

# Same *MSH2* Gene Mutation But Variable Phenotypes in 2 Families With Lynch Syndrome: Two Case Reports and Review of Genotype-Phenotype Correlation

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**ABSTRACT:** Lynch syndrome is an autosomal dominant syndrome that can be subdivided into Lynch syndrome I, or site-specific colonic cancer, and Lynch syndrome II, or extracolonic cancers, particularly carcinomas of the stomach, endometrium, biliary and pancreatic systems, and urinary tract. Lynch syndrome is associated with point mutations and large rearrangements in DNA Mismatch Repair (*MMR*) genes. This syndrome shows a variable phenotypic expression in people who carry pathogenetic mutations. So far, a correlation in genotype-phenotype has not been definitely established. In this study, we describe 2 Lynch syndrome cases presenting with the same genotype but different phenotypes and discuss possible reasons for this.

**KEYWORDS:** Lynch syndrome, correlation genotype-phenotype, *MMR* genes, *MSH2* gene

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## Introduction

Hereditary colon rectal cancer (CRC) syndromes account for up to 5% to 10% of total cases of CRC. Hereditary CRCs are divided into polyposis syndromes, such as familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), and *PTEN* hamartoma tumor syndrome,<sup>1–3</sup> and nonpolyposis syndromes, such as Lynch syndrome (LS).<sup>4</sup> Lynch syndrome is an autosomal dominant syndrome that can, in turn, be subdivided into LS I, or site-specific colonic cancer, and LS II, or extracolonic cancer, particularly carcinoma of the stomach, endometrium, biliary and pancreatic systems, and the urinary tract.<sup>5</sup> Lynch syndrome is associated with point mutations and large rearrangements in DNA Mismatch Repair (*MMR*) genes, such as *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MLH3*, and *MSH3*.<sup>6,7</sup> The loss of function of the *MMR* complex translates at a somatic level (in tumor tissue) into genetic instability known as microsatellite instability (MSI).<sup>8</sup> Microsatellite instability analysis was performed on DNA extracted from tumor tissue embedded in paraffin to allow the classification of the tumor tissue with a status of either high MSI (MSI-H) or low MSI (MSI-L).<sup>9</sup> Moreover, *MMR* immunodeficiency at the somatic level showed the loss of expression of one or more *MMR* proteins as detected by immunohistochemistry (IHC).<sup>10</sup> The identification of an inherited predisposition is important because it enables targeted clinical surveillance that significantly reduces cancer morbidity and mortality in LS families.<sup>11</sup> Mutation detection analysis allows us to identify the mutation responsible for an LS phenotype. However, the type of mutation and the *MMR* gene involved do not provide information about the age of onset the tumor or the type of cancer that will develop. Understanding the molecular mechanisms underlying

genotype-phenotype correlation in LS may provide very useful information for genetic counseling.

In this study, we report the description of 2 LS cases presenting with the same genotype but a different phenotype and discuss possible reasons for this.

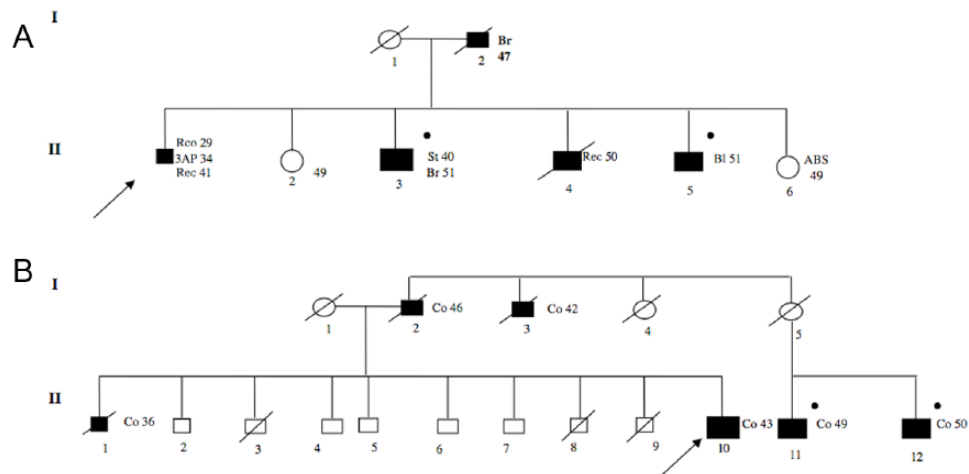
## Case Report

Samples from all subjects were collected after being granted authorization from the local ethics committee “Comitato Etico per le Attività Biomediche Carlo Romano” of the University of Naples “Federico II” (protocol no. 120/10). Once authorization was obtained, the study received ethics approval, and participants’ informed and written consent was obtained.

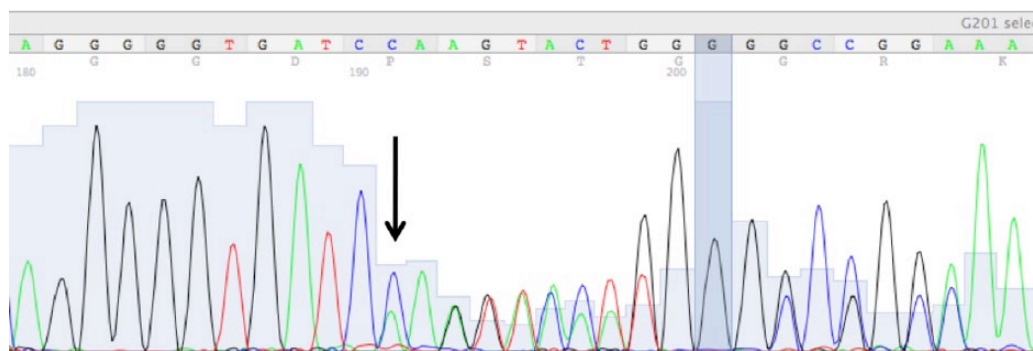
A 48-year-old man, who had previously undergone a total colectomy for a colon cancer, was referred for advice on a specific intervention on the basis of a molecular diagnosis of LS. Our reference number for this patient was 0212. His past history included right colon cancer (at age 29 years), 3 adenomatous polyps in the remaining part of the colon (at age 34 years), and a rectal adenocarcinoma (at age 41 years). The latter tumor, an adenocarcinoma (pT2 G1 N0 diameter of 2 cm) in ulcerative lesion, showed an absence of *MSH2* and *MSH6* protein expressions after IHC and MSI-H of tumor DNA; subsequently, the patient underwent a prophylactic total proctocolectomy (at age 48 years). The patient’s family history included brain, stomach, and bladder cancers in several first-degree relatives (Figure 1A); therefore, this family showed a tumor spectrum of LS II form.<sup>5</sup>

A second patient, a 43-year-old man with a right colon cancer, was also referred for advice on the basis of a molecular





**Figure 1.** The family pedigrees of index case (A) 0212 and (B) 0421. Arrow represents index case; black symbol represents colorectal cancer or tumors associated with LS. AP indicates adenomatous polyp; BI, bladder cancer; Br, brain cancer; RCo, right colon cancer; Rec, rectal cancer; St, stomach cancer. Numbers next to each diagnosis denote age at onset; • represents carrier mutation.



**Figure 2.** Sequence analysis of exon 1 of the *MSH2* gene revealed the mutation, namely, c.192dupC, in both index cases, 0212 and 0421. The mutated base is indicated by the arrow.

diagnosis of LS. Our reference number for this patient was 0421. The adenocarcinoma at right colon cancer (pT3 G3 N2 of 8 cm × 5 cm) in ulcerated sleeve lesion showed the absence of expression of MSH2 and MSH6 proteins after IHC and MSI-H of tumor DNA. The patient underwent a right hemicolectomy of 36 cm. The patient's family history included only colon cancer in several first-degree relatives (Figure 1B); therefore, this family showed a tumor spectrum typical of LS I form.<sup>5</sup>

The phenotypes of both patients and their families were consistent with a deficiency of DNA mismatch repair and with LS. We performed mutation detection analysis for *MMR* genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *MLH3*, and *MSH3*) using denaturing high-performance liquid chromatography (dHPLC) and direct sequencing techniques for both our index cases, according to the procedures described by us previously.<sup>6,7,9,12-14</sup> These patients belonged to 2 families who were apparently unrelated.

For both patients (0212 and 0421), DNA analysis found a variation in an exon 1 sequence that was a duplication of one nucleotide (c.192dupC) in *MSH2*, as detected by dHPLC and

DNA sequencing analysis (Figure 2). This mutation at the protein level determined a premature stop codon, 17 codons downstream of the mutated base, namely, p.Lys65Glnfs\*17.

Mutations were not identified in any other *MMR* genes (*MLH1*, *MSH6*, *PMS2*, *MLH3*, and *MSH3*) analyzed in this study in these 2 index cases.

## Discussion

Their recognition through molecular screening allows us to differentiate several hereditary CRC syndromes. Clearly, once the mutated gene is identified, it is possible to identify candidates for molecular genetic testing find carriers of a specific mutation.<sup>11</sup> This is essential for putting in place a lifetime screening and management program for the carrier patient. In particular, because LS lacks well-defined premonitory symptoms and markers, the molecular diagnosis of this disease plays a very important role. Being able to undertake a genetic test to identify a genetic predisposition to the development of colorectal cancer and related LS cancers in asymptomatic people belonging to at-risk families allows us to reduce the high morbidity and mortality typical of this condition. Indeed, LS

guidelines outline a specific surveillance protocol to follow for *MMR* gene mutation carriers.<sup>15</sup>

Lynch syndrome is the most common form of hereditary CRC, with a frequency of about 2% to 7% of total CRC cases.<sup>4</sup> Predisposed individuals not only have a 50% to 80% risk of developing colon cancer but also have a 20% to 60% increased risk of endometrial cancer, 4% to 30% increased risk of prostate cancer, and a 15% increased risk of developing other cancers, in total.<sup>16</sup>

Identifying the molecular diagnostic pathway, currently performed in our laboratory, allows the analysis of point mutations by dHPLC and sequencing in *MMR* genes, followed by the detection of large rearrangements in these same genes by multiplex ligation-dependent probe amplification.<sup>6,7,9,12–14</sup> Thus, detection mutation analysis of *MMR* genes is important to confirm the clinical diagnosis of LS and to perform a risk prevention program for at-risk people; however, it is unable to predict the disease phenotype. Indeed, unlike other CRC hereditary syndromes, such as FAP where mutations predict the phenotypic features of the disease, the type of mutation indicates little regarding the phenotype of the syndrome in LS.

In this study, we described 2 interesting cases of LS. The first case concerned a man with a history of multiple tumors and who belonged to a family with LS type II, where cancer cases described were located in the brain, stomach, and bladder beyond the colon. This index case (0212) had developed a right colon cancer at age 29 years, 3 adenomatous polyps at age 34 years, and a rectal adenocarcinoma at age 41 years. The latest tumor showed a loss of expression of *MSH2* and *MSH6* by IHC that usually indicates a germline *MSH2* mutation<sup>17</sup>; moreover, DNA extracted from cancer tissue embedded in paraffin showed an MSI-H status. Molecular analysis confirmed the presence of a mutation in the *MSH2* gene (c.192dupC), already reported in Insight databases<sup>18</sup> as a pathogenetic variant (Class 5). This mutation was also identified in other affected patients in family 1, as shown in Figure 1A.

This same mutation was also identified in our second case (0421). This patient had also developed a right colon cancer that showed a loss of expression of *MSH2* and *MSH6* by IHC and an MSI-H status; however, the onset age was a more advanced 43 years against 29 years for the first case. Moreover, the index case, 0421, belonged to a family with LS type I, where affected members showed only colon cancers (Figure 1B), which, on average, showed an age of onset at a more advanced age than that of the family of the index case, 0212. Therefore, the 2 cases reported in this study presented a phenotypic heterogeneity; nevertheless, they had the same genotype. Such variability has been shown both in the increased risk of extracolonic neoplasms, as in the family of index case 0212, and in the different ages of tumor onset between the 2 families. With the introduction of genome-wide association studies, it has become possible to evaluate the role of common low-penetrance genetic modifiers and how they can affect disease expression that occurs

both within and between families or individuals with similar *MMR* gene profiles.<sup>19</sup>

Previous studies have shown the impact of low-penetrance genetic modifiers in CRC; however, genotype-phenotype correlation was not identified for LS. Recently, a systematic literature review and meta-analysis were conducted to evaluate the role and effects of common low-penetrance genetic polymorphisms in modifier genes for a better understanding of their association with CRC development risk in patients with LS. In 10 polymorphisms analyzed, no statistically significant association was identified in the development of CRC in patients with LS.<sup>20</sup> Therefore, further studies in these areas are needed, perhaps with more polymorphisms analyzed to better define the genotype-phenotype correlation in LS. Many other factors may be influencing the high variability of the disease, such as environmental factors, copy number variants, and epigenetic alterations, as well as the gut microbiota. In future, it will be interesting to perform, using next-generation sequencing techniques, the complete exome sequencing of the DNA of these 2 patients to compare the 2 genetic profiles and to determine the likely genetic variants responsible for this extreme phenotypic variability typical of LS. It is known that the molecular characterization of cancer-associated mutations can provide valuable information on disease prognosis and patient response to therapy.<sup>21</sup>

In our opinion, a better understanding of the genotype-phenotype correlation in LS may lead to more effective counseling of patients with specific genetic profiles in terms of their disease prognosis and course.

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### Author Contributions

FD conceived and designed the experiments, analyzed the data, wrote the first draft of the manuscript and developed the structure and arguments for the paper. FD, RL, and MDR agreed with manuscript results and conclusions, made critical revisions, and approved the final version. All authors reviewed and approved the final manuscript.

### REFERENCES

1. Dodaro C, Grifasi C, Florio J, et al. The role of mutation analysis of the APC gene in the management of FAP patients: a controversial issue. *Ann Ital Chir*. 2016;87:321–325.
2. De Rosa M, Galatola M, Borriello S, Duraturo F, Masone S, Izzo P. Implication of adenomatous polyposis coli and MUTYH mutations in familial colorectal polyposis. *Dis Colon Rectum*. 2009;52:268–274.
3. Paparo L, Rossi GB, Delrio P, et al. Differential expression of PTEN gene correlates with phenotypic heterogeneity in three cases of patients showing clinical manifestations of PTEN hamartoma tumour syndrome. *Heredit Cancer Clin Pract*. 2013;11:8.
4. Liccardo R, De Rosa M, Izzo P, Duraturo F. Novel implications in molecular diagnosis of Lynch syndrome. *Gastroenterol Res Pract*. 2017;2017:2595098.
5. Mecklin JP, Järvinen HJ. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). *Cancer*. 1991;68:1109–1112.

6. Duraturo F, Liccardo R, Cavallo A, De Rosa M, Rossi GB, Izzo P. Multivariate analysis as a method for evaluating the pathogenicity of novel genetic MLH1 variants in patients with colorectal cancer and microsatellite instability. *Int J Mol Med*. 2015;36:511–517.
7. Duraturo F, Cavallo A, Liccardo R, et al. Contribution of large genomic rearrangements in Italian Lynch syndrome patients: characterization of a novel Alu-mediated deletion. *Biomed Res Int*. 2013;2013:219897.
8. Gologan A, Sepulveda AR. Microsatellite instability and DNA mismatch repair deficiency testing in hereditary and sporadic gastrointestinal cancers. *Clin Lab Med*. 2005;25:179–196.
9. Duraturo F, Liccardo R, Cavallo A, De Rosa M, Grosso M, Izzo P. Association of low-risk MSH3 and MSH2 variant alleles with Lynch syndrome: probability of synergistic effects. *Int J Cancer*. 2011;129:1643–1650.
10. Shia J, Klimstra DS, Nafa K, et al. Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms. *Am J Surg Pathol*. 2005;29:96–104.
11. Cohen SA, Leininger A. The genetic basis of Lynch syndrome and its implications for clinical practice and risk management. *Appl Clin Genet*. 2014;7:147–158. doi:10.2147/TACG.S51483.
12. Liccardo R, De Rosa M, Rossi GB, Carlomagno N, Izzo P, Duraturo F. Incomplete segregation of MSH6 frameshift variants with phenotype of Lynch syndrome. *Int J Mol Sci*. 2017;18:999. doi:10.3390/ijms18050999.
13. Duraturo F, Liccardo R, Izzo P. Coexistence of MLH3 germline variants in colon cancer patients belonging to families with Lynch syndrome-associated brain tumors. *J Neurooncol*. 2016;129:577–578. doi:10.1007/s11060-016-2203-0.
14. Liccardo R, De Rosa M, Rossi GB, Rigler G, Izzo P, Duraturo F. Characterization of novel, large duplications in the MSH2 gene of three unrelated Lynch syndrome patients. *Cancer Genet*. 2017;221:19–24. doi:10.1016/j.cancergen.2017.11.008.
15. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. *Am J Gastroenterol*. 2014;109:1159–1179. doi:10.1038/ajg.2014.186.
16. Lang M, Gasche C. Chemoprevention of colorectal cancer. *Dig Dis*. 2015;33:58–67.
17. van Lier MG, Wagner A, van Leerdam ME, et al. A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory. *J Cell Mol Med*. 2010;14:181–197. doi:10.1111/j.1582-4934.2009.00977.x.
18. <https://www.insight-group.org/variants/>.
19. De Rosa M, Pace U, Rega D, et al. Genetics, diagnosis and management of colorectal cancer. *Oncology Rep*. 34:1087–1096.
20. Donald N, Malik S, McGuire JL, Monahan KJ. The association of low penetrance genetic risk modifiers with colorectal cancer in Lynch syndrome patients: a systematic review and meta-analysis [published online ahead of print May 15, 2017]. *Fam Cancer*. doi:10.1007/s10689-017-9995-8.
21. De Rosa M, Rega D, Costabile V, et al. The biological complexity of colorectal cancer: insights into biomarkers for early detection and personalized care. *Therap Adv Gastroenterol*. 2016;9:861–886.