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Title: High prevalence of mismatch repair deficiency in prostate cancers diagnosed in mismatch repair gene mutation carriers from the Colon Cancer Family Registry

Running Title: Prostate Cancers in Lynch Syndrome

Christophe Rosty[1,2,3,4≠], Michael D. Walsh[1,5≠], Noralane M. Lindor[6], Stephen N. Thibodeau[7], Erin Mundt[8], Steven Gallinger[9,10,11], Melyssa Aronson[11], Aaron Pollett[11], John A. Baron[12], Sally Pearson[2], Mark Clendenning[1,2], Rhiannon J. Walters[2], Belinda N. Nagler[2], William J. Crawford[2], Joanne P. Young[2], Ingrid Winship[13,14], Aung Ko Win[15], John L. Hopper[15,16], Mark A. Jenkins[15], Daniel D. Buchanan[1,2,15] for the Colon Cancer Family Registry

[≠]These two authors contributed equally to this work

[1]Oncogenomics Group, Genetic Epidemiology Laboratory, Department of Pathology, University of Melbourne, Parkville VIC 3010, Australia

[2]Cancer and Population Studies Group, Queensland Institute of Medical Research, Bancroft Centre, Herston QLD 4006, Australia

[3]Envoi Specialist Pathologists, Herston QLD 4006, Australia

[4]University of Queensland, School of Medicine, Herston, QLD 4006, Australia

[5]Department of Histopathology, Sullivan Nicolaides Pathology, Taringa QLD 4068, Australia

[6]Department of Health Science Research, Mayo Clinic Arizona, Scottsdale, AZ 85259, USA

[7]Department of Laboratory Medicine, Mayo Clinic, Rochester, MN 59095, USA

[8]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

[9]Cancer Care Ontario, Toronto, Ontario, Canada

[10]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

[11]Zane Cohen Centre for Digestive Diseases, Mount Sinai Hospital, Toronto, Ontario, Canada

[12] Department of Medicine, University of North Carolina at Chapel Hill, NC 27599, USA

[13]Department of Medicine, The University of Melbourne, Parkville, VIC 3010, Australia

[14]Genetic Medicine, The Royal Melbourne Hospital, Parkville, VIC 3010, Australia

[15] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville VIC 3010, Australia

[16] Seoul National University, Seoul, Korea

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Corresponding Author:
Daniel Buchanan PhD
Senior Research Fellow
Head, Oncogenomics Group
Genetic Epidemiology Laboratory
Department of Pathology and
Centre for Epidemiology and Biostatistics
University of Melbourne
Parkville, VIC, 3010 Australia
Ph: +61 3 8344 1240
Email: daniel.buchanan@unimelb.edu.au

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Abstract

Background: The question of whether prostate cancer is part of the Lynch syndrome spectrum of tumors is unresolved. We investigated the mismatch repair (MMR) status and pathologic features of prostate cancers diagnosed in MMR gene mutation carriers.

Methods: Prostate cancers (mean age at diagnosis= 62 ± SD=8 years) from 32 MMR mutation carriers (23 *MSH2*, 5 *MLH1* and 4 *MSH6*) enrolled in the Australasian, Mayo Clinic and Ontario sites of the Colon Cancer Family Registry were examined for clinico-pathologic features and MMR-deficiency (immunohistochemical loss of MMR protein expression and high levels of microsatellite instability; MSI-H).

Results: Tumor MMR-deficiency was observed for 22 cases (69%; 95% confidence interval (CI)=50-83%), with the highest prevalence of MMR-deficiency in tumors from *MSH2* mutation carriers (19/23, 83%) compared with *MLH1* and *MSH6* carriers combined (3/9, 33%; p=0.01). MMR-deficient tumors had increased levels of tumor infiltrating lymphocytes (TILs) compared with tumors without MMR-deficiency (p=0.04). Under the assumption that tumour MMR-deficiency occurred only because the cancer was caused by the germline mutation, mutation carriers are at 3.2-fold (95% CI=2.0-6.3) increased risk of prostate cancer, and when assessed by gene, the relative risk was greatest for *MSH2* carriers (5.8, 95% CI=2.6-20.9). Prostate cancer was the first or only diagnosed tumor in 37% of carriers.

Conclusions: MMR gene mutation carriers have at least a 2-fold or greater increased risk of developing MMR-deficient prostate cancer where the risk is highest for *MSH2* mutation carriers. MMR IHC screening of prostate cancers will aid in identifying MMR gene mutation carriers.

Introduction

Lynch syndrome, formerly known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant disorder caused by germline mutations in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Recently, we have shown that MMR gene mutation carriers are at increased risk of developing cancers of the colorectum and endometrium, as well as cancers of the ovary, kidney, pancreas, stomach, urinary bladder and breast [1]. They are also at an increased risk of developing second primary cancers, including those in the breast and prostate [2, 3]. Over 80% of colorectal cancers diagnosed in individuals with Lynch syndrome have tumor microsatellite instability (MSI) or loss of expression of one or more of the MMR proteins by immunohistochemistry (collectively termed MMR-deficiency) [4, 5]. Morphologically, colorectal cancers in people with Lynch syndrome frequently demonstrate high histologic grade, solid growth pattern and conspicuous lymphocytic infiltration [6].

Recently, sufficient data on Lynch syndrome has been collected to allow rigorous investigation of associations of MMR gene mutations with the more common cancers. Newer molecular and risk estimation studies support the inclusion of breast cancer as part of the Lynch syndrome-associated tumor spectrum [1, 7]. Case reports of uncommon tumors continue to emerge, including sarcomas [8, 9], peritoneal mesothelioma, adrenocortical carcinoma, anaplastic thyroid carcinoma, or neuroendocrine pancreatic tumors [10, 11]. Prostate cancer has not traditionally been considered part of the spectrum of tumors associated with Lynch syndrome, but recent small studies have suggested an increased risk of prostate cancer for people with Lynch syndrome, in particular for *MSH2* mutation carriers [3, 12-15]. In addition, MMR-deficiency assessed by loss of immunohistochemical expression or by polymerase chain reaction-based methods has been reported several times in prostate

cancers in a small number of MMR gene mutation carriers [12, 16-18]. However, to date, no large studies have examined the expression of MMR proteins and pathology features of prostate cancers diagnosed in MMR gene mutation carriers. Consequently, the question of whether prostate cancer is part of the spectrum of tumors is unresolved.

The aim of this study was to investigate the histological features, MSI and MMR immunohistochemical expression of prostate cancers in proven MMR gene mutation carriers from the Colon Cancer Family Registry.

Materials and Methods

Study sample

Participants were from families recruited between 1997 and 2010 to the Colon Cancer Family Registry via probands who were either recently diagnosed colorectal cancer cases ascertained through the Victorian population-cancer registry in Australia (population-based recruitment) and a state-based population-based registry in the USA (Minnesota Cancer Surveillance System) or they were persons from multiple-case families referred to family cancer clinics in Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney), New Zealand (Auckland), the Mayo Clinic, Rochester, Minnesota, USA (clinic-based recruitment) or the Mount Sinai Hospital, Toronto, Ontario, Canada[19]. Inclusion criteria for this study were: (a) proven to be carrying a pathogenic germline mutation in one of the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*, (b) having a diagnosis of prostate carcinoma confirmed by histological examination, and (c) the availability of archival tissue blocks for additional laboratory testing. Ethics approval was obtained from the relevant institutional Human Research Ethics Committees at recruiting centers including the Queensland Institute of Medical Research under project approval P628.

Germline mutation testing

Mutation testing for *MLH1*, *MSH2*, and *MSH6* was performed by Sanger sequencing or denaturing high performance liquid chromatography (dHPLC), followed by confirmatory DNA sequencing [7, 19]. Large duplication and deletion mutations were detected by Multiplex Ligation Dependent Probe Amplification (MLPA). *PMS2* mutation testing was performed using long-range PCR and MLPA as previously described [20] on individuals demonstrating solitary loss of PMS2 protein expression in a tumor. All donated samples from participants who were relatives of probands with a pathogenic mutation were tested for the same mutation identified in the proband. A pathogenic germline mutation in a DNA mismatch repair genes was defined as a variant causing a stop codon, a large duplication or deletion, a frameshift mutation or a missense mutation previously reported in the scientific literature as being pathogenic [1].

Pathology review

Paraffin-embedded tissue blocks containing prostate cancer were obtained from relevant clinical pathology departments. Hematoxylin and eosin stained sections were reviewed by one pathologist (CR) to assess tumor histologic type, Gleason score, the presence of capsular and perineural invasion and locoregional lymph node metastases. For four of nine tumors diagnosed in Ontario, pathology review was performed on a digitally scanned hematoxylin and eosin stained section. Tumor infiltrating lymphocytes (TILs) were counted and considered to be ‘significant’ when >4 TILs were identified by high power field [21]. Information on pre-operative prostate specific antigen (PSA) levels were abstracted from the clinical notes on pathology reports or obtained from diagnostic laboratories’ records.

Mismatch repair deficiency testing

Sections from formalin fixed paraffin embedded tissue blocks were used for immunohistochemical (IHC) assessment of the expression of MLH1, MSH2, MSH6 and PMS2 as previously described[22]. For tumors not from Ontario, MSI status was determined by using a 10-loci panel of microsatellite markers in tumor DNA [23] and tumors were deemed to have high levels of microsatellite instability (MSI-H) if ≥30% of markers were unstable. For tumors from Ontario, MSI was assessed using 2 mononucleotide markers BAT-25 and BAT-26 and tumors were deemed to be MSI-H if at least one marker was unstable. MMR-deficiency was defined as loss of protein expression by IHC with or without MSI-H where tested. A tumor was defined to be MMR-proficient if it had no loss of MMR protein expression by IHC and, when tested, was microsatellite stable (MSS).

Statistical analysis

Pearson's chi-squared tests or Fisher's exact tests were used to test the statistical significance of differences in contingency tables as appropriate. Student's *t*-test was used to test the statistical significance of differences in the means of continuous variables. Following convention, statistical significance was considered as $p<0.05$. 95% confidence intervals (CIs) of proportions were estimated using binomial exact method. Under the assumption that a MMR-deficient prostate cancer was caused by the MMR gene mutation, the relative risk (RR) of MMR-deficient prostate cancer for men with a germline MMR gene mutation can be estimated by back calculation from the attributable fraction as $RR = N / (N - n)$, where N is the total number of prostate cancer-affected mutation carriers and n is the number of these for

which their tumor exhibited MMR-deficiency. The 95% CI was estimated by assuming that n has a Binomial (N;p) distribution with P = n / N [24, 25].

Results

Clinical and pathological characteristics of prostate cancers in MMR gene mutation carriers

A total of 32 men from 31 families fulfilled the selection criteria and were included in the study as prostate cancer cases. The Amsterdam II criteria (ACII) were met by 25/31 families (81%). There were 23 x *MSH2* mutation carriers (72%; two from the same family), 5 x *MLH1* mutation carriers (16%), and 4 x *MSH6* mutation carriers (12%; **Table 1**). No *PMS2* gene mutation carriers diagnosed with prostate cancer were identified. Of the 147 population-based families with MMR gene mutations from the Australasian, Ontario and Mayo sites, the distribution of *MLH1*, *MSH2* and *MSH6* mutations was 43% (n=63), 43% (n=63) and 14% (n=21) respectively. In these families, there were 351 (151 male) carriers of mutation in a MMR gene (148 x *MLH1*, 170 x *MSH2* and 33 x *MSH6*) with 58 (39%), 75 (44%) and 18 (54%) males, respectively. Given this distribution of mutation carriers, there was an over-representation of male *MSH2* mutation carriers (23/75, 31%) and an under-representation of male *MLH1* mutation carriers (5/58, 9%) with prostate cancer from these 147 families (p=0.002). The mean age at diagnosis of prostate cancer was 62 ± 8 years (range 45 – 74). Information on pre-operative PSA was available for eight carriers with a mean level of 38µg/l (standard deviation (SD) = 31µg/l; range 4 – 81µg/l). Two other carriers were reported as having "rising" and "elevated" PSA values without quantified scores.

The pathology specimens were transrectal ultrasound biopsies (TRUS Bx; n = 9), transurethral resection of the prostate (TURP) specimens (n = 5) and radical prostatectomy specimens (n = 18) (**Table 2**). All tumors were prostatic adenocarcinomas of acinar type.

Total Gleason scores (GS) ranged from 5 to 10; two tumors had a GS of 5, twenty two had a GS of 6 or 7, and eight had a GS ≥ 8 (including one case reported as poorly differentiated). There was some evidence for an association between the gene mutated (*MSH2* versus *MLH1* and *MSH6* combined) and a GS ≥ 8, however, this was not nominally significant (8/23 versus 0/9; p=0.07). Of the assessable tumors, perineural invasion was identified in 12/18 (67%) and extracapsular invasion was identified in 9/19 (47%). The nodal status was known for nine carriers, one of whom had metastatic disease (11%).

Mismatch repair status of prostate cancers

IHC expression of MMR proteins was assessed for all 32 prostate cancer tumors and MSI status was determined for 10 tumors (**Table 2**). Loss of expression of MMR proteins by IHC was observed for 22 tumors (69%; 95% confidence interval CI 50-84%) and, when evident, the pattern of loss of protein expression was 100% concordant with that of the underlying germline mutation (**Figure 1**). The tumors from *MSH2* mutation carriers had the highest proportion of MMR-deficiency (19 in 23 (83%; 95% CI= 61-95%)) compared with tumors in *MLH1* mutation carriers (2 in 5 (40%; 95% CI= 5-85%)) and tumors in *MSH6* carriers (1 in 4 (25%; 95% CI= 1-81%)). This variation was inconsistent with chance (p=0.01).

Under the assumption that tumor MMR-deficiency occurs only because the cancer was caused by the underlying germline mutation, the relative risk of prostate cancer for all MMR gene mutation carriers was estimated to be 3.2-fold (95% CI=2.0-6.3). When broken down by gene, the relative risk was estimated to be 5.8-fold (95% CI=2.6-20.9) for *MSH2* mutation carriers, 1.7-fold (95% CI=1.1-6.7) for *MLH1* mutation carriers and 1.3-fold (95% CI=1.1-5.3) for *MSH6* mutation carriers. The difference in relative risk between *MSH2* and other gene mutation carriers was significant (p=0.01).

The prostate tumor from one *MSH6* mutation carrier also had loss of MSH2 expression which was consistently shown on repeated testing. A subsequent colorectal carcinoma from this carrier had loss of expression of MSH6 only. Of the 10 tumors tested for MSI, five were MSI-H and also had loss of expression by IHC and five were not MSI-H of which 3 were MMR-proficient by IHC. There were 2 carriers whose tumors had loss of concordant MMR proteins that were not MSI-H.

There was no difference in the mean age at diagnosis of prostate cancer between carriers with a MMR-deficient tumor compared with those with a MMR-proficient tumor (63 ± 8 years vs 60 ± 8 years; p = 0.4) (**Table 3**). Compared with MMR-proficient tumors, MMR-deficient tumors were more likely to have tumor infiltrating lymphocytes (p = 0.04) but there was no difference in the presence of high histologic grade (GS ≥8) (p = 0.4), perineural invasion (p = 0.1) or capsular invasion (p = 0.2). All the high grade prostate cancers were diagnosed in *MSH2* mutation carriers. Regional lymph node status was assessed for only seven carriers, and the single tumor with involved lymph nodes was MMR-deficient.

Personal history of other malignancies

Twenty three carriers (72%, 95% CI= 53-86%) had a diagnosis of colorectal cancer (**Table 1**). For twenty of these the colorectal cancer preceded the prostate cancer, by on average 16 ± 8 years (range 2 – 34). The prostate cancer was diagnosed 2 years prior to the colorectal cancer for two carriers, and 4 years prior for one carrier. Prostate cancer was the first (n = 5) or only (n = 7) tumor diagnosed for 37% of carriers (95% CI= 22-56%). There was no difference in the history of other malignancies between carriers with a MMR-deficient tumor compared with carriers with a MMR-proficient tumor (p = 0.7) (**Table 3**).

Discussion

We observed that 69% of 32 prostate cancers diagnosed in MMR gene mutation carriers had MMR-deficiency, consistent with the 88% and 100% reported by two previous studies of a total of 10 MMR gene mutation carriers[12, 18]. MSI has been detected in prostate cancer cell lines and in some studies of primary tumors with a wide range of frequencies (20-65%) [26-29]. MMR-deficiency and/or high levels of MSI are the hallmarks of Lynch syndrome-associated tumors. Our demonstration of this phenotype in a large proportion of prostate cancers from mutation carriers adds weight to the argument that prostate cancers can develop as a result of MMR gene mutations. However, further evidence is needed to determine whether MMR-deficiency is a driver that initiates the carcinogenesis of these tumors or is a passenger molecular alteration with little effect on tumor initiation and development.

We observed an equal proportion of families with mutations in *MSH2* and *MLH1* overall from the Australasian, Ontario and Mayo sites of 43%. However, when comparing the prevalence of mutation carriers with prostate cancer with male mutation carriers overall, we identified a significant over-representation of prostate cancer-affected *MSH2* mutation carriers (31%) while prostate cancer-affected *MLH1* mutation carriers were under-represented (9%). *MSH2* mutation carriers also demonstrated a higher prevalence of tumor MMR-deficiency when compared with *MLH1* and *MSH6* mutation carriers. Previous studies have also reported an over-representation of *MSH2* mutations in carriers with a prostate cancer [12-14, 16, 17]. Among MMR gene mutation carriers with a diagnosis of prostate cancer, the *MSH2* mutation has been reported as the putative cause for 6/9 tumors by Grindedal et al [12] and 7/8 by Barrow et al [14]. However, unlike these studies, we found that prostate cancer with MMR-deficiency was not restricted to *MSH2* and *MSH6* mutation carriers: we found five cases in *MLH1* mutation carriers, two of which had loss of MLH1

protein expression in tumor cells. Together these data suggest gene specific differences in the risk of prostate cancer with *MSH2* mutation carriers more likely to develop prostate cancer. We did not find any case of prostate cancer in *PMS2* mutation carriers. Most published studies did not include *PMS2* mutation carriers in their Lynch syndrome patient cohorts. Only one prostate cancer in an obligate *PMS2* mutation carrier has been reported [12]; however, immunohistochemistry has not been performed to demonstrate loss of PMS2 expression in tumor cells.

In addition to MMR-deficiency, tumors associated with Lynch syndrome often have a particular pathological phenotype including high histological grade and a pronounced lymphocytic immune response with the presence of tumor infiltrating lymphocyte. These morphological characteristics are exemplified in colorectal and endometrial carcinomas and can be used to predict MMR-deficiency in these tumor types [21, 22]. This study is the first to demonstrate that prostate cancers with MMR-deficiency more frequently showed tumor infiltrating lymphocytes than tumors that did not display MMR-deficiency. However, the prevalence of high histological grade (Gleason score ≥8) was not significantly different between the two groups. In the series of prostate cancers in proven or obligate MMR gene mutation carriers reported by Grindedal et al, all 5 tumors with a Gleason score of 8 or more were identified in *MSH2* mutation carriers [12]. Similarly, we found that all 6 MMR-deficient prostate cancers with a Gleason score ≥8 were diagnosed in *MSH2* mutation carriers. However, having a *MSH2* mutation or MMR-deficiency was not associated with a high Gleason score in prostate cancers in our study.

Previous studies utilizing the Colon Cancer Family Registry have investigated the risk of prostate cancer for MMR gene mutation carriers compared with men from the general population. In a retrospective study, Dowty et al. observed no evidence of an increased risk of prostate cancer as a first cancer diagnosis in mutation carriers: hazard ratio (HR) of 0.79

(95% CI, 0.25-2.5) for men with *MLH1* mutations and 1.0 (95% CI 0.47-2.3) for men with *MSH2* mutations [30]. In a prospective study, Win et al. estimated the increased risk of prostate cancer for mutation carriers by a standardized incidence ratio (SIR) of 2.49 (95% CI 0.51-7.28) [1]. However, for men with Lynch syndrome with a previous diagnosis of colorectal cancer, Win et al. estimated a two-fold increased risk of prostate cancer for all mutation carriers combined, compared with the general population (SIR, 2.05; 95% CI, 1.23-3.01)[3]. In that study, most prostate cancers (15/19) were in men with *MSH2* mutations, for whom the SIR was 3.62 (95% CI, 2.07-5.36) compared with 0.87 (95% CI, 0.00-2.19) for men with *MLH1* mutations. Three other independent studies found an increased risk of prostate cancer for MMR gene mutation carriers compared with the general population with SIRs of 2.5 (95% CI, 1.2-4.0) [13] and 5.1 (95% CI, 4.1-17.1) [12] and a relative risk estimated to 10.4 (95% CI, 2.80-26.65) for *MSH2* mutation carriers [14]. A further recent study of 198 families carrying MMR gene mutations reported a two-fold increased risk of prostate cancer in mutation carriers compared with the general population (HR=1.99, 95% CI, 1.31-3.03, p=0.0013) [15]. We observed that 69% of prostate cancers in carriers of MMR gene mutations had MMR-deficient tumors, and thus demonstrated a potential link between the germline mutation and prostate tumor initiation. Based on this high prevalence of MMR-deficiency and the assumption that tumors with MMR-deficiency were caused by the underlying germline mutation (and a somatic mutation as the second hit), we estimated the relative risk of MMR-deficient prostate cancer for all mutation carriers combined and for *MSH2* mutation carriers alone to be 3.2 (95% CI=2.0-6.3) and 5.8 (95% CI=2.6-20.9), respectively, providing further support for the inclusion of prostate cancer as part of the Lynch syndrome-associated tumor spectrum. However, the issue of whether prostate cancer risk is increased for men with Lynch syndrome is still debatable as other studies have not found evidence for an increased risk [31, 32]. Therefore, future studies using large

prospective studies of known mutation carriers with long follow-up will be needed to conclusively resolve the issue of risk of prostate cancer for MMR gene mutation carriers.

An interesting finding from this study was the diagnosis of prostate cancer in 12 of 32 mutation carriers (37%) as the first or only diagnosed malignancy. A similar finding was reported for a series of breast cancers diagnosed in women with Lynch syndrome, in which 44% of those with a MMR-deficient tumor had no previous history of malignancy [7]. This suggests that testing for MMR protein expression in tumors currently not considered part of the Lynch syndrome spectrum, such as breast or prostate cancers, can identify people with Lynch syndrome, even when there is no suspicion of Lynch syndrome, as well as in families with a known or suspected MMR gene germline mutation when no colorectal tumors are available for testing.

This study has some limitations. We selected only cases for which paraffin tissue blocks were available for additional testing and, therefore, were not able to assess all the prostate tumors from mutation carriers within the Colon Cancer Family Registry. MSI status by PCR-based methods was determined for only 10 tumors. It is possible that some additional MMR-deficient tumors not tested for MSI may have been missed. Two tumors showed loss of MSH2/MSH6 by immunohistochemistry but no evidence of MSI-H. This discordance may be caused by insufficient proportion of tumor cells in DNA to demonstrate the MSI-H phenotype. Also, our relative risk calculations were based on the assumption that tumors with MMR-deficiency were caused by the underlying germline mutation and an unmeasured second somatic hit. We did not confirm the presence or type of this second hit, however, the fact that inactivation of both alleles is needed to cause loss of MMR function is a well-established tumorigenic mechanism in Lynch syndrome. Given that all the men in the study were MMR gene mutation carriers, other mechanisms of MMR-deficiency such as tumor DNA promoter methylation is less likely. For 14 of the 32 prostate cancers (44%), the

pathologic evaluation was performed from biopsy specimens (TRUS or TURP) which may affect the overall Gleason score, and this precluded a complete assessment of other pathologic features in relation to the MMR status of the tumor.

In conclusion, to the best of our knowledge this is the largest study of prostate cancers in proven MMR gene mutation carriers for whom pathology and MMR status has been characterized. We found MMR protein loss of expression in 69% of tumors. We observed, for the first time, tumor infiltrating lymphocytes more often in MMR-deficient tumors than in MMR-proficient prostate tumors, similar to what is observed for other Lynch syndrome spectrum tumors. These findings suggest that defective mismatch repair is involved in prostate cancer development in men who carry a MMR gene mutation, in particular a *MSH2* gene mutation, and together with other recent evidence of an increased risk of prostate cancer for mutation carriers suggests that this malignancy be considered part of the spectrum of tumors in Lynch syndrome. Furthermore, screening for MMR-deficiency in men presenting with prostate cancer, especially those with other indications of Lynch syndrome, could identify MMR gene mutation carriers and provide the opportunity to target cancer prevention strategies to carriers and their relatives.

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Figure 1. Prostate carcinoma from carrier #4 who had a pathogenic germline mutation in *MSH2* (*MSH2* del x1-8). A: Hematoxylin and eosin stained sections showing Gleason 8 adenocarcinoma with tumor infiltrating lymphocytes; B and C: Immunohistochemistry showing normal nuclear expression of MLH1 in tumor cells (B) and loss of nuclear expression of MSH2 in tumor cells (C). All images magnification x400.

References

1. Win AK, Young JP, Lindor NM, et al. (2012) Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol 30: 958-64 DOI 10.1200/JCO.2011.39.5590

2. Win AK, Lindor NM, Winship I, et al. (2013) Risks of colorectal and other cancers after endometrial cancer for women with Lynch syndrome. J Natl Cancer Inst 105: 274-9 DOI 10.1093/jnci/djs525

3. Win AK, Lindor NM, Young JP, et al. (2012) Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. J Natl Cancer Inst 104: 1363-72 DOI 10.1093/jnci/djs351

4. Umar A, Boland CR, Terdiman JP, et al. (2004) Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 96: 261-8

5. Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN (2009) EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 11: 42-65 DOI 10.1097/GIM.0b013e31818fa2db

6. Jenkins MA, Hayashi S, O'Shea AM, et al. (2007) Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology 133: 48-56 DOI S0016-5085(07)00830-X [pii]

10.1053/j.gastro.2007.04.044

7. Walsh MD, Buchanan DD, Cummings MC, et al. (2010) Lynch syndrome-associated breast cancers: clinicopathologic characteristics of a case series from the colon cancer family registry. Clin Cancer Res 16: 2214-24 DOI 1078-0432.CCR-09-3058 [pii]

10.1158/1078-0432.CCR-09-3058

8. Brieger A, Engels K, Schaefer D, et al. (2011) Malignant fibrous histiocytoma is a rare Lynch syndrome-associated tumor in two German families. Fam Cancer 10: 591-5 DOI 10.1007/s10689-011-9455-9

9. Nilbert M, Therkildsen C, Nissen A, Akerman M, Bernstein I (2009) Sarcomas associated with hereditary nonpolyposis colorectal cancer: broad anatomical and morphological spectrum. Fam Cancer 8: 209-13 DOI 10.1007/s10689-008-9230-8

10. Broaddus RR, Lynch PM, Lu KH, Luthra R, Michelson SJ (2004) Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. Mod Pathol 17: 981-9

11. Karamurzin Y, Zeng Z, Stadler ZK, et al. (2012) Unusual DNA mismatch repair-deficient tumors in Lynch syndrome: a report of new cases and review of the literature. Hum Pathol 43: 1677-87 DOI 10.1016/j.humpath.2011.12.012

12. Grindedal EM, Moller P, Eeles R, et al. (2009) Germ-line mutations in mismatch repair genes associated with prostate cancer. Cancer Epidemiol Biomarkers Prev 18: 2460-7 DOI 10.1158/1055-9965.EPI-09-0058

13. Engel C, Loeffler M, Steinke V, et al. (2012) Risks of less common cancers in proven mutation carriers with lynch syndrome. J Clin Oncol 30: 4409-15 DOI 10.1200/JCO.2012.43.2278

14. Barrow PJ, Ingham S, O'Hara C, et al. (2013) The spectrum of urological malignancy in Lynch syndrome. Fam Cancer 12: 57-63 DOI 10.1007/s10689-012-9573-z

15. Raymond VM, Mukherjee B, Wang F, et al. (2013) Elevated risk of prostate cancer among men with lynch syndrome. J Clin Oncol 31: 1713-8 DOI 10.1200/JCO.2012.44.1238

16. Soravia C, van der Klift H, Brundler MA, et al. (2003) Prostate cancer is part of the hereditary non-polyposis colorectal cancer (HNPCC) tumor spectrum. Am J Med Genet A 121A: 159-62 DOI 10.1002/ajmg.a.20106

17. Wagner DG, Gatalica Z, Lynch HT, Kohl S, Johansson SL, Lele SM (2010) Neuroendocrine-type prostatic adenocarcinoma with microsatellite instability in a patient with lynch syndrome. Int J Surg Pathol 18: 550-3 DOI 10.1177/1066896910379406

18. Bauer CM, Ray AM, Halstead-Nussloch BA, et al. (2011) Hereditary prostate cancer as a feature of Lynch syndrome. Fam Cancer 10: 37-42 DOI 10.1007/s10689-010-9388-8

19. Newcomb PA, Baron J, Cotterchio M, et al. (2007) Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 16: 2331-43 DOI 1055-9965.EPI-07-0648 [pii]

10.1158/1055-9965.EPI-07-0648

20. Clendenning M, Walsh MD, Gelpi JB, et al. (2013) Detection of large scale 3' deletions in the PMS2 gene amongst Colon-CFR participants: have we been missing anything? Fam Cancer 12: 563-6 DOI 10.1007/s10689-012-9597-4

21. Young J, Simms LA, Biden KG, et al. (2001) Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. Am J Pathol 159: 2107-16

22. Walsh MD, Cummings MC, Buchanan DD, et al. (2008) Molecular, pathologic, and clinical features of early-onset endometrial cancer: identifying presumptive Lynch syndrome patients. Clin Cancer Res 14: 1692-700 DOI 1078-0432.CCR-07-1849 [pii]

10.1158/1078-0432.CCR-07-1849

23. Lindor NM, Burgart LJ, Leontovich O, et al. (2002) Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol 20: 1043-8

24. Rothman KJ, Greenland S, Lash TL (2008) Modern Epidemiology 3rd Edition. Philadelphia Lippincott: 54

25. Ahlbom A, Norell S (1990) Introduction to Modern Epidemiology. Epidemiological Resources Inc: 33

26. Chen Y, Wang J, Fraig MM, et al. (2001) Defects of DNA mismatch repair in human prostate cancer. Cancer Res 61: 4112-21

27. Cunningham JM, Shan A, Wick MJ, et al. (1996) Allelic imbalance and microsatellite instability in prostatic adenocarcinoma. Cancer Res 56: 4475-82

28. Egawa S, Uchida T, Suyama K, et al. (1995) Genomic instability of microsatellite repeats in prostate cancer: relationship to clinicopathological variables. Cancer Res 55: 2418-21

29. Gao X, Wu N, Grignon D, et al. (1994) High frequency of mutator phenotype in human prostatic adenocarcinoma. Oncogene 9: 2999-3003

30. Dowty JG, Win AK, Buchanan DD, et al. (2013) Cancer risks for MLH1 and MSH2 mutation carriers. Hum Mutat 34: 490-7 DOI 10.1002/humu.22262

31. Pande M, Wei C, Chen J, et al. (2012) Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based Lynch syndrome registry. Fam Cancer 11: 441-7 DOI 10.1007/s10689-012-9534-6

32. Scott RJ, McPhillips M, Meldrum CJ, et al. (2001) Hereditary nonpolyposis colorectal cancer in 95 families: differences and similarities between mutation-positive and mutation-negative kindreds. Am J Hum Genet 68: 118-27

Table 1. Clinical and genetic characteristics of 32 men with a germline mutation in a mismatch repair gene diagnosed with prostate carcinoma.

Carrier #	**Gene**	**Variant**	**Amsterdam II criteria**	**Age at diagnosis**	**PSA levels (ug/L)**	**Other malignancy* (age at diagnosis)**

1	*MSH2*	c.1865C>T p.Pro622Leu	Yes	71	NA	Colorectal x2 (44)
2	*MSH2*	c.892C>T p.Gln298X	Yes	58	NA	Colorectal x4 (38, 45, 51, 68); Small intestinal (64); Renal pelvis (68)
3	*MSH2*	c.892C>T p.Gln298X	Yes	73	81	Pancreatic (75)
4	*MSH2*	c.1-?_1386+?del p.Met1_Gln462del	Yes	53	35	
5	*MSH6*	c.3439-1 G>T r.spl? p.?	Yes	64	3.6	
6	*MSH2*	c.942+3A>T r.793_942del p.Val265_Gln314del	Yes	63	54	
7	*MSH2*	c.645+1G>A r.spl? p.?	Yes	59	NA	Colorectal (54); Melanoma (57)
8	*MSH2*	c.1165C>T p.Arg389X	Yes	70	“rising”	Colorectal x2 (50, 61)
9	*MLH1*	c.588delA p.Lys196AsnfsX6	Yes	68	6.9	Colorectal x2 (34, 59)
10	*MSH2*	c.1277-?_1386+?del p.Lys427GlyfsX4	Yes	69	NA	Colorectal (61); Bladder (69)
11	*MSH2*	c.792+1G>A r.spl? p.?	Yes	55	26	Colorectal (42)
12	*MLH1*	c.117-2A>G r.spl?p.?	Yes	68	NA	Colorectal (44)
13	*MSH2*	c.1147C>T p.Arg383X	Yes	71	NA	Melanoma (50); Renal pelvis (59); Colorectal x2 (64, 68)
14	*MS*	c.942+3A>T r.793_942del	Yes	61	NA	

	H2	p.Val265_Gln314del				
15	*MSH2*	c.1046C>T p.Pro349Leu	Yes	47	NA	
16	*MSH2*	c.645-1967_1076+5075del10166 p.Ile216_Arg359>IlefsX29	Yes	57	NA	Colorectal (50); sebaceous skin tumor (63)
17	*MLH1*	c. 350C>T p.Thr117Met	Yes	62	NA	Colorectal (46)
18	*MSH6*	c.3261_3262insC p.Phe1088LeufsX5	No	59	NA	Colorectal (61); sebaceous skin tumor (65)
19	*MSH6*	c.2731C>T p.Arg911X	No	60	NA	
20	*MLH1*	c. 1852_1854delAAG p.Lys618del	No	61	NA	Colorectal x2 (34, 59); Leiomyosarcoma (67)
21	*MSH2*	c.942+3A>T r.793_942del p.Val265_Gln314del	Yes	50	"elevated"	Colorectal (52)
22	*MSH2*	c.1889_1892delGAAG p.Gly630GlufsX4	Yes	45	80	
23	*MSH2*	c.1865C>T p.Pro622Leu	Yes	72	NA	Colorectal (54)
24	*MSH2*	c.1591_1611del p.Lys531_537del	Yes	73	NA	Jejunal (46), Colorectal x 4 (49, 63, 63, 63), sebaceous adenoma (63)
25	*MSH2*	c.1786_1788delAAT p.Asn596del	No	74	NA	Colorectal x3 (60, 61, 61), ureter (73)
26	*MS*	c.1864C>A p.Pro622Thr	Yes	68	NA	Colorect

	H2					al (54), Bladder (67), ureter (78)
27	*MSH6*	c.3336_3337insATGA p.Ile1113MetfsX7	Yes	70	19	Colorectal x2 (61, 65)
28	*MSH2*	c.475_476insA p.Arg159LysfsX19	Yes	53	NA	Colorectal (51)
29	*MSH2*	del exons 9-12	No	59	NA	Sebaceous adenoma (61)
30	*MSH2*	c.1906G>C p.Ala636Pro	No	60	NA	Colorectal (64)
31	*MLH1*	c.2038_2063del p.Cys680AlafsX5	Yes	47	NA	Colorectal x4 (35, 35, 51, 53), Melanoma (53)
32	*MSH2*	c.1-?_2805+?del	Yes	60	NA	Colorectal x2 (45, 61), Basal cell carcinoma (69), Sebaceous adenoma (70)

*Other malignancies that are underlined have been tested by immunohistochemistry for mismatch repair proteins and demonstrated pattern of loss of protein concordant with underlying germline mutation. This information was not available on cases 24 to 32 from Ontario.

Table 2. Pathological features and mismatch repair status of men with prostate carcinoma.

Carrier #	**Gene**	**Specimen type**	**Gleason score**	**TILs**	**Perineural invasion**	**Capsular invasion**	**Node metastasis**	**Pattern of MMR protein loss by IHC**	**MSI status**	**MMR status**
1	*MSH*	TURP	9	Presen	NA	NA	NA	MSH2/MSH6 loss	NT	**MMR**

	2			t						**-deficient**
2	*MSH2*	TRUS Bx	7	Present	NA	NA	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
3	*MSH2*	TRUS Bx	7	NA	NA	NA	NA	MSH2/MSH6 loss	MSI-H	**MMR-deficient**
4	*MSH2*	Radical prostatectomy	8	Present	Present	Present	Present	MSH2/MSH6 loss	NT	**MMR-deficient**
5	*MSH6*	TRUS Bx	6	Absent	NA	NA	NA	Normal expression	NT	MMR-proficient
6	*MSH2*	TURP	10	Present	NA	NA	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
7	*MSH2*	TRUS Bx	7	Present	Absent	NA	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
8	*MSH2*	TURP	9	Absent	NA	NA	NA	Normal expression	MSS	MMR-proficient
9	*MLH1*	TRUS Bx	7	Absent	Present	NA	NA	MLH1/PMS2 loss	NT	**MMR-deficient**
10	*MSH2*	Radical prostatectomy	9	Present	NA	Present	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
11	*MSH2*	TRUS Bx	6	Absent	NA	NA	NA	MSH2/MSH6 loss	NT	**MMR-defi**

										cient
12	*MLH1*	Radical prostatectomy	7	Present	NA	Present	Absent	MLH1 loss*	MSI-H	**MMR-deficient**
13	*MSH2*	Radical prostatectomy	7	Present	Present	Present	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
14	*MSH2*	Radical prostatectomy	7	Present	Present	Present	Absent	MSH2/MSH6 loss	NT	**MMR-deficient**
15	*MSH2*	Radical prostatectomy	7	Absent	Absent	Absent	Absent	Normal expression	NT	MMR-proficient
16	*MSH2*	Radical prostatectomy	6	Present	Absent	Absent	Absent	Normal expression	NT	MMR-proficient
17	*MLH1*	Radical prostatectomy	7	Absent	Absent	Absent	NA	Normal expression	NT	MMR-proficient
18	*MSH6*	Radical prostatectomy	5	Present	Present	Absent	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
19	*MSH6*	Radical prostatectomy	7	Absent	NA	Absent	Absent	Normal expression	NT	MMR-proficient
20	*MLH1*	Radical prostatectomy	5	Present	Present	Present	Absent	Normal expression	NT	MMR-proficient
21	*MSH2*	Radical prostatectomy	7	Present	Present	Absent	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
22	*MSH2*	TRUS Bx	9	Absent	Present	NA	NA	MSH2/MSH6 loss	NT	**MMR-**

										deficient
23	*MSH2*	TURP	7	Absent	NA	NA	NA	MSH2/MSH6 loss	NT	**MMR-deficient**
24	*MSH2*	TRUS Bx	Poor diff	NA	NA	NA	NA	MSH2/MSH6 loss	MSI-H	**MMR-deficient**
25	*MSH2*	TURP	9	NA	NA	NA	NA	MSH2/MSH6 loss	MSI-H	**MMR-deficient**
26	*MSH2*	Radical prostatectomy	7	NA	Present	Present	NA	MSH2/MSH6 loss	MSI-H	**MMR-deficient**
27	*MSH6*	TRUS Bx	6	Absent	Absent	Absent	NA	Normal expression*	NT	MMR-proficient
28	*MSH2*	Radical prostatectomy	7	NA	NA	No	NA	MSH2/MSH6 loss	NA	**MMR-deficient**
29	*MSH2*	Radical prostatectomy	7	Present	Present	Present	Absent	MSH2/MSH6 loss	MSS	**MMR-deficient**
30	*MSH2*	Radical prostatectomy	7	Present	Present	Present	Absent	Normal expression	MSS	MMR-proficient
31	*MLH1*	Radical prostatectomy	7	Absent	Present	Absent	NA	Normal expression	MSS	MMR-proficient
32	*MSH2*	Radical prostatectomy	7	NA	Absent	Absent	NA	MSH2/MSH6 loss	MSS	**MMR-deficient**

*PMS2 not tested. TRUS Bx: transrectal ultrasound biopsy, TURP: transurethral resection of the prostate, TILs: tumor infiltrating lymphocytes, MMR IHC: mismatch repair immunohistochemistry, MSI: microsatellite instability, MSS: microsatellite stable, NA: not available, NT: not tested.

Table 3. Comparison between mismatch repair (MMR) deficient and MMR proficient prostate carcinomas

Features	**MMR-deficient tumors n/N(%)**	**MMR-proficient tumors n/N(%)**	**P values**
Age at diagnosis, years (mean ± SD)	63 ± 8	60 ± 8	0.35
Gleason score ≥8	7/22 (32%)	1/10 (10%)	0.38
Presence of TILs	12/16 (75%)	3/10 (30%)	0.04
Perineural invasion	9/11 (82%)	3/7 (43%)	0.14
Capsular invasion	7/11 (64%)	2/8 (25%)	0.17
History of other malignancies	18/22 (82%)	7/10 (70%)	0.65

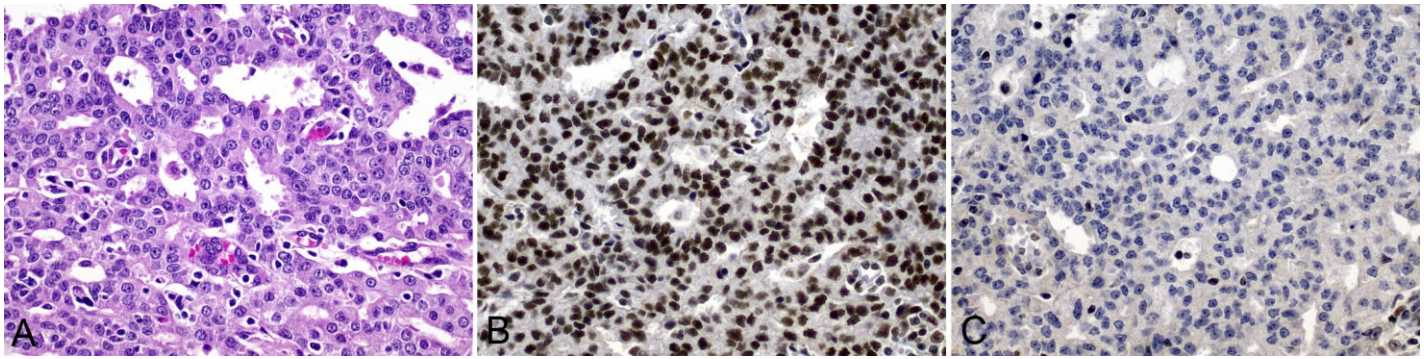


Figure 1. Prostate carcinoma from carrier #4 who had a pathogenic germline mutation in *MSH2* (*MSH2* del x1-8). **A**: Hematoxylin and eosin stained sections showing Gleason 8 adenocarcinoma with tumor infiltrating lymphocytes; **B** and **C**: Immunohistochemistry showing normal nuclear expression of MLH1 in tumor cells (**B**) and loss of nuclear expression of MSH2 in tumor cells (**C**). All images magnification x400.