Published in final edited form as:
Clin Cancer Res. 2010 April 1; 16(7): 2214–2224. doi:10.1158/1078-0432.CCR-09-3058.

Lynch Syndrome-Associated Breast Cancers: Clinicopathological Characteristics of a Case Series from the Colon CFR

Michael D Walsh[1,2,#], **Daniel D Buchanan**[1,2], **Margaret C Cummings**[3], **Sally-Ann Pearson**[1], **Sven T Arnold**[1], **Mark Clendenning**[1], **Rhiannon Walters**[1], **Diane M McKeone**[1], **Amanda B Spurdle**[4], **John L Hopper**[5], **Mark A Jenkins**[5], **Kerry D Phillips**[6], **Graeme K Suthers**[6,7], **Jill George**[8], **Jack Goldblatt**[8,9], **Amanda Muir**[10], **Kathy Tucker**[10], **Elise Pelzer**[11], **Michael R Gattas**[11], **Sonja Woodall**[12], **Susan Parry**[12,13], **Finlay A Macrae**[14], **Robert W Haile**[15], **John A Baron**[16], **John D Potter**[17], **Loic Le Marchand**[18], **Bharati Bapat**[19], **Stephen N Thibodeau**[20], **Noralane M Lindor**[20], **Michael A McGuckin**[21], and **Joanne P Young**[1,2]

[1]Familial Cancer Laboratory, Queensland Institute of Medical Research, Herston QLD, Australia [2]University of Queensland School of Medicine, Herston, QLD, Australia [3]University of Queensland Centre for Clinical Research, Herston, QLD, Australia [4]Molecular Cancer Epidemiology Laboratory, Queensland Institute of Medical Research, Herston QLD, Australia [5]University of Melbourne, Centre for MEGA Epidemiology, School of Population Health, Melbourne, VIC, Australia [6]South Australian Clinical Genetics Service, North Adelaide, SA, Australia [7]Department of Paediatrics, University of Adelaide, SA, Australia [8]Genetic Services of Western Australia, King Edward Memorial Hospital, Subiaco, WA, Australia [9]School of Paediatrics and Child Health University of Western Australia, Nedlands, WA, Australia [10]Clinical Genetics Service, Prince of Wales Hospital, Randwick, NSW, Australia [11]Genetic Health Queensland, Royal Brisbane and Women's Hospital, Herston, QLD, Australia [12]Northern Regional Genetics, Auckland Hospital, Auckland, New Zealand [13]University of Auckland, Auckland, New Zealand [14]Department of Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, VIC, Australia [15]Keck School of Medicine, University of Southern California, Los Angeles, CA, USA [16]Dartmouth Medical School, Hanover, NH, USA [17]Fred Hutchinson Cancer Research Center, Seattle, WA, USA [18]Cancer Research Center of Hawaii, University of Hawaii at Manoa, Honolulu, HI, USA [19]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Canada [20]Mayo Clinic, Rochester, MN, USA [21]Mater Medical Research Institute, South Brisbane, QLD, Australia

Abstract

PURPOSE—The recognition of breast cancer (BC) as a spectrum tumor in Lynch syndrome remains controversial. The aim of this study was to explore features of breast cancers arising in Lynch syndrome families.

EXPERIMENTAL DESIGN—This observational study involved 107 cases of BC identified from the Colorectal Cancer Family Registry (Colon CFR) from 90 families where 1) both breast and colon

[1]http://epi.grants.cancer.gov/CFR

#Correspondending author: Michael D Walsh, Familial Cancer Laboratory, I Floor, Bancroft Centre, Queensland Institute of Medical Research, Herston Road, Herston, QLD 4006, AUSTRALIA, Phone: +61-7-38453775, Fax: +61-7-33620107, Michael.Walsh@qimr.edu.au.

A subset of this work, namely pathology features of 94 breast cancers, has been presented as a poster at the XXVII Congress of the International Academy of Pathology, Athens, GREECE, 2008

[DNA Mismatch Repair Deficiency in Breast Cancers Arising in Breast/Colon Families. Walsh M, Cummings M, Buchanan D, Arnold S, McKeone D, Walters R, Jass J, Hopper J, Jenkins M, Spurdle A, McGuckin M, and Young J. *Histopathology* (2008) 53 (Suppl. 1): 71.].

cancer co-occurred, 2) families met either modified Amsterdam criteria, or had at least one early onset (<50 years) colorectal cancer, and 3) breast tissue was available within the biospecimen repository for mismatch repair (MMR) testing. Eligibility criteria for enrolment in the Colon CFR are available online1. Breast cancers were reviewed by one pathologist. Tumor sections were stained for MLH1, PMS2, MSH2 and MSH6, and underwent MSI testing.

RESULTS—BC arose in 35 mutation carriers and of these, 18 (51%) demonstrated immunohistochemical absence of MMR protein corresponding to the MMR gene mutation segregating in the family. MMR-deficient BCs were more likely to be poorly differentiated (p=0.005) with a high mitotic index (p=0.002), steroid hormone receptor negative (ER p=0.031; PR p=0.022), and to have peritumoral lymphocytes (p=0.015), confluent necrosis (p=0.002), and growth in solid sheets (p<0.001) similar to their colorectal counterparts. No difference in age of onset was noted between the MMR deficient and intact groups.

CONCLUSIONS—MMR deficiency was identified in 51% of BC arising in known mutation carriers. BC therefore may represent a valid tissue option for the detection of MMR deficiency where spectrum tumors are lacking.

Introduction

Defects in autosomal dominant genes are suspected to be responsible for up to 10% of all breast cancers (BCs) (1;2). The most commonly affected known genes are *BRCA1* and *BRCA2* while other genes such as p53, *PTEN*, *STK11/LKB1* and *CDH1* are implicated in fewer cases (3;4). There are, in addition, a significant number of cases of breast cancer that present at an earlier than usual age of onset and with a conspicuous family history for which the causative gene(s) has not been identified (5).

Lynch syndrome, or hereditary non-polyposis colorectal cancer (HNPCC) (6), was first identified nearly a century ago as familial clustering of cancers, particularly of the colon, small intestine, stomach, endometrium, upper urinary tract and sebaceous tumors of the skin. Approximately 80 % of Lynch-syndrome associated cancers are attributable to defects in the DNA mismatch repair (MMR) genes *MLH1* and *MSH2*, with the majority of the remaining cases occurring in carriers of mutations in *MSH6* and, to a lesser extent, *PMS2* (7). The consideration of breast cancer as a spectrum tumor in Lynch syndrome has been controversial with evidence for and against constituting a rigorous debate over time. An extensive study published in 2002 excluded breast cancer as part of the Lynch syndrome spectrum of tumors (8). Another study, whilst demonstrating no increased risk for developing mammary cancers found that tumors in known carriers presented at an earlier age (9). This led de Leeuw *et al* to postulate that, whilst MMR deficiency does not in itself initiate breast tumors, the increased rate of mutation accelerates their progress leading to an earlier presentation (10). In contrast to these observations, Scott *et al* reported a significant 15-fold increased *risk* of breast cancer in *MLH1* mutation carriers (but not *MSH2* carriers) (11), and a study of Brazilian Lynch syndrome families showed an increased incidence of breast cancer equal in incidence to endometrial cancer cases (12), though this study used clinical criteria to define Lynch syndrome. In more recent times, both case reports (13;14) and statistical studies (15;16) respectively have shown that MMR-deficient breast cancers can and do arise in mutation carriers.

Immunohistochemistry (IHC) screening of tumors for deficiency in MMR proteins is currently the most efficacious method for the recognition of Lynch syndrome (17). IHC allows not only identification of potential Lynch syndrome patients, but also by its pattern of staining, the most likely causative gene. This is important because tracking down a mutation in MMR genes is not a trivial exercise. IHC is generally applied to recognized Lynch syndrome spectrum tumors such as those from the colorectum, endometrium, ovary, stomach and urothelial tissues. This

has also become increasingly important as family size decreases and screening becomes more widespread, lessening the power of clinical criteria to detect Lynch syndrome, and highlighting the need to increase the potential sources of tissue that may be utilized for diagnosis. Breast cancers with both MSI and/or immunohistochemical absence of MMR proteins have been reported by many authors (13;14;18–23), though many of these reports contain small numbers of cases. In this study, we sought to establish the frequency with which breast cancers occurring in mutation carriers for Lynch syndrome display microsatellite instability as a consequence of loss of DNA MMR proteins, and to examine the clinicopathological features of such tumors. This study represents the largest series of breast cancers with MMR deficiency reported to date, and attests to the utility of breast tissue as a diagnostic sample in suspected Lynch syndrome when colon or endometrial tissue is unavailable (13).

Patients and Methods

Patients

One hundred and seven cases of breast cancer (arising in 102 females and 2 males) from 90 colorectal cancer families were identified from the Colorectal Cancer Family Registry (Colon CFR), a National Cancer Institute–supported consortium established in 1997 to create a comprehensive collaborative infrastructure for interdisciplinary studies of the genetic and molecular epidemiology of colorectal cancer (see detailed information about the registry at the CFR website[2]) (24). All patients in this study had institutional review board approval under the policies and procedures of the Colon CFR for recruitment of participants and protocols for carrying out research projects. The average age of patients with breast cancer was 56.1 ± 11.3 years, ranging from 36.1 to 86.7 years of age.

Families were selected for study in which 1) both breast and colon cancer co-occurred, with at least one breast cancer regardless of age at diagnosis 2) families met either modified Amsterdam criteria, or had at least one early onset (<50 years) colorectal cancer, and 3) breast tissue was available within the biospecimen repository for mismatch repair (MMR) testing thereby limiting the number of families which could be analysed. Comprehensive cancer histories *and* tissue were available for 90 families recruited through the Australasian Colorectal Family Registry and the Mayo Clinic Cooperative Family Registry for Colon Cancer Studies. The majority of families (n=86) were enrolled in the Colon CFR on the basis of a strong family history of colorectal cancers compatible with Lynch syndrome, and the remaining four families were identified following enrollment of participants with early onset (<50 years) colorectal cancer.

Fifty-four of ninety (60%) families met modified Amsterdam criteria, and the remainder had multiple cancers including at least one early onset (<50 years) colorectal cancer per family. In 13 cases (13%) the breast cancer patient was also affected with early onset colorectal cancer, 58 individuals (56%) were first degree relatives of an individual with early onset CRC, 25 individuals (24%) were second degree relatives, and the remaining 8 cases (8%) were more distantly related. All reported tumors (breast and other sites) were verified by either examination of the original histopathology material or histopathology reports for all affected kindred members where possible. In three cases, bilateral metachronous tumors were available for testing. In no instances was there evidence of familial adenomatous polyposis (FAP). No deleterious mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* were detected in the small minority of families (n = 7) tested. As the Colon CFR recruits families on the basis of CRC, it is unlikely that families enrolled in this registry would be of the type of configuration that would trigger BRCA1 testing.

[2]http://epi.grants.cancer.gov/CFR

Histopathological Review

One consultant histopathologist (MCC) reviewed material from 104 of the 107 cases of breast cancer to confirm diagnosis and score histopathological features. Clinicopathological data for the remaining three cases was abstracted from histopathology reports. Note was made of the following features: tumor location, size, primary histological type, tumor grade (using the Nottingham modification of Bloom Richardson system (25)), tumor margin, confluent necrosis, calcification, presence of tumor infiltrating and peritumoral lymphocytes, presence of *in situ* carcinoma and atypical ductal hyperplasia and axillary lymph node status. While note was also made of the steroid hormone receptor status as originally reported, these markers were reassessed by immunohistochemistry in our laboratory owing to the large number of incomplete reports. *HER2/neu* status was extracted, where available, from original laboratory reports.

Immunohistochemistry

for DNA mismatch repair proteins was performed as previously described (26). A subset of tumors was stained for ER and PR, and p53. Paraffin sections (4μm) were subjected to heat induced epitope retrieval in High pH Target Retrieval Solution (Dako Corporation, Carpinteria, CA) for ER and PR, and Reveal Decloaker solution™ (BioCare Medical, Concord, CA) for p53. Sections were stained with rabbit monoclonal anti-human ER (clone SP1) or rabbit monoclonal anti-human PR (clone SP2) at 1/2500 or mouse monoclonal anti-p53 antibody at 1/100 followed by the EnVision Plus Mouse HRP detection system (Dako) for p53, or MACH3 Rabbit HRP polymer kit (BioCare Medical) for ER and PR. The proportion of positive cancer cell staining was graded as follows: 0 (negative), less than 10% (1+), 11–25% (2+), 26–50% (3+), 51–75% (4+) and more than 75% (5+). Tumors were scored as positive where there was strong expression in >10% tumor cells. Histologically normal breast epithelium present within tumor blocks served as the positive control for ER and PR, and sections from known p53-overexpressing colorectal cancers as the positive control when staining for p53 in breast cancers.

Assessment of Tumors for Microsatellite Instability and MLH1 Methylation

Tumors were assessed for microsatellite instability using a panel of 10 microsatellite markers and classified as MSI-H (MSI high) if ≥ 30% of the markers showed instability, MSI-L (MSI low) if one or more markers but < 30% of all markers demonstrated instability and MSS (microsatellite stable) if no marker exhibited instability as has been previously reported (17). Only cases with 5 or more evaluable markers were considered. Methylation of the *MLH1* promoter was detected using the Methylight assay as has been recently described (27).

BRAF V600E Allele Specific PCR Assay

The somatic T>A mutation at nucleotide 1799 causing the V600E mutation in the BRAF gene was determined using a fluorescent allele specific PCR assay. Briefly, 20–50ng of DNA, extracted from formalin-fixed paraffin embedded tumour tissue, was amplified in a 25μl reaction containing 100nM each of allele specific primers tagged with differing fluorophores (Mutant Primer (F1): 6-Fam-5'-**CAGT**GATTTTGGTCTAGCTTCAGA-3' Wild Type Primer (F2): NED - 5'- **T**GATTTTGGTCTAGCTACAGT-3' and a common reverse primer (Reverse Primer (REV): 5'-CTCAATTCTTACCATCCACAAAATG-3'), together with 2.5units of Taq polymerase (Eppendorf), 1x buffer and 200μM of dNTPs. The cycling conditions consisted of an initial denaturation of 95°C for 2mins followed by 35 cycles of 94°C for 30sec, 59°C for 30sec and 65°C for 30sec then a final extension of 65°C for 10mins. After amplification 1μl of the PCR product was added to an 8.7μl mix of HiDi formamide and ROX Genescan 500 size marker (Applied Biosystems, Foster City, CA). The mutant allele (A1799) primer generated a PCR product of 97bp, 3bp larger than the wildtype PCR product after separation

on an ABI 3100 genetic analyser. GeneMarker (SoftGenetics) software was used to identify the different size and fluorescent allele PCR products. Positive and negative controls were run in each experiment and 10% of samples were replicated with 100% concordance.

Mutation Testing

DNA (10ng) was amplified in 25μl reactions using HotMaster Taq and buffer (Eppendorf, Hamberg, Germany) with 20pmol of each primer. Cycling protocols were applied according to previously established conditions for each primer set and amplicon, verified to selectively amplify the target amplicon only. PCR products were cleaned using Millipore Montage PCR96 Cleanup Plates (Millipore, Bedford, MA, USA). Cleaned PCR product (1μl) was used in a 12μl sequencing reaction utilizing the BigDye Terminator v3.1 reagents and protocol (Applied Biosystems, Foster City, CA, USA) and 2pmol of primer. Sequencing product was cleaned with the DyeEx 96 Kit (Qiagen, Hilden, Germany) using the recommended protocol. The product was dried, resuspended in HiDi Formamide (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI PRISM 3100 (Applied Biosystems, Foster City, CA, USA). Bi-directional sequencing was performed throughout. Results were compared to reference sequence NC_000003.10 (genomic) and NM_000249.2 (cDNA) for *MLH1*, and NC_000002.10 (genomic) and NM_000251.1 (cDNA) for *MSH2*, NC_000002.11 (genomic) and NM_000179.2 (cDNA) for *MSH6*, and NC_000007.13 (genomic) and NM_000535.3 (cDNA) for *PMS2*. MLPA was used to detect large exonic deletions and duplications in the four MMR genes, using the Salsa MLPA P003 and P248 kits for *MLH1* and *MSH2* and the P008 kit for *MSH6* and *PMS2* (MRC-Holland, Amsterdam, The Netherlands) according to manufacturer's protocol.

Statistical Analysis

was carried out using Statistical Package for Social Sciences (SPSS, version 17.0). Contingency tables were assessed using Chi-squared or Fisher's exact test as appropriate. Differences between means were assessed using a t-test after testing to ensure equality of the variance in groups using probability plots and an F-test. P values < 0.05 were considered significant. Sensitivity and specificity calculations were performed using VassarStats [3].

Results

Of 90 families with breast and colorectal cancer, 53 families (59%) were classified as Lynch syndrome on the basis of a germline mutation (n=52) or multiple MSH2/MSH6 deficient tumors within the family (n=1). Table 1 shows the distribution of families amongst the four causative MMR genes (*MLH1, MSH2, MSH6* and *PMS2)*. In thirty-seven remaining families, Lynch syndrome could be excluded as no evidence of MMR deficiency from IHC or MSI testing was found, nor were any MMR mutations identified where tested. In 30 families Lynch syndrome was excluded on the basis of IHC and MSI testing of several family members with no evidence of MMR deficiency. The remaining seven families showed multiple MLH1-deficient colorectal and/or endometrial cancers associated with *MLH1* methylation and/or somatic *BRAF* mutation (CRC only) with no evidence of germline mutations in *MLH1* either by direct sequencing or MLPA. Only one of these seven families met modified Amsterdam criteria (ACII). None of the 61 breast cancers in this study, which could be analyzed, demonstrated the V600E activating mutation in *BRAF*.

Abnormal immunostaining for MMR protein was observed for 18/107 breast cancers (17%) (Figure 1 and Supplementary Figure 1 and Supplementary Figure 2). In twelve cases, tumors showed loss of MSH2 and MSH6 proteins, in five cases MLH1 and PMS2 were absent, and

[3]http://faculty.vassar.edu/lowry/VassarStats.html

in one case, MSH6 only was absent. Microsatellite instability testing was performed for 89 breast cancers and was concordant with IHC results in 85 cases (96%). All but one of the MMR deficient breast cancers arose in families meeting ACII. Of the 18 participants whose breast cancers showed loss of one or more MMR proteins on IHC, 16 tested positive for germline mutations in the DNA MMR genes *MLH1*, *MSH2* or *MSH6* consistent with both their tumor immunodeficiency as well as their respective family mutation, whilst a further individual who was deceased was found to be an obligate carrier of her family mutation (Table 2). The remaining case demonstrating immunohistochemical loss of MSH2 and MSH6 arose in a family in which no *MSH2* mutation has been identified to date but which has three affected kindred members where their tumors demonstrate commensurate loss of MMR proteins.

Overall, 18/35 known MMR mutation carriers with breast cancer (51%) produced a breast cancer that was MMR deficient. Of the 89 breast cancers with normal immunohistochemistry, 13 arose in individuals from families where *MLH1* mutations were identified, 20 in individuals with a family *MSH2* mutation, 5 with *MSH6* mutations and 3 with *PMS2*. Of these 41, 17 individuals tested carried the family mutation, suggesting that only a proportion of breast cancers in mutation carriers are associated with MMR deficiency.

Ten of the 18 individuals showing loss of one or more MMR proteins in their breast cancers had other primary tumors as summarized in Table 3. In three of the ten cases, the breast cancer was the first diagnosed malignancy, preceding the second cancer by between 2 and 27 years. In the other patients, the breast cancers were diagnosed between 1 and 42 years after the first cancer. In all cases but one (a meningioma), the non-breast cancers tested showed the same pattern of MMR protein deficiency as the breast tumors, a finding which supports the premise of this report, namely that the breast cancers in mutation carriers that are MMR deficient are likely to have developed in association with the germline mutation carried. Importantly, in eight cases of breast cancer in a proven mutation carrier, breast cancer was the *only* cancer documented.

There was no statistical difference in age of presentation between the MMR deficient breast cancers (mean = 57.5 ± 8.1 yr, range 43.4 – 75.0 yr) and MMR intact BCs (mean = 55.8 ± 11.9 yr, range 36.1 – 86.7 yr) (p=0.56), nor between the MMR deficient BC group and MMR intact known mutation carriers (57.1 ± 12.0 yr, range 36.1 – 80.5 yr) (p=0.90). Similarly, no difference in mean age of presentation was observed between the five BC cases which were MMR deficient in *MLH1* germline mutation carriers and the twelve cases occurring in *MSH2* carriers (58.0 yr vs. 57.5 yr) (p=0.90). The average age of the 11 individuals with the primary or only cancer being a breast cancer with MMR deficiency was 53.7 ± 6.0 yr.

In 104 breast cancers that underwent pathology review, histological differences for invasive BCs only were compared, with twelve cases of ductal carcinoma *in* situ excluded from analysis. Specifically, MMR deficient invasive breast cancers (n=16) were more likely to be estrogen- and progesterone receptor negative (p=0.031 and p=0.022 respectively), have peritumoral lymphocytes (p=0.015), to have confluent necrosis (p=0.002), to have growth in solid sheets (p<0.001), and to have a higher mitotic rate (p=0.002) when compared to MMR-proficient BCs (n=79). In addition, MMR deficient breast cancers less frequently had contiguous *in situ* disease (p=0.038). No statistically significant association was seen between MMR status and tumor type, size, lymphovascular invasion, node status, prominent eosinophilic nucleoli, or tumoral calcification (Table 4 and Supplementary Table 1). No statistical differences were observed for clinicopathological features between the MMR-proficient invasive breast tumors arising in known carriers of germline MMR gene mutations and tumors from the non-Lynch syndrome group (data not shown), and therefore all MMR-intact tumors were considered together as the reference group for comparison with MMR-deficient invasive cancers. There were, however, significant differences in growth in solid sheets (p=0.002), and the presence of pushing margins

(p=0.042), and confluent necrosis (p=0.017) and residual carcinoma *in situ* (p=0.004) between MMR deficient and intact invasive cancers amongst proven carriers of MMR gene germline mutations (Table 5).

Four tumors displayed typical *BRCA1* histological phenotype (characterized by high grade, high mitotic index, pushing margin, growth in solid sheets, and the presence of lymphocytic infiltrate and tumor necrosis (28)), and two of these tumors (50%) showed loss of MSH2 and MSH6.

The two cases of ductal carcinoma *in situ* which exhibited loss of MMR expression were both of solid type, but there was no significant difference overall between DCIS type (cribriform, solid, papillary or clinging) and MMR expression when including *in situ* disease accompanied by an invasive component (p = 0.45) (data not shown). Lobular carcinoma *in situ* was present in eight cases accompanying invasive disease. There was no statistically significant difference between MMR deficient and proficient breast cancers and over-expression of p53 (p = 0.39).

There was a trend for individuals with a MMR deficient breast cancer to have also developed an early onset colorectal cancer, or was a first degree relative of someone so affected (n= 15) when compared to individuals with a MMR proficient BC having more distantly related cases of early-onset CRC (n=3) (p=0.11, 21% vs. 9%, respectively). Of the thirteen cases in which the same individual had both early-onset colorectal cancer *and* breast cancer, five (39%) showed mismatch repair deficiency. There was no statistical difference between degree of kinship between the breast and early onset CRC patients within individual families and whether the pedigree satisfied the modified Amsterdam criteria (p = 0.17).

Discussion

In the present study we have examined the incidence of mismatch repair deficiency occurring in breast cancers from families with a history of early onset (<50 yr) colorectal cancer and breast cancer occurring at any age. Of the 104 individuals investigated, 35 were found to harbor deleterious mutations in one of the DNA mismatch repair genes, and of these, 18 individuals (51%) were found to have loss of MMR expression consistent with their respective germline mutations. This study follows previous reports where several groups have examined breast cancers with varying panels of markers in order to assess the extent of instability in tumors from this site. Many, however, have yielded disappointing results where little or no instability could be detected in the majority of tumors (22;29–32) although these studies were not specifically designed to detect Lynch syndrome. In 1996, Risinger and colleagues described breast cancer occurring in Lynch syndrome kindreds that showed high-level microsatellite instability. On the basis of this, it was suggested that breast cancer might be included in the tumor spectrum of Lynch Syndrome II (21). A subsequent study failed to show an increased risk for breast cancer in Lynch syndrome, and further suggested that sporadic breast cancer with MMR deficiency may be exceedingly rare (8). However, reports of breast cancer with MMR deficient phenotypes continued to be presented (9;10;33) with more targeted studies subsequently demonstrating that MSI is indeed a common feature of breast cancers occurring in known mutation carriers, being found in up to 60% of cases (10;34;35). In our study, where MSI and IHC results were highly correlated, we returned a figure of 51% for the proportion of breast cancers (which included a male breast cancer) arising in MMR mutation carriers that demonstrated MMR deficiency, commensurate with this previously reported figure.

A study by Vasen and co-workers of 200 putative Lynch syndrome families has shown that though breast cancer occurs at an early age in Lynch syndrome, there is no elevated risk *per se* (9). This suggested that MMR deficiency may accelerate tumorigenesis in breast cancers which occur in Lynch syndrome mutation carriers but is unlikely to be the initiating event

(10). We found no significant difference between MMR proficient and MMR deficient breast cancers when age of onset was analyzed. The mean age at diagnosis was 57 years in our study. Vasen *et al.* reported a mean age of 46 years for seven cases of breast cancer arising in mutation carriers (9), or 50 years reported by both Stupart *et al.* who examined incidence of breast cancers in women carrying in common a single mutation in *MLH1* (c. C1528T)(36) and Jensen *et al.* for a series of breast cancers arising in 20 mutation carriers reported recently (35). The numbers of cases in all studies are, however, small, and recruitment biases in different studies may account for any differences.

Medullary carcinomas of the breast show morphological similarities to the MSI-high colorectal tumors (poor differentiation and lymphocytic infiltrate) and a proportion of these are MSI-high (37). Many of the studies to date investigating the issue of breast carcinomas arising in the setting of Lynch syndrome have been anecdotal case reports of one or two patients who have shown loss of appropriate MMR proteins by immunohistochemistry and/or high levels of microsatellite instability in tumors arising in proven mutation carriers (19;38;39). With such small numbers in any given study, it has been difficult to determine whether there is an “MSI” phenotype associated with such cancers, although Yee *et al* described higher levels of MSI in lobular carcinomas (39%) than in infiltrating ductal cancers (13.5%) (40). The issue has been made all the more difficult in that, whilst some breast cancers in mutation carriers have appropriate MMR protein loss with resultant MSI, there are commensurate numbers of reports of breast tumors arising in proven carriers which have competent MMR (8;39;41). In this report, we found that breast cancers with proven MMR deficiency are significantly more likely than those with proficient MMR to demonstrate hormone receptor negativity, poor differentiation, a solid growth pattern, lymphocytic infiltrate, high mitotic rate, confluent necrosis and vesicular tumor nuclei. In common with the study by Jensen *et al.*, we found that ductal carcinoma NOS was the predominant histotype (35) with no evidence of over-representation of specific types such as medullary or invasive lobular carcinoma.

Several of these features including poor differentiation and lymphocytic infiltrate are also reported features of Lynch syndrome colorectal cancers (42;43), and a dense lymphocytic infiltrate has been previously shown in a breast cancer case arising in a Lynch syndrome mutation carrier (14), and more recently in a larger series of MMR deficient BCs arising in mutation carriers where half of the six tumors were reported to have both TILs and PTLs (35).

The presence of breast cancer in Lynch syndrome has been reported to be over-represented in Lynch syndrome families with *MLH1* mutations (11;15). Rarer causes of Lynch syndrome such as germ line mutations in *MSH6* have also been associated with synchronous breast and colon cancers (44). However, due to our limited study design, we are unable to offer any comments regarding whether or not the risk of breast cancer is increased in Lynch syndrome mutation carriers.

Familial aggregation of cancers from different anatomical sites has been previously documented (45–47). Such clustering may arise from shared environmental risk factors common to the cancers, inherited defects in cancer susceptibility genes or interaction between the two. A series of large studies utilizing the Swedish Family-Cancer Database, concluded that in the absence of known, strong environmental risk factors, most of the familial aggregation that is observed is likely due to genetic factors that increase the risk of cancer at more than one site (48). In addition, it has become increasingly apparent that most inherited cancer susceptibilities confer a risk for cancer at a range of sites, suggesting that mechanisms of carcinogenesis are shared by different tissues. This is true of Lynch syndrome, where a defect in DNA MMR clearly confers an increased risk for cancers of the colorectum, endometrium, ovary, stomach, bladder and renal pelvis. As described in multiple previous reports, we found

gene-appropriate MMR deficiency to be also readily demonstrable in approximately half of the breast cancers arising in Lynch syndrome mutation carriers using MMR protein immunohistochemistry, thereby confirming breast cancer tissue as a valid screening option for Lynch syndrome diagnosis. A caveat to testing breast cancers for Lynch syndrome however is that, whilst in this study we demonstrated that loss of MMR by IHC is 100% specific for Lynch syndrome, the sensitivity for detecting a mutation carrier is only 51.4%. This compares with a much higher sensitivity for colorectal cancers where MMR proficient phenocopies are relatively rare in mutation carriers.

Further, we found that MMR deficient tumors demonstrate certain histological features significantly more often than would be expected thus increasing confidence in the selection and use of particular breast cancers for this purpose. It is worth noting that, of four cancers that displayed the "*BRCA1*" histological phenotype, two showed loss of MMR proteins suggesting that screening of such cases for Lynch syndrome might be considered where *BRCA1* mutation testing has proved fruitless. The finding that breast carcinoma was the only malignancy reported for half of the women with MMR deficient BCs is consistent with the findings of Jensen *et al.* who reported no other cancer types in three of seven such cases (35).

Translational Relevance

Lynch syndrome predisposes individuals to increased rates of colorectal and endometrial cancer. Lynch syndrome tumors are characterized by loss of DNA mismatch repair proteins, and it is this feature which can be used for recognition of Lynch syndrome amongst incident cancers in a population. Whether breast cancers constitute part of the tumor spectrum in this syndrome, and thus could be used for molecular diagnosis of Lynch syndrome, remains controversial. In this report, we demonstrate that not only do 50% of breast cancers arising in Lynch syndrome mutation carriers show loss of mismatch repair proteins, but also, that they have histological features which further alert pathologists to the possibility that a breast cancer may be arising in a person who has Lynch syndrome. With families becoming smaller the addition of breast cancer to the repertoire of tissues which can be used to identify patients and families at risk is likely to improve detection rates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Cancer Institute, National Institutes of Health under RFA # CA-95-011 and through cooperative agreements: Australasian Colorectal Cancer Family Registry (U01 CA097735) and Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (U01 CA074800). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the CFRs, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the CFR. A portion of archival tissue for this project was obtained from *The Jeremy Jass Memorial Pathology Bank*. The authors are also grateful to the many pathology laboratories involved for supply of extra archived breast tissue for analysis. Thanks are due to Erika Pavluk, Judi Maskiell, Leanne Prior and Maggie Angelakos for data and pedigree retrieval, and to members of the families who have given significant time and effort to the contribution of data. Joanne Young is a Cancer Council Queensland Senior Research Fellow.

Funding: This work was supported by the National Cancer Institute, National Institutes of Health under RFA # CA-95-011 and through cooperative agreements: Australasian Colorectal Cancer Family Registry (U01 CA097735) and Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (U01 CA074800).

Reference List

1. Anonymous. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 2001;358:1389–1399. [PubMed: 11705483]
2. Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer 1996;77:2318–2324. [PubMed: 8635102]
3. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med 2008;359:2143–2153. [PubMed: 19005198]
4. Tan DS, Marchio C, Reis-Filho JS. Hereditary breast cancer: from molecular pathology to tailored therapies. J Clin Pathol 2008;61:1073–1082. [PubMed: 18682420]
5. Claus EB, Risch NJ, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. Am J Epidemiol 1990;131:961–972. [PubMed: 2188501]
6. Jass JR. Hereditary Non-Polyposis Colorectal Cancer: the rise and fall of a confusing term. World J Gastroenterol 2006;12:4943–4950. [PubMed: 16937488]
7. Woods MO, Williams P, Careen A, et al. A new variant database for mismatch repair genes associated with Lynch syndrome. Hum Mutat 2007;28:669–673. [PubMed: 17347989]
8. Müller A, Edmonston TB, Corao DA, et al. Exclusion of breast cancer as an integral tumor of hereditary nonpolyposis colorectal cancer. Cancer Res 2002;62:1014–1019. [PubMed: 11861375]
9. Vasen HF, Morreau H, Nortier JW. Is breast cancer part of the tumor spectrum of hereditary nonpolyposis colorectal cancer? Am J Hum Genet 2001;68:1533–1535. [PubMed: 11359214]
10. de Leeuw WJ, van Puijenbroek M, Tollenaar RA, Cornelisse CJ, Vasen HF, Morreau H, Müller A Correspondence re, et al. Exclusion of breast cancer as an integral tumor of hereditary nonpolyposis colorectal cancer. Cancer Res 2002;62:1014–1019. Cancer Res. 2003;**63**: 1148–9. [PubMed: 11861375]
11. Scott RJ, McPhillips M, Meldrum CJ, et al. Hereditary nonpolyposis colorectal cancer in 95 families: differences and similarities between mutation-positive and mutation-negative kindreds. Am J Hum Genet 2001;68:118–127. [PubMed: 11112663]
12. Oliveira, Ferreira F.; Napoli Ferreira, CC.; Rossi, BM., et al. Frequency of extra-colonic tumors in hereditary nonpolyposis colorectal cancer (HNPCC) and familial colorectal cancer (FCC) Brazilian families: An analysis by a Brazilian Hereditary Colorectal Cancer Institutional Registry. Fam Cancer 2004;3:41–47. [PubMed: 15131405]
13. Shanley S, Fung C, Milliken J, et al. Breast cancer immunohistochemistry can be useful in triage of some HNPCC families. Fam Cancer 2009;8:251–255. [PubMed: 19123071]
14. Westenend PJ, Schutte R, Hoogmans MM, Wagner A, Dinjens WN. Breast cancer in an MSH2 gene mutation carrier. Hum Pathol 2005;36:1322–1326. [PubMed: 16311127]
15. Barrow E, Robinson L, Alduaij W, et al. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin Genet 2009;75:141–149. [PubMed: 19215248]
16. Geary J, Sasieni P, Houlston R, et al. Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (HNPCC). Fam Cancer 2008;7:163–172. [PubMed: 17939062]
17. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus Microsatellite Instability Testing in Phenotyping Colorectal Tumors. J Clin Oncol 2002;20:1043–1048. [PubMed: 11844828]
18. Bergthorsson JT, Egilsson V, Gudmundsson J, Arason A, Ingvarsson S. Identification of a breast tumor with microsatellite instability in a potential carrier of the hereditary non-polyposis colon cancer trait. Clin Genet 1995;47:305–310. [PubMed: 7554364]
19. Boyd J, Rhei E, Federici MG, et al. Male breast cancer in the hereditary nonpolyposis colorectal cancer syndrome. Breast Cancer Res Treat 1999;53:87–91. [PubMed: 10206076]
20. Caluseriu O, Cordisco EL, Viel A, et al. Four novel MSH2 and MLH1 frameshift mutations and occurrence of a breast cancer phenocopy in hereditary nonpolyposis colorectal cancer. Hum Mutat 2001;17:521–525. [PubMed: 11385712]
21. Risinger JI, Barrett JC, Watson P, Lynch HT, Boyd J. Molecular genetic evidence of the occurrence of breast cancer as an integral tumor in patients with the hereditary nonpolyposis colorectal carcinoma syndrome. Cancer 1996;77:1836–1843. [PubMed: 8646682]

22. Siah SP, Quinn DM, Bennett GD, et al. Microsatellite instability markers in breast cancer: a review and study showing MSI was not detected at 'BAT 25' and 'BAT 26' microsatellite markers in early-onset breast cancer. Breast Cancer Res Treat 2000;60:135–142. [PubMed: 10845276]
23. Spagnoletti I, Pizzi C, Galietta A, et al. Loss of hMSH2 expression in primary breast cancer with p53 alterations. Oncol Rep 2004;11:845–851. [PubMed: 15010883]
24. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: An International Resource for Studies of the Genetic Epidemiology of Colon Cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2331–2243. [PubMed: 17982118]
25. Elston CW. The assessment of histological differentiation in breast cancer. A N Z J Surg 1984;54:11–15.
26. Walsh MD, Cummings MC, Buchanan DD, et al. Molecular, pathologic, and clinical features of early-onset endometrial cancer: identifying presumptive Lynch syndrome patients. Clin Cancer Res 2008;14:1692–1700. [PubMed: 18310315]
27. Poynter JN, Siegmund KD, Weisenberger DJ, et al. Molecular Characterization of MSI-H Colorectal Cancer by MLHI Promoter Methylation, Immunohistochemistry, and Mismatch Repair Germline Mutation Screening. Cancer Epidemiol Biomarkers Prev 2008;17:3208–3215. [PubMed: 18990764]
28. Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. J Natl Cancer Inst 1998;90:1138–1145. [PubMed: 9701363]
29. Jönsson M, Johannsson O, Borg Å. Infrequent occurrence of microsatellite instability in sporadic and familial breast cancer. Eur J Cancer 1995;31A:2330–2334. [PubMed: 8652265]
30. Lee SC, Berg KD, Sherman ME, Griffin CA, Eshleman JR. Microsatellite instability is infrequent in medullary breast cancer. Am J Clin Pathol 2001;115:823–827. [PubMed: 11392877]
31. Özer E, Yuksel E, Kizildag S, et al. Microsatellite instability in early-onset breast cancer. Pathol Res Pract 2002;198:525–530. [PubMed: 12389995]
32. Zhou XP, Hoang JM, Li YJ, et al. Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites. Genes Chromosomes Cancer 1998;21:101–107. [PubMed: 9491320]
33. Walsh T, Chappell SA, Shaw JA, Walker RA. Microsatellite instability in ductal carcinoma in situ of the breast. J Pathol 1998;185:18–24. [PubMed: 9713355]
34. Jeon HM, Lynch PM, Howard L, Ajani J, Levin B, Frazier ML. Mutation of the hMSH2 gene in two families with hereditary nonpolyposis colorectal cancer. Hum Mutat 1996;7:327–333. [PubMed: 8723682]
35. Jensen UB, Sunde L, Timshel S, et al. Mismatch repair defective breast cancer in the hereditary nonpolyposis colorectal cancer syndrome. Breast Cancer Res Treat. 2009
36. Stupart DA, Goldberg PA, Algar U, Ramesar R. Cancer risk in a cohort of subjects carrying a single mismatch repair gene mutation. Fam Cancer 2009;8:519–523. [PubMed: 19688281]
37. Schmitt FC, Soares R, Gobbi H, et al. Microsatellite instability in medullary breast carcinomas. Int J Cancer 1999;82:644–647. [PubMed: 10417760]
38. González-Aguilera JJ, Nejda N, Fernández FJ, et al. Genetic alterations and MSI status in primary, synchronous, and metachronous tumors in a family with hereditary nonpolyposis colorectal cancer (HNPCC). Am J Clin Oncol 2003;26:386–391. [PubMed: 12902892]
39. Müller A, Beyser K, Arps H, Bolander S, Becker H, Rüschhoff J. Genotype and phenotype of a new 2-bp deletion of hMSH2 at codon 233. Virchows Arch 2001;439:191–195. [PubMed: 11561760]
40. Yee CJ, Roodi N, Verrier CS, Parl FF. Microsatellite instability and loss of heterozygosity in breast cancer. Cancer Res 1994;54:1641–1644. [PubMed: 8137273]
41. Cederquist K, Emanuelsson M, Wiklund F, Golovleva I, Palmqvist R, Grönberg H. Two Swedish founder MSH6 mutations, one nonsense and one missense, conferring high cumulative risk of Lynch syndrome. Clin Genet 2005;68:533–541. [PubMed: 16283884]
42. Jenkins MA, Hayashi S, O'Shea AM, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterol 2007;133:48–56.
43. Young J, Simms LA, Biden KG, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. Am J Pathol 2001;159:2107–2116. [PubMed: 11733361]

44. Plaschke J, Kruppa C, Tischler R, et al. Sequence analysis of the mismatch repair gene hMSH6 in the germline of patients with familial and sporadic colorectal cancer. Int J Cancer 2000;85:606–613. [PubMed: 10699937]

45. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692–695. [PubMed: 7907678]

46. Cannon-Albright LA, Thomas A, Goldgar DE, et al. Familiality of cancer in Utah. Cancer Res 1994;54:2378–2385. [PubMed: 8162584]

47. Hemminki K, Vaittinen P. Familial risks in in situ cancers from the Family-Cancer Database. Cancer Epidemiol Biomarkers Prev 1998;7:865–868. [PubMed: 9796630]

48. Dong C, Hemminki K. Second primary neoplasms in 633,964 cancer patients in Sweden, 1958–1996. Int J Cancer 2001;93:155–161. [PubMed: 11410860]

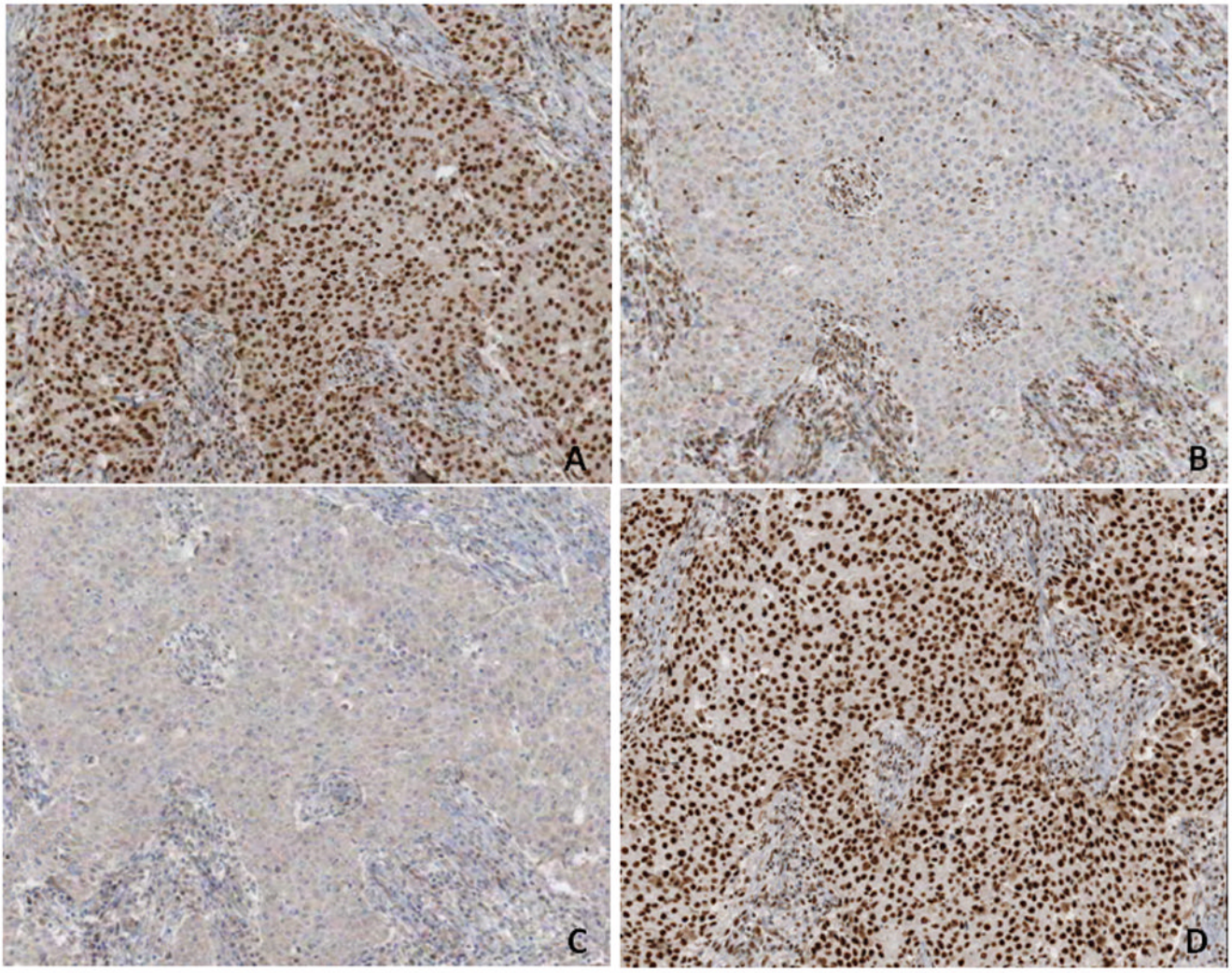

Figure 1.
(A) – (D) illustrate a poorly differentiated ductal carcinoma showing retention of MLH1 (A) and PMS2 (D) expression but loss of MSH2 (B) and MSH6 (C) staining in tumor cells.

Table 1

Characteristics of families related to mutation in a particular MMR gene

Gene	Families	ACII	% Families Meeting ACII Criteria	Breast Cancers	Individuals
MLH1	16	12	75%	19	18
MSH2	30	24	80%	33	32
MSH6	5	4	80%	6	6
PMS2	2	1	50%	3	3
Total Lynch syndrome	53	41	77%	61	59
MLH1 Methylators	7	1	14%	7	7
Other	30	12	40%	39	38
Total Non-Lynch	37	13	35%	46	45

Table 2

Mutation status of individuals with MMR deficient breast cancers

Individual	Sex	IHC absent	Family mutation	Individual status
FN4770-02	F	MLH1 and PMS2	MLH1: c.76C>T p.G26X	POSITIVE
FN2100-09	M	MLH1 and PMS2	MLH1: c.1946delC p.P649fs	POSITIVE
FN3321-01	F	MLH1 and PMS2	MLH1: c.1954G>T p.G652X	POSITIVE
FN3321-10	F	MLH1 and PMS2	MLH1: c.1954G>T p.G652X	POSITIVE
FN1120-34	F	MLH1 and PMS2	MLH1: c.350C>T p.T117M	POSITIVE
FN1710-01	F	MSH2 & MSH6	Not found	*
FN5122-12	F	MSH2 and MSH6	MSH2 del x2-8	POSITIVE
FN6930-01	F	MSH2 and MSH6	MSH2: dup x9-11	POSITIVE
FN4340-01	F	MSH2 and MSH6	MSH2: c.508C>T p.Q170X	POSITIVE
FN1750-01	F	MSH2 and MSH6	MSH2: c.685A>T p.K229X	POSITIVE
FN1010-16	F	MSH2 and MSH6	MSH2: c.892C>T p.G298X	POSITIVE
FN1601-01	F	MSH2 and MSH6	MSH2: c.892C>T p.Q298X	POSITIVE
FN7310-02	F	MSH2 and MSH6	MSH2: c.545+1G>A r.spl.	POSITIVE#
FN4610-14	F	MSH2 and MSH6	MSH2: c.1165C>T p.R389X	POSITIVE
FN5055-01	F	MSH2 and MSH6	MSH2:c.1704_5delAG p.Glu569IlefsX2	POSITIVE
FN3992-11	F	MSH2 and MSH6	MSH2: c.2038C>T p.R680X	POSITIVE
FN6003-02	F	MSH2 and MSH6	MSH2: c.1886_1889delAAGG p.Gln629fs	POSITIVE
FN4430-01	F	MSH6	MSH6: c.1628_1629delAA p.K543fs	POSITIVE

* Multiple affected family members with MSH2/MSH6 deficient tumors but no germline mutation found in either MSH2 or MSH6, and also negative for large rearrangements in *EpCAM (TACSTD1)*

Obligate carrier

Table 3

Multiple MMR deficient tumors in MMR deficient breast cancer patients

Individual	Age (yr)	Tumor site	MMR IHC
FN1601-01	48	**Breast**	MSH2 and MSH6 absent
	75	Meningioma	IHC normal
FN1120-34	54	**Breast**	MLH1 and PMS2 absent
	58	Endometrial	MLH1 and PMS2 absent
	60	Colorectal	MLH1 and PMS2 absent
	64	Stromal sarcoma (post XRT)	PMS2 only absent
FN5055-01	49	**Breast**	MSH2 and MSH6 absent
	51	Sebaceomas	MSH2 and MSH6 absent
	52	Endometrial	MSH2 and MSH6 absent
FN1750-01	40	Colorectal	MSH2 and MSH6 absent
	51	**Breast #1**	MSH2 and MSH6 absent
	57	**Breast #2**	Not tested
FN4610-14	43	Colorectal	MSH2 and MSH6 absent
	50	Colorectal	MSH2 and MSH6 absent
	65	**Breast**	MSH2 and MSH6 absent
FN4340-01	55	Colorectal	MSH2 and MSH6 absent
	56	**Breast**	MSH2 and MSH6 absent
FN4770-02	53	Endometrial	MLH1 and PMS2 absent
	63	Colorectal	MLH1 and PMS2 absent
	64	**Breast**	MLH1 and PMS2 absent
FN7310-02	61	Endometrial	MSH2 and MSH6 absent
	66	**Breast**	MSH2 and MSH6 absent
FN6003-02	52	Colorectal	MSH2 and MSH6 absent
	55	Endometrial	MSH2 absent
	64	**Breast**	MSH2 and MSH6 absent
FN5122-12	33	Endometrial	Not tested
	55	Colorectal	Not tested
	56	Colorectal	Not tested
	62	Colorectal	Not tested
	63	Colorectal	MSH2 and MSH6 absent
	75	**Breast**	MSH2 and MSH6 absent
	75	Bladder	MSH2 and MSH6 absent

Table 4

Histological features in MMR deficient vs. MMR proficient invasive breast cancers

	MMR deficient	MMR intact	p-value*	OR	95% CI
Size (>20mm)	8/15 (50%)	26/73 (36%)	0.248	2.07	0.67 – 6.34
Grade 3 (poorly differentiated)	**9/15 (60%)**	**17/78 (22%)**	***0.005***	***5.38***	***1.68 – 17.25***
Poor tubule formation (<10%)	**14/15 (93%)**	**48/78 (62%)**	***0.017***	***8.75***	***1.09 – 70.00***
Marked nuclear pleiomorphism	9/15 (60%)	28/78 (36%)	0.093	2.68	0.86 – 8.31
High mitotic index (=3)	**8/15 (53%)**	**11/78 (14%)**	***0.002***	***6.96***	***2.10 – 23.07***
Growth in solid sheets (>25%)	**8/15 (53%)**	**3/77 (4%)**	***<0.001***	***28.19***	***6.06 – 131.06***
Pushing margin present	**5/15 (33%)**	**9/76 (12%)**	***0.05***	***3.72***	***1.04 – 13.38***
Vesicular nuclei	5/15 (33%)	11/77 (14%)	0.128	3.00	0.86 – 10.46
Eosinophilic nucleoli	4/15 (27%)	11/77 (14%)	0.258	2.18	0.59 – 8.09
Confluent necrosis	**6/15 (40%)**	**5/76 (7%)**	***0.002***	***9.47***	***2.40 – 37.43***
Calcification	3/15 (20%)	26/78 (33%)	0.376	0.86	0.10 – 7.68
Lymphovascular invasion	0/14 (0%)	15/75 (20%)	0.115	0.80	0.71 – 0.90
Lymph node metastases	3/16 (19%)	20/53 (38%)	0.229	0.38	0.10 – 1.50
Discernible cell borders	1/14 (7%)	10/77 (13%)	1.000	0.52	0.06 – 4.38
TILs	6/15 (40%)	16/77 (21%)	0.182	2.54	0.79 – 8.20
PTLs	**9/15 (60%)**	**20/77 (26%)**	***0.015***	***4.28***	***1.35 – 13.52***
ER Positive	**5/12 (42%)**	**44/57 (77%)**	***0.031***	***0.21***	***0.06 – 0.78***
PR Positive	**4/12 (33%)**	**36/51 (71%)**	***0.022***	***0.21***	***0.05 – 0.80***
p53 Positive	3/10 (30%)	10/57 (18%)	0.394	2.01	0.44 – 9.16
HER2/*neu* Positive	0/5 (0%)	5/21 (19%)	0.555	0.76	0.60 – 0.97
Contiguous *in situ* carcinoma	**5/14 (36%)**	**52/78 (67%)**	***0.038***	***0.28***	***0.08 – 0.91***

TILs: Tumor infiltrating lymphocytes

PTLs: Peritumoral lymphocytes

*Fisher's exact test

Table 5

Histological features in MMR deficient vs. MMR proficient invasive breast cancers from MMR germline mutation carriers

	MMR deficient	MMR intact	p-value*	OR	95% CI
Size (>20mm)	8/15 (53%)	6/13 (46%)	1.000	1.33	0.30 – 5.91
Grade 3 (poorly diff.)	9/15 (60%)	6/15 (40%)	0.466	2.25	0.52 – 9.70
Poor tubule formation (<10%)	14/15 (93%)	11/15 (73%)	0.330	5.10	0.50 – 52.29
Marked nuclear pleiomorphism	9/15 (60%)	6/15 (40%)	0.466	2.25	0.52 – 9.70
High mitotic index (=3)	8/15 (53%)	3/15 (20%)	0.128	4.57	0.90 – 23.14
Growth in solid sheets (>25%)	**8/15 (53%)**	**0/14 (0%)**	***0.002***	***2.14***	***1.25 – 3.68***
Pushing margin present	**5/15 (33%)**	**0/14 (0%)**	***0.042***	***1.50***	***1.05 – 2.14***
Vesicular nuclei	5/15 (33%)	2/15 (13%)	0.390	3.25	0.52 – 20.37
Eosinophilic nucleoli	4/15 (27%)	4/15 (27%)	1.000	1.00	0.20 – 5.05
Confluent necrosis	**6/15 (40%)**	**0/14 (0%)**	***0.017***	***1.67***	***1.10 – 2.52***
Calcification	3/15 (20%)	4/15 (27%)	1.000	0.69	0.13 – 3.79
Lymphovascular invasion	0/14 (7%)	1/14 (7%)	1.000	0.93	0.80 – 1.07
Lymph node metastases	3/16 (19%)	4/11 (36%)	0.391	0.40	0.07 – 2.34
Discernible cell borders	1/14 (7%)	0/15 (0%)	0.483	1.08	0.93 – 1.25
TILs	6/15 (40%)	5/15 (33%)	1.000	1.33	0.30 – 5.92
PTLs	9/15 (60%)	6/15 (40%)	0.466	2.25	0.52 – 9.70
ER Positive	5/12 (42%)	9/13 (69%)	0.238	0.32	0.06 – 1.64
PR Positive	4/12 (33%)	8/12 (67%)	0.220	0.25	0.05 – 1.37
p53 Positive	3/10 (30%)	1/10 (10%)	0.582	3.86	0.33 – 45.57
HER2/*neu* Positive	0/6 (0%)	1/5 (20%)	0.455	0.80	0.52 – 1.24
Contiguous *in situ* carcinoma	5/14 (36%)	13/14 (93%)	***0.004***	***0.04***	***0.00 – 0.43***

TILs: Tumor infiltrating lymphocytes

PTLs: Peritumoral lymphocytes

*Fisher's exact test