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The Changing Landscape of Lynch Syndrome due to *PMS2* Mutations

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Abstract

DNA repair pathways are essential for cellular survival as our DNA is constantly under assault from both exogenous and endogenous DNA damaging agents. Five major mammalian DNA repair pathways exist within a cell to maintain genomic integrity. Of these, the DNA mismatch repair (MMR) pathway is highly conserved among species and is well documented in bacteria. In humans, the importance of MMR is underscored by the discovery that a single mutation in any one of four genes within the MMR pathway (*MLH1*, *MSH2*, *MSH6* and *PMS2*) results in Lynch syndrome (LS). LS is an autosomal dominant condition that predisposes individuals to a higher incidence of many malignancies including colorectal, endometrial, ovarian, and gastric cancers. In this review, we discuss the role of *PMS2* in the MMR pathway, the evolving testing criteria used to identify variants in the *PMS2* gene, the LS phenotype as well as the autosomal recessive condition called Constitutional Mismatch Repair Deficiency syndrome, and current methods used to elucidate the clinical impact of *PMS2* mutations.

Graphical abstract

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PMS2; Lynch syndrome; CMMRD; variants of uncertain significance; pseudogene; DNA mismatch repair

1. Introduction and Role of PMS2 in Mismatch Repair

Prior to the ability to perform molecular genetic testing, the transmission of an increased cancer risk throughout generations of some families was recognized by multiple clinicians.^{1–5} In 1913, Dr. Aldred Scott Warthin was the first to describe a hereditary cancer syndrome.^{1–5} In 1913, Dr. Aldred Scott Warthin was the first to describe a hereditary cancer syndrome.^{1–5} In studying a large family of German descendants, Warthin observed and documented a high rate of uterine, gastric, and colon cancer incidence starting with a sibship of ten individuals and continuing throughout succeeding generations; he called this family “Family G”.² In the 1960s, Dr. Henry Lynch began to document cancers from large families with pedigrees that demonstrated an increased incidence of colon, gastric, and endometrial cancer.^{4,6} Initially termed “Cancer Family Syndrome” by Dr. Lynch, this condition was later renamed Hereditary Nonpolyposis Colorectal Cancer syndrome (HNPCC) to distinguish it from other hereditary cancer syndromes. Currently, the term Lynch syndrome (LS) is used to describe families with germline mutations that disrupt the DNA mismatch repair (MMR) pathway, while HNPCC is now reserved for families effected by a familial colorectal cancer syndrome with features of LS that lack a germline mutation in any of the MMR genes.^{7,8} Monoallelic mutations in the four main MMR genes: *MSH2*, *MSH6*, *MLH1*, and *PMS2*, are believed to account for >97% of all LS cases.⁹ Most recently, a small percentage (~1–3%) of LS families were found to have large deletions in the 3′ terminus of *EPCAM* (EPithelial Cellular Adhesion Molecule) disrupting transcription of *MSH2*, which lies downstream of *EPCAM*.¹⁰ For the remainder of this review, we will focus on *PMS2*. We discuss the current knowledge regarding the role of *PMS2* in the MMR pathway, describe the identification of germline *PMS2* variants, discuss the clinical phenotype of two distinct conditions caused by

pathogenic mutations in this gene, and review current testing methods, which have impacted our understanding of *PMS2* mutations.

The DNA MMR pathway is one of five major DNA repair pathways in humans and is highly conserved from prokaryotes to eukaryotes.^{11–15} Mismatches and slippage errors commonly arise during DNA replication and result in non-Watson Crick base pairs or insertion/deletion loops (IDLs).^{11–15} The MMR pathway increases replication fidelity by 100 – 1000 fold and is essential for the maintenance of genomic integrity.^{13,14, 16} MMR proceeds as four distinct steps: mismatch recognition (by the MutS complexes), recruitment of downstream proteins (MutL complex) to the site of the mismatch, excision of the DNA strand that contains the mismatch, and resynthesis by a replicative DNA polymerase.^{11–15} In humans, the MutS homolog (MSH) MSH2-MSH6 complex, also referred to as MutS α , initiates the repair of single base mismatches and short 1 – 2 nt IDLs.^{11–15} Mismatch recognition and ATP binding induce a conformational change in MutS that is required for the recruitment of the MutL α complex that initiates repair.^{11–15} MutL α is the predominant MutL heterodimer, which is formed by the MutL homolog 1 (MLH1) and the postmeiotic segregation increased 2 (PMS2) proteins.^{17,18} *PMS2* is located on chromosome 7 and spans ~38,000 base pairs (Figure 1A).¹⁹ The full-length *PMS2* protein product is translated from 15 exons and is 862 amino acids in length (Figure 1A).¹⁹ *PMS2* forms a heterodimer with MLH1 via a C-terminal interaction domain (Figure 1B). This interaction is absolutely essential for the stability of *PMS2* *in vivo*.²⁰ In addition, *PMS2* functions as a latent endonuclease and is required for 3' nick-directed MMR.^{15,21} The crystal structure of the N-terminal 364 amino acids of human *PMS2* reveals the ATPase domain of the protein that is able to hydrolyze ATP as well as bind to DNA (Figure 1B).²² While there is no structural information for the C-terminal domain of *PMS2*, this region harbors an endonuclease domain that is required for protein function during MMR.^{17,23}

2. *PMS2* and Genetic Testing

Of the approximately 3,300 unique MMR gene variants listed in the International Society for Gastrointestinal Hereditary Tumors (InSiGHT) database, only 9% are reported in *PMS2*, compared to 39% in *MLH1*, 33% in *MSH2* and approximately 19% reported in *MSH6* (<http://www.insight-database.org/genes>). This lower observed frequency of germline variants in *PMS2* may be due to the fact that *PMS2* has not been studied as rigorously as the other MMR genes. Analysis of *PMS2* in clinical settings has been impacted by the presence of *PMS2* pseudogenes (Figure 1A) as well as a lack of genetic testing in families who have a low penetrance of cancer.^{24–26}

Pseudogenes

Clinical genetic testing of *PMS2* was not offered until 2009, nine years after testing for *MLH1* and *MSH2* was available.²⁴ Analysis of the *PMS2* gene has been complicated by the presence of a large family of pseudogenes that are highly homologous to *PMS2* (Figure 1A).^{19,25} All *PMS2*-associated pseudogenes are found on chromosome 7.¹⁹ It is the presence of these pseudogenes that complicates accurate sequencing of *PMS2* to identify variants within this gene. Fourteen pseudogenes ($\psi 1 - 14$) overlap with some or all of exons 1 – 5 of *PMS2*

and vary in length (Figure 1A) whereas the *PMS2CL* pseudogene (formerly $\psi 0$) possesses high sequence similarity to exons 9 and 11–15 (Figure 1A).^{25,27} While avoiding pseudogenes has presented a challenge in the accurate detection of *PMS2* variants, various tactics to circumvent sequencing of these regions during *PMS2* analysis have been employed. For instance, a modified long-range PCR method has been used successfully to preferentially amplify *PMS2* using a forward primer within exon 10 that does not overlap with the *PMS2CL* pseudogene.²⁸ The PCR products are sequenced and further compared to the NCBI reference sequence for *PMS2* (NT_007819.16) to confirm that the sequence amplified is not from the pseudogene. However, this method alone is not fully reliable for the detection of large-scale deletions.²⁹ To detect large-scale deletions, a combination of the modified long-range PCR method developed by Vaughn et al., and a multiplex ligation-dependent PCR amplification (or MLPA), can be used.^{28,30} Another approach using long-range RT-PCR followed by sequencing appears to be largely successful at avoiding the detection of variants within the *PMS2* pseudogenes.³¹ A method using direct cDNA sequencing has also been employed to identify splice-site mutations, insertions, and deletions that are often overlooked using traditional PCR-based protocols.^{32,33}

Identification of Families with LS

The low frequency of observed *PMS2* mutations is in part due to the ascertainment bias inherent in choosing individuals who should undergo testing for mutations in the MMR genes. Families that meet the most stringent of criteria used to identify LS will most often have a germline mutation in *MLH1* or *MSH2* causing 40% and 34% of LS cases, respectively.^{34,35} On the other hand, mutations in *PMS2* were once thought to account for only 8–15% of LS cases (Figure 2).^{26,34,35} Traditionally, clinicians recommended molecular genetic testing for LS based on a patient's personal and/or family history of cancer. Clinical genetic testing for LS first began in families that met Amsterdam I criteria (Box 1).³⁶ The expanded understanding of the clinical presentation of LS led to the introduction of the Amsterdam II criteria which allowed for the inclusion of multiple LS-associated cancers (Box 1).³⁷ Use of the Amsterdam II testing guidelines facilitated the identification of multiple individuals and families that had LS but did not present with a predominance of early-onset colorectal cancers.³⁸ Based on the discovery that MSI was a hallmark of LS tumors, the National Cancer Institute (NCI) set forth to develop criteria for selecting colon and rectal tumors that warranted MSI testing during a meeting in Bethesda, Maryland, and published in 1997 (Box 2).^{39,40} Following a cost-effective analysis on the use of MSI and immunohistochemistry (IHC) to screen colorectal cancers for LS, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group published a recommended tumor testing strategy in 2009.⁴¹ EGAPP recommended that all newly diagnosed colorectal cancers be screened for LS using preliminary tests that include MSI and/or tumor IHC, allowing for the identification of LS regardless of age at time of cancer diagnosis or family history. The suggested testing algorithms begin with IHC and/or MSI testing of the tumor followed by germline sequencing and deletion/duplication analysis of the LS-associated genes.⁴¹ The sensitivity of MSI testing or IHC to screen for the presence of a germline *PMS2* mutation is 67% and 75%, respectively.^{42,43} Therefore, MSI and IHC screening has been found to be helpful in identifying individuals who should further undergo

germline testing. The American College of Obstetrics and Gynecology (ACOG) later recommended that all endometrial tumors be screened for LS using MSI/IHC.⁴⁴

Box 1

Amsterdam Criteria

Amsterdam I Criteria

There should be at least three relatives with histologically verified colorectal cancer.

Additional considerations:

- One should be a first-degree relative to the other two
- At least two successive generations should be affected
- At least one relative should be diagnosed with colorectal cancer before age 50
- Familial adenomatous polyposis should be excluded

Amsterdam II Criteria

There should be at least three relatives with an LS-associated cancer. This includes CRC, EC, gastric, ovarian, small bowel, ureter or renal pelvis.

Additional considerations:

- One should be a first-degree relative to the other two
- At least two successive generations should be affected
- At least one should be diagnosed before age 50
- Familial adenomatous polyposis should be excluded
- Tumors should be verified by pathological examination

Box 2

Revised Bethesda Guidelines: Tumors from individuals should be tested for MSI in the following situations

- Colorectal cancer diagnosed in a patient <50 years of age
- Presence of synchronous, metachronous colorectal or other LS-associated tumors regardless of age. These include CRC, EC, gastric, ovarian, pancreatic, ureter/renal pelvis, biliary tract
- CRC with the MSI-high histology* diagnosed in a patient (<60 years of age)
- CRC diagnosed and one or more first-degree relatives with an LS-related tumor, with one of the cancers being diagnosed < 50 years of age.
- CRC diagnosed in two or more first- or second-degree relatives with LS-related tumors, regardless of age

*Histological features that are common in tumors with high microsatellite instability include tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous or signet-ring differentiation, or medullary growth pattern

With each evolving set of guidelines, more families with LS were identified. The diagnosis of LS was further aided by the advent of next-generation sequencing (NGS).⁴⁵ This technology allows clinicians to simultaneously test multiple genes with massive parallel sequencing in a cost-effective manner. The number of genes in a panel range from two to >100 and the use of gene panels to test for hereditary cancer syndromes became integrated into standard clinical practice starting in 2012.⁴⁶ *PMS2* is included within many NGS panels although the highly homologous *PMS2* pseudogenes present a technical challenge even for NGS. To perform accurate mutation detection of *PMS2*, the genomic testing methods described above are still used including long-range PCR for sequencing and MLPA for large deletion and duplications analyses.^{46–48} Utilization of these panels has led to the discovery that many families who did not meet any of the previously described guidelines for testing for LS have deleterious mutations in *PMS2*. Published data from multiple laboratories, clinics, and academic centers now show that *PMS2* deleterious mutations are much more common than initially thought.^{46–50} A comparison of initial MMR mutation frequencies to updated mutation frequencies is available in Figure 2. Recent data suggests that pathogenic mutations in *PMS2* are just as frequent as mutations in the other three MMR genes associated with LS (Figure 2A, B) with a population-based carrier frequency of 1 in 714.⁵¹

3. The *PMS2* Phenotype

Lynch syndrome

Initial studies, which focused primarily on individuals with mutations in *MLH1* and *MSH2*, estimated a 57–80% lifetime risk of colorectal cancer and 32–42% lifetime risk of endometrial cancer for LS.^{52–55} Individuals with LS had also been shown to be at an increased risk for cancers of the stomach, ovaries, small intestine, biliary tract, brain and urinary tract.^{52–55} We are now beginning to understand the lower penetrance in LS families with *PMS2* mutations. Pathogenic mutations in *PMS2* cause an estimated 25 – 32% lifetime risk of LS-associated cancers.^{26,56} According to a recent report, the age-related risk for colon cancer in *PMS2* carriers is 8% for individuals in their 20s, 20% for those in their 30s, 31% for individuals in their 40s, 22% for those in their 50s, and 19% for individuals aged 60 or higher.⁵⁷ In those diagnosed with cancer, the mean age of diagnosis of the first colorectal cancer is estimated to be 48 years.⁵⁷ One study, which compared the lifetime risk of colorectal cancer based on gender, reported a higher risk in males (18.75%) compared to females (10.56%).⁵⁶ In individuals with an identified germline *PMS2* mutation, uterine cancer was the second most common cancer diagnosis, with an estimated lifetime risk of 12%–15%.^{26,56,57} The cancer risks listed above may be overestimated given that many of the cases were based on selecting tumors that were *PMS2* deficient by IHC.^{26,57} Other cancers that have been documented in *PMS2* heterozygotes include ovarian, breast, duodenal, small intestine, gastric, urinary tract, and central nervous system cancers.⁵⁷ Counseling individuals with *PMS2* mutations on the risk for cancers beyond colorectal and endometrial is challenging given that most studies performed to elucidate LS cancer risks

either do not report data for *PMS2* alone or may not have included *PMS2* mutations in their analysis. One study looking at 98 families with confirmed pathogenic mutations in *PMS2* reported a significant standardized incidence ratio (SIR) for malignancies of the small bowel, renal pelvis, ovaries, and breast.⁵⁶ An earlier study found that *PMS2* carriers have a combined 6% lifetime risk of developing cancer of the renal pelvis, stomach, ovary, small bowel, ureter, and brain.²⁶ Additional studies need to be performed to further understand risks beyond colorectal cancer and endometrial cancer in these patients.

Frequently updated guidelines on the medical management recommendations for individuals with a *PMS2* mutation can be found at the National Comprehensive Cancer Network (NCCN) website.⁵⁸ NCCN guidelines originally recommended colonoscopies beginning at age 25 with repeat scope every 1–2 years for any man or woman with LS, regardless of the gene harboring a mutation. The 2.2015 version veered from this initial guideline with respect to timing of initial colonoscopy for those with a *PMS2* mutation, suggesting that these individuals begin colonoscopies at age 35. Data indicating a lower overall risk for colorectal cancer and later average age of onset for individuals with *PMS2* mutations supports the rationale for delayed colonoscopies.^{26,56} An expert *ad hoc* working group later acknowledged that colon cancer is diagnosed prior to age 30 in multiple *PMS2* heterozygotes, and concluded that the most appropriate medical management decision would be to initiate colonoscopies at age 25–30 or 2–5 years prior to the earliest diagnosis of colorectal cancer in the family (if it was diagnosed prior to age 30).⁵⁷

Additional medical management recommendations for carriers of *PMS2* mutations are not as clear.⁵⁸ Clinicians can consider screening for endometrial cancer with endometrial biopsy every one to two years starting at age 30–35 then adding transvaginal ultrasound after menopause.⁵⁸ While these screening recommendations can be considered, they have not been proven to reduce mortality from endometrial cancer in women with *PMS2* mutations.⁵⁸ Prophylactic hysterectomy and bilateral salpingo-oophorectomy (BSO) should also be discussed as an option. The NCCN guidelines aptly state that the recommendation and timing of prophylactic hysterectomy and BSO should reflect the following: (1) the woman's desire to have more children, (2) menopause status, (3) comorbidities, (4) presence of gynecologic cancers in the family, and (5) the lower risk of endometrial and ovarian cancer in *PMS2* mutations carriers compared to the other MMR genes.⁵⁸ Screening for any of the other LS-associated cancers can be considered on a case-by-case basis to reflect the types of cancers diagnosed in a family and anticipated efficacy of available screening protocols.⁵⁸

Owing to phenotypic heterogeneity, it should be acknowledged that families with *PMS2* mutations might be harder to identify than previously thought. In Figure 3, we provide examples of three pedigrees, which harbor *PMS2* variants to demonstrate this phenotypic heterogeneity. The first family (Figure 3A) meets Amsterdam II criteria where the proband (family member who first presents for genetic analysis) also meets Bethesda criteria. In this family, targeted testing for LS identified a *PMS2* mutation (Figure 3A). However, many families harboring *PMS2* mutations present with a lack of LS-associated cancers and do not meet Amsterdam criteria, leading their clinician to suspect a different hereditary cancer syndrome. The proband in Figure 3B, initially underwent *BRCA1* and *BRCA2* analysis because she only met testing criteria for Hereditary Breast and Ovarian Cancer syndrome.

Due to the suspicious family history, panel testing was later offered, at which point, a pathogenic *PMS2* mutation was identified. Figure 3C provides an example of a family in which the proband underwent panel testing following her early-onset breast cancer diagnosis and the only mutation identified was a *PMS2* variant of uncertain significance (VUS) where there is a lack of data available regarding the clinical impact the specific mutation. It is encouraging to see that prediction models are now taking into account the lower penetrance of cancer seen in individuals with a *PMS2* mutation compared to other LS families. The PREdiction Model for gene Mutations (PREMM_{1,2,6}), developed to predict the likelihood of mutations in *MLH1*, *MSH2* and *MSH6*, was updated to PREMM₅ to allow for identification of families with a mutation in any of the five LS-associated genes.⁵⁹

Constitutional Mismatch Repair Deficiency syndrome

While LS is an autosomal dominant condition, biallelic germline mutations in *PMS2* (in addition to *MLH1*, *MSH2*, and *MSH6*) have been observed in an autosomal recessive condition called Constitutional Mismatch Repair Deficiency syndrome (CMMRD), a rare condition that was first described in 1999 within two articles.^{60,61} It should be noted that over 50% of individuals with molecularly confirmed CMMRD have biallelic mutations in *PMS2*, a larger percentage compared to the other MMR genes.⁶² As discussed above, the carrier frequency of *PMS2* mutations may be much higher than previously reported.⁵¹ In studying CMMRD cases, it was noted that the heterozygous *PMS2* family members did not appear to be at an increased risk of cancer, drawing attention to the lower penetrance of cancer in *PMS2* heterozygotes.⁶³ The higher incidence of *PMS2* mutations in CMMRD patients may also be explained by the severity of disease associated with *MLH1* and *MSH2* biallelic mutations. These patients may die before age 10 due to their first primary cancer and therefore prior to proper diagnosis of CMMRD. Furthermore, many CMMRD patients are known to be homozygous for a Pakistani founder mutation arising in families with consanguinity.⁶³ Although not yet well studied, it has been suggested that individuals with biallelic mutations in *MLH1* or *MSH2* may have at least one allele that leads to reduced penetrance whereas inheriting two highly penetrant *MLH1* or *MSH2* mutations will be embryonic lethal.⁶²

Individuals who are either homozygotes for *PMS2* mutations or compound heterozygotes are affected by CMMRD, which primarily increases the risk of childhood-onset hematologic malignancies, brain tumors, and early-onset gastrointestinal tumors.^{64,65} T-cell lymphoma is the most commonly diagnosed hematological malignancy in CMMRD and typically the first malignancy to present in affected individuals.⁶⁴ Brain tumors diagnosed in individuals with CMMRD are primarily glioblastomas.⁶⁶ Colorectal cancers are also common, although they occur at a much younger age compared to LS patients with a mean age of diagnosis at 16.4 years.⁶⁷ Individuals with CMMRD are also at an increased risk of early-onset duodenal and gastric cancers.⁶⁸ CMMRD can manifest with non-malignant features that share clinical overlap with Neurofibromatosis Type 1 (NF1) and frequently presents with café au lait macules as well as less common NF1 features such as axillary freckling, Lisch nodules, and plexiform neurofibromas.^{64,69} Unlike with the tumor-screening algorithm developed for LS patients, MSI is neither sensitive nor specific in diagnosing CMMRD.^{62,66,67} However, in

patients with CMMRD, IHC shows loss of the affected MMR protein in up to 100% of cases.^{62,66,67}

Although the efficacy of surveillance protocols is still not clear, several groups have proposed screening recommendations for patients with CMMRD.^{66,67,70} A consensus surveillance protocol for individuals with CMMRD recommends the use of brain MRIs starting at the time of diagnosis (as early as birth) and repeated every six months.⁷¹ Due to the wide range of tumors that can develop in children with CMMRD, these authors also advocate for the use of annual whole-body MRIs starting at age six or once anesthesia is not needed. Gastrointestinal surveillance should include annual colonoscopy starting between the ages of four and six and annual upper endoscopies with video capsule endoscopy starting at age eight. The frequency of endoscopy should increase to every six months upon identification of polyps and a colectomy should be considered once any polyps display high-grade dysplasia or if the polyp burden cannot be managed with endoscopic excision. Recommendations regarding screening for leukemia and lymphoma include consideration of complete blood count (CBC) and abdominal ultrasounds every six months. Beginning at age 20, genitourinary cancer screening should include a gynecological exam, transvaginal ultrasound, endometrial sampling, and urinalysis with dipstick analysis and urine cytology performed on an annual basis.⁷¹

4. Current Methods Used to Classify *PMS2* Variants

Germline genetic testing reveals one of three outcomes: a known pathogenic mutation that might explain cancer formation and progression, a benign variant that does not result in a malignant phenotype, or a VUS where little to no data are available regarding the variant.^{72,73} A majority of mutations found in LS families are pathogenic resulting in an LS-associated cancer due to deficient MMR. Pathogenic variants typically result in premature truncation, deletion, or a deleterious mutation within the particular MMR protein product.⁷³

Approximately 20–30% of missense mutations identified in the MMR genes are VUSs and because they are uncharacterized, pose a challenge to the diagnosis and medical management of LS patients.^{72,74–77} Some VUSs result in altered, non-functional proteins while others do not result in a change in protein function lending importance to the proper characterization of VUSs as pathogenic or benign in diagnosing LS.⁷⁸ VUSs include nonsynonymous and synonymous variants that can be single nucleotide changes, insertions or deletions, and splice-site variants. Depending on the location of the VUSs, functional assays can be performed to properly designate these variants and several strategies have been previously proposed to assess the functional impact of VUSs.⁷⁹ Nonsynonymous variants that alter the amino acid sequence of the resulting PMS2 protein can be scrutinized using *in vitro* molecular, biochemical, and structural methods.⁷⁵

The use of recombinant proteins to study *in vitro* function has become increasingly common.⁸⁰ For PMS2, while production of the full-length protein requires expression in insect cells, both the N-terminal ATPase domain (residues 1 – 365) and the C-terminal endonuclease domain (residues 506 – 862) in a complex with MLH1 (residues 474 – 756) can be readily expressed in bacteria (*E. coli*).^{22,23,81} The ATPase and DNA binding activities

of the N-terminal domain of PMS2 have been previously documented.²² Residues in the endonuclease domain of PMS2 are involved with coordinating metal binding (likely zinc) and are necessary for the endonucleolytic activity of PMS2 *in vitro*.^{17,23} ATPase, DNA-binding, and endonuclease activities using suitable substrates, as well as complex formation with MLH1 can be measured to compare wild-type (WT) and variant proteins. Structural techniques including small-angle X-ray scattering, and X-ray crystallography can also be further used to assign a functional role for the variant enzymes.^{82,83} Variant proteins that display aberrant activity compared to the WT protein are more likely to be pathogenic.

While it is useful to study protein function outside of the cell, the recombinant proteins do not recapitulate a cellular environment. Cell-based studies to assess PMS2 protein expression, stability (half-life), and MMR reconstitution from extracts have been used with much success to analyze the impact of a variant on protein function.^{75,84,85} The latter makes use of plasmid DNA containing a single mismatch that is either repaired or remains unrepaired after treatment with cell extracts containing WT or variant PMS2.⁸⁶ Furthermore genome-editing tools can be used to directly “knockin” a particular variant to study its function at the endogenous level.^{87,88} This latter technique is advantageous because it does not involve procedures to first knockdown gene expression prior to transfection with a plasmid harboring the variant. More recently, a cell-free system using *in vitro* transcription/translation to create the MMR protein variants was used to study the impact of a variant on protein function.^{89,90} Therefore, a combination of cell-based and *in vitro* biochemical assays is powerful and will aid in the reclassification of a variant as pathogenic or benign.

Other aspects of MMR function that can be hindered as a result of a VUS include protein localization and mRNA splicing that are essential for maintaining genomic integrity.^{91,92} Both PMS2 and MLH1 possess nuclear localization signals that are required for import into this organelle where nuclear import appears to be dependent upon heterodimerization.⁹³ A VUS that disrupts heterodimerization will also likely interrupt nuclear localization thereby causing an MMR defect. For the identification of pathogenic variants that impact mRNA splicing, minigene reporter assays can be performed where the splicing patterns of transcripts generated after transient transfection of PCR amplified genomic DNA from the WT and variant constructs are compared.^{91,94} Sixteen PMS2 variants were previously tested using this assay, where several of the variants displayed aberrant splicing patterns.⁹¹ Taken together, these assays are essential to the understanding of the regulation of MMR proteins and are required for the proper designation of a particular VUS as pathogenic or benign.

5. Concluding Remarks

It is of utmost importance when recommending genetic testing of *PMS2* to select a laboratory that uses techniques, which provide a comprehensive analysis of this gene (sequencing and deletion/duplication analysis) while also taking into account the hindrance of pseudogenes. Since many LS families with *PMS2* mutations remain undiagnosed, panel testing for individuals with a strong family history of cancer is recommended. Panel testing has led to higher detection rates of *PMS2* mutations, therefore informing probands as well as family members faced with medical management decisions that are meant to aid in early diagnosis or to prevent tumor formation altogether. These medical interventions would

include more frequent colonoscopies or, in some cases, prophylactic surgeries. While *PMS2* mutations are associated with a much lower lifetime risk of cancer compared to mutations in the other LS-associated genes, knowledge of a germline *PMS2* mutation will also help couples make informed-decisions regarding reproductive risk when both parents are *PMS2* heterozygotes.

While universal IHC and/or MSI are widely used as a first step to diagnosing LS, these techniques are not useful in patients not yet diagnosed with cancer. In an effort to provide preventative care, population screening for LS could be considered. Currently, there are several barriers to general population screening: (1) out of pocket expenses for molecular analysis, (2) preventative care costs, (3) interpreting mutations that are low penetrance, and (4) access to genetic experts/testing. The clinical impact of testing for LS is highest when cascade testing is performed throughout a family that allows healthcare providers to implement preventative medical management strategies.⁹⁵

It is hypothesized that many *PMS2* heterozygotes remain undiagnosed because: (1) most *PMS2* carriers are not diagnosed with cancer and (2) *PMS2* carriers that are affected by a particular cancer do not undergo genetic testing, as they do not meet current LS-testing guidelines.⁵⁷ Keeping in mind the low penetrance of cancer in some families with a *PMS2* germline mutation, it may be reasonable to counsel patients on the phenotypic heterogeneity seen amongst LS families, particularly if the *PMS2* mutation was identified through universal tumor screening or panel testing.

Several laboratories are in the process of evaluating the pathogenicity of VUSs in *PMS2* and characterizing their impact on MMR function. Reclassification of *PMS2* VUSs is hindered by the low penetrance of classic LS-associated tumors and potential inability to use co-segregation studies to calculate likelihood that a variant is pathogenic. Furthermore, some *PMS2* variants may be family-specific, and therefore exceedingly rare, leading to the prolonged classification of the variant as a VUS. Since co-segregation studies cannot be solely relied on when analyzing a *PMS2* mutation that has low penetrance, we must rely on functional studies to determine the clinical impact of VUSs. Thorough and rigorous analyses of these variants is necessary to aid clinicians in making medical management plans for patients and their families. This would include combining biochemical, molecular, and cell-based data with co-segregation analysis.

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Figure 1. Domain map and known structure of PMS2

(A) PMS2 exons (not to scale) and pseudogenes (ψ). The PMS2 protein spans 15 exons and is 862 aa in length. Portions of pseudogenes $\psi 1 - 14$ overlap with exons 1 – 5 while $\psi 0$ covers exons 9 and 11 – 15. (B) The N-terminal ~364 residues of PMS2 constitute an ATPase domain whose structure has been determined previously,²² whereas the C-terminal end of PMS2 possesses an endonuclease domain that forms a heterodimer with MLH1.

Figure 2. Pie chart displaying the percent mutation in genes causing LS
(A) LS incidence in individuals ascertained based on meeting Amsterdam or Bethesda criteria (NCBI database).⁹ (B) LS incidence when ascertained by multigene panel testing, regardless of personal or family history of cancer.⁴⁶

Figure 3. Examples of families presenting with *PMS2* mutations

(A) Pedigree of a family with a *PMS2* mutation (c.2117delA) that meets Amsterdam II criteria. (B) Pedigree of a family with a *PMS2* mutation (c.730C>G) that does not meet Amsterdam II criteria; detected using multi-gene panel next generation sequencing. (C) Family that presents with a *PMS2* variant of uncertain significance (c.1420G>T). A circle represents a female and a square represents a male. Black squares/circles represent individuals diagnosed with cancer and a strike-through represents individuals who are deceased. The arrow points to the proband (first person in the family who underwent genetic testing) and the "+" refers to family members who have tested positive for the mutation or VUS.