Several case-control studies have reported an association (6). From this sample we selected the 53 individuals and family members and exclusion criteria were as follows: presence of the TGFBR1*6A allele increasing the risk of colorectal neoplasia (7, 8). To determine whether the TGFBR1*6A allele is responsible for the observed linkage at 9q22.2-3.1, we genotyped 53 kindreds from the Colon Neoplasia Sibling Study with colon cancer or advanced adenomas for the presence of the TGFBR1*6A or TGFBR1*9A allele. We then examined the evidence for linkage and allelic association with the TGFBR1*6A allele.

Ascertainment and Phenotyping. Ascertainment of individuals and family members and exclusion criteria were as previously described (6). From this sample we selected the 53 original kindreds used to identify the linkage signal on chromosome 9 (6). We utilized all siblings within a sibship and classified the individuals as affected, unaffected, or unlinked as previously described (6).

Statistical Methods. Genotypes from the TGFBR1 locus (genotyping details available from the authors) along with the markers from the original genome scan (6) were used to obtain multipoint probabilities that sib-pairs shared zero, one, or two alleles identical by descent. These were used to determine whether study subjects were “linked” or “unlinked” to chromosome 9q22.2-3.1. In the absence of linkage, all sibship pairs are expected to share one allele identical by descent; in the presence of linkage, we expect increased allele sharing between concordant sib-pairs (i.e., >1) and decreased allele sharing between discordant sib-pairs (i.e., <1). We considered affected individuals as linked if they shared two alleles identical by descent with another affected sib with probability of ≥0.85 or shared zero or one allele identical by descent with another affected sibling with probability of ≥0.95 or if they shared two alleles identical by descent with an unlinked sibling with a probability of ≥0.70 (n = 16). A final group comprised the individuals for whom identity by descent could not be so clearly distinguished, and these individuals were classified as unlinked (n = 63).

We refined our Haseman-Elston regression analysis (9-11) by including the number of TGFBR1*6A alleles (0, 1, 2, 3, or 4) for each pair of sibs as a covariate in a multiple regression equation to determine if the TGFBR1*6A genotypes could account for the observed linkage signal. This provides a direct means to test for allelic association that, if there is linkage, is attributable to TGFBR1*6A or an allele in linkage disequilibrium with it. Reported P values were confirmed by comparison to a Monte Carlo sample of the permutation distribution created by permuting the allele sharing values relative to the pair labels (concordant or discordant). The number of permutations done, both across sibships of the same size and within sibships (always between 2,308 and 126,580), was sufficient to assure with 95% confidence that the estimated P value was within 5% of the true P value. Family-based tests of association (12-14) were also done.

Results and Discussion

The TGFBR1*6A allele was identified in 21% of affected siblings and in 28% of unaffected siblings (Table 1). The distribution of the TGFBR1*6A genotypes in the linked and unlinked affected individuals showed that proportionately more 9A/6A heterozygotes (4 of 16, 25%) were in the affected unlinked group than in the affected linked group (4 of 36, 11%; Table 1). This proportion changed little when individuals with small adenomas or late onset colon cancer were classified as affected (25% unlinked group versus 16% linked; see Table 1). Among all affected and unaffected individuals (parents and offspring) there is no significant difference in the percentage of subjects with one or more TGFBR1*6A alleles (Table 1). In fact, more siblings who had normal colon screening examinations had one or more TGFBR1*6A alleles than the siblings affected with colorectal cancer or advanced adenomas (28% versus 21%, respectively).

Inclusion of the TGFBR1 genotypes into the Haseman-Elston regression analysis resulted in a reduction of the linkage signal at D17S1786, which is the most significant marker from the...
original genome scan and is within 2cM of $TGFBR1$ (Table 2a and b) rather than as an increase, as would be expected for a susceptibility variant. To determine whether including individuals with small adenomas ($n = 27$) or colon cancer after the age of 65 ($n = 2$) would change our results, we repeated the analysis with them as affected. This resulted in further loss of signal at the $TGFBR1^*6A$ site ($P = 0.16$).

The results of adding the number of $TGFBR1^*6A$ alleles as a pair-specific covariate in the Haseman-Elston regression analysis are shown in Table 2c.

The allele sharing for $TGFBR1$ alleles was the sole disease-causing variant in our sample, which is the hypothesis being tested. Although our analysis cannot exclude the possibility of the $TGFBR1^*6A$ allele increasing the risk of colorectal neoplasia in some populations, it can definitely be excluded as a susceptibility allele at the nearby linked locus we found on chromosome 9. A recent study validating linkage of colorectal cancer to chromosome 9q also excluded the $TGFBR1^*6A$ allele as a disease-causing variant in that sample (8). We conclude that there remains an unidentified susceptibility locus in the region 9q22.2-31.2.

Acknowledgments

We thank Christine Ticknor for the development of the assay used to genotype and distinguish the $TGFBR1^*6A$ and $*9A$ alleles.

References


Table 1. Distribution of genotypes among linked and unlinked affected siblings and among affected and unaffected individuals

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>9A/9A</th>
<th>9A/6A</th>
<th>6A/6A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linked</td>
<td>31 (86%)</td>
<td>4 (11%)</td>
<td>1 (3%)</td>
<td>36</td>
</tr>
<tr>
<td>Unlinked</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Unknown</td>
<td>53 (87%)</td>
<td>8 (13%)</td>
<td>0</td>
<td>61*</td>
</tr>
<tr>
<td>Total</td>
<td>96 (85%)</td>
<td>16 (14%)</td>
<td>1 (1%)</td>
<td>113</td>
</tr>
</tbody>
</table>

Distribution including individuals with small adenomas or late onset colon cancer as affected individuals

<table>
<thead>
<tr>
<th>Genotypes in affected individuals</th>
<th>9A/9A</th>
<th>9A/6A</th>
<th>6A/6A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linked</td>
<td>42 (82%)</td>
<td>8 (16%)</td>
<td>1 (2%)</td>
<td>51</td>
</tr>
<tr>
<td>Unlinked</td>
<td>21 (75%)</td>
<td>7 (25%)</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Unknown</td>
<td>33 (97%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>96 (85%)</td>
<td>16 (14%)</td>
<td>1 (1%)</td>
<td>113</td>
</tr>
</tbody>
</table>

Genotypes in affected and unaffected individuals

<table>
<thead>
<tr>
<th>Genotypes in affected individuals</th>
<th>9A/9A</th>
<th>9A/6A</th>
<th>6A/6A</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected individuals</td>
<td>90</td>
<td>24</td>
<td>1</td>
<td>115</td>
</tr>
<tr>
<td>All unaffected individuals</td>
<td>36</td>
<td>12</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>Affected siblings</td>
<td>88</td>
<td>23</td>
<td>1</td>
<td>112</td>
</tr>
<tr>
<td>Unaffected siblings</td>
<td>26</td>
<td>10</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>

*Two individuals in the unknown subgroup failed for genotyping at the $TGFBR1$ gene.

† Indicates percentage of sample with one or more 6A alleles.

Table 2. Haseman-Elston regression linkage analysis of 9q22.2-31.2 in 53 kindreds with colon cancer and colon adenomatous polyps

<table>
<thead>
<tr>
<th>Marker</th>
<th>(a) Original estimates*</th>
<th>(b) Including $TBFBR1$ genotypes †</th>
<th>(c) Including $TGFBR1^*6A$ alleles (0, 1, 2, 3, or 4) as a pair-specific covariate at D9S1786</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>SE</td>
<td>$P$</td>
</tr>
<tr>
<td>D9S283</td>
<td>0.1584</td>
<td>0.0583</td>
<td>0.0036</td>
</tr>
<tr>
<td>D9S1786</td>
<td>0.1796</td>
<td>0.0539</td>
<td>0.0005</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>0.1772</td>
<td>0.0554</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

NOTE: (a) original multipoint identical by descent estimates without the $TGFBR1^*6A$ and $TGFBR1^*9A$ genotypes. (b) multipoint identical by descent estimates with the $TGFBR1^*6A$ and $TGFBR1^*9A$ genotypes. (c) regression analysis including $TGFBR1^*6A$ alleles as a covariate.

Abbreviation: NA, not available.

*Analysis included the 17 polymorphic markers used in the original linkage study of this region to estimate the Haseman-Elston regression coefficient $\beta$.

†Assessment using the original 17 polymorphic region and the $TBFBR1^*6A$ and $9A$ genotypes to estimate $\beta$ (7).

The Haseman-Elston regression coefficient $\beta$ estimates the amount of trait variation due to a gene at this location. The covariate regression coefficient $\beta$ in (c) estimates the effect of the number of $TBFBR1*6A$ alleles from 0 to 4 that are carried by a pair of sibs on being affected.

SE associated with the estimate of $\beta$.

The allele sharing for $TGFBR1$ is interpolated based upon the allele sharing at the 17 polymorphic markers but does not actually use the $TGFBR1$ genotypes in the original estimates.
Is TGFBR1*6A a Susceptibility Allele for Nonsyndromic Familial Colorectal Neoplasia?

Denise Daley, Wendi Morgan, Susan Lewis, et al.


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