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Reduced Fhit Expression in Sporadic and *BRCA2*-linked Breast Carcinomas[1]

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ABSTRACT

Evidence for alteration of the *FHIT* gene in a significant fraction of breast carcinomas has been reported, in apparent concordance with loss of heterozygosity (LOH) at chromosome region 3p14.2 in breast cancer and benign proliferative breast disease. A significantly higher frequency of LOH at the *FHIT* locus was reported for *BRCA2*$^{-/-}$ tumors, possibly due to misrepaired double-strand breaks at this common fragile region. To determine whether such genomic alterations lead to Fhit inactivation, we have assessed the level of Fhit expression by immunohistochemical detection in sporadic tumors and cancers occurring in *BRCA2* 999del5 carriers. To determine whether Fhit inactivation may have prognostic significance, we have also assessed expression of breast cancer markers and clinical features in sporadic tumors relative to Fhit expression. Of 40 consecutive sporadic breast carcinomas studied for tumor markers, 50% showed reduced Fhit expression. In these sporadic cancers, loss of Fhit expression was not correlated significantly with the presence or absence of other tumor markers. In a study of 58 sporadic and 34 *BRCA2* 999del5 Icelandic invasive cancers, there was a significant association of LOH at 3p14.2 with reduced expression of Fhit ($P = 0.001$); also the lower expression of Fhit and higher LOH at 3p14.2 in *BRCA2* 999del5 tumors relative to sporadic cancers was significant ($P = 0.002$). Thus, genetic alteration at the fragile site within the *FHIT* gene leads to loss of Fhit protein in a significant fraction of sporadic breast cancers and a much larger fraction of familial breast cancers with an inherited *BRCA2* mutation, consistent with the idea that loss of *BRCA2* function affects stability of the *FHIT/FRA3B* locus.

INTRODUCTION

A complex set of genetic abnormalities has been detected in breast tumors, including amplification of oncogenes, mutation of the tumor suppressor gene *TP53* and LOH[3] from chromosomes 1, 3p, 6q, 7q, 8p, 11, 13q, 16q, 17, 18q, and 22q (reviewed in Ref. 1). The regions of LOH may target tumor suppressor genes. Furthermore, germ-line mutations of the *BRCA1* and *BRCA2* genes have been implicated in inherited breast cancer (2, 3). Somatic loss of the wild-type chromosome in tumors of *BRCA1* and *BRCA2* mutation carriers suggests that both alleles of the corresponding gene are inactivated in cancer, a pattern expected of a tumor suppressor gene (4). A germ-line mutation in the *BRCA2* gene, termed 999del5, has been detected in Icelandic breast cancer families, and a common haplotype in the vicinity of the gene in these families suggests a founder effect (5–7). The *BRCA2* 999del5 germ-line mutation has been found in 8% of patients diagnosed with breast cancer in Iceland (8, 9). A higher frequency of aberrations at several chromosome arms in tumors of *BRCA2* germline mutation carriers compared with sporadic tumors has been reported (10–12). These results suggested a specific or more aggressive tumor progression pathway in breast cancers due to a *BRCA2* mutation.

In sporadic breast cancer, interstitial deletions of the short arm of chromosome 3, with involvement of several 3p subbands, have commonly been observed by studies of cytogenetics and loss of heterozygosity (reviewed in Ref. 1). Band 3p14 has been observed to be the minimal common deleted segment (13–15), and similar deletions have been observed in benign proliferative breast disease (16, 17), suggesting that 3p14 deletions could be an early event in a significant fraction of mammary carcinomas. After description of the *FHIT* gene, encompassing the common chromosomal fragile region (*FRA3B*) at band 3p14.2 (18), Panagopoulos *et al.* (19) showed that the *FHIT* gene was homozygously deleted in two cases of benign proliferative breast disease associated with 3p14 cytogenetic rearrangements and familial breast cancer. Additionally, homozygous deletions within the *FHIT* gene have been observed in some breast cancer-derived cell lines, and ~20% of primary breast carcinomas were reported to exhibit altered Fhit transcripts by reverse transcription-PCR analysis (20, 21); altered reverse transcription-PCR products are frequently due to internal deletions within FHIT, which appears to be inactivated by deletions rather than point mutations (reviewed in Ref. 22).

In familial breast cancer kindreds, early studies by Bergthorsson *et al.* (23) showed a high frequency of allelic imbalance at 3p14 relative to the frequency of allelic imbalance in the same region in sporadic breast cancers. In a follow-up study, Bergthorsson *et al.* (10) mapped the 3p regions of imbalance in known *BRCA2* carrier tumors relative to sporadic tumors. The degree of allelic imbalance for some 3p markers was significantly higher than in tumors from noncarriers; in both groups, elevated 3p14 imbalance was noted in patients with bilateral disease, and allelic imbalance was observed most frequently near marker *D3S1217* (centromeric to *FHIT*) and in the *FHIT* gene. The authors concluded that genomic aberrations in 3p14 are especially frequent in tumors with *BRCA2* gene defects and suggested that they were caused by regional loss of chromosome stability rather than selection; that is, loss of *BRCA2* function may affect 3p14 stability in the tumors.

Because the *BRCA2* gene is involved in DNA repair (24–26) and because the *FHIT* gene encompasses the common fragile region *FRA3B*, it seems likely that the frequent alterations at 3p14.2 in tumors of *BRCA2* carriers could be initiated by faulty repair of fragile region double-strand breaks. This does not preclude the possibility that such alterations, which would frequently inactivate the *FHIT* gene, could impart a growth advantage to the affected cell. The very fact that these genetic alterations can be detected strongly suggests clonal (mono or oligoclonal) expansion of cells with *FHIT* locus alterations. To determine the effect of such 3p14.2 alterations on expression of the *FHIT* gene, we have studied expression of the Fhit protein by immunohistochemical detection in sporadic breast cancers and compared it with Fhit expression in cancers of *BRCA2* mutation carriers.

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[3] The abbreviations used are: LOH, loss of heterozygosity; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor; HNPCC, hereditary nonpolyposis colorectal cancer.

MATERIALS AND METHODS

Tissues. The 50 sporadic breast cancers studied for tumor marker expression were paraffin-embedded normal and malignant tissues obtained for analysis from the Department of Pathology, Anatomy and Cell Biology at Thomas Jefferson University of Philadelphia; 10 cases were DCIS and were excluded from statistical analyses. The remaining 40 cases were invasive carcinomas. Clinical features of the invasive cases are listed in Table 1.

The Icelandic sporadic and familial (*BRCA2* 999del5) breast cancer tissues have been described previously (8). Twenty-nine sporadic tumors (average age at diagnosis, 47 years; range, 29–79) and 29 familial tumors (average age at diagnosis, 51 years; range, 33–78) were studied for 3p14.2 LOH within the *FHIT* gene. Fifty-eight sporadic and 34 familial cases were analyzed for expression of Fhit.

Immunohistochemistry. Fhit expression was analyzed by standard staining protocols, as described previously (27, 28). Briefly, deparaffinization from xylene to 95% alcohol and rehydration prior to microwave antigen recovery were carried out on a Leica Autostainer (Leica, Inc., Deerfield, IL). The deparaffinization process included a 30-min methanolic peroxide block for endogenous peroxidase activity. The antigen recovery step was carried out in an 800-W microwave oven in 200 ml of citrate buffer (0.01 M, pH 5.5–5.7). The immunostaining was performed on a Techmate 1000 (Ventana Medical Systems, Tucson, AZ) using capillary gap technology. Tissue sections were exposed for 10 min to normal horse serum (1:50), followed by overnight primary antibody incubation (1:2000). The primary antibody was a polyclonal rabbit immunoglobulin raised against gstFhit protein (29). The next day, slides were washed, incubated for 30 min with a biotinylated goat anti-rabbit IgG secondary antibody (1:200), and exposed to avidin-biotin complex for 45 min. After a wash, slides were subjected to three consecutive 8-min applications of DAB/peroxide solution. Treated slides were counterstained with hematoxylin and coverslipped prior to evaluation.

Immunohistochemical detection of p53, ER, PR, proliferation antigen Ki67, and ErbB2 receptor expression was by the same method using the following antisera and dilutions: ER, mouse monoclonal antibody ID5 from Dako Corp. diluted 1:80; PR, monoclonal antibody IA6 from Novocastra, 1:80; Ki67, antibody clone MIB1 from Coulter-Immunotech at 1:100; p53, clone 1801 from Novocastra, 1:80; and ErbB2, clone CB11 from Signet Laboratories, 1:100.

Expression of Fhit in sporadic and familial breast cancers from Iceland was studied using a tissue microarray method (30). This technique has the advantage of minimal tissue requirements and is therefore especially useful for studying rare and valuable tissue specimens, such as tumors from patients carrying mutations in cancer susceptibility genes. Briefly, small tissue cores (diameter, 0.6 mm; length, 2–3 mm) were retrieved from morphologically representative sites in the tumors and subsequently arrayed to a blank paraffin block at a high density using a custom-built instrument. Sections from this block were cut with an adhesive-coated tape sectioning system (Instrumedics, Inc., Hackensack, NJ) and stained with the Fhit antibody. Sections were deparaffinized, rehydrated, and rinsed in tap water before antigen retrieval by heating in a 0.01 M citrate buffer (pH 6.0) twice for 5 min at 850 W. Sections were incubated with the gstFhit antibody overnight at room temperature. Immunohistochemical staining was visualized using the StreptABComplex/HRP Duet (Mouse/rabbit from DAKO) according to the manufacturer's instructions. Fhit expression in normal breast epithelial cells and stromal cells, respectively, served as positive and negative controls. Tumors were graded by

Table 1 *Summary of clinical features and expression of markers in sporadic breast carcinomas*

Case	Diagnosis[a]	Nuclear grade	% + ER[b]	% + PR[c]	% + Ki67	% + p53	Erb B2[d]	Fhit[e]
18914	IDC	III	0	<5	10–20	80	+	+++
18996	IDC	I	90	80	5	5	+	+++
18852	IDC	III	0	<5	25–80	0	−	+++
18832	MET	III	0	<5	50–90	0	+	+++
18752	IDC	II	95	0	2	0	+	+++
17932	IDC	I–II	95	<5	1–2	0	−	+++
17875	IDC	II	95	70–75	5–10	0	−	+++
3472	IDC	II	70	95	25	0	−	+++
2696	IDC	II	90	75	2	0	+	+++
1956	IDC	II	100	95	2	1–2	++	+++
1928	IDC	II	95	50	2–5	1	++	+++
1916	LC	I	80	10–20	1–2	0	+	+++
878	IDC	I	100	5	2	0	+	+++
574	LC	I	95	40	2–5	0	+	+++
573	IDC	II	95	75	15–20	0	+	+++
18811	IDC	II	95	85	15	0	+	++
3786	IDC	II	95	85	5	0	−	++
1742	IDC	II	95	0	5–10	0	++	++
1704	IDC	I–II	95	75	1–2	0	++	++
985	IDC	II	90	60	2	0	−	++
17905	IDC	II	0	0	80	<2	+	+++/−
18325	IDC	II	>95	90	2–5	0	+	++/−
19147	MET	II	<2	0	70	95	+++	++/−
18182	IDC	III	0	0	50	95	−	++/−
18160	IDC	II	90	20	15–40	0	−	++/−
18148	IDC	II	90	75–90	10–40	0	+/−	++/−
18072	IDC	II–III	0	0	80	30	+	++/−
3573	IDC	II	90	25	1–2	0	+	++/−
3478	MET	III	0	5	30	0	+++	++/−
2640	LC	I	90	30	2–5	0	+	++/−
1047	IDC	II	85	35	2	4	++	++/−
968	IDC	II	95	50	20–30	0	−	++/−
821	IDC	II	90	70	2	30	+	++/−
578	IDC	II	95	90	5–10	0	+	++/−
201	IDC	II–III	75	10	10	0	+	++/−
3105	IDC	I	95	2	2–5	0	+	+/−
1040	IDC	II	0	0	70–80	0	−	−/++
3708	IDC	II	5	5	30	0	+++	−/++
19065	IDC	III	60	20	25	0	−	−/+
715	IDC	I	95	75	1	0	+	−/+

[a] IDC, invasive ductal carcinoma; LC, lobular carcinoma; MET, metastasis.
[b] ER, estrogen receptor; PR, progesterone receptor.
[c] For PR, Ki67, and p53, if percent positive was a range, the higher number was used in statistical calculations.
[d] +++, strong positive, ++, moderate positive; +, weak positive.
[e] +++/−, ++/−, +/−, more than 50% positive; −/+, −/++, more than 50% negative.

intensity of staining as negative (−), weakly positive (+), moderately positive (++), and strongly positive (+++). The standard tissue sections of the 50 sporadic Philadelphia cases frequently showed heterogeneity for Fhit expression and were thus scored as heterogeneous (++/−, +/−, −/++, −/+, etc.; if the plus sign occurs first, >50% of the cancer cells were positive for Fhit expression; if the minus sign occurs first, the majority of cancer cells were Fhit negative). Examples of the scoring system were exchanged between laboratories to assure consistency in scoring for the study.

Microsatellite Marker Analysis. The PCR primers used for the microsatellite marker analysis were localized at 3p14.2 within the *FHIT* gene: *D3S4260* (intron 3), *D3S1461* (intron 4), *D3S2757* (intron 4), *D3S1300* (intron 5), and *D3S1234* (intron 5). DNA samples (25 ng) were subjected to PCR analysis in a total volume of 25 μl. DynaZyme™ polymerase (Finnzymes Oy, Espoo, Finland) was used in the buffer solution provided by the manufacturer with 100 mM of each deoxynucleotide triphosphate and 0.25 mM of primers. After 5 min denaturation at 94°C, samples were subjected to 35 cycles of amplification, consisting of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by a final extension for 10 min at 72°C. The PCR products were denatured in formamide buffer, separated on 6.5% polyacrylamide sequencing gels, and transferred to a Hybond-N+ nylon membrane (Amersham, Aylesbury, United Kingdom). Hybridization and visualization of the PCR products were based on a peroxidase-labeled probe (ECL kit; Amersham). LOH was evaluated visually by comparing the intensity of alleles from normal and tumor DNA. Absence or significant decrease in the intensity of one allele relative to the other was considered an allelic imbalance.

Statistical Analyses. For comparison of Fhit expression with other tumor markers (ErbB2, ER, PR, Ki67, and p53) in sporadic breast cancers, the Fhit scores were correlated using Spearman correlations. The ranking of the Fhit expression codes was as in Table 1. Spearman (nonparametric) correlations assess monotinicity of relationship, not necessarily linearity. The *P*s are two-tailed and used the asymptotic method but were checked by an approximate method for small sample sizes (StatXact 3.1; Cytel Corp., Cambridge, MA).

Wilcoxon rank sum tests were used to compare Fhit expression observed in the *BRCA2* 999del5 group to the control group of sporadic tumors and to compare Fhit expression in tumors with 3p14.2 LOH to those retaining LOH. *P*s were calculated using a method appropriate for small sample sizes with numerous tied scores (StatXact).

RESULTS

Fhit Expression in Sporadic Breast Cancer. To determine whether the *FHIT* gene might be a target of breast cancer-specific chromosome 3p alterations, we have examined formalin-fixed tissue sections of 50 consecutive breast cancers, 10 DCIS, and 40 invasive cancers, which were resected in 1997 at the Thomas Jefferson University Hospital in Philadelphia. Sections from each case were tested for expression of ER, PR, Ki67, p53, ErbB2, and Fhit by standard immunohistochemical detection using antisera specific for the individual proteins. Because normal breast tissue is included in most of these sections, it serves as internal control and showed that normal breast epithelial cells are uniformly strongly positive for Fhit expression (+++). Results for the 40 invasive carcinomas are summarized in Table 1, which lists each case individually with diagnosis, grade, and expression level of the six proteins tested. The cases have been listed in descending order of Fhit expression, with those expressing levels equal to that observed in normal breast ductal epithelium (+++) at the top and those expressing very little Fhit (−/++ or −/+) at the bottom. Four cases or 10%, shown at the bottom of Table 1, were mostly negative for Fhit expression [60, 70, 85, and 90% of cells were completely negative; the remaining cells were weakly (+) or moderately positive (++)]. Another 16 cases (40%), shown below the horizontal line in Table 1, were partially negative (10–40% negative, with the remaining cells weakly or moderately positive) for Fhit expression. Cases that were uniformly strongly positive (+++, 38%) are shown at the top of Table 1 and are cases in which the *FHIT* locus is unaltered. The uniformly moderately positive cases (++; Table 1) we have considered unaltered in our statistical analysis, although it is possible that one *FHIT* allele has been inactivated in some of these five cases. Examples of expression of Fhit in selected cases are shown in Fig. 1. For the 10 noninvasive DCIS cases (data not shown), only one was mostly negative (−/++, 80% negative), one case was +++/−, and the others were all +++ (five cases) or ++ (three cases).

To determine whether absence or decrease of Fhit expression in invasive breast cancers correlates with expression of other tumor marker proteins or with clinical features, we have compared Fhit expression, by Spearman correlation, with expression of each of the other proteins tested (shown in Table 1) and have then determined whether diagnosis or grade affected the correlation. As shown in Table 2, when Fhit expression was compared with expression of the five biological tumor markers, no significant correlations were observed, although there was a trend toward higher expression of Ki67, a proliferation marker. In addition, although not evident in the correlation analysis, four of the five invasive tumors with significant expression of p53 were tumors that were partially Fhit negative.

Fhit Expression in Breast Carcinomas from BRCA2 Mutation Carriers. As an initial characterization of the regulation and function of the *FHIT* gene in tumors from individuals carrying the *BRCA2* 999del5 germ-line mutation, we have compared these tumors with Icelandic sporadic tumors. In all, 92 Icelandic primary invasive breast tumors, 58 sporadic and 34 *BRCA2* 999del5, were stained with the Fhit antibody. Fig. 2 shows examples of Fhit antibody staining, and Table 3 summarizes the results. The tumors in individuals carrying the *BRCA2* germ-line mutation showed reduced Fhit expression ($P = 0.002$). They were more frequently negative or weakly positive with respect to Fhit antibody staining than sporadic tumors, 32 and 14%, respectively, and only 18% of the *BRCA2* 999del5 tumors but 48% of sporadic tumors show strongly positive Fhit expression.

Of the 92 samples subjected to immunohistochemical analysis, DNA from 58 of them was available for LOH analysis. LOH was detected in 7 of 29 (24%) sporadic tumors and in 22 of 29 (76%) *BRCA2* tumors. Among BRCA2 tumors, none of the seven tumors with retention of heterozygosity exhibited negative or weakly positive Fhit expression compared with 10 of 22 (45%) with LOH. A significant difference ($P = 0.001$) in levels of Fhit expression was observed in tumors with loss and retention of heterozygosity (summarized in Table 4). Forty-eight % of tumors with retention of heterozygosity at the *FHIT* locus showed the strongly positive staining pattern typical of normal breast epithelium, whereas only 14% of the tumors showing LOH were strongly positive. Similarly, only 8% of tumors with retention of both *FHIT* alleles exhibited negative or weakly positive Fhit expression, whereas 38% of tumors with LOH have this pattern of staining. Tumor DNA was necessarily isolated from a different population of tumor cells than were tested for Fhit expression. Because there is heterogeneity in expression of Fhit in subpopulations of tumor cells, as shown above for sporadic breast cancers, such heterogeneity probably accounts for the lack of concordance between LOH and Fhit expression results in individual tumors.

The microsatellite markers used, *D3S1234*, *D3S1300*, *D3S2757*, *D3S4260*, and *D3S1481*, are located at 3p14.2 within the *FHIT* gene. Fig. 3 is an example of LOH analysis showing results from tumors where breakpoints are detected within the *FHIT* gene, and Fig. 4 shows complete results of tumors showing LOH in comparison with Fhit expression. In most tumors showing LOH with intragenic *FHIT* microsatellite markers, there is concordance among markers in the sense that LOH is detected at all informative

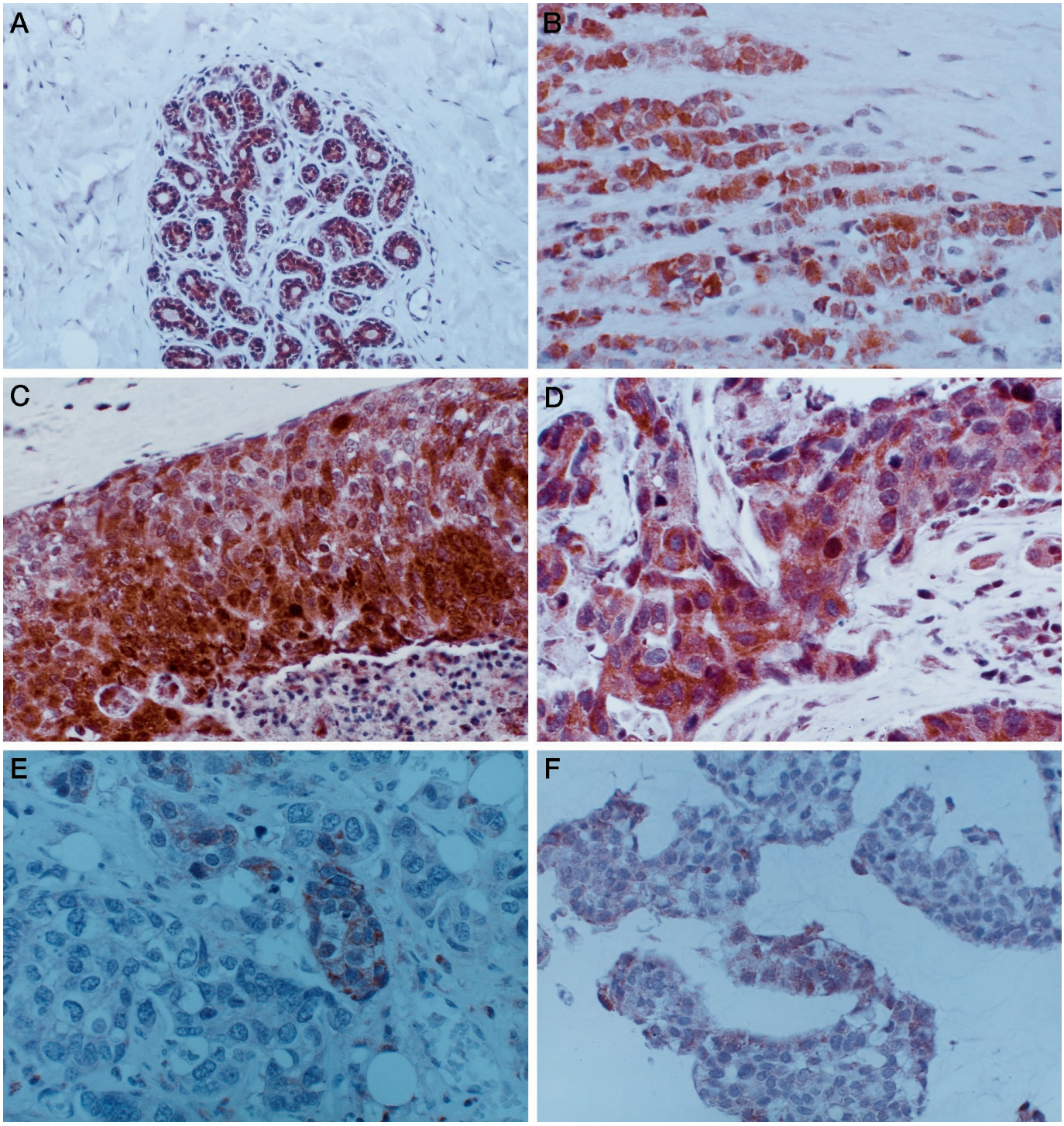

Fig. 1. Immunohistochemical detection of Fhit protein in sporadic breast cancer. *A*, normal breast (×200); *B*, section from lobular carcinoma case 574, scored Fhit +++ (×400); *C*, section from case 3472, a DCIS scored Fhit +++ (×400); *D*, section from case 18832, an IDC scored Fhit +++ (×400); *E*, case 1040, an IDC scored −/+ for Fhit (×400); *F*, case 3105, an IDC scored +/− for Fhit (×400).

loci. Two tumors show retention of both *D3S1481* alleles and LOH with other informative markers; two tumors show LOH with the *D3S1481* marker and retention of both alleles at other informative loci within the *FHIT* gene, indicating breakpoints within the *FHIT* gene in these tumors.

Thus, we observed association of 3p14.2 LOH with reduced Fhit expression, as well as lower expression and higher 3p14.2 LOH in *BRCA2* 999del5 tumors than in sporadic tumors.

DISCUSSION

In this study, we report elevation in LOH at the *FHIT* locus at chromosome 3p14.2 and reduced Fhit antibody staining in tumors of *BRCA2* 999del5 carriers, compared with tumors from individuals without this mutation. We found reduced Fhit expression levels in both sporadic and *BRCA2* 999del5 tumors but significantly lower levels in the *BRCA2* 999del5 group. Elevation of 3p14.2 LOH in *BRCA2* 999del5 carriers is in line with earlier

Table 2 *Correlation of expression of Fhit and tumor markers in sporadic cancers*

	Invasive cancers (n = 40), correlation with Fhit	
Tumor marker	95% Confidence interval	P
ErbB2	0.025 (−0.296, 0.347)	0.876
ER	0.245 (−0.065, 0.555)	0.128
PR	0.177 (−0.110, 0.463)	0.276
Ki67	−0.209 (−0.535, 0.117)	0.196
p53	0.014 (−0.275, 0.304)	0.931

findings (1, 10, 11). Decreased Fhit expression was associated with LOH in the *FHIT* region. Our results suggest that *FHIT* could play an important role in hereditary breast cancer due to loss of gene copy number, with resulting loss of Fhit protein expression from deleted alleles.

Although the discovery of the *BRCA1* and *BRCA2* genes for familial breast cancer has led to exciting new clues into normal cell proliferation and double-strand break repair, it was a surprise that mutations in these two genes are not observed frequently in sporadic breast cancers (31), as might have been expected by parallels to previously described germ-line tumor suppressor gene mutants. Because of the association of these gene products with repair complexes (Refs. 24–26; reviewed in Ref. 32) and with each other (33), it is likely that these genes have a caretaker function, much as mismatch repair genes, *MSH2* and *MLH1*, perform a caretaker function in prevention of HNPCC. Absence of Msh2 or Mlh1 protein in HNPCCs

Table 3 *Expression of Fhit in sporadic and BRCA2999del5 breast tumors*

Microarrayed tissues were assessed by immunohistochemistry for expression of Fhit. In all, 58 sporadic and 34 familial tumors were analyzed; P = 0.002 by Wilcoxon analysis.

	No. of tumors	
Fhit expression	Sporadic (%)	Familial (%)
−	1 (2)	1 (3)
+	7 (12)	10 (29)
++	22 (38)	17 (50)
+++	28 (48)	6 (18)

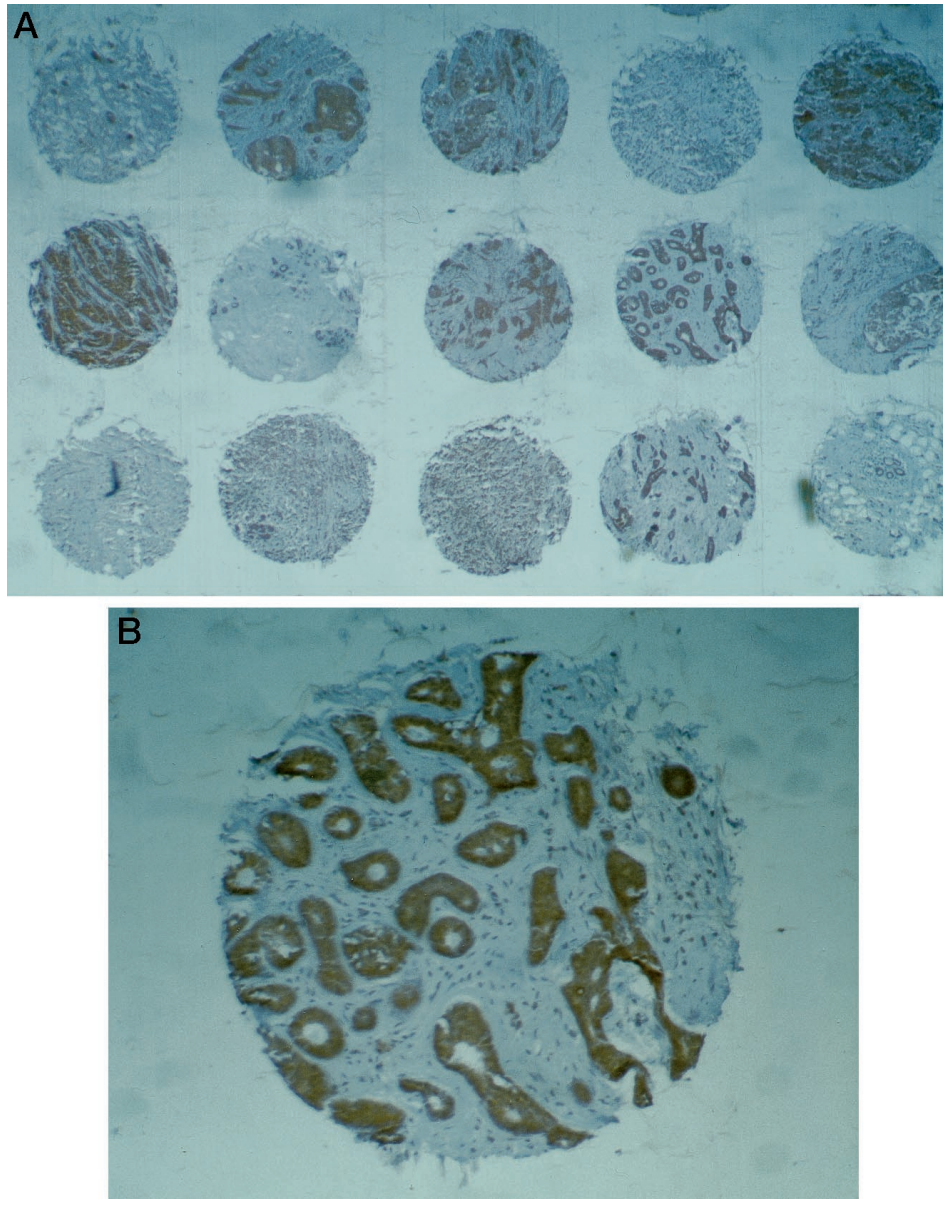

Fig. 2. Immunohistochemical detection of Fhit expression in micoarrayed Icelandic sporadic and familial breast cancers. *A*, section of slide showing numerous cases at low power (×25); *B*, an IDC scored Fhit +++ (×100).

Table 4 *Expression of Fhit in Icelandic breast tumors with and without LOH in the FHIT gene*

Microsatellite markers within the *FHIT* gene were used to assess loss of alleles in microdissected tumor DNA, and microarrayed tissues were assessed by immunohistochemistry for expression of Fhit. A total of 58 cases (29 sporadic and 29 familial) were assessed for LOH at *FHIT*. The difference in Fhit expression between all tumors with LOH and all other tumors is significant ($P = 0.001$) by Wilcoxon analysis.

	No. of tumors					
	Retention (%)			Loss (%)		
Fhit expression	Sporadic (%)	*BRCA2* (%)	All (%)	Sporadic (%)	*BRCA2* (%)	All (%)
−	1 (5)	0	1 (4)	0	1 (5)	1 (4)
+	1 (5)	0	1 (4)	1 (14)	9 (41)	10 (34)
++	8 (36)	5 (71)	13 (44)	5 (71)	9 (41)	14 (48)
+++	12 (55)	2 (29)	14 (48)	1 (14)	3 (14)	4 (14)

leads to genome-wide microsatellite instability that can be detected as shifts in mobility of microsatellite alleles on polyacrylamide gels, compared with constitutive alleles. Loss of Msh2 or Mlh1 protein thus causes HNPCC indirectly through unrepaired or misrepaired damage to genes involved in regulation of cellular growth or invasion. It is possible that loss of wild-type *BRCA2* also contributes to cancer indirectly by failure to correctly repair double-strand chromosome breaks. Breast carcinomas from individuals carrying germ-line *BRCA1* or *BRCA2* mutations show increased frequencies of chromosomal abnormalities when compared with sporadic breast carcinomas. In a genome-wide survey by comparative genome hybridization, Tirkkonen *et al.* (12) showed distinct somatic alterations in tumors of *BRCA1* and *BRCA2* carriers compared with unselected sporadic breast cancers. There were about twice as many chromosomal changes in the hereditary cancers, but different chromosome arms were affected in the two types of familial cancers, with losses of 5q, 11q, 4p, 2q, and 12q occurring much more frequently in *BRCA1* tumors than in sporadic and a higher frequency of 13q and 6q losses in *BRCA2* tumors. *BRCA2* tumors also showed elevated frequency of gains of 17q22–24 and 20q13. Small allelic deletions would not be detected by comparative genome hybridization analysis, but LOH analyses comparing *BRCA2* cancers to sporadic cancers showed that the frequency of LOH at some chromosome regions was similar (1p, 16q) and at others significantly different (3p, 6q, 11p, 11q, 13q, and 17p; Ref. 11). These chromosome regions have been implicated in sporadic breast cancers at lower frequency (11). A majority of *BRCA2* mutant carrier cancers show LOH at each of the loci. Interestingly, the chromosome arms 6q, 11p, 11q, and 13q each show three common fragile sites, whereas 3p has one in 3p14.2 within the region of highest loss on 3p; 17p does not exhibit a common fragile site. It is likely that each of these regions of high LOH contains at least one gene that is a target of loss; perhaps *TP53* on 17p, *BRCA2* at 13q, and *FHIT* at 3p14.2. Conversely, 16q exhibits two common fragile sites near a region of loss in sporadic breast cancers, but 16q LOH was not elevated in the *BRCA2* mutant cases. Thus, presence of a common fragile site alone cannot explain elevated LOH frequencies.

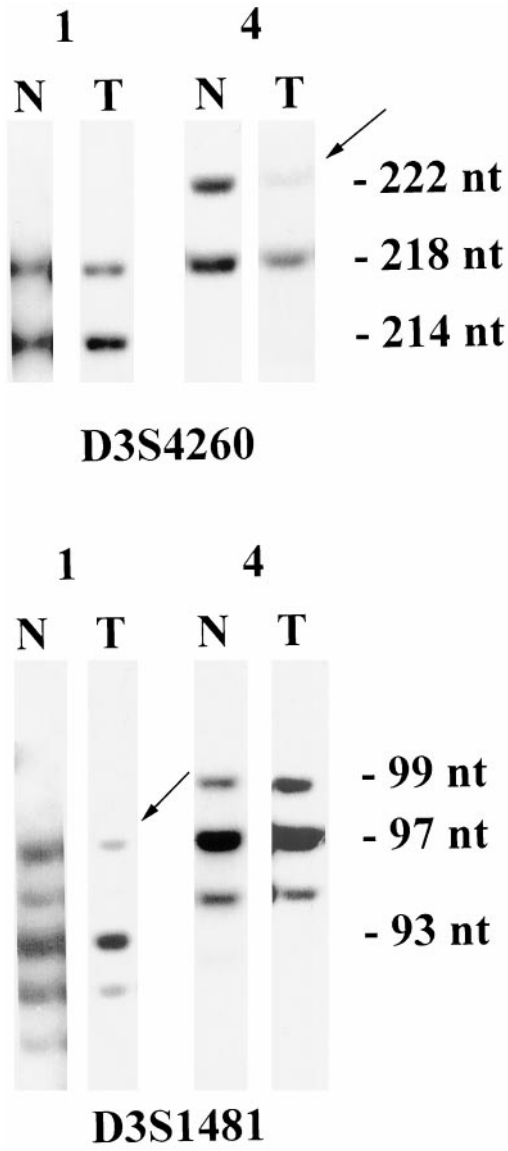

Fig. 3. LOH at the *FHIT* locus in matched normal (*N*) and tumor (*T*) tissue from cancer patients. *Top*, case numbers. *Bottom*, markers used. *Right*, size in nucleotides of the DNA products. Tumor 1 shows retention of both alleles with marker *D3S4260* but loss with marker *D3S1481*, and tumor 4 shows retention with mrker *D3S1481* and loss with marker *D3S4260*.

We have examined LOH and expression of the *FHIT* gene in the *BRCA2* 999del5 familial *versus* sporadic cancers and have found that Fhit expression is reduced in a large fraction of sporadic breast cancers and in at least twice as many familial cancers. Our results may reflect the inability of mutated Brca2 to participate in the Rad51-mediated repair. Possibly due to infidelity in mitotic recombination and corresponding DNA repair, the *BRCA2* 999del5 cases follow a more aggressive pathway of chromosome damage, where fragile sites in the genome could be a hot-spot target. The numerous somatic *FHIT* gene deletions detected in *BRCA2* carriers indicate that germ-line mutation of the *BRCA2* gene results in accelerated accumulation of secondary somatic genetic changes at this locus in the tumors. These genetic aberrations result in reduced expression of a putative tumor suppressor gene, *FHIT*.

In sporadic breast cancer, ~40% showed reduced expression of Fhit. In the study of familial *versus* sporadic cancer, Fhit expression was assessed on microarrays constructed using one sampling site per tumor. Therefore, it was not possible to evaluate the regional heterogeneity of Fhit expression. It is likely that some of these tumors are

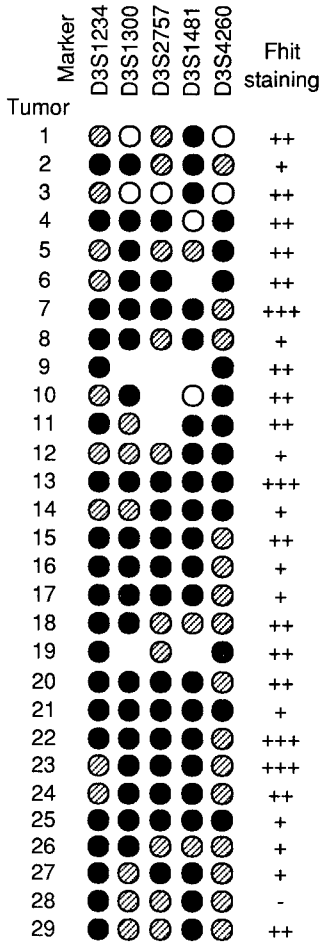

Fig. 4. Summary of LOH within the *FHIT* gene. Tumors 1–7 were sporadic, and tumors 8–29 were from *BRCA2* 999del5 carriers. ●, LOH; ▨, homozygosity; ○, retention of heterozygosity. Tumors 1, 3, 4, and 10 show breakpoints within the *FHIT* gene.

heterogeneous for expression of Fhit, as are the sporadic Philadelphia breast cancers, which could explain why the LOH data are not completely concordant with the Fhit expression data (shown in Fig. 4), *i.e.*, one portion of a tumor could show LOH with concomitant Fhit reduction, whereas another portion might show very strong Fhit expression. In this study, in agreement with the previous LOH study, expression of Fhit was reduced in a significantly higher fraction of familial cancers relative to sporadic cancers analyzed in the same microarray experiments. Thus, it is likely that *FHIT* inactivation contributes to clonal expansion in both sporadic and familial breast cancer. Because there is a trend toward higher Ki67 and p53 values in breast cancers with reduced Fhit expression, it is possible that Fhit loss contributes to aggressivity of these cancers; however, studies of larger panels of breast cancers will be necessary to confirm these trends.

In a previous study of lung carcinoids (28), we have shown that normal bronchial epithelium is strongly positive for Fhit, typical (benign) carcinoids with minimal LOH on 3p were strongly positive, small cell lung cancers were completely negative, whereas atypical carcinoids (malignant) show extensive LOH in *FHIT* and moderate to weak Fhit expression. We concluded that loss of one *FHIT* allele leads to reduced Fhit expression. Similarly, weak/moderate Fhit expression may result from allelic loss at the *FHIT* locus in sporadic and familial breast cancers, suggesting that loss of one *FHIT* allele may contribute to clonal expansion in breast tissue.

Thus, our interpretation of molecular events at the *FHIT* locus in breast cancer is: one fragile *FHIT* allele is broken and misrepaired, resulting in deletion within one *FHIT* gene and down-regulation or loss of expression of that allele; reduction in Fhit expression allows a slight clonal expansion of Fhit ++ or + cells, increasing the opportunity for breakage and misrepair of the second *FHIT* allele and resulting in expansion of a subpopulation of Fhit negative cells. The tumor in this example would then be a ++/− or +/− tumor.

The defects in the *FHIT* gene described in this study are likely to be helpful in the understanding of the somatic genetic progression pathways that contribute to the development of malignancy in genetically predisposed individuals.

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