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Frequency of mismatch repair deficiency in ovarian cancer: A systematic review

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Abstract

Loss of mismatch repair (MMR) capacity may represent an important tumor initiating mechanism in ovarian cancer. We conducted a systematic review to analyze the frequency of microsatellite instability (MSI), immunohistochemical (IHC) staining for MMR proteins, and hypermethylation of the *MLH1* promoter region in ovarian cancers. Studies examining MSI, loss of MMR gene expression by IHC staining and *MLH1* promoter hypermethylation in ovarian cancer were identified by a systematic literature search of the PubMed electronic database through August 31, 2009. Pertinent data was extracted from eligible studies and estimates for pooled proportions were computed using random effects models. The pooled proportion of MSI detection was 0.10 (95% CI, 0.06–0.14) among 1234 cases in 22 studies. Dinonucleotide markers had a higher frequency of instability than mononucleotide markers. The pooled proportion of MLH1 or MSH2 staining loss was 0.06 (95% CI, 0.01–0.17) among 474 cases in 3 studies, with a higher frequency of loss in MLH1. The pooled proportion of *MLH1* methylation was 0.10 (95% CI, 0.06–0.15) among 672 cases in 7 studies. Data reporting MSI and loss of MMR staining in the same cases was limited. Although MMR deficiency was found in all histologic subtypes, endometrioid cancers had the highest proportions. Approximately 10% of unselected ovarian cancers are related to MMR deficiency. While MMR deficiency is associated with improved survival in other MMR-deficiency related cancer sites, epidemiological and clinical factors related to the MMR-deficient phenotype have not been adequately studied in ovarian cancer to date.

Keywords

ovarian cancer; mismatch repair deficiency; microsatellite instability; immunohistochemical staining; *MLH1* methylation

Introduction

Ovarian cancer is responsible for more deaths annually than any other female reproductive cancer. In the United States, one out of seventy females will develop this malignancy in their lifetime. Because early detection is difficult, most women present with late-stage disease, which contributes to a low overall 5-year survival rate, of only 45%. A limited number of ovarian cancer risk factors have been identified to date; neither the etiology nor the pathogenesis is well understood. While several histologic subtypes can be distinguished, it is not clear how these subtypes relate to risk factors or molecular pathways.

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¹Ovarian cancer has a poorly understood and highly heterogeneous etiology. We show that approximately 10% of ovarian cancers are related to mismatch repair deficiency, a well defined molecular pathway which may be a clinically and epidemiologically distinct phenotype with marked public health importance.

Mismatch repair (MMR) deficiency is a distinct carcinogenic mechanism. Following its discovery in the hereditary non-polyposis colorectal cancer (HNPCC) syndrome in the early 1990s 1, MMR deficiency was identified as a potential cancer-initiating pathway in several other cancer sites, including the uterus and the ovaries. MMR-deficient colorectal cancers have distinct clinical features: they are preferentially located in the proximal colon and have a better prognosis than their MMR-proficient counterparts 1. There is some evidence that standard chemotherapy regimens are associated with worse outcomes in MMR-deficient cancers 2. Furthermore, it has been demonstrated that MMR-deficient cancers elicit strong anti-tumoral immune responses and use specific immune evasion strategies 3. It is not clear whether MMR deficiency in ovarian cancer is associated with similar characteristics.

Microsatellite instability (MSI) occurs when mismatch repair genes are compromised due to mutations or methylation. Small deletion/insertion mutations occur frequently during DNA replication 4. These small mutations are repaired by a complex of MMR proteins including MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2 5· 6. DNA sequences with mono- or dinucleotide repeats (microsatellites) are especially susceptible to insertion/deletion mutations and can serve as sentinel markers for MMR deficiency 4. At an NCI-consensus workshop in 1997, a set of microsatellite markers for assessment of MMR deficiency in colorectal cancer was defined. The marker panel consists of two mononucleotide markers (BAT25 and BAT26) and three dinucleotide markers (D2S123, D5S346 and D17S250); these five markers are referred to as the Bethesda Panel 7. It is unclear whether the markers recommended for MSI assessment in colorectal cancers have similar performance at other cancer sites.

Deleterious mutations or methylation of mismatch repair genes usually result in loss of mismatch repair protein expression; therefore, immunohistochemical (IHC) staining of mismatch repair proteins is an alternative approach to detect MMR deficiency. While more than seven mismatch repair genes have been identified so far, most MMR-deficient cancers have mutations in *MLH1* or *MSH2* 7· 8. Hypermethylation of the *MLH1* promoter has also been identified as a causal event in sporadic MMR-deficient cancers 4

Here, we updated recent summaries of MSI frequencies in ovarian cancers 9 and added a detailed analysis of MSI frequencies for specific mononucleotide and dinucleotide markers. We also extended our systematic review to two other markers of MMR deficiency, IHC staining of mismatch repair proteins and *MLH1* promoter methylation. We analyzed the frequency of MMR deficient ovarian cancers in unselected case series and the association of the MMR deficient phenotype with histologic subtypes based on MSI, MMR staining, and MLH1 methylation.

Materials and Methods

Identification of Studies

All articles examining MSI, MMR deficiency by IHC staining and *MLH1* promoter hypermethylation in unselected ovarian cancers or in histologically selected ovarian cancers published through August 31, 2009 in the English language on the PubMed electronic database were obtained through systematic keyword searches, using the following terms: (“ovarian cancer” or “ovarian carcinoma”) and (“MMR,” “microsatellite instability,” “MMR,” “mismatch repair,” “IHC,” “immunohistochemistry,” “methylation,” or “MSP”). Additional studies were identified through review of references in articles identified through the above described search. A total of 2,151 studies were identified.

Inclusion and Exclusion Criteria

Only original articles contributing at least five participants were included in this review. To determine the overall percentage of MMR-deficient ovarian cancers, only studies with case series not selected for specific histologic subtypes or hereditary cases were included. In addition, studies reporting findings for a specific histologic subtype were included in analyses focusing on that subtype only. After initial exclusion of ineligible studies using these criteria by a single reviewer (MAM), a set of 54 potentially eligible studies was reviewed independently by two reviewers (MAM, NW). Studies without agreement were discussed to reach a final discussion about eligibility. A total of 38 studies were included in these analyses.

Data Extraction

First author, date of publication, study sample size, histology distribution, listing of MSI markers used and instability status, detailed instability results using Bethesda Panel markers (BAT25, BAT26, D2S123, D5S346, and D17S250), IHC staining results for expression of MMR proteins, MLH1 and MSH2, and *MLH1* methylation status were recorded.

Analysis

Detection of MMR deficiency using MSI was examined using multiple approaches. Pooled proportions estimates and accompanying confidence intervals were computed for crude MSI, using published MSI results from all eligible studies that assessed MMR deficiency by any two or more MSI markers. Analysis was completed in all unselected cases and in serous, endometrioid, clear cell and mucinous subtypes. A refined MSI estimate was calculated utilizing published MSI results from eligible studies with unselected case populations that assessed MMR deficiency by two or more of the 24 markers recommended at the Bethesda workshop. These loci comprised BAT25, BAT26, D2S123, D5S346, D17S250, BAT40, BAT34C4, TGFBR2, ACTC, D3S1029, D5S107, D7S519, D8S87, D10S197, D13S153, D13S175, D17S588, D17S787, D18S55, D18S58, D18S61, D18S64, D18S69, and D20S100. A specific MSI estimate was also calculated using published MSI results from all studies that utilized the five Bethesda Panel markers to identify MSI. These five loci comprised: BAT25, BAT26, D2S123, D5S346 and D17S250. Studies containing pooled MSI results were included if the results incorporated only these five markers. Studies including results separated by marker were incorporated only if data were available for each of the five markers. MSI was determined using the definitions established in the Bethesda Guidelines, defining MSI positivity for $\geq 2/5$ markers showing instability [7].

Pooled proportions estimates and accompanying confidence intervals for Bethesda Panel mononucleotide and dinucleotide markers were calculated using published MSI results for individual markers from studies with unselected case populations. The two mononucleotide and three dinucleotide markers comprised BAT25 and BAT26, and, D2S123, D5S346 and D17S250, respectively. Studies including individually reported results for any of these markers were included.

Estimates and confidence intervals for pooled proportions of loss of MLH1 and MSH2 protein expression using IHC staining were computed from published study results. Studies included in this analysis utilized unselected case populations assessing one or both of these proteins. An analysis was completed in specific histologic subtypes (serous, endometrioid, clear cell and mucinous types) in addition to the unselected cases. Estimates for pooled proportions of *MLH1* methylation detected by methylation specific PCR were also computed utilizing studies which included unselected cases.

Statistical Analysis

Pooled proportion estimates with confidence intervals were computed for all variables using independent binomial proportions from included studies. Data transformations were employed to stabilize variance and to improve distribution normality in non-normally distributed data. The Freeman-Tukey transformation, a double arcsine transformation, was used to convert binomial counts to working quantities prior to analyses designed to fit normally distributed data [10]. Independent binomial proportions were transformed prior to calculation of a pooled proportions estimate. Transformed pooled proportions and 95% confidence intervals were calculated using a random effects model to account for in-study and between-study error. True pooled proportions were determined using a back-transformation of the mean transformed pooled proportion and the corresponding confidence limits. A forest plot was used to illustrate the effect of the individual studies associated with the pooled proportions estimate for the crude MSI estimate. Although the random effects model accounts for between-study error in addition to in-study error, an indicator of heterogeneity between studies can provide an easily interpretable measure of variability. Traditionally, the Cochran's Q test has been used to assess whether heterogeneity is present in a set of samples. In recent years, the I^2 test has replaced the Q test because it measures the extent of heterogeneity as opposed to presence of heterogeneity alone. The I^2 statistic is expressed as a percent of total between-study variability, ranging from 0–100%. The I^2 test was used to determine heterogeneity of all subgroups analyzed; heterogeneity among samples was interpreted using a previously described scale: high heterogeneity was observed in samples with $I^2 \geq 75\%$, moderate heterogeneity in samples with $50\% < I^2 < 75\%$; low heterogeneity in samples with $I^2 < 50\%$ [11].

Results

MSI frequency in unselected ovarian cancer cases

A total of 22 studies including 1,234 unselected participants were identified. A crude MSI proportions estimate of 0.10 (95% CI, 0.06–0.14) was calculated using published MSI results from the 22 studies varying in number and type of markers used. Heterogeneity among the results published in these 22 studies was high ($I^2 = 80\%$). [Figure 1, Table 1]

For a refined MSI analysis studies using less than two of the 24 recommended markers were excluded, yielding a total of 16 studies including 1,027 participants. The refined analysis demonstrated an estimated MSI proportion of 0.09 (95% CI, 0.05–0.14, $I^2 = 84\%$) using published MSI frequencies from all pertinent studies. [Table 1]

Only four studies (245 participants) included distinct results for all five Bethesda Panel markers (BAT25, BAT26, D2S123, D5S346 and D17S250). The four pertinent studies yielded a pooled MSI proportion of 0.09 (95% CI, 0.06–0.13, $I^2 = 0\%$). When analyzed individually, dinucleotide markers D5S346 and D17S250, yielded the highest proportions of instability at 0.12 (95% CI, 0.04–0.23; $I^2 = 85\%$) and 0.08 (95% CI, 0.02–0.17; $I^2 = 90\%$), respectively. The dinucleotide marker, D2S123, yielded an estimated proportion of 0.06 (95% CI, 0.02–0.11; $I^2 = 77\%$), and the mononucleotide markers, BAT25 and BAT26, showed an estimated proportion of 0.07 (95% CI, 0.03–0.12; $I^2 = 70\%$) and 0.05 (95% CI, 0.02–0.09; $I^2 = 62\%$), respectively. [Table 1, Table 2]

Five studies (309 participants) reported individual results for the two mononucleotide markers included in the Bethesda Panel (BAT25 and BAT26). These studies indicated that an estimated proportion of 0.02 (95% CI, 0.01–0.04, $I^2 = 9\%$) cases exhibited MSI in both markers; an estimated proportion of 0.09 (95% CI, 0.02–0.18, $I^2 = 83\%$) cases exhibited MSI in either of the two markers. Four studies (245 participants) reported individual results for the three dinucleotide markers associated with the Bethesda Panel (D2S123, D5S346 and

D17S250). These studies indicated that an estimated proportion of 0.02 (95% CI, 0.00–0.05, $I^2 = 33\%$) cases exhibited MSI in all three markers; an estimated proportion of 0.07 (95% CI, 0.01–0.17, $I^2 = 82\%$) showed MSI in at least 2 markers and a proportion of 0.13 (95% CI, 0.06–0.23, $I^2 = 72\%$) cases exhibited MSI in any of the three markers. [Supplemental Table 1]

MSI frequency in ovarian cancer subtypes

MSI data for serous, endometrioid, mucinous and clear cell cancers were used in a crude subtype analysis. Sixteen studies including 564 serous cases, 17 studies including 314 endometrioid cases, 12 studies including 117 clear cell cases and 12 studies including 101 mucinous cases were identified. Published MSI frequencies from all pertinent studies were used to calculate pooled estimates. The frequency of MSI by histologic subtypes was: 0.08 (95% CI, 0.05–0.12; $I^2 = 64\%$) serous; 0.19 (95% CI, 0.13–0.26; $I^2 = 47\%$) endometrioid; 0.12 (95% CI, 0.07–0.18; $I^2 = 0\%$) clear cell; 0.17 (95% CI, 0.07–0.30; $I^2 = 53\%$) mucinous. [Table 3]

Frequency of MMR deficiency detected by IHC staining loss and MLH1 promoter methylation

Four studies (498 participants) measuring MLH1 deficiency in unselected populations were identified; of these four studies, three (474 participants) also measured MSH2 deficiency. Loss of MLH1 expression was detected in 0.05 (95% CI, 0.01–0.10; $I^2 = 73\%$), loss of MSH2 expression in 0.02 (95% CI, 0–0.09; $I^2 = 85\%$), and loss of either protein in 0.06 (95% CI, 0.01–0.17; $I^2 = 88\%$) of the cases. [Table 4]

The paucity of data available for MLH1 and MSH2 deficiency in histologic subtypes did not allow for a comprehensive subtype analysis and was restricted to endometrioid and clear cell subtypes. Endometrioid subtypes demonstrated an estimated pooled proportion for overall loss of expression of 0.10 (95% CI, 0.01–0.26, $I^2 = 77\%$), for MLH1 deficiency of 0.06 (95% CI, 0.01–0.16, $I^2 = 70\%$), and for MSH2 deficiency of 0.05 (95% CI, 0.01–0.09, $I^2 = 0\%$). Clear cell subtypes demonstrated an estimated pooled proportion for overall loss of expression of 0.12 (95% CI, 0.06–0.20, $I^2 = 0\%$), for MLH1 deficiency of 0.07 (95% CI, 0.03–0.12, $I^2 = 0\%$), and for MSH2 deficiency of 0.06 (95% CI, 0.01–0.15, $I^2 = 41\%$). [Table 5]

Data related to *MLH1* promoter methylation were reported for 672 tumors. The pooled proportion of *MLH1* promoter methylation was 0.10 (95% CI, 0.06–0.15; $I^2 = 72\%$). [Table 4]

Discussion

Previous studies have provided varying estimates for MMR deficiency in ovarian cancer; our analysis indicates that about 10% of ovarian cancers are related to this molecular pathway. We analyzed three indicators of MMR deficiency: MSI, MMR protein staining, and *MLH1* methylation.

Our crude MSI analysis included data from studies which used any number or type of microsatellite markers; our refined analysis drew from studies including at least two markers from the Bethesda Panel in conjunction with any number or type of additional markers. In a further analysis, we computed MSI exclusively using the Bethesda Panel. These three analyses, which varied by type and number of markers used, all yielded a proportion between 9–10% MSI in ovarian cancers. Heterogeneity in both crude and refined MSI estimates was high, while results using the Bethesda Panel were more homogeneous, albeit in a very small set of studies. Interestingly, analysis of studies that assessed MSI by tumor

histology yielded more homogeneous results; moderate heterogeneity for the frequency of MSI was reported for serous and mucinous carcinomas, while low heterogeneity was reported for endometrioid and clear cell carcinomas. This suggests that heterogeneity is at least partly related to a variable distribution of tumor histology in the different case series. Relatively high proportions for MSI estimates were observed in endometrioid (19%) and mucinous (17%) subtypes, while serous and clear cell subtypes yielded lower proportion estimates.

Since publication of the Bethesda Guidelines in November, 1998, the use of the Bethesda Panel markers in studies assessing MMR deficiency in ovarian cancer has increased significantly ($p < 0.01$). Still, it remains unclear as to whether the Bethesda Panel includes the most appropriate markers for detection of MMR deficiency in ovarian cancer. Although the Panel markers are regularly used to assess MSI in ovarian and other tumor types, they were only validated in colorectal tumors prior to selection 47, 48. In colorectal cancer, the relative proportion of instability of mononucleotide markers BAT25 and BAT26 is higher than the proportion of instability of dinucleotide markers D5S346 and D17S250. In contrast, we found MSI more frequently in dinucleotide markers, D5S346 and D17S250, than in mononucleotide markers, BAT25 and BAT26. Due to the paucity of concomitant MSI analyses and MMR staining or mutation data in the literature, we cannot determine whether dinucleotide markers are more sensitive for detection of MMR in ovarian cancers, or whether they are less specific as suggested in other cancer sites.

In unselected series of colorectal cancers, at least 70–90% of MMR deficient cases show loss of expression of one of two MMR genes, *MLH1* and *MSH2* 49, 50. Due to the lack of parallel MSI testing, we cannot directly estimate the proportion of *MLH1* and *MSH2* loss in MMR-deficient ovarian cancer. However, comparing the percentage of staining loss with the MSI frequency suggests a similar proportion (6.4% loss of *MLH1* or *MSH2* staining vs. 9.6% MSI) in ovarian cancers. The different distribution of instability in mononucleotide vs. dinucleotide markers between colorectal and ovarian cancers suggest that there could be biological differences of MMR deficient cancers at the two sites 47. However, studies of unselected ovarian cancers including MSI analysis, MMR protein staining, as well as clinical and histological data are required to further explore this notion.

While we report that MMR deficiency occurs in all histologic subtypes of ovarian cancer, proportions varied by subtype with endometrioid and mucinous cancers being most frequently affected. Of note, these two subtypes occasionally are metastases of colorectal and endometrial cancers rather than ovarian primaries and both cancer sites have a higher frequency of MMR deficient cancers compared to ovaries. We assume that MMR deficiency is tumor initiating in a subset of ovarian cancers, similar to colorectal and endometrial cancers. To date, MMR deficient ovarian cancers have been only poorly characterized with respect to their epidemiological, molecular and clinical features. Current literature findings are limited by small sample sizes and poorly standardized assays. Few studies reported any risk factor information; lack of basic epidemiologic and clinical data, such as age, race/ethnicity and common risk factors as well as grade, stage, and survival, limited the scope of our study.

Due to the lack of epidemiological and clinical data, the relevance of detecting the MMR-deficient ovarian cancer subtype is currently unclear. Given the limited knowledge about ovarian cancer etiology and the dismal prognosis of advanced ovarian cancer it seems important to study risk factors and prognosis associated with this clearly definable molecular subtype. Similar to MMR-deficient subtypes at other cancer sites, MMR-deficient ovarian cancers may have distinct features with public health importance, e.g. different age of onset, different survival, and different response to chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

MMR	mismatch repair
MSI	microsatellite instability
IHC	immunohistochemical
HNPCC	hereditary non-polyposis colorectal cancer

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Figure 1.

Forest plot displaying individual proportions associated with the crude pooled proportion of MSI in unselected ovarian cancer cases 5, 6, 8, 12-30

Comparison of the proportion of ovarian cancer cases exhibiting microsatellite instability in an unselected population using three approaches. The crude approach includes studies with any type and number of MSI markers in the pooled analysis; the refined approach includes studies with two or more recommended markers in the study; the specific approach only includes studies using the Bethesda Panel markers. Italicized markers are NCI-recommended markers (of which there are 19); markers which are italicized and underlined are included in the Bethesda Panel (of which there are 5). S indicates the number of studies included in the analysis; N indicates the total number of cases included in the study; MSI indicates the number of cases exhibiting microsatellite instability in the study; prop indicates the frequency proportion estimate for MSI in the study.

Table 1

Author	Year	Markers Used [†]	Crude Estimate (S = 22)			Refined Estimate (S = 16)			Specific Estimate		
			N	MSI	Prop	N	MSI	prop	N	MSI	prop
Han12	1993	<i>D2S123</i> , D3S1067, D2S136, TP53	19	3	0.16	--	--	--	--	--	--
Fujita13	1995	MFD26, <i>MFD27</i> , MFD41, MFD49	47	8	0.17	--	--	--	--	--	--
King14	1995	GABARB1, <i>MDF27</i>	41	7	0.17	--	--	--	--	--	--
Kobayashi15	1995	D1S226, D2S393, D3S1067, D5S644, TP53	68	2	0.03	--	--	--	--	--	--
Arzimanogloul16	1996	<i>D2S123</i> , D2S119, <i>D10S197</i>	90	11	0.12	90	11	0.12	--	--	--
Krajnovic17	1998	DMD, <i>BAT26</i> , D3S1611, D3S1286, D9S169, D12S320, THRA, D21S1436	12	2	0.17	--	--	--	--	--	--
Hickey18	1999	<i>D5S346</i> , D7S486, D7S522, D11S904, P53, D17S855	20	2	0.1	--	--	--	--	--	--
Allen19	2000	<i>D2S123</i> , <i>D18S58</i> , <i>BAT26</i> , <i>BAT40</i>	26	1	0.04	26	1	0.04	--	--	--
Johannsdottir20	2000	<i>D2S123</i> , D2S382, <i>BAT26</i> , <i>D3S1210</i> , <i>D3S1217</i> , <i>D6S292</i> , <i>D7S480</i> , <i>D9S157</i> , <i>D13S153</i>	33	1	0.03	33	1	0.03	--	--	--
Alvi21	2001	<i>BAT26</i> , <i>BAT40</i> , <i>D2S123</i> , D8S255, <i>D13S175</i>	43	3	0.07	43	3	0.07	--	--	--
Chiaravalli8	2001	<i>BAT25</i> , <i>BAT26</i> , <i>BAT40</i>	14	4	0.29	14	4	0.29	--	--	--
Gras22	2001	<i>BAT25</i> , <i>BAT26</i> , <i>D2S123</i> , <i>D5S346</i> , <i>D17S250</i>	35	2	0.06	35	2	0.06	35	2	0.06
Sood23	2001	<i>D2S123</i> , <i>BAT25</i> , <i>D17S250</i> , <i>BAT26</i> , <i>D5S346</i> , NME1, <i>D10S197</i> , D11S175, DXS981, DXS6800, DXS6807, AR, D3S1611	109	13	0.12	109	13	0.12	109	12	0.11
Watanabe24	2001	<i>BAT25</i> , <i>BAT26</i> , <i>BAT40</i> , <i>D2S123</i> , D3S1612, <i>D5S346</i> , D11S904, <i>D18S58</i> , TP53	24	2	0.08	24	2	0.08	--	--	--
Geisler25	2003	<i>D2S123</i> , D5S346, <i>D17S250</i> , <i>BAT25</i> , <i>BAT26</i> , NM23	125	21	0.17	125	21	0.17	--	--	--
Dellas26	2004	<i>BAT25</i> , <i>BAT26</i> , <i>D5S346</i> , <i>D2S123</i> , <i>D17S250</i> , <i>BAT40</i> , MYCL1	66	20	0.3	66	20	0.3	--	--	--
Singer27	2004	<i>BAT25</i> , <i>BAT26</i> , <i>D2S123</i> , <i>D5S346</i> , <i>D17S250</i> , D2S442, D21S1436, UT5320, D8S321, D20S82, UT5037	75	11	0.15	75	11	0.15	75	6	0.08
Helleman28	2006	<i>BAT25</i> , <i>BAT26</i> , <i>BAT40</i> , <i>D2S123</i>	75	0	0	75	0	0	--	--	--
Begum6	2008	TP53, <i>D17S250</i> , CACNLB1, <i>D18S58</i> , D19S49, DXS538, DXS454, D5S117, <i>D5S107</i> , D6S284, D6S305, D9S171, D9S15, D11S554, D11S29, D13S272	171	4	0.02	171	4	0.02	--	--	--

Author	Year	Markers Used [†]	Crude Estimate (S = 22)			Refined Estimate (S = 16)			Specific Estimate		
			N	MSI	Prop	N	MSI	prop	N	MSI	prop
Huan29	2008	<i>D2S123, D5S346, D8S87, D13S153, D13S175, D18S55, D20S100, BAT25</i>	51	7	0.14	51	7	0.14	--	--	--
Plistecka-Halasa30	2008	<i>D17S926, TP53, TP53-Alu, D17S261, RB, D3S1611, D8S1811, D11S904, D10S197, D2S123, BAT26, BAX</i>	64	0	0	64	0	0	--	--	--
Yoon5	2008	<i>BAT26, D5S346, BAT25, D17S250, D2S123, NME1, D10S197, DXS981, AR, D3S1611, D13S175, D11S904, DXS6807, DXS6800</i>	26	0	0	26	0	0	26	1	0.04
Overall Value			1234	124	0.1	1027	100	0.1	245	21	0.09
Estimate of Proportion			9.6%			8.6%			9.1%		
95% CI			(6.1%, 13.8%)			(4.7%, 13.5%)			(5.8%, 13.0%)		
I ² [‡]			80.1%			83.9%			0.0%		

[†] All markers are listed by the name under which they were reported. MFD26, MFD27, MFD41 and MFD49 are more commonly known as D18S34, D5S107, D17S261 and D15S87, respectively.

[‡] I² 75% is interpreted as highly heterogeneous; 75% > I² 50% is interpreted as moderate heterogeneity; I² < 50% is interpreted as low heterogeneity.

Proportion of microsatellite instability detection by individual Bethesda Panel markers compared to microsatellite instability detection by the full panel of Bethesda markers in an unselected population, derived from thirteen studies 5, 6, 16-19, 22-24, 26-28, 30.

Table 2

	Studies Included	Instability	N	Estimate of Proportion	95% CI	I ² [†]
Mononucleotide Markers						
BAT25	6	26	374	6.7%	(2.7%, 12.2%)	69.5%
BAT26	9	20	471	4.8%	(2.0%, 8.6%)	62.3%
Dinucleotide Markers						
D2S123	10	39	584	6.0%	(2.6%, 10.8%)	76.5%
D5S346	6	51	334	12.1%	(4.4%, 23.0%)	84.9%
D17S250	7	41	498	7.8%	(1.9%, 17.1%)	89.5%

[†]I² 75% is interpreted as highly heterogeneous; 75% > I² 50% is interpreted as moderate heterogeneity; I² < 50% is interpreted as low heterogeneity.

Table 3

Comparison of the proportion of ovarian cancer cases exhibiting microsatellite instability in serous, endometrioid, mucinous and clear cell subtypes, derived from twenty three studies 5, 6, 13, 14, 18, 19, 22, 23, 25–39.

Histology	Studies Included	MSI	N	Estimate of Proportion	95% CI	I ² [†]
Serous	16	48	564	7.9%	(4.5%, 12.3%)	64.0%
Endometrioid	17	64	314	19.2%	(13.2%, 26.0%)	46.8%
Clear Cell	12	11	117	11.5%	(6.5%, 17.7%)	0.0%
Mucinous	12	20	101	16.9%	(7.1%, 29.7%)	52.5%

[†]I² 75% is interpreted as highly heterogeneous; 75% > I² 50% is interpreted as moderate heterogeneity; I² < 50% is interpreted as low heterogeneity.

Estimate of the proportion of ovarian cancer cases exhibiting loss of MLH1 or MSH2 expression by immunohistochemical staining and *MLH1* promoter hypermethylation in unselected populations, derived from four 24·40–42 and seven 25·28·41·43–46 studies, respectively.

Table 4

	Studies Included	Loss of staining	N	Estimate of Proportion	95% CI	I ² †
IHC Staining						
Any Loss	3	16	474	6.4%	(0.8%, 16.6%)	88.4%
MLH1	4	13	498	4.7%	(1.2%, 10.2%)	72.8%
MSH2	3	6	474	2.3%	(0%, 8.5%)	84.5%
Promoter Hypermethylation						
<i>MLH1</i>	7	65	672	10.4%	(6.3%, 15.4%)	71.6%

†I² 75% is interpreted as highly heterogeneous; 75% > I² 50% is interpreted as moderate heterogeneity; I² < 50% is interpreted as low heterogeneity.

Estimate of the proportion of ovarian cancer cases exhibiting loss of MLH1 or MSH2 expression by immunohistochemical staining in endometrioid and clear cell subtypes, derived from four 36, 37, 40, 42 and five studies 35, 36, 38, 40, 42, respectively.

Table 5

	Studies Included	Loss of staining	N	Estimate of Proportion	95% CI	I ² †
Endometrioid						
Any Loss	2	13	108	10.0%	(1.3%, 25.7%)	77.0%
MLH1	4	12	145	6.2%	(0.8%, 16.1%)	70.3%
MSH2	2	4	108	4.5%	(1.4%, 9.2%)	0.0%
Clear Cell						
Any Loss	3	9	82	11.9%	(5.9%, 19.7%)	0.0%
MLH1	5	5	102	6.6%	(2.7%, 12.1%)	0.0%
MSH2	3	5	82	6.0%	(1.1%, 14.6%)	40.9%