Meta-Analysis of Mismatch Repair Polymorphisms within the Cogent Consortium for Colorectal Cancer Susceptibility

Citation for published version:

Digital Object Identifier (DOI):
10.1371/journal.pone.0072091

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
PLoS One

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Meta-Analysis of Mismatch Repair Polymorphisms within the Cogent Consortium for Colorectal Cancer Susceptibility

Simone Picelli1,2,*, Justo Lorenzo Bermejo3,4,9, Jenny Chang-Claude5, Michael Hoffmeister6, Ceres Fernández-Rozadilla7, Angel Carracedo7, Antoni Castells8, Sergi Castellví-Bel8, The EPICOLON Consortium9, Alessio Naccaрат9, Barbara Pardini9, Ludmila Vodickova9,10, Heiko Müller11, Bente A. Talseth-Palmer12, Geoffrey Stibbard13, Paolo Peterlongo14,15, Carmela Nic11, Silvia Veneroni16, Li Li17, Graham Casey18, Albert Tenesa19, Susan M. Farrington19, Ian Tomlinson20, Victor Moreno21, Tom van Wezel22, Juul Wijnen23, Malcolm Dunlop19, Paolo Radice14,15, Rodney J. Scott12,24, Pavel Vodicka9,10, Clara Ruiz-Ponte7, Hermann Brenner11, Stephan Buch25, Henry Völzke26, Jochen Hampe25, Clemens Schafmayer27, Annika Lindblom1

1 Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden, 2 Ludwig Institute for Cancer Research – Stockholm branch, Stockholm, Sweden, 3 Institute of Medical Biometry and Informatics, University Hospital Heidelberg, Heidelberg, Germany, 4 Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, 5 Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, 6 Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany, 7 Galician Public Foundation of Genomic Medicine (PFGMX), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Genomics Medicine Group, Hospital Clínic, Santiago de Compostela, University of Santiago de Compostela, Galicia, Spain, 8 Department of Gastroenterology, Hospital Clinic, The Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Institut d’Investigacions Biomèdiques August Pi i Sanyor (IDIBAPS), University of Barcelona, Barcelona, Catalonia, Spain, 9 Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic, 10 First Medical Faculty of the Charles University, Prague, Czech Republic, 11 Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany, 12 School of Biomedical Science and Pharmacy, University of Newcastle, and the Hunter Medical Research Institute, Newcastle, Czech Republic, 13 School of Science and IT, University of Newcastle, Newcastle, Australia, 14 Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 15 Fondazione IFOM, Istituto FIRC di Oncologia Molecolare, Milan, Italy, 16 Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 17 Department of Family Medicine, Case Center for Transdisciplinary Research on Energetics and Cancer, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, United States of America, 18 University of Southern California, Norris Comprehensive Cancer Centre, Los Angeles, California, United States of America, 19 Colon Cancer Genetics Group, Institute of Genetics and Molecular Medicine, University of Edinburgh and MRC Human Genetics Unit, Edinburgh, United Kingdom, 20 Oxford NIHR Comprehensive Biomedical Research Centre, Oxford, United Kingdom, 21 IDIBELL-Institut Català d’Onccologia (ICO), CIBER Epidemiologia y Salud Pública (CIBERESP) and University of Barcelona, L’Hospitalet de Llobregat, Barcelona, Spain, 22 Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, 23 Department of Human Genetics and Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, 24 Division of Genetics, Hunter Area Pathology Service, John Hunter Hospital, Newcastle, NSW Australia, 25 Department of General Internal Medicine, University Hospital Schleswig-Holstein, Kiel, Germany, 26 Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany, 27 Department of General and Thoracic Surgery, Christian-Albrechts-University, Kiel, Germany

Abstract

In the last four years, Genome-Wide Association Studies (GWAS) have identified sixteen low-penetration polymorphisms on fourteen different loci associated with colorectal cancer (CRC). Due to the low risks conferred by known common variants, most of the 35% broad-sense heritability estimated by twin studies remains unexplained. Recently our group performed a case-control study for eight Single Nucleotide Polymorphisms (SNPs) in 4 CRC genes. The present investigation is a follow-up of that study. We have genotyped six SNPs that showed a positive association and carried out a meta-analysis based on eight additional studies comprising in total more than 8000 cases and 6000 controls. The estimated recessive odds ratio for one of the SNPs, rs3219489 (MUTYH Q338H), decreased from 1.52 in the original Swedish study, to 1.18 in the Swedish replication, and to 1.08 in the initial meta-analysis. Since the corresponding summary probability value was 0.06, we decided to retrieve additional information for this polymorphism. The incorporation of six further studies resulted in around 13000 cases and 13000 controls. The newly updated OR was 1.03. The results from the present large, multicenter study illustrate the possibility of decreasing effect sizes with increasing samples sizes. Phenotypic heterogeneity, differential environmental exposures, and population specific linkage disequilibrium patterns may explain the observed difference of genetic effects between Sweden and the other investigated cohorts.


Editor: Nathan A. Ellis, University of Illinois at Chicago, United States of America

Received January 10, 2013; Accepted July 6, 2013; Published September 6, 2013

Copyright: © 2013 Picelli et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
**Introduction**

In recent years low-risk common alleles have attracted increasing attention in the search for the “missing heritability” in colorectal cancer (CRC). It concerns the part of heritability that cannot be explained by mutations in already known high-risk genes but should, according to twin studies, account for about 35% [1]. Known high-penetration germline mutations in CRC genes contribute for less than 6% of the observed cases [2]. Therefore, much of the remaining inherited variation in genetic susceptibility is probably due to multiple low-penetration variants, both common and rare.

To date sixteen common variants have been identified through large multi-centre genome-wide association studies (GWAS) [3]. Taken together, however, they only explain a small proportion of familial CRC cases. Although the risk associated with each of these variants is modest, they contribute to the disease burden due to their high frequency in the population and the possibility of acting in concert with each other, which may increase the individual’s risk of developing CRC [4].

Against this background, a few years ago we attempted to assess the role of eight SNPs in four already known CRC genes (APC, MLH1, MSH6 and MUTYH) through a case-control association study in the Swedish population [5]. These 8 SNPs had been previously studied, but their pathogenicity was unknown and they were assumed to constitute polymorphisms. In our first study several positive associations were detected but, due to limited sample size (1785 cases and 1722 controls) [5], the results needed to be validated in a follow-up study.

**Funding:** The authors are grateful to: the Spanish National Genotyping Center (CEGEN-ISICII)-USC and the UPF nodes, as well as the Institutional Tumor Bank of Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. The work was carried out (in part) at the Esther Koplowitz Centre, Barcelona. This study was supported by Instituto de Salud Carlos III, Spanish Ministry of Health, (grants FIS PI06-1635 and PS09-1037, VM), Cancer Research UK Programme Grant (C348/A12076) and a Centre Grant from the CORE Charity (MD); CZGA CRGAP304/10/1286 and CZGA CRGA310/07/1430 (PV); Hunter Medical Research Institute research grant and Priority Research Centre for Information Based Medicine (RS); German Research Council, German Federal Ministry of Research and Education (HB, HV); Baden Württemberg State Ministry of Research, Science and Arts (HB); Fondo de Investigación Sanitaria/FEDER (08/0024, 08/1276, PS09/02368, 11/00219, 11/00681); Instituto de Salud Carlos III (Acción Transversal de Cáncer, Xunta de Galicia (RIH07/04 and 08CSA005208P); Ministerio de Ciencia e Innovación (SAF2010-19273); Asociación Española contra el Cáncer (Fundación Científica y Junta de Barcelona); Fundación Olga Torres (CRP), and FP7 CHIBCHA Consortium (AC and SCB); Fondo de Investigación Sanitaria (CP 03-0070 to SCB and PS09/02368 to CFR). CIBERehd and CIBERER are funded by the Instituto de Salud Carlos III. JLB is partially supported by a grant of the Deutsche Forschungsgemeinschaft (DFG, SFB/TR77, project Z2). LL is supported by a grant from National Cancer Institute R01 CA136726. SB, JH, WvS and CS were supported by the German Ministry for Education and Research through the German National Genome Research Network (NGFNplus) Colon Cancer Network (CCN). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

* E-mail: simone.picelli@licr.ki.se

* These authors contributed equally to this work.

* Membership of The EPICOLON Consortium is provided in the Acknowledgments.

**Mutation screening**

Six SNPs in four different CRC genes were included in the analysis: rs459552: T>A (APC D1822V), rs1799977: A>G (MLH1 I219V), rs1800932: A>G (MSH6 P92P), rs1800933: T>C (MSH6 D180D), rs3219484: G>A (MUTYH V22M) and rs3219489: G>C (MUTYH Q338H). MUTYH Q338H corresponds to Q324H in our first study [5]. The SNP nomenclature was modified to meet the largest theoretically known transcript. For MUTYH this corresponds to NM_001128423.1 and NP_001121897.1 for mRNA and protein, respectively [7,8,9].

**Subjects**

Details regarding the number of cases and controls in all fourteen studies are summarized in Table S1. One SNP, rs459552 (APC D1822V), was genotyped in seven studies, for a total of 8654 cases and 7731 controls. Four SNPs, rs1799977 (MLH1 I219V), rs1800932 (MSH6 P92P), rs1800933 (MSH6 D180D) and rs3219484 (MUTYH V22M) were genotyped in 8 studies for a total of 8308 cases and 7434 controls. The SNP with rs number 3219489 (MUTYH Q338H) was genotyped in 13 cohorts for a total of 12902 cases and 14602 controls.

For all the subjects genomic DNA was extracted from peripheral blood by standard procedures. Additional information regarding localization of the tumor, age at diagnosis, gender and ethnicity was retrieved whenever possible. Out of 5770 controls with ethnicity information, 3647 were of Caucasian origin, the rest being mostly African American.

**Genotyping**

In studies 1, 5, 6, 7, 8, 9 and 10 SNPs were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystem, Foster City, CA). Genotyping in study 2 and 12 (controls only) was carried out by using the KASP chemistry of the K-bioscience (Hoddesdon, Herts, UK) [http://www.kbioscience.co.uk/reagents/KASP_manual.pdf], which is a competitive allele-specific PCR SNP genotyping system that uses FRET quencher cassette oligos. Study 3 genotyped with the MassARRAY (Sequenom Inc., San Diego, USA) technology. Study 4 genotyped by means of fluorescent hybridization probe melting curves using the Light Cycler instrument (Roche). Study 11 genotyped using Illumina HumanHap 550 Bead Arrays. Study 12 was genotyped by Sanger sequencing (cases only). Studies 13 and 14 were genotyped using Illumina HumanHap300 and Illumina HumanHap240S.
Statistical analysis

Deviation of observed genotype frequencies in controls from those expected under Hardy-Weinberg equilibrium were assessed by $\chi^2$ tests. Risks of CRC associated with genotypes were compared by odds ratios (ORs) with corresponding confidence intervals (CIs) based on logistic regression. Study heterogeneity was summarized using a Mantel-Haenszel test but we assumed that the studies were random samples from a general population and used a random effect model to summarize OR estimates under dominant, recessive and additive penetrance models in the meta-analyses. Results were represented by forest plots as follows: confidence intervals for each individual study were indicated by horizontal lines, single ORs by squares and summary estimates by diamonds with horizontal limits at confidence limits and width inversely proportional to the standard error. Meta-analyses were performed using the package meta in the free software environment for statistical computing R.

Results

The distribution of the genotypes in controls did not deviate from Hardy-Weinberg equilibrium in any study. Mantel-Haenszel tests identified study heterogeneity for rs1800932 (MSH6 P92P) under recessive and additive penetrance, with $p$-values equal to 0.04 and 0.03, respectively (Table S2). This does not constitute a major issue since this SNP showed no differences between the genotype distributions of cases and controls either in single studies or in the global analysis. Study heterogeneity was not found for any other SNP. Genotyping results for the 6 SNPs based on studies 1–8 are presented in Table S2.

The only SNP that was marginally significant in the meta-analysis was rs3219489 (MUTYH Q338H), both under a recessive model (summary OR = 1.08, 95% CI 1.00 to 1.17; $p$ = 0.05) and assuming additive allelic effects (summary OR = 1.07, 95% CI 1.00 to 1.14; $p$ = 0.06). We ascribe the combined result mainly to the Swedish study, with individual ORs of 1.18 (95% CI = 1.01–1.38, recessive model) and 1.19 (95% CI = 1.05–1.35, additive model) (Table S2). The goodness of fit was slightly better for the recessive than for the additive model, and the recessive and additive models clearly outperformed the dominant model.

In an attempt to validate the findings under recessive inheritance, we set up collaborations with additional groups and requested to genotype rs3219489 in their cohorts. In the end, additional 4234 cases and 6800 controls were included, adding up to a total of 12232 cases and 13380 controls (Table S3).

We updated the meta-analysis once more considering all samples regardless of tumor localization as well as stratifying them for colon and rectal tumors. As shown in Table S4, data were available for 4573 colon and 1774 rectal cancer cases. Results from the updated meta-analyses are presented in Figure 1. The new summary OR for colorectal cancer was 1.06 (95% CI 0.94 to 1.19, probability values 0.09 (OR = 1) and 0.37 (study heterogeneity) (Figure 1B) and for rectal cancer was 1.06 (95% CI 0.94 to 1.19, probability values 0.37 (OR = 1) and 0.31 (study heterogeneity) (Figure 1C).

Discussion

In the present investigation we performed a case-control association study for six out of eight previously investigated SNPs [5]. For five of them, rs459552 (APC D1822V), rs1799977 (MLH1 P219V), rs1000932 (MSH6 P92P), rs1000935 (MSH6 D180D) and rs3219404 (MUTYH V22M) samples were retrieved from eight additional studies totaling 8306 cases and 7434 controls. For the sixth SNP, rs3219489 (MUTYH Q338H), which was selected based on promising results from two samples of Swedish origin (study 8 in the present manuscript and reference [5]), we set up an even larger replication dataset comprising 14 different studies with a total of 12232 cases and 13380 controls.

For all SNPs included in the analysis we were unable to confirm the associations with CRC risk found in the Swedish population. In particular, the recessive ORs of CRC for rs3219489 decreased from 1.52 in the original Swedish study to 1.18 in the Swedish replication cohort, to 1.08 (95% CI 1.00 to 1.17) in the first meta-analysis and to 1.03 (95% CI 0.97 to 1.10) in the updated meta-analysis (Table S2). The summary ORs in the extended meta-analyses were 1.07 (95% CI 0.99 to 1.16) for colon cancer and 1.06 (95% CI 0.94 to 1.19) for rectal cancer, in contrast with results based on Swedish samples. The updated meta-analysis had statistical power of 99% to detect a recessive OR of 1.52 and a power of 89% to detect a recessive OR of 1.18 (Type I error rate 5% and prevalence of CC genotypes among controls 5.6%). Biological plausibility was also existent. MUTYH Q338H is interesting because it represents a missense change in the MUTYH protein, which is involved in the base excision repair (BER) pathway. A common product of oxidative damage to 2’-deoxyguanosine is 7,8-dihydro-8-oxo-2’-deoxyguanosine (OG) [10,11]. In mammalian cells OG has been shown to be highly mutagenic and leading to an increased rate of G→T transversions, due to its miscoding properties that cause a mispairing with an adenine during DNA replication to form a stable OGG1 mismatch [11,12]. The BER pathway plays an important role in repairing this type of DNA damage through the action of the mutY homolog MUTYH, in concert with OGG1 and MTH1 [11,13]. It is well established that bi-allelic mutations in MUTYH gene introduce G:C to T:A transversions also in the adenomatous polyposis coli (APC) gene, leading to genomic instability and abnormal and disregulated cell proliferation in the colonic epithelium [14,15]. Patients with two mutations in the MUTYH gene develop the MUTYH-associated polyposis (MAP) syndrome [13].

To date, 85 different MAP-associated mutations have been found [16], scattered throughout the entire length of the protein, but only 3 (including Q338H) map within putative protein interaction domains as revealed by the recently solved crystal structure of hMUTYH [17]. It is tempting to speculate that Q338H might affect this protein-protein interaction, but additional experimental support is warranted.

The contrasting results on rs3219489 and its association with CRC risk in the Swedish versus other populations might suggest that the effect of this variant is specific for the Swedish population or not large enough in the other populations to be detected with the present sample size. For example, the statistical power of the updated meta-analysis was only 43% to detect a recessive OR of 1.10 (Type I error rate 5% and prevalence of CC genotypes among controls 5.6%). A closer look at the data actually shows that one of the German cohorts (ESTHER) gave results in concert with our Swedish results, with OR = 1.36 (95% CI 1.00 to 1.86) for colorectal cancer (Figure 1A) and OR = 1.61 (95% CI 1.08 to 2.40) for rectal cancer (Figure 1C). This is likely a spurious result due to the small size of that cohort (318 cases and 365 controls).

On the other hand, in agreement with Swedish results, rs3219489 has also been shown to be associated with CRC risk in three independent studies in the Japanese population [18,19,20] and among African-Americans [Yuan et al., 2nd InSiGHT].
meeting, Yokohama, Japan, unpublished) even though all these studies have a limited sample size and the results need further validation.

It is also possible that rs3219489 represents a risk-associated variant in the Swedish population in combination with environmental factors in the broad sense. For example, screening programs for CRC in Sweden could result in a diagnosis earlier in life, thus inflating the ORs estimated in Sweden. Another alternative is that the polymorphism is in linkage disequilibrium with other unidentified causal variants. The marker and the causal variant could be located on the same risk haplotype in the Swedish population and on different haplotypes in other populations.

Independently of the unknown reason for replication failure, the results from the present study clearly illustrate the possibility of

<table>
<thead>
<tr>
<th>Study</th>
<th>OR</th>
<th>95%</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>0.93</td>
<td>0.67</td>
<td>1.30</td>
</tr>
<tr>
<td>Czech_Republic</td>
<td>1.16</td>
<td>0.94</td>
<td>1.42</td>
</tr>
<tr>
<td>Spain_EPICOLON</td>
<td>1.04</td>
<td>0.88</td>
<td>1.24</td>
</tr>
<tr>
<td>Germany_POPGEN-SHIP</td>
<td>0.97</td>
<td>0.85</td>
<td>1.11</td>
</tr>
<tr>
<td>Germany_DACHS</td>
<td>1.06</td>
<td>0.91</td>
<td>1.24</td>
</tr>
<tr>
<td>Germany_ESTHER</td>
<td>1.36</td>
<td>1.00</td>
<td>1.86</td>
</tr>
<tr>
<td>Sweden</td>
<td>1.18</td>
<td>1.01</td>
<td>1.38</td>
</tr>
<tr>
<td>USA</td>
<td>0.97</td>
<td>0.83</td>
<td>1.13</td>
</tr>
<tr>
<td>Italy</td>
<td>0.99</td>
<td>0.82</td>
<td>1.18</td>
</tr>
<tr>
<td>UK_CORGI</td>
<td>0.83</td>
<td>0.60</td>
<td>1.15</td>
</tr>
<tr>
<td>Scotland_1</td>
<td>0.94</td>
<td>0.78</td>
<td>1.12</td>
</tr>
<tr>
<td>Scotland_2</td>
<td>1.09</td>
<td>0.88</td>
<td>1.35</td>
</tr>
<tr>
<td>Summary</td>
<td>1.03</td>
<td>0.97</td>
<td>1.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>OR</th>
<th>95%</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czech_Republic</td>
<td>1.15</td>
<td>0.92</td>
<td>1.44</td>
</tr>
<tr>
<td>Spain_EPICOLON</td>
<td>1.08</td>
<td>0.86</td>
<td>1.35</td>
</tr>
<tr>
<td>Germany_ESTHER</td>
<td>1.20</td>
<td>0.83</td>
<td>1.73</td>
</tr>
<tr>
<td>Sweden</td>
<td>1.26</td>
<td>1.05</td>
<td>1.50</td>
</tr>
<tr>
<td>USA</td>
<td>0.97</td>
<td>0.83</td>
<td>1.13</td>
</tr>
<tr>
<td>Italy</td>
<td>0.96</td>
<td>0.75</td>
<td>1.24</td>
</tr>
<tr>
<td>Scotland_1</td>
<td>0.96</td>
<td>0.77</td>
<td>1.19</td>
</tr>
<tr>
<td>Scotland_2</td>
<td>1.11</td>
<td>0.86</td>
<td>1.44</td>
</tr>
<tr>
<td>Summary</td>
<td>1.07</td>
<td>0.99</td>
<td>1.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>OR</th>
<th>95%</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czech_Republic</td>
<td>1.18</td>
<td>0.89</td>
<td>1.55</td>
</tr>
<tr>
<td>Spain_EPICOLON</td>
<td>0.91</td>
<td>0.67</td>
<td>1.24</td>
</tr>
<tr>
<td>Germany_ESTHER</td>
<td>1.61</td>
<td>1.08</td>
<td>2.4</td>
</tr>
<tr>
<td>Sweden</td>
<td>1.02</td>
<td>0.82</td>
<td>1.27</td>
</tr>
<tr>
<td>Italy</td>
<td>0.93</td>
<td>0.66</td>
<td>1.33</td>
</tr>
<tr>
<td>Scotland_1</td>
<td>0.97</td>
<td>0.74</td>
<td>1.25</td>
</tr>
<tr>
<td>Scotland_2</td>
<td>1.09</td>
<td>0.77</td>
<td>1.55</td>
</tr>
<tr>
<td>Summary</td>
<td>1.06</td>
<td>0.94</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Figure 1. Forest plots with observed odds ratios and 95% confidence intervals for rs3219489 (MUTYH Q338H) under a recessive penetrance model in colorectal cancer (A), colon cancer only (B) and rectal cancer only (C).

doi:10.1371/journal.pone.0072091.g001
decreasing effect sizes with increasing collections of individuals, a phenomenon well-known in the field of genetic epidemiology denominated the winner’s curse [21]. It should be kept in mind that this outcome is rather expected in association studies, in particular those dealing with regionally heterogeneous complex diseases.

Supporting Information

Table S1 Number of cases and controls genotyped in the fourteen studies.

Table S2 Genotype counts and allele frequencies for rs459552 (APC D1822V), rs1799977 (MLH I219V), rs1800932 (MSH6 P92P), rs1800935 (MSH6 D180D), rs3219484 (MUTYH V22M) and rs3219489 (MUTYH Q338H).

Table S3 Genotype counts and allele frequencies for rs3219489 (MUTYH Q338H).

Table S4 Genotype counts for colon and rectal cancer cases in studies with available information on tumor location.

Acknowledgments

We thank all the participants that participated in this study. Members of the EPICOLON Consortium (Gastrointestinal Oncology Group of the Spanish Gastroenterological Association): Hospital de 2 de Octubre, Madrid: Juan Diego Morillas (local coordinator), Raquel Muñoz, Marisa Manzano, Francisco Colina, Jose Diaz, Carolina Ibarrola, Guadalupe López, Alberto Ibáñez; Hospital Clinic, Barcelona: Antoni Castells (local coordinator), Virginia Pitiló, Sergi Castellví-Bel, Francesc Balaguer, Victoria Gonzalez, Teresa Ocaña, María Dolores Giraldez, María Pallós, Anna Serresdeanfar, Leticia Morcillo, Miriam Cuartecasas, Josep M. Piqué; Hospital Clínico Universitario, Zaragoza: Ángel Lanas (local coordinator), Javier Alcedo, Javier Ortego; Hospital Cristal-Piñol, Complejo Hospitalario de Ourense: Joaquín Cubiella (local coordinator), Mª Soledad Diez, Merced Salgado, Eloy Sánchez, Mariano Vega; Parc de Salut Mar, Barcelona: Montserrat Andreu (local coordinator), Anna Abuli, Xavier Bessa, Mar Iglesias, Agustín Seoane, Felipe Bory, Gemma Navarro, Beatrix Bellosillo, Josep Mª Dedou, Cristina Álvarez, Marc Puigvéhi; Hospital San Eloy, Baracaldo and Hospital Donostia, CIBERech, University of Basque Country, San Sebastián: Luis Bujanda (local coordinator) Ángel Cosme, Inés Gil, Mikel Larrazabal, Carlos Placer, María del Mar Ramírez, Elisabet Hijona, Jose M. Enriquez-Navascués, José L. Elosegui; Hospital General Universitario de Alicante: Artémio Payá (EPICOLON I local coordinator), Rodrigo Jover (EPICOLON II local coordinator), Cristina Alenda, Laura Sempere, Nuria Acane, Estefanía Rojas, Lucía Pérez-Carbonell; Hospital General de Granollers: Joaquim Rigau (local coordinator), Ángel Serrano, Anna Giménez; Hospital General de Vic: Joan Saló (local coordinator), Eduard Batiste-Alentorn, Josefina Autonell, Ramon Barniol; Hospital General Universitario de Guadalajara and Fundación para la Formación e Investigación Sanitarias Murcia: Ana María García (local coordinator), Fernando Carballo, Antonio Bienvenido, Eduardo Sanz, Fernando González, Jaume Sánchez, Akiko Ono; Hospital General Universitario de Valencia: Mercedes Latorre (local coordinator), Enrique Medina, Jaime Cuquerella, Pilar Canelles, Miguel Martorell, José Ángel García, Francisco Quiles, Elisa Ortiz; CHUVI-Hospital Meixoeiro, Vigo: EPICOLON I: Juan Clófent (local coordinator), Jaime Seoane, Antoni Tardó, Eugenia Sánchez; EPICOLON II Mª Luisa de Castro (local coordinator), Antoni Tardó, Juan Clófent, Vicent Hernández; Hospital Universitari Germans Trias i Pujol, Badalona and Section of Digestive Diseases and Nutrition, University of Illinois at Chicago, IL, USA: Xavier LLor (local coordinator), Rosa M. Nicolà, Marta Piñol, Mercè Rosinach, Anna Roca, Elisenda Pons, José M. Hernández, Miquel A. Gassull; Hospital Universitari Mutua de Terrassa: Fernanndo Fernández-Bañares (local coordinator), Josep M. Viver, Antonio Salas, Jorge Espiñós, Montserrat Forne, María Esteve; Hospital Universitari Arnau de Vilanova, Lleida: Josep M. Reñé (local coordinator), Carmen Piñol, Juan Buenestado, Joan Viñas; Hospital Universitario de Canarias: Enrique Quintero (local coordinator), David Nicolás, Adolfo Parra, Antonio Martí; Hospital Universitario La Fe, Valencia: Lidia Argüello (local coordinator), Vicente Pons, Virginia Pertejo, Teresa Sala; Hospital Sant Pau, Barcelona: Dolors González (local coordinator) Eva Roman, Teresa Ramon, María Poca, Mª Mar Concepción, Marta Martín, Lourdes Pérez; Hospital Xeral Cies, Vigo: Daniel Martínez (local coordinator); Fundación Pública Galega de Medicina Xenomica (FPGMX), CIBERER, Genomic Medicine Group-University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain: Ángel Carracedo (local coordinator), Clara Ruiz-Ponce, Ceres Fernández-Rozadilla, Mª Magdalena Castro; Hospital Universitario Central de Asturias: Sabino Riestra (local coordinator), Luis Rodrigo; Hospital de Galdacano, Vizcaya: Javier Fernández (local coordinator), José Luis Cabria; Fundación Hospital de Calahorra (La Rioja) La Rioja: Luis Carreño (local coordinator), Susana Quiníñena, Federico Bolado; Hospital Río Villaviciosa, Ramaguna: Elena Peña (local coordinator), José Manuel Blas, Gloria Cefía, Juan José Sebastián; Hospital Universitari Reina Sofia, Córdoba: Antonio Naranjo (local coordinator).

Author Contributions

Conceived and designed the experiments: AL. Performed the experiments: SP, JCC, MH. Contributed reagents/materials/analysis tools: AL. Analyzed the data: SP, JLB. Contributed reagents/materials/analysis tools: SP, JCC, MH, CFR, A. Carracedo, A. Castells, SCB, AN, BP, LV, HM, BTP, GS, PP, CN, SV, LL, GC, AT, SMF, IT, VM, TVW.

References

5. Larrazabal, Carlos Placer, María del Mar Ramírez, Elisabet Hijona, Jose M. Enriquez-Navascués, José L. Elosegui; Hospital General Universitario de Alicante: Artémio Payá (EPICOLON I local coordinator), Rodrigo Jover (EPICOLON II local coordinator), Cristina Alenda, Laura Sempere, Nuria Acane, Estefanía Rojas, Lucía Pérez-Carbonell; Hospital General de Granollers: Joaquim Rigau (local coordinator), Ángel Serrano, Anna Giménez; Hospital General de Vic: Joan Saló (local coordinator), Eduard Batiste-Alentorn, Josefina Autonell, Ramon Barniol; Hospital General Universitario de Guadalajara and Fundación para la Formación e Investigación Sanitarias Murcia: Ana María García (local coordinator), Fernando Carballo, Antonio Bienvenido, Eduardo Sanz, Fernando González, Jaume Sánchez, Akiko Ono; Hospital General Universitario de Valencia: Mercedes Latorre (local coordinator), Enrique Medina, Jaime Cuquerella, Pilar Canelles, Miguel Martorell, José Ángel García, Francisco Quiles, Elisa Ortiz; CHUVI-Hospital Meixoeiro, Vigo: EPICOLON I: Juan Clófent (local coordinator), Jaime Seoane, Antoni Tardó, Eugenia Sánchez; EPICOLON II Mª Luisa de Castro (local coordinator), Antoni Tardó, Juan Clófent, Vicent Hernández; Hospital Universitari Germans Trias i Pujol, Badalona and Section of Digestive Diseases and Nutrition, University of Illinois at Chicago, IL, USA: Xavier LLor (local coordinator), Rosa M. Nicolà, Marta Piñol, Mercè Rosinach, Anna Roca, Elisenda Pons, José M. Hernández, Miquel A. Gassull; Hospital Universitari Mutua de Terrassa: Fernanndo Fernández-Bañares (local coordinator), Josep M. Viver, Antonio Salas, Jorge Espiñós, Montserrat Forne, María Esteve; Hospital Universitari Arnau de Vilanova, Lleida: Josep M. Reñé (local coordinator), Carmen Piñol, Juan Buenestado, Joan Viñas; Hospital Universitario de Canarias: Enrique Quintero (local coordinator), David Nicolás, Adolfo Parra, Antonio Martí; Hospital Universitario La Fe, Valencia: Lidia Argüello (local coordinator), Vicente Pons, Virginia Pertejo, Teresa Sala; Hospital Sant Pau, Barcelona: Dolors González (local coordinator) Eva Roman, Teresa Ramon, María Poca, Mª Mar Concepción, Marta Martín, Lourdes Pérez; Hospital Xeral Cies, Vigo: Daniel Martínez (local coordinator); Fundación Pública Galega de Medicina Xenomica (FPGMX), CIBERER, Genomic Medicine Group-University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain: Ángel Carracedo (local coordinator), Clara Ruiz-Ponce, Ceres Fernández-Rozadilla, Mª Magdalena Castro; Hospital Universitario Central de Asturias: Sabino Riestra (local coordinator), Luis Rodrigo; Hospital de Galdacano, Vizcaya: Javier Fernández (local coordinator), José Luis Cabria; Fundación Hospital de Calahorra (La Rioja) La Rioja: Luis Carreño (local coordinator), Susana Quiníñena, Federico Bolado; Hospital Río Villaviciosa, Ramaguna: Elena Peña (local coordinator), José Manuel Blas, Gloria Cefía, Juan José Sebastián; Hospital Universitari Reina Sofia, Córdoba: Antonio Naranjo (local coordinator).

Author Contributions

Conceived and designed the experiments: AL. Performed the experiments: SP, JCC, MH. Contributed reagents/materials/analysis tools: AL. Analyzed the data: SP, JLB. Contributed reagents/materials/analysis tools: SP, JCC, MH, CFR, A. Carracedo, A. Castells. SCB, AN, BP, LV, HM, BTP, GS, PP, CN, SV, LL, GC, AT, SMF, IT, VM, TVW.

Received: 30 August 2013; Accepted: 22 September 2013; Published: 15 October 2013

PLOS ONE | www.plosone.org 5 September 2013 | Volume 8 | Issue 9 | e72091


