Int J Colorectal Dis (2014) 29:1019–1020
DOI 10.1007/s00384-014-1890-2

LETTER TO THE EDITOR

Confirmed pathogenic effect of a splice site variation in the MLH1 gene causing Lynch syndrome

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Accepted: 27 April 2014 / Published online: 9 May 2014
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Dear editor:

DNA mismatch repair (MMR) deficiency is associated with increased risk of developing several types of cancer (mainly colorectal and ovarian). In the case of hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome), the identification of MMR gene mutations is considered as an essential tool for genetic counseling and for follow-up aimed at cancer prevention in affected family members. Nowadays, there are many described germline mutations in the main MMR genes MLH1 (c.655A>A/G, p.Leu555Pro), MSH2 (R406X), and MSH6 (p.Leu435Pro) and also, recently, somatic mutations in MLH1 and MSH2 genes [1]. Most of these mutations are insertions, deletions, or nucleotide substitutions that create a premature stop codon or nucleotide changes at the consensus splice site that affect mRNA splicing. However, there are still many mutations in MMR gene that are not clearly classified as pathogenic or their clinical implications have not yet been described like some missense or silent variants or mutations close to splice sites are. Therefore, those variants are classified as variants of unknown significance (VUS) and represent up to 30 % of the identified DNA changes in MMR genes [1]. We have reported a family case with nine family members affected by Lynch syndrome, all of them with the presence of a not previously described as pathogenic variation 117-1 G>K in MLH1 gene. There are other studies that have attributed to c.117-2A>T DNA change as possible pathogenic; however, there are not many data allowed.

A genetic analysis performed in 19 members of a family with historical medical cases of LS was performed. After the genetic analysis, it was confirmed that all of them have 117-1 G>K variation. After this genetic study, unaffected members of this family were classified as high risk, and they will follow frequent clinical examination for colorectal cancer (CRC).

To confirm as pathogenic and the effect of this splicing variation, the variant c.117–1G>T was evaluated by a functional analysis as it is recommended by the practice guidelines for VUS. These guidelines include functional assays as one of the best means of confirming pathogenicity. We have performed the following analyses: the use of bioinformatics analysis with softwares NNSPLICE and Human Splicing Finder, the amplification of exon 2 and intronic flanking sequences of MLH1 (total length 703 bp), the whole clonation of the splicing vector pSAD®v6.0, the whole sequencing of the inserted region, the introduction by site-directed mutagenesis of DNA variant c.117–1G>T, functional assay in eukaryotic cells of wild-type minigenes and c.117–1G>T by RNA extraction and quantification, RT-PCR in exons for Psad vector and analysis of the fragments in ABI3130 sequencer, and sequencing of RT-PCR products. All these analyses finally confirmed that variant c.117-1G>T in MLH1 gene removes the natural acceptor site by splicing and it appears as an alternative one 5 nucleotides downstream (NNSPLICE score=0.79). The affection of splicing effect of this variation is also confirmed by minigene analysis (MGmlh1_EX2) that in variant c.117-1G>T produced an aberrant transcript with a deletion of 5 nucleotides. The effect in mRNA is known as

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r.117_121del, and the effect in the protein is the production of a truncating protein in codon 39 (p.Cys39X).

Carriers of gene mutations in the MMR genes have a 50–80 % lifetime risk of developing CRC. Identifying gene mutation carriers improves the efficiency of cancer surveillance for affected individuals and helps distinguish family members who require intense management. Sequencing of the MMR genes is not possible for all CRC patients, so there are different approaches for the identification of hereditary nonpolyposis colorectal cancer. The candidates for testing have personal or family cancer histories or CRC at an early age, parameters which are included in clinical criteria as Amsterdam criteria or Bethesda guidelines. They are also used as prediction models which are easy to apply and which are validated recently. Molecular tumor testing, microsatellite instability (MSI), and immunohistochemistry (IHC) identify mutation carriers among those patients with a CRC diagnosis. IHC further identifies loss of expression of any of the MMR proteins associated with Lynch syndrome, facilitating the germline mutation analysis.

To date, there are many described mutations in MMR genes related to increased risk of developing CRC,due to SNPs with a small effect or it is also possible that a combination of several variants in many gene works synergistically to increase the risk of CRC [1]. In MLH1, there are many confirmed variants with a pathogenic effect such as p.Arg182Gly (c.544A>G) [2], p.Leu555Pro, and p.Leu622His [3], and we have recently reported 117-1 G>K variation. In our patients, a total of nine members of the same family, some of them affected by Lynch syndrome, have the presence of 117-1 G>K variation. According to Boland, most MMR gene deletions and nonsense are classified as mutations with pathologic consequences, but missense mutations are not always interpretable. Most of the mutations cause a disruption of regions of protein–protein interaction by a non-conservative amino acid change that could disrupt complex formation and function [4].

Finally, we can conclude that this variant has an effect on the splicing site. Thanks to the discovering of a new variation and a combination of genetic analysis in MMR genes, this family could benefit for genetic counseling and clinical-specific management that could not be discover by other kinds of analyses. In this family, clinical criteria for Lynch syndrome have been identified subjects with cancer and MLH1 mutation and other younger family members with the mutation but without cancer, which should be closely monitored respect to CRC and other cancers associated with LS.

Acknowledgments We thank all the donors and the Service of Gastroenterology of the University "Hospital San Cecilio" (Granada, Spain) for making this study possible. We also want to thank the "Instituto de Biologia y Genetica Molecular, Grupo de Splicing y Cancer, CSIC" for performing the functional analysis of this variation.

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