Selection criteria for search for germ mutations in colorectal cancer hereditary nonpolyposis

Dear Editor,

Members of families affected by hereditary nonpolyposis colorectal cancer (HNPPC), or Lynch Syndrome, may benefit from clinical examination programs. However, for these programs to be effective, they must be provided to those patients that are carriers of germline mutations in one of the mismatch repair (MMR) system genes. Therefore, when programmes that identify syndrome carriers do not preselect subjects to be studied, the low detection rates obtained make these programmes economically unfeasible. The application of the classic “Amsterdam criteria” as a selective pattern before genetic analysis is excessively strict since only 50% of mutation carriers comply with it. In addition, when the family history is not considered, the frequency of mutation detection is very variable (2–63%) and depends on the selection criteria applied, such as age at tumour diagnosis or the presence of microsatellite instability (MSI) in the tumour tissue.

Our objectives were to analyse patients with primary colorectal carcinomas (CRC) diagnosed before 51 years of age, and: (1) the utility of MSI phenotype as an indicator of germline mutations in hMLH1 and hMSH2; (2) the utility of family cancer history and tumour location as parameters to indicate the existence of germline mutations in the aforementioned MMR system genes; and (3) to establish the efficacy of the combination of MSI phenotype expression in tumour tissue and early age at diagnosis to detect such germline mutations.

We selected a total of 44 patients diagnosed with CRC before the age of 51. A tumour tissue sample was obtained from each; DNA was extracted from peripheral blood (germline) and from tumour tissue (tumour line). The presence of germline mutations in all exon sections of hMLH1 and hMSH2 genes was determined by means of the denaturing gradient gel electrophoresis (DGGE) technique. International guidelines for MSI evaluation in colorectal cancer have been used to determine the existence and the degree of instability in repetitive DNA sequences. These guidelines established a panel of 5 microsatellites to define the MSI phenotype. Based on clinical and family history, the patients were classified as Bethesda 1, 2 or 3 (bt1, bt2 or bt3), according to their compliance with Bethesda guidelines: (a) Criterion 1: individuals who met Amsterdam Criteria; (b) Criterion 2: individuals who had 2 synchronous and metachronous tumours associated with CRC; and (c) Criterion 3: individuals who had a first-degree relative with CRC diagnosed before the age of 45, or colorectal adenoma diagnosed before the age of 40.

Regarding MSI, 43% of the cases (n = 19) showed microsatellite instability (MSI) phenotype, and the other 57% (n = 25), had a stable phenotype. All tumours with any alteration in the germline in hMLH1 or hMSH2 genes showed MSI phenotype. In addition, there was an association between the expression of the MSI phenotype and the identification of germline mutations in the hMLH1 and hMSH2 genes (p = 0.004). The frequency of germline mutations in these genes was superior to 13% (6/44), whereas, in the samples that expressed MSI phenotype, the frequency was superior to 31% (6/19). The “sensitivity” of the MSI phenotype to detect germline mutations was 100% (6/6).

With regards to family history, 9 cases (20%) met at least one of the Bethesda guideline requirements. The 4 patients classified as bt1 developed highly unstable tumours, and 3 of them showed germline mutations in hMLH1 or hMSH2 genes. Both bt2 presented germline mutations in hMSH2 genes together with high MSI. Finally, from the 3 patients classified as bt3, one showed high MSI and the other 2 were stable. Altogether, there was a correlation between family history (bt1, bt2 or bt3) and the presence of mutation in hMLH1 or hMSH2 genes (p < 0.001). This correlation became more significant with requirements 1 and 2, since 5 out of 6 cases that met those requirements showed mutations in one of these genes.

Tumour location was similar in both stable and unstable tumours.

In humans, CRC are tumours that most frequently express instability in the microsatellite sequences; this phenotype is present in around 13% of these tumours. The frequency of this instability varies depending on age at diagnosis and is higher in young individuals. When studying the instability of microsatellite sequences in the tumour tissue of patients aged <30 with CRC, Farrington et al. observed an incidence of almost 50%.

The frequency of germline mutations found in hMLH1 or hMSH2 genes in CRC varies depending on the selection criteria of the samples to be analysed. Thus, there is an increase in the mutation detection rate when the expression of MSI phenotype is combined with young ages. Along this line, Farrington et al. showed that when young ages were combined with MSI phenotype, the rate rose to 63%, as opposed to a rate of just 28% when only age was considered. In our study, we observed that when age was considered as the only factor of selection to find germline mutations in hMLH1 or hMSH2 genes, the detection rate dropped to 13%, versus 31% when combined with the presence of MSI phenotype in tumour tissue.

As for the sensitivity of the MSI phenotype as a selection factor, most studies indicate that it is high and in our series it is

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100%. Some authors have questioned its usefulness, as there are descriptions of some germ-line mutation carriers in the sequence of hMLH1 or hMSH2 genes and whose tumour tissue does not express MSI, although the proportion of patients with this characteristic is minimal.

The existence of a family history of cancers associated with HNPCC is considered to be an important indicator of germ-line mutations in hMLH1 or hMSH2 genes. Mutations in one of these genes are present in 50–70% of affected individuals from families that completely meet the Amsterdam Criteria. Overall, there is a clear relationship between family history (bt 1, 2 or 3) and the presence of mutations in hMLH1 or hMSH2 genes (p < 0.001). This correlation is particularly evident with criteria 1 and 2.

The location of the CRC is proximal in 70% of HNPCC cases with MSI phenotype and hMLH1 or hMSH2 gene mutations, although the location of the tumour on the right cannot be considered a specific distinctive characteristic of the syndrome and, therefore, it is not useful as an indicator of germ-line mutations in hMLH1 or hMSH2 genes in individuals affected by CRC.

Finally, it is important to mention that in this study we have used samples of paraffin-embedded tumour tissues. This technique has some disadvantages: the poor quality of the extracted DNA and the impossibility to analyse mRNA or proteins, with the exception, in some cases, of the application of immunohistochemistry techniques. For all these reasons, the present study has not analysed the existence of somatic mutations in the APC gene or in genes of the MMR system. However, this kind of material has allowed us to select with more detail the different areas of tumour tissue and, in some cases, to tackle the sequencing of shorter DNA fragments.

In conclusion, we can affirm that the combination of MSI presence in tumour tissue and young age at CRC diagnosis is a good indicator of germ-line mutations in hMLH1 and hMSH2 genes. According to our results, the selection of tumours with MSI phenotype in individuals diagnosed with CRC at age ≤ 50 years can obtain mutation detection rates in these genes higher than 30%.

References


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