Short Report

Frequency of hereditary non-polyposis colorectal cancer among Uruguayan patients with colorectal cancer


Few studies have investigated the frequency of hereditary non-polyposis colorectal cancer (HNPCC) in patients with colorectal cancer (CRC), and these have shown marked geographic variations. The aim of this study was to estimate the frequency of HNPCC in a cohort of Uruguayan CRC patients. We included all patients operated consecutively for CRC in the Hospital Central de las Fuerzas Armadas (Uruguay) between 1987 and 2003. Cases were classified into three groups: (i) those fulfilling Amsterdam criteria; (ii) those not fulfilling Amsterdam criteria but considered as a population at increased risk of cancer; and (iii) sporadic CRC. Genetic analysis to detect point mutations in \textit{hMLH1}/\textit{hMSH2}/\textit{hMSH6} genes was performed in group 1 patients. Cases not showing mutations were tested by multiplex ligation-dependent probe amplification. Among 461 patients, group 1 represented 2.6%, group 2 represented 5.6%, and sporadic cases 91.8%. \textit{hMLH1}/\textit{hMSH2}/\textit{hMSH6} mutations were found in 25% of cases classified as HNPCC (two in \textit{hMLH1} and one in \textit{hMSH2}). No mutations were detected in \textit{hMSH6} gene. The proportion of CRC patients that fulfilled Amsterdam criteria agrees with other reports. However, the percentage of HNPCC cases with identified mutations (25%) may be lower than that reported from other populations. This may reflect, among other possible causes, a different genetic profile in the Uruguayan population.

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited cancer syndrome characterized by a high risk and early onset of colorectal cancer (CRC) and other related cancers (e.g. endometrium, ovary, stomach, pancreas, small bowel, and uro-epithelial and biliary tract cancers). This syndrome is expressed by genomic fluctuations, which results in microsatellite instability (MSI) in the tumor tissue as a consequence of germline mutations in DNA repair genes (1).

The molecular environment of HNPCC has been recently clarified (2, 3). Defects in seven mismatch repair genes (i.e. \textit{hMLH1}, \textit{hMSH2}, \textit{hPMS1}, \textit{hMSH2}, \textit{hMSH6}, \textit{hMLH3}, and \textit{hMSH3}) have been associated with this syndrome (4–8). Mutations in \textit{hMSH2}/\textit{hMLH1} account for the majority of cases of HNPCC (5–8). These mutations induce a generalized genomic instability, clearly evident in microsatellites (phenotype RER\textsuperscript{+}, replication errors) (9, 10), which is used to define these defective genes. It has been shown in CRC patients that they are associated with a longer survival time than those not presenting this instability (11). Susceptible individuals could clearly benefit from a monitoring program for these genetic markers and by the vigilance and monitoring of target organs.

Uruguay has an epidemiological behavior similar to that observed in countries in the developed world. Cancer is considered as a major health issue, because it is responsible for almost 25% of deaths. Presently, the risk of developing CRC is estimated at 5.8% at 70 years of age (12), and
the same is observed in most Western industrialized countries. The incidence rates of CRC in Uruguay are 33.83/100,000 for men and 26.31/100,000 for women, while the mortality rates are 17.57/100,000 and 13.94/100,000, respectively. As in many other countries, the actual incidence of HNPCC is unknown.

The few epidemiological studies, which have analyzed the frequency of HNPCC in patients who have undergone surgery for CRC, showed a marked geographic variability. The incidence of this condition reported in international studies has varied between 0.3 and 13% (13–22). This variability may reflect on the strategies used for follow-up and treatment. It appears that these strategies must be tailored to the incidence of this condition in each particular region (23–27). For example, Cornaggia (15) states that ‘the data underline the importance of a precise knowledge of actual HNPCC incidence in different populations in order to optimize effectiveness and efficiency of screening programs for the disease’.

Our purpose is to estimate the frequency of HNPCC among non-selected Uruguayan patients having undergone surgery for CRC in a single institution, by using the family history approach, followed by genetic testing in families suspected of having HNPCC.

Patients and methods

Patients

A retrospective analysis was performed on a sample of non-selected patients operated consecutively for CRC in the Central Armed Forces Hospital (CAFH) of Montevideo, Uruguay. Inclusion criteria were as follows: (i) histologically confirmed CRC; (ii) surgery performed in the CAFH between January 1985 and January 2003; and (iii) completion of a registration chart which included personal and family history of cancer, polyps, and inflammatory bowel disease, causes of morbidity and mortality of first- and second-degree relatives, surgical procedures performed for CCR treatment in probed, surgical findings, and pathology reports. All the data were entered in a database using EPI-INF 6.04c (Centers for Disease Control and Prevention, Atlanta, GA). After surgery, patients were followed as recommended (28). Informed consent was obtained from all participants.

Description of the population

Uruguay has a total population of 3,163,763 inhabitants (29). The CAFH is responsible for the care of 5% of the entire country population (170,000 persons). Patients from all social levels, residing in the capital city (Montevideo), in small towns and in rural areas are treated in this hospital.

Classification of patients according to family history

Each patient was included in one of the three groups:

1. Group 1: those who fulfilled Amsterdam criteria I or II (Tables 1 and 2) categorized as HNPCC (30, 31).
2. Group 2: those patients with a family history of cancer for two consecutive generations and/or a total of at least three relatives in the first degree, not taking into account the site of the tumor or age at diagnosis, as these were considered to be a population at increased risk of cancer (PIR). They are defined as a subgroup of the general population of a country or geographic region that – through several generations and because of genetic mechanisms – has an increased incidence of cancer than that calculated for the general population of the respective region (32, 33). This group has an elevated cancer aggregation but does not fulfill Amsterdam criteria I or II. The phenotypic characteristics of the individuals, in connection with CRC, are similar to those of the general population. However, through the investigation of the family history, an increased frequency of different types of cancer is found.
3. Group 3: this includes patients with sporadic cancers who do not fulfill the above-mentioned criteria for groups 1 and 2.

For every patient in groups at increased risk (1, 2), a new family protocol was opened, including the following information: site of the tumor in relative(s), age at the onset of CRC or other HNPCC-related cancer in relative(s), the date of birth and death of the individual(s), pathology

<table>
<thead>
<tr>
<th>Table 1. Amsterdam criteria I (30)</th>
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<tbody>
<tr>
<td>There must be at least three relatives with colorectal cancer.</td>
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<td>The following criteria must all be present:</td>
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<tr>
<td>One affected member directly related to two other affected relatives</td>
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<td>At least two successive generations must be affected</td>
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<td>At least one individual diagnosed with colorectal cancer before the age of 50 years</td>
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<tr>
<td>Familial adenomatous polyposis must be ruled out</td>
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<td>Tumors must have pathologic confirmation</td>
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reports, endoscopic studies, institution where they were admitted, and name of referring physicians. Detailed pedigrees were constructed for each of the patients in groups 1 and 2. Whenever a case of CRC or any other HNPCC-related cancer in family members was reported, this was confirmed by pathology reports. When it was not available, we resorted to the death certificate.

Mutation detection

Samples containing 20 ml of peripheral blood drawn from all patients in group 1 (HNPCC) at the CAFH were sent to P. Peltomäki. These samples were first screened for possible point mutations in the three major HNPCC-associated genes hMLH1, hMSH2, and hMSH6. The first two genes were analyzed by denaturing gradient gel electrophoresis of genomic DNA (mainly) or by the in vitro transcription and translation analysis of RNA, followed by automated sequencing of genomic DNA in cases showing aberrant fragments. The methodology has been described previously (34, 35). hMSH6 was studied by direct exon-specific sequencing using published primers (36). Cases that did not show mutations were tested for possible large deletions and amplifications by multiplex ligation-dependent probe amplification (MLPA) (37), using kits (SALSA P003 and SALSA P008) and protocols provided by the manufacturer (MRC-Holland, Amsterdam, The Netherlands).

Statistical analysis

Non-parametric tests were employed to compare age medians between different groups.

Survival time was measured as the interval between surgery and the day of death or last control. Kaplan–Meier’s method was applied for drawing and analyzing survival curves. The log-rank test was used for univariate comparisons. sss software was used to perform these calculations (version 11.0.1 for Windows, SPSS Inc., Chicago, IL).

Results

This study sample comprised a total of 461 patients fulfilling the inclusion criteria: 251 men and 210 women with a median age at diagnosis of 66 years (range: 18–96). No patients were excluded, and the necessary forms were completed in all cases. In total, 12 cases [2.6%; 95% confidence interval (95% CI): 1.1–4.1%] were classified as HNPCC according to the above-mentioned criteria, 26 cases as PIR (5.6%; 95% CI: 3.5–7.7), and 423 as sporadic (91.8%; 95% CI: 89.3–94.3). Table 3 summarizes the main clinical and pathologic characteristics of the patients included in the study.

Median age at diagnosis was 45 years (range: 26–65) for HNPCC (75% of this group were less than 55 years of age), 70 years for PIR (range: 36–88), and 67 years (range: 18–96) for sporadic cases. There was a statistically significant difference between median age of HNPCC and those of the other two groups (p = 0.0003). Sixteen percent of the patients (95% CI: 14.85–17.50) were classified as HNPCC according to the above-mentioned criteria, 26 cases as PIR (5.6%; 95% CI: 3.5–7.7), and 423 as sporadic (91.8%; 95% CI: 89.3–94.3). Table 3 summarizes the main clinical and pathologic characteristics of the patients included in the study.

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Table 3. Summary of clinical and pathological characteristics according to group

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (HNPCC)</th>
<th>Group 2 (PIR)</th>
<th>Group 3 (Sporadic)</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>12 (2.6%)</td>
<td>26 (5.6%)</td>
<td>423 (91.8%)</td>
</tr>
<tr>
<td>Median age</td>
<td>45</td>
<td>70</td>
<td>67</td>
</tr>
<tr>
<td>Colon affected</td>
<td>9/12 (75%)</td>
<td>15/26 (58%)</td>
<td>254/423 (60%)</td>
</tr>
<tr>
<td>Dukes stage B</td>
<td>10/12 (83.3%)</td>
<td>14/26 (53.8%)</td>
<td>209/423 (49.4%)</td>
</tr>
<tr>
<td>Five-year survival</td>
<td>11/12 (91.7%)</td>
<td>17/26 (65.4%)</td>
<td>252/423 (59.6%)</td>
</tr>
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</table>

HNPCC, hereditary non-polyposis colorectal cancer; PIR, population at increased risk of cancer.
Colon cancer represented 75% (9/12; 95% CI: 50.00–100.00) of tumors in patients classified as HNPCC, 58% (15/26; 95% CI: 39.03–76.97) in PIR, and 60% (254/423; 95% CI: 55.33–64.67) in sporadic cases.

With regard to Dukes staging system modified by Astler and Coller (38), a predominance of stage B was observed in HNPCC, representing 83.3% (10/12; 95% CI: 65.64–100.00) of all cases. In the PIR group, 14 patients were of stage B (53.8%; 95% CI: 34.2–72.96) and 209 in the group 3 (49.4%; 95% CI: 44.64–54.16).

Five-year survival rates for the different groups were 91.7% (11/12; 95% CI: 76.09–100) for HNPCC, 65.4% (17/26; 95% CI: 47.11–83.63) for PIR, and 59.6% (252/423; 95% CI: 54.92–64.28) for sporadic cases. Table 4 summarizes mean survival according to Dukes stage for each group of patients, whereas Fig. 1 shows survival curves for patients whose tumor were staged as Dukes B and C.

In two of the 12 cases classified as HNPCC (16.6%), a germline mutation in hMLH1 gene was found (deletion resulting in a frameshift and a premature stop codon -codon 228-). These two patients were cousins (family no. 4082) and were affected by the same mutation, as has been previously reported by us (34, 39). Both patients are undergoing a strict follow-up protocol and have presented no malignancies in more than 5 years. In another patient, member of family no. 8161, a nonsense mutation, R406X, in hMSH2 was found (Fig. 3). No mutations were detected in hMSH6 gene. Finally, no deletions were found using MLPA.

### Table 4. Mean survival in each group according to Dukes stage

<table>
<thead>
<tr>
<th>Dukes A</th>
<th>Mean survival</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>HNPCC</td>
<td>No patients</td>
<td></td>
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<tr>
<td>PIR</td>
<td>All alive</td>
<td></td>
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<tr>
<td></td>
<td>157.55</td>
<td>128.00–187.10</td>
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<tr>
<td></td>
<td>Dukes B</td>
<td></td>
</tr>
<tr>
<td>HNPCC</td>
<td>All alive</td>
<td></td>
</tr>
<tr>
<td>PIR</td>
<td>150.21</td>
<td>110.71–189.71</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
<td>140.48</td>
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<tr>
<td></td>
<td>Dukes C</td>
<td></td>
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<tr>
<td>HNPCC</td>
<td>All alive</td>
<td></td>
</tr>
<tr>
<td>PIR</td>
<td>83.21</td>
<td>55.09–111.32</td>
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<tr>
<td></td>
<td>Sporadic</td>
<td>123.91</td>
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<td></td>
<td>Dukes D</td>
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<tr>
<td>HNPCC</td>
<td>All alive</td>
<td></td>
</tr>
<tr>
<td>PIR</td>
<td>48.49</td>
<td>33.75–63.23</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
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</table>

HNPCC, hereditary non-polyposis colorectal cancer; PIR, population at increased risk of cancer.

### Discussion

This is the first South American study analyzing the frequency of HNPCC in patients with CRC, by means of a clinical evaluation as well as genetic studies in patients fulfilling Amsterdam criteria for this condition.

Of the 461 patients included, 16% (n = 74) were less than 50 years old; this is greater than the percentage reported for the entire Uruguayan population in the 2-year period between 1996 and 1997, in which for all cases of CRC, 7.9% were below 50 years of age (40). Seventy-five percent (9/12) of patients classified as HNPCC were less than 55 years old. This supports the finding that age is an important factor for increased risk of HNPCC. Therefore, it is critical to obtain a detailed family history in young patients with CRC.

There is marked geographic variability in the results of studies investigating the frequency of HNPCC in patients having undergone surgery for CRC in that its incidence have ranged from 0.3 to 13% (13, 22). These studies differ in methodological design, inclusion and exclusion criteria, and methods for detecting mutations, all of which may partially explain the observed differences. All of these studies have estimated the frequency of HNPCC in consecutive cases of CRC based on family history. In many of these studies, no molecular analyses were performed for hMLH1, hMSH2, and/or hMSH6 gene mutation detection. For instance, Evans et al. (13) studied 1137 consecutive patients with CRC, of which only 0.3% fulfilled Amsterdam criteria and another 1.4% fulfilled less stringent criteria.

Reliability of family histories in retrospective studies with long observational periods can be called in question, and in part it depends on how researchers confirm the data of interest (in this case colorectal and other HNPCC-related cancers in relatives). In our study, during the 15-year inclusion period, all cases were treated, followed, and analyzed by the same group of investigators; every diagnosis of CRC or other HNPCC-related cancer was confirmed by pathology report, and if this was not possible, by the death certificate. In Uruguay, death certificates are easily available for researchers, making it simple to verify the cause of death of all relatives, in case pathology report was not available.

About 2.6% of all surgical patients with CRC in our study fulfilled Amsterdam criteria; this agrees with the frequency observed by other authors (Table 5) (13, 22).

Although variations in the estimated frequency of HNPCC based only on family history may reflect true differences in the populations studied,
they may also be the result of differences in the degree of certainty required by researchers to confirm every diagnosis of CRC in the family members. The performance of genetic studies in patients fulfilling Amsterdam criteria may attain greater certainty with regard to the true frequency of HNPCC caused by mutations in \textit{hMLH1}, \textit{hMSH2}, and \textit{hMSH6} genes.

Despite the use of a comprehensive methodology for mutation detection covering point mutations and large rearrangements in the three major HNPCC-associated genes, our study detected germline mutations only in 25% (3/12) of patients classified as HNPCC according to Amsterdam criteria, which represent 0.6% of all CRC patients included. While the proportion of

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**Fig. 1.** Survival curves for patients with stages B and C colorectal cancer (mean survival times and 95% CI in Table 4). HNPCC, hereditary non-polyposis colorectal cancer; PIR, population at increased risk of cancer.

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**Fig. 2.** Genealogy of family no. 4082. A mutation in \textit{hMLH1} was found in two of its members (cousins).
mutations detected is clearly lower than that reported in studies using MSI testing or DNA mismatch repair protein expression for preselection (up to 70%) (41, 42), our figure may or may not differ from studies using the fulfillment of Amsterdam criteria alone as the starting point (25–60%) (43–48). This difference could reflect a differing genetic profile in the Uruguayan population compared with other published studies. However, this would not be expected considering that the Uruguayan population is composed predominantly of people of Spanish, Italian, French, and Portuguese origin (49), and most of the studies evaluating hMLH1 and hMSH2 gene mutations in patients with CRC were conducted in Europe. Besides, it must be considered that this is a hospital-based series and it may not reflect the actual frequency of HNPCC throughout Uruguay.

The high frequency of cases fulfilling Amsterdam criteria but with no mutations in the above-mentioned genes may be the result of random family aggregations, shared environmental carcinogens, unusual types of mutations not detectable by the present methods, or mutations in other genes which were not studied. Some authors have suggested that most families fulfilling Amsterdam criteria and presenting an aggregation of left colon tumors and/or absence of multiple HNPCC-related cancers have a lower probability of presenting mutations in the genes studied than those with a predominance of right colon tumors and/or presence of other cancers related to HNPCC (1). In our study, all patients with detected mutations presented tumors of the right colon, and the same propensity for tumors in that site was observed in other affected members of family no. 4082, in which right colon cancers where diagnosed in 68.6% of all cases (24/35 CRC). It is likely that families who met Amsterdam criteria with predominance of left-sided colon cancer and without other HNPCC-related cancer, fulfilled the criteria due to other causes than mutations in hMLH1, hMSH2, or hMSH6.

The estimated proportion of CRC attributable to HNPCC due to mutations in mismatch repair genes may be an underestimate of the actual proportion of this condition in the population studied. Using Bethesda criteria for categorizing CRC patients (50) would have led us to perform molecular analysis in a large number of patients due to the wider inclusion criteria of this system, and therein, it could have resulted in more families with detected mutations. Some hMLH1/hMSH2/hMSH6 mutation carriers could have been identified among families who did not meet the Amsterdam criteria but belonged to the PIR group, if we had performed molecular analyses in this group. These cases would have been categorized as HNPCC. However, we must not expect a large number of mutation carriers among families not categorized as HNPCC based on the family history (1). Besides, the identification of new mutants would not have been possible in our study.

When interpreting our results, the low number of cases in the HNPCC group must also be
considered, because additional cases could have had a great effect on the results. We expect to continue including cases in the future in order to enlarge the series.

In addition to the 2.6% of patients included in group 1, another 5.6% were classified as PIR – patients not fulfilling Amsterdam criteria but presenting a significant family accumulation of CRC. In these cases, molecular studies were not performed. If both groups (HNPCC and PIR) are considered as having an increased risk for developing cancer, 8.2% of patients included in this study may be considered as carriers for an increased family risk of CRC.

The favorable prognoses found in HNPCC patients agree with other studies (51, 52). This outcome may be independent of the pathologic characteristics of the tumor. This indicates that the early detection of this group of patients is an important issue because it may change the treatment strategy (53, 54).

In conclusion, we have presented the only existing South American study thus far that estimates the frequency of HNPCC patients having undergone surgery for CRC. This estimation was based on clinical criteria as well as molecular studies of patients fulfilling Amsterdam criteria. While the percentage of cases fulfilling these criteria was similar to that observed in other studies, the percentage of patients with germline mutations in DNA mismatch repair genes may be lower than that reported from other populations, which needs to be examined further by additional investigations.

References

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