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SMAD7 Variant rs4939827 Is Associated with Colorectal Cancer Risk in Croatian Population

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Abstract

Background: Twenty common genetic variants have been associated with risk of developing colorectal cancer (CRC) in genome wide association studies to date. Since large differences between populations exist, generalisability of findings to any specific population needs to be confirmed.

Aim: The aim of this study was to perform an association study between risk variants: rs10795668, rs16892766, rs3802842 and rs4939827 and CRC risk in Croatian population.

Methods: An association study was performed on 320 colorectal cancer cases and 594 controls recruited in Croatia. We genotyped four variants previously associated with CRC: rs10795668, rs16892766, rs3802842 and rs4939827.

Results: SMAD7 variant rs4939827 (18q21.1) was significantly associated with CRC risk in Croatian population. C allele was associated with a decreased risk, odds ratio (OR): 0.70 (95% CI: 0.57-0.85, P=3.5E-04). Compared to TT homozygotes, risk was reduced by 34% in heterozygotes (OR=0.66, 95% CI: 0.47-0.92) and by 52% in CC homozygotes (OR=0.48, 95% CI: 0.33-0.72).

Conclusion: Our results show association of rs4939827 with colorectal cancer risk in Croatian population. The higher strength of the association in comparison to other studies suggests population-specific environmental or genetic factors may be modifying the association. More studies are needed to further describe role of rs4939827 in CRC. Likely reason for failure of replication for other 3 loci is inadequate study power.


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Introduction

Colorectal cancer (CRC) affects over one million people worldwide each year [1]. It is the third most common cancer and fourth leading cause of cancer mortality [2]. Whilst colorectal cancer mortality has been declining over the last two decades in Europe [3], an increase in both mortality and incidence has been reported for Croatia [4]. When compared to other European countries, Croatia is ranked 4th for mortality in both men (age-standardised rate, ASR: 25.4/100,000) and women (ASR: 13.1/100,000), but according to CRC incidence it is ranked 9th in men (ASR: 44.4/100,000) and 15th in women (ASR: 24.3/100,000) [5]. Colorectal cancer is the 2nd most common cancer and 2nd commonest cause of cancer death in Croatia [6]. Screening programs have been initiated on National level in 2005, their full impact is still expected [7].

It has been estimated that over 30% of the variation in colorectal cancer susceptibility is attributable to genetic factors and majority of this seems to be due to multiple low risk mutations [8]. To date, genome-wide association studies...
SMAD7 Polymorphism and Colorectal Cancer

(GWAS) have identified 20 independent single nucleotide polymorphisms (SNP) associated with colorectal cancer risk [9-15]. It is known that the contribution of risk alleles to CRC risk may vary between populations, for example due to allele frequencies or specific linkage disequilibrium (LD) structure, or because of particular genetic and environmental backgrounds may modify the effect of the variants [16,17]. Understanding differential effects in different populations may aid in uncovering disease mechanism, and is important for the translation of results to risk prediction in different populations.

The role of risk loci in colorectal cancer occurrence is largely unexplored in Croatian population. In this paper, we set out to investigate the role of four risk loci: rs4939827, rs10795668, rs3802842 and rs16892766, on colorectal cancer risk in Croatian population.

Methods

In the period from November 2008 to July 2009, a total of 338 colorectal cancer cases were recruited at departments of surgery in two hospitals in Zagreb, Croatia. Although both are located in Zagreb, these tertiary care hospitals attract patients from across the country. Data on age at diagnosis, sex, tumour histology and TNM stage were collected from CRC patients’ medical records and histopathology reports. Eighteen cases were excluded from the analysis because diagnosis of adenocarcinoma was not confirmed in histopathology report. Metastasis identified intraoperatively or confirmed by imaging methods were considered in evaluating disease progression. Colorectal cancer cases were classified into stages according to American Joint Committee on Cancer (AJCC) 7th edition [18].

594 controls were selected from the ‘10,001 Dalmatians’ study, an ongoing genetic study in Croatia. This study includes approximately three thousand individuals from the general population of Split and from Vis and Korčula Islands in Croatia. Controls were picked randomly, but with constrains regarding age (+/− 5 years) and sex. Participants with personal history of malignant disease were excluded from the control group.

Informed consent was obtained in writing from all participants. The study was approved by the appropriate ethics committees: the Ethics Committee of the University of Split Medical School and Ethical Board of the University Hospital Center Sestre milosrdnice.

At recruitment, peripheral blood sample was collected from each subject and stored in EDTA coated vials. DNA was extracted using standard procedures. In cases, variants rs4939827, rs3802842, rs10795668 and rs16892766 were genotyped using the TaqMan SNP Genotyping Assay. DNA samples of controls were genotyped according to the manufacturer’s instructions on Illumina Infinium SNP bead microarrays (HumanHap300v1 or HumanCNV370-Quad). Genotypes were determined using Illumina BeadStudio software. Samples with a call rate below 97% were excluded. Variants rs4939827 and rs3802842 and were represented on the array and directly genotyped while rs10795668 and rs16892766 were imputed. Imputation with reference to HapMap CEU build 36 release 22 was performed using the software MACH v1.0.15 after exclusion of SNP with MAF <0.01, call rate <98% and HWE deviation p <10-6. Imputation quality was 99% for rs10795668 and 98% for rs16892766.

Statistical Analysis

R software was used for analyses and R package GenABEL was used for manipulating genetic data. χ² test was used to assess deviations from Hardy-Weinberg equilibrium. Age- and sex-adjusted logistic regression analysis of CRC on allele dosage (0, 1 or 2) was performed to assess the association between four variants and colorectal cancer risk, under the assumption of additive genetic model. Multiple testing corrected P-value of P<0.0125 was considered representative of statistical significance.

Results

A total of 320 (40% female) colorectal cancer cases and 594 (45% female) controls were included in this study. Mean age of cases was 68.91 ± 10.08 y and of controls 66.12 ± 10.23 y. Where tumour site was known, 56% of cases had colon cancer and 44% had rectal or rectosigmoid tumour. Majority of cases had disease in AJCC stages 2 and 3 (33% and 42%, respectively). The characteristics of cases and controls are detailed in Table 1.

### Table 1. Characteristics of study population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>320 (35%)</td>
<td>594 (65%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>192 (60%)</td>
<td>324 (55%)</td>
</tr>
<tr>
<td>Female</td>
<td>128 (40%)</td>
<td>270 (45%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.91 ± 10.08</td>
<td>66.12 ± 10.23</td>
</tr>
<tr>
<td>AJCC stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>56 (18%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>107 (33%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>134 (42%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>21 (7%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (1%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>320 (100%)</td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>128 (40%)</td>
<td></td>
</tr>
<tr>
<td>rectosigmoid</td>
<td>7 (2%)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>94 (29%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>91 (28%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>320 (100%)</td>
<td></td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td>5.0 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>range (cm)</td>
<td>1-13</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampled</td>
<td>15 (IQR: 14-19)</td>
<td></td>
</tr>
<tr>
<td>positive (%)</td>
<td>5 (IQR: 0-27)</td>
<td></td>
</tr>
<tr>
<td>positive (% if 0 positive)</td>
<td>27 (IQR: 12-49)</td>
<td></td>
</tr>
</tbody>
</table>

a. only cases with 12 or more lymph nodes sampled are included (N=156)

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All four variants conformed to the Hardy-Weinberg equilibrium. In the entire sample (cases and controls) allele frequency of T allele was 49.55%. In the logistic regression model we found statistically significant association between rs4939827 and CRC risk. The proportion of each of the tumour characteristics: AJCC stage, tumour grade, tumour size and apoptosis and plays an important role in cancer initiation and progressions [21,22]. TGF-β pathway regulates growth inhibition and apoptosis and plays an important role in cancer initiation and progressions [21,22].

The mechanism by which rs4939827 may influence colorectal cancer involves inhibitory role of the SMAD7 protein and significant deviation from HWE was reported) [10]. The allele frequencies we observed at rs4939827 locus in Croatian population. This finding warrants larger studies to explore the effect allele in this population may help uncover mechanisms of colorectal cancer occurrence and progression, and may subsequently lead to translation of findings into more accurate risk prediction and better informed prevention should the interaction with environment be found.

We failed to replicate associations between CRC risk and rs10795668, rs16892766 and rs3802842; however, this is may be due to the relatively small sample size [26-29].

**Discussion**

In this study we found a significant association between SMAD7 variant rs4939827 (18q21.1) and risk of colorectal cancer in Croatian population. Compared to CC homozygotes, risk was increased by 58% in heterozygotes (OR=0.66, 95% CI: 0.47-0.92) and by 34% in heterozygotes (OR=0.66, 95% CI: 0.47-0.92) and by 52% in CC homozygotes (OR=0.48, 95% CI: 0.33-0.72) (Table 2).

We next investigated the association between genotypes at rs4939827, rs10795668, rs3802842 and tumour characteristics in an age and sex adjusted multivariate linear regression model. No statistically significant association was observed between selected variants and the following tumour characteristics: AJCC stage, tumour grade, tumour size or proportion of positive lymph nodes.

<table>
<thead>
<tr>
<th>SNP</th>
<th>genotypes</th>
<th>cases</th>
<th>controls</th>
<th>effect allele</th>
<th>OR</th>
<th>95% CI:</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10795668</td>
<td>AA/AG/GG</td>
<td>34/123/148</td>
<td>48/276/270</td>
<td>A</td>
<td>0.99</td>
<td>0.80-1.23</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16892766</td>
<td>AA/AC/CC</td>
<td>248/42/1</td>
<td>502/86/6</td>
<td>A</td>
<td>1.06</td>
<td>0.73-1.54</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>29</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3802842</td>
<td>AA/AC/CC</td>
<td>132/122/17</td>
<td>280/252/62</td>
<td>A</td>
<td>1.15</td>
<td>0.92-1.45</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>49</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4939827</td>
<td>CC/CT/TT</td>
<td>63/143/96</td>
<td>172/291/131</td>
<td>C</td>
<td>0.7</td>
<td>0.57-0.85</td>
<td>3.5x10-04</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>18</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

doi: 10.1371/journal.pone.0074042.t002
small sample size may be the reason why we fail to detect significant associations between rs4939827, rs10795668, rs3802842 and rs16892766 and tumour characteristics (AJCC stage, tumour grade, tumour size or proportion of positive lymph nodes). With the exception of rs1321311, rs3824999 and rs5934683 [15], the role of other known CRC risk loci on susceptibility to CRC in Croatian population is otherwise unexplored [9-14].

Limitations of the study include relatively small sample size that may have contributed to our limited power to detect weak associations. Secondly, while controls were mainly from Split and islands of Vis and Korčula, cases were sampled from tertiary hospitals in Zagreb. However, this is not a major issue for this study; firstly, Croatian population is small and homogeneous; secondly, Zagreb and Split represent urban centres that attract immigration from other regions in Croatia, and lastly, patients would seek treatment in Zagreb due to perceived superiority of health care irrespective of their residence.

In conclusion, a common variant rs4939827 is associated with colorectal cancer risk in Croatian population. Understanding differential effects in different populations may aid in uncovering disease mechanism and is important for the translation of results to risk prediction in different populations.

Author Contributions

Conceived and designed the experiments: IK HC DVV LZ. Performed the experiments: IK VH IS CH SŽ. Analyzed the data: IK PM GA LZ. Contributed reagents/materials/analysis tools: CH MGD DK IR. Wrote the manuscript: IK PM GA LZ. Helped with interpretation of results: NA IR DK LZ MGD.

References


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