European Journal of Obstetrics & Gynecology and Reproductive Biology 238 (2019) 54–57

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

In-silico analysis of Thr767Ile pathogenic variant in the *MSH6* gene in family with endometrial cancer

Agnieszka Stembalska[a], Jakub Klapecki[b], Andrzej Pławski[c,d], Paweł Karpinski[a,e,*]

[a] Department of Genetics, Wroclaw Medical University, Marcinkowskiego 1, 50-368 Wroclaw, Poland
[b] Medgen Genetics Clinic, Orzycka 27, Warsaw, Poland
[c] Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland
[d] Department of General, Endocrinological Surgery and Gastroenterological Oncology, Poznan University of Medical Sciences, Poznan, Poland
[e] Laboratory of Genomics & Bioinformatics, Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland

ARTICLE INFO

Article history:
Received 7 January 2019
Received in revised form 15 April 2019
Accepted 20 April 2019

Keywords:
MSH6
Pathogenic variant
Endometrial cancer
Genetic predisposition
Bioinformatic

ABSTRACT

Objective: To examine the mechanism of pathogenity of Thr767Ile variant on MSH6 protein.
Study design: We describe a family diagnosed with endometrial cancer in two generations associated with variant in the *MSH6* gene (p. Thr767Ile / c. 2300C>T) (rs587781462). *MSH6* c. 2300C>T was associated with autosomal-dominant pattern of inheritance. *MSH6* c. 2300C>T has pathogenic status in ClinVar and LOVD3 databases but it has never been described in context of hereditary endometrial cancer. We utilized a number of *in-silico* bioinformatic approaches using MSH6 protein sequence and structural information to assess influence of Thr767Ile on MSH6 properties.
Results: MSH6 Thr767 is highly conservative amino acid among various kingdoms of organisms. Thr767Ile was predicted deleterious and likely decreases affinity of MSH2-MSH6 complex to DNA but not affect interaction between MSH2 and MSH6.
Conclusions: To the best of our knowledge, this is the first description of *MSH6* T767I pathogenic variant that could be associated with a hereditary endometrial cancer. Bioinformatic analyses showed that T767I substitution most likely affects the MSH6 most important role, which is a DNA binding.

Introduction

Uterine carcinoma is one of the six most prevalent malignancies in women worldwide [1]. Most endometrial cancers are sporadic adenocarcinomas [2]. One of the factors increasing risk for endometrial cancer is positive family history [3]. Endometrial cancer is notably a component malignancy of two cancer predispositions syndromes: Lynch syndrome (hereditary non-polyposis colorectal cancer syndrome, HNPCC) and Cowden syndrome (CS). Lynch syndrome demostrates an autosomal dominant pattern of inheritance with varying degree of penetrance. Cancer spectrum of Lynch syndrome includes colorectal cancer and numerous extracolonic cancers such as: endometrial, ovarian, gastric, pancreatic, hepatobiliary tract, urothelial and small intestine. CS is a multiple hamartoma syndrome characterized by an increased risk of breast, thyroid and endometrial cancer. The cause of Lynch syndrome includes germline pathogenic variants in mismatch repair genes, such as *MSH6, MSH2, MLH1* and *PMS2* (in descending frequency) or pathogenic variants in *EPCAM* gene (1% of cases), whereas CS is caused by germline pathogenic variants in *PTEN* gene [4–6].

Herein, we describe familial endometrial cancer presenting in two generations, that was caused by a variant (p. Thr767Ile; p. T767I; c.2300C>T; rs587781462) in the *MSH6* gene. Influence of Thr767Ile variant on MSH6 properties has been assessed by the number of bioinformatic approaches.

Materials and methods

Case report

A Caucasian 52-year-old woman (V:1, Fig. 1) presented with a history of endometrial cancer (papillary adenocarcinoma G1) at the age of 45. The woman was not known to have any other medical problems, except for Gilbert syndrome. Her 58-year-old sister, 76-year-old mother and 2 sisters of the mother: aged 74 and 71 had been diagnosed with endometrial cancer (adenocarcinoma) at the ages of 58, 52, 70 and 67, accordingly (V:3, IV:4, IV:6, IV:8, Fig. 1). The proband has two children: a son and a daughter (VI:1, VI:2, Fig. 1). Extended family history revealed other cancer cases. The

* Corresponding author.
E-mail address: polemiraza@poczta.fm (P. Karpinski).

https://doi.org/10.1016/j.ejogrb.2019.04.035
0301-2115/

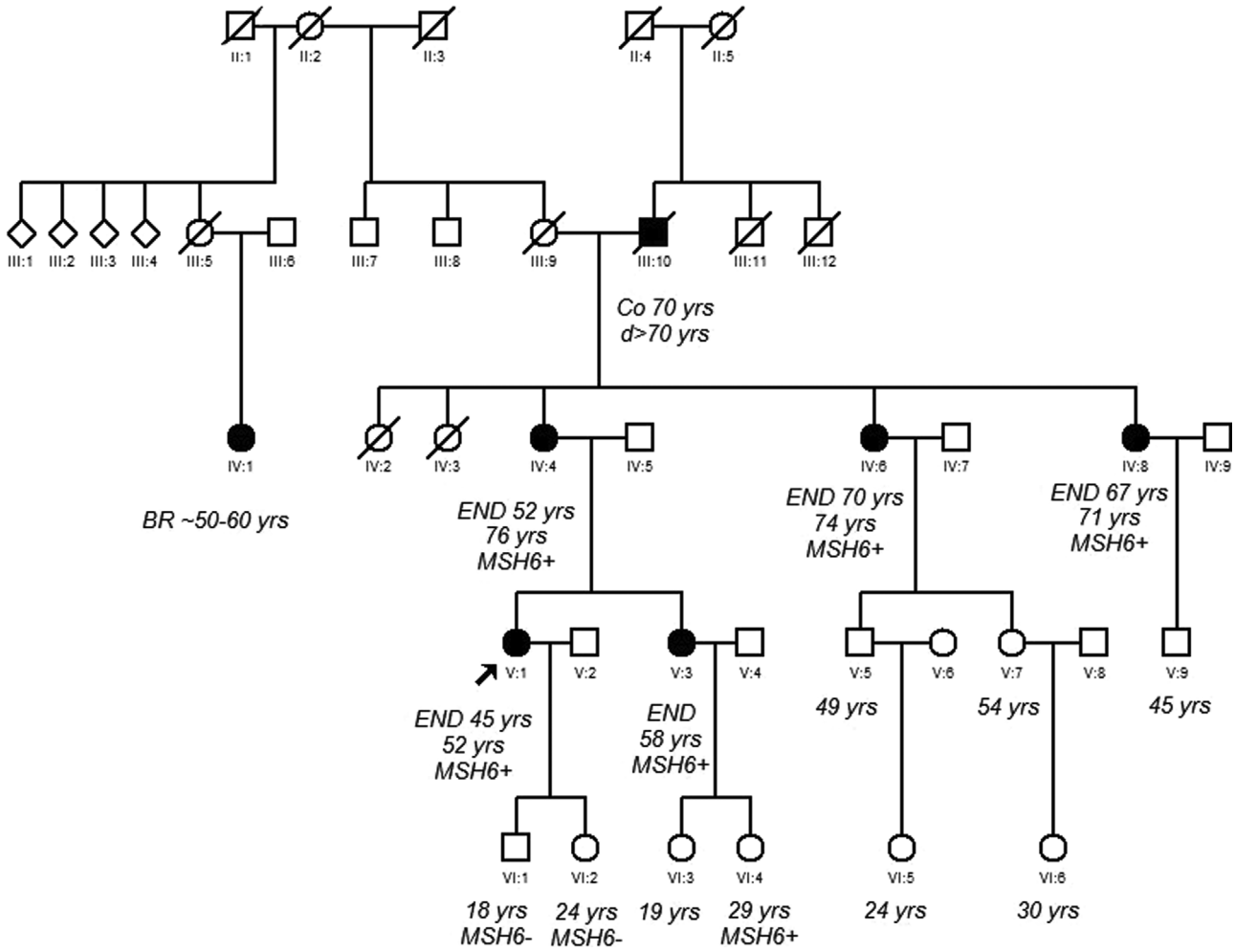

Fig. 1. Pedigree of the family with *MSH6* pathogenic variant c. 2300C > T (*MSH6*+). END – endometrial cancer, BR – breast cancer, ST – stomach cancer, MM – malignant melanoma, "MSH6+" - presence of *MSH6* pathogenic variant, "MSH6-" wild-type *MSH6*, d - death.

maternal grandfather of the proband (III:10, Fig. 1) was diagnosed at the age of 70 with a stomach cancer (he died in his seventies), and a living daughter of grandmother half sister (IV:1, Fig. 1) with breast cancer in her fifties. The family pedigree is presented in Fig. 1.

Subjects

After immunohistochemistry staining performed on a paraffin-embeded endometrial cancer section (MLH1+, MSH2+, MSH6-), *MSH6* sequence analysis was done initially in the proband, then followed by testing in all women with endometrial cancer (V:1, V:3, IV:4, IV:6, IV:8, Fig. 1) and in three healthy relatives (VI:1, VI:2, VI:4, Fig. 1). Microsatellite analysis has not been assessed in the tumors.

Whole blood was collected in EDTA tubes and DNA was isolated using a standard extraction protocol with phenol and chloroform followed by precipitation with ethanol. DNA fragments encompassing entire coding sequence of *MSH6* gene were amplified using primers and PCR conditions described previously [7]. The PCR products were purified by precipitation and sequenced on both strands using the Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems). The sequence products were purified and separated on an automated sequencer (ABI 3130xl Genetic Analyzer, Applied Biosystems).

Bioinformatic analysis

Evolutionary conservation of MSH6 T767 residue was assessed using MSH6 sequences from organisms belonging to the various kingdoms. The alignments were calculated using ClustalW [8].

Sequence-based predictions were determined using MSH6 protein sequence (accession number P52701.2) as a template for a number of algorithms including Pol-Phen-2, Provean, and specifically dedicated to MSH6 protein substitutions – CoDP [9–12].

For structure-based predictions we used as an input crystal structure of MSH2-MSH6 complex bound to ADP and AGT mispair (pdb code: 2o8b) [13]. Analyses were performed in mCSM and mCSM-NA tools [14,15].

Finally, molecular modelling of MSH6 wild-type and MSH6 T767I substitution were performed on Chimera software [16]. *In silico* mutagenesis was carried out using Durbrack rotamer library [17]. Ile rotamer with highest probability and lowest clashes score were selected. We used default Chimera clashes/contacts parameters.

Results and discussion

Endometrial cancers are relatively rarely explained by genetic factors but in about 10% of familial cases an inherited predisposition is suggested [18]. In at least 1,8% of newly diagnosed

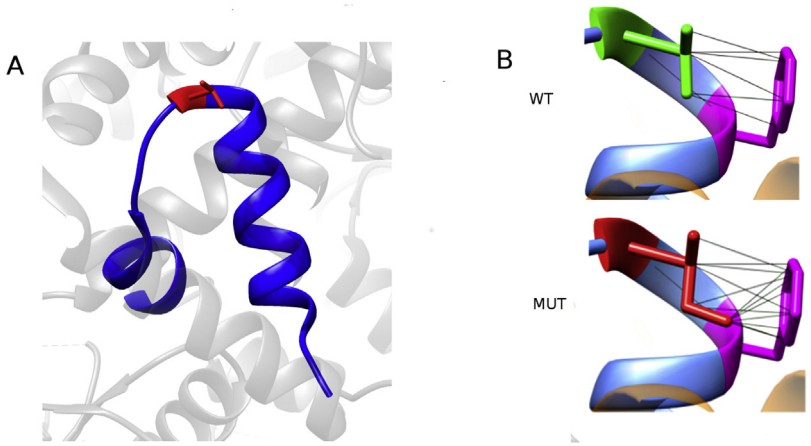

Fig. 2. A - Localization of T767 residue in domain 3 of MSH6 protein. Red - T767 residue; blue - conserved loop that comprise amino acids 757–782.
B - The structural analysis of the MSH6 pathogenic variant T767I. Green - T767 residue; magenta - F769 residue; red - I767 residue; black lines - pseudo bonds

endometrial carcinomas, Lynch syndrome is diagnosed [19]. In the described family the Amsterdam criteria II for Lynch syndrome were fulfilled [20]. Because of the hereditary nature of cancer predisposition syndromes, genetic counselling was offered to all of the family members.

Pathogenic variants in *MSH6* acount for about 10–20% of Lynch syndrome [21]. Most of the female carriers of *MSH6* pathogenic variant will be affected by endometrial carcinoma during their lifetime [22]. The risk of developing endometrial cancer increases with age. In these women the frequency of uterine cancer outweights the frequency of colon cancer occurrence as well as the frequency of uterine cancer in *MSH2* or *MLH1* pathogenic variant carriers [21–23]. A lifetime risk of endometrial cancer may be up to 60%, with the highest incidence between ages 40 and 60 [5].

Sequencing the *MSH6* gene on genomic DNA revealed a heterozygous substitution (c. 2300C > T;) in exon 4 (Supplementary Fig. 1) in the proband (V:1, Fig. 1). The same *MSH6* gene variant was found in all affected members of the described family and in one healthy relative (Fig. 1). Given that, c. 2300C > T variant appeared in two generations in five women with endometrial cancer we assume autosomal-dominant pattern of its inheritance. The *MSH6* c. 2300C > T results in Thr767Ile (p.T767I) (threonine to isoleucine) missense substitution and has been recorded in dbSNP under rs587781462 (allele ID: 150772). According to ClinVar and LOVD3 databases MSH6 T767I is classified as "pathogenic" and conditions under which c. 2300C > T variant was found were described as "colorectal cancer", "hereditary nonpolyposis colon cancer" and "Lynch syndrome I" [24,25]. Taken above to account, we conclude that *MSH6* c. 2300C > T has not previously been reported in the context of hereditary endometrial cancer.

T767I is located in MutSIII domain (PF05192) of MSH6 within a conserved loop that comprises of amino acids 757–782 (Fig. 2A) [13]. It has been suggested that due to its location this loop may be involved in signal transduction between ATPase and DNA binding domains of MSH6 [13].

To examine the mechanism of pathogenity of MSH6 p. T767I substitution we utilized a number of computational approaches. Initially, we assessed evolutionary conservation of MSH6 p.T767. This analysis revealed that T767 residue is evolutionarily conserved throughout species from prokaryota (*Escherichia coli*) to human (Supplementary Fig. 2). Sequence-based predictions of p. T767I substitution based on a number of algorithms recognized p. T767I as non-neutral/damaging substitution (Table 1).

Structural-based predictions revealed that T767I substitution likely decreases affinity of MSH2-MSH6 complex to DNA but does not affect interaction between MSH2 and MSH6 (Table 1). Finally, molecular modelling of MSH6 wild-type and MSH6 T767I substitution on Chimera software revealed atomic clashes between I767 and F769 residues (Fig. 2B). It would be plausible to expect that the defective interaction of these two residues would unfavourably affect the function of conserved loop that comprises of amino acids 757–782 [13]. Structural changes observed in MSH6 T767I could also contribute to negative IHC staining by affecting the MSH6 epitope. This phenomenon has been previously reported for a number of MSH6 missense variants. In summary, our bioinformatic analysis of MSH6 T767I substitution revealed that T767 residue is highly conserved and is located in MSH6 loop that plays an important role in MSH6 activity. Both structural-based and modelling analyses showed that T767I substitution most likely affects the MSH6 most important role, which is DNA binding. On the other hand, possibility of aberrant splicing due to c. 2300C > T variant could not be ruled out due to inaccuracy of current prediction methods. Therefore, additional experiments are required to investigate the possible involvement of c. 2300C > T variant in aberrant splicing. While computational approaches might help in pathogenic variant interpretation functional assays for MSH6 c. 2300C > T (T767I) variant are necessary to verify our *in-silico* analysis [26].

Table 1
Results of sequence-based and structural based analysis of *MSH6* p. T767I substitution.

Software	Input	Prediction	Specific output
Pol-Phen-2	Protein aa sequence	probably damaging	score = 1
Provean	Protein aa sequence	detelerious	score = 0.001
CoDP	Protein aa sequence	Impair molecular function	score = 0.956
MutaBind	Protein structure	Does not affect MSH2-MSH6 interaction	ΔΔGBind (kcal/mol) = 0.47
mCSM-NA	Protein structure	Reduced DNA binding affinity	Affinity change ΔΔG = -2.31 Kcal/mol
Chimera	Protein structure	Atomic clashes between I767 and F769 residues	none

Concluding, we characterized a pathogenic variant in *MSH6* gene which has not previously been reported in the context of hereditary endometrial cancer. MSH6 T767I was found in two generations of a family, in all of the five women with endometrial cancer and in one unaffected individual. As shown by computational approaches, this variant is non-neutral and is predicted to decrease binding affinity of MSH6 to DNA, thus, it likely affects DNA repair which in turn increases the risk of developing endometrial cancer.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2019.04.035.

References

[1] Henley SJ, Miller JW, Dowling NF, Benard VB, Richardson LC. Uterine cancer incidence and mortality – United States, 1999-2016. MMWR Morb Mortal Wkly Rep 2018;67(48):1333–8, doi:http://dx.doi.org/10.15585/mmwr.mm6748a1.

[2] Kurman R, Ellenson L, Ronnett B. Blaustein's pathology of the female genital tract. 6 ed. New York: Springer; 2011.

[3] Cook LS, Nelson HE, Stidley CA, et al. Endometrial cancer and a family history of cancer. Gynecol Oncol 2013;130(2):334–9, doi:http://dx.doi.org/10.1016/j.ygyno.2013.04.053.

[4] Garg K, Soslow RA. Lynch syndrome (hereditary non-polyposis colorectal cancer) and endometrial carcinoma. J Clin Pathol 2009;62(8):679–84, doi:http://dx.doi.org/10.1136/jcp.2009.064949.

[5] Daniels MS. Genetic testing by cancer site: uterus. Cancer J 2012;18(4):338–42, doi:http://dx.doi.org/10.1097/PPO.0b013e3182610cc2.

[6] Zhao YS, Hu FL, Wang F, et al. Meta-analysis of MSH6 gene mutation frequency in colorectal and endometrial cancers. J Toxicol Environ Health A 2009;72(11–12):690–7, doi:http://dx.doi.org/10.1080/15287390902841003.

[7] Kolodner RD, Tytell JD, Schmeits JL, et al. Germ-line msh6 mutations in colorectal cancer families. Cancer Res 1999;59(20):5068–74.

[8] Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007;23(21)2947–8 DOI btm404 [pii]/bioinformatics/btm404.

[9] Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248–9, doi:http://dx.doi.org/10.1038/nmeth0410-248.

[10] Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. PLoS One 2012;7(10)e46688, doi:http://dx.doi.org/10.1371/journal.pone.0046688.

[11] Bromberg Y, Rost B. SNAP: predict effect of non-synonymous polymorphisms on function. Nucleic Acids Res 2007;35(11)3823–35 DOI gkm238 [pii]/nar/gkm238.

[12] Terui H, Akagi K, Kawame H, Yura K. CoDP: predicting the impact of unclassified genetic variants in MSH6 by the combination of different properties of the protein. J Biomed Sci 2013;20(25), doi:http://dx.doi.org/10.1186/1423-0127-20-25.

[13] Warren JJ, Pohlhaus TJ, Changela A, Iyer RR, Modrich PL, Beese LS. Structure of the human MutSalpha DNA lesion recognition complex. Mol Cell 2007;26(4):579–92, doi:http://dx.doi.org/10.1016/j.molcel.2007.04.018.

[14] Pires DE, Ascher DB, Blundell TL. mCSM: predicting the effects of mutations in proteins using graph-based signatures. Bioinformatics 2014;30(3):335–42, doi:http://dx.doi.org/10.1093/bioinformatics/btt691.

[15] Li M, Simonetti FL, Goncearenco A, Panchenko AR. MutaBind estimates and interprets the effects of sequence variants on protein-protein interactions. Nucleic Acids Res 2016;44(W1):W494–501, doi:http://dx.doi.org/10.1093/nar/gkw374.

[16] Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera–a visualization system for exploratory research and analysis. J Comput Chem 2004;25(13):1605–12, doi:http://dx.doi.org/10.1002/jcc.20084.

[17] Shapovalov MV, Dunbrack RL. A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. Structure 2011;19(6):844–58, doi:http://dx.doi.org/10.1016/j.str.2011.03.019.

[18] Barak F, Milgrom R, Laitman Y, et al. The rate of the predominant Jewish mutations in the BRCA1, BRCA2, MSH2 and MSH6 genes in unselected Jewish endometrial cancer patients. Gynecol Oncol 2010;119(3):511–5, doi:http://dx.doi.org/10.1016/j.ygyno.2010.08.027.

[19] Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66(15):7810–7, doi:http://dx.doi.org/10.1158/0008-5472.CAN-06-1114.

[20] Samadder NJ, Smith KR, Wong J, et al. Cancer risk in families fulfilling the Amsterdam Criteria for lynch syndrome. JAMA Oncol 2017;3(12):1697–701, doi:http://dx.doi.org/10.1001/jamaoncol.2017.0769.

[21] Baglietto L, Lindor NM, Dowty JG, et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst 2010;102(3):193–201, doi:http://dx.doi.org/10.1093/jnci/djp473.

[22] Devlin LA, Graham CA, Price JH, Morrison PJ. Germline MSH6 mutations are more prevalent in endometrial cancer patient cohorts than hereditary non polyposis colorectal cancer cohorts. Ulster Med J 2008;77(1):25–30.

[23] Watson P, Vasen HFA, Mecklin JP, et al. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer 2008;123(2):444–9, doi:http://dx.doi.org/10.1002/ijc.23508.

[24] Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, et al. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 2011;32(5):557–63, doi:http://dx.doi.org/10.1002/humu.21438.

[25] Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46(D1), doi:http://dx.doi.org/10.1093/nar/gkx1153 D1062-D7.

[26] Houlleberghs H, Goverde A, Lusseveld J, et al. Suspected Lynch syndrome associated MSH6 variants: a functional assay to determine their pathogenicity. PLoS Genet 2017;13(5), doi:http://dx.doi.org/10.1371/journal.pgen.1006765 e1006765.