematological cancers (transplant cohort). Demographic, clinical, and tumor characteristics were reviewed in breast cancer patients. The frequency of *C282Y* genotypes in breast cancers was compared with the frequency in nonbreast cancers, an outpatient sample from Tennessee ($n = 169$), and a published United States national sample. The frequency of at least one *C282Y* allele in breast cancers was higher (36.6%, 5 homozygotes/10 heterozygotes) than frequencies in Tennessee (12.7%, $P < 0.001$), the general population (12.4%, $P < 0.001$), and similarly selected nonbreast cancers (17.0%, $P = 0.008$). The likelihood of breast cancer in the transplant cohort increased with *C282Y* allele dose ($P_{trend} = 0.010$). These results were supported by the finding in a nontransplant cohort of a higher frequency of *C282Y* mutations in Caucasian (18.4%, $P = 0.039$) and African-American (8.5%, $P = 0.005$) women with breast cancer than race-specific national frequency estimates. A high prevalence of *C282Y* alleles in women with breast cancer with and without poor risk features suggests that altered iron metabolism in *C282Y* carriers may promote the development of breast cancer and/or more aggressive forms of the disease.

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Introduction

Elevated iron stores are linked to an increased risk of cancer and cancer mortality. Iron is an essential micronutrient for both benign and neoplastic cells and is required for the activity of ribonucleotide reductase, a key enzyme in DNA synthesis, as well as for respiratory and oxidative cell metabolism. However, free (reactive) iron is highly toxic to cells because it catalyzes the generation of potent free-radical species such as the hydroxyl radical via the Fenton and Haber-Weiss reactions (Fig. 1). A balance between the low-level formation of free radicals during normal metabolism and their scavenging by natural antioxidants is necessary to prevent damage to DNA, lipids, and proteins. A relative increase in free radical-mediated damage, termed *oxidative stress*, is thought to be involved in many disease states and in carcinogenesis. Therefore, the tight sequestration of excess iron (under physiological conditions) in nonreactive forms such as ferritin and the regulation of iron uptake by cells through modulation of their transferrin receptors are crucial mechanisms of antioxidant defense (1, 2).

Hereditary hemochromatosis is an iron overload disorder associated in the vast majority of cases with homozygosity for a missense mutation (the substitution of a tyrosine for a cysteine residue at position 282 or *C282Y*) in the *HFE* gene on chromosome 6*p* (3). Normal *HFE* protein, stabilized by β-2-microglobulin, binds the cell-surface transferrin receptor and facilitates the uptake of iron-bound transferrin, the major iron transport protein in blood (4, 5). This action enables *HFE* to regulate duodenal iron absorption by an incompletely understood mechanism (6, 7). In *C282Y* homozygotes, unregulated iron absorption over time leads to toxic iron deposition in the liver, heart, anterior pituitary, skin, and joints. Another *HFE* mutation (the substitution of aspartate for histidine at position 63, or *H63D*) has minor effects on iron transport when present alone but increases iron loading in *C282Y* heterozygotes (8). Although *C282Y* heterozygotes rarely develop iron overload, they also have higher mean body iron stores than *HFE* wild-type individuals and significantly increased circulating levels of reactive iron (9–11). Because the prevalence of heterozygosity for the *C282Y* allele among white Caucasians in North America, Europe, Australia, and New Zealand is ~10%, it is important to determine whether these biochemical differences have health consequences (12).

Patients with hereditary hemochromatosis and cirrhosis because of iron overload are at a 200-fold increased risk of

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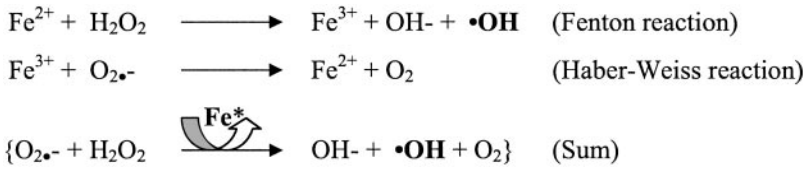

Fig. 1. Mechanism of *in vivo* free radical generation by iron. *Only trace amounts of iron (Fe) are necessary to produce abundant quantities of the hydroxyl radical (·OH) from less reactive oxygen species (*e.g.*, the superoxide anion, O_2·-). The hydroxyl radical causes irreversible oxidative damage to DNA, lipids, and proteins.

developing hepatocellular carcinoma (13). An increased risk of extrahepatic neoplasms, including hematological, gastric, and colorectal tumors, has also been reported previously (14, 15). In *C282Y* heterozygotes, the risk of cancer has been less clear (16–19). An association between breast cancer and *HFE* mutations has not been previously shown, to our knowledge, although local iron accumulation and oxidative stress have been postulated to play a role in breast carcinogenesis (20, 21).

We report a high prevalence of *C282Y* alleles in a cohort of breast cancer patients who underwent high-dose chemotherapy and blood cell transplantation (BCT) for poor-prognosis disease. An increased prevalence of this allele, although less pronounced, was also observed in a nontransplant sample of women with primary invasive breast cancer. These findings suggest a possible link between altered iron metabolism in *C282Y* carriers and the pathophysiology of breast cancer.

Patients and Methods

Patients. The transplant cohort consisted of 168 adults who received high-dose chemotherapy and BCT at Vanderbilt University Medical Center between 1995 and 1998 and who were also enrolled in an observational study of transplant-associated lung injury (the Lung Injury after Hematopoietic Stem Cell Engraftment Study). Adults considered eligible for autologous or allogeneic BCT at Vanderbilt included those with high-risk hematological malignancies, aplastic anemia, poor-prognosis breast cancer, and relapsed or refractory germ cell tumors. Poor-prognosis breast cancer was defined as (*a*) stage II disease with ≥10 involved axillary lymph nodes or (*b*) measurable stage III or metastatic (stage IV) disease that was responsive to chemotherapy. Exclusion criteria for the Lung Injury after Hematopoietic Stem Cell Engraftment Study included active viral hepatitis and a history of prior high-dose chemotherapy with BCT; 2 patients with blood malignancies and no breast cancer patients were excluded. A nontransplant cohort, consisting of breast cancer patients who did not undergo BCT, is described under "Comparison Populations."

All patients provided informed consent for review of their medical records and for broadly defined DNA analyses. The Vanderbilt Institutional Review Board approved these studies.

Comparison Populations. Several comparison populations were used. Because the same nondisease selection criteria for BCT were applied to all patients in the transplant cohort, nonbreast cancer cases within this cohort constituted an internal comparison group. A second comparison population was based on published data from Phase II of the Third National Health and Nutrition Examination Survey (NHANES III) conducted by the National Center for Health Statistics from 1992 through 1994 (22). That cross-sectional study of 5171 samples from the NHANES III DNA bank yielded prevalence estimates for *HFE* genotypes in the United States population that are considered nationally representative because adjustment was made for oversampling of certain racial/ethnic groups. Another comparison population consisted of 169 unrelated Tennesseans (mean age 49 years, range 43–60 years), recruited without regard to race/ethnicity for an epidemiological study of *HFE* mutations, who attended Vanderbilt-affiliated outpatient medicine and general gastroenterology clinics from 1999 to 2002.[9] This population was predominantly male. No significant gender differences exist in the distribution of *C282Y* genotypes (22).

Finally, a previously assembled nontransplant cohort, comprised of 141 Caucasian (non-Hispanic white) and 59 African-American (non-Hispanic black) women with primary invasive breast cancer, was studied. These women were evaluated and treated at our institution between 1982 and 1996 and had participated in other genetic studies for which the only exclusion criterion was insufficient tissue for DNA extraction (tumors < 1.0 cm); 7 patients were excluded (23).

Histopathology, Staging, and Clinical Characteristics of Breast Cancer Cases. Pathologists at Vanderbilt reviewed all breast and lymph node biopsies performed before BCT and confirmed the cancer diagnoses in both transplant and nontransplant cohorts. Information regarding patient race/ethnicity, age, staging evaluations, prior chemotherapy for breast cancer, tumor pathology and hormone receptor studies, and tobacco and alcohol use was obtained from patients' medical records by an investigator blinded to *HFE* genotypes. Certain clinical data such as parity and age at menarche were available only in the nontransplant cohort.

Detection of HFE Mutations. Genotypes at the *C282Y* locus (both cohorts) and *H63D* locus (transplant cohort only) were determined by single-strand conformation polymorphism analysis (24). Genomic DNA was isolated either from whole blood that was collected from transplant recipients before initiating high-dose chemotherapy or from breast biopsy specimens in the nontransplant cases. All banked DNA samples were stored at −70°C. Oligonucleotide primers, based on the published *HFE* sequence, and PCR were used to amplify a 158-bp fragment encompassing the G-to-A nucleotide transition at position 845 in exon 4 of the *HFE* gene (primers: 5′-TGGATGCCAAGGAGTTCGA-3′ and 5′-ACCCCAGATCACAATGAGGG-3′; Ref. 25). In *C282Y* heterozygotes, a separate PCR was also performed to amplify a 131-bp fragment containing the C-to-G transversion at nucleotide 187 (primers: 5′-TTCATGGGTGCCTCAGAGCA-3′ and 5′-CTGGAAACCCATGGAGTTCG-3′; *H63D* allele). The PCR reactions from cancer patients were treated with formamide and subjected alongside sequenced heterozygous mutant, homozygous mutant, and normal controls, as well as blank PCR controls, to nondenaturing gel electrophoresis for 9 h at 4°C in a mutation detection enhancement gel matrix (FMC, Rockland, ME). Readers blinded to clinical parameters scored *HFE* genotypes after gels were stained with silver nitrate to detect DNA bands. Direct sequencing of the *C282Y* locus in randomly sampled single-strand conformation polymorphism-determined heterozygotes provided independent genotype validation.

[9] A. Kallianpur, M. Bumbalough, L. Hall, M. Yadav, S. Summar, J. Awad, A. Mushawar, and M. Summar, unpublished data.

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Estimation of Iron Stores. Iron studies were not obtained in the transplant or nontransplant cohorts at enrollment. Only frozen serum in the former group, collected in tubes containing EDTA (an iron chelator) and stored at −80°C, was available for analysis. We measured levels of soluble transferrin receptor (STR), which are inversely proportional to body iron stores, in banked serum samples that were available in 30 patients (15 randomly selected *C282Y* carriers and 15 HFE *wild-type* patients; Refs. 26, 27). The Chemiluminescence Immunoassay was used to measure STR levels (Nichols Research Corp., El Segundo, CA).

Statistical Analysis. Demographic and clinical characteristics of breast cancer patients were compared using Fisher's exact test (discrete variables) or the Student's two-sample *t* test with unequal variances (continuous variables). Results of *t* tests were confirmed with the Mann-Whitney *U* test. The proportions of patients with breast and nonbreast cancers who carried at least one *C282Y* allele (*i.e.*, *C282Y* heterozygotes plus homozygotes) are presented along with 95% confidence intervals (CIs). Results were compared between breast cancers in the transplant cohort and (*a*) nonbreast cancers in the cohort, (*b*) the Tennessee sample, and (*c*) published figures from the NHANES III, stratified by race/ethnicity (22).[9] The frequencies of *C282Y* genotypes observed in the nontransplant cohort were compared with race- and gender-specific NHANES III figures. Using Pearson's χ^2 test or Fisher's exact test for these comparisons did not alter the results (two-sided *P*s < 0.05 considered significant). The Hardy-Weinberg law states that $p^2 + 2pq + q^2 = 1$, where *p* and *q* are allele frequencies in a two-allele system. We used Pearson's χ^2 test in this study to assess Hardy-Weinberg equilibrium of *C282Y* and *wild-type* alleles. Expected values for each cohort were derived from the non-Hispanic white (NHW), non-Hispanic black (NHB), or both subsets of the NHANES III population sample, as appropriate. The *P*s for comparisons within individual *C282Y* genotype categories (observed *versus* expected homozygous and heterozygous frequencies) were obtained using an exact binomial test because of the small number in one homozygote cell. The *P* for trend in likelihood of breast cancer with increasing number of *C282Y* alleles was calculated with the Cuzick nonparametric test for trend across ordered groups, an extension of the Wilcoxon rank-sum test (28). All statistical analyses were performed with STATA statistical software (version 7.0, Stata Corp., College Station, TX, 1984–2001).

Results

Characteristics of Breast Cancer Patients in the Transplant Cohort. Forty-two women (mean age 48 years) with previously diagnosed breast cancer were enrolled in the Lung Injury after Hematopoietic Stem Cell Engraftment Study. Thirty-nine of these women underwent escalated-dose chemotherapy and BCT for poor-prognosis, limited-stage (stages I-III) breast cancer, or responding metastatic disease, accepted indications for autologous BCT at that time. Two women underwent BCT for acute leukemia caused by chemotherapy they had received for breast cancer (secondary leukemia). Ninety-six percent of the entire transplant cohort was NHW, and race/ethnicity did not differ between breast cancer and nonbreast cancer patients (data not shown). Data on tumor size at diagnosis (6 stage IV patients), lymph node involvement (1 stage IV patient), and hormone receptor expression (1 stage IV breast cancer) were not documented in the medical record. Overall, 36 women (88%) had limited-stage but poor-risk disease, and 5 (12%) had distant metastases at diagnosis. Women had received an average of eight cycles of chemotherapy and four different drugs for breast cancer before BCT; mean interval between diagnosis and BCT was 2.3 years. Twenty-nine tumors (71%) expressed estrogen receptor with or without progesterone receptor. The histories taken on admission for BCT revealed no heavy alcohol use in any patients; 14 women (34%) were current or former smokers. There were no statistically significant differences between breast cancer patients with and without the *C282Y* allele with respect to age, menopausal status, stage or tumor size at diagnosis, lymph node involvement, estrogen receptor, or progesterone receptor expression. The number of prior cytotoxic agents or cycles of chemotherapy received, interval between diagnosis and transplant, and smoking history was also similar (Table 1). Smoking is not a risk factor for breast cancer (23, 29). Family cancer histories were variably recorded and therefore not analyzed.

HFE Mutations in the Transplant Cohort. Genotyping at the *HFE C282Y* locus was successful in 41 of 42 women who carried a breast cancer diagnosis and in 127 other patients in the transplant cohort with predominantly hematological malignancies. Of the 169 patients in this study, PCR amplification was unsuccessful in 1 woman with stage III breast cancer. Direct sequence analysis confirmed *HFE* genotypes in all randomly selected, single-strand conformation polymorphism-determined heterozygotes. The distribution of *HFE* genotypes for the *C282Y* mutation in breast cancer cases was significantly different from expected values based on the general population ($P < 0.001$ for deviation from Hardy-Weinberg equilibrium;

Table 1 Characteristics of breast cancer patients with poor-prognosis disease (transplant cohort)

Variable	*HFE* genotype	
	≥1 *C282Y* allele[a] $n = 15$ (*n*, %)[b]	*Wt/Wt* $n = 26$ (*n*, %)[b]
Mean age at diagnosis in years (range)	50.1 (36–54)	48 (36–59)
Premenopausal at diagnosis[c]	8 (53)	19 (73)
Primary reason for BCT[d]		
Breast cancer	15	24
Secondary malignancy	1	1
Limited stage at diagnosis[e]	13 (87)	23 (88)
Metastatic disease at diagnosis	3 (20)	2 (8)
Tumor size at diagnosis		
<5.0 cm	8 (46)	15 (58)
≥5.0 cm	4 (27)	9 (34)
N/A	4 (27)	2 (8)
ER ± PR tumor expression		
Positive	11 (73)	18 (69)
Negative	4 (27)	7 (27)
N/A	0 (0)	1 (4)
Lymph node involvement at staging		
≤5 LN+	6 (40)	8 (35)
>5 LN+	8 (53)	17 (65)
N/A	1 (7)	0 (0)
Mean time from diagnosis to BCT years (range)	2.8 (1–8)	2.0 (0–14)
Smoking	7 (47)	7 (27)
Chemotherapy cycles (mean no., range)	8 (4–13)	6 (4–16)
Chemotherapeutic agents (mean no., range)	4 (2–6)	8 (2–6)

[a] ≥1 *C282Y* allele, homozygotes + heterozygotes; *Wt/Wt*, homozygous *wild-type*.
[b] All *P*s for comparisons between genotype groups were insignificant (>0.05).
[c] Ovarian function present.
[d] BCT, blood cell transplantation; ER/PR, estrogen receptors/progesterone receptors; N/A, data not available; LN, lymph node(s).
[e] Includes two cases of inflammatory breast cancer.

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Table 2 Prevalence of at least one *C282Y* allele[a] in breast and nonbreast cancer cases in the transplant cohort compared with other population samples

Transplant cohort		United States population[b]			Tennessee[c]	
Breast cancer[d] (n = 41) % (95% CI)[e]	Nonbreast cancer[d] (n = 129) % (95% CI)	Overall (n = 5171) % (95% CI)	All women (n = 2884) % (95% CI)	Non-Hispanic white (n = 2016) % (95% CI)	Overall (n = 169) % (95% CI)	Non-Hispanic white (n = 118) % (95% CI)
36.6 (22.0–51.3)	17.0 (10.5–23.4)	6.6 (5.9–7.3)	7.2 (6.3–8.1)	12.4 (11.0–13.8)	10.6 (6.0–15.2)	12.7 (6.7–18.7)

[a] Includes *C282Y* homozygotes and heterozygotes (heterozygotes include *C282Y/Wt* + *C282Y/H63D* genotypes).
[b] Data from Steinberg *et al.* (22).
[c] Kallianpur *et al.*[9]
[d] Both transplant categories include 2 patients who each had both breast cancer and leukemia diagnoses; nonbreast cancers include all hematological malignancies, two germ cell cancers and 1 aplastic anemia. The transplant cohort was 96% non-Hispanic whites.
[e] Values are percentage of patients with 95% confidence intervals (95% CI).

Table 2). We anticipated finding no *C282Y* homozygotes and up to 4 heterozygotes among breast cancer cases but found 5 ($P < 0.001$) and 10 ($P = 0.027$), respectively. The 95% CI for the proportion of breast cancer patients carrying at least one *C282Y* allele did not overlap those calculated for the general United States population (based on published NHANES III data) or Tennessee, even when stratified by race/ethnicity. Comparison of our group of women with breast cancer to only women in the general population (NHANES III, stratified by sex) led to the same conclusion (Table 2). The distribution of *HFE* genotypes was significantly associated with a diagnosis of breast as compared with nonbreast cancer ($\chi^2 = 6.97$, $P = 008$) in the cohort, despite a small overlap in the 95% CIs for the proportion of patients with at least one *C282Y* mutation in these groups (Table 2). (The χ^2 test has greater power to discern differences because it does not assume a normal distribution of the data and uses observations in all possible genotype categories.) A trend toward a breast cancer diagnosis was also observed with increasing dosage of the *C282Y* allele, *i.e.*, 0, 1, or 2 *C282Y* alleles ($P = 0.010$; Table 3).

Breast cancer cases comprised only 24% of the transplant cohort, but 45% of all *C282Y* homozygotes and 40% of all heterozygotes in this group had a prior diagnosis of breast cancer. The distribution of *C282Y* genotypes is shown in Table 3. Genotyping at the *H63D* locus was not done in *C282Y* homozygotes, and no *H63D* mutations were identified in breast cancer patients heterozygous for the *C282Y* allele.

Genotype-Phenotype Correlations. The mean STR levels in 15 randomly selected patients with and 15 patients without the *C282Y* allele (12 heterozygotes and 3 homozygotes) were 16.6 nmol/liter (95% CI 13.9–25.8) and 23.5 nmol/liter (95% CI 11.2–35.8), respectively. The mean STR level was significantly lower, however, in the 10 patients in this group with breast cancer (13.6 nmol/liter) than in the 20 patients with other diagnoses (23.0 nmol/liter, P for the difference 0.048). The STR level is inversely proportional to body iron stores and is generally unaffected by illness (27).

Findings in a Nontransplant Cohort. In contrast to women in the transplant cohort, 59 women in this cohort (29.5%) were NHB. The mean ages of the NHW and NHB women were 58 and 56 years, respectively, and the distribution of *HFE C282Y* genotypes in the two subgroups was analyzed separately. Demographic and clinical features (including tumor type and variables often associated with breast cancer risk such as parity, contraceptive hormone use, and age at menarche) were not significantly different in NHW women with and without the *C282Y* allele (data not shown). The number of NHB women was too small for meaningful analysis of differences between *C282Y* genotype categories. There were no *C282Y* homozy-

Table 3 Distribution of *C282Y* genotypes in the transplant cohort by diagnosis

Diagnosis	*HFE* genotype			Total	P (trend)
	Wt/Wt n = 132 (n, %)[a]	*C282Y/Wt* n = 25 (n, %)[a]	*C282Y/C282Y* n = 11 (n, %)[a]		
Breast cancers	26 (20)[b]	10 (40)	5 (45)[b]	41[c]	0.010
Hematological cancers					0.040
Acute leukemias	22 (17)[b]	6 (24)	3 (27)[b]	31[c]	
Chronic leukemias	28 (21)	2 (8)	1 (9)	31	
Myelodysplasia	7 (5)	1 (4)	0 (0)	8	
Multiple myeloma	12 (9)	0 (0)	1 (9)	13	
Non-Hodgkin lymphoma	25 (19)	5 (20)	1 (9)	31	
Hodgkin disease	10 (8)	1 (4)	1 (9)	12	
(Subtotal)	(104)	(15)	(7)	(126)[c]	
Miscellaneous					—[d]
Aplastic anemia	1 (<1)	0	0	1	
Germ cell cancer	2 (<2)	0	0	2	
(Subtotal)	(2)	(0)	(0)	(3)	
(Total cancers)				(170)[c]	

[a] Percentage of patients in each genotype category with the indicated diagnosis.
[b] Each of these categories includes 1 patient with both breast cancer and leukemia.
[c] Includes 2 patients with both breast cancer and leukemia.
[d] Insufficient number for analysis.

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Table 4 Prevalence of at least one HFE *C282Y* allele[a] in a nontransplant cohort with primary invasive breast cancer compared with United States population-based estimates

Women with invasive breast cancer		United States population by race/ethnicity and sex		
NHW[b] women (n = 141) % (95% CI)[c]	NHB women (n = 59) % (95% CI)	All NHW (n = 2016) % (95% CI)	All NHB (n = 1600) % (95% CI)	All women (n = 2884) % (95% CI)
18.4 (12.0–24.9)	8.5 (1.4–15.6)	12.4 (11.0–13.8)	2.5 (1.7–3.3)	7.2 (6.3–8.1)

[a] Includes *C282Y* homozygotes and heterozygotes (heterozygotes include *C282Y/Wt* + *C282Y/H63D* genotypes); data from Steinberg *et al.* 22.
[b] NHW, Non-Hispanic white(s); NHB, Non-Hispanic black(s).
[c] Values are percentage of patients with 95% confidence intervals (95% CIs). There were no *C282Y* homozygotes among breast cancer cases in the nontransplant cohort.

gotes in the nontransplant cohort, but the distributions of genotypes deviated significantly from Hardy-Weinberg equilibrium in both NHW ($P = 0.020$) and NHB ($P < 0.001$) women. The frequency of *C282Y* heterozygotes was higher among NHW women (18.4%, $P = 0.039$) and NHB women (8.5%, $P = 0.005$) than published estimates (from NHANES III data) for all NHW or all NHB persons in the United States; it was also higher than the frequency for all women in the United States (Table 4). National frequency estimates specifically for NHW women and NHB women were not available for head-to-head comparison of these subpopulations.

Discussion

We found an unexpectedly high prevalence of *HFE C282Y* alleles in women with breast cancer that may have important implications for the pathophysiology of this devastating disease. Breast cancer is the most common malignancy among women in the United States and the leading cause of cancer death among women worldwide. Despite extensive research, little is currently known about modifiable risk factors for breast cancer. Mutations in the *BRCA1* and *BRCA2* genes account for ~5% of all breast cancers and explain 20–25% of familial cases overall (29, 30). However, significant variability even in women with *BRCA1/2* mutations with respect to cancer risk, cancer site, and the age at which tumors manifest suggests that other gene loci and exogenous factors are important risk modifiers (31, 32). Given the high prevalence of *HFE* mutations in the general population and the ability to modify iron stores, any procarcinogenic effects of *C282Y* could substantially influence both the burden of cancer and the development of novel treatment strategies.

There are several possible explanations for the high prevalence of the *C282Y* allele that we observed in this breast cancer cohort. The *C282Y* allele may lead to one or a combination of the following reasons: (*a*) breast carcinogenesis; (*b*) increased resistance of breast cancer to chemotherapy (possibly because of earlier metastasis); (*c*) an increase in benign, nonproliferative breast disease that enhances detection of cancer in adjacent breast tissue; or (*d*) improved survival of women with breast cancer. A causal link between altered iron metabolism and breast cancer is highly plausible. Iron catalyzes the formation of mutagenic hydroxyl radicals, acts as a growth factor for proliferating neoplastic cells, and may suppress host antitumor immunity (33, 34). Compelling data from *in vitro* and *in vivo* studies also favors a role for iron in the pathogenesis or behavior of breast cancer. The density of transferrin receptors is significantly elevated in breast carcinoma cells (particularly those with higher metastatic potential) as compared with normal or benign cells, and the concentration of ferritin, the principal iron storage protein, in breast carcinoma is several-fold that of benign breast tissue. Ferritin concentration also correlates with aggressive growth features and prognosis in breast cancer (21). Recently, Yang *et al.* (35) reported that the growth of human breast carcinoma cells is inhibited by an antisense oligonucleotide targeted to the transferrin receptor (*TFRC*) gene. Evidence that iron in malignant breast tissue may not be an innocent bystander comes from the observation that mammary carcinomas develop at much higher rates in rats that are injected with iron or fed high-iron diets, as compared with rats that are untreated or fed standard/low-iron diets (36). This observation holds whether or not chemical carcinogens are used. Other investigators have also shown reproducible and progressive growth inhibition of transplanted mammary carcinomas in rodents with increasing dietary iron deprivation; a maximal inhibitory effect occurred in rats given low-iron diets plus the iron chelator, deferoxamine (36). Finally, evaluation of the *HFE*-knockout mouse model of hemochromatosis recently revealed evidence of oxidative damage in mammary tissue when mice were fed standard iron as opposed to low-iron diets (37). This observation supports the theory that dietary iron content may modify the phenotype caused by *HFE C282Y*.

Breast cancer is associated with high levels of hydroxyl-radical-modified DNA, and most *in vivo* hydroxyl radical formation occurs via the iron-catalyzed conversion of hydrogen peroxide (20). Estrogen, a relatively weak breast carcinogen by an unknown mechanism, has iron-regulatory properties. One proposed mechanism for its carcinogenic effects, supported by experimental evidence, is the avid reaction of iron with redox-cycling estrogen metabolites to produce hydroxyl radicals (38, 39). Hydroxyl radical formation is also an important mechanism of radiation-induced DNA damage and carcinogenesis (40). Hence, iron and *HFE* mutations may contribute directly or indirectly to the development of radiation- and/or estrogen-inducible cancers.

A significantly increased risk of cancer was reported among women who participated in the first NHANES (NHANES I 1971–1975) whose transferrin saturations exceeded 36.7% (41). The types of cancers that developed in women were not reported. Many heterozygotes for *C282Y* have transferrin saturations in this range (9). Therefore, even the modest increases in body iron stores seen in *C282Y* heterozygotes are associated with elevated cancer risk. A reduction in iron stores also appears to be protective: a significant reduction in overall cancer incidence (relative risk ratio 0.79) was observed in a large study of Swedish blood donors that was designed to test this hypothesis (42). The prescription of iron supplements and iron-containing multivitamins for real or perceived iron deficiency and the fortification of foods with iron are routine in the United States. If heterozygosity for the *C282Y* allele has adverse health consequences, such practices should perhaps be reconsidered. Concern about the potential ill effects of mandatory iron-fortification of foods on the part of the Danish and Swedish governments led to its discontinuation in those countries in 1987 and 1995, respectively (43, 44).

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Indirect evidence exists for increased resistance of breast cancer to chemotherapy when excess iron is present. As previously noted, more highly metastatic variants of breast carcinoma exhibit increased cellular iron stores and are primed for iron uptake. Breast cancer cells also show marked resistance to some chemotherapeutic agents such as bleomycin after chronic exposure to iron *in vitro* (2). The degree of resistance is proportional to cellular iron content.

Increased benign breast disease among *C282Y* carriers is possible, but women taking oral contraceptives experience a relative increase in iron stores and a reduced incidence of benign, nonproliferative breast disease (45, 46). Stimulation of proliferative breast changes, with or without atypia, that increase the risk of subsequent breast cancer is possible in *C282Y* heterozygotes (47). This question warrants further study.

Few studies specifically address the effects of *HFE* mutations on overall survival. However, cancer mortality is modestly increased in both men and women with elevated iron stores, and cardiovascular deaths in women have been associated with heterozygosity for the *C282Y* mutation (41, 48). A large, population-based study in Denmark also documented an age-related reduction in the frequency of *C282Y* heterozygotes, suggesting that carrier status was associated with shortened life expectancy (49). Collectively, these studies do not favor increased survival in breast cancer patients with the *C282Y* allele as an explanation of the observed association.

Our results differ from two previous studies that sought but did not find an association between *HFE* mutations and breast cancer. Beckman *et al.* (16) reported an increased risk of several cancers, including breast cancer (odds ratio 2.2, 95% CI 1.0–4.8), in Swedish cancer patients homozygous for the *TFRC Ser142 allele (S142G* polymorphism) who also carried the *HFE C282Y* allele. When tested separately, however, neither allele was associated with risk of any cancer (frequency of *C282Y* mutations 15.8% in breast cancer cases, as compared with 13.3% in the control population). Because cases and controls were not matched on age or sex and came from two distinct populations, the power to detect an association in that study may have been limited. A community-based survey study compared the risk of neoplastic diseases between parents of clinically diagnosed hemochromatosis patients and parents of their spouses (controls). Age-adjusted relative risks of colonic adenoma and gastric cancer were significantly elevated in women but not cancers of the lung, breast, and cervix. However, the authors acknowledge that inaccuracy of disease reporting, the low survey response rate, and the high expected prevalence of *C282Y* heterozygosity in the control population may have led to an underestimation of disease risk. Also, no mention was made of genotype confirmation in probands or their spouses (17).

Limited availability of banked DNA prevented the determination of *BRCA1*, *BRCA2*, or *TFRC* genotypes in our breast cancer patients. Because of the original intention of studying lung injury in this cohort, data on some variables (*e.g.*, age at menarche, hormone replacement therapy, and parity) was not obtained (50, 51). These women received high-dose chemotherapy and BCT for high-risk disease and are therefore not representative of all women at risk for breast cancer (52). Despite the underrepresentation of African-American, Asian, and Hispanic women in this sample, we believe that the homogeneity of our transplant cohort may have facilitated the detection of an important association in a subset of women with breast cancer, particularly those with more aggressive disease. The prevalence of at least one *C282Y* allele was higher in breast cancer cases than in similarly selected nonbreast (largely hematological) malignancies, although *HFE* mutations may be more prevalent in some hematological malignancies than in the general population (17, 19, 53, 54). We also observed an increased frequency of the *C282Y* allele in our patients with hematological malignancies (Table 2). Finally, a significant trend favoring a breast cancer diagnosis occurred in this cohort with increasing dosage of the *C282Y* allele (0, 1, or 2 alleles). Routine iron studies are difficult to interpret in patients with malignancies and were not obtained in this transplant cohort. The levels of STR, measured retrospectively in serum samples from these patients, however, were consistent with higher body iron stores in *C282Y* carriers as compared with noncarriers and with significantly higher iron stores in breast cancer cases as compared with other cancers, providing evidence of a genotype-phenotype correlation. Nevertheless, to assess the generalizability of our results in this highly selected group of breast cancers, we determined *C282Y* genotypes in a larger population of 200 women with breast cancer who did not undergo transplantation. This nontransplant cohort included both African-American and Caucasian women with a spectrum of primary invasive breast cancer. Studies of *HFE* mutations specifically in African Americans and other ethnic minorities have consistently reported very low carriage rates for the *C282Y* allele (55, 56). The observed *C282Y* genotype frequencies in both NHW and NHB women in the nontransplant cohort were higher than anticipated from published national estimates stratified by gender or race/ethnicity. Not unexpectedly, the frequency of at least one *C282Y* allele in NHW women in the nontransplant cohort was lower than the frequency in transplanted breast cancers selected for poor-prognosis disease but similar to the frequency in transplanted nonbreast cancers. The NHW breast cancer cases in the nontransplant cohort were an average of 9 years older than NHW cases in the transplant cohort, and this difference may have influenced the observed frequencies. Overall, these findings support the validity of the association we detected, but stronger conclusions are premature. Precise estimates of the risk of breast cancer associated with the *C282Y* allele will require analyses in larger numbers of women with this disease that include appropriate age-, sex-, and race-matched controls.

In conclusion, we believe this study is the first to report an association between the *C282Y* allele (heterozygous or homozygous genotype) and breast cancer. The possibility that a prevalent allele like *HFE C282Y* may increase the burden of breast cancer or high-risk breast cancer, particularly when superimposed on an iron-replete diet, is a significant public health concern that deserves careful investigation. Confirmation of this finding in a larger breast cancer sample, comparison to women with benign breast evaluations, and multivariate analysis of potential modifiers of a *C282Y* effect, including *BRCA* and *TFRC* gene mutations, smoking, and hormone replacement therapy, are needed.

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