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The role of *MYH* gene in genetic predisposition to colorectal cancer: Another piece of the puzzle

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Abstract

Biallelic germline mutations in the *MYH* gene cause *MYH*-Associated Polyposis but patients with a single mutation possibly have an increased colorectal cancer (CRC) risk. Using DNA from consecutive CRC patients we carried out a case-control study, with the aim to contribute data on the Italian population. Genotyping of four *MYH* mutations found two biallelic and two monoallelic carriers among 439 cases, and only one heterozygous individual among 247 age-matched controls. The frequencies of the mutant alleles were 0.68% (6/878) and 0.20% (1/494), respectively. These differences were not statistically significant. Results on the monoallelic carriers were combined with those from 11 studies on other populations, and the risk of developing a CRC was estimated with an OR = 1.11 (95% CI = 0.90; 1.36), yet not reaching a significant evidence of increased CRC risk.

Keywords: *MYH*; Colorectal cancer; Polyposis

1. Introduction

Familial Adenomatous Polyposis and Hereditary Non Polyposis Colorectal Cancer are defined by clinical and genetic criteria whose application has shown that these two syndromes together account for approximately 3–5% of the total colorectal cancer (CRC) cases [1]. Hence, the molecular genetic basis of the majority of hereditary and familial CRC cases is yet to be elucidated.

Recent developments in the genetic analysis of hereditary CRC demonstrated that loss of base excision repair (BER) function might play an important

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role [2–4]. The BER pathway, repairing free-radical damaged purine and pyrimidine bases, abasic sites and single strand breaks, is highly conserved from bacteria to human and, together with other DNA repair pathways, is essential for maintaining genome stability [5]. Biallelic germline mutations in the *MYH* gene were shown to cause multiple colorectal adenomas and carcinomas because loss of *MYH* function results in increased frequency of G:C>T:A somatic mutations in *APC* and other genes [2,6,7].

Y165C and G382D are the most frequent mutations found in the Caucasian population. On the basis of studies published so far, a clinical-genetic picture of *MYH*-Associated Polyposis (MAP) has been outlined: a variable number of polyps (ranging from 10–20 to >100) and early onset CRC; absence of vertical transmission from parent to offspring; sporadic or multiple-case presentations within one generation [8–13]. These features are characteristic of an autosomal recessive pattern of inheritance.

Case-control studies based upon a population of patients with CRC confirmed the well established increased risk associated with biallelic *MYH* mutations and a few of them indicated that patients with a single mutation may possibly have an increased CRC risk [9,10,12,14–17], a suggestion also supported by a population-based family study [18]. If this last assumption were further proved, *MYH* might play a relevant role in CRC onset within the general population, and the identification of mono- or bi-allelic mutations would represent an important step in CRC screening. Therefore, in order to define the effective contribution of *MYH* in CRC susceptibility, and quantify the related risk, it is fundamental to obtain additional data from other population studies.

From the above mentioned findings, a number of specific questions that the scientific community would like to address have emerged: what is the role of *MYH* in predisposing the general population to CRC? Does the loss of *MYH* functions trigger tumorigenesis exclusively in a recessive condition/way or are heterozygotes also at an increased cancer risk? Are the biallelic *MYH* mutations invariably associated with a polyposis phenotype?

These questions led us to inquire into the role played by *MYH* following the experimental approach of the mutation analysis of the *MYH* gene in consecutive CRC cases. Several groups have already reported similar analyses on specific populations and we now intend to contribute our data on the Italian patients.

2. Materials and methods

2.1. Colorectal cancer cases and controls

The study was carried out on 478 unrelated and consecutive cases of CRC operated between October, 2002 and December, 2003 at Padova's general hospital and ULSS 16. All patients underwent a standardized family history reconstruction by a restricted pool of surgeons and gave informed consent for genetic analyses. There was no selection of cases on the basis of their familial history or clinical phenotype, thus polyposis and HNPCC patients were not excluded from enrolment. Thirty-nine cases were excluded owing to PCR reaction failure and the analysis was conducted on a total of 439 cases (168 females and 271 males) with a mean age of 67 ± 11.42 years (range 32–92). Three hundred sixty patients had colon cancer, 78 had rectal cancer and for 1 patient the localization was not specified.

DNA was also collected from 247 control subjects, shared out between healthy blood donors (167) and individuals with documented clean colon (80), age-matched with cases (±5 years and with 2 cases/1 control ratio) and representing a mean age of 64.03 ± 10.51 years (range 32–88).

Genomic DNA was extracted from peripheral blood or paraffin-embedded normal tissues.

2.2. Analysis of MYH variants Y165C, G382D, IVS10+3A>C, 1395-7delGGA

Genotyping for four mutations was performed by pyrosequencing on a PyroMark ID instrument. Exons 7, 10, 13 and 14 were amplified by PCR and sequenced using the primers listed in Table 1 that were designed using the PSQ Assay Design software to specifically detect the Y165C, G382D, IVS10+3A>C, 1395-7delGGA *MYH* variants. Mutated samples were confirmed by direct bidirectional sequencing with the di-deoxy method using different sets of primers (available upon request).

2.3. Statistical analysis

The association of the *MYH* mutations and the *MYH*-mutated genotypes with CRC was tested by means of standard two-tailed Fisher's exact tests. Odds Ratios (ORs) and 95% CIs were also calculated for biallelic, monoallelic and any mutation carriers.

A meta-analysis including all previous case-control studies evaluating the CRC risk associated to the monoallelic *MYH* mutations was performed. The homogeneity throughout studies was quantified by using a homogeneity test based on the χ^2 test. In addition, the I^2 statistics were calculated to assess the impact of heterogeneity on the results. These statistics describe the percentage of total variation in study estimates that is due to heterogeneity