Research Article

Novel Mutations in MLH1 and MSH2 Genes in Mexican Patients with Lynch Syndrome

Jose Miguel Moreno-Ortiz, 1,2 María de la Luz Ayala-Madrigal, 1 Jorge Román Corona-Rivera, 1,3 Manuel Centeno-Flores, 3 Víctor Maciel-Gutiérrez, 3 Ramón Antonio Franco-Topete, 3 Juan Armendáriz-Borunda, 4 Erin Hotchkiss, 5 Lucia Pérez-Carbonell, 5 Jennifer Rhees, 5 Clement Richard Boland, 5 and Melva Gutiérrez-Angulo 1,6

1 Instituto de Genética Humana, CUCS, Universidad de Guadalajara, Sierra Mojada 950, Colonia Independencia, 44340 Guadalajara, JAL, Mexico
2 Trayectoria de Genómica Alimentaria, Universidad de la Ciéncia del Estado de Michoacán de Ocampo, Avenida Universidad 3000, Colonia Lomas de la Universidad, 59103 Sahuayo, MICH, Mexico
3 Hospital Civil de Guadalajara “Dr. Juan I. Menchaca”, Salvador de Quevedo y Zubieta 750, 44340 Guadalajara, JAL, Mexico
4 Departamento de Biología Molecular y Genómica, CUCS, Universidad de Guadalajara, Sierra Mojada 950, Colonia Independencia, 44340 Guadalajara, JAL, Mexico
5 GI Cancer Research Laboratory, Baylor University Medical Center, 3500 Gaston Avenue, Suite 250鸿利兹, Dallas, TX 75204, USA
6 Departamento de Clínicas, CUAltos, Universidad de Guadalajara, Carretera a Yahualica Km. 7.5, 47600 Tepatitlán de Morelos, JAL, Mexico

Correspondence should be addressed to Melva Gutiérrez-Angulo; melva73@hotmail.com

Received 8 February 2016; Accepted 14 April 2016

Academic Editor: Hubert E. Blum

Copyright © 2016 Jose Miguel Moreno-Ortiz et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Lynch Syndrome (LS) is characterized by germline mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2. This syndrome is inherited in an autosomal dominant pattern and is characterized by early onset colorectal cancer (CRC) and extracolonic tumors. The aim of this study was to identify mutations in MMR genes in three Mexican patients with LS. Methods. Immunohistochemical analysis was performed as a prescreening method to identify absent protein expression. PCR, Denaturing High Performance Liquid Chromatography (dHPLC), and Sanger sequencing complemented the analysis. Results. Two samples showed the absence of nuclear staining for MLH1 and one sample showed loss of nuclear staining for MSH2. The mutations found in MLH1 gene were c.2103+1G>C in intron 18 and compound heterozygous mutants c.1852_1854delAAG (p.K618del) and c.1852_1853delinsGC (p.K618A) in exon 16. In the MSH2 gene, we identified mutation c.638dupT (p.L213fs) in exon 3. Conclusions. This is the first report of mutations in MMR genes in Mexican patients with LS and these appear to be novel.

1. Introduction

Lynch Syndrome (LS), previously called hereditary non-polyposis colorectal cancer (HNPPC), was described in the early twentieth century by A. S. Warthin and was further characterized in the second half of the twentieth century by Lynch [1]. This syndrome is inherited as an autosomal dominant pattern and is characterized by early onset CRC and other specific extracolonic tumors [2]. Most patients with LS have heterozygous mutations in MLH1, MSH2, MSH6, or PMS2, the principal genes in the DNA mismatch repair (MMR) system [3, 4]. The proteins encoded by MMR
genes are involved in the identification and repair of errors occurring during S phase, and repetitive sequences called microsatellites are particularly vulnerable to mutation in the absence of DNA MMR activity [4–6]. The diagnosis of LS is suspected clinically using the Revised Bethesda Guidelines, and a definitive diagnosis requires the identification of mutations in one of the LS associated genes [7]. A large number of unique mutations are found in LS families, and founder mutations are commonly found in relatively isolated populations [8]. The goal of this study was to identify mutations in MMR genes in Mexican patients with LS.

2. Material and Methods

2.1. Patients. The study utilized peripheral blood and tumor samples obtained from three unrelated patients diagnosed clinically with LS at the “Dr. Juan I. Menchaca” Civil Hospital of Guadalajara, Jalisco, Mexico (LS-23, LS-41, and LS-52). The genealogies including age and cancers type associated are shown in Figure 1. Two additional family members affected with CRC were analyzed in association with the proband LS-23 (II-2 and II-5). All the patients provided informed consent. The study was performed in accordance with the Helsinki Declaration and considered the ethical aspects of research involving human subjects according to the Mexican General Health Law. This research was approved by the ethics committee of Centro Universitario de los Altos, Universidad de Guadalajara (CUA/CINV/494/2009).

2.2. Immunohistochemistry. Protein expression of MLH1, MSH2, MSH6, and PMS2 was analyzed by immunohistochemistry (IHC) staining with the DAKO EnVision System-HRP polymer system kit (DakoCytomation Inc., Carpinteria, CA). The following antibodies were used: for MLH1, clone 13271A, BD Pharmingen, San Diego, CA; for MSH2, clone FEH, Oncogene Research Products, Boston, MA; for MSH6, clone 44, BD Transduction Laboratories, Lexington, KY; and, for PMS2, clone A37, BD Pharmingen. One block of formalin-fixed paraffin-embedded tumor tissue was selected per case. IHC was performed as previously reported [9]. Briefly, 5μm thick tissues were cut, dewaxed in xylene, and rehydrated in graded alcohol concentrations to buffer. The slides were incubated for one hour with the appropriate dilutions of mouse monoclonal antibodies. The peroxidase reaction was developed using diaminobenzidine tetrachloride as the chromogen. Normal expression of protein was indicated by the presence of nuclear staining in colon cells, and loss of staining in tumor cells was determined only if nonneoplastic colonocytes and stromal cells were positively stained.

2.3. DNA Extraction. Genomic DNA was extracted from peripheral blood samples of patients as previously reported [10]. The DNA concentration was measured by spectrophotometry and the quality was evaluated in 1% agarose gel electrophoresis.

2.4. dHPLC and DNA Sequencing. Specific primers were used to amplify all 19 exons of MLH1 gene and 16 exons of MSH2 gene by polymerase chain reaction (PCR) according to conditions established by the laboratory. The fragments were then visualized by gel electrophoresis in a 2% agarose gel, stained with ethidium bromide.

Denaturing High Performance Liquid Chromatography (dHPLC) was undertaken using the Hitachi WAVE® DNA fragment analysis system HSX-3500 (Transgenomic™). An aliquot (5μL) of the PCR product was directly injected into a DNA Sep column. The samples were analyzed under the optimum melting temperature determined in the GI Cancer Research Laboratory at Baylor University Medical Center, Dallas. The chromatograms of each fragment were compared with those of the wild type, and fragments containing heteroduplexes and shorter retention times compared to wild type fragments underwent Sanger sequencing to confirm putative sequence variations.

PCR products were purified using the QuickStep™ 2 PCR Purification Kit (EdgeBio, Gaithersburg, MD) and PERFORMA® DTR Gel Filtration Cartridges Kit (EdgeBio). The successive purified products were subjected to cycle sequencing in the forward and reverse directions using a BigDye Terminator v1.1 Kit (Applied Biosystems, Life Technologies) on the automated PRISM 3100-Avant DNA Sequencer (Applied Biosystems, Life Technologies). The sequences for each fragment were analyzed in the genome browser: http://genome.ucsc.edu/.

2.5. PCR-RFLP. PCR-RFLP was done for a variant localized in 18 exon-intron boundary of MLH1 using standard PCR conditions in samples of family members of proband LS-23 (II-2 and II-5, Figure 1). The primers were as follows: forward 5’-TTT TGA GGT ATT GAA ‘TTT CTG TGG-3’ and reverse 5’-TGA CCT GCT GTC CTA GTC CTG-3’. A PCR fragment of 191bp was used for digestion with the Alul restriction enzyme. This cuts the mutant allele and the fragments generated for heterozygous individuals were 191 bp, 144 bp, and 47 bp.

3. Results

MLH1 and MSH2 protein expression were altered in the tissue samples evaluated. LS-23 and LS-41 showed the absence of nuclear staining for MLH1, and sample LS-52 showed loss of nuclear staining for MSH2.

All the exons of MLH1 and MSH2 genes were successfully amplified and screened by dHPLC. Samples showing heteroduplex peaks were subjected to DNA sequencing to characterize the germline mutations. Patient LS-23 had a substitution at a splice donor site, located in the first nucleotide base of intron 18 of MLH1, c.2103+1G>C. Once we identified the mutation in this patient, two additional members with CRC in this family (II-2 and II-5, Figure 1) were screened by PCR-RFLP as the mutation created a novel restriction site (data not shown). The results showed a heterozygous genotype for the mutation in the analyzed individuals. In patient LS-41, two different sequence variations were found
in exon 16 of \textit{MLH1}. One allele had an in-frame deletion of a codon, c.1852_1854delAAG (p.K618del), and the other displayed a deletion/insertion of two bases, c.1852_1853delinsGC (p.K618A), which resulted in a lysine to alanine mutation at codon 618. The third sequence variation was duplication c.638dupT in codon 213 in exon 3 of the \textit{MSH2} gene, in patient LS-52. This resulted in the frameshift mutation, p.L213fs (Figure 2). These data were presented at American Society of Human Genetics Annual Meeting in 2014 [11].

4. Discussion

The Revised Bethesda Guidelines recommend MSI analysis and/or IHC analysis of tumor tissue to select patients for definitive DNA sequence analysis [12]. Our study identified three putative LS patients from Mexico, initially suspected based upon family history and screened by IHC and confirmed with DNA analyses. In the initial screening by IHC, we identified two patients with absent expression of \textit{MLH1} and one with absent expression of \textit{MSH2}.

Patient LS-23 had a substitution at a splice donor site, located in the first nucleotide base of intron 18 of \textit{MLH1}, c.2103+1G>C. This change is located in a canonical GT/AG splice site. The phyloP and phastCons values were 4.281 and 1, respectively, suggesting strong evolutionary conservation [13]. This mutation is included in the SNP database as rs267607888 whereas the changes G>A and G>T (c.2103+1G>A and c.2103+1G>T) have been reported in patients with LS in the PubMed database [14–20]. Variations in this position are classified as likely pathogenic or
pathogenic by the InSiGHT Variant Interpretation Committee, respectively [21]. Mutation G>C found in this patient (c.2103+1G>C) of MLH1 has apparently been reported only in the InSiGHT database [21]. The mutational effect on the MLH1 protein is unclear since mutations in splice site sequences could promote exon skipping, intron retention, or activation of cryptic splice sites [22].

Patient LS-41 had a c.1852_1853delinsGC (p.K618A) (rs35502531; c.1852_1853delAinsGC) variant in one allele and a c.1852_1854delAAG (p.K618del) (rs63751247) sequence variation in the other allele of MLH1. The K618A variant is classified as nonpathogenic in the InSiGHT database [21]. The Human Gene Mutation Database (http://insight-group.org/) [21], indicated that the duplication of T generates a protein of 230 amino acids instead of the wild type protein of 934 amino acids, due to the premature stop in codon 231. Mangold et al. analyzed 1721 German patients and detected a frameshift mutation in three patients in this specific region; however the alteration in the DNA coding sequence was different, c.638dupT [30]. Although frameshift mutations in codon 213 of exon four of MSH2 gene have been described previously [30, 31], the c.638dupT alteration has not been reported in any LS database, including the International Society for Gastrointestinal Hereditary Tumors Mutation Database (http://insight-group.org/) [21], the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) [32], or the MMR Genes Variant Database (http://www.med.mun.ca/variants) [33]. Therefore, this is likely a novel mutation.

LS is the most common form of inherited CRC and accounts for about 3% of all CRC cases [34]. In Mexico, CRC has a lifetime incidence of 5.8% according to Globocan 2012 [35]. The search for MMR genes mutations in CRC patients is essential for making the diagnosis in LS families [1]. The individuals positive for these mutations will have a surveillance according to international guidelines [36] in order to avoid the cancer development or progression.

5. Conclusions

This study reports the c.638dupT in MSH2 and the compound heterozygous alteration c.1852_1853delinsGC/c.1852_1854delAAG in MLH1 as novel findings in LS. Moreover, these findings highlight the importance of identifying novel genetic alterations populations that have not been previously studied.

Competing Interests

The authors declare that they have no competing interests.
Acknowledgments

The authors thank Nurse Elisa Andrade for her technical assistance at the “Dr. Juan I. Menchaca” Civil Hospital. This work was supported by a COECYTJAL-UDG Grant PS-2009-494. Jose Miguel Moreno-Ortiz received a Ph.D. CONACyT Fellowship.

References

[1] C. R. Boland and H. T. Lynch, “The history of Lynch syndrome,” Familial Cancer, vol. 12, no. 2, pp. 145–157, 2013.

[2] H. F. A. Vasen, “Clinical description of the Lynch syndrome [hereditary nonpolyposis colorectal cancer (HNPPC)],” Familial Cancer, vol. 4, no. 3, pp. 219–225, 2005.

[3] K. Lagerstedt Robinson, T. Liu, J. Vandrovocva et al., “Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics,” Journal of the National Cancer Institute, vol. 99, no. 4, pp. 291–299, 2007.

[4] J. V. Martin-Lopez and R. Fishel, “The mechanism of mismatch repair and the functional analysis of mismatch repair defects in Lynch syndrome,” Familial Cancer, vol. 12, no. 2, pp. 159–168, 2013.

[5] E. Vilar and S. B. Gruber, “Microsatellite instability in colorectal cancer: the stable evidence,” Nature Reviews Clinical Oncology, vol. 7, no. 3, pp. 153–162, 2010.

[6] C. R. Boland and A. Goel, “Microsatellite instability in colorectal cancer,” Gastroenterology, vol. 138, no. 6, pp. 2073–2087, 2010.

[7] R. H. Sijmons and R. M. Hofstra, “Review: clinical aspects of hereditary DNA Mismatch repair gene mutations,” DNA Repair, vol. 38, pp. 155–162, 2016.

[8] H. T. Lynch, A. de la Chapelle, H. Hampel et al., “American founder mutation for Lynch syndrome. Prevalence estimates and implications,” Cancer, vol. 106, no. 2, pp. 448–452, 2006.

[9] A. Goel, T. Nagasaka, J. Spiegel, R. Meyer, W. E. Lichtler, and C. R. Boland, “Low frequency of Lynch syndrome among young patients with non-familial colorectal cancer,” Clinical Gastroenterology and Hepatology, vol. 8, no. 11, pp. 966–971.e1, 2010.

[10] S. Gustinich, G. Manfioletti, G. del Sal, C. Schneider, and P. Carinchi, “A fast method for high-quality genomic DNA extraction from whole blood,” BioTechniques, vol. 11, no. 3, pp. 298–300, 1991.

[11] J. M. Moreno-Ortiz, M. L. Ayala-Madrigal, J. R. Corona-Rivera et al., “Germline mutational analysis in Mexican patients with Lynch syndrome,” in Proceedings of the 64th Annual Meeting of the American Society of Human Genetics, San Diego, Calif, USA, October 2014.

[12] F. M. Giardiello, J. I. Allen, J. E. Axilbund et al., “Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the us multi-society task force on colorectal cancer,” Diseases of the Colon and Rectum, vol. 57, no. 8, pp. 1025–1048, 2014.

[13] J. M. Schwarz, C. Rödelsperger, M. Schuelke, and D. Seelow, “MutationTaster evaluates disease-causing potential of sequence alterations,” Nature Methods, vol. 7, no. 8, pp. 575–576, 2010.

[14] J. Wijnen, P. Meera Khan, H. Vasen et al., “Majority of hMLH1 mutations responsible for hereditary nonpolyposis colorectal cancer cluster at the exonic region 15-16,” American Journal of Human Genetics, vol. 58, no. 2, pp. 300–307, 1996.

[15] M. J. W. Berends, H. Hollema, Y. Wu et al., “MLH1 and MSH2 protein expression as a pre-screening marker in hereditary and non-hereditary endometrial hyperplasia and cancer,” International Journal of Cancer, vol. 92, no. 3, pp. 398–403, 2001.

[16] Y. Hendriks, P. Franken, J. W. Dierssen et al., “Conventional and tissue microaray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors,” The American Journal of Pathology, vol. 162, no. 2, pp. 469–477, 2003.

[17] A. Wagner, A. Barrows, J. T. Wijnen et al., “Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene,” The American Journal of Human Genetics, vol. 72, no. 5, pp. 1088–1100, 2003.

[18] E. Domingo, P. Laiho, M. Ollikainen et al., “BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing,” Journal of Medical Genetics, vol. 41, no. 9, pp. 664–668, 2004.

[19] I. H. Overbeek, C. M. Kets, K. M. Hebeda et al., “Patients with an unexplained microsatellite instable tumour have a low risk of familial cancer,” British Journal of Cancer, vol. 96, no. 10, pp. 1605–1612, 2007.

[20] M. Bujalkova, K. Zavadna, T. Krivulcik et al., “Multiplex SNaPshot genotyping for detecting loss of heterozygosity in the mismatch-repair genes MLH1 and MSH2 in microsatellite-unstable tumors,” Clinical Chemistry, vol. 54, no. 11, pp. 1844–1854, 2008.

[21] International Society for Gastrointestinal Hereditary tumours, 2016, http://insight-group.org/.

[22] R. K. Singh and T. A. Cooper, “Pre-mRNA splicing in disease and therapeutics,” Trends in Molecular Medicine, vol. 18, no. 8, pp. 472–482, 2012.

[23] J. Kosinski, I. Hinrichsen, J. M. Bujnicki, P. Friedhoff, and G. Plotz, “Identification of Lynch syndrome mutations in the MLH1-PMS2 interface that disturb dimerization and mismatch repair,” Human Mutation, vol. 31, no. 8, pp. 975–982, 2010.

[24] B. A. Thompson, D. E. Goldgar, C. Paterson et al., “A multifactorial likelihood model for MMR gene variant classification incorporating probabilities based on sequence bioinformatics and tumor characteristics: a report from the colon cancer family registry,” Human Mutation, vol. 34, no. 1, pp. 200–209, 2013.

[25] A. Abuli, L. Bujanda, J. Muñoz et al., “The MLH1 c.1852A>G (p.Arg265Cys) variant in colorectal cancer: genetic association study in 18,732 individuals,” Public Library of Science One, vol. 9, no. 4, Article ID e95022, 2014.

[26] M. Takahashi, H. Shimodaira, C. Andreutti-Zaugg, R. Iggo, R. D. Kolodner, and C. Ishioka, “Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays,” Cancer Research, vol. 67, no. 10, pp. 4595–4604, 2007.

[27] A. Castillejo, C. Guarinos, A. Martínez-Canto et al., “Evidence for classification of c.1852A>G (p.K618A) variant in colorectal cancer: genetic association study in 18,732 individuals,” BMC Medical Genetics, vol. 6, article 12, 2011.

[28] S. Perera and B. Bapat, “The MLH1 variants p.Arg265Cys and p.Lys618Ala affect protein stability while p.Leu749Gln affects heterodimer formation,” Human mutation, vol. 29, no. 2, p. 332, 2008.

[29] The molecular toolkit program of Colorado State University, April 2015, http://www.vivo.colostate.edu/.

[30] E. Mangold, C. Pagenstecher, W. Friedl et al., “Spectrum and frequencies of mutations in MSH2 and MLH1 identified in
1,721 German families suspected of hereditary nonpolyposis colorectal cancer," *International Journal of Cancer*, vol. 116, no. 5, pp. 692–702, 2005.

[31] N. Rahner, N. Friedrichs, M. Wehner et al., "Nine novel pathogenic germline mutations in MLH1, MSH2, MSH6 and PMS2 in families with Lynch syndrome," *Acta Oncologica*, vol. 46, no. 6, pp. 763–769, 2007.

[32] P. D. Stenson, E. V. Ball, M. Mort et al., "Human Gene Mutation Database (HGMD®): 2003 update," *Human Mutation*, vol. 21, no. 6, pp. 577–581, 2003.

[33] M. O. Woods, P. Williams, A. Careen et al., "A new variant database for mismatch repair genes associated with Lynch syndrome," *Human Mutation*, vol. 28, no. 7, pp. 669–673, 2007.

[34] H. T. Lynch and A. de la Chapelle, "Hereditary colorectal cancer," *The New England Journal of Medicine*, vol. 348, no. 10, pp. 919–932, 2003.

[35] J. Ferlay, H. Shin, F. Bray, D. Forman, C. Mathers, and D. Parkin, GLOBOCAN 2012. v1.0, *Cancer Incidence and Mortality Worldwide*, IARC Cancer Base No. 11. International Agency for Research on Cancer, Lyon, France, 2013, http://globocan.iarc.fr.

[36] S. Syngal, R. E. Brand, J. M. Church, F. M. Giardiello, H. L. Hampel, and R. W. Burt, "ACG Clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes," *The American Journal of Gastroenterology*, vol. 110, no. 2, pp. 223–262, 2015.