Investigation of Genetic Variants of Genes of the Hemochromatosis Pathway and Their Role in Breast Cancer

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Abstract

Iron overload has been noticed as a feature of human breast cancer. Cellular iron uptake is regulated by the hemochromatosis and transferrin receptor system, mutations of which cause the iron storage disease hereditary hemochromatosis. To understand the role of hemochromatosis and transferrin receptor system mutations in breast cancer, we analyzed 19 sequence variations at *HFE*, *TFR1*, *TFR2*, and *FPN1* and compared genotype frequencies between cases and controls in a German population. There were 688 breast cancer patients and 724 population-based and age-matched controls. For genotyping, we applied the Hemochromatosis Strip Assay and TaqMan allelic discrimination analyses. In addition to genotype frequencies, we established frequencies of compound genotypes. The frequencies of *HFE* at His63Asp, Ser65Cys, and Cys282Tyr, and of *TFR1* at Ser142Gly minor alleles in this German population were 15.9%, 1.8%, 5.6%, and 46.0%, respectively. No rare variants at 15 more loci at *HFE*, *TFR2*, and *FPN1* were observed in breast cancer patients. There were no significant differences of allele and genotype frequencies between cases and controls. Triple and quadruple compound genotypes at *HFE*_His63_Cys282-*TFR1*_Ser142Gly and *HFE*_His63_Ser65_Cys282-*TFR1*_Ser142Gly showed a nonsignificant increase in cases. Although limited by low numbers, an increased prevalence of the *HFE* Tyr282 minor allele was observed in breast cancer cases with a high number of affected lymph nodes ($P = 0.032$). Our data suggest that variants of the hemochromatosis-transferrin receptor system have no direct effect on the incidence of breast cancer in Germany. Possible effects on tumor progression and prognosis remain elusive. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1102–7)

Introduction

Iron is an important micronutrient required for ribonucleotide reductase, a key enzyme in DNA synthesis as well as respiratory and oxidative cell metabolism. In mammals iron homeostasis is regulated at the level of uptake because there is no excretory route. The precise mechanism and regulation of absorption is attributed to the transferrin receptor system, but another yet not completely understood system is likely to play a role (1). There is epidemiologic, clinical, and experimental evidence for iron to favor neoplastic growth (2). The free metal is carcinogenic due to its catalytic effect on the formation of mutagenic hydroxyl radicals, the suppression of host defense cells, and being a nutrient for unrestricted cancer cell multiplication (2). In animals and humans, primary neoplasms develop at extensive sites of iron deposits. In a carcinogen-induced experimental breast cancer model, rats developed sooner and twice as many mammary tumors on excess dietary iron administration compared with controls on a normal diet (3). In human breast cancer tissues iron overload has been noticed and may reflect a hunger for iron in those cancer cells. Particularly, transferrin receptor expression has been noticed as a feature of clinical breast cancer samples (4, 5) and specific transferrin receptor binding capacity was identified in microsomal preparations (6, 7). Also, elevated ferritin concentrations were identified in breast carcinomas (8), which were assigned to the malignant epithelium (9).

Insight into the molecular basis of iron storage disorders comes from hereditary hemochromatosis (OMIM 235200) that develops on germline mutations within the hemochromatosis pathway comprising genes involved in iron absorption and cellular uptake. These involve the hemochromatosis gene *HFE*, the ferroprotein 1 gene *FPN1*, and the transferrin receptor gene *TFR2* (10-12). Variations of genes causing dysregulation of iron uptake may serve as corollaries for an increased breast cancer risk. More than 35 *HFE* allelic variants are known but most hereditary hemochromatosis cases develop on homozygosity of the *HFE*_845_G > A variant that substitutes tyrosine to cytosine at residue 282 (Cys282Tyr; ref. 13). Hemochromatosis is a MHC class I–like molecule that regulates duodenal iron absorption when stabilized by β2-miocroglobulin and bound to cell surface–associated transferrin receptor (14). Hemochromatosis Tyr282 homozygotes fail to properly assemble the HFE-β2m-TFR complex and are at increased risk of iron accumulation and its sequelae (15).

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This iron storage disorder eventually may lead to liver cirrhosis and hepatocellular carcinoma, diabetes mellitus, hypogonadism, cardiac manifestations including cardiomyopathy and arrhythmias, hyperpigmentation, arthritis, and premature death (16).

Hereditary hemochromatosis is the commonest autosomal recessive condition in individuals of Northern European descent and the prevalence of *HFE* Cys282Tyr heterozygotes among white Caucasians of Northern Europe, North America, South Africa, and New Zealand is about 10% (13). Increased cancer risks for extrahepatic neoplasms have been reported including that for hematologic, gastric, and colorectal tumors (17-19). Recently, an increased prevalence of the *HFE* Tyr282 allele has been reported for women with breast cancer (20), however, other previous breast cancer studies did not identify such an association (21, 22). With breast cancer being the most frequent female neoplasm in the western hemisphere and the *HFE* Cys282Tyr polymorphism being the commonest disease causing variation in Caucasian populations, it is important to clarify a possible relationship. Here we report the prevalence of 19 variations within *HFE* and *TFR* genes in a German population and discuss the role of *HFE*, *FPN1*, *TFR1*, and *TFR2* in breast cancer susceptibility.

Materials and Methods

Subjects. We analyzed incident breast cancer cases and population controls of the GENICA study population recruited between August 2000 and October 2002 from the Greater Bonn Region in Germany, an area of more than 1 million inhabitants. This is part of a wider effort of the Interdisciplinary Study Group on Gene Environment Interactions and Breast Cancer in Germany (GENICA) which focuses on the identification of breast cancer risks (23). Works are therefore referred to as the GENICA study. The study includes 688 breast cancer cases with a first time diagnosis of primary breast cancer that was histologically confirmed within 6 months of enrollment, and 724 population-based controls matched by age in 5-year classes. Cases and controls were included when they were below the age of 80 years, of Caucasian ethnicity, and residing in the study region. Risk factor information was collected via in-person interviews and included assessment of menopausal status, menstrual cycle, lifestyle factors, medical history, as well as personal and family history of cancer. Clinical and histopathologic variables were collected from medical records and pathology reports. The response rate was 88% for cases and 67% for controls. Study participants provided a blood sample drawn into heparin tubes (no. 367680 green, Becton Dickinson, Franklin Lake, NJ). The GENICA study was approved by the Ethics Committee of the University of Bonn. All study participants gave written informed consent.

DNA Samples. Genomic DNA was extracted from 20 mL heparin blood samples using the Puregene kit (Gentra Systems, Inc., Mineapolis, MN) according to the instructions of the manufacturer. DNA samples were available from 610 of 688 (89%) cases and from 651 of 724 (90%) controls (23).

Allelic Discrimination by TaqMan Analysis. For genotyping, we applied TaqMan allelic discrimination (Perkin-Elmer ABI Prism 7700 Sequence Detection System; Applied Biosystems, Foster City, CA) and Hemochromatosis Strip Assay analyses (ViennaLab, Vienna, Austria). All DNA samples were genotyped at *HFE*_187_C > G (His63Asp, rs1799945), *HFE*_193_A > T (Ser65Cys, rs1800730), *HFE*_845_G > A (Cys282Tyr, rs1800562) and *TFR1*_424_A > G (Ser142Gly, rs3817672) using the TaqMan methodology. PCRs were carried out in reaction volumes of 25 μL containing 50 ng DNA, 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 280 nmol/L of each primer, 6-carboxyfluorescein–labeled minor groove binder probe, and VIC-labeled minor groove binder probe (200 nmol/L for *HFE*_193_A > T and *TFR1*_424_A > G, 400 nmol/L for *HFE*_187_C > G and *HFE*_845_G > A). Primers were synthesized by MWG-Biotech AG (Ebersberg, Germany) and minor groove binder probes were from Applied Biosystems. Primer sequences and minor groove binder probes are given in Table 1. PCR conditions included an initial incubation at 50°C for 2 minutes, 95°C for 10 minutes followed by 35 cycles of 15 seconds at 92°C and 1 minute at 60°C. Genotype analysis was done using the ABI Prism 7700 SDS software (Applied Biosystems).

For controls, homozygote DNA samples were included that had been verified before genotyping by sequence analysis using Big-Dye Terminator mix and ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Allele Assignment by Hemochromatosis Strip Assay. DNA samples of 321 cases were genotyped at 18 polymorphic sites at *HFE* (Val53Met, Val59Met, His63Asp, His63His, Ser65Cys, Gln127His, Pro160delC, Glu168Gln, Glu168Stop, Trp169Stop, Cys282Tyr, and Gln283Pro), *TFR2* (Glu60Stop, Met172Lys, Tyr250Stop, and AlaValAlaGln594-597del), and *FPN1* (Asn144His and Val162del) using the Hemochromatosis Strip Assay (ViennaLab). *HFE*, *TFR2*, and *FPN1* polymorphic sequences were amplified simultaneously in multiplex PCR using biotin-labeled oligonucleotides. Genotyping was done by reverse hybridization with immobilized corresponding oligonucleotides using streptavidin-alkaline phosphatase and color substrates according to a published protocol (12).

Statistical Analysis. Statistical analyses of differences between breast cancer cases and controls with respect to epidemiologic characteristics were done using Fishers' exact test. Genotype frequencies were tested for deviations from Hardy-Weinberg equilibrium by Pearson χ^2 square test. Data were considered in Hardy-Weinberg equilibrium when P values were >0.05. Epidemiologic risk factors, genotype frequencies, and adjusted odds ratios were calculated using

Table 1. Primers and probes of allelic discrimination analyses at polymorphic loci of *HFE* and *TFR1*

Single nucleotide polymorphism	Primer	Fluorescence-labeled MGB probe
*HFE*_187_C > G	F 5′-GAAGCTTTGGGCTACGTGGAT-3′	VIC-TCGTGTTCTATGATGATGA-MGB
	R 5′-TCTACTGGAAACCCATGGAGTTC-3′	6-FAM-CGTGTTCTATGATCATGA-MGB
*HFE*_193_A > T	F 5′-GAAGCTTTGGGCTACGTGGAT-3′	VIC-TGAGTGTCGCCGTGTG-MGB
	R 5′-TCTACTGGAAACCCATGGAGTTC-3′	6-FAM-ATGAGAGTCGCCGTGTG-MGB
*HFE*_845_G > A	F 5′-GGCTGGATAACCTTGGCTGTAC-3′	VIC-TATACGTACCAGGTGGAG-MGB
	R 5′-TCACATACCCCAGATCACAATGA-3′	6-FAM-ATATACGTGCCAGGTGG-MGB
*TFR1*_424_A > G	F 5′-CCTGCAGCACGTCGCTTATAT-3′	VIC-CTTCACCGGCACCAT-MGB
	R 5′-AGATACTTGCACAGCAGCTGG-3′	6-FAM-AGACTTCACCAGCACC-MGB

NOTE: F, forward; R, reverse; 6-FAM, 6-carboxyfluorescein; MGB, minor groove binder.

SAS/STAT software version 8.02. Risk estimates for the development of breast cancer were calculated as odds ratios with 95% confidence interval using logistic regression analysis, conditional on age (<45, 45-50, 50-55, 55-60, 60-65, 65<75, ≥70 years). Additionally, we adjusted the risks for potential breast cancer risk factors such as smoking (never, ever), history of cancer in mother or sisters, and hormone replacement treatment in 10-year groups (0, <10, ≥10 years) referred to as adjusted odds ratios. Statistical analyses of differences in genotype frequencies between cases and controls or between studies were done by χ^2 or Fishers' exact test where applicable. Fishers' exact test for count data was used for the classification of *HFE* and *TFR1* genotypes by nodal status of breast cancer cases.

Results

Characteristics of GENICA Study Subjects. Baseline characteristics of the GENICA controls and breast cancer cases are given in Table 2. These include age, menopausal status, menstrual cycle, potential breast cancer risk factors, iron storage disorder–related conditions, personal and family history of cancer, as well as histopathologic variables. There were no significant differences between patients and controls with the exception of known risk factors such as breast cancer in mother and sisters ($P = 0.01$) as well as hormone replacement treatment use ($P = 0.047$).

Allelic Prevalence and Genotype Frequencies in the GENICA Case-Control Population and Comparisons with Published Breast Cancer Cohorts. Study participants were genotyped for 19 polymorphic loci at *HFE* (Val53Met, Val59Met, His63Asp, His63His, Ser65Cys, Gln127His, Pro160-delC, Glu168Gln, Glu168Stop, Trp169Stop, Cys282Tyr, and Gln283Pro), *TFR1* (Ser142Gly), *TFR2* (Glu60Stop, Met172Lys, Tyr250Stop, and AlaValAlaGln594-597del), and *FPN1* (Asn144His and Val162del). Overall success rate with respect to genotype calling was 97.3%. Frequent polymorphisms *HFE*_187_C > G (His63Asp), *HFE*_193_A > T (Ser65Cys), and *HFE*_845_G > A (Cys282Tyr) were analyzed in duplicate by TaqMan and Strip Assay analyses for 321 DNA patient samples. Both assays showed concordance of results in 100% of samples. Because *TFR1*_424_A > G (Ser142Gly) was not included in the Strip Assay, we obtained genotypes by TaqMan analysis.

HFE_187_C > G (His63Asp). In GENICA population controls, the frequency of the *C* allele was 84.1% and the frequency of the *G* allele was 15.9%. Observed genotype frequencies were in Hardy-Weinberg equilibrium with 70.7% *CC*, 26.8% *CG*, and 2.5% *GG*. In cases, allele and genotype frequencies were comparable with those of controls (Table 3).

HFE_193_A > T (Ser65Cys). In GENICA population controls, the frequency of the *A* allele was 98.1% and the frequency of the *T* allele was 1.9%. Observed genotype frequencies were in Hardy-Weinberg equilibrium with 96.3% *AA*, 3.7% *AT*, and 0% *TT*. In cases, allele and genotype frequencies were comparable with those of controls (Table 3).

HFE_845_G > A (Cys282Tyr). In GENICA population controls, the frequency of the *G* allele was 94.4% and the frequency of the *A* allele was 5.6%. Observed genotype frequencies were in Hardy-Weinberg equilibrium with 88.9% *GG*, 10.9% *GA*, and 0.2% *AA*. Allele and genotype frequencies were comparable between cases and controls (Table 3). When we compared both genotype and allele frequencies of the GENICA breast cancer patients with those published by others (Table 4), our data were not significantly different ($P = 0.15$) from a Swedish breast cancer cohort but differed significantly ($P < 0.0001$) from an American breast cancer cohort. The latter showed an increased frequency of the minor *A* allele, which was responsible for an overall percentage of 24.4% for the heterozygote *GA* and 12.2% for the homozygote *AA* genotypes.

TFR1_424_A > G (Ser142Gly). In GENICA population controls, the frequency of the *A* allele was 53.9% and the frequency of the *G* allele was 46.1%. Observed genotype frequencies were in Hardy-Weinberg equilibrium with 29.7% *AA*, 48.5% *AG*, and 21.8% *GG*. Allele and genotype frequencies as well as Hardy-Weinberg equilibrium in the group of breast

Table 2. Characteristics of the GENICA study population

Characteristic	Cases (n = 605) n (%)	Controls (n = 651) n (%)	P*
Age (y)			
≤39	36 (6.0)	38 (5.8)	
40-49	103 (17.0)	112 (17.2)	
50-59	174 (28.8)	187 (28.7)	
60-69	185 (30.6)	194 (29.8)	
≥70	107 (17.7)	120 (18.4)	
Menopausal status			
Premenopause	143 (23.9)	149 (23.1)	
Natural postmenopause	255 (42.6)	288 (44.7)	
Surgical postmenopause	38 (6.4)	40 (6.2)	
Other postmenopause	162 (27.1)	167 (25.9)	0.91
Menstrual cycle			
Regularly, short cycle	26 (4.3)	18 (2.8)	
Regularly, normal cycle	446 (73.8)	486 (74.9)	
Regularly, long cycle	15 (2.5)	28 (4.3)	
Not regularly	117 (19.4)	117 (18.0)	0.14
Years of HRT† use			
None	291 (48.7)	328 (50.8)	
<10	152 (25.5)	188 (29.1)	
>10	154 (25.8)	130 (20.1)	0.047
History of smoking			
Ever	348 (57.6)	362 (55.6)	
Never	256 (42.4)	289 (44.4)	0.49
Chronic anemia			
None or over short period	517 (86.9)	550 (85.0)	
Without medical treatment	7 (1.2)	11 (1.7)	
With medical treatment	71 (11.9)	86 (13.3)	0.59
Diabetes			
Not diabetic	573 (95.5)	608 (94.0)	
Diabetic, type I	0 (0)	4 (0.6)	
Diabetic, type II	27 (4.5)	35 (5.4)	0.15
History of cancer			
Personal	41 (6.8)	63 (9.7)	0.07
Family‡	338 (55.9)	334 (51.3)	0.11
Breast cancer in Mother and sisters	71 (11.7)	49 (7.5)	0.01
Histology			
Ductal	375 (67.0)		
Lobular	82 (14.6)		
Others	103 (18.4)		
Tumor size			
T_1	361 (60.8)		
T_2	169 (28.5)		
T_3	16 (2.7)		
T_4	28 (4.7)		
Carcinoma *in situ*	20 (3.4)		
Nodal status			
N_0	325 (62.7)		
N_1	177 (34.2)		
N_2	14 (2.7)		
N_3	2 (0.4)		
Grading			
G1	60 (10.6)		
G2	349 (61.7)		
G3	152 (26.9)		
Carcinoma *in situ*	5 (0.8)		

*Fisher's exact test; there were no significant differences between patients and controls with the exception of breast cancer in mother and sisters as well as HRT use.
†HRT, hormone replacement therapy.
‡Cancer in parents, siblings, or children.

Table 3. Association analyses of hemochromatosis related *HFE* and *TFR1* genotypes in GENICA breast cases and controls

Factor	Cases, *n* (%)	Controls, *n* (%)	OR_{adj}* (95% CI)
*HFE*_187_C > G			
CC	421 (73.7)	457 (70.7)	1
CG	138 (24.2)	173 (26.8)	0.87 (0.67-1.13)
GG	12 (2.1)	16 (2.5)	0.79 (0.37-1.71)
*HFE*_193_A > T			
AA	561 (95.3)	617 (96.3)	1
AT	28 (4.8)	24 (3.7)	1.26 (0.72-2.21)
TT	0 (0)	0 (0)	—
*HFE*_845_G > A			
GG	505 (89.2)	577 (88.9)	1
GA	59 (10.4)	71 (10.9)	0.92 (0.65-1.37)
AA	2 (0.4)	1 (0.2)	—
*TFR1*_424_A > G			
AA	178 (30.1)	192 (29.7)	1
AG	289 (48.9)	314 (48.5)	0.99 (0.76-1.29)
GG	124 (21.0)	141 (21.8)	0.95 (0.69-1.30)

NOTE: OR_{adj}, adjusted odds ratios; 95% CI, 95% confidence interval.
*Logistic regression conditional on 5-year age groups, adjustment for family history of breast cancer in mother or sisters, HRT use, and smoking.

Table 5. Genotypes in GENICA breast cancer cases at sites of 15 rare mutations reported in hemochromatosis

Sequence variation	Homozygous genotypes identified, *n* = 321
*HFE*_157_G > A (V53M)	*GG*
*HFE*_175_G > A (V59M)	*GG*
*HFE*_189_T > C (H63H)	*TT*
*HFE*_381_A > C (Q127H)	*AA*
*HFE*_480_478-480_delC (P160delC)	*CC*
*HFE*_502_G > C (E168Q)	*GG*
*HFE*_502_G > T (E168X)	*GG*
*HFE*_506_G > A (W169X)	*GG*
*HFE*_848_A > C (Q283P)	*AA*
*TFR2*_84-88_insC (E60X)	No insertion
*TFR2*_515_T > A (M172K)	*TT*
*TFR2*_750_C > G (Y250X)	*CC*
*TFR2*_1780-1791del (AVAQ594-597del)	No deletion
*FPN1*_734_A > C (N144H)	*AA*
*FPN1*_779-790_delTTG (V162del)	No deletion

cancer patients were comparable with controls (Table 3). When we compared both allele and genotype frequencies of the GENICA breast cancer patients with those published by others (Table 4), our data were not significantly different ($P = 0.22$) from a Swedish breast cancer cohort.

Rare Variants at HFE, TFR2, and FPN1. Among GENICA breast cancer patients none of the known rare variants at *HFE*, *TFR2*, and *FPN1* were identified (Table 5) in patients tested. All 321 patients were carriers of frequent homozygous genotypes at all loci.

Combinations of Frequent *HFE* and *TFR1* Genotypes in GENICA Population Controls and Breast Cancer Patients. Because no significant differences in allele and genotype frequencies were identified between controls and breast cancer patients of the GENICA study population, we extended the search for a putative genetic breast cancer susceptibility to the compound *HFE-TFR1* genotypes. The combination of *HFE*_845_G > A (Cys282Tyr) and *TFR1*_424_A > G (Ser142Gly) genotypes did not show significant differences between patients and controls (not shown). In a next step, we established compound genotypes of two *HFE* loci *HFE*_187_C > G (His63Asp) and *HFE*_845_G > A (Cys282Tyr) together with the corresponding genotype at *TFR1*_424_A > G (Ser142Gly) locus. We observed a minor increase of 2.9% with respect to the frequency of the compound genotype *HFE*_187_CC/*HFE*_845_GG/*TFR1*_424_AG in cases ($n = 181$, 32.9%) when compared with controls ($n = 191$, 30.0%; not shown). This difference was not significant but remained stable (2.8%) when we further extended this compound genotype by a third *HFE* polymorphism, *HFE*_193_A > T (Ser65Cys). Accordingly, the compound quadruple genotype *HFE*_187_CC/*HFE*_193_AA/*HFE*_845_GG/*TFR1*_424_AG was identified in 172 (31.3%) patients and 181 (28.5%) controls (Table 6). All other differences in compound genotype frequencies were lesser and ranged between 0% and 1.6% (Table 6).

Stratification of *HFE* and *TFR1* Genotypes according to Factors Potentially Related to Breast Cancer or Hemochromatosis. *HFE*_187_C > G (His63Asp), *HFE*_193_A > T (Ser65Cys), *HFE*_845_G > A (Cys282Tyr), and *TFR1*_424_A > G (Ser142Gly) genotypes were stratified by menopausal status, chronic anemia, and diabetes. No significant increases of breast cancer risks were observed (not shown).

Classification of *HFE* and *TFR1* Genotypes by Histopathologic Variables in Breast Cancer Patients. For patients, genotypes at *HFE*_187_C > G (His63Asp), *HFE*_193_A > T (Ser65Cys), *HFE*_845_G > A (Cys282Tyr), and *TFR1*_424_A > G (Ser142Gly) were classified by tumor type, size, and grade as well as nodal status. With the exception of nodal status, histopathologic variables did not influence the homogeneity of distribution of genotypes among patient groups. However, when we tested for the distribution of genotypes among patients with different nodal status ($N_0 + N_1$ versus $N_2 + N_3$)

Table 4. Genotype and allele frequencies at frequent *HFE* and *TFR1* polymorphic sites in GENICA breast cancer patients compared with other published data

Genotype	GENICA study, North Rhine-Westphalia Germany, *n* (%)	Beckman et al., 1999; North Sweden, *n* (%)	Kallianpur et al., 2004; Tennessee, United States, *n* (%)
*HFE*_845_G > A* (C282Y)	*n* = 566	*n* = 165	*n* = 41
GG	505 (89.2)	139 (84.2)	26 (63.4)
GA	59 (10.4)	25 (15.2)	10 (24.4)
AA	2 (0.4)	1 (0.6)	5 (12.2)
A allele	63 (5.6)	27 (8.2)	20 (24.4)
95% CI	4.3-7.1	5.5-11.7	15.6-35.1
*TFR1*_424_A > G (S142G)	*n* = 592	*n* = 165	NI
AA	177 (29.9)	61 (37.0)	
AG	290 (49.0)	71 (43.0)	
GG	125 (21.1)	33 (20.0)	
G allele	540 (45.6)	137 (41.5)	
95% CI	42.6-48.3	41.5-47.0	

NOTE: NI, not investigated.
*Genotype frequencies of GENICA cases differ significantly from those reported by Kallianpur et al., 2004 ($P < 0.0001$).

Table 6. Frequencies of *HFE* and *TFR1* genotype combinations in GENICA breast cancer cases (*n* = 550) and controls (*n* = 636)

*HFE*_187_C > G (H63D)	*HFE*_193_A > T (S65C)	*HFE*_845_G > A (C282Y)	*TFR1*_424_AA (S142S)		*TFR1*_424_AG (S142G)		*TFR1*_424_GG (G142G)	
			Cases, *n* (%)	Controls, *n* (%)	Cases, *n* (%)	Controls, *n* (%)	Cases, *n* (%)	Controls, *n* (%)
CC	AA	GG	93 (16.9)	104 (16.4)	172 (31.3)	181 (28.5)	69 (12.6)	89 (14.0)
CC	AA	GA	12 (2.2)	21 (3.3)	18 (3.3)	26 (4.1)	16 (2.9)	8 (1.3)
CC	AA	AA	—	—	1 (0.2)	1 (0.2)	—	—
CC	AT	GG	7 (1.3)	4 (0.6)	9 (1.6)	10 (1.6)	5 (0.9)	5 (0.8)
CC	AT	GA	1 (0.2)	—	1 (0.2)	1 (0.2)	—	—
CG	AA	GG	41 (7.5)	48 (7.6)	56 (10.2)	75 (11.8)	23 (4.2)	30 (4.7)
CG	AA	GA	2 (0.4)	6 (0.9)	5 (0.9)	4 (0.6)	3 (0.6)	3 (0.5)
CG	AT	GG	1 (0.2)	—	3 (0.6)	3 (0.5)	—	1 (0.2)
GG	AA	GG	3 (0.6)	6 (0.9)	7 (1.3)	7 (1.1)	2 (0.4)	3 (0.5)
Total			160	189	272	308	118	139

there was a significant association of the *HFE*_845_G > A (Cys282Tyr) polymorphism with high nodal status ($P = 0.032$, data not shown). Similarly, when we compared the frequencies of the combined heterozygous (*HFE*_845_GA) and homozygous (*HFE*_845_AA) variant genotypes with the more prevalent *HFE*_845_GG genotype, we observed an increased risk for breast cancer of high nodal status with an odds ratio of 2.6 (95% confidence interval, 0.6-9.0). This difference was not observed with the other genotypes.

Discussion

One of the cellular features of breast cancer is iron overload, a reason why the hemochromatosis and transferrin receptor complex attracted interest as potential contributors to breast cancer susceptibility. If dysregulation of iron uptake would be involved in a breast cancer risk, carriers of variations within the hemochromatosis and transferrin receptor systems should be more susceptible to breast cancer than noncarriers. Such a risk should be particularly obvious in populations of Northern European descent with a reputedly high prevalence of *HFE* mutations. Our contribution to this issue is the investigation of the prevalence of 19 known *HFE*, *TFR1*, *TFR2*, and *FPN1* variations in a female German population and their possible effects on the incidence of breast cancer. Whereas investigations of the frequent *HFE* Cys282Tyr and *TFR1* Ser142Gly polymorphisms in breast cancer populations have been previously reported, nothing is known on the putative health consequences of other known hemochromatosis and transferrin receptor pathway–related mutations. This is the first report of a comprehensive analysis of these genes and their possible role in breast cancer susceptibility.

Our population from Germany showed *HFE* and *TFR1* variants at four loci including the three most frequent *HFE* polymorphisms, Cys282Tyr, His63Asp, and Ser65Cys, as well as the *TFR1* Ser142Gly polymorphism. No other known variants at *HFE*, *TFR1*, *TFR2*, and *FPN1* were identified. Frequencies of the minor alleles at *HFE* and *TFR1* loci were similar in controls and cases. Particularly, the frequency of the major *HFE* Tyr282 allele was 5.6% in both controls and cases. This frequency obtained by us from a population in North Rhine-Westphalia (GENICA study population) matches that previously reported for a Bavarian population (24), but exceeds frequencies reported in other German studies ranging from 1.9% to 4.8% (13). Owing to sufficient size and population-based design as well as unlikely gender-specific contributions, we expect the GENICA study population to provide reliable data with respect to the prevalence of population-specific *HFE* and *TFR* variations. Our findings are in agreement with a Viking origin (25) of the *HFE* Cys282Tyr polymorphism and are also reminiscent to observed frequencies in Celtics (6.9%), Nordics (6.5%), and Anglo Saxons (6.0%), but differ with respect to observed frequencies in Southern Europeans (2.5%) and Russians (1.8%; ref. 26). As expected, frequencies of the *HFE* Asp63 allele were higher and those of the *HFE* Cys65 allele were lower. Case-control comparisons with respect to individual *HFE* loci and compound *HFE* genotypes did not indicate an association with breast cancer. To further explore possible relationships, we included the *TFR1* Ser142Gly polymorphism in our analysis, which was previously suggested to cooperate with the *HFE* Tyr282 variant to increase breast cancer susceptibility (21). Although the prevalence of the *TFR1* Gly142 variant was high, there was no difference between breast cancer cases and controls with respect to allele and genotype distributions.

We compared our results with those of the two other studies that previously investigated the possible role of *HFE* and *TFR1* variants in breast cancer risk. A study from North Sweden did not find an obvious association between the *HFE* Tyr282 allele and breast cancer risk (21). However, when carriers of at least one *HFE* 282Tyr allele were also carriers of Ser142 at *TFR1*, the risk to develop breast cancer was more than 2-fold increased. This risk was even higher in compound *HFE* Cys282Tyr and His63Asp heterozygotes, but not significant. When we tested for similar relationships in our larger population-based breast cancer study, we observed a nonsignificant increase of compound heterozygotes in breast cancer patients for the triple compound *HFE*_His63_Cys282-*TFR1*_Ser142Gly and the quadruple compound *HFE*_His63_Ser65_Cys282-*TFR1*_Ser142Gly genotypes. Reasons for the differences in observations may be attributed to the large study size and population-based design of our study as well as possible variations of genotype frequencies between the Swedish and German populations.

A recent report of an American breast cancer study from Tennessee showed a high increase in the prevalence of the *HFE* Tyr282 allele in a highly selected series of breast cancer patients when compared with female populations from Tennessee or the United States (20). Although this study included a total of 182 breast cancer patients, this observation was solely attributed to a subgroup of 41 recipients of escalated-dose chemotherapy and blood cell transplantation. Accordingly, patients were selected on the basis of accepted indications for autologous blood cell transfusion reflecting breast cancers of poor prognosis. Moreover, the mean age at diagnosis of patients was below 50 years, indicating a large proportion of early-onset types of breast cancer in that group of patients. The strong association observed in this study may be influenced by the preselection and small numbers of breast cancer cases, issues that are part of an ongoing debate in

molecular epidemiologic studies (27). Yet, there is a chance that socioeconomic effects and differences in ethnicity between the North American and European populations might have influenced the outcome of that study. In contrast, the incidental breast cancer cases of the GENICA study were not preselected and therefore represent a normal distribution of breast cancer patients. Kallianpur et al. (20) suggested that their transplant cohort may have facilitated the detection of an association of the *HFE* Tyr282 allele with aggressiveness of the disease. Interestingly, we also observed a 2.6-fold increased prevalence of at least one *HFE* Tyr282 allele in patients with four or more affected lymph nodes. A supportive biological rationale for this observation may be inferred from the known increased iron uptake associated with the *HFE* Tyr282 allele (15) and a high iron content in breast cancer cells (4, 5). However, this conclusion is limited by the low numbers of patients with high nodal status in our study as shown by the wide confidence interval.

Based on our data obtained from a population-based study with high response rates for cases and controls as well as accurate genotyping by diagnostic and state-of-the-art procedures, we consider it rather unlikely that variants of the hemochromatosis-transferrin receptor system have an immediate effect on the incidence of breast cancer in Germany. The high breast cancer incidence in this population may therefore not be attributable to these genetic variants. Beyond that, putative effects on breast cancer progression and prognosis may be possible but remain elusive.

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