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Common variants in human CRC genes as low-risk alleles

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ABSTRACT

The genetic susceptibility to colorectal cancer (CRC) has been estimated to be around 35% and yet high-penetrance germline mutations found so far explain less than 5% of all cases. Much of the remaining variations could be due to the co-inheritance of multiple low penetrant variants. The identification of all the susceptibility alleles could have public health relevance in the near future. To test the hypothesis that what are considered polymorphisms in human CRC genes could constitute low-risk alleles, we selected eight common SNPs for a pilot association study in 1785 cases and 1722 controls. One SNP, rs3219489:G>C (MUTYH Q324H) seemed to confer an increased risk of rectal cancer in homozygous status (OR = 1.52; CI = 1.06–2.17). When the analysis was restricted to our 'super-controls', healthy individuals with no family history for cancer, also rs1799977:A>G (MLH1 I219V) was associated with an increased risk in both colon and rectum patients with an odds ratio of 1.28 (CI = 1.02–1.60) and 1.34 (CI = 1.05–1.72), respectively (under the dominant model); while 2 SNPs, rs1800932:A>G (MSH6 P92P) and rs459552:T>A (APC D1822V) seemed to confer a protective effect. The latter, in particular showed an odds ratio of 0.76 (CI = 0.60–0.97) among colon patients and 0.73 (CI = 0.56–0.95) among rectal patients. In conclusion, our study suggests that common variants in human CRC genes could constitute low-risk alleles.

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1. Introduction

Colorectal cancer (CRC) is a complex disease caused by genetic and environmental factors, with the former accounting for a third of the cases and the latter for the remaining two-thirds.[1] Being able to divide the population into risk categories would allow tailored prevention programmes according to the risk of each individual. In order to do so, it is important to understand how the genetic background is affected by environmental factors and how this complex interplay of genetic and non-genetic factors contributes to the CRC development and progression.[2,3]

Several familial syndromes are known: Familial Adenomatous Polyposis (FAP), caused by a germline mutation in APC[4,5] and Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC), caused by a germline mutation in one of the mismatch repair genes (MMR) MLH1, MSH2, MSH6 and PMS2.[6–9] However, they together account for only approximately 5% of the cases.[10] MUTYH Polyposis (MAP) is usually phenotypically identical to a classic or mild form of FAP, but the difference stands in its recessive mode of inheritance.[11]

In the past 2 years Genome-Wide Association Studies (GWAS) have led to the discovery of several susceptibility alleles.[12–18] However, the risk associated with each variant is very low and these SNPs are suggested to explain only a small part of the remaining unknown genetic contribution.[19]

Similarly, further high-penetrance alleles are probably rare and restricted to families with multiple cases. Several genetic models have been proposed to explain the aetiology of CRC, ranging from few common alleles conferring a modest risk (the so-called 'common disease-common variant hypothesis') to a very large number of alleles conferring a higher risk (the 'common disease-rare variant hypothesis').[20,21]

Our hypothesis is that variants in genes already known to be involved in CRC development could explain at least part of the sporadic cases without family history and clearly pathogenic mutations and, perhaps, also partly some familial cases. These variants could act as low-penetrance alleles and it seems reasonable to assume that several of them together could drive the cell towards the tumourigenic process.

In the past years many SNPs of unknown pathogenicity were found in the MMR genes, APC and MUTYH by our group and in many other laboratories around the world.[22–24] In order to test our hypothesis we conducted a pilot study on eight of the most common SNPs found in our studies in the Swedish population: one in MLH1, one in APC, two in MUTYH and four in MSH6.

These variants are considered polymorphisms but could nonetheless act as low-risk alleles in CRC.

2. Materials and methods

The case cohort used in this study was composed of 1785 consecutive colorectal cancer patients of Swedish origin divided as follows: 1103 individuals with a diagnosis of colon cancer, 637 with a diagnosis of rectal cancer and 45 individuals whose exact tumour location was not specified. All cases were collected through the Family Cancer Clinic at the Karolinska Hospital (Stockholm, Sweden) and were recruited by 14 different hospitals from central Sweden. The control cohort was composed of 1306 blood donors from the general population between the age of 18 and 65 and 416 so-called 'super-controls'. The super-controls are unaffected spouses of CRC patients, which are cancer-free at the moment of the diagnosis and do not have a family history of any type of cancer. In principle, the super-controls constitute a better cohort to be used for these types of case–control studies, since they should carry even less susceptibility factors than blood donors, who we consider to represent the general population. We investigated eight SNPs in four different genes (Table 1): rs459552:T>A (APC D1822V), rs1799977:A>G (MLH1 I219V), rs1042821:G>A (MSH6 G39E), rs1800932:A>G (MSH6 P92P), rs1800935:T>C (MSH6 D180D), rs1800937:C>T (MSH6 Y214Y), rs3219484:G>A (MUTYH V22M) and rs3219489:G>C (MUTYH Q324H).

Informed consent was obtained from all participants. The study was undertaken in accordance with the Swedish legislation of ethical permission (02/489). Genomic DNA was extracted from peripheral blood by standard procedures. Genotyping and a first-quality check of rs459552:T>A (RefSeq NM_000038.4), rs1799977:A>G (RefSeq NM_000249.2), rs1800932:A>G and rs1800935:T>C (RefSeq NM_000179.2), rs3219484:G>A and rs3219489:G>C (RefSeq NM_001048171.1) were done at deCode Genetics (Reykjavik, Iceland). The remaining two SNPs in the MSH6 gene, rs1042821 and rs1800937 (RefSeq NM_000179.2) were genotyped using TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA). Deviations of the genotype frequency in cases and controls from those expected under Hardy–Weinberg equilibrium were calculated by χ^2 tests (1 degree of freedom). Allelic frequencies of the SNPs in the case and control groups were compared using a χ^2 test (allele 1 [common] versus allele 2 [minor]). Analyses were also performed under various types of genetic contrasts including the contrast of homozygotes (genotype 11 versus genotype 22), the dominant (genotype 11 versus genotype [12 + 22]) and recessive [genotype (11 + 12) versus genotype 22] models. In addition, Armitage's trend test, which takes into account the individuals' genotypes rather than just alleles,[25] was performed using the DeFinetti program provided as an online source (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The significance level for statistical tests was set at 0.05. Odds ratios (ORs), their 95% confidence intervals (CIs) and their corresponding p-values were calculated using the same program. These analyses were performed on the entire cohort of CRC cases as well as for colon and rectum only.

3. Results

The results of the case–control association study in the whole cohort as well as the analyses stratified by tumour location are reported in Table 2.

APC D1822V did not show any difference between cases and controls, but when we considered only the super-controls (n = 344), the heterozygous carrier status was associated with an odds ratio of 0.77 (CI = 0.60–0.97, p = 0.03) and the homozygous status with an odds ratio of 0.67 (CI = 0.43–1.05; p = 0.08). If a dominant model was assumed, the combined