Lack of an Association between the TGFBR1*6A Variant and Colorectal Cancer Risk

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Abstract Purpose: Recently a common variant of the TGFBR1 gene, TGFBR1*6A, has been proposed to act as a low-penetrance tumor susceptibility allele for colorectal cancer, but data from published studies with individually low statistical power are conflicting. To further evaluate the relationship between TGFBR1*6A and colorectal cancer risk, we have conducted a large case-control study and a meta-analysis of previously published studies.

Experimental Design: A total of 1,042 colorectal cancer cases and 856 population controls were genotyped for the TGFBR1*6A polymorphism. Previously published case-control studies of the relationship between TGFBR1*6A and colorectal cancer were identified, and a meta-analysis was conducted.

Results: We found no evidence that homozygosity, heterozygosity or carrier status for the TGFBR1*6A allele confers an increased risk of colorectal cancer; respective odds ratios (OR) were 1.05 [95% confidence interval (95% CI), 0.83-1.32], 0.82 (95% CI, 0.34-1.99), and 0.92 (95% CI, 0.74-1.15), respectively. A meta-analysis of our case-control study and seven other studies that provided data on 2,627 colorectal cancer cases and 3,387 controls also yielded no evidence that possession of the TGFBR1*6A allele is associated with an elevated risk of colorectal cancer; pooled estimate of the OR were 1.20 (95% CI, 0.64-2.24) for homozygosity, 1.11 (95% CI, 0.96-1.29) for heterozygosity, and 1.13 (95% CI, 0.98-1.30) for carriers of TGFBR1*6A.

Conclusion: Current data provide limited support for the hypothesis that sequence variation in TGFBR1 defined by the TGFBR1*6A allele confers an elevated risk of colorectal cancer.

Colorectal cancer is the third most common cause of cancer-related mortality in the Western world, and in the United States, it represents the second most common cause of cancer death (1). A recent twin study indicates that about 35% of all colorectal cancers can be ascribed to an inherited susceptibility (2). Mendelian predisposition syndromes associated with mutations in known genes (such as APC, DNA mismatch repair genes, MTH, SMAD4, BMPR1A/ALK3, and STK11/LKB1), however, together account for only ~5% of the overall incidence (3). The nature of the remaining heritability is at present undefined, but a model in which high-risk alleles account for all of the excess inherited risk seems improbable. An attractive hypothesis concerning the allelic architecture of colorectal cancer susceptibility proposes that part of the inherited risk is conferred by common, low-penetration alleles.

Transforming growth factor β (TGF-β) is a potent inhibitor of cell growth influencing the behavior of a number of cancers (4). TGF-β mediates its action through a heteromeric cell-surface complex of two types of transmembrane serine/threonine kinases; TGF-β receptor type 1 (TGFBR1) and type 2 (TGFBR2; refs. 5, 6). Unrestricted cell growth due to lack of growth inhibitory activity. The nature of the remaining heritability is at present undefined, but a model in which high-risk alleles account for all of the excess inherited risk seems improbable. An attractive hypothesis concerning the allelic architecture of colorectal cancer susceptibility proposes that part of the inherited risk is conferred by common, low-penetrance alleles.

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Materials and Methods

Patients
A total of 1,042 unselected colorectal cancer cases were consecutively ascertained through 12 hospitals serving the Stockholm-Gotland and Uppsala-Orebro health-care regions in Sweden. Eight hundred and fifty-six blood donors from the same geographic area were used as a source of population controls. All blood samples and clinical information were obtained from subjects with informed consent and local ethical review board approval in accordance with the tenets of the Declaration of Helsinki.

Genotyping
The TGFBR1*6A variant was determined by PCR amplification using fluorescent primers Fwd 5’-GAGCCAGGTGGTCTGGGTACGG-3’ and Rev 5’-CATGGTGGAGAAGGACAGGGAGG-3’. Amplification was done using the Platinum Taq DNA polymerase and supplied protocol for GC-rich fragments (Invitrogen). Amplified fragments were separated by electrophoresis on an ABI 377 semiautomated DNA Sequencer (Applied Biosystems) and genotypes assigned using GENESCAN and GENOTYPER software (Applied Biosystems). A product size of ~247 bp corresponded to the *6A allele (Fig. 1). All *6A homozygotes and *6A/*9A heterozygotes and all samples with rare alleles were retyped after a second independent PCR amplification to confirm the allele calling. Further details on genotyping can be obtained from the authors.

Systematic review and meta-analysis

Selection criteria. Studies were eligible if they were based on unrelated individuals and examined the association between colorectal cancer and the presence of the TGFBR1*6A variant. Only studies published as full-length articles or letters in peer-reviewed journals in English were included in the analysis. For duplicate publications the smaller data set was excluded.

Data extraction. Data for analyses, including study design, sample size, ethnicity, and allele and genotype frequencies were abstracted from published articles and summarized in a consistent manner to aid comparison. Authors of published articles were contacted to resolve ambiguity regarding distribution of genotypes in cases or controls.

Statistical methods. Risks associated with TGFBR1 genotypes were estimated by odds ratios (ORs) using unconditional logistic regression, and associated 95% confidence intervals (95% CI) were computed. To test for population stratification, the distribution of genotypes in controls was tested for a departure from Hardy-Weinberg equilibrium. Differences in the distribution of proportions were assessed by Fisher’s exact test. Meta-analysis was conducted using standard methods for combining estimates of ORs based on the weighted sum of the log estimates with the inverse of the variance of the estimate of the weight. Cochran’s Q statistic to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated. To incorporate within-study and between-study variability, we used DerSimonian and Laird’s method (10) for calculating random-effects summary ORs and their associated 95% CIs. The presence of publication bias was examined by visual inspection of Forrest plots and formally evaluated with Egger’s regression asymmetry test (11), based on inverse-variance−weighted regression of the effect sizes on their precision (the inverse of SE) testing whether the intercept deviates significantly from zero. Estimates of study power were done on the basis of the method published by Fleiss et al. (12). A P value of 0.05 was considered statistically significant in all analyses. Computations were undertaken using the statistical software STATA Version 7.0 (Stata Corporation).

Results

Case-control study. The frequency of *9A/*9A, *9A/*6A, and *6A/*6A genotypes in cases and controls were not significantly different between cases and controls, 827 (79.4%), 203 (19.5%), and 10 (1.0%) and 682 (79.7%), 160 (18.7%), and 10 (1.2%) respectively (P = 0.78). Four rare alleles, *5A, *7A, *10A, and *11A, all reported previously (13, 14), were detected in six individuals, both cases and controls. The observed frequencies of TGFBR1*6A genotypes in controls were in accordance with Hardy-Weinberg laws of equilibrium (P = 0.86, respectively), providing no evidence of population stratification within the data set. ORs associated with hetero- and homozygosity for the TGFBR1*6A allele and carrier status were 1.05 (95% CI, 0.83-1.32), 0.82 (95% CI, 0.34-1.99), and 0.92 (95% CI, 0.74-1.15), respectively. Genotype distribution among cases with documented family history of colorectal cancer did not differ from the whole data set.

Fig. 1. Representative electropherograms of TGFBR1 genotypes. From top, *9A/*9A, *6A/*9A, and *6A/*6A. Shown below each peak is the fragment length in base pairs and corresponding number of alanine repeats. Right, vertical scale displays fragment quantity in terms of peak height.
Systematic review and meta-analysis. Four previously published articles reporting TGFBR1*6A genotype and risk of colorectal cancer were identified from the literature (7–9, 13). Two of these provided data on more than one case-control study. The study by Pasche et al. (13) provided data on two separate case-control studies, the first based on subjects from New York City and the second on subjects from northern Italy. A subsequent meta-analysis reported by Pasche et al. in 2004 (7)

Table 1. TGFBR1 exon 1 genotypes in colorectal cancer cases and controls from all studies

<table>
<thead>
<tr>
<th>Place of study</th>
<th>Ethnicity</th>
<th>TGFBR1 exon 1 genotype</th>
<th>Cases (n = 112)</th>
<th>Controls (n = 735)</th>
<th>Cases (n = 65)</th>
<th>Controls (n = 50)</th>
<th>Cases (n = 252)</th>
<th>Controls (n = 362)</th>
<th>Cases (n = 117)</th>
<th>Controls (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasche et al. (US) (13)</td>
<td>New York, NY</td>
<td>9A/9A, number (%)</td>
<td>90 (80.4)</td>
<td>654 (89.0)</td>
<td>57 (87.7)</td>
<td>38 (76.0)</td>
<td>202 (80.2)</td>
<td>295 (81.5)</td>
<td>108 (92.3)</td>
<td>179 (89.5)</td>
</tr>
<tr>
<td>Pasche et al. (It) (13)</td>
<td>Northern Italy</td>
<td>9A/6A, number (%)</td>
<td>17 (15.2)</td>
<td>78 (10.6)</td>
<td>8 (12.3)</td>
<td>12 (24.0)</td>
<td>46 (18.3)</td>
<td>58 (16.0)</td>
<td>8 (0.6)</td>
<td>20 (10.0)</td>
</tr>
<tr>
<td>Stefanovska et al. (8)</td>
<td>Celtic</td>
<td>6A/6A, number (%)</td>
<td>4 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (0.8)</td>
<td>5 (1.4)</td>
<td>1 (0.9)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Samowitz et al. (9)</td>
<td>NA</td>
<td>9A/9A, number (%)</td>
<td>90 (87.7)</td>
<td>654 (89.0)</td>
<td>57 (87.7)</td>
<td>38 (76.0)</td>
<td>202 (80.2)</td>
<td>295 (81.5)</td>
<td>108 (92.3)</td>
<td>179 (89.5)</td>
</tr>
<tr>
<td>Samowitz et al. (9)</td>
<td>NA</td>
<td>9A/6A, number (%)</td>
<td>17 (15.2)</td>
<td>78 (10.6)</td>
<td>8 (12.3)</td>
<td>12 (24.0)</td>
<td>46 (18.3)</td>
<td>58 (16.0)</td>
<td>8 (0.6)</td>
<td>20 (10.0)</td>
</tr>
<tr>
<td>Stefanovska et al. (8)</td>
<td>Macedonian</td>
<td>6A/6A, number (%)</td>
<td>4 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (0.8)</td>
<td>5 (1.4)</td>
<td>1 (0.9)</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available.
*Ethnicity; 80% Caucasian, 10% Hispanic, 5% African-American, 5% Asian.
Ethnicity; 90% Swedish origin, 10% non-Swedish origin (suggested by name).
Included in the total number are rare TGFBR1 alleles such as *5A, *7A, *8A, *10A, *11A, and *12A.

Fig. 2. Forrest plot of the risk of colorectal cancer associated with (A) TGFBR1*6A homozygosity (from comparison of *6A/*6A and *9A/*9A); (B) TGFBR1*6A heterozygosity.
included data on three studies; by Ellis et al., Caldes et al., and from the Northwestern Cancer Genetics Program. Together with our study, the final data set for the meta-analyses thus included eight case-control studies (Table 1). These studies provided data on TGFBR1*6A genotypes in a total of 2,627 colorectal cancer cases and 3,387 controls. Four of the eight studies provided ethnicity data on study participants (Table 1). Two studies (8, 13) recruited hospital-based rather than population-based controls. There was a wide variation in the allele frequency in the control groups across different populations, ranging from 0.05 in the United States to 0.12 in an Italian population (Table 1).

Figure 2 shows estimates of the risk of colorectal cancer associated with TGFBR1*6A homozygosity, heterozygosity, and carrier status in each study. Overall, only two of the eight case-control studies included show an association of the TGFBR1*6A variant with an increased risk of colorectal cancer. In the U.S. study reported by Pasche et al. (13), an increased risk was seen in homozygous, but not in heterozygous carriers of the variant. In the study by Caldes et al., reported by Pasche et al. (7), an increased risk was seen in heterozygous variant carriers only. No association with any increased risk was seen in European populations.

Figure 2 also shows the pooled OR estimates of colorectal cancer under a fixed-effects model. The pooled estimate of the OR under the fixed-effect model was 1.20 (95% CI, 0.64-2.24; Cochran’s Q = 9.89; P = 0.129; I² = 39%) for homozygosity, 1.11 (95% CI, 0.96-1.29; Cochran’s Q = 9.28, P = 0.23, I² = 25%) for heterozygosity, and 1.13 (95% CI, 0.98-1.30; Cochran’s Q = 12.63; P = 0.082; I² = 45%) for carriers of TGFBR1*6A alleles. Under a random-effects model, summary estimates changed very little; corresponding pooled ORs were 1.58 (95% CI, 0.61-4.06), 1.12 (95% CI, 0.93-1.35), and 1.15 (95% CI, 0.92-1.43), respectively. Visual inspection of funnel plots for publication bias provided little evidence of overt publication bias toward studies reporting increased risk of colorectal cancer associated with the TGFBR1*6A genotype, a conclusion supported by Egger’s test (P values of 0.12, 0.63 and 0.81, respectively, for risk estimates associated with homo-, heterozygosity, and carrier status in the studies).

**Discussion**

Given the pivotal role of TGFBR1 in cellular function and impact of perturbation of this axis in colorectal cancer, it is entirely plausible that variation in TGFBR1 may influence colorectal cancer risk. Based on data analysis of colorectal cancer cases and controls from the United States, it was originally proposed that the TGFBR1*6A variant confers a 1.6-fold increase in risk of colorectal cancer. Substantial research has been carried out on this topic, including a comprehensive meta-analysis of eight case-control studies (7, 8, 13). These studies provided data on TGFBR1*6A genotypes in a total of 2,627 colorectal cancer cases and 3,387 controls. Four of the eight studies provided ethnicity data on study participants, with wide variation in allele frequency across different populations.

Figure 2 illustrates the risk estimates of colorectal cancer associated with TGFBR1*6A homozygosity, heterozygosity, and carrier status in each study. Only two studies showed an association of the TGFBR1*6A variant with increased risk of colorectal cancer. In the U.S. study by Pasche et al. (13), increased risk was observed only in homozygous carriers. In the study by Caldes et al., reported by Pasche et al. (7), increased risk was observed only in heterozygous carriers. No association was observed in European populations.

The pooled OR estimates under a fixed-effects model showed a pooled OR of 1.20 (95% CI, 0.64-2.24) for homozygosity, 1.11 (95% CI, 0.96-1.29) for heterozygosity, and 1.13 (95% CI, 0.98-1.30) for carriers of TGFBR1*6A alleles. Under a random-effects model, these estimates changed minimally, with corresponding ORs of 1.58, 1.12, and 1.15, respectively. Funnel plots for publication bias did not show significant evidence of publication bias toward studies reporting increased risk.

**Table 1.** TGFBR1 exon 1 genotypes in colorectal cancer cases and controls from all studies (Cont’d)

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases (n = 767)</th>
<th>Controls (n = 767)</th>
<th>Cases (n = 237)</th>
<th>Controls (n = 294)</th>
<th>Cases (n = 1,042)</th>
<th>Controls (n = 856)</th>
<th>Cases (n = 2,627)</th>
<th>Controls (n = 3,387)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellis et al. (7)</td>
<td>655 (85.4)</td>
<td>663 (82.5)</td>
<td>183 (77.2)</td>
<td>250 (85.0)</td>
<td>105 (85.4)</td>
<td>682 (79.7)</td>
<td>2,152 (81.9)</td>
<td>2,866 (84.6)</td>
</tr>
<tr>
<td>Caldes et al. (7)</td>
<td>108 (14.1)</td>
<td>100 (13.0)</td>
<td>50 (21.1)</td>
<td>42 (14.3)</td>
<td>5 (14.3)</td>
<td>17 (13.8)</td>
<td>445 (16.9)</td>
<td>487 (14.4)</td>
</tr>
<tr>
<td>Caldes et al. (7)</td>
<td>4 (0.5)</td>
<td>2 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (1.0)</td>
<td>23 (0.9)</td>
<td>20 (0.6)</td>
</tr>
</tbody>
</table>

Fig. 2 Continued. C. TGFBR1*6A carrier status. Shaded squares, ORs, with their size being proportional to the inverse variance of the study-specific estimate. Horizontal lines, 95% CIs. Diamond, combined effects estimate and its associated 95% CI. Vertical line, null effect (OR = 1.0). No *6A/6A* genotypes were observed by Pasche et al. (Italy), and therefore, no OR could be calculated for homozygosity (A).

C

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds ratio (95% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northwestern 2006</td>
<td>0.97 (0.33,2.84)</td>
<td>1.8</td>
</tr>
<tr>
<td>Pasche et al 199 Italy</td>
<td>0.44 (0.17,1.19)</td>
<td>2.1</td>
</tr>
<tr>
<td>Stefanovskaya et al 2001</td>
<td>0.71 (0.31,1.61)</td>
<td>3.0</td>
</tr>
<tr>
<td>Pasche et al 199 US</td>
<td>1.56 (1.15,3.32)</td>
<td>7.2</td>
</tr>
<tr>
<td>Caldes et al 2006</td>
<td>1.69 (1.08,2.85)</td>
<td>10.0</td>
</tr>
<tr>
<td>Samowitz et al 2001</td>
<td>1.11 (0.73,1.69)</td>
<td>11.7</td>
</tr>
<tr>
<td>Ellis et al 2006</td>
<td>1.10 (0.82,1.47)</td>
<td>24.3</td>
</tr>
<tr>
<td>This study</td>
<td>1.03 (0.82,1.29)</td>
<td>39.8</td>
</tr>
<tr>
<td>Overall (95% CI)</td>
<td>1.13 (0.98,1.30)</td>
<td></td>
</tr>
</tbody>
</table>
out evaluating the relationship between polymorphic variation in TGFBR1 and colorectal cancer risk. Has this been worthwhile? It is not uncommon for the first small published studies to report overinflated estimates of risk or effects, which subsequent larger studies cannot replicate. Although our study had ~80% power to detect a 1.4-fold increase in risk at a significance level of 5%, we found no evidence to support the hypothesis that TGFBR1*6A acts as a colorectal cancer susceptibility allele. Moreover, the meta-analysis we conducted of 5,993 subjects also provides no evidence of an increased colorectal cancer risk associated with the TGFBR1*6A variant.

The frequency of the TGFBR1*6A allele is different between populations; hence, it may be inappropriate to regard the different ethnic groups genetically similar and population-specific linkage disequilibrium with a functional variant could be operating. It is, however, noteworthy that much of the evidence for the proposal that the TGFBR1*6A allele influences colorectal cancer risk comes from the U.S. study reported by Pasche et al. (13), which is based on an analysis of only 112 cases of mixed ethnicity. Population stratification is acknowledged to be a source of confounding in association studies and is a particular issue in studies of low-frequency variants when based on relatively small sample sets. Finally, as with all association studies, a simple but unattractive explanation for the observed heterogeneity is publication bias.

A recent study found the TGFBR1*6A variant to be somatically acquired in 29.5% of liver metastases from colorectal cancer (15). From the patients used in our case-control study, no tumor tissue was available for genotype analysis. Therefore, although our study does not provide evidence for any increased risk of colorectal cancer development in germ line carriers, we cannot rule out an effect on growth and metastasizing capabilities in tumors.

In summary, collectively, current data provide no support for the hypothesis that sequence variation in TGFBR1 defined by the TGFBR1*6A allele confers an elevated risk of colorectal cancer per se.

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References

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