Genomic rearrangements in *MSH2* and *MLH1* are rare mutational events in Spanish patients with hereditary nonpolyposis colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the most common neoplasms and a leading cause of death related to cancer worldwide. Hereditary nonpolyposis colorectal cancer (HNPCC) is the most frequent autosomal dominant predisposition to the development of CRC, accounting for approximately 2.5% of the total CRC burden in Spain. Genomic rearrangements in the *MSH2* and *MLH1* genes have been reported to account for an important proportion of the mutation spectrum in HNPCC, and DNA dosage techniques have been developed facilitating molecular screening of such deletions/duplications. We screened for *MSH2* and *MLH1* genomic rearrangements by multiplex ligation-dependent probe amplification (MLPA) in 142 Spanish patients at risk for HNPCC prior to the exon-by-exon mutation scanning and found a deletion encompassing exons 9–16 of *MSH2* and a duplication encompassing exons 11–16 of *MSH2*, both only in one case. These results showed that *MSH2*/*MLH1* rearrangements in Spanish patients at risk for HNPCC seem to be a less frequent mutational event than in other populations.

Keywords: Colorectal cancer; HNPPC; *MLH1*; *MSH2*; Genomic rearrangement; MLPA

1. Introduction

Colorectal cancer (CRC) is the second most common neoplasm and the third leading cause of death related to cancer in Spain [1]. The lifetime risk of developing CRC in the general population is...
around 5%, but it is much higher for individuals with familial CRC aggregation. Among them, hereditary nonpolyposis colorectal cancer (HNPCC) is the most frequent autosomal dominant predisposition to the development of CRC [2], and it seems to account for 2.5% of CRC cases in Spain [3]. It is caused by mutations in one of the DNA mismatch repair (MMR) genes, the majority of which occur in the MSH2 and MLH1 genes [4]. Virtually all CRC tumors from MSH2 or MLH1 mutation carriers show microsatellite instability (MSI), which reflects the MMR defect. HNPCC clinical diagnosis is usually based on the Amsterdam criteria [5], although less stringent criteria have been also developed (Amsterdam II criteria, Bethesda guidelines and revised Bethesda guidelines).

Several germline mutations have been detected in HNPCC families including truncating, frameshift, splicing or missense mutations [6]. Recently, genomic rearrangements in the MSH2 and MLH1 genes have been reported to account for an important proportion of the mutation spectrum in these MMR genes in other populations [7–12], making advisable to begin the molecular analysis by screening for them previous to the exon-by-exon mutation scanning. Furthermore, DNA dosage techniques have been developed [13], which facilitate molecular screening of such rearrangements in HNPCC patients.

In the present study, we screened for MSH2 and MLH1 genomic rearrangements by multiplex ligation-dependent probe amplification (MLPA) in 142 Spanish patients at risk for HNPCC prior to the exon-by-exon mutation scanning and found a deletion encompassing exons 9–16 of MSH2 and a duplication encompassing exons 11–16 of MSH2, both only in one case, showing that MSH2/MLH1 rearrangements in Spanish patients at risk for HNPCC seem to be a less frequent mutational event than in other populations.

2. Patients and methods

2.1. Patients

In the setting of the EPICOLON study, a general population-based, multicenter study aimed at determining HNPCC frequency in Spain [3], 1222 incident CRC patients were enrolled over 1-year period. Patients were clinically characterized and molecular studies (MSI detection and MSH2/MLH1 immunostaining) were performed in all of them. Patients whose tumor showed MSI or did not express MSH2/MLH1 (n = 91) were selected to undergo further germline genetic testing in MSH2/MLH1 genes. Additionally, 51 additional HNPCC patients fulfilling the Amsterdam criteria referred to our centers for genetic counseling were also included, completing a total of 142 unrelated CRC cases.

The study was approved by the institutional Ethics Committee of each participating hospital, and written informed consent was obtained from all patients.

2.2. DNA and RNA isolation

Genomic DNA was extracted from normal-appearing colonic mucosa in those patients included in the EPICOLON study [3]. In some of these patients, DNA and RNA were also isolated from peripheral blood in order to confirm suspected rearrangements observed in tissue samples. DNA isolation was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. RNA isolation was performed using the RNeasy Blood Module and the RNeasy-4PCR kits (Ambion, Austin, USA) following the manufacturer’s protocol. On the other hand, in patients referred to our centers for HNPCC genetic counseling, DNA was extracted from peripheral blood.

2.3. Multiplex ligation-dependent probe amplification (MLPA)

Genomic rearrangements in MLH1 and MSH2 genes were evaluated by means of the MLH1/MSH2 exon deletion MLPA test (MRC-Holland, Amsterdam, The Netherlands) and performed according to the supplied protocol. The probe mix contains one probe for each MLH1/MSH2 exon, and 7 control probes specific for DNA sequences outside those genes. Briefly, 50–100 ng of genomic DNA in 5 μl Tris–EDTA were denatured at 95 °C for 5 min, cooled, and incubated with the probe mix for 16 h at 60 °C. Following probe hybridization, ligation proceeded for 15 min at 54 °C. The ligation products were then amplified by PCR using one primer labeled with 6-FAM. PCR product (1 μl) was then mixed with 12 μl
of deionized formamide and 0.5 μl of fluorescent size standard and analyzed in either an ABI 3100 sequencer using Genescan and Genotyper software (Applied Biosystems, Foster City, CA, USA) or in a megaBACE 500 sequencer using Fragment Profiler software (Amersham Biosciences, Uppsala, Sweden). Specific peaks corresponding to each exon were identified according to their migration relative to the size standards. Peak heights of each fragment were compared to those of a control sample and deletions or duplications were suspected when peak height differed by more than 30%. Control DNA samples with known genomic rearrangements in MSH2 or MLH1 were available and included in each batch of experiments.

2.4. RT-PCR

RT-PCR was performed using the Titan One Tube RT-PCR kit (Roche Diagnostics, Manheim, Germany) according to the manufacturer’s protocol and using primers del12F (5'-ATGTTAATGTCACCCCAC-3') and del12R (5'-CCCTGCTCATTAATTTCTTCT-3').
3. Results and discussion

After completing the MLPA assay in 142 samples, we obtained chromatogram patterns showing an abnormal height reduction in one peak or contiguous peaks in 13 cases and an abnormal height increase in contiguous peaks in one additional case. One of the abnormal chromatogram patterns corresponded to a contiguous deletion of exons 9–16, and other to a duplication of exons 11–16, both in the MSH2 gene (Fig. 1). The other abnormal chromatogram pattern present in the remaining 12 samples was consistent with a deletion of exon 12 in the MLH1 gene. This deletion pattern was consistently detected after repeating experiments with the initial DNA samples. MLPA assay also detected all specific deletions in positive control DNA samples with previously known MSH2 or MLH1 genomic rearrangements.

In order to further confirm and characterize the presumptive deletion of exon 12 in MLH1, we proceeded to isolate DNA and RNA from peripheral blood since the initial DNA was isolated from paraffin-embedded tissue in all these patients. Seven out of these 12 patients were available for this purpose. MLPA assays using peripheral blood DNA of those cases with the presumptive deletion revealed a normal pattern showing no deletion (Fig. 2). Likewise, the RT-PCR experiments encompassing exons 11–13 of the MLH1 gene detected a normal band of 640 bp in all cases but failed to detect a shorter band which would be in agreement with a deletion of exon 12 in MLH1 (data not shown). Therefore, the suspected deletion of exon 12 in MLH1 was not confirmed in any of the analyzed samples. Regarding this matter, we should conclude that this presumptive deletion corresponded most likely to artifacts detected in the DNA extracted from paraffin-embedded tissue, probably due to its high degree of degradation. Indeed, one of the disadvantages of MLPA is that reactions are more sensitive to contaminants and, accordingly, results obtained in DNA extracted from paraffin-embedded tissues should be confirmed using a DNA sample from a healthy tissue (MLPA protocol, MRC-Holland; J. Schouten, personal communication).

Concerning the deletion of exons 9–16 of the MSH2 gene observed in one patient, she fulfilled the Amsterdam criteria and the corresponding tumor showed, in agreement, absence of MSH2 protein expression and MSI. Moreover, this genomic rearrangement has also been reported recently in the English population [12]. On the other hand, the duplication of exons 11–16 of the MSH2 gene was present in a patient also fulfilling the Amsterdam criteria, and it corresponds to a novel MSH2 mutation. Duplications in the MMR genes seem to be less frequent mutational event than deletions, existing only one previous reported duplication in the MSH2 gene involving exons 9 and 10 [8].

Fig. 2. Partial MLPA chromatogram patterns in one of the patients with a presumptive deletion of exon 12 in MLH1. (A) DNA sample from paraffin embedded tissue, and (B) DNA sample from peripheral blood. Asterisk indicates the peak height reduction detected in sample (A).
MSH2/MLH1 genomic rearrangements are frequent mutational events in a significant proportion of patients at risk for HNPCC in other populations [7–12]. Regarding our results, its frequency in the Spanish population seems to be rather low (<1.5%). On the other hand, genetic heterogeneity for HNPCC is confirmed worldwide, and it has been demonstrated to be remarkably high in the Spanish population for other genetic diseases, such as cystic fibrosis [14], which could explain this discrepancy of results in the different geographical areas.

In summary, MLPA has proven to be a simple, efficient and robust technique to measure gene dosage in patients at risk for HNPCC prior to the cumbersome exon-by-exon mutation scanning of the MSH2/MLH1 genes. Despite its apparent low frequency in Spanish population, we consider it is still advisable to include screening for such rearrangements in the MSH2/MLH1 genetic testing, specially considering the straightforwardness of the MLPA assay.

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Appendix. Investigators from the Gastrointestinal Oncology Group of the Spanish Gastroenterological Association who participated in the EPICOLON study

All participants listed below were fully involved in the study:
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References


