Null Results in Brief

Colorectal Cancer Susceptibility Quantitative Trait Loci in Mice as a Novel Approach to Detect Low-Penetrance Variants in Humans: A Two-Stage Case-Control Study

Ceres Fernández-Rozadilla1, Rosa Tarrio1, Juan Clofent2, Luisa de Castro3, Alejandro Brea-Fernández1, Xavier Bessa4, Anna Abuli5, Montserrat Andreu4, Rodrigo Jover5, Rosa Xicola6, Xavier Llor6, Antoni Castells7, Sergi Castellvi-Bel7, Angel Carracedo1, and Clara Ruiz-Ponte1 for the Gastrointestinal Oncology Group of the Spanish Gastroenterological Association

Abstract

Thirty-five percent of colorectal cancer (CRC) susceptibility is thought to be attributable to genetics, but only a small proportion of the cases (<6%) can be explained by highly penetrant mutations. The rest of the susceptibility could be explained by a number of low-penetrance variants following a polygenic model of inheritance. Genetic modeling in rodents has been successful for the unraveling of the genetic basis of diseases. The investigation of mouse quantitative trait loci led to the discovery of 15 “susceptibility to colorectal cancer” (Scc) loci. Thus, we aimed to analyze the human-mouse syntenic regions defined by these Scc loci and select human candidate genes within. Twenty-one genes were chosen and their single-nucleotide polymorphisms were tested as possible low-penetrance variants predisposing to CRC risk. Our most strongly associated single-nucleotide polymorphism, rs954353, seems to be in the 5′ region of the CYR61 gene, which could implicate it in terms of the cis-regulation of the gene. CYR61 has been proposed as a connection point among signaling pathways and a probable marker for early CRC detection. However, we could not replicate the association. Despite our negative results, we believe that our candidate gene selection strategy could be quite useful in the future determination of variants predisposing to disease. Cancer Epidemiol Biomarkers Prev; 19(2); 619–23. ©2010 AACR.

Introduction

Colorectal cancer (CRC) is the second most frequent neoplasm and one of the most important morbidity causes in the developed world (1). Despite the fact that 35% of CRC susceptibility could be attributable to genetics, only a small proportion of the cases (<6%) can be explained by highly penetrant mutations, suggesting that the rest of the susceptibility should exist in the form of low-penetrance variants following a polygenic model of inheritance (2).

Genetic modeling in rodents has been proved to be an important tool in the unraveling of the genetic basis of diseases. The investigation of mouse quantitative trait loci (QTL) to identify chromosomal regions harboring genetic variants that affect susceptibility successfully led to the discovery of 15 “susceptibility to colorectal cancer” (Scc) loci (3, 4). Because there is increasing evidence that causal genes underlying disease QTLs are conserved between rodents and humans (5), a sensible approach to identify these genes would be to map them in mice and, subsequently, investigate the role of their human homologues. Hence, our aim is to analyze the human-mouse syntenic regions defined by these Scc loci and select human candidate genes to screen their single-nucleotide polymorphisms (SNP) and test them as possible low-penetrance variants predisposing to CRC risk in a two-stage case-control study.

Materials and Methods

Study Populations

Subjects on stage I were 515 CRC cases and 515 controls from EPICOLON I, a prospective, multicenter, population-based epidemiology study (6). Subjects on stage II (933 cases and 955 controls) belonged to...
<table>
<thead>
<tr>
<th>QTL</th>
<th>Mouse chr</th>
<th>Human gene</th>
<th>Human mapping</th>
<th>Gene description</th>
<th>Gene ontology</th>
<th>SNPs analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scc1</td>
<td>2</td>
<td>PTPRJ</td>
<td>11p11.2</td>
<td>Protein tyrosine-phosphatase receptor type J</td>
<td>Regulation of cellular growth, differentiation and oncogenic transformation</td>
<td>rs10742827; rs100838801; rs10838810; rs11039519; rs1503185; rs1566734; rs2270992; rs2270993; rs4752904; rs7117386; rs7123436; rs7947811</td>
</tr>
<tr>
<td>Scc2</td>
<td>2</td>
<td>CRB2</td>
<td>9q33.2</td>
<td>Crumbs homolog 2</td>
<td>Polarized cell morphogenesis</td>
<td>rs10818812; rs1105223; rs1891632; rs1891638; rs33984675; rs4838051; rs7033144; rs884320</td>
</tr>
<tr>
<td>Scc3</td>
<td>1</td>
<td>TGFB2</td>
<td>1q41</td>
<td>Transforming growth factor β2</td>
<td>Suppressive effects on interleukin-2–dependent T-cell growth</td>
<td>rs10863396; rs1539399; rs17558745; rs1890994; rs1891467; rs2000220; rs2796821; rs4846476; rs4846479</td>
</tr>
<tr>
<td>Scc4</td>
<td>17</td>
<td>PRKD3</td>
<td>2p22-p21</td>
<td>Protein kinase D3</td>
<td>Receptor of phorbol esters: a class of tumor promoters</td>
<td>rs10177176; rs10460627; rs1056021; rs11124575; rs11887618; rs2300880; rs2300771; rs2300894; rs2302650; rs3770761</td>
</tr>
<tr>
<td>Scc5</td>
<td>18</td>
<td>TNFAIP8</td>
<td>5q23.1</td>
<td>Tumor necrosis factor α–induced protein 8</td>
<td>Negative mediator of apoptosis with a role in tumor progression</td>
<td>rs10077888; rs1045241; rs1045242; rs11064; rs17385413; rs3203922; rs32658; rs3797339; rs3797345</td>
</tr>
<tr>
<td>Scc6</td>
<td>11</td>
<td>EGFR</td>
<td>7p12</td>
<td>Epidermal growth factor receptor</td>
<td>Cell growth and differentiation control</td>
<td>rs1015793; rs1050171; rs1140475; rs11487218; rs11971997; rs12538489; rs12671550; rs17172446; rs17290169; rs17337023; rs2072454; rs2293347; rs3800827; rs4947942; rs4947971; rs6593205; rs6972246; rs759170; rs759171; rs7796139; rs7809394; rs88425</td>
</tr>
<tr>
<td>Scc7</td>
<td>3</td>
<td>CYR61</td>
<td>1p31-p32</td>
<td>Cysteine-rich 61</td>
<td>Promotes cell proliferation, chemotaxis, angiogenesis, and cell adhesion</td>
<td>rs12086058; rs12239954; rs1576424; rs3753793; rs721471; rs954353; rs9658584</td>
</tr>
<tr>
<td>Scc8</td>
<td>8</td>
<td>TFDP1</td>
<td>13q34</td>
<td>Transcription factor Dp-1</td>
<td>Regulation of the expression of cellular promoters</td>
<td>rs2316121; rs6577068; rs9577286</td>
</tr>
<tr>
<td>CDC16</td>
<td>13q34</td>
<td>Cell division cycle 16 homolog</td>
<td>Ubiquitin ligase with role in cell cycle control</td>
<td>rs3211416; rs7318644; rs7994151; rs8002514; rs9590408; rs9590409</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
### Table 1. Description of the 15 Scc loci and the selected genes within the human-mouse QTL syntenic regions (Cont’d)

<table>
<thead>
<tr>
<th>QTL</th>
<th>Mouse chr</th>
<th>Human gene</th>
<th>Human mapping</th>
<th>Gene description</th>
<th>Gene ontology</th>
<th>SNPs analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scc9</td>
<td>10</td>
<td>MDM2</td>
<td>12q14.3-q15</td>
<td>Transformed 3T3 cell double minute 2</td>
<td>p53 inhibitor</td>
<td>rs1470383; rs1795481; rs769412</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LGR5</td>
<td>12q22-q23</td>
<td>Leucine-rich repeat–containing G-protein–coupled receptor 5</td>
<td>Overexpressed in human colon tumors</td>
<td>rs10748178; rs10784923; rs11178798; rs11178832; rs11178845; rs1148985; rs12422259; rs12829521; rs17109799; rs17109924; rs17109926; rs1880892; rs3803033; rs389150; rs3923863; rs7298504; rs941197</td>
</tr>
<tr>
<td>Scc11</td>
<td>4</td>
<td>HEYL</td>
<td>1p34.3</td>
<td>Hairy/enhancer-of-split related with YRPW motif-like</td>
<td>Downstream effector of Notch signaling that networks together with Wnt</td>
<td>rs1180320; rs4660892; rs784622</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MYCL1</td>
<td>1p34.2</td>
<td>V-myc myelocytomatosis viral oncogene homolog 1</td>
<td>Loss of heterozygosity at MYCL1 is a marker for poor prognosis in CRC</td>
<td>rs3117088; rs3134614; rs3134615</td>
</tr>
<tr>
<td>Scc12</td>
<td>7</td>
<td>DMBT1</td>
<td>10q25.3-26</td>
<td>Deleted in malignant brain tumors 1</td>
<td>Role in the interaction of tumor cells and the immune system</td>
<td>rs1051715; rs2981783; rs3013236</td>
</tr>
<tr>
<td>Scc13</td>
<td>6</td>
<td>TRAF2</td>
<td>9q34</td>
<td>TNF receptor–associated factor 2</td>
<td>Regulates TNF-induced apoptosis</td>
<td>rs10870140; rs2784078; rs2784075; rs908831</td>
</tr>
<tr>
<td>Scc14</td>
<td>10</td>
<td>LATS1</td>
<td>6q24-q25.1</td>
<td>Large tumor suppressor homolog 1 (Drosophila)</td>
<td>Maintenance of ploidy and tumor suppressor activity through regulation of p53</td>
<td>rs3798761; rs3924871</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIP</td>
<td>6q25</td>
<td>Vasoactive intestinal peptide</td>
<td>Proangiogenic factor</td>
<td>rs12212849; rs3823082; rs637572; rs671330; rs680314; rs688136</td>
</tr>
<tr>
<td>Scc15</td>
<td>11</td>
<td>LLGL1</td>
<td>17p11.2</td>
<td>Lethal giant larvae homolog 1 (Drosophila)</td>
<td>Reduced expression related to progression of colon cancer; similar to a tumor suppressor in Drosophila</td>
<td>rs11869582; rs2245430; rs2245737; rs2290505; rs2746027; rs8821</td>
</tr>
<tr>
<td>Ccs1</td>
<td>12</td>
<td>FOS</td>
<td>14q24.3</td>
<td>v-fos FBJ murine osteosarcoma viral oncogene homolog</td>
<td>Signal transduction protein implicated in cell proliferation and differentiation</td>
<td>rs1046117; rs1569328; rs3742769; rs7101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JDP2</td>
<td>14q24.3</td>
<td>Jun dimerization protein 2</td>
<td>Mediator in UV-induced apoptosis, cell differentiation, tumorigenesis, and angiogenesis</td>
<td>rs10057; rs10873278; rs1474503; rs175644; rs4899566; rs84044</td>
</tr>
</tbody>
</table>

**NOTE:** For some of the Scc loci, more than one gene was selected because of their possible functional implications.

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**EPICOLON II**, an extension of EPICOLON I. Cases and controls were matched for sex and age. All samples were obtained with informed consent reviewed by the ethical board of the corresponding hospital.

**Candidate Gene Selection**

QTLs were defined by their flanking markers by revision of the author’s data and the MGI (7). Genes within each human-mouse syntenic region showing enriched...
expression in primary affected tissues in mice were selected with ExQuest (8). Finally, 21 human genes were chosen from the 15 Scn (Table 1; ref. 9).

SNP Selection and Genotyping

One hundred forty-seven SNPs were selected from the 21 genes with PupaSuite (10), FESD (11), dbSNP (12), and HapMap Phase II (genome build 36; ref. 13). SNPs with unadjusted P values <0.01 were replicated in an independent case-control series. Genotyping was done in the SNPlex (Applied Biosystems), MassARRAY (Sequenom, Inc.), and TaqMan (Applied Biosystems) platforms at the Santiago de Compostela node of the Spanish Genotyping Center.

Statistical Analyses

Quality control was assessed with the Genotyping Data Filter (14) and Structure v2.2 (15). Genotypic distributions in controls followed Hardy-Weinberg equilibrium, and there was no sign of underlying population stratification. Association was evaluated for every single SNP and all possible haplotypes in each gene with Haploview v4.0 (16) and Unphased (17). Permutation tests and Bonferroni were used for multiple-testing corrections. Odds ratio (OR) and 95% confidence intervals were calculated with PLINK v1.03 (18). Descriptive information and association data for all the SNPs that passed quality control are shown in Supplementary Table S1.

Results

Allelic association tests revealed only one significant SNP after multiple-testing correction: rs12086058, lying in an intergenic region 6.4 kb upstream the CYR61 gene (1p31-p22). The OR value for this SNP showed a protective effect of the minor allele (Table 2). Haplotypes in an intergenic region 6.4 kb upstream the CYR61 gene (1p31-p22). The OR value for this SNP showed a protective effect of the minor allele (Table 2). Haplotype analysis was performed in the SNPlex (Applied Biosystems), MassARRAY (Sequenom, Inc.), and TaqMan (Applied Biosystems) platforms at the Santiago de Compostela node of the Spanish Genotyping Center.

To verify the results, SNPs with nominal $P < 0.01$ (rs12086058, rs954353, and rs10077888) were further replicated on an independent sample. Nevertheless, none of the associations could be replicated (Table 2).

Discussion

Our study combines the advances in CRC genetics in animal models with the investigation of the variation underlying the disease in humans. We selected 21 genes identified from synteny regions defined by mouse QTLs to screen their SNP variability in a two-stage case-control association study. However, we did not find any replicable association. Our study had enough power to detect OR ≥1.3, assuming allelic association and $\alpha = 0.05$ (19). Results in stage I were therefore simply due to chance or to type I error.

Nevertheless, our most strongly associated SNP, rs954353, seems to be in the 5′ region of the CYR61 gene, which could still implicate it in terms of cis-regulation. We analyzed the region harboring rs954353 and found it to be lying very close to two transcription factor binding site sequences. The direct sequencing of these failed to find any common variants within the consensus target that could explain the association signal found in stage I. However, we did find a 6-bp insertion polymorphism 38 bp upstream the first transcription factor binding site. This variant showed significant differences in frequencies between cases and controls ($P = 0.0236$, although no further implications could be stated about its relationship with CRC susceptibility (data not shown).

CYR61 has been proposed as a connection point among signaling pathways and a probable marker for early CRC detection (20). Besides, it has been extensively implicated in carcinogenesis-related events such as angiogenesis (21), tissue invasion (22), cell migration, and metastasis (23), although no association studies have been published thus far that analyze its relationship with CRC.

Despite our negative results, we believe that our candidate gene selection, through the identification of genes or regions conferring susceptibility to other species, could be quite useful in the future determination of variants predisposing to disease. Our QTLs analyses proved to be very helpful as a starting point in the search for candidate genes affecting CRC susceptibility because all the genes identified were somehow related to carcinogenic events.

Table 2. Association analyses for the three SNPs selected for replication on stage II

<table>
<thead>
<tr>
<th>SNP_ID</th>
<th>Gene</th>
<th>Relevance</th>
<th>Alleles</th>
<th>Observed MAF</th>
<th>OR (95% CI)</th>
<th>Stage I $\chi^2$ df $P$</th>
<th>Stage II permutations $P$</th>
<th>Bonferroni $P$</th>
<th>Stage II $\chi^2$ df $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12086058</td>
<td>CYR61</td>
<td>5′ UTR</td>
<td>A/G</td>
<td>0.428</td>
<td>0.71 (0.59-0.86)</td>
<td>0.0005</td>
<td>0.0326</td>
<td>0.0405</td>
<td>0.4099</td>
</tr>
<tr>
<td>rs954353</td>
<td>CYR61</td>
<td>5′ UTR</td>
<td>A/G</td>
<td>0.434</td>
<td>0.70 (0.59-0.84)</td>
<td>0.0002</td>
<td>0.0246</td>
<td>0.027</td>
<td>0.3917</td>
</tr>
<tr>
<td>rs10077888</td>
<td>TNFAIP8</td>
<td>Intronic</td>
<td>C/G</td>
<td>0.302</td>
<td>0.75 (0.61-0.92)</td>
<td>0.0019</td>
<td>0.2058</td>
<td>0.2565</td>
<td>0.8188</td>
</tr>
</tbody>
</table>

Abbreviations: MAF, minor allele frequency; 95% CI, 95% confidence interval; UTR, untranslated region.
In fact, although this approach has not been successful thus far for CRC, it positively identified a haplotype in PTPrj as a breast cancer genetic susceptibility low-penetration allele (24). Hence, we encourage future efforts in this field and believe that the relationship between CYR61 and CRC should be studied in other populations to fully discard a putative genetic association.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

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