Hereditary Non-polyposis Colorectal Cancer/Lynch Syndrome in Korean Patients with Endometrial Cancer

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Objective: We investigate the frequency of hereditary non-polyposis colorectal cancer among Korean endometrial cancer patients according to two clinical criteria and the uptake rate of a genetic test and genetic status of such patients in routine clinical practice.

Methods: This was a retrospective study involving 161 consecutive endometrial cancer patients. Patients were classified into clinical and suspected hereditary non-polyposis colorectal cancer. Using direct sequencing, germline mutations were analyzed in the MLH1 and MSH2 genes.

Results: There were four (2.5%) clinical hereditary non-polyposis colorectal cancer patients, three of whom underwent genetic testing, and a mutation (c.882delT) in the MSH2 gene was identified in one patient. There were also 14 (8.7%) suspected hereditary non-polyposis colorectal cancer patients, 6 of whom underwent genetic testing; 1 [1/6 (16.7%)] patient had a mutation (c.1757_1758insC) in the MLH1 gene and 1 patient had a sequence variant of unknown significance (c.1886A>G) in the MSH2 gene. Half of the patients (9 of 18) who met clinical or suspected hereditary non-polyposis colorectal cancer criteria declined genetic testing mainly for the reason of financial factor (8 of 9).

Conclusions: The proportion of hereditary non-polyposis colorectal cancer [11.2% (18 of 161)] was significant to offer genetic counseling and genetic testing in Korean endometrial cancer patients. Optimal financial support is crucial to increase the uptake rate of a genetic test.

Key words: MLH1 – MSH2 – Koreans – hereditary disease – endometrial cancer

INTRODUCTION

The incidence of endometrial cancer has been increasing over the past decade in Korea (1). Inherited factors are suggested as one of the most important risk factors for endometrial cancer (2). In addition to rare Mendelian-inherited syndromes with a predisposition to endometrial cancer, such as Muir Torre, Cowden and Turcot syndromes, hereditary non-polyposis colorectal cancer (HNPCC) is a common autosomal dominant condition characterized by the development of colon and endometrial cancers. The genes that are responsible for repair of mismatched DNA are defective in families with this syndrome (2). HNPCC is caused by several germline mutations in the DNA mismatch repair (MMR) genes, MLH1, MSH2, PMS1, PMS2 and MSH6 (3–6).

The Amsterdam criteria, the first clinical criteria for the diagnosis of HNPCC (c-HNPCC), were established in 1991...
based on colorectal cancers (7). In 1999, the Amsterdam criteria were revised (8). The new criteria, the Amsterdam criteria II, include colorectal cancers and cancers of the endometrium, small bowel, renal pelvis and ureters (8). The Amsterdam criteria II are so strict that many patients with MMR gene mutations are not included according to these criteria (9). The suspected HNPCC (s-HNPCC) criteria include families that do not fulfill the Amsterdam criteria, but in whom HNPCC is nevertheless strongly suspected (9). Women with mutations in DNA MMR genes have a risk for endometrial cancer by age 70 of ~42–60% in Western populations (10,11). In the Korean population, very few reports on this issue in endometrial cancer patients exist (12,13).

Therefore, we undertook this study with two objectives. The first was to elucidate the frequency of HNPCC in Korean endometrial cancer patients and determine the genetic status in such patients from our routine clinical practice. The second was to investigate the uptake rate of genetic testing for HNPCC and the cause of non-uptake of a genetic test in such patients.

PATIENTS AND METHODS

This retrospective study was approved by an institutional review board. Between January 2005 and June 2008, 161 endometrial cancer patients who were treated at the National Cancer Center, Korea, and the members of their families were interviewed for information on family history of any cancers, with specific reference to any history of HNPCC-associated cancers, such as cancer of the colon, endometrium, small intestine and urinary tract (Tables 1 and 2). All patients were confirmed pathologically to have endometrial cancer. Patients who understood that they would receive the results and counseling regarding the implications underwent genetic testing. They were informed of the possibility that the genetic testing and the results could lead to psychological distress.

Written informed consent was obtained from each patient who agreed to the genetic testing. Ethylenediaminetetraacetic acid-anticoagulated blood samples were obtained by phlebotomy from each patient. The entire coding region and the exon–intron boundaries of the MLH1 and MSH2 genes in patients with endometrial cancer have been scanned at Green Cross Reference Laboratory, Yongin-si, Korea.

DIRECT SEQUENCING

Genetic DNA was isolated from white blood cells of peripheral blood using a Puregene DNA Purification kit (Gentra System, Minneapolis, MN, USA), following the manufacturer’s instructions. DNA concentrations were determined using a DU 800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Whole exon and exon–intron boundaries were included in direct sequencing. The PCR mixture (10 μl) contained 1.0 μl of 10 × PCR buffer (Takara, Tokyo, Japan), 0.7 μl each of 2.5 mM dNTP (Takara), 0.3 μM each primer (Bioneer Corp., Chongwon, Korea), 0.5 U of Taq-DNA polymerase (Takara) and 1 μl (0.5 μg) of genomic DNA. The thermal cycler (Biometra T Gradient PCR, Gottingen, Germany) protocol was as follows: 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 60°C (up to 65°C) and 30 s extension at 72°C. There was a 5 min pre-incubation at 95°C before commencing a cycle and a 10 min additional extension at 72°C after completion of the cycles. Amplified DNA (1.5 μl) was incubated with 2 U of shrimp alkaline phosphatase and 5 U of exonuclease I (USB Corp., Cleveland, OH, USA) at 37°C for 15 min. The enzymes were inactivated by incubation at 80°C for 15 min, after which the DNA was denatured at 95°C for 15 min. The presence of a PCR product was determined using agarose gel electrophoresis. Cycle sequencing was performed using a BigDye Terminator Cycle Sequencing Ready Reaction kit (v3.0; Applied Biosystems, Foster City, CA, USA) and an automated ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

RESULTS

Of the 161 patients, there were four (2.5%) c-HNPCC patients fulfilling the Amsterdam criteria II (Figure 1). Three of the four c-HNPCC patients underwent genetic testing. A germline mutation in the MSH2 gene (c.882delT) was identified in one patient (Table 3).

Table 1. Revised criteria for clinical HNPCC/Lynch syndrome (Amsterdam criteria II)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Adopted from Vasen et al. (8)</th>
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<tbody>
<tr>
<td>At least three relatives with a HNPCC-associated cancer (colorectal,</td>
<td></td>
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<tr>
<td>endometrial, small bowel, ureter or renal pelvis cancers)</td>
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<tr>
<td>One should be a first-degree relative of the other two</td>
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<tr>
<td>At least two successive generations should be affected</td>
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<tr>
<td>At least one should be diagnosed before age 50 years</td>
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<tr>
<td>Familial adenomatous polyposis should be excluded in the colorectal</td>
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<tr>
<td>cancer(s), if any</td>
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<tr>
<td>Tumors should be verified by pathologic examination</td>
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</table>

HNPCC, hereditary non-polyposis colorectal cancer. Adopted from Vasen et al. (8).

Table 2. Revised criteria for suspected HNPCC/Lynch syndrome

| At least two HNPCC-associated cancers in first-degree relatives (colorectum, endometrium, small intestine and urinary tract), and | adopted from Park et al. (9). |
| Multiple colorectal tumors, or | Development of accompanying cancer in family members (stomach, biliary, ovary and pancreas) |
| At least one HNPCC-associated cancer diagnosed before age 50 years or | |

Adopted from Park et al. (9).
Fourteen (8.7%) patients met the revised criteria for s-HNPCC based on family history (Figure 1). Colon cancer and endometrial cancer were identified in the first-degree relatives of eight and six patients, respectively. Renal cell cancer and cholangiocarcinoma existed with colon cancer at the same time in one first-degree relative. Gastric cancer \((n = 2)\), renal cell cancer \((n = 1)\), pancreatic cancer \((n = 1)\), hepatoma \((n = 1)\) and cervical cancer \((n = 1)\) were identified in the second-degree relatives of the patients. Six of 14 patients (43%) underwent genetic testing. One patient had a germline mutation \((c.1757_1758\text{ins}C)\) in the \(MLH1\) gene, and one patient had a sequence variant of undetermined significance \((c.1886A \rightarrow G)\) in the \(MSH2\) gene (Table 3).

Of 18 patients who met c-HNPCC or s-HNPCC criteria, 9 patients accepted genetic testing. Of nine patients who decline the genetic testing, eight patients revealed the financial factor for the main barrier of genetic testing and one patient did not revealed the reason for the non-uptake of a genetic test. Predictive factors such as clinical factors of endometrial cancer including stage and disease status, offspring, religion, economic status and education to uptake a genetic test was not identified.

Three of the 143 patients who were not classified as c-HNPCC or s-HNPCC wanted to undergo genetic testing and no sequence variants were identified (Table 4).

**DISCUSSION**

The objective of the current study was to investigate the frequency of HNPCC in Korean endometrial cancer patients according to the two clinical criteria (c-HNPCC and s-HNPCC) and to determine the genetic status of the \(MLH1\) and \(MSH2\) genes of such patients. We also evaluated the uptake rate of a genetic test and the reason for the non-uptake of a genetic test in Korean endometrial cancer patients from routine clinical practice for the first time as far as we know. Eighteen of the 161 endometrial cancer patients (8.7%) met one of the two clinical criteria for HNPCC, and deleterious mutations in \(MLH1\) and \(MLH2\) genes were identified in two of nine HNPCC patients (22.2%). Our results for the frequency of HNPCC in patients with endometrial cancer are consistent with a previous study (12).

In the current study, the incidence of patients with c-HNPCC fulfilling Amsterdam criteria II was 2.5% (4 of 161). This is within the range of previous results in Asian women (0.5% in Japanese and 6.4% in Chinese) (14,15). The explanation for the different incidence in Asian countries might be explained as follows. First, the different
<table>
<thead>
<tr>
<th>Genetic test</th>
<th>Incidence of deleterious MMR mutations</th>
<th>Institution/country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unselected patients with endometrial cancer</strong></td>
<td></td>
<td></td>
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<tr>
<td>Screening method</td>
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<tr>
<td>23.4% (18 of 77) MSI high</td>
<td>MLH1 and MSH2</td>
<td>0% (0 of 18)</td>
<td>Tokyo University/Japan</td>
</tr>
<tr>
<td>32.1% (9 of 28) MSI high</td>
<td>MLH1 and MSH2</td>
<td>0% (0 of 28) and 0% (0 of 9)</td>
<td>Mayo Clinic/USA</td>
</tr>
<tr>
<td>30.8% (12 of 39) MSI high</td>
<td>MLH1 and MSH2</td>
<td>2.6% (1 of 39) and 8.3% (1 of 12)</td>
<td>Keio University/Japan</td>
</tr>
<tr>
<td>22.9% (17 of 74) MSI high</td>
<td>MLH1, MSH2 and MSH6</td>
<td>1.4% (1 of 74) and 5.8% (1 of 17)</td>
<td>Ohio University/Japan</td>
</tr>
<tr>
<td>13.9% (9 of 65) MSI high</td>
<td>MSH2, MLH1, PMS1 and PMS2</td>
<td>3.1% (2 of 65) and 22.2% (2 of 9)</td>
<td>Baltimore/USA</td>
</tr>
<tr>
<td>21.7% (118 of 543) MSI high or low (98 of 118 MSI high and 20 of 118 MSI low)</td>
<td>MLH1, MSH2, MSH6 and PMS2</td>
<td>1.8% (10 of 543) and 7.6% (9 of 118 MSI+)</td>
<td>Ohio population/USA</td>
</tr>
<tr>
<td>28.8% (127 of 441) MSI high</td>
<td>MSH6</td>
<td>1.6% (7 of 441) and 5.5% (7 of 127)</td>
<td>University of Washington and Indiana University/USA</td>
</tr>
<tr>
<td><strong>Unselected cohort of endometrial cancer patients</strong></td>
<td>MSH6</td>
<td>3.8% (4 of 105)</td>
<td>Belfast City Hospital/UK</td>
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<tr>
<td><strong>Endometrial cancer patients with high risk (familial history, age or tumor location)</strong></td>
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<tr>
<td>Definition of high risk population</td>
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<tr>
<td>Amsterdam criteria II, 2.7% (4 of 113)</td>
<td>MLH1, MSH2 and MSH6</td>
<td>2.7% (3 of 113)</td>
<td>Seoul National University Hospital/Korea</td>
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<tr>
<td>Suspected HNPCC criteria, 7.1% (8 of 113)</td>
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<tr>
<td>MSI high with loss of MMR protein expression after screening of familial history, 8.9% (101 of 113)</td>
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<tr>
<td>Group 1: Patients with ≥2 first-degree relatives with HNPCC-related tumors (colorectal cancer, endometrial cancer, small intestinal cancer, urethral or renal pelvic cancer, gastric cancer, ovarian cancer or breast cancer) and an age at onset of &lt;50 years for at least one tumor</td>
<td>MLH1, MSH2 and MSH6</td>
<td>15.0% (18 of 120): Group A, 15.8% (9 of 57); Group B, 8.3% (4 of 48); Group A&amp;B, 33.3% (5 of 15)</td>
<td>Five multicenters/Japan</td>
</tr>
<tr>
<td>Group 2: Patients with ≥2 synchronous or metachronous HNPCC-related tumors (colorectal, endometrial, small intestinal, urinary system or renal pelvis, gastric, breast or ovarian cancers), regardless of age at onset</td>
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<td>Familial site-specific endometrial cancer defined as occasional families showing clustering of endometrial cancer, without colon or other cancers [6.4% (33 of 519)]: 33 EC among 23 families</td>
<td>MLH1, MSH2 and MSH6</td>
<td>8.7% (2 of 23)</td>
<td>Helsinki University/Finnland</td>
</tr>
<tr>
<td>Unselected cohort of women diagnosed with endometrial cancer at &lt;50 years of age</td>
<td>MLH1, MSH2 and MSH6</td>
<td>9.0% (9 of 100)</td>
<td>Three multicenters/USA</td>
</tr>
<tr>
<td>Unselected cohort of women diagnosed with endometrial cancer at &lt;50 years of age (n = 58): with HNPCC-related cancers (n = 22)</td>
<td>MLH1, MSH2 and MSH6</td>
<td>8.6% (5 of 58) and 22.7% (5 of 22)</td>
<td>Comprehensive Cancer Center North/Netherlands</td>
</tr>
<tr>
<td>Endometrial cancer in the lower uterine segment [3.5% (35 of 1009)]</td>
<td>MLH1, MSH2, MSH6 and PMS2</td>
<td>29% (10 of 35)</td>
<td>M. D. Anderson Cancer Center/USA</td>
</tr>
</tbody>
</table>

EC, endometrial cancer; MMR, mismatch repair; MSI, microsatellite instability.

*aIncidence of MMR gene mutation in patients with MSI high and/or low.

*bOne MSH6 mutation in patients with MSI negative and abnormal immunohistochemical staining for MSH6.
incidence might reflect genetic differences among the three countries. Second, a limited family structure due to a smaller family significantly affects such an incidence (16). The family size is typically decreasing in developed countries. Third, recall bias might be one of the causes. Family history is usually identified based on the memory of patient and/or the family member. The family history in colorectal cancer patients with HNPCC is not reliable to a certain degree because the false-positive rate reaches 21% (17). In particular, the sensitivity of uterine cancer based on the self-reported family history is quite low (30%) compared with a database-linked family history (18). Further studies are warranted to determine the exact causes for the different incidence in endometrial cancer patients with HNPCC.

The incidence of MMR gene mutations in patients with sporadic endometrial cancer ranges from 0 to 3.1% after microsatellite instability (MSI) screening (19–27). Of those patients with a positive MSI, the incidence of MMR gene mutations has been reported to be 0–22.2% (19–26). Even in an unselected cohort of patients with endometrial cancer, the incidence of mutated MLH6 was reported to be 3.8% (27). Currently, high risks suggesting hereditary endometrial cancer may be involved in a family history of HNPCC-related cancer, age and tumor location (12,28–32). In patients with a family history, the incidence of MMR gene mutations (MLH1, MSH2 and MSH6) ranged from 2.7 to 33.3% based on criteria (12,29,31). Also, the incidence of MMR gene mutations is high in young endometrial cancer patients <50 years of age (8.6–9.0%) (30,32). Endometrial cancer in the lower uterine segment is suggestive of hereditary cancer; the incidence of MMR gene mutations in such patients is 29% (28). We can expect a higher incidence of MMR gene mutations than current outcomes in endometrial cancer patients because only parts of the MMR genes (MLH1, MSH2, MSH6, PMS2 and PMS1) were investigated in previous studies.

In patients with endometrial cancer, MMR gene mutations are most prevalent in the MSH2 gene (5.2–7.0%) (30,32). One MSH2 mutation (c.882delT) was identified in our study. This mutation was first identified in not only Korean but also in all races. The loss of MSH2 immunohistochemical (IHC) expression is highly predictive of identifying an MSH2 mutation (30). One mutation (c.1757_1758insC) in the MLH1 gene was identified in the current study; Shin et al. (33) reported that the mutation is a founder mutation inherited from a common Korean ancestor. The prevalence of MLH1 mutations in endometrial cancer patients was not high in a previous study (1–1.7%) (30,32). The loss of MLH1 IHC expression is less predictive of identifying an MLH1 mutation because a significant number of MSI-positive endometrial cancers have MLH1 promoter hypermethylation (12).

MSH2 c.1886A > G is one of the well-known missense variants. The variant has been reported as a pathologic variant in Koreans (33,34). There are web tools to presume the functional effect of novel missense variants, such as PolyPhen (http://genetics.bwh.harvard.edu/pph/), SIFT (http://sift.jcvi.org/) and PMut (http://mmb2.pcb.ub.es:8080/PMut/). According to these web analyses, PolyPhen is thought to be potentially damaging, whereas SIFT and PMut are thought to be benign. Therefore, there was no clear evidence that the mutation was a pathologic variant, and also there was no evidence that the mutation was a neutral mutation. Thus, we can classify the MSH2 c.1886A > G as a variant of unknown significance.

Mutation analysis in the MSH6 and PMS2 genes was not performed in the current study. The prevalence of MSH6 mutations in endometrial cancer patients was 1–3.8% in previous studies (27,30,32). Hirai et al. (31) reported the importance of genetic studies of MSH6 in the evaluation of Japanese patients with endometrial cancer and HNPCC (31). One-half (9 of 18) of such patients had MSH6 mutations of the evaluated MLH1, MSH2 and MSH6. It has been recently reported that pathogenic PMS2 mutation is more frequently identified (4%) than originally expected (35). Therefore, in the future study on HNPCC, MSH6 and PMS2 should be considered to be included.

The decision to undergo genetic testing is influenced by a complex mix of familial, cultural and social life experiences (36). It has been published that the actual uptake rate of genetic testing is lower than the rate of interest in genetic testing among populations at risk for hereditary cancer (36,37). Although the predictors of the actual uptake of genetic testing are indefinite, women who have the additional risk of endometrial cancer are more interested in testing for HNPCC than men and subjects with children compared with subjects without children, who are more likely to seek genetic testing (36). Lerman et al. (38) reported that 43% (90 of 208) of HNPCC family members accept genetic testing and barriers to test acceptance seem to be a less formal education and the presence of symptoms of depression. Although most of the patients who met the criteria of HNPCC are actually want to know the endometrial cancer is hereditary or not, only half of the patients with endometrial cancer underwent genetic testing in this study. Main barrier to undergo genetic testing is cost. We believe insurance coverage for genetic counseling, genetic testing and genetic prescreening will offer better medical environment for endometrial cancer patients and their progenies. Clinical predictors of genetic testing may differ according to gender, offspring, type of disease, disease status, study population, country and study methods. Therefore, further studies in larger patients groups are needed to clarify this issue.

There were several limitations mainly originated from study design, retrospective review of routine clinical practice. First, selection bias and other confounders found in a retrospective study were also possibilities in this study. We made an effort to minimize the selection bias and confounders as much as possible. Second, IHC staining and MSI were not analyzed in this study, because these tests were performed inconsistently in 33.5% (54 of 161) of the study population and 52.6% (10 of 19) of c-HNPCC or s-HNPCC patients. If the stringent clinical criteria (c-HNPCC) was satisfied,
Although IHC staining offers cost-effective strategy to direct genetic test without a screening test could be revised (39). The direct genetic test without a screening test could be recommended even in s-HNPCC patients from small family (12). Although IHC staining offers cost-effective strategy to find patients with mismatch gene mutation (39), trivial limitations exist to select a specific gene because of concurrent loss of MSH2 with MSH6: these act together due to abrogation of the MutSα complex formed by MSH2 and MSH6 proteins (40). And MLH1 protein expression from MLH1-promoter hypermethylation without germline MLH1 mutation in 15% of the patients with endometrial cancer cause an unnecessary genetic test (41). Accuracy of MSI-H as a surrogate marker for HNPCC is not perfect and reaches ~90% (42,43). Although we did not evaluate the non-uptake of IHC staining and MSI analysis specifically, significant portion of the patients chose a direct genetic test to avoid revisit of hospital after the positive result of a screening test for convenience and shortening the waiting time for the final outcome. Generally, the mutational genetic test is quite expensive (> $2000 USD just for MSH2 and MLH1) and three-step process (IHC staining → MSI analysis → genetic test) is accepted as a cost-effective approach in USA (39,44). Although cost-effectiveness was not investigated in Korea, patients favor a direct genetic test: one of the causes is relatively cheap cost of the genetic test (< $600 USD for MSH2 and MLH1) in Korea. Third, genetic testing of the MMR gene was limited to the MLH1 and MSH2 genes. Clinical usefulness of MSH6 was belatedly identified in 1999 compared with MLH1 and MSH2 genes which were identified in 1994 and 1993, respectively (45–47). It is in this context that MSH6 was recently available in clinical practice field, and currently, we have included MSH6 for one of the genetic tests in patients with HNPCC. Fourth, the separation of family due to the Korean War was sometimes an obstruction of the completeness of pedigree analysis (48). Some patients did not know their parents’ causes of death. Therefore, the real proportion of HNPCC in Korean endometrial cancer patients may be more than that revealed in the current study.

In conclusion, this study provides the first clinical practice-based result for the frequency of HNPCC and the acceptance rate of genetic testing in Korean endometrial cancer patients. We found that 11.2% of Korean endometrial cancer patients met the criteria for c-HNPCC or s-HNPCC. Half of the c- and s-HNPCC patients accept genetic testing; main reason of the non-uptake of a genetic test is cost. Two deleterious germline mutations in the MLH1 and MSH2 genes were identified among three clinical HNPCC and six s-HNPCC patients (22%). Until more definitive, larger prospective studies are made available; our study results may assist gynecologic oncologists and gynecologists in their approach at the time of genetic counseling and genetic testing in Korean patients with endometrial cancer. Financial support from government healthcare system and private health insurance is essential for optimal approach to genetic screening (IHC staining and MSI analysis) and genetic testing.

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**Conflict of interest statement**

None declared.

**References**