Title

Morphological and genomic characteristics of breast cancers occurring in individuals with Lynch Syndrome

Journal

Clinical Cancer Research, 28(2)

ISSN

1078-0432

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Publication Date

2022-01-15

DOI

10.1158/1078-0432.ccr-21-2027

Peer reviewed

HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2022 June 15.

Published in final edited form as:

Clin Cancer Res. 2022 January 15; 28(2): 404–413. doi:10.1158/1078-0432.CCR-21-2027.

Breast cancer in Lynch Syndrome patients: genomic and pathologic evidence in support of a potential causal relationship

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AUTHOR CONTRIBUTIONS

CJS & HZ conceived the work. Conceptualization and methodology: All authors. Funding acquisition: FP, BW and JSR-F. Supervision: JS, JSR-F and HZ. Data curation and formal analysis: CJS, EMdS, AM, AMG, PS, VR, DM & JS. Writing – original draft: CJS, JSR-F and HZ. Writing – review & editing: All authors.

Conflict of Interest

JSR-F reports receiving personal/consultancy fees from Goldman Sachs, REPARE Therapeutics, Paige.AI and Eli Lilly, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis, Genentech and InVicro, outside the scope of this study. JZD reports receiving honorarium from OncLive. P.R. Received institutional grant/funding from Grail, Illumina, Novartis, Epic Sciences, ArcherDx and Consultation/Ad board/Honoraria from Novartis, Foundation Medicine, AstraZeneca, Epic Sciences, Inivata, Natera, and Tempus. HZ receives consultancy fees from Roche/Genetech. All remaining authors have declared no conflicts of interest.

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Abstract

Purpose: Lynch syndrome (LS) is defined by germline pathogenic mutations involving DNA Mismatch Repair (MMR) genes and linked with the development of MMR-deficient (MMRd) colon and endometrial cancers. Whether breast cancers (BC) developing in context of LS are causally related to MMR deficiency (MMRd), remains controversial. Thus, we explored the morphological and genomic characteristics of BCs occurring in LS individuals.

Experimental Design: A retrospective analysis of 20,110 cancer patients who underwent multigene panel genetic testing was performed to identify individuals with a likely pathogenic/pathogenic germline variant in *MLH1*, *MSH2*, *MSH6* or *PMS2* who developed BCs. The histological characteristics and immunohistochemical (IHC) assessment of BCs for MMR proteins and programmed death-ligand 1 (PD-L1) expression were assessed on cases with available materials. DNA samples from paired tumors and blood were sequenced with MSK-IMPACT (≥468 key cancer genes). MSI status was assessed utilizing MSISensor. Mutational signatures were defined using SigMA.

Results: 272 LS individuals were identified, 13 (5%) of whom had primary BCs. The majority of BCs (92%) were hormone receptor positive tumors. Five (42%) of 12 BCs displayed loss of MMR proteins by IHC. Four (36%) of 11 BCs subjected to tumor-normal sequencing showed dominant microsatellite instability mutational signatures, high tumor mutational burden and indeterminate (27%) or high MSISensor scores (9%). One patient with metastatic MMRd BC received anti-PD1 therapy and achieved a robust and durable response.

Conclusions: A subset of BCs developing in LS individuals are etiologically linked to MMRd and may benefit from anti-PD1/PD-L1 immunotherapy.

Statement of translational relevance:

The association of Lynch syndrome (LS) and breast cancer has been a widely debated and controversial topic in past decades. Here we characterized the pathologic and genomic features of breast cancers (BCs) occurring in LS patients and investigated whether these breast cancers are casually linked to the mismatch repair deficiency (MMRd) that defines LS-related cancers. Out of 20,110 cancer patients who underwent multigene genetic testing on tumor and matched normal DNA samples, 272 LS individuals were identified, 13 of whom had primary breast cancers. Five of these 13 BCs displayed MMRd by immunohistochemistry and/or dominant MSI-related mutational signatures as defined by tumor-normal sequencing. One patient with widely metastatic ER positive/HER2 negative MMRd BC achieved a robust and durable response upon treatment with anti-PD1 immunotherapy. Although LS-associated BCs are rare, this study highlights a subset that are etiologically linked to underlying MMRd and may be candidates for immunotherapy.

Keywords

breast cancer; microsatellite instability; mismatch repair; Lynch syndrome; genomics; immunohistochemistry; immunotherapy

INTRODUCTION

Lynch syndrome (LS) is a cancer predisposition syndrome characterized by germline mutations in major DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6* or *PMS2*) and associated with a significantly increased risk of developing colorectal, endometrial, ovarian, small bowel and ureteral cancers (1-3). The hallmark of LS spectrum cancers is defective DNA mismatch repair (MMRd) and high levels of microsatellite instability (MSI-H), which occur upon loss of the expression of MMR genes through a germline mutation and inactivation of the second allele through somatic mutation or loss-of-heterozygosity, and, more rarely, due to *MLH1* gene promoter methylation.

Whether LS individuals are at increased risk of developing breast cancer (BC) remains controversial (2, 4-12). Although previous studies have suggested a lack of association between pathogenic germline variants affecting MMR genes and breast cancer risk (7, 11, 12), whether breast cancers occurring in LS individuals are causally related to the genetic alteration of MMR genes is unclear (2). Loss of MMR protein expression has been reported in up to 50% of BCs from LS patients (8, 9). Davies et al. reported 2 of 11 patients with MMRd BCs identified by whole genome sequencing occurring in LS patients (10). Other studies have found no evidence of MMRd in breast tumors derived from LS patients or individuals with synchronous or metachronous breast plus colorectal cancer (11). In fact, a recent pan-cancer survey of over 15,000 tumors subjected to Memorial Sloan Kettering Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) found no evidence of MMRd BC occurring in LS patients (12).

Defining whether breast cancers developing in the context of LS display MMRd is not a mere academic exercise, given that MSI-high has been approved as a pan-cancer biomarker of clinical benefit from immune check-point inhibitors (13). Hence, the identification of MMRd/MSI-high BCs in LS patients has a direct therapeutic implication. Here, we sought to characterize the pathologic and genomic features of BCs occurring in LS patients and to explore whether these BCs would be causally linked to the MMRd characteristic of LS-related cancers. By expanding on the analysis by Latham et al. (12), including ≥20,000 patients subjected to multigene germline genetic testing to identify primary breast cancers in LS patients and focusing on the histologic, immunohistochemical and genomic features of these cancers, we provide direct evidence supporting MMRd/MSI-high in a substantial subset of breast cancers in patients with LS.

METHODS

Study population

The cohort consisted of 20,110 cancer patients, who underwent multigene panel genetic testing for germline mutations at Memorial Sloan Kettering Cancer Center (MSKCC) from 2015 to present day, as previously described (14). The cohort includes 3583 patients reported previously from January 2014 to June 2017 by Latham et al (12), and additional 16,527 unique patients derived from the germline sequencing pool. The tumor somatic alterations by MSK-IMPACT with > 468 gene panels of the identified BCs were also analyzed.

Individuals with likely pathogenic/pathogenic germline variant in *MLH1*, *MSH2*, *MSH6* or *PMS2* who developed BCs were retrospectively identified. The genetic testing was performed in a CLIA certified laboratory using tumor/normal sequencing. All germline LS variants for patients in this cohort were reviewed by a board-certified molecular pathologist (D.L.M.) and classified according to the American College of Medical Genetics and Genomics (ACMG) criteria (15) as likely pathogenic/pathogenic.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC). Every patient included in this study signed a written informed consent form according to the protocol approved by the MSKCC IRB.

Study design and immunohistochemistry analysis

Histology of all breast cancers were reviewed by two pathologists (C.J.S. & H.Z.) and graded according to the Nottingham grading index (16). Clinicopathologic data were retrieved from the electronic medical records and curated by C.J.S.

Representative formalin-fixed paraffin embedded (FFPE) tissue blocks of invasive carcinomas from patients with Lynch Syndrome (LS) were retrieved from the archive at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY). Estrogen receptor (ER) and human epidermal growth factor-2 (HER2) status were assessed by immunohistochemistry (IHC) and/or fluorescence *in situ* hybridization (FISH) as previously described (17), following the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline recommendations (18,19). Immunohistochemical staining for mismatch repair proteins (MLH1, PMS2, MSH2 and MSH6) were performed according to methods previously described (20). Positive and negative controls, including internal positive controls, were assessed in every run and every case, respectively. Tumors with positive nuclear expression were classified as normal/retained MMR protein expression. All immunohistochemical reactions were reviewed by 3 pathologists (C.J.S., J.S. & H.Z.). PDL-1 (SP142) IHC was performed using Ventana Benchmark Ultra System (Ventana Medical Systems, Tucson, AZ, USA) with antibody detection using the OptiView DAB IHC Detection Kit (Ventana Medical Systems), according to the manufacturer's manual (21). PD-L1 was assessed by two pathologists (C.J.S. & H.Z.) and scored as expression on stromal tumor-infiltrating immune cells occupying ≥1% of the tumor area as previously described (22,23).

Tumor infiltrating lymphocytes (TILs)

All LS breast cancers were evaluated for the presence of stromal TILs following the recommendations by the International TILs Working Group 2014 (24). Three pathologists (C.S., H.W., and H.Z.) quantified the percentage of stromal area within the borders of the invasive tumor covered by TILs, and the average was reported. TILs outside of the tumor border, around ductal carcinoma *in situ* (DCIS) and normal lobules and in tumor zones with crush artifacts, necrosis and regressive hyalinization were excluded.

Massively parallel sequencing

Adequate tumor and normal tissue samples were available from 11 patients and underwent targeted massively parallel sequencing (n=11) using the FDA-cleared MSK-IMPACT assay, which targets all exons and selected introns of 468 (n=7) or 505 (n=4) cancer genes as previously described (25). Of the 11 cases, 8 had sequencing performed at the time of diagnosis, and 3 were among the patients identified from the germline mutation database and were sequenced retrospectively at the time of this study.

In brief, somatic single nucleotide variants (SNVs) were detected using MuTect (v1.17) (26) and small insertions and deletions (indels) were identified using a combination of Strelka (v1.0.15) (27), VarScan2 (v2.3.7) (28), Lancet (v1.0.0) (29) and/or Scalpel (v0.5.3) (30) with further curation using visual inspection as previously described (31). Copy number alterations (CNAs) in the tumors were identified using FACETS (32) as previously described (31). Mutational hotspots were classified according to Chang et al (33).

Analysis of microsatellite instability (MSI) by PCR

Analysis of MSI status by PCR in tumors (n=11) was performed using the MSI Analysis System, version 1.2 kit (Promega, Madison, WI). In brief, this assay analyzes five mononucleotide microsatellite loci, including NR-21, BAT-25, MONO-27, NR-24, and BAT-26, in both tumor and normal DNA. A ≥ 3 base pair shift in the tumor DNA relative to the matched normal tissue constituted instability at one locus. Instability at ≥2 of the 5 microsatellite loci defines MSI-H status, with fewer than two unstable loci classified as microsatellite stable (MSS), according to methods described previously (34). One microliter of amplified PCR product was applied to the Applied Biosystems 3730 DNA Analyzer. Automatic fragment analysis was carried out with GeneMapper 6.0 Software (Applied Biosystems).

Mutational signatures

The mutational signatures exposures were obtained using Signature Multivariate Analysis (SigMA), a tool for the decomposition of mutational signatures that can be optimized for the analysis of targeted capture sequencing panel results obtained from formalin-fixed paraffin embedded tissue samples. It has been demonstrated that when only a few single nucleotide variants per sample are available, SigMA outperforms other state of the art methods (35). SigMA computes signature exposures for samples with at least 5 single nucleotide variants, while other tools (e.g. DeconstructSig) require ≥10 variants (36). The SigMA was set with customized settings (i.e. data type: msk, cancer type: breast, check msi: true). The exposures obtained were converted using the NNLS algorithm by SigMA into percentages per signature as previously described (36). Additional analysis was performed using the tool SigProfiler, another state-of-the-art tool that identifies the probability for each signature to cause a specific mutation type in a cancer sample. The python package SigProfilerPlotting was used for the visualization of the mutational spectrum (figure 2G) (37).

Comparison of LS sequenced breast cancers to matched sequenced breast cancers

ER-positive/HER2 negative (n=9), ER-positive/HER2 positive (n=1), and ER-negative/HER2-negative (n=1) LS BCs were compared to 1,918 BCs subjected to MSK-IMPACT

targeted sequencing from the study by Razavi et al (38). Tumors were matched by hormone receptor (HR) status, tumor type (invasive ductal (n=9) or lobular carcinoma (n=2), respectively), age (10-year intervals) and menopausal status at a 1:3 ratio (n=33), as previously described (31). Comparison of frequencies of genes altered by somatic genetic alterations between LS BCs (n=11) and control-matched BCs (n=33) was performed using Fisher's exact test. Multiple testing correction using the Benjamini-Hochberg method was applied to control for the false discovery rate whenever appropriate. Tumor mutational burdens (TMB) and MSISensor scores were compared between LS BCs (n=11) and control-matched BCs (n=33) by the Mann-Whitney *U* test. Dominant mutational signatures of sequenced tumors with at least 5 SNVs were compared between LS BCs (n=9) and control-matched BCs (n=16) by Fisher's exact test. *P* values < 0.05 were considered significant. Statistical analysis was performed using R (version 3.1.2).

RESULTS

Clinical characteristics of study cohort

Two hundred seventy-two (1.4%) MMR gene pathogenic variants indicating Lynch syndrome (LS) were identified from the total cohort of 20,110 patients. This included 57 (21%), 96 (35%), 63 (23%) and 56 (21%) variants affecting *MLH1*, *MSH2*, *MSH6* and *PMS2*, respectively. Thirteen patients in this cohort (5%) had been diagnosed with primary BCs (Table 1), all of whom had confirmatory germline testing using a multigene inherited cancer susceptibility panel comprised of 76 (n=1) or 88 genes (n=12). Two of these patients (cases 2 and 3) overlapped with a previous study (14).

Of the thirteen patients, pathogenic germline variants were identified in *MLH1* (n=2, 15%), *MSH2* (n=2, 15%), *MSH6* (n=6, 46%) and *PMS2* (n=3, 23%). Seven patients (54%) had BCs as their sole malignancy, five patients (38%) had other LS-related cancers, and one patient was diagnosed with synchronous uterine and breast carcinomas (8%, Table 1). Eleven patients (84%) had a family history of LS-related neoplasia. The median age of breast cancer diagnosis was 47 years (range 33-74).

Tumor characteristics and clinical follow-up

Histologically, ten BCs (77%) were invasive ductal carcinomas and three (23%) were invasive lobular carcinomas (Table 2). All BCs were of histologic grade 2 (38%) or 3 (62%). The median tumor size was 2.6cm (range 1.0-10.0) Of the thirteen tumors, eleven tumors (84%) were estrogen receptor (ER)-positive/HER2-negative, one tumor (case 8) was ER-positive/HER2-positive, and the remaining one tumor (case 7) was ER-negative/HER2-negative. Stromal TILs ranged from 1% to 25%. PDL-1 (SP142) staining was positive (>1%) in 5 of 9 tumors (55%, Table 2).

In terms of locoregional disease control, 69% of patients received total mastectomy and 15.5% were treated with breast conservation therapy. The remaining 15.5% had metastatic disease at presentation. The median follow-up is 36 months (range 3-240, Table 2). At the time of study, the majority of patients were either alive with disease (46%) or dead of disease (15%). The remaining cohort showed no evidence of disease (39%) (Table 2).

Immunohistochemical evidence of MMRd

We next sought to define whether BCs developing in the context of LS germline variants would harbor the hallmark features of MMRd. Immunohistochemical analysis (IHC) of 12 BCs (Table 1) with available material revealed loss of MMR proteins in 5 cases (42%), all of which are all ER-positive/HER-2 negative BCs. Case 4, harboring an *MLH1* c.954delC frameshift deletion, demonstrated loss of MLH1 and PMS2 with partial loss of MSH6 protein (Figure 1A-1F). Loss of MLH1 and PMS2 protein was also observed in case 13, harboring an *MLH1* deletion. Deficiency of MSH2 and MSH6 protein was seen in two cases (cases 10 and 11), with missense mutations in *MSH2*. Isolated protein loss of MSH6 was observed in case 6, harboring a frameshift mutation resulting in a premature termination codon 12 amino acids downstream of p. Leu1330Valfs*12.

Genomic evidence of MMRd

MSK-IMPACT analysis of 11 BCs revealed a repertoire of somatic mutations consistent with that reported for invasive breast cancers, including *TP53* (54%), *PIK3CA* (45%) and *GATA3* (27%, Figure 1G and Supplementary Table S1). Three of four BCs demonstrated MMR protein loss by IHC showed two hits in the proposed LS gene. Two cases (cases 4 and 13) showed loss of heterozygosity in an MMR gene, whereas case 6 harbored a second somatic inactivating mutation in *MSH6* (p. Phe1088Leufs, Figure 1G). Case 10 (germline *MSH2*) lacked evidence of biallelic inactivation despite loss of MSH2 and MSH6 proteins by IHC. Thus, inactivation of the gene may be the result of another inactivating regulatory process such as somatic hypermethylation, which has been reported to occur in LS individuals with *MSH2* germline variants (39).

Of the four cases demonstrating MMR protein loss by IHC with adequate materials for sequencing, three showed evidence of MSI by PCR analysis of 5 microsatellite loci and one yielded an inconclusive result (Figure 1G and Table 3). Three of four cases displayed high tumor mutational burden (i.e. > 10 mutations per Mb) (40) and either indeterminate (3 cases) or high (1 case) MSISensor scores (Figure 1G and Table 3). The sole case (case 10) deemed MSI-H (score of > 10 by MSISensor) is a 63-year-old female with a missense mutation in the *MSH2* gene. The tumor was also MSI by PCR (Table 3). Both patient and family members had history of LS-related cancers. The tumor was a moderately differentiated ER-positive/HER2-negative invasive ductal carcinoma with low TILs (2%). Immunohistochemical analysis for PD-L1 was negative. The patient underwent breast conservation surgery followed by radiation therapy and hormonal therapy. The patient currently has no evidence of disease after 144 months of clinical follow-up.

Nine cases were found to have a least 5 single nucleotide variants (81%) and were subjected to mutational signature analysis with SigMA. All cases with MMR protein loss and indeterminate or high MSISensor scores demonstrated dominant microsatellite instability signatures. Notably, three of these MMRd BCs displayed expression of PD-L1 (>1%) in the tumor-associated inflammatory cells with variable percentages of tumor stromal infiltrating lymphocytes (mean 14.3, range 2-25%, Figure 1G).

Given the higher frequency of MMRd/MSI (4/11, 36%) observed in the LS BC cohort, and prior observations of MMRd in <2% of all BCs (10), we compared the TMBs, MSISensor scores and dominant mutational signatures to a 1:3 cohort of BCs (n=33) matched according to age, tumor type, HR profile and menopausal status, respectively (Supplementary Table S2). BCs in the LS cohort (n=11) displayed a higher TMB (p value = 0.003, Mann-Whitney U test, Supplementary Figure 1A), had MSI scores showing a positive trend toward significance (p value = 0.069, Mann Whitney U Test, Supplementary Figure 1B) when compared to control-matched BCs (n=33). We also observed an enrichment in dominant MSI mutational signatures in tumors harboring at least 5 SNVs were also enriched in LS BCs (n=11) relative to control-matched BCs (n=16, p value=0.040, Fisher's exact test, Supplementary Figure 1C). We next compared the genomic characteristics of LS BCs relative to the control-matched BC cohort (Supplementary Figure 2). No statistically significant differences in single gene comparisons were identified. Given the small sample size of LS BCs included in this study, further analyses to define differences in mutational frequencies between LS BCs and non LS BCs are warranted.

In summary, 5 of 13 cases (38.5%) with tissue available for immunohistochemistry and/or MSK-IMPACT displayed features of MMRd or MSI-H (Summarized in Table 3).

Immunotherapy in LS patient with metastatic MMRd BC

Given that MSI-high has been approved as a pan-cancer biomarker of benefit from check-point inhibitors (41, 42) we queried if any LS patients with MMRd tumors had received immunotherapy. A 64-year-old female (case 6), with a germline *MSH6* frameshift mutation and remote history of an ER-positive BC 18 years prior, developed a contralateral ER-positive/HER2-negative/MMRd/PD-L1 positive BC with synchronous lymph node and extensive bone metastases involving both the pelvis and lumbar spine (Figures 2A-2F). Anti-PD1 therapy (Pembrolizumab) was initiated in the palliative setting with complete pathologic response at 5 months on PET imaging (Figure 2G). The patient has no radiologic evidence of disease 13 months after initiation of therapy.

DISCUSSION

We explored the clinical, morphological, and genomic characteristics of breast cancers occurring in LS individuals from a single tertiary cancer center. Our study revealed 5 out of 13 BCs in LS individuals demonstrating MMRd by either immunohistochemistry, PCR, and/or multigene panel studies. We were able to identify these BCs using a large sequencing pool from >20,000 patients who underwent germline testing at MSKCC. The majority were ER-positive/HER2 negative (85%), moderately to poorly differentiated BCs (100%). At a median follow up of 36 months, the patients were AWD (2/5) or NED (3/5). One patient with advanced MMRd BC and distant metastasis demonstrated durable and complete clinical response to immunotherapy administered in the palliative setting and currently is NED after 13 months of the initiation of immunotherapy.

Our findings provide direct evidence that a subset of primary BCs developing in the context of a LS germline pathogenic variant harbors features of MMRd, including MSI-high, in line with the previous observations showing MMRd BCs in the context of LS (8,10). MSI-high

has been approved as a pan-cancer biomarker of benefit from immune check-point inhibitors (41, 42). Consistent with the notion that some BCs developing in the context of LS germline pathogenic variants are bona fide MSI-high cancers, benefit from immune check-point inhibition was observed in case 6, as expected. Further studies beyond the case highlighted here are warranted.

MSISensor scores of ≥ 10 have been shown to reliably identify MSI-H status in solid tumors from various primary sites (34, 43). Applying this criterion in identifying MSI-H breast cancers has been explored. Latham et al (12) identified the Lynch Syndrome in 15,045 patients who had matched tumor/normal DNA sequencing encompassing over 50 cancer types through MSK-IMPACT platform in MSKCC from January 1, 2014 to June 30, 2017. They identified LS in 0.3% (7 out of 2,371) of breast cancer patients and all 7 cases were microsatellite stable (MSS) tumor based on MSISensor scores of < 3. In our current study, we identified 272 LS individuals among 20,110 cancer patients who had consecutive genetic testing performed through MSKCC clinical pipeline with a cutoff date of November 30, 2020. Only 3,583 patients included in our study had the clinical genetic testing performed before the cut-off date of June 30, 2017 employed in Latham's study, representing the overlapping patient population between these two cohorts (up to 3,583 out of 20,110; 18%). Two patients in the population included in both studies had developed primary breast cancers (cases 2 and 3 in our study) showing no evidence of MMRd, Including the overlapping 2 patients included in Latham's and additionally identified 11 patients unique to our study, 5 patients were identified to have MMRd breast cancers based on loss of MMR proteins by IHC (5/13) and/or tumor-normal sequencing studies on 11 cases with available tissue materials (4/11) showing dominant microsatellite instability mutational signatures, high tumor mutational burden and indeterminate or high MSISensor scores. Of the nine cases subjected to mutational signature analysis by SigMA, the four MMRd breast cancers harbored 57, 52, 45, and 17 SNVs, well above the 5 SNV threshold required for analysis (35). These cases were found to harbor a dominant MSI mutational signature and a mutational spectrum consistent with that of MSI-H cancers (i.e. mutational signatures 20 and 21). Only 1 MMRd breast cancer (case #10) in our study had a MSISensor score of >10, suggesting that MMRd BCs may have a lower MSISensor threshold than other MSI/MMRd cancers, such as colorectal and endometrial cancers, for which the assay was initially intended to identify (34). In line with the study from Latham et al. (12), our results suggest that further evaluation might be indicated to determine whether current MSISensor cut-off values can faithfully identify MMRd/MSI-H BCs.

This study has limitations. Despite the large cohort of patients subjected to tumor-normal MSK-IMPACT sequencing, the sample size of BCs developing in the context of LS was small, consistent with the rarity of MMRd and/or MSI-high in primary BCs. MMRd breast cancer is reported to represent around 1.7% of all BCs, with a much smaller fraction involving LS individuals (10). Signature analysis could only be performed in 9 out of 11 patients, given that only MSK-IMPACT sequencing was performed. Mutational signature decomposition when the number of SNVs is low (e.g. in the case of cancers subjected to MSK-IMPACT containing 5-10 somatic SNVs) may have suboptimal robustness. Although a substantial proportion of breast cancers occurring in LS patients likely constitute sporadic breast cancers developing in the context of LS, whole-genome sequencing, to ascertain the

frequency of MMRd mutational signatures, as well as MMR immunohistochemical analysis, to define the loss of MMR proteins, of consecutive breast cancers from LS patients is warranted.

Although our study is not intended to address if BC belongs in the spectrum of LS-related cancers, it is important to note that at the population level, there appears to be no increased risk for breast cancer in LS individuals with *MLH1*, *MSH2* and *PMS2* variants (44, 45). Individuals with *MSH6* variants may confer a slightly higher risk of developing BC, however significance was not achieved in a large case-control study (44). Currently, BCs are not considered LS-associated cancers per current NCCN guidelines due to confounding data regarding risk in LS patients (46), and BCs developing in the context of LS are currently not included in the special management of hereditary breast cancers (47). It is plausible that the remaining tumors in our cohort likely correspond to sporadic BCs arising in LS individuals, as previously reported (48). With recent studies supporting universal genetic testing for breast cancer patients ≤60 (49), it remains uncertain if MMR genes should be added to the testing panel. Our results emphasize, however, that in up to four in ten LS patients who develop breast cancers, their tumors may be etiologically linked with the MMRd caused by the pathogenic germline variant affecting *MLH1*, *MSH2*, *MSH6* or *PMS2* genes. These findings support the contention that MMR immunohistochemical analysis and/or MSI assessment by PCR or multigene sequencing should be performed in the tumor specimens from LS patients who develop breast cancer.

In conclusion, we demonstrated pathologic and genomic features of MMRd/MSI-high in a subset of breast cancers arising in LS patients, establishing a likely etiologic link between the germline alteration and hallmark features of MMRd/MSI in this context. BCs occurring in LS should be tested for MMRd by either IHC or genomic sequencing to identify the patients who may benefit from immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was funded in part by the Marie-Josée and Henry R. Kravis Center for Molecular Oncology and the National Cancer Institute Cancer Center Core Grant No. P30-CA008748. F.P. is partially funded by an NIH K12 CA184746 grant. FP, BW and JSR-F are funded in part by the NIH/NCI P50 CA247749 01 grant. E.M. dS is partially funded by the MSK-MIND grant. JSR-F is funded in part by a grant from the Breast Cancer Research Foundation.

The funders had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript.

Data availability statement

Data are available in a repository (cBioPortal for Cancer Genomics) and can be accessed via a DOI link.

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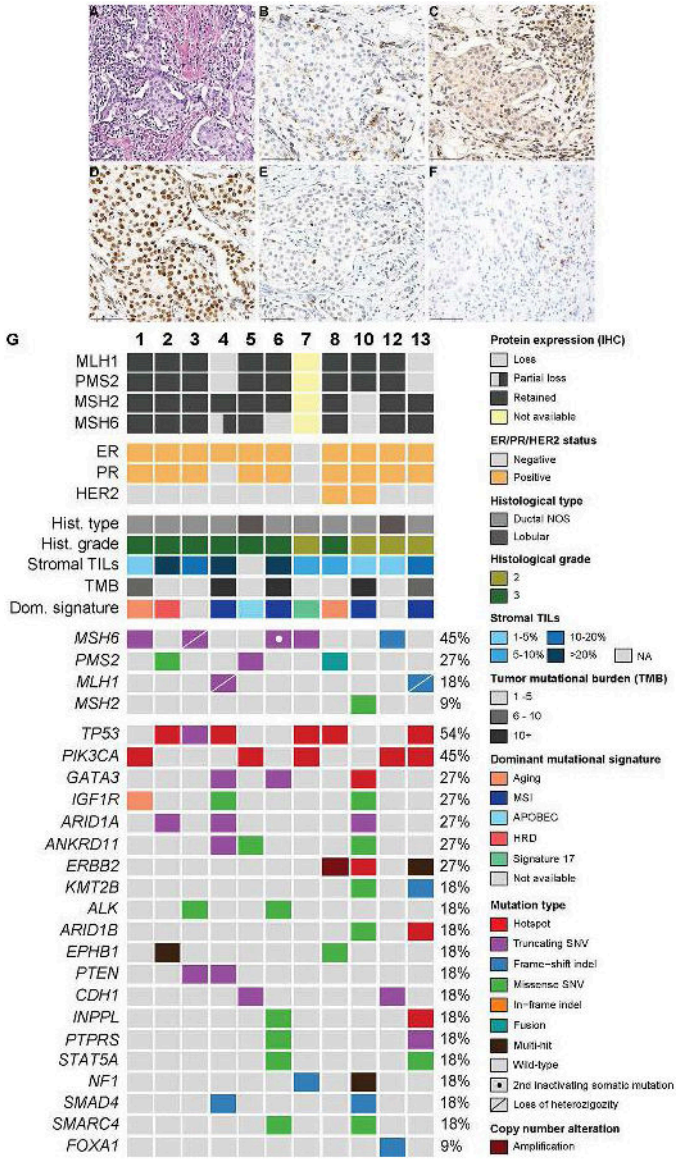

Figure 1. Clinicopathologic and genomic characterization of breast cancers (BCs) in patients with Lynch Syndrome (LS) germline pathogenic variants.

(A) representative photomicrographs of Case 4, including immunohistochemical analysis of MLH1 **(B)**, PMS2 **(C)**, MSH2 **(D)**, MSH6 **(E)** and PD-L1 (SP142) **(F)**. Scale bars=100 μM. **(G)**, non-synonymous somatic mutations and gene copy number alterations identified by MSK-IMPACT targeted sequencing. Cases are shown in columns and genes in rows. Phenobars (top) provides information about DNA mismatch repair (MMR) protein expression, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status, histologic type, histologic grade, stromal tumor infiltrating-lymphocytes (TILs), tumor mutational burden (TMB) and dominant mutational signatures. Germline and somatic mutations are color-coded according to the legend (right).

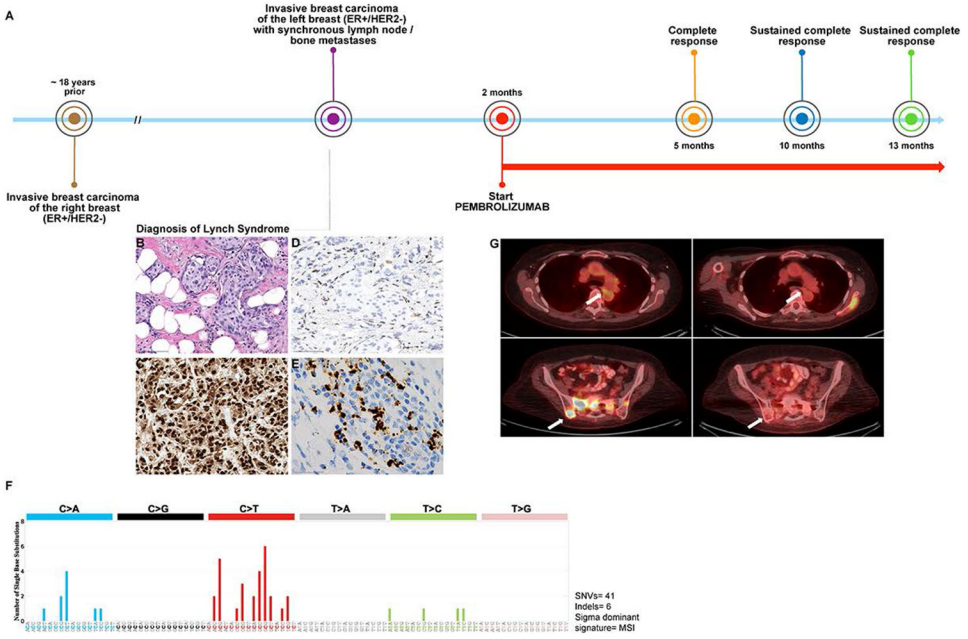

Figure 2. Metastatic breast cancer (case 6) in Lynch Syndrome (LS) patient treated with immune-checkpoint inhibitor.

(A) Timeline of disease progression, including initial breast cancer diagnosis, development of contralateral breast cancer/metastatic disease and diagnosis of Lynch syndrome, and treatment interval with anti-PD-1therapy. Representative hematoxylin-and-eosin photomicrographs of the tumor **(B)**, ER **(C)**, MSH6 **(D)** and PD-L1 (SP142) **(E)**. Scale bars=100 μM. **(F)** Representation of the mutational spectrum using the conventional 96 codon-contexts mutation type classification. This classification is based on the six substitution subtypes: C>A, C>G, C>T, T>A, T>C, and T>G, as well as the nucleotides immediately 5’ and 3’ to the mutation. **(G)** Positron emission tomography scan (PET) of lymph node (top left panel) and pelvic metastasis before treatment (bottom left panel). Complete PET response of lymph node (top right panel) and pelvis (bottom right panel) after treatment.

Table 1:

Summary of Germline Alterations and Cancer History in Lynch Syndrome Cohort

Case	Age	Germline Alteration	Type of Mutation	Family History	Other Cancer History
1	46	*MSH6* c.3103C>T (p.R1035*)	Nonsense	(F, CC, 66), (M, BC, 73) (MGM, BC, 63), (MA, OC, 66)	None
2	43	*PMS2* c.137G.T(p. Ser46IIe)	Missense	(PGM, UC, 73)	None
3	36	*MSH6* c.3959_3962delCAAG (p. Ala1320Glufs*6)	Frameshift Deletion	(MGM, UC, 63)	None
4	33	*MLH1* c.954delC (p. His318Glnfs*49)	Deletion	(F, CC, 58) (PA, OC, 41; CC, 62)	(UC,33)
5	47	*PMS2* c.248T>G (p. Leu83*)	Nonsense	(MGM, BC, 74)	(BC,38)
6	64	*MSH6* c.3984_3987dup (p. Leu1330Valfs*12)	Frameshift Deletion	(M, BC, 43), (D, BC, 35), (MA, BC 54, 65), (MA, UC, 63)	(BC, 46), (FGP with LGD, 66)
7	59	*MSH6* c.3513+3514delTA (p. Asp117Glufs*5)	Frameshift Deletion	None	(UC, 58)
8	38	*PMS2* exon 11-12 duplication	Rearrangement	(M, BC, 51)	None
9	47	*MSH6* c.2906A>G (p. Tyr969Cys)	Missense	None	None
10	63	*MSH2* c.484G>A (p. Gly162Arg)	Missense	(M, CC, 40), (MA, CC, 59), (S,CC,56), (D, SBC, 51)	(SBC, 56), (LC, 66), (RC, 73)
11	74	*MSH2* c.942+3A>T	Missense	(F, CC, 71), (PGM, CC, 66)	(UC, 46), (UrC, 81) (DCIS, 75)
12	56	*MSH6* c.3485_3487delCTG (p. Ala1162del)	Deletion	(M, UC, 63), (MA, CC, 70), (PGM, BC,63)	(UC, 53)
13	42	*MLH1* c.1852-1854delAAG (p. Lys1852del3)	Deletion	(PA, CC, 49), (PA, OC, 50), (PA, UrC, 70)	None

Abbreviations: F, father; M, mother; MGM, maternal grandmother; MA, maternal aunt; PA, paternal aunt; PGM, paternal grandmother; S, son; D, daughter, CC, colon cancer; BC, breast cancer; OC, ovarian cancer; UC, uterine cancer; FGP, fundic gland polyp; LGD, low grade dysplasia; SBC, small bowel cancer; UrC, urothelial cancer; LC, lung cancer; RC, rectal cancer; DCIS, ductal carcinoma in situ.

Table 2:

Clinicopathologic characteristics of breast cancers in Lynch Syndrome cohort

Case	Age	Presentation	Tumor Type	Grade	Size (cm)	LVI	ER (%)	PR (%)	HER2*	Stromal TILS (%)	PDL-1 (SP142)	Surgery	Metastasis	TTP (mos.)	Foll Up (mo
1	46	Self-palpated	Ductal	3	3.5	Yes	97	99	2+	2%	N/A	TM	Yes	43	17
2	43	Self-palpated	Ductal	3	3.2	Yes	80	90	0/1+	20%	Negative	TM	Yes	55	6
3	36	Palpated on physical exam	Ductal	3	3.5	Yes	90	95	0	10%	Negative	TM	Yes	1	4
4	33	Screening Mammography	Ductal	3	3.3	Yes	99	1	0	25%	Positive	TM	No	N/A	3
5	47	Screening Mammography	Lobular	3	N/A	N/A	100	95	1+	N/A	N/A	TM	Yes	108	24
6	64	Screening Mammography	Ductal	3	1.3	Yes	99	30	0	10%	Positive	TM	Yes	12	1
7	59	Screening Mammography	Ductal	2	N/A	N/A	0	0	2+	10%	Positive	N/A	Yes	0	1
8	38	Self-palpated	Ductal	3	2	N/A	99	99	3+	10%	N/A	N/A	Yes	N/A	1
9	47	Self-palpated	Ductal	2,2	1.0,1.0	Yes	100	70	1+	10%	Positive	TM	No	N/A	3
10	63	Palpated on physical exam	Ductal	2	1	No	90	2	1/2+	2%	Negative	BCS	No	N/a	14
11	74	Screening Mammography	Lobular	2	1.15	No	95	90	1/2+	N/A	N/A	TM	No	N/a	11
12	56	Screening Mammography	Lobular	2	10	Suspicious	90	60	0	1%	Negative	TM	No	N/a	2
13	42	Screening Mammography	Ductal	2	1.9	Yes	70	80	0	20%	Positive	BCS	Yes	34	3

*All tumors with 2+ or 1/2 + staining for HER2 IHC were negative for amplification by fluorescent in situ hybridization.

Abbreviations: LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TILs, tumor infiltrating lymphocytes; TTP, time to disease progression; AWD, alive with disease; DOD, died of disease; NED, no evidence of disease; N/A, not available.

Table 3:

Summary of MMR immunohistochemistry, MSI polymerase chain reaction, tumor mutational burden and MSI Score/Sensor in Lynch Syndrome cohort

Case	MMR Status (IHC)	Second Hit in MMR genes (MSK-IMPACT)	MSI Status (PCR)	Signature Category by Exposure_SigMA	TMB Score (mut/Mb)	MSI Score	MSI Sensor
1	Retained	No	MSS	Aging	5.3	1.07	Stable (Liver Metastasis)
2	Retained	No	MSS	HRD	3.5	0.05	Stable
3	Retained	Yes (LOH)	MSS	N/A	2.6	0.58	Stable
4	Loss of MLH1 and PMS2, partial loss of MSH6	Yes (LOH)	MSI	MSI	16.7	7.24	Indeterminate
5	Retained	No	MSS	APOBEC	3.5	0	N/A
6	Loss of MSH6	Yes (Somatic)	Indeterminate	MSI	20.2	3.56	Indeterminate
7	N/A	N/A	MSS	Sig 17	3.5	0.25	Stable
8	Retained	No	MSS	Aging	3.5	0.12	Stable
9	Retained	N/A	N/A	N/A	N/A	N/A	N/A
10	Loss of MSH2 and MSH6	No	MSI	MSI	24.84	11.5	High
11	Loss of MSH2 and MSH6	N/A	N/A	N/A	N/A	N/A	N/A
12	Retained	No	MSS	N/A	1.05	0.57	Stable
13	Loss of MLH1 and PMS2	Yes (LOH)	MSI	MSI	8.39	7.68	Indeterminate

Abbreviations: MMR, mismatch repair protein; IHC, immunohistochemistry; MSI, microsatellite instability; PCR, polymerase chain reaction; TMB, tumor mutational burden (somatic mutations per Megabase (mut/Mb); MSS, Microsatellite stable; LOH, loss of heterozygosity; HRD; Homologous repair deficiency; N/A, not available.