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Universal molecular screening does not effectively detect Lynch syndrome in clinical practice

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Abstract

Background: Lynch syndrome (LS) due to an inherited damaging mutation in mismatch repair (MMR) genes comprises 3% of all incident colorectal cancer (CRC). Molecular testing using immunohistochemistry (IHC) for MMR proteins is a recommended screening tool to identify LS in incident CRC. This study assessed outcomes of population-based routine molecular screening for diagnosis of LS in a regional center.

Methods: We conducted a prospective, consecutive case series study of universal IHC testing on cases of resected CRC from September 2004–December 2013. Referred cases with abnormal IHC results that attended a familial cancer clinic were assessed according to modified Bethesda criteria (until 2009) or molecular criteria (from 2009).

Results: 1612 individuals underwent resection for CRC in the study period and had MMR testing by IHC. Of these, 274 cases (16.9%) exhibited loss of expression of MMR genes. The mean age at CRC diagnosis was 68.1 years (± standard deviation 12.7) and the mean age of those with an IHC abnormality was 71.6 (± 11.8). A total of 82 (29.9%) patients with an abnormal result were seen in a subspecialty familial cancer clinic. Patients aged under 50 ($p = 0.009$) and those with loss of MSH6 staining ($p = 0.027$) were more likely to be referred and to attend. After germ-line sequencing, 0.6% (10 of 82) were identified as having a clinically significant abnormality. A further eight probands with pathogenic germ-line mutations were identified from other referrals to the service over the same time period.

Conclusions: While technically accurate, the yield of 'universal' IHC in detecting new Lynch probands is limited by real-world factors that reduce referrals and genetic testing. We propose an alternative approach for universal, incident case detection of Lynch syndrome with 'one-stop' MMR testing and sequencing.

Keywords: colorectal neoplasms, DNA mismatch repair, immunohistochemistry, Lynch syndrome

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Introduction

Lynch syndrome (LS) is the most common inherited colorectal cancer (CRC) syndrome, accounting for 3% of colorectal cancers.[1,2] Affected pedigrees bear a deleterious mutation in one allele of a DNA mismatch repair (MMR) gene – MLH1, MSH2, MSH6 or PMS2, or a mutation of EPCAM, which leads to MSH2 silencing.[3,4] These defects predispose affected individuals to CRC as well as endometrial, pancreatic, genitourinary and other cancers.[5,6] These cancers arise in adult life after acquired (somatic) inactivation of the wild type MMR allele and loss of MMR function. Loss of MMR can be detected within tumor tissue as microsatellite instability (MSI)[7] or by loss of MMR protein expression.[8]

The high penetrance of CRC, endometrial and other cancers in those with Lynch syndrome has driven efforts to diagnose the disorder at the preclinical stage. Confirmation of LS by genetic testing leads to enhanced surveillance and cancer prevention[9,10,11] reduces anxiety,[12] the need for surveillance in family members not bearing the familial mutation, and is cost effective.[13,14] In 1991, clinical criteria for the definition of LS were published[15] and were revised in 1999.[16] While highly specific, these criteria lack sensitivity, leading to underdiagnosis of LS.[17,18] This experience has led to proposals for routine molecular screening for diagnosis of LS in incident CRC[17,8] and incident adenoma,[19] as well as hybrid schemes utilizing clinical and molecular criteria.[20] The aim of these schemes is to limit the number of cases of cancer in the affected family to the incident case. As these schemata rely on clinicians to recognize and apply the criteria, conduct genetic counselling, order genetic tests where indicated, interpret and communicate the results, and perform follow up and surveillance in probands and affected family members,[21,22,23] all such schemes will underdiagnose LS. Those that live in regions lacking formal cancer genetic services may be particularly vulnerable to underdiagnosis.

After Lynch and de la Chappelle[8] and without a dedicated cancer genetic service, we commenced molecular screening for LS in incident CRC cases undergoing resection in the Australian Capital Territory (ACT). As refinements of molecular screening strategies were developed, these were incorporated into our clinical practice. We present the outcome of 8 years of molecular screening for LS, show that significant underdiagnosis occurs, and propose a change in practice to address this underdiagnosis.

Methods

Population: The Australian Capital Territory (ACT) has a population of 390,000 but the hospitals draw additional patients from the surrounding New South Wales region to service approximately 550,000 people.

All CRC cases were drawn from three hospitals with pathology services provided by one public provider (ACT Pathology) and one private provider (Capital Pathology). For retrospective analysis, CRC cases were retrieved from the pathology providers' databases. We included all resected colorectal adenocarcinomas identified in the ACT from September 2004 until December 2012. We also retrieved index cases of LS diagnosed in the ACT from September 2004 until December 2012 from the ACT Genetic Service Database. We identified the reason for referral and means of identifying the patients as having LS. The study was approved by the ACT Health Human Research Ethics Committee (ETH.10/02.376).

Screening strategy: The study aim was that all resected CRC in the study period would be prospectively screened for LS using immunohistochemistry (IHC) as a routine diagnostic test. Study investigators wrote to all treating surgeons at the study commencement providing an 'opt out' from universal testing. MMR IHC staining results were reported to requesting doctors in the body of the histopathology report, together with an advisory for referral to the sub-speciality clinic of the gastroenterology unit. This advisory recommended referral for cases with abnormal MMR, in a patient aged less than 50, or a prior history of any LS cancer, first-degree relative with any LS cancer, or synchronous cancers. There was no involvement of the study team in the decision to refer to the service.

Immunohistochemistry: MMR IHC was performed on relevant formalin-fixed, paraffin-embedded (FFPE) tumour blocks and reported in line with accepted criteria by pathologists expert in interpretation of MMR staining.[24] From September 2004, specimens were analyzed using anti-MLH1 and anti-MSH2. Staining for MSH6 expression was introduced in 2005 and for PMS2 in 2008 in the public sector, while MSH6 and PMS2 tests were introduced in 2009 in the private sector. Expanded testing was initiated collaboratively between the investigators and providers. We retrospectively tested any cases that were not tested initially for MMR abnormality using the four MMR protein stains.

Clinical Assessment: Clinic attendance at a subspecialty gastroenterology clinic or to a general genetics service was recorded until 31 December 2013, allowing at least 1 year from cancer diagnosis for attendance to occur. For each patient, we recorded the specialty of the referring clinician. Patients who attended the service were offered genetic counseling and given the option for further genetic testing, when appropriate. In referred patients with loss of MLH1 staining, somatic loss

Table 1. Characteristics of individuals described in the study.

Total number of individuals with resected CRC	1612
Average age (years ± SD)	68.1 ± 12.7
Males	824 (51%)
Females	788 (49%)

CRC, colorectal cancer; SD, standard deviation.

was further assessed by V600E BRAF and MLH1 methylation assays from 2009. Prior to 2009, a detailed family history was taken and patients assessed as high risk using the modified Bethesda criteria[16] were offered germ-line testing. Patients with loss of expression of MSH2, MSH6 and PMS2 were given genetic counseling before genetic testing and offered a consultation with a cancer geneticist by videoconference. Testing was conducted by an accredited diagnostic molecular service (Hunter Genetics Lab, New England, NSW). MMR alterations were classified according to the International Society for Gastrointestinal Hereditary Tumors (InSIGHT) Variant Interpretation Committee Classification.[25,26]

Statistical analysis: Demographic data was recorded and summarised as mean (± SD). The effect of individual characteristics on clinic attendance was estimated with clinic attendance as the outcome and the factors as predictors. Fisher's exact test was used to test the effect of the predictors. Age was analyzed as a categorized variable, grouping individuals by age (<50, 50–70, and >70). All statistical analyses were performed using SPSS (version 20; SPSS Chicago, IL). Statistical significance was defined as $p < 0.05$.

Results

Patient characteristics: A total of 1612 individuals underwent resection for CRC in the study period and had MMR testing by IHC. Of these, 274 individuals (17.0%) had loss of expression of at least one of the four mismatch repair genes. The mean age at CRC diagnosis was 68.0 (range 22–95) and 49% of individuals were female (Table 1). Most patients were aged over 70 (175, 68.9%). The mean age of those with an IHC abnormality was 71.6 (± 11.8) and 58.4% were female (Table 2).

Table 2. Characteristics of the 274 individuals with abnormal mismatch repair testing.

Age ± SD	71.6 ± 11.8*
Sex (M:F)	114:160 (41.6 : 58.4%)
Proximal§	223 (81.3%)
Mucinous	51 (18.6%)
Tumor stage	
I–III	213
IV	61
IHC staining pattern	
Absence MLH1 ± PMS2	211
Absence MSH2 ± MSH6	18
Absence PMS2	13
Absence MSH6	19
Absence all	5
Mispaired loss#	8

*Significantly different to group without abnormal MMR testing, $p = 0.007$.
§Proximal CRC were defined as those up to but not including the splenic flexure.
#Mispaired loss included MLH1/MSH2, MLH1/MSH2/MSH6, MLH1/MSH6/PMS2; all were BRAF V600E + or exhibited MLH1 hypermethylation.
SD, standard deviation; IHC, immunohistochemistry; CRC, colorectal cancer; MMR, mismatch repair.

Result of immunohistochemistry: Of individuals with abnormal staining, the majority as expected (211) had loss of expression of MLH1 with or without PMS2 (77.0%). There were 18 (6.6%) with loss of MSH2 with or without MSH6 loss, 19 (6.9%) with MSH6 only loss, 13 (4.7%) with PMS2 loss, 5 (1.8%) with loss of all 4 MMR and 8 with atypical staining patterns or mispaired loss (e.g. MSH2/PMS2, MLH1/MSH6: Table 2). Of cancers with atypical staining patterns or mispaired loss, all were found to be either MLH1 negative or BRAF positive. 145 individuals (9%) did not have IHC performed at the time of resection. Of these, 18 (1.1% of CRC resections for the period) had abnormal staining on retrospective analysis and all of those were found to have loss of MLH1 staining.

Pathological findings: Of those with an abnormality on IHC, 24.3% were mucinous adenocarcinomas and 81% of the tumors were proximal (Table 2). Of tumors with MMR abnormality, 78% (213) were stage I–III while 61 (22%) were stage IV. Seven individuals had synchronous tumors at the time of resection.

Figure 1. Flow diagram of the mismatch repair screening results in the study, and results of further testing.
IHC, immunohistochemistry.

Table 3. Factors associated with attendance at a familial cancer clinic.

	Attendance at familial cancer clinic		*p* value (Fishers exact)
	YES	NO	
Sex (M:F)	32.5: 28.1%	67.5: 71.9%	0.504
Tumour site (proximal: distal)	30.6: 27.3%	69.4: 72.7%	0.742
Site of resection (public: private hospital)	29.9: 30.0%	70.1: 70.0%	0.55
Mucinous histology	45.3: 26.2%	54.7: 73.8%	**0.012**
Age < 50	62%	39%	
Age $\geqslant 50 < 70$	36%	64%	**0.009**
Age $\geqslant 70$	25%	75%	
MMR staining pattern			
Absence MLH1 ± PMS2	28.4%	71.6%	0.202
Absence MSH2 ± MSH6	44.4%	55.6%	0.186
Absence PMS2	15.4%	84.6%	0.356
Absence MSH6	52.6%	47.4%	0.027
Mispaired loss	40.0%	60.0%	0.637

Table 4. Source of referrals to familial cancer clinic for the 82 individuals seen with an immunohistochemistry abnormality.

Specialist group	Percentage (%)
Surgeon	52.4
Medical Oncologist	23.2
Gastroenterologist	15.9
General practitioner	7.3
General physician	1.2

Results of Screening Strategy: Figure 1 summarises the results of the screening strategy. A total of 145 (9.0%) did not have IHC staining done at the time of resection due to oversight or the preference of the reporting pathologist. A total of 82 (29.9%) patients with an abnormal result were seen in a familial cancer clinic; 77 in a subspecialty gastroenterology clinic and 5 in a collaborating general genetics service (Table 3). Factors predicting referral and attendance were age under 50 ($p = 0.009$) and individuals with tumors having a significant mucinous component ($p = 0.012$). Of all IHC abnormalities, individuals with loss of MSH6 staining were more likely to attend ($p = 0.027$). Rates of clinic attendance were not associated with gender, tumor site or place of surgery (public *versus* private hospital). Patients were most likely to be referred to the clinic by treating surgeons and oncologists (Table 4).

Outcomes of germ-line testing: A total of 10 individuals of the 82 with abnormal MMR IHC and who underwent MMR germ-line testing were considered likely to have LS by combined molecular and clinical criteria (Table 5). The mean age of these 10 cases was 52.1 years (range 19–80). Only 7 had any germ-line alteration identified; of these, 3 had a pathogenic (Class 5) mutation using InSIGHT criteria allowing for predictive testing in the proband's family. One had a variant of uncertain significance, one had compound MSH6 variants (Class 3/Class 1), two had variants that are novel at the time of submission and one had a benign (intronic, single nucleotide missense) variant. A further three cases did not have a germ-line mutation confirmed. One was not tested due to the restricted pedigree and patient preference, and two cases had no mutation identified after sequencing but were considered as likely LS because of compelling clinical features.

Alternate Index case identification: Eight probands with pathogenic (Class 5) germ line mutations were identified after referral to the familial cancer service ($n = 4$) or a general genetics service ($n = 4$, Table 6). These patients were referred for previous history of other cancer,

Table 5. Characteristics of individuals considered Lynch syndrome on combined clinical and molecular criteria.

Patient no.	Sex	Age at first appointment	CRC site	IHC Result	Gene	Exon	Mutation	Variant ID	Class
1	F	19	Synchronous ascending colon	MLH1 negative	*MLH1*	17	1975_1976 delCG	MLH1_00694	5
2	M	54	Ascending colon	MSH2/MSH6 negative	*MSH6*		No mutation identified*		
3	M	80	Rectal	MSH6 negative	*MSH6*	7	c.3583A > G	Novel	N/A
4	F	59	Transverse colon	MSH2/MSH6 negative	*MSH2*	7	c.1216C > T	MSH2_00312	5
5	M	33	Splenic flexure	MLH1/PMS2 negative	*MLH1*		No mutation identified§		
6	M	56	Rectal	MSH6 negative	*MSH6*	4	1186 C > G	MSH6_00118	1
						8	3694_369delGTT	MSH6_00721	3
7	M	51	Rectal	MHS6 negative	*MSH6*	5	c.3261dup	MSH6_00201	5
8	M	33	Caecum	MSH2 negative	*MSH2*	6	c.1042C > T	Novel	N/A
9	M	67	Sigmoid	MLH1/PMS2 negative	*MLH1*	12	c.1039-8T > A		Benign
10	M	69	Transverse colon	MLH1 and PMS2 negative	*MLH1*		Not tested#		

*No mutation in MSH2 and MSH6 or EPCAM on sequencing or MSH2 rearrangement by MLPA. Family history of CRC and uterine cancer.
§MLH1 – BRAF negative, MLH1 methylation neg, no mutation on MLH1 sequencing.
#Not tested due to restricted pedigree and patient preference.
CRC, colorectal cancer, IHC, immunohistochemistry.

Table 6. Characteristics of the eight individuals diagnosed with Lynch syndrome but not presenting with colorectal cancer in the study period.

Patient no.	Sex	Age at first appointment	Reason for assessment	Use of IHC	Gene	Exon	Mutation	Variant ID	Class
1	M	21	Family history	Loss of PMS2 restrospectively analyzed cancer tissue from a relative	PMS2	1	C1A>g	PMS2_00130	5
3	M	53	Age at CRC diagnosis, family history	IHC on CRC specimen diagnosed before universal screening	MSH2	8	del1277-1386	MSH2_01582	5
4	F	37	Age at CRC diagnosis	IHC on endometrial cancer specimen	MSH2	1 – 16	Gene deletion	MSH2_00043	5
5	M	59	Multiple cancers	IHC on sebaceous adenoma and renal tract tumour specimen	MSH2	2, 6	Exon 2 – 6 inversion	MSH2_01579	5
6	F	55	Multiple cancers	IHC on small intestinal cancer	MSH2	14	c2228c>G	MSH2_00646	5
7	F	38	Family history	IHC on adenomatous polyp	MSH2	3	c484G>A	MSH2_00152	5
8	F	66	Multiple cancers	IHC on ovarian cancer specimen	MSH2	4 – 16	DEL 646-2674	MSH2_01255	5
9	F	57	Age at CRC diagnosis, family history	IHC on CRC specimen diagnosed before universal screening	MLH1	16	In-frame deletion 618	MLH1_00652	5

IHC, immunohistochemistry;CRC, colorectal cancer.

family history of CRC, or a resected colorectal cancer that fell outside the study period. MMR IHC was used retrospectively in these eight patients and led to identification of the pathogenic mutation (Table 6).

Discussion

The yield of universal IHC in detecting new Lynch probands may be limited by factors that reduce referral for genetic assessment and testing.[27,28] We detected an abnormal IHC result in 16.9% a population-based sample of incident CRC of all ages undergoing resection during the study period. This result is similar to other screening studies with differing methodologies and populations.[29,3,30,31,24,6] Only 10/274, or 0.6% of our study population, were considered likely to have a germ-line MMR mutation, a figure similar to that from another Australian genetic service,[28] although a germ-line alteration was only detected in 7 of these 10 cases. Other studies with universal MMR analysis have detected LS in between 0.7% and 3.1% of individuals with newly diagnosed CRC and at a higher rate, 4.5%, when only those under 70 are included for routine testing.[6,29,24,32]

Our study confirms that implementation of universal IHC/MSI screening does not on its own lead to diagnosis of the majority of LS, which accounts for 3% of all CRC.[3,6] We identified the steps at which cases escaped detection. We identified 145 cases where the intended IHC screening was not performed at the time of resection, due to an oversight of the reporting pathologist. All of these cases were in the public sector, and on retrospective analysis, 18 patients cases exhibited loss of MLH1 expression. The majority of these cases were considered by the investigators to have somatic loss of MLH1; however, it is possible that some of these cases are LS.

Of patients who had contemporaneous MMR testing and returned an abnormal result, 30% attended further assessment at either specialty service. Referral to appropriate genetics review is highly variable, both locally and internationally,[33,27,28,34] and is the subject of a current research intervention.[35] In our study, one factor may have been the advisory attached to the MMR result, which may have created a referral bias; patients attending the services were more likely to be under the age of 50. Prior to 2009, we also used the modified Bethesda criteria[16] in ordering mutation testing after abnormal MMR immunohistochemistry. In addition, some patients may be reluctant to undergo further assessment. A review of screening programmes in the Lynch Syndrome Screening Network found that the rate that patients proceed to further testing following a positive screen ranges from 10–85%.[22] The yield of universal screening was enhanced when automatic testing of BRAF and MLH1 methylation was done in MLH1-negative cases, a measure we adopted in referred cases during the study and have now also automated.

The availability of expert cancer geneticists is a barrier to case detection. In Australia, expert cancer geneticists are located only in major population centers. While patients living in remote and regional Australia may access services remotely, distance, and the lack of genetic workforce, is a proven barrier to genetic testing.[36] In response to this, we operate a subspecialty gastroenterology service, which the majority of new probands in this study attended. Patients were given informal counseling and those with abnormal sequencing were offered consultation with a cancer geneticist by videoconference. Our approach yielded a similar case detection rate for LS as a program from a major genetics service,[28] noting, however, the same barriers to case detection. We conclude that in centers where cancer geneticists do not practice, that assessment for highly penetrant cancer syndromes can be performed adequately by nongeneticists.

We detected an abnormal IHC result in 16.9% of a population-based sample of incident CRC of all ages undergoing resection during the study period. This result is similar to other screening studies with differing methodologies and populations. The negative predictive value of MMR IHC has not been assessed in any population-based prospective series against a standard of germ-line testing, to the best of our knowledge, although smaller series indicate a relative lack of sensitivity for MSH6 in late-onset cancers (reviewed in Resnick[37]).

Our study evolved due to expansion of MMR tests available, and this may have limited the study by failing to detect LS in unscreened individuals. Offsetting this, an alternate referral pathway based on clinical criteria was available, in line with previous practice.

The detection rate of LS from MMR screening is unsatisfactory, considering the potential cancer

burden that flows from failure to detect LS. This detection rate could greatly increase if the analysis were rapid, decisive and required less of the patients and their referring doctors. A 'one-stop' approach of initial MMR screening by immunohistochemistry, complemented by sequencing of blood for MMR genes at diagnosis is feasible and affordable.[38] The use of parallel sequencing of cancer susceptibility genes through multigene panels has become widespread. Its value is great if there are clear indications, a focused genomic panel and appropriate follow up.[39] One challenge these panels pose to clinicians is the interpretation of unexpected findings in low-penetrance genes[40] but this issue does not arise in MMR mutations, which are both penetrant and well curated. The ability to gain truly informed consent for these tests is acknowledged.[41] The broad ethical principles governing point-of-care sequencing in cancers have been developed in this country and elsewhere.[42,43] We propose that such an approach be adopted in a pilot, clinical setting.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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