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REVIEW ARTICLE

MUTYH the base excision repair gene family member associated with colorectal cancer polyposis

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ABSTRACT

Colorectal cancer is classified in to three forms: sporadic (70-75%), familial (20-25%) and hereditary (5-10%). hereditary colorectal cancer syndromes classified into two different subtypes: polyposis and non polyposis. Familial Adenomatous polyposis (FAP; OMIM #175100) is the most common polyposis syndrome, account for <1% of colorectal cancer incidence and characterized by germline mutations in the Adenomatous polyposis coli (APC, 5q21-q22; OMIM #175100). FAP is a dominant cancer predisposing syndrome which 20-25% cases are *de novo*. There is also another polyposis syndrome; *MUTYH* associated polyposis (MAP, OMIM 608456) which it is caused by mutation in *human Mut Y homologue MUTYH (MUTYH*; OMIM 604933) and it is associated with multiple (15-100) colonic adenomas. In this paper we discuss *MUTYH* mechanism as an important member of Base Excision Repair (BER) family and its important role in polyposis condition.

Keywords: Colorectal cancer, MAP, *MUTYH*, Base excision repair (BER).

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Introduction

It is estimated that 20,000 DNA damages occur in every cell per day (1). Gastrointestinal tract is a main target for oxidising elements which are highly mutationic (2). So colorectal cancer considered as a main cancer arises from exposure to this kind of agents. Beside Mismatch Repair (MMR) and Nucleotide excision repair (NER) Pathways which are the fundamental repair pathways interact with the mismatch pairs and aberrant nucleotide occurs in replication process, respectively, the base excision repair (BER) pathway is one of the main and primary DNA repair mechanisms that is involved in correcting the base mutations arised from oxidative, alkylation, deamination and depurination/depyrimidination damages (3). *MUTYH* is a DNA glycosylase and it belongs to BER family. The *MUTYH* protein is involved in the repair of post-replicative mispairs within DNA replication (4,5).

MUTYH function and interactions:

A human homologue of the *Escherichia coli* (*E. coli*) *mutY* gene was first cloned in 1996 (6), while

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the identification of the functional activity of the *MUTYH* gene first back in 2000 (7). This gene is called *MUTYH* and often known as *hMYH* or *MYH*, although this is not a correct name, because *MYH* is the gene symbol for the myosin heavy-chain gene. *MUTYH* is located on the short arm of chromosome 1(1p34.1) and spans 11.2 kb. This gene is a DNA glycosylase that is involved in the repair of post-replicative mispairs and plays a critical role in base excision repair (BER) pathway (4,5) .The oxidized form of guanine is 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxoG) which is considered as a frequent and stable element (8). In replication process 8-oxoG can pair with adenine as well as cytosine. The modified guanine (8-oxoG) is replicated in each round and the failure to remove the oxidized nucleotides before replication results in G: C to T: A transversion mutation (9,10).

MUTYH mediates to remove A from A: 8-oxoG mispairs (7, 11) and OGG1 the other member of BER pathway detects and then removes 8-oxoG opposite cytosine (8-oxoG: C) (12-13). Thus the cooperation of OGG1 and *MUTYH* together prevents G: C to T: A transversion mutation due to oxidative damages within replication process.

When an aberrant base is incised and then removed, it produce a gap called Apurinic/ A pyrimidinic (AP) sites which are mutagenic and should be corrected quickly (14).Completion of the repair process requires involvement of many additional proteins. More than 8 specific proteins detect specific DNA mutations which produce a basic or Apurinic/ A pyrimidinic (AP) sites (15).

Based on proteins involved the process of BER activity, there are two main mechanisms to repair AP site created by DNA damage: short patch repair pathway and long patch repair pathway (16).

Short-patch repair pathway involves making the association between *POLB*, *APE1*, *XRCC1*, *PARP1*, and either *LIG1* or *LIG3* genes. These related genes are activated when a single nucleotide insertion occurs and an AP endonuclease (also known as *APE1* or *APEX*) incises the incorrect matched DNA at the AP site resulting in the formation of a 3´-hydroxyl end (3´OH) and a 5´ a basic sugar phosphate end (5´dRP) (17) Since *MUTYH* has no AP-lyase activity *APE1* detects a basic site and then proceed the excision process. At the end, repair procedure of an aberrant nucleotide is accomplished by DNA ligase III (16).

A long-patch repair pathway requires *PCNA, APE1, RFC, RPA, PARP1, FEN1, POLD/POLE* and *LIG1* for BER activity and they involve when 2-10 nucleotides mispaired in a DNA strand genes. In long patch repair pathway cleavage process accomplished by AP endonuclease (*APEX1*) and repair process is completed by proliferating cell nuclear antigen (*PCNA*) which has different types of functions include in DNA repair as well as cell cycle and DNA replication (3,18).

Among repair genes, *MUTYH* is the main protein that detects peculiar A:G and A: 8-oxoG mispairs on DNA helix (19).

The *MUTYH* protein structure consists of many functional domains such as the N-terminal domain on the 5' side and the C-terminal domain on the 3' side. The N-terminal domain contains the catalytic region and includes a helix-hairpin-helix (HhH), pseudo HhH and an iron-sulfur cluster loop motif, which are also common region in other BER glycosylases; the C-terminal domain on the 3' side of the *MUTYH* protein structure reported to have a role in recognition of 8-oxoG and shares homology with *MTH1* (member of the BER family) (20-22).

Association between *MUTYH* and Replication Protein A (*RPA*), Proliferating Cell Nuclear Antigen (*PCNA*), p73, p53 and APE1has also reported in several studies (18, 23). Many papers suggested that in the damage condition, *PCNA* increases *MUTYH* activity (24-25). Association of *MUTYH* gene and MMR genes such as *MSH6*,

MSH2 and *MLH1* has also been discussed (26-28). Although Most of the *APC* mutations produce truncated proteins, most pathogenic *MUTYH* variants are missense and splice site mutations and only a minority of variants are truncating mutations (29). The distribution of *MUTYH* mutations in MAP patients shows ethnic differences. Some variants are more common in other populations including: E480X in Indian (30), Y104X in Pakistani (31), c.1437_1439delGGA in Italian (32), c.1228_1229insGG in Portuguese (33), Q498H in German (34), and G25D and P18L in Chinese populations (35). Also, Y179C and G396D (previously known as Y165C and G382D) are two most common *MUTYH* mutations (80%) in Caucasian populations (30). Since only a few variants of *MUTYH* gene analyzed for their repair activity so far, it is recommended that other variants of *MUTYH* need to be examined for their involvement in pathogenesis of MAP (36).

Frequency of large deletions in *MUTYH* gene seem to be low and just Two papers revealed the presence of large deletions in *MUTYH* gene so far(5,37). Loss of heterozygosity (LOH) of 1p is frequently happening in CRC tumors with chromosomal instability (CIN) (38). Since LOH is a common event in CRC tumors with CIN, LOH in MAP tumors display a distinct pattern of loss of heterozygosity with loss of parts of chromosomes without copy number alterations termed copy-neutral loss of heterozygosity which is not a frequent event in CRC tumors with CIN (39-40). Croitoru et al showed that LOH detected in 20% of biallelic and 47% of monoallelic *MUTYH* mutation carriers (41). As demonstrated in several studies, microsatellite stable (MSS) is a dominant pattern of MSI in MAP tumors (42,43).

MUTYH association polyposis characterizations

Mutation in *MUTYH* gene causes a predisposing condition to CRC termed *MUTYH* association polyposis (MAP) (2,30). MAP was first reported by Al Tassan et al while they were evaluating 'family N'. In this family three of seven siblings had a phenotype resemble with AFAP without aberrant mutation in *APC* gene, instead they observed that 11 tumors from three affected siblings had 18 somatic *APC* mutations which 15 mutations were G:C to T:A transversion mutations, this finding highlighted the possibility of deficiency in repair process of 8-oxoG mutations. They also reported that all three affecting siblings had biallelic mutation in *MUTYH* gene since it wasn't detected in rest of four siblings (2). Mean age at diagnosis of MAP patients is 48 and patients have between 10 and 100 colorectal polyps. The penetrance of this syndrome is 20–80% between 50 and 80 years (42-44). The phenotype of MAP patients resembles with Attenuated Familial Adenomatous polyposis coli AFAP (AFAP; OMIM #175100) individuals (30, 44).

Diagnosis of MAP patients with cases present overlapping features or AFAP patients is difficult since they share some similarities such as number of polyps, proximal location of polyps and early onset of CRC (42-45). In MAP patients Polyps are frequently small and mostly located left-side of the colon (42). Proximal location of polyps is the key point to distinguish cases with moderate adenomatous polyps from those of sporadic (42,46). In comparison with the general population CRC risk in MAP patients, associated with 28- to 93-fold (42, 47). Histopathologically Adenomas (tubular or tubulo-villous) are detected in entire colon consider as predominant lesions in AFAP/FAP and also in MAP patients. Since serrated polyps: hyperplastic polyps, sessile serrated polyps (also referred to as sessile serrated adenomas) and traditional serrated adenomas are not present in Affected harbouring mutation in *APC* gene, they are common types of lesions in MAP patients (48). Finally *APC* genetic testing for this group of patients with serrated polyps

wouldn't be informative. Tumors in MAP patients show a high frequency of distinctive somatic G:C to T:A mutations in the *APC* and *Kras* genes (2,30). GAA sequences in *APC* gene are the target sites for truncating mutations and this site is frequently mutated during tumorigenesis (2, 30,49). *APC* has 216 GAA sites in which G:C→T:A mutations could happen and result in a termination codons (2). In contrast *TP53*, *PTCH*, *RB1*, *NF1* and *VHL* have fewer target sites and this makes the APC the best target than the other genes for mutagenesis in MAP (tumors (49). It is notable that, in *Kras* gene the hot spot codon is c.34G>T at codon12 (50,51).

Screening and Management

Early detection, genetic counseling and *MUTYH* mutation screening are important in affected individuals and their siblings. Based on National Comprehensive Cancer Network NCCN recommendation, Colonoscopy starts at age 25 years (52) and patients with more than 10 adenomas should be referred for genetic counseling and testing procedure (52). Patients with less than 10 adenomas should be referred for follow up screening and genetic testing for this group of patients is not necessary.

Other surveillance protocol for MAP patients recommends similar screening program similar AFAP patients. Patients undergo colonoscopy every 2 years starting at 18 - 20 years and upper gastrointestinal endoscopy starts when affected is between 25 and 30 years of age (53,54).

MUTYH mutation screening is recommended for people who are diagnosed with MAP and patients who have a recessive mutation transmission and phenotype similar to AFAP. The affected may not be seen in every generation and usually have a normal parents (55,56).

First of all, the two putative codons Y165C or G382D are examined for their high incidence rate in majority of populations. Then PCR sequencing is performed for the entire coding region and intron–exon boundaries of *MUTYH.* The genetic testing for *MUTYH* in patients with multiple serrated polyps without adenomas is not recommended by NCCN (52).

There haven't been any reports to define molecular screening in patients with MAP in Iran yet and Research is ongoing to determine APC and *MUTYH* variants in FAP patients. But screening of mutations in other Genes associated with CRC carried out and other repair genes like *MLH1* and *MSH6* have been studied (57-60).

Prevalence

Approximately 0.3%–1% of all colorectal cancers is associated with MAP (41, 42).

It is estimated that 1% to 2% of the general population has a mutation in *MUTYH.* There isn't a peculiar criterion to classify nonpolyposis *MUTYH*-associated CRC phenotype, so it has been recommended that all early-onset CRC cases should be evaluated for *MUTYH* mutations (61). Several Studies demonstrated that up to 30% of biallelic *MUTYH* mutation carriers develop CRC although they do not present a polyposis condition (62). There has also been reported some cases with MAP and no polyps whereas in some cases more than 500 colorectal polyps observed (44). *APC* germline mutations are not present in 10–30% of FAP patients and in up to 90% of AFAP patients (63). In another word, *APC* mutations are detected in 10–22% of AFAP cases and biallelic germline mutation of *MUTYH* were identified in 15–30% of AFAP patients and approximately 7–22% of FAP patients. Biallelic *MUTYH* mutations can be detected in 30% of *APC* mutation-negative patients. (30, 55, 64, 65).

Biallelic germline mutations in *MUTYH* are common in patients with negative *APC* related FAP patients and in MAP cases but recently studies are focused on monoallelic *MUTYH* variants in CRC patients and try to identify the

association between monoallelic mutation susceptibility to CRC (66-68).

Monoallelic *MUTYH* mutation carrier's account for 1% to 2% of the general population since Biallelic mutations observed in less than 1% of all CRCs and the frequency of this mutation in patients with10 to 100 polyps are 28% and in individuals with 100 to 1000 polyps are 14% (44, 69).

First degree relatives of MAP patients with biallelic mutation in *MUTYH* gene are considered as obligate carriers who carry at least one *MUTYH* mutation. Both parents are carriers of a biallelic mutation and each child has 25% chance of inheriting two mutations. Whether monoallelic *MUTYH* carriers (heterozygote) are at high risk for developing CRC is still not clear: compared with the general population There is evidence that obligate monoallelic *MUTYH* mutation carriers have a modest risk for colorectal cancer (47,66). Some authors believe that heterozygote mutation carriers should consider as low penetrance alleles, although consensus surveillance guidelines for this subgroup need to be developed.

MUTYH and other cancers

Extracolonic manifestations are common in patients with MAP which include: duodenal cancer and related polyposis, cancers such as gastric, small intestinal, endometrial, liver, ovarian, bladder, thyroid, Breast and skin cancers including melanoma, squamous epithelial, and basal cell carcinomas (70,71). Other manifestations such as osteomas, dental cysts and congenital hypertrophy of the retinal pigment epithelium (CHRPE) are also seen in this group of patients (32, 71, 72). These extra colonic manifestations are also reported in FAP patients and the occurrence is less in MAP than in FAP or AFAP patients (73). The association between breast cancer and *MUTYH* gene is not defined clearly so far (71). Since Frequency of biallelic *MUTYH* mutations in breast cancer seem to be low (74,75) in valuable paper by Wasielewski et al. reported heterozygote mutations in *MUTYH* gene in families with both CRC and breast cancer moreover they have also reported that there was an increased risk for breast cancer in Female MAP patients (76).The association of many malignancy such as endometrial (77,78), Gastric(79,80), Bladder(81) lung(82) and Diabetes(83) with MAP have reported respectively. Screening of Somatic *MUTYH* gene Mutations in sporadic CRC patients doesn't seem to be informative since the majority of papers revealed no association between *MUTYH* and sporadic colorectal cancer (84, 85) except one paper detected two *MUTYH* mutations in Tunisian patients (86).

Immune system response

The immune system of patients with deficiency in DNA mismatch repair genes is more active than individuals without DNA repair defects. It is proposed that the accumulation of peptide elements and mutant proteins in surface of the tumor cells in patients with high MSI or aberrant expression of MMR genes, makes the immune system more active then this results in better diagnosis and survival (87, 88). This active immune system could affect tumorigenesis while Antigens presenting in the tumor cell surface (89). Infact The accumulation of peptides and neoantigenes in such patients simulate anti-tumor immune response (90) and in comparison with the other lesions they show increased survival rates (91). High Infiltration of lymphocytes in MAP tumors reported in several studies previously (43, 51). Human leukocyte antigen class I complexes mediates in targeting of tumor cells by CD8+ and loss of expression of this antigen is a common event in MSI-H and MAP tumors (92-94). When HLA is lost then tumors hide from immune system due to deficiency in recognition and elimination (95, 96).

Conclusion

Although the *MUTYH* mutations gaining attention in diagnosis and counseling of patients with CRC and polyposis, many questions about diagnostic and screening protocols still remained unanswered. Detection of *MUTYH* gene variants and their association with polyposis and non polyposis CRC and study of immune system molecules and their involvements in tumorigenesis of MAP patients will be worthwhile for better diagnosis and further screening schedule for MAP patients.

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References

1. Drabløs F, Feyzi E, Aas PA, Vaagbø CB, Kavli B, Bratlie MS, et al. Alkylation damage in DNA and RNA—repair mechanisms and medical significance. DNA Repair 2004; 3:1389-407.

2. Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, et al. Inherited variants of *MYH* associated with somatic G:C-->T:A mutations in colorectal tumors. Nat Genet 2002; 30:227-32.

3. Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells: Base excision repair: the long and short of it. Cell Mol Life Sci 2009; 66:981-93.

4. Nghiem Y, Cabrera M, Cupples CG, Miller JH. The mutY gene: a mutator locus in Escherichia coli that generates G. C----T.A transversions. Proc Natl Acad Sci USA 1988; 85:2709-13.

5. Torrezan GT, da Silva FC, Krepischi AC, Santos ÉM, Ferreira Fde O, Rossi BM, et al. Breakpoint characterization of a novel large intragenic deletion of *MUTYH* detected in a MAP patient: case report. BMC Med Genet 2011; 12:128.

6. Slupska MM, Baikalov C, Luther WM, Chiang JH, Wei YF, Miller JH. Cloning and sequencing a human homolog (*hMYH*) of the Escherichia coli mutY gene whose function is required for the repair of oxidative DNA damage. J Bacteriol 1996; 178:3885-92.

7. Shinmura K, Yamaguchi S, Saitoh T, Takeuchi-Sasaki M, Kim SR, Nohmi T, et al. Adenine excisional repair function of *MYH* protein on the adenine: 8-hydroxyguanine base pair in double-stranded DNA. Nucleic Acids Res 2000; 28:4912-8.

8. Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res 1984; 12:2137-45.

9. Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry 1990; 29:7024-32.

10. Moriya M, Ou C, Bodepudi V, Johnson F, Takeshita M, Grollman AP. Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in E. coli. Mutat Res 1991; 254:281-88.

11. Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. Functional expression of *hMYH*, a human homolog of the Escherichia coli *MutY* protein. J Bacteriol 1999; 181:6210-13.

12. Shinmura K, Yokota J. The *OGG1* gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. Antioxid Redox Signal 2001; 3:597-609.

13. Klungland A, Bjelland S. Oxidative damage to purines in DNA: role of mammalian *Ogg1*. DNA Repair (Amst) 2007; 6:481-88.

14. Nilsen H, Krokan HE. Base excision repair in a network of defence and tolerance. Carcinogenesis 2001; 22:987-98.

15. Thyagarajan B, Lindgren B, Basu S, Nagaraj S, Gross MD, Weisdorf DJ, et al. Association between genetic variants in the base excision repair pathway and outcomes after hematopoietic cell transplantations. Biol Blood Marrow Transplant 2010; 16:1084-89.

16. van Loon B, Hübscher U. An 8-oxo-guanine repair pathway coordinated by *MUTYH* glycosylase and DNA polymerase lambda. Proc Natl Acad Sci USA 2009; 106:18201-206.

17. Memisoglu A, Samson L. Base excision repair in yeast and mammals. Mutat Res 2000; 451:39-51.

18. Parker A, Gu Y, Mahoney W, Lee SH, Singh KK, Lu AL. Human homolog of the MutY repair protein (*hMYH*) physically interacts with proteins involved in long patch DNA base excision repair. J Biol Chem 2001; 276:5547-55.

19. Gu Y, Lu AL. Differential DNA recognition and glycosylase activity of the native human MutY homolog (*hMYH*) and recombinant *hMYH* expressed in bacteria. Nucleic Acids Res 2001; 29:2666-74.

20. Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. Orphanet J Rare Dis 2009; 4:22.

21. Sieber OM, Lipton L, Crabtree M, Heinimann K, Fidalgo P, Phillips RK, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in *MYH*. N Engl J Med 2003; 348:791-99.

22. Noll DM, Gogos A, Granek JA, Clarke ND. The C-terminal domain of the adenine-DNA glycosylase *MutY* confers specificity for 8-oxoguanine.adenine mispairs and may have evolved from *MutT*, an 8-oxo-dGTPase. Biochemistry 1999; 38:6374-79.

23. Zaika E, Wei J, Yin D, Andl C, Moll U, El-Rifai W, et al. p73 protein regulates DNA damage repair. FASEB J 2011; 25:4406-14.

24. Shi G, Chang DY, Cheng CC, Guan X, Venclovas C, Lu AL. Physical and functional interactions between MutY glycosylase homologue (*MYH*) and checkpoint proteins Rad9-Rad1-Hus1. Biochem J 2006; 400:53-62.

25. Gembka A, Toueille M, Smirnova E, Poltz R, Ferrari E, Villani G, et al. The checkpoint clamp, *Rad9-Rad1-Hus1* complex, preferentially stimulates the activity of apurinic/apyrimidinic endonuclease 1 and DNA polymerase beta in long patch base excision repair. Nucleic Acids Res 2007; 35:2596-608.

26. Giráldez MD, Balaguer F, Bujanda L, Cuatrecasas M, Muñoz J, Alonso-Espinaco V, et al. *MSH6* and *MUTYH* deficiency is a frequent event in early-onset colorectal cancer. Clin Cancer Res 2010; 16:5402-13.

27. GU Y, Parker a, Wilson TM, Bai H, Chang DY, Lu AL. Human *MutY* homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human *MutS* homolog 2/human *MutS* homolog 6. J Biol Chem 2002; 277:11135-42.

28. Lefevre JH, Colas C, Coulet F, Bonilla C, Mourra N, Flejou JF, et al. *MYH* biallelic mutation can inactivate the two genetic pathways of colorectal cancer by *APC* or *MLH1* transversions. Fam Cancer 2010; 9:589-94.

29. Out AA, Tops CM, Nielsen M, Weiss MM, van Minderhout IJ, Fokkema IF, et al. Leiden Open Variation Database of the *MUTYH* gene. Hum Mutat 2010; 31:1205-15.

30. Jones S, Emmerson P, Maynard J, Best JM, Jordan S, Williams GT, et al. Biallelic germline mutations in *MYH* predispose to multiple colorectal adenoma and somatic G:C-->T:A mutations. Hum Mol Genet 2002; 11:2961-67.

31. Dolwani S, Williams GT, West KP, Newman J, Stock D, Griffiths AP, et al. Analysis of inherited *MYH*/(*MutYH*) mutations in British Asian patients with colorectal cancer. Gut 2007; 56:593.

32. Gismondi V, Meta M, Bonelli L, Radice P, Sala P, Bertario L, et al. Prevalence of the Y165C, G382D and 1395delGGA germline mutations of the *MYH* gene in Italian patients with adenomatous polyposis coli and colorectal adenomas. Int J Cancer 2004; 109: 680-84.

33. Isidro G, Laranjeira F, Pires A, Leite J, Regateiro F, Castro e Sousa F, et al. Germline *MUTYH* (*MYH*) mutations in Portuguese individuals with multiple colorectal adenomas. Hum Mutat 2004; 24:353-54.

34. Görgens H, Krüger S, Kuhlisch E, Pagenstecher C, Höhl R, Schackert HK, et al. Microsatellite stable colorectal cancers in clinically suspected hereditary nonpolyposis colorectal cancer patients without vertical transmission of disease are unlikely to be caused by biallelic germline mutations in *MYH*. J Mol Diagn 2006; 8:178-82.

35. Chen H, Xu L, Qi Q, Yao Y, Zhu M, Wang Y. A haplotype variation affecting the mitochondrial transportation of *hMYH* protein could be a risk factor for colorectal cancer in Chinese. BMC Cancer 2008; 8:269.

36. Shinmura K, Goto M, Tao H, Sugimura H. Role of Base Excision Repair Enzyme MUTYH in the Repair of 8-Hydroxyguanine and MUTYH-Associated Polyposis (MAP). Hereditary Genet 2012; 10: 2161-41.

37. Rouleau E, Zattara H, Lefol C, Noguchi T, Briaux A, Buecher B, et al. First large rearrangement in the *MUTYH* gene and attenuated familial adenomatous polyposis syndrome. Clin Genet 2011; 80:301-303.

38. Thorstensen L, Qvist H, Heim S, Liefers GJ, Nesland JM, Giercksky KE, et al. Evaluation of 1p losses in primary carcinomas, local recurrences and peripheral metastases from colorectal cancer patients. Neoplasia 2000; 2:514-22.

39. Middeldorp A, van Puijenbroek M, Nielsen M, Corver WE, Jordanova ES, ter Haar N, et al. High frequency of copy-neutral LOH in *MUTYH*-associated polyposis carcinomas. J Pathol 2008; 216:25-31.

40. Melcher R, Hartmann E, Zopf W, Herterich S, Wilke P, Müller L, et al. LOH and copy neutral LOH (cnLOH) act as alternative mechanism in sporadic

colorectal cancers with chromosomal and microsatellite instability. Carcinogenesis 2011; 32:636-42.

41. Croitoru ME, Cleary SP, Di Nicola N, Manno M, Selander T, Aronson M, Redston M, et al. Association between biallelic and monoallelic germline *MYH* gene mutations and colorectal cancer risk. J Natl Cancer Inst 2004; 96:1631-34.

42. Lubbe SJ, Di Bernardo MC, Chandler IP, Houlston RS. Clinical implications of the colorectal cancer risk associated with *MUTYH* mutation. J Clin Oncol 2009; 27:3975-80.

43. Nielsen M, de Miranda NF, van Puijenbroek M, Jordanova ES, Middeldorp A, van Wezel T, et al. Colorectal carcinomas in *MUTYH*-associated polyposis display histopathological similarities to microsatellite unstable carcinomas. BMC Cancer 2009; 9:184.

44. Nielsen M, Morreau H, Vasen HF, Hes FJ. *MUTYH*-associated polyposis (MAP). Crit Rev Oncol Hematol 2011; 79:1-16.

45. Burt RW, Leppert MF, Slattery ML, Samowitz WS, Spirio LN, Kerber RA, et al. Genetic testing and phenotype in a large kindred with attenuated familial adenomatous polyposis. Gastroenterology 2004; 127:444-51.

46. O'Shea AM, Cleary SP, Croitoru MA, Kim H, Berk T, Monga N, et al. Pathological features of colorectal carcinomas in *MYH*-associated polyposis. Histopathology 2008; 53:184-94.

47. Farrington SM, Tenesa A, Barnetson R, Wiltshire A, Prendergast J, Porteous M, et al. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. Am J Hum Genet 2005; 77:112-19.

48. Snover DC, Ahnen DJ, Burt RW, et al. Serrated polyps of the colon and rectum and serrated polyposis. In: Bosman FT, Carneiro F, Hruban RH, et al, eds. WHO Classification of Tumours of the Digestive System. 4th ed. Lyon, France: IARC; 2010:160Y165.

49. Cheadle JP, Sampson JR. Exposing the *MYtH* about base excision repair and human inherited disease. Hum Mol Genet 2003; 12:R159-65.

50. van Puijenbroek M, Nielsen M, Tops CM, Halfwerk H, Vasen HF, Weiss MM, et al. Identification of patients with (atypical) *MUTYH*-associated polyposis by *KRAS2* c.34G > T prescreening followed by *MUTYH* hotspot analysis in formalin-fixed paraffin-embedded tissue. Clin Cancer Res 2008 J; 14:139-42.

51. Lipton L, Halford SE, Johnson V, Novelli MR, Jones A, Cummings C, et al. Carcinogenesis in *MYH*-associated polyposis follows a distinct genetic pathway. Cancer Res 2003; 63:7595-99.

52. NCCN clinical practice guidelines in oncology. Colorectal cancer screening; V1.2012. 2012. Available at: http://www.nccn.org. Accessed April 3, 2012.

53. Groves CJ, Saunders BP, Spigelman AD, Phillips RK. Duodenal cancer in patients with familial adenomatous polyposis (FAP): results of a 10 year prospective study. Gut 2002; 50:636-41.

54. Bülow S, Björk J, Christensen IJ, Fausa O, Järvinen H, Moesgaard F, et al. Duodenal adenomatosis in familial adenomatous polyposis. Gut 2004; 53:381-86.

55. Aretz S, Uhlhaas S, Goergens H, Siberg K, Vogel M, Pagenstecher C, et al. *MUTYH*-associated polyposis: 70 of 71 patients with biallelic mutations present with an attenuated or atypical phenotype. Int J Cancer 2006; 119:807-14.

56. Olschwang S, Blanché H, de Moncuit C, Thomas G. Similar colorectal cancer risk in patients with monoallelic and biallelic mutations in the *MYH* gene identified in a population with adenomatous polyposis. Genet Test 2007; 11:315-20.

57. Pourhoseingholi MA, Zali MR. Colorectal cancer screening: Time for action in Iran. World J Gastrointest Oncol 2012; 4:82-3.

58. Montazer Haghighi M, Radpour R, Aghajani K, Zali N, Molaei M, Zali MR. Four novel germline mutations in the *MLH1* and *PMS2* mismatch repair genes in patients with hereditary nonpolyposis colorectal cancer. Int J Colorectal Dis 2009; 24:885-93.

59. Shahmoradi S, Bidmeshkipour A, Salamian A, Emami MH, Kazemi Z, Salehi M. Two novel mutations in *hMLH1* gene in Iranian hereditary non-polyposis colorectal cancer patients. Fam Cancer 2012; 11:13-17.

60. Khodadoostan M, Fatemi R, Maserat E, Hooshang A, Alizade M, Molaie M, et al. Clinical and pathological characteristics of colorectal polyps in Iranian population. East Afr J Public Health 2010; 7:157-59.

61. Riegert-Johnson DL, Johnson RA, Rabe KG, Wang L, Thomas B, Baudhuin LM, et al. The value of *MUTYH* testing in patients with early onset microsatellite stable colorectal cancer referred for hereditary nonpolyposis colon cancer syndrome testing. *Genet Test* 2007; 11:361-65.

62. Balaguer F, Castellví-Bel S, Castells A, Andreu M, Muñoz J, Gisbert JP, et al. Identification of *MYH* mutation carriers in colorectal cancer: a multicenter, case-control, population-based study. Clin Gastroenterol Hepatol 2007; 5:379-87.

63. Armstrong JG, Davies DR, Guy SP, Frayling IM, Evans DG. *APC* mutations in familial adenomatous

polyposis families in the Northwest of England. Hum Mutat 1997; 10:376-80.

64. Spirio L, Olschwang S, Groden J, Robertson M, Samowitz W, Joslyn G, et al. Alleles of the *APC* gene: an attenuated form of familial polyposis. Cell 1993; 75:951-57.

65. Friedl W, Caspari R, Sengteller M, Uhlhaas S, Lamberti C, Jungck M, et al. Can *APC* mutation analysis contribute to therapeutic decisions in familial adenomatous polyposis? Experience from 680 FAP families. Gut 2001; 48:515-21.

66. Jones N, Vogt S, Nielsen M, Christian D, Wark PA, Eccles D, et al. Increased colorectal cancer incidence in obligate carriers of heterozygous mutations in *MUTYH*. Gastroenterology 2009; 137:489-94, 494.e167.

67.Win AK, Hopper JL, Jenkins MA. Association between monoallelic *MUTYH* mutation and colorectal cancer risk: a meta-regression analysis. Fam Cancer 2011; 10:1-9.

68. Theodoratou E, Campbell H, Tenesa A, Houlston R, Webb E, Lubbe Set al. A large-scale meta-analysis to refine colorectal cancer risk estimates associated with *MUTYH* variants. Br J Cancer 2010; 103: 1875-84.

69. Cleary SP, Cotterchio M, Jenkins MA, Kim H, Bristow R, Green R, et al. Germline *MutY* human homologue mutations and colorectal cancer: a multisite case-control study. Gastroenterology 2009; 136: 1251-60.

70. Win AK, Cleary SP, Dowty JG, Baron JA, Young JP, Buchanan DD, et al. Cancer risks for monoallelic *MUTYH* mutation carriers with a family history of colorectal cancer. Int J Cancer 2011; 129:2256-62.

71. Vogt S, Jones N, Christian D, Engel C, Nielsen M, Kaufmann A, et al. Expanded extracolonic tumor spectrum in *MUTYH*-associated polyposis. Gastroenterology 2009; 137:1976-85.e1-10.

72. Nielsen M, Franken PF, Reinards TH, Weiss MM, Wagner A, van der Klift H, et al. Multiplicity in polyp count and extracolonic manifestations in 40 Dutch patients with MYH associated polyposis coli (MAP). J Med Genet 2005; 42:e54.

73. Poulsen ML, Bisgaard ML. *MUTYH* Associated Polyposis (MAP). Curr Genomics. 2008; 9:420-35.

74. Beiner ME, Zhang WW, Zhang S, Gallinger S, Sun P, Narod SA. Mutations of the *MYH* gene do not substantially contribute to the risk of breast cancer. Breast Cancer Res Treat 2009; 114:575-78.

75. Out AA, Wasielewski M, Huijts PE, van Minderhout IJ, Houwing-Duistermaat JJ, Tops CM, et al. *MUTYH* gene variants and breast cancer in a Dutch case–control study. Breast Cancer Res Treat 2012; 134:219-27.

76. Wasielewski M, Out AA, Vermeulen J, Nielsen M, van den Ouweland A, Tops CM, et al. Increased *MUTYH* mutation frequency among Dutch families with breast cancer and colorectal cancer. Breast Cancer Res Treat 2010; 124:635-41.

77. Barnetson RA, Devlin L, Miller J, Farrington SM, Slater S, Drake AC, et al. Germline mutation prevalence in the base excision repair gene, *MYH*, in patients with endometrial cancer. Clin Genet 2007; 72:551-55.

78. Ashton KA, Proietto A, Otton G, Symonds I, Scott RJ. Genetic variants in *MUTYH* are not associated with endometrial cancer risk. Hered Cancer Clin Pract 2009; 7:3.

79. Zhang Y, Liu X, Fan Y, Ding J, Xu A, Zhou X, et al. Germline mutations and polymorphic variants in MMR, E-cadherin and *MYH* genes associated with familial gastric cancer in Jiangsu of China. Int J Cancer 2006; 119:2592-96.

80. Zhu M, Chen X, Zhang H, Xiao N, Zhu C, He Q, et al. AluYb8 insertion in the *MUTYH* gene and risk of early-onset breast and gastric cancers in the Chinese population. Asian Pac J Cancer Prev 2011; 12:1451-55.

81. Figueroa JD, Malats N, Real FX, Silverman D, Kogevinas M, Chanock S, et al. Genetic variation in the base excision repair pathway and bladder cancer risk. Hum Genet 2007; 121:233-42.

82. Miyaishi A, Osawa K, Osawa Y, Inoue N, Yoshida K, Kasahara M, et al. *MUTYH* Gln324His gene polymorphism and genetic susceptibility for lung cancer in a Japanese population. J Exp Clin Cancer Res 2009; 28:10.

83. Chen H, Sun C, Guo W, Meng R, Du H, Qi Q, et al. AluYb8 insertion in the *MUTYH* gene is related to increased 8-OHdG in genomic DNA and could be a risk factor for type 2 diabetes in a Chinese population. Mol Cell Endocrinol 2011; 332:301-305.

84. Halford SE, Rowan AJ, Lipton L, Sieber OM, Pack K, Thomas HJ, et al. Germline mutations but not somatic changes at the *MYH* locus contribute to the pathogenesis of unselected colorectal cancers. Am J Pathol 2003; 162(5):1545-8.

85. Vasovcak P, Pavlikova K, Sedlacek Z, Skapa P, Kouda M, Hoch J, et al. Molecular genetic analysis of 103 sporadic colorectal tumours in Czech patients. PLoS One 2011; 6:e24114.

86. Bougatef K, Marrakchi R, Kourda N, Ben Lahely YB, Jileni SB, et al. Somatic mutation of *MUTYH* in

Tunisian patients with sporadic colorectal cancer. J Clin Lab Anal 2007; 21: 372-74.

87. Guidoboni M, Gafà R, Viel A, Doglioni C, Russo A, Santini A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. Am J Pathol 2001; 159:297-304.

88. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer 2001

15; 91:2417-22.

89. Cheadle JP, Sampson JR. *MUTYH*-associated polyposis--from defect in base excision repair to clinical genetic testing. DNA Repair 2007 1; 6:274-79.

90. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002; 3:991-98.

91. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005; 23:609-18.

92. Dierssen JW, de Miranda NF, Ferrone S, van Puijenbroek M, Cornelisse CJ, Fleuren GJ, et al. HNPCC versus sporadic microsatellite-unstable colon cancers follow different routes toward loss of HLA class I expression. BMC Cancer 2007; 7:33.

93. Kloor M, Becker C, Benner A, Woerner SM, Gebert J, Ferrone S, et al. Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. Cancer Res 2005; 65:6418-24.

94. de Miranda NF, Hes FJ, van Wezel T, Morreau H. Role of the microenvironment in the tumourigenesis of microsatellite unstable and *MUTYH*-associated polyposis colorectal cancers. Mutagenesis 2012; 27:247-53.

95. Algarra I, García-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother 2004; 53:904-10.

96. Klein J, Sato A. The HLA system: First of two parts. N Engl J Med 2000; 343:702-709.