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The proportion of endometrial cancers associated with Lynch syndrome: a systematic review of the literature and meta-analysis

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Purpose: Endometrial cancer (EC) is often the sentinel cancer in women with Lynch syndrome (LS). However, efforts to implement universal LS screening in EC patients have been hampered by a lack of evidence detailing the proportion of EC patients that would be expected to screen positive for LS.

Methods: Studies were identified by electronic searches of Medline, Embase, Cochrane CENTRAL and Web of Science. Proportions of test positivity were calculated by random and fixed-effects meta-analysis models. I^2 score was used to assess heterogeneity across studies.

Results: Fifty-three studies, including 12,633 EC patients, met the inclusion criteria. The overall proportion of endometrial tumors with microsatellite instability or mismatch repair (MMR) deficiency by immunohistochemistry (IHC) was 0.27 (95% confidence interval [CI] 0.25-0.28, I^2: 71%) and 0.26 (95% CI 0.25-0.27, I^2: 88%), respectively. Of those women with abnormal tumor testing, 0.29 (95% CI 0.25-0.33, I^2: 83%) had LS-associated pathogenic variants on germline testing; therefore around 3% of ECs can be attributed to LS. Preselection of EC cases did increase the proportion of germline LS diagnoses.

Conclusion: The current study suggests that prevalence of LS in EC patients is approximately 3%, similar to that of colorectal cancer patients; therefore our data support the implementation of universal EC screening for LS.

Keywords: systematic review; Lynch syndrome; endometrial cancer; mismatch repair (MMR) immunohistochemistry; microsatellite instability (MSI)

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant cancer predisposition syndrome. Those affected most commonly inherit an inactivating variant in one of the four mismatch repair system (MMR) genes: MLH1, MSH2, MSH6, or PMS2. This highly conserved system is responsible for correcting insertion and deletion errors that occur during genomic replication.1 Loss of MMR functioning, termed MMR deficiency (MMRd), leads to microsatellite instability (MSI),2 a hypermutated phenotype, and increased cancer susceptibility. LS patients are at an increased risk for a number of different malignancies, but most commonly develop colorectal and endometrial cancer.3,4 As a result, patients with LS have a decreased life expectancy compared to nonaffected individuals.5

Diagnosing LS in endometrial cancer (EC) patients is an important step in clinical management. It allows for cascade testing to diagnose family members who may also have the disease.6 Furthermore, timely LS diagnosis allows for the initiation of lifestyle modification such as weight loss, chemoprophylaxis, and cancer site surveillance to prevent the development of further LS-related malignancies, most notably colorectal cancer (CRC).4,7 Annual colonoscopy has been shown to improve overall survival in LS patients through the detection and removal of adenomatous polyps.4 There is a growing drive for universal screening of CRC patients for LS.8-10 Indeed, the National Institute of Health and Care Excellence (NICE) in the United Kingdom has recently introduced a LS screening pathway for all CRC patients, alongside numerous institutions in the United States.11 LS

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screening pathways utilize tumor-based testing (immunohistochemistry [IHC] for MMR protein loss, MSI testing or MLH1 promoter methylation testing) to triage cases to undergo germline testing to identify a pathogenic variant in one of the MMR genes.

Universal screening of EC patients for LS has been recommended by numerous experts and specialist societies.12 Such practice has already been adopted in several cancer centers across the world.13–15 Proponents suggest a similar proportion of EC is related to LS as seen in CRC. Furthermore, there is evidence that EC is often the sentinel cancer in women with LS.16 Therefore, a diagnosis of LS at the time of EC diagnosis could afford earlier CRC surveillance and achieve greater survival benefit. However, the true proportion of EC associated with LS remains unclear. Published proportions vary greatly, with estimates ranging from 1% to around 10%.17,18 Such variation in estimates is in part due to variable testing strategies employed across different studies.

In this systematic review we sought to provide accurate data estimating the outcomes of testing for LS in EC patients. Specifically, we asked what proportion of EC patients would be expected to be put forward for definitive germline testing following initial tumor-based tests (namely IHC, MSI with or without MLH1 promoter methylation analysis), and secondly, what proportion of these would be confirmed Lynch syndrome by next-generation sequencing (NGS). The results of this study may be of benefit in informing the planning and implementation of universal LS screening in EC patients.

MATERIALS AND METHODS

Search strategy and study identification
A systematic literature search devised by a specialist librarian, following PRISMA guidelines,19 was undertaken. Medline, Embase, Cochrane CENTRAL, and Web of Science were searched. The gray literature and nonelectronic literature were not included. Search terms were “colorectal neoplasms, hereditary nonpolyposis” and “endometrial cancer” with associated Medical Subject Headings (MeSH). In addition, a secondary search was conducted using “Lynch syndrome” as a multipurpose term and “endometrial cancer” as a MeSH term. The search included all studies from source commencement to the end of July 2018. Citation searching was utilized to augment the initial results.

Studies found to have inconsistent results were excluded after unanimous review and agreement between all authors. Assessment of bias analysis was conducted by three reviewers (N.A.J.R., D.B., and M.C.D.) independently using Review Manager (RevMan) (Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Disagreements were resolved by either unanimous agreement after rereview or by decision of the senior author (E.J.C.).

Selection criteria
The protocol for this systematic review was preregistered with the PROSPERO database registration (ID: CRD42017081707) and has been published.20 Only studies investigating LS in an EC population were included. Initial searches were limited by English language, human adults (>18 years), and female subjects. Only studies that used either direct germline analysis for pathogenic variants of MMR genes or proxy tumor-based molecular diagnostic methods (IHC, MSI with or without MLH1 promoter hypermethylation), or any combination of these were included. Microsatellite instability-high (MSI-H) was defined, where possible, as involving ≥30% of the included microsatellite markers. An IHC positive result was taken as loss of expression of one of the MMR proteins. Pathogenic variants of MMR genes were defined as per the authors’ analysis. To avoid double counting data, authors of more than one study were contacted for clarification and/or registry analysis was crosschecked. Where there was overlapping data, the larger study was included and the smaller excluded. Only articles that contributed at least 15 participants were included.

Data extraction
The results from the initial search were combined. The titles and abstracts were collated in a spreadsheet template downloaded from http://libguides.sph.uth.tmc.edu/excel_workbook_home. This is available from the authors on request. Duplicates were removed with the use of Endnote X7 (Thompson Reuters, New York, NY). All titles and abstracts where initially screened independently by three authors (N.A,J.R., D.B., and M.C.D.). Conflicts were resolved by unanimous agreement between the three observers. Where unanimous agreement could not be reached a senior author (E.J.C.) made the final decision. Those studies identified as meeting the inclusion criteria underwent full article review and data extraction. Those excluded at full manuscript review are detailed in supplementary materials appendix 1.

Baseline data extracted included type of study, selection criteria, number of participants, country of origin, demographic data, type of initial screening method for LS, gold standard test, pathogenic variant distribution, and pathology distribution.

Statistical analysis
The primary outcome measure was the proportion of EC patients who were identified as being likely LS (aberrant MMR IHC expression, MSI-H with or without MLH1 promoter hypermethylation) or as carrying a germline MMR pathogenic variant. The Freeman–Tukey (a double arcsine transformation) transformed proportions of LS positive EC patients were pooled using the inverse variance heterogeneity model.21 To aid interpretation, all results were presented after back transformation to natural proportions. A quantification of heterogeneity across studies was presented as an $I^2$ score (with $I^2$ score of 25%, 50%, and 75% representing low, moderate, and high levels of heterogeneity respectively).22 All statistical analyses were performed R, Version 3.3.1 (https://cran.r-project.org), using the package “meta.”
RESULTS

Search results
The combined search terms yielded 1119 articles. Primary review of titles and abstracts identified 83 articles that warranted full manuscript review. Of these, 56 studies met the inclusion criteria. At data extraction and quality assessment, three studies were removed due to incomplete data (n = 1) (ref. 25), inconsistent presentation of results (n = 1) (ref. 24), or an inappropriate population (n = 1) (ref. 25) (Fig. 1). Bias scores for each of the studies are outlined in supplementary appendix 2.

Fifty-three papers were included for the final analyses.13–15,17,18,26–73 These studies included 12,633 participants with EC. The majority of studies were conducted in North America (n = 33), with relatively small numbers carried out in Europe (n = 6), Southeast Asia (n = 7), Australasia (n = 4), and South America (n = 3). Twenty-three (43%) populations were preselected by age, family history, or other clinical parameters before analysis. Primary testing included MSI (n = 9), IHC (n = 28), MSI and IHC (n = 13), or germline testing (n = 3). Studies are summarized in Table 1.

All studies originated from specialist tertiary referral centers or their biobanks. Histological features were reported in 20 papers. Type 1/endometrioid tumors constituted 79.3% of tumors, consistent with the literature.74 There were insufficient data to describe the histological breakdown of tumors diagnosed in women who were found to have abnormal tumor triage or LS. From the studies that included age data (n = 43), the median age of subjects tested was 59.5 years (interquartile range [IQR:] 53–62).

Immunohistochemical analysis
In total, 42 papers reported the outcome of IHC analysis. These studies include 10,683 participants, 10,460 with completed IHC analysis. Of these, 2563 (25%) were found to have absent expression of at least one of the MMR proteins on IHC. This represents an overall proportion of 0.26 to have absent expression of at least one of the MMR proteins. Of these, 2563 (25%) were found to have absent expression of at least one of the MMR proteins. These studies include 10,683 participants, 10,460 with completed IHC analysis. Of these, 2563 (25%) were found to have absent expression of at least one of the MMR proteins. Of these, 2563 (25%) were found to have absent expression of at least one of the MMR proteins.

Microsatellite instability analysis
MSI data were available for 4310 tumors, 2580 of which were also tested by IHC. Of these, 1133 (26.3%) were MSI-H. When studies that used preselective criteria were excluded, the total number of participants was 2890, of whom 768 were positive (26.6%) The overall proportion of MSI-H ECs was 0.27 (CI 95% 0.25–0.29, I²: 71%). This was 0.27 (CI 95% 0.25–0.29, I²: 75%) and 0.26 (CI 95% 0.24–0.29, I²: 68%) for unselected and preselected populations, respectively (Fig. 3). The results of MLH1 methylation analysis in conjunction with MSI testing are shown in supplementary appendix 4.

There was no significant difference between the proportion of positive test results if IHC or MSI was used as the initial tumor triage (0.25 vs. 0.27 p value = 0.5 [Student’s t test]). Analysis of MSI proportions pre-2011 vs. post-2011 did not find a significant difference (t test p value = 0.11).

Germline analysis
In total, 23 studies used some form of germline analysis to establish a diagnosis of LS. There was a wide degree of variation in the completeness of germline testing, with few studies (n = 9) testing all those they intended to. Furthermore, 12 studies that used a tumor-based triage test did not perform MLH1 promoter methylation testing. Two studies failed to report the outcome of their MLH1 methylation testing.14,54 The majority of these studies used another means of excluding a proportion of their MLH1 IHC positive results, for example a negative family history. Therefore, the population that went on to have definitive germline analysis is heterogeneous.
Fig. 1 Flowchart detailing study identification, study selection, and characteristics of included studies. IHC immunohistochemistry, MSI microsatellite instability.
| Author            | Study year | Country     | Selection       | Initial tumor screen | Number of participants | Proportion of positive IHC | Proportion of positive MSI | Proportion of negative methylation after positive tumor triage | Proportion of positive germline samples after positive tumor-based triage | Comments                                                                                                                                 |
|-------------------|------------|-------------|-----------------|----------------------|------------------------|---------------------------|----------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Backes et al.     | 2009       | USA         | None            | IHC                  | 385                    | 0.12                      | NA                         | NA                                                                     | 0.37                                                                    | IHC results not described by gene; MSI not clearly described                                                              |
| Batte et al.      | 2014       | USA         | None            | IHC                  | 579                    | 0.07                      | NA                         | NA                                                                     | 0.8                                                                    | Only 36 tumors had MSH6 IHC testing; PMS2 not tested; 20 tumors were MSI-H; 37 were MSI-L                               |
| Berends et al.    | 2003       | USA         | <50 years       | IHC and MSI         | 58                     | 0.49                      | 0.35                       | NA                                                                     | 0.09                                                                   | None                                                                                                                          |
| Bruegl et al.     | 2014       | USA         | None            | IHC                  | 412                    | 0.29                      | NA                         | 0.11                      | NA                                                                     | Only one MS region investigated; only MLH1 and MSH2 IHC carried out; results quoted in the paper are not consistent |
| Buchanan et al.   | 2013       | Australia   | None            | IHC                  | 702                    | 0.24                      | NA                         | 0.09                      | 0.14                                                                   | Not clear how IHC was applied                                                                                    |
| Buttin et al.     | 2004       | USA         | None            | MSI                  | 413                    | NA                        | 0.27                       | 0.19                      | NA                                                                     | None                                                                  |
| Catsas et al.     | 1998       | Spain       | None            | MSI                  | 42                     | NA                        | 0.29                       | NA                                                                     | NA                                                                     | PMS2 germline testing not performed; all MSI had GL                                                                       |
| Chadwick et al.   | 2001       | USA         | None            | MSI                  | 74                     | NA                        | 0.23                       | NA                                                                     | 0.12                                                                   | None                                                                                                                          |
| Cook et al.       | 2013       | Canada      | <80 years       | MSI                  | 480                    | NA                        | 0.27                       | NA                                                                     | NA                                                                     | Analysis includes 2 known LS carriers                                                                                 |
| Cossio et al.     | 2010       | Brazil      | <50 years of strong FHx | IHC and MSI | 30                     | 0.33                      | 0.23                       | NA                                                                     | NA                                                                     | None                                                                                                                          |
| Dillon et al.     | 2017       | USA         | None            | IHC                  | 233                    | 0.26                      | NA                         | 0.05                      | 0.45                                                                   | None                                                                                                                          |
| Djordjevic et al. | 2013       | USA         | None            | IHC                  | 154                    | 0.30                      | NA                         | 0.14                      | NA                                                                     | None                                                                                                                          |
| Egoavil et al.    | 2013       | Spain       | None            | IHC and MSI         | 173                    | 0.34                      | 0.27                       | 0.14                      | 0.42                                                                   | 89 had unselected GL analysis; 4 GL results not available; those MSI +ve or MLH1 deficient without FHx did not have GL analysis; numbers of IHC MLH1 loss not clear |
| Author          | Study year | Country     | Selection | Initial tumor screen | Number of participants | Proportion of positive IHC | Proportion of positive MSI | Proportion of negative methylation after positive tumor triage | Proportion of positive germline samples after positive tumor-based triage | Comments                                                                                                                                                                                                 |
|-----------------|------------|-------------|-----------|----------------------|------------------------|-----------------------------|-----------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ferguson et al. | 2014       | Canada      | None      | IHC and MSI          | 119                    | 0.29                        | 0.23                        | NA                                                                             | 0.08                                                                            | A pre universal screening cohort, described in this paper, was not included in this analysis; those who underwent testing not as a result of universal screening were also excluded                                           |
| Frolova et al.  | 2015       | USA         | None      | IHC                  | 234                    | 0.22                        | NA                          | 0.09                                                                          | 0.31                                                                            | None                                                                                                                                                                                                  |
| Garg et al.     | 2009       | USA         | <50 years or LS morphology | IHC                  | 71                      | 0.45                        | NA                          | NA                                                                            | NA                                                                             | PMS2 not tested                                                                                                                                                                         |
| González et al. | 2012       | Puerto Rico | None      | IHC                  | 20                     | 0.25                        | NA                          | NA                                                                            | NA                                                                             | None                                                                                                                                                                                                  |
| Goodfellow et al.| 2015       | USA         | Endometrioid only | IHC and MSI          | 1002                   | 0.36                        | 0.30                        | 0.11                                                                          | 0.4                                                                            | Not all variants detected could be classified as pathogenic                                                                                                                                       |
| Hampel et al.   | 2006       | USA         | None      | MSI                  | 543                    | 0.34                        | 0.22                        | 0.06                                                                          | 0.02                                                                            | Germline pathogenic variants are not described; the PMS2 deficient case on IHC also lacked MSH6 expression                                                                                     |
| Hartnett et al. | 2015       | USA         | None      | IHC                  | 205                    | 0.21                        | NA                          | 0.03                                                                          | 0.01                                                                            | Of the 24 tumors tested 5 did not have sufficient material for any MSI; a further 4 had sufficient material for limited analysis                                                                    |
| Hewitt et al.   | 2006       | UK          | FHx       | MSI                  | 17                     | 0.18                        | NA                          | NA                                                                            | NA                                                                             | None                                                                                                                                                                                                  |
| Joehlin-Price et al. | 2014 | USA         | None      | IHC                  | 1054                   | 0.22                        | NA                          | NA                                                                            | NA                                                                             | None                                                                                                                                                                                                  |
| Kato et al.     | 2016       | Japan       | None      | IHC                  | 360                    | 0.03                        | NA                          | 0.02                                                                          | 0.25                                                                            | Only PMS2 results clearly reported                                                                                                                                                                   |
| Kost et al.     | 2016       | USA         | <50 years | IHC                  | 83                     | 0.24                        | NA                          | 0.16                                                                          | None                                                                            | None                                                                                                                                                                                                  |
| Lee et al.      | 2018       | Singapore   | <50 years | IHC                  | 315                    | 0.21                        | NA                          | NA                                                                            | NA                                                                             | None                                                                                                                                                                                                  |
| Leenen et al.   | 2012       | Netherlands | <70 years | IHC and MSI          | 179                    | 0.23                        | 0.23                        | 0.06                                                                          | 0.7                                                                            | Only MLH1 and MSH2 on IHC                                                                                                                                                                          |
| Author          | Study year | Country | Selection | Initial tumor screen | Number of participants | Proportion of positive IHC | Proportion of positive MSI | Proportion of negative methylation after positive tumor triage | Proportion of positive germline samples after positive tumor-based triage | Comments |
|-----------------|------------|---------|-----------|----------------------|------------------------|---------------------------|----------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------|----------|
| Lim et al.      | 1996       | USA     | None      | MSI                  | 28                     | NA                        | 0.32                       | NA                                                            | NA                                                          | Two additional patients with previously known Lynch syndrome added to final results; only 3 germline results are clearly defined |
| Lin et al.      | 2016       | USA     | Mixed     | IHC                  | 76                     | 0.22                      | NA                         | 0.08                                                          | 0.6                                                         | 21 of 41 showed MSH2/MSH6 and 10 of 41 showed MLH1/PMS2; however authors also report individual protein loss consisting of 72 in total |
| Long et al.     | 2014       | China   | None      | IHC                  | 173                    | 0.24                      | NA                         | NA                                                            | NA                                                          | PMS2 not tested |
| Lu et al.       | 2007       | USA     | <50 years | GL                   | 100                    | 0.34                      | 0.33                       | 0.22                                                          | 0.09                                                        | None |
| Chu et al.      | 2015       | Hong Kong | <45 years | IHC                  | 67                     | 0.33                      | 0.23                       | NA                                                            | NA                                                          | None |
| Mas-Moya et al. | 2015       | USA     | None      | IHC                  | 215                    | 0.33                      | NA                         | 0.17                                                          | 0.65                                                        | Only those with IHC loss had MSI |
| Matthews et al. | 2008       | USA     | <50 years | IHC                  | 61                     | 0.34                      | 0.34                       | NA                                                            | NA                                                          | MMR IHC done on TMA |
| McConechy et al.| 2015       | Canada  | None      | MSI                  | 89                     | NA                        | 0.26                       | NA                                                            | NA                                                          | MSI results are not clear |
| Mills et al.    | 2014       | USA     | None      | IHC and MSI          | 604                    | 0.25                      | NA                         | 0.09                                                          | 0.81                                                        | Three phases of testing using different tests and referral criteria; 4 had multiple gene loss on IHC; methylation testing not universally applied and therefore cannot make any sensible deductions from it |
| Moline et al.   | 2013       | USA     | Mixed     | IHC or MSI or IHC with Methylation | 245 | 0.25 | 0.13 | NA | 0.24 | None |
| Najdawi et al.  | 2017       | Australia | None     | IHC                  | 124                    | 0.24                      | NA                         | NA                                                            | 0.33                                                        | Only 9 of 11 of the Lynch-like tumors had germline testing |
| Author          | Study year | Country   | Selection     | Initial tumor screen | Number of participants | Proportion of positive IHC | Proportion of positive MSI | Proportion of negative methylation after positive tumor triage | Proportion of positive germline samples after positive tumor-based triage | Comments                                                                 |
|-----------------|------------|-----------|---------------|-----------------------|------------------------|----------------------------|----------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Parc et al.      | 2000       | USA       | <52 years     | IHC and MSI           | 62                     | 0.24                       | 0.34                       | NA                                                            | NA                                                        | MSH2 and MSH6 IHC outcomes not reported                                |
| Pecorino et al.  | 2017       | Italy     | <50 years     | IHC                   | 41                     | 0.31                       | 0.42                       | NA                                                            | NA                                                        | None                                                                   |
| Pennington et al.| 2013       | USA       | Serous only   | GL                    | 151                    | NA                         | NA                         | NA                                                            | NA                                                        | 0.00                                                                   |
| Rabban et al.    | 2014       | USA       | >50 years     | IHC                   | 273                    | 0.15                       | NA                         | 0.04                           | 0.29                       | None                                                                   |
| Resnick et al.   | 2009       | USA       | None          | IHC                   | 477                    | 0.28                       | NA                         | NA                                                            | NA                                                        | None                                                                   |
| Riggi et al.     | 2016       | Argentina | None          | IHC                   | 84                     | 0.33                       | NA                         | NA                                                            | NA                                                        | None                                                                   |
| Ring et al.      | 2013       | USA       | <50 years     | IHC                   | 111                    | 0.26                       | NA                         | NA                                                            | NA                                                        | None                                                                   |
| Ring et al.      | 2016       | USA       | None          | IHC or MSI or IHC with methylation | 381 | NK | NK | NK | 0.06 | Tumor-based molecular triage was used but the results are not detailed |
| Rubio et al.     | 2016       | Spain     | None          | IHC and MSI           | 94                     | 0.33                       | 0.27                       | NA                                                            | 0.15                       | GL result not clear                                                  |
| Sugawara et al.  | 2015       | Japan     | None          | IHC                   | 182                    | 0.30                       | NA                         | 0.22                           | NA                                                        | Of all samples 55% not tested                                         |
| Tan et al.       | 2013       | Australia | <80 years     | IHC                   | 246                    | 0.24                       | NA                         | NA                                                            | NA                                                        | None                                                                   |
| Walsh et al.     | 2008       | Australia | <50 years     | IHC and MSI           | 146                    | 0.26                       | 0.35                       | 0.18                           | NA                                                        | None                                                                   |
| Watkins et al.   | 2016       | USA       | None          | IHC                   | 242                    | 0.20                       | NA                         | 0.05                           | 0.4                        | None                                                                   |
| Woo et al.       | 2014       | Malaysia  | Endometrioid only | IHC             | 77                     | 0.19                       | NA                         | NA                                                            | NA                                                        | PMS2 not tested                                                        |
| Yoon et al.      | 2007       | Korea     | None          | IHC and MSI           | 113                    | 0.23                       | 0.44                       | 0.17                           | 0.04                       | None                                                                   |
| Zauber et al.    | 2010       | USA       | None          | MSI                   | 213                    | NA                         | 0.26                       | 0.09                           | NA                                                        | None                                                                   |

FHx family history, GL germline, IHC immunohistochemistry, LS Lynch syndrome, MSI microsatellite instability, MSI-H microsatellite instability high, MSI-L microsatellite instability low. TMA tissue microarray, NA not applicable, NK not known, AFP age, family and personal history of cancer criteria.
Fig. 2 Forest plot and meta-analysis of the proportion of endometrial tumors with mismatch repair deficiency by immunohistochemistry, including those that did and did not preselect tumors for testing. CI confidence interval.

In total, 14,770 tumors underwent tumor-based triage with IHC \((n = 10,460)\) and/or MSI \((n = 4310)\). Concurrent testing with both IHC and MSI was sufficiently reported in ten studies \(16,27,34,36,37,40,47,51,63,68\) and enabled removal of duplicates with positive concordant IHC and MSI data. Of the remaining 14,293 tumors, 1133 were MSI-H and 2563 had aberrant IHC. Studies that reported MLH1-specific IHC and MLH1 promoter methylation tumor outcomes
(n = 5594) allowed further triage by removing tumors with likely somatic MLH1 loss. In total, 1005 women were eligible for and 700 women underwent germline testing for Lynch syndrome following tumor-based triage. A total of 181 (26%) were positive. When studies that preselected their population were excluded, the combined population was 5882, of whom, upon removal of methylated results, 821 (14%) were Lynch-like on the basis of their tumor analyses and therefore should have undergone germline testing. Around 3% of ECs can be attributed to LS. The gene breakdown from NGS is shown in supplementary appendix 3.

Four studies, which examined unselected populations of EC, had complete germline testing of cases suggestive of LS on the basis of their tumor analysis. Focusing on these studies, 180 tumors were suggestive of LS of which 32 were found to have a pathological variant in one of the MMR genes. This represents a proportion of 0.21 (CI 95% 0.15–0.28, I²: 79%). Therefore, around 3% of ECs can be attributed to LS. The gene breakdown from NGS is shown in supplementary appendix 3.

**Fig. 3** Forest plot and meta-analysis of the proportion of endometrial tumors showing microsatellite instability, including those that did and did not preselect tumors for testing. CI confidence interval.

| Study                  | Events | Total | Proportion | 95%–CI       | Weight (fixed) | Weight (random) |
|------------------------|--------|-------|------------|--------------|----------------|-----------------|
| **Unselected**         |        |       |            |              |                |                 |
| Lim et al.48           | 9      | 28    | 0.32       | [0.16; 0.52] | 0.7%           | 2.3%            |
| Catasus et al.31       | 12     | 42    | 0.29       | [0.16; 0.45] | 1.0%           | 2.8%            |
| Chadwick et al.32      | 17     | 74    | 0.23       | [0.14; 0.34] | 1.6%           | 3.6%            |
| McConehy et al.52      | 23     | 89    | 0.26       | [0.17; 0.36] | 2.1%           | 4.0%            |
| Egoavil et al.36       | 47     | 173   | 0.27       | [0.21; 0.34] | 4.2%           | 5.2%            |
| Yoon et al.63          | 50     | 113   | 0.44       | [0.35; 0.54] | 3.4%           | 4.9%            |
| Zauber et al.64        | 56     | 213   | 0.26       | [0.21; 0.33] | 5.0%           | 5.4%            |
| Hapuri et al.61        | 98     | 543   | 0.18       | [0.15; 0.22] | 9.8%           | 6.2%            |
| Buttin et al.30        | 111    | 413   | 0.27       | [0.23; 0.31] | 9.9%           | 6.2%            |
| Goodfellow et al.40    | 296    | 1002  | 0.30       | [0.27; 0.32] | 25.5%          | 6.8%            |
| Rubio et al.60         | 22     | 83    | 0.27       | [0.17; 0.37] | 2.0%           | 3.9%            |
| Ferguson et al.37      | 27     | 117   | 0.23       | [0.16; 0.32] | 2.5%           | 4.4%            |
| Fixed effect model     | 2890   |       | 0.27       | [0.25; 0.29] | 67.8%          |                 |
| Random effects model   |        |       |            |              | 55.6%          |                 |

Heterogeneity: $I^2 = 75\%, \tau^2 = 0.0724, p < 0.01$

| Selected               |        |       |            |              |                |                 |
| Hewitt et al.43        | 3      | 17    | 0.18       | [0.04; 0.43] | 0.3%           | 1.1%            |
| Matthews et al.51      | 21     | 61    | 0.34       | [0.23; 0.48] | 1.7%           | 3.7%            |
| Parc et al.55          | 21     | 62    | 0.34       | [0.22; 0.47] | 1.7%           | 3.7%            |
| Moline et al.54        | 33     | 245   | 0.13       | [0.09; 0.16] | 3.5%           | 4.9%            |
| Leenen et al.47        | 42     | 179   | 0.23       | [0.17; 0.30] | 3.9%           | 5.1%            |
| Cook et al.53          | 129    | 480   | 0.27       | [0.23; 0.31] | 11.5%          | 6.3%            |
| Chu et al.58           | 14     | 62    | 0.23       | [0.13; 0.35] | 1.9%           | 3.2%            |
| Berends et al.27       | 20     | 57    | 0.35       | [0.23; 0.49] | 1.6%           | 3.6%            |
| Lu et al.50            | 25     | 95    | 0.26       | [0.18; 0.36] | 2.2%           | 4.2%            |
| Walsh et al.62         | 42     | 120   | 0.35       | [0.27; 0.44] | 3.3%           | 4.8%            |
| Cosio et al.34         | 7      | 23    | 0.30       | [0.13; 0.53] | 0.6%           | 1.9%            |
| Pecorino et al.56      | 8      | 19    | 0.42       | [0.20; 0.67] | 0.6%           | 1.9%            |
| Fixed effect model     | 1420   |       | 0.26       | [0.24; 0.29] | 32.2%          |                 |
| Random effects model   |        |       |            |              | 44.4%          |                 |

Heterogeneity: $I^2 = 68\%, \tau^2 = 0.1099, p < 0.01$

| Fixed effect model     | 4310   |       | 0.27       | [0.25; 0.28] | 100.0%         | ---              |
| Random effects model   | 0.27   | [0.24; 0.30] | 100.0% | ---              |

Heterogeneity: $I^2 = 71\%, \tau^2 = 0.0756, p < 0.01$
appendix 5. Subgroup analysis of pre-2011 vs. post-2011 proportions of germline pathogenic variants found after tumor triage was not significant (t test \( p = 0.14 \)).

Further subgroup analysis was carried out to explore the relationship between potential confounding factors; these are shown in supplementary appendix 5. Of note, those studies that did not use a priori tumor-based triage, using instead direct germline sequencing of all samples, found a higher proportion of LS carriers (0.06); however, the number of studies is limited (\( n = 3 \)). In addition, limiting testing to individuals less than 50 years yielded higher levels of MSI (0.31) and aberrant IHC (0.28).

**DISCUSSION**

Here we present a systematic review and meta-analysis to define the proportion of EC patients who test positive for LS. This work includes data from 53 studies and 12,633 participants with EC who underwent IHC, MSI, methylation, or germline analysis to diagnose LS. From these data, of 100 unselected cases of EC, approximately 3 people are estimated to have LS, consistent with current literature.\(^{66,67}\) While this is a modest percentage of positive tests, each diagnosis allows for prevent the development of other LS cancers, most notably CRC. The results of this meta-analysis are summarized in supplementary figure S12 showing the estimated outcome from each stage of the LS diagnostic pathway.

Our results do not support the use of a particular tumor triage method. The proportion of positive test results if IHC or MSI was used as the initial tumor test (0.26 vs. 0.27 \( p = 0.5 \) [Student’s t test]) was similar. This small difference could be explained by the commonly used Bethesda panel for the detection of MSI, which has only been validated in CRC and not EC.\(^{69}\) In addition, MSH6 deficient EC can be microsatellite low or stable.\(^{80}\) That said, IHC does enable a more targeted application of \textit{MLH1} promoter
hypermethylation testing, given that it need only be applied to MLH1 deficient tumors. In addition, germline analysis could be limited to the gene(s) that corresponds to the protein lost; this has potential cost saving implications.

Preselecting EC populations by age or clinical criteria did not significantly change the proportion of positive IHC or MSI results, although we did find higher proportions in these subgroups. This is somewhat surprising, as preselected populations would be expected to harbor more women with LS. This may be partly explained by the later age of onset seen in older (unselected) populations. In other words, the application of age cutoffs reduces specificity without a significant increase in sensitivity. However, universal testing does seem to decrease the yield of pathogenic variant carriers; this may arise from somatic events that lead to false positives at the tumor triage stage. MLH1 methylation is associated with increased age and so is more common in older (unselected) populations. Subgroup analysis of studies that did not use a tumor triage stage, instead using direct germline sequencing, found a higher proportion of LS carriers (0.06). This could suggest that tumor triage itself misses potentially 50% of LS carriers. However, there were only three studies in the subgroup analysis. In addition, one of the studies preselected those who had germline testing on the basis of age (<50 years). However, this finding should encourage debate as to the application of NGS without tumor-based triage in EC populations; even more so given the decreasing cost of this technology.

Our work has several key strengths. Our conclusions are based on the results of over 50 studies and 12,600 participants; the search criteria were purposely broad as to capture the maximum number of studies. During the screening phase, three independent reviewers ensured the accuracy of study selection and data capture. Therefore, the foundations of our meta-analysis were robust. In addition, we have estimated the proportions of positive results seen in IHC, MSI, MLH1 methylation, and germline testing with a high degree of precision, as reflected in the narrow 95% confidence intervals in our meta-analysis.

The heterogeneity across the studies included in our review was high, and limits the strength of our conclusions. This is a reflection of the varying quality and rigor of the included studies, some of which had small numbers of participants, and were subject to bias. The majority of studies used retrospective cohorts. Furthermore, many studies failed to complete the indicated testing in their cohorts, leading to ambiguity in their conclusions. This is evidenced by the lower proportion of LS pathological variants in those studies with complete germline analysis of Lynch-like tumors versus those with partial germline analysis (0.21 vs. 0.29). To allow for the pooling of such heterogeneous data, studies were grouped according to selection and diagnostic method. All studies that were grouped reported the same endpoint.

Another potential weakness of this study is the evolution in diagnostic technology over time. The included studies were published between 1996 and 2017. During this time diagnostic technology and guidelines have evolved significantly. Although IHC based diagnostics has remained relatively constant, MSI diagnostics has been informed by the adoption of the Bethesda panel in 1998 and the development of the more modern panels such as the pentaplex and hexaplex panels, which became widely applied to clinical practice after 2011. Even so, analysis of MSI testing results pre- vs. post-2011 did not find a significant difference (t test p value = 0.11). Only one study predates the Bethesda guidelines. The area of germline diagnostics remains innovative, but again analysis of pre- vs. post-2011 proportions of germline pathogenic variants found after tumor triage was not significant (t test p value = 0.14). Generalizability is limited by the predominance of North American and European populations in our study. Most took place in insurance-based health-care systems, which impact negatively on the uptake of genetic testing. Therefore, it could be that the proportions are an underestimate due to reduced uptake of testing, especially in high-risk groups such as the young or those with a strong family history.

In summary, ours is the first meta-analysis to examine the proportion of EC cases that are associated with LS. Different tumor triage methods did not affect estimates of the proportion of EC associated with LS, which remained constant at around 3%. Our findings suggest that a similar proportion of EC patients will test positive for LS as seen in CRC LS screening. This supports the move toward the introduction of universal screening for LS in EC.

SUPPLEMENTARY INFORMATION
The online version of this article (https://doi.org/10.1038/s41436-019-0536-8) contains supplementary material, which is available to authorized users.

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DISCLOSURE
The authors declare no conflicts of interest.
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