gibco

24 days of stem cells

Shape the future of stem cell innovation

October 1- November 1, 2019

Join us for 24 Days of Stem Cells; a premiere virtual event featuring the latest advances in stem cell research.

This year's format will feature a new hour of cutting edge content every week day starting October 1st. Attend the sessions that are most relevant to your work - at your convenience and at your pace.

During the 24-day long event, you can:

- Access leading scientific presentations from thought leaders around the world
- Watch live training demonstrations from our stem cell experts
- Download key stem cell tools and resources
- Complete weekly challenges to earn points towards certification and prizes

Register today at
www.24daysofstemcells.com

ThermoFisher SCIENTIFIC

WILEY

Received: 30 November 2018 | Revised: 15 March 2019 | Accepted: 24 March 2019

DOI: 10.1002/humu.23756

BRIEF REPORT

WILEY Human Mutation HGVS HUMAN GENOME VARIATION SOCIETY

PMS2 expression decrease causes severe problems in mismatch repair

Mariann Kasela | Minna Nyström | Minttu Kansikas

Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

Correspondence
Minttu Kansikas, Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, PO Box 56 (Viikinkaari 9), FI-00014 Helsinki, Finland.
Email: minttu.kansikas@helsinki.fi

Funding information
Jane ja Aatos Erkon Säätiö

Abstract

PMS2 is one of the four susceptibility genes in Lynch syndrome (LS), the most common cancer syndrome in the world. Inherited mutations in DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, and *MSH6*, account for approximately 90% of LS, while a relatively small number of LS families segregate a *PMS2* mutation. This and the low cancer penetrance in *PMS2* families suggest that *PMS2* is only a moderate or low-risk susceptibility gene. We have previously shown that even a partial expression decrease in *MLH1*, *MSH2*, or *MSH6* suggests that heterozygous LS mutation carriers have MMR malfunction in constitutive tissues. Whether and how *PMS2* expression decrease affects the repair capability is not known. Here, we show that *PMS2* knockdown cells retaining 19%, 33%, or 53% of *PMS2* expression all have significantly reduced MMR efficiency. Surprisingly, the cells retaining expression levels comparable to *PMS2* mutation carriers indicate the lowest repair efficiency.

KEYWORDS
colorectal cancer, Lynch syndrome, mismatch repair, mRNA expression, PMS2

As a component of the MutLα (MLH1 + PMS2) heterodimer, PMS2 (postmeiotic segregation increased 2) is central in the postreplicative human DNA mismatch repair (MMR) mechanism (Kunkel & Erie, 2005). Heterozygous germline mutations in MMR genes cause Lynch syndrome (LS; MIM# 120435), the most common cancer predisposition syndrome in human (de la Chapelle, 2004; Lynch, Snyder, Shaw, Heinen, & Hitchins, 2015). Mutations in *MLH1* (MIM# 120436) and *MSH2* (MIM# 609309) are the most prevalent in known LS families (36.2% and 35.3%, respectively), while the number of *MSH6* (MIM# 600678) and *PMS2* (MIM# 600259) mutations is lower (19.4% and 9.1%, respectively; www.insight-database.org, data accessed January 22, 2019). The absence of functional MMR protein results in DNA repair malfunction, which is the cause of early-onset colorectal and endometrial cancers and less frequently cancer of the ovaries, stomach, pancreas, prostate, and breast in LS families (Lynch et al., 2015). However, in *PMS2* families the prevalence of stomach, prostate, and breast cancers is unusually high and compared to *MLH1* and *MSH2* families, also the mean age of cancer onset is higher and the penetrance of colorectal and endometrial cancers lower, resembling the situation in *MSH6* families (Hendriks et al., 2006; Roberts et al., 2018; Senter et al., 2008; ten Broeke et al., 2015; Truninger et al., 2005). One explanation for the lower cancer penetrance in *PMS2* families might be the protein homologue MLH3, which is also able to bind and partially function with MLH1 (Cannavo et al., 2005; Korhonen, Raevaara, Lohi, & Nystrom, 2007; Senter et al., 2008; ten Broeke et al., 2015).

The diagnostic workflow to identify Lynch syndrome typically involves tumor studies and DNA analyses. The first clinical step in diagnosing LS includes tumor immunohistochemistry (IHC) and microsatellite instability (MSI) analyses followed by mutation screening dictated by the IHC and MSI results (Kansikas, Kariola, & Nystrom, 2011; Yurgelun & Hampel, 2018). While IHC is a valuable method for distinguishing *PMS2* carriers, who generally lose only PMS2, from *MLH1* carriers, who frequently lose both MLH1 and PMS2 protein expressions

in a tumor (Niessen et al., 2009), it took a decade to differentiate sequence between *PMS2* and its 15 pseudogenes for reliable sequencing and mutation detection (De Vos, Hayward, Picton, Sheridan, & Bonthron, 2004; van der Klift et al., 2010; Vaughn et al., 2010). As an outcome, the prevalence of *PMS2* mutations in suspected LS families has increased according to the InSiGHT database, from approximately 2–9% during the last two decades (www.insight-database.org). Furthermore, the population-based *PMS2* variant prevalence was recently reported to be relatively high (1:714) compared to the variant prevalence in *MLH1* (1:1946) and *MSH2* (1:2841; Win et al., 2017) and a strikingly high prevalence (96%) was reported from an Icelandic population using whole genome sequencing data of a colorectal cancer cohort (Haraldsdottir et al., 2017).

Nevertheless, atypical clinical phenotypes and a low cancer penetrance in *PMS2* families still question its role in Lynch syndrome. Hence, this study aimed to determine the significance of *PMS2* as an LS susceptibility gene by analyzing whether a partial *PMS2* expression decrease, compared to the expression decrease in a Lynch syndrome mutation carrier, causes mismatch repair malfunction, as was previously shown to be the case with the susceptibility genes *MLH1*, *MSH2*, and *MSH6*.

Accordingly, stable short hairpin RNA (shRNA) mediated *PMS2* knockdown (KD) clones were established and characterized by real-time polymerase chain reaction (RT-qPCR), and the clones with reduced *PMS2* expression were included in functional MMR analyses (Supporting Information Materials, Materials & Methods). Each KD clone had its own specific control across all studies to avoid experimental artifacts. A prerequisite for the functional analysis was that the *PMS2* messenger RNA (mRNA) expression level should be at most ~50% of the normal level, a level comparable to that of an LS mutation carrier. From a total of 16 clones, the three retaining 19%, 33%, and 53% of *PMS2* mRNA expression met the criteria for further MMR analysis.

To be able to assess the repair efficiency of each clone, the nuclear proteins of the cells in chosen clones and their controls were extracted in parallel as described previously (Holmes, Clark, & Modrich, 1990; Kantelinen et al., 2010). The nuclear protein expression was analyzed by western blot analysis (WB; Supporting Information Materials, Materials & Methods; Figure 1). Despite the semiquantitative nature of the method, each KD clone clearly showed decreased PMS2 protein expression compared to the level in its respective control (NC), indicating that the amount of protein reflects the reduced mRNA expression in the cell extract. Importantly, the MLH1 protein expression was shown to be normal in the KD extracts.

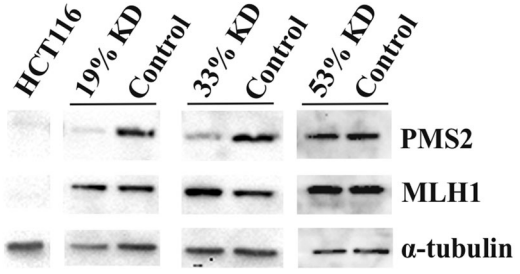

FIGURE 1 Western blot analysis of KD extracts with reduced *PMS2* mRNA expression. Western blot analysis was used to evaluate the PMS2 protein expression of KD extracts and their controls. Commercial HCT116 cell line (Manassas, Virginia; ATCC® CCL-247™) lacks both MLH1 and PMS2 protein and served as a negative control, α-tubulin was used as a loading control. The results demonstrated decreased PMS2 protein expression in KD extracts, while the amount of MLH1 remained normal. Moreover, PMS2 protein expression increased according to mRNA expression in the clones. KD: knockdown; mRNA: messenger RNA

For a long time, *PMS2* mutation prevalence was underestimated, suggesting that *PMS2* is not an important LS susceptibility gene. Its significance in LS has been questioned by the atypically low cancer penetrance in *PMS2* families (Senter et al., 2008; ten Broeke et al., 2015), an unclear risk for extra-colonic cancers (Roberts et al., 2018; ten Broeke et al., 2018), as well as inconsistencies across studies due to variable cohort sizes and carrier ascertainment (compliant with Amsterdam II criteria and/or revised Bethesda Guidelines; Senter et al., 2008; ten Broeke et al., 2015; ten Broeke et al., 2018; ten Broeke, Suerink, & Nielsen, 2018). For these reasons, *PMS2*-specific surveillance methods remain under debate (Espenschied et al., 2017; ten Broeke et al., 2018).

Nevertheless, over 20% of *PMS2* carriers are still estimated to be missed with the conventional criteria used for LS identification (Senter et al., 2008; ten Broeke et al., 2015). The IHC- and MSI-studies help the pathogenicity assessment since up to 99% of *PMS2* carriers have been shown to lack PMS2 in tumor tissue, and 98% of tumors have been characterized as MSI-high (Goodenberger et al., 2016). Since both these tumor phenotypes signal MMR deficiency, it was anticipated that *PMS2* expression decrease should also interfere with MMR capability of the cells, as was previously shown to be the case with the other LS susceptibility genes (Kansikas, Kasela, Kantelinen, & Nystrom, 2014). Indeed, the functional *in vitro* MMR assay (Nyström-Lahti et al., 2002; Raevaara et al., 2003; Supporting Information Materials, Materials & Methods) demonstrated lowered MMR efficiency in cells with reduced *PMS2* mRNA expression, while all controls maintained higher repair proficiencies throughout three independent assays (Figure 2a). The reduction in MMR capability in cells retaining 19%, 33%, or 53% of *PMS2* mRNA expression was statistically significant (according to the Student's *t* test) in all, showing 21%, 15%, and 12% of average repair efficiency, while their controls showed 38%, 55%, and 48% of repair capability, respectively ($P = 0.002$, 0.001, and 0.0002; Figure 2a). Interestingly, the most severe repair defect was in cells retaining 53% of *PMS2* expression. The relative repair efficiencies (RR%) of cells retaining 19%, 33%, and 53% of *PMS2* mRNA expression were 55%, 27%, and 25%, respectively (Figure 2b), confirming the finding that the KD cells with 53% *PMS2* expression had the lowest repair capability.

Previously, a significant reduction in MMR efficiency was detected in cells retaining 75% of *MSH6*, 50% of *MSH2*, and 25% of *MLH1* mRNA expression (Kansikas et al., 2014). In this study, we show that *PMS2* expression decrease also affects MMR capability but unexpectedly in a different way than what was observed with the

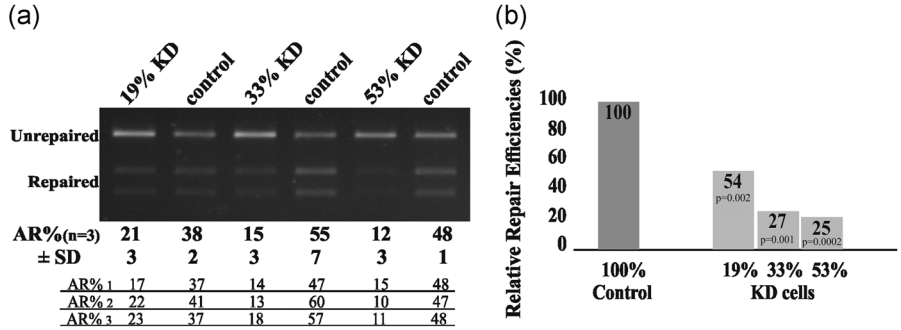

FIGURE 2 Functional MMR efficiency of KD extracts with reduced *PMS2* mRNA expression. (a) To investigate the effects of reduced *PMS2* mRNA expression on the MMR capability, nuclear proteins extracted from KD cells were analyzed in the functional MMR assay. The average of absolute repair efficiencies (AR%, $_{n=3}$) of KD extracts and their controls were calculated from three repetitions and are illustrated below the gel electrophoresis picture. A significant decrease in the MMR efficiency was observed in all extracts retaining 19%, 33%, or 53% of *PMS2* mRNA expression compared with their respective controls ($P = 0.002$; 0.001; 0.0002, respectively). (b) Relative repair efficiencies (RR%) were calculated from the AR% values of the KD extracts relative to the AR% values of their respective controls (set to 100). These results further confirm the finding that the cells with 53% *PMS2* expression have the lowest repair capability. KD: knockdown; MMR: mismatch repair; mRNA: messenger RNA; *SD*: standard deviation

other MMR genes. Surprisingly, the lower *PMS2* expression levels (19% and 33%) had higher repair efficiencies than the carrier-like level (53%), while in *MLH1* and *MSH2*, 25% expression level was not enough to maintain any repair capability (25% level in *MSH6* was not studied; Kansikas et al., 2014). The severity of *MLH1* and *MSH2* expression decrease may be due to their obligatory roles in the heterodimeric protein complexes (Acharya et al., 1996; Kondo, Horii, & Fukushige, 2001), while such a low level of *PMS2* may be compensated by some homologous protein. Compensation of MutLα with MutLγ (MLH1 + MLH3) has been suggested to explain the low penetrance of *PMS2* mutations (Cannavo et al., 2005; Korhonen et al., 2007). Although PMS2 is the main partner of MLH1 among MutL homologues, MutLγ has shown at least low MMR capability (Cannavo et al., 2005; Raschle, Marra, Nystrom-Lahti, Schar, & Jiricny, 1999). The nuclear localization of MLH3 seems to be dependent on the absence of PMS2 in the cell (Korhonen et al., 2007), and while excess of MLH3 has been shown to reduce PMS2 binding to MLH1, most probably MLH3 is not a significant counterpart to MLH1 in a normal cell due to the 60-fold higher expression of PMS2 (Cannavo et al., 2005; Kondo et al., 2001). To determine whether MLH3 could compensate MMR efficiency in 19% and 33% *PMS2* KD clones and improve their repair capability compared to the 53% clone, we further studied *MLH3* mRNA expression and protein expression in the clones. Although, the *MLH3* mRNA expression levels were decreased, they did not differ between the KD cells (Figure S1), even though slightly lowered MLH3 protein expression in 53% KD clone compared to 19% clone was detected by WB (Figure S2). While MLH3 expression differences do not explain the lowered MMR efficiency in KD clones, it is still possible that the cells expressing only 19% or 33% of *PMS2* are able to activate a "survival mechanism" that sustains MMR capability.

By using a functional approach to assess the significance of *PMS2* in Lynch syndrome, we show that *PMS2* expression decrease causes severe problems in mismatch repair, which is the hallmark of LS susceptibility genes.

ACKNOWLEDGEMENT

This study was supported by contract grant sponsors Jane and Aatos Erkko Foundation.

CONFLICT OF INTERESTS

Nyström and Kansikas are inventors on patent number PCT/EP2012/062708. Nyström is a shareholder and a board member and Kansikas and Kasela are employees of LS CancerDiag Ltd.

ORCID

Mariann Kasela http://orcid.org/0000-0001-9969-4361
Minttu Kansikas http://orcid.org/0000-0003-1482-9031

REFERENCES

Acharya, S., Wilson, T., Gradia, S., Kane, M. F., Guerrette, S., Marsischky, G. T., … Fishel, R. (1996). hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(24), 13629–13634.

Cannavo, E., Marra, G., Sabates-Bellver, J., Menigatti, M., Lipkin, S. M., Fischer, F., & Jiricny, J. (2005). Expression of the MutL homologue hMLH3 in human cells and its role in DNA mismatch repair. *Cancer Research*, *65*(23), 10759–10766. https://doi.org/65/23/10759

de la Chapelle, A. (2004). Genetic predisposition to colorectal cancer. *Nature Reviews.Cancer*, *4*(10), 769–780. https://doi.org/nrc1453

De Vos, M., Hayward, B. E., Picton, S., Sheridan, E., & Bonthron, D. T. (2004). Novel PMS2 pseudogenes can conceal recessive mutations causing a distinctive childhood cancer syndrome. *American Journal of Human Genetics*, *74*(5), 954–964. https://doi.org/10.1086/420796

Espenschied, C. R., LaDuca, H., Li, S., McFarland, R., Gau, C. L., & Hampel, H. (2017). Multigene panel testing provides a new perspective on

Lynch syndrome. *Journal of Clinical Oncology*, *35*(22), 2568–2575. https://doi.org/10.1200/JCO.2016.71.9260

Goodenberger, M. L., Thomas, B. C., Riegert-Johnson, D., Boland, C. R., Plon, S. E., Clendenning, M., … Lindor, N. M. (2016). PMS2 monoallelic mutation carriers: The known unknown. *Genetics in Medicine*, *18*(1), 13–19. https://doi.org/10.1038/gim.2015.27

Haraldsdottir, S., Rafnar, T., Frankel, W. L., Einarsdottir, S., Sigurdsson, A., Hampel, H., … Stefansson, K. (2017). Comprehensive population-wide analysis of Lynch syndrome in iceland reveals founder mutations in MSH6 and PMS2. *Nature Communications*, *8*, 14755. https://doi.org/10.1038/ncomms14755

Hendriks, Y. M. C., Jagmohan–Changur, S., van der Klift, H. M., Morreau, H., van Puijenbroek, M., Tops, C., … Wijnen, J. T. (2006). Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). *Gastroenterology*, *130*(2), 312–322. https://doi.org/S0016-5085(05)02232-8

Holmes, J., Jr, Clark, S., & Modrich, P. (1990). Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(15), 5837–5841.

Kansikas, M., Kariola, R., & Nystrom, M. (2011). Verification of the three-step model in assessing the pathogenicity of mismatch repair gene variants. *Human Mutation*, *32*(1), 107–115. https://doi.org/10.1002/humu.21409

Kansikas, M., Kasela, M., Kantelinen, J., & Nystrom, M. (2014). Assessing how reduced expression levels of the mismatch repair genes MLH1, MSH2, and MSH6 affect repair efficiency. *Human Mutation*, *35*(9), 1123–1127. https://doi.org/10.1002/humu.22605

Kantelinen, J., Kansikas, M., Korhonen, M. K., Ollila, S., Heinimann, K., Kariola, R., & Nystrom, M. (2010). MutSbeta exceeds MutSalpha in dinucleotide loop repair. *British Journal of Cancer*, *102*(6), 1068–1073. https://doi.org/10.1038/sj.bjc.6605531

Kondo, E., Horii, A., & Fukushige, S. (2001). The interacting domains of three MutL heterodimers in man: HMLH1 interacts with 36 homologous amino acid residues within hMLH3, hPMS1 and hPMS2. *Nucleic Acids Research*, *29*(8), 1695–1702.

Korhonen, M. K., Raevaara, T. E., Lohi, H., & Nystrom, M. (2007). Conditional nuclear localization of hMLH3 suggests a minor activity in mismatch repair and supports its role as a low-risk gene in HNPCC. *Oncology Reports*, *17*(2), 351–354.

Kunkel, T. A., & Erie, D. A. (2005). DNA mismatch repair. *Annual Review of Biochemistry*, *74*, 681–710. https://doi.org/10.1146/annurev.biochem.74.082803.133243

Lynch, H. T., Snyder, C. L., Shaw, T. G., Heinen, C. D., & Hitchins, M. P. (2015). Milestones of Lynch syndrome: 1895–2015. *Nature Reviews Cancer*, *15*(3), 181–194. https://doi.org/10.1038/nrc3878

Niessen, R. C., Kleibeuker, J. H., Westers, H., Jager, P. O., Rozeveld, D., Bos, K. K., … Hofstra, R. M. (2009). PMS2 involvement in patients suspected of Lynch syndrome . *Genes, Chromosomes & Cancer*, *48*(4), 322–329. https://doi.org/10.1002/gcc.20642

Nyström-Lahti, M., Perrera, C., Räschle, M., Panyushkina-Seiler, E., Marra, G., Curci, A., … Jiricny, J. (2002). Functional analysis of MLH1 mutations linked to hereditary nonpolyposis colon cancer. *Genes, Chromosomes & Cancer*, *33*(2), 160–167. https://doi.org/10.1002/gcc.1225

Raevaara, T. E., Vaccaro, C., Abdel-Rahman, W. M., Mocetti, E., Bala, S., Lönnqvist, K. E., … Nyström-Lahti, M. (2003). Pathogenicity of the hereditary colorectal cancer mutation hMLH1 del616 linked to shortage of the functional protein. *Gastroenterology*, *125*(2), 501–509. https://doi.org/S0016508503009053

Raschle, M., Marra, G., Nystrom-Lahti, M., Schar, P., & Jiricny, J. (1999). Identification of hMutLbeta, a heterodimer of hMLH1 and hPMS1. *The Journal of Biological Chemistry*, *274*(45), 32368–32375.

Roberts, M. E., Jackson, S. A., Susswein, L. R., Zeinomar, N., Ma, X., Marshall, M. L., … Chung, W. K. (2018). MSH6 and PMS2 germ-line pathogenic variants implicated in Lynch syndrome are associated with breast cancer. *Genetics in Medicine*, *20*, 1167–1174. https://doi.org/10.1038/gim.2017.254

Senter, L., Clendenning, M., Sotamaa, K., Hampel, H., Green, J., Potter, J. D., … de la Chapelle, A. (2008). The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology*, *135*(2), 419–428. https://doi.org/10.1053/j.gastro.2008.04.026

ten Broeke, S. W., Brohet, R. M., Tops, C. M., van der Klift, H. M., Velthuizen, M. E., Bernstein, I., … Wijnen, J. T. (2015). Lynch syndrome caused by germline PMS2 mutations: Delineating the cancer risk. *Journal of Clinical Oncology*, *33*(4), 319–325. https://doi.org/10.1200/JCO.2014.57.8088

ten Broeke, S. W., Suerink, M., & Nielsen, M. (2018). Response to roberts et al. 2018: Is breast cancer truly caused by MSH6 and PMS2 variants or is it simply due to a high prevalence of these variants in the population? *Genetics in Medicine*, *21*, 256–257. https://doi.org/10.1038/s41436-018-0029-1

ten Broeke, S. W., van der Klift, H. M., Tops, C. M. J., Aretz, S., Bernstein, I., Buchanan, D. D., … Win, A. K. (2018). Cancer risks for PMS2-associated lynch syndrome. *Journal of Clinical Oncology*, *36*(29), 2961–2968. https://doi.org/10.1200/JCO.2018.78.4777

Truninger, K., Menigatti, M., Luz, J., Russell, A., Haider, R., Gebbers, J. O., … Marra, G. (2005). Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. *Gastroenterology*, *128*(5), 1160–1171. https://doi.org/S001650850500168X

van der Klift, H. M., Tops, C. M. J., Bik, E. C., Boogaard, M. W., Borgstein, A. M., Hansson, K. B. M., … Wijnen, J. T. (2010). Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. *Human Mutation*, *31*(5), 578–587. https://doi.org/10.1002/humu.21229

Vaughn, C. P., Robles, J., Swensen, J. J., Miller, C. E., Lyon, E., Mao, R., … Samowitz, W. S. (2010). Clinical analysis of PMS2: Mutation detection and avoidance of pseudogenes. *Human Mutation*, *31*(5), 588–593. https://doi.org/10.1002/humu.21230

Win, A. K., Jenkins, M. A., Dowty, J. G., Antoniou, A. C., Lee, A., Giles, G. G., & MacInnis, R. J. (2017). Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *26*(3), 404–412. https://doi.org/10.1158/1055-9965.EPI-16-0693

Yurgelun, M. B., & Hampel, H. (2018). Recent advances in Lynch syndrome: Diagnosis, treatment, and cancer prevention. *American Society of Clinical Oncology Educational Book. American Society of Clinical Oncology. Annual Meeting*, *38*, 101–109. 101-109. https://doi.org/10.1200/EDBK_208341

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kasela M, Nyström M, Kansikas M. *PMS2* expression decrease causes severe problems in mismatch repair. *Human Mutation*. 2019;40:904–907. https://doi.org/10.1002/humu.23756