Renal cell carcinoma accounts for ~3% of all human malignancies and is the 10th leading cause of male cancer death in the United States (1). Genetic aberrations have been associated with the etiology of sporadic renal cell carcinoma. For example, loss of chromosome 3p and VHL gene mutations were frequently identified in conventional renal cell carcinomas, and MET mutations were observed in papillary type renal cell carcinomas (2). However, renal cell carcinoma is recognized as a heterogeneous disease, in terms of its presentation, pathology, and clinical course. Moreover, the underlying molecular and genetic mechanisms for renal cell carcinoma initiation and development have largely remained unclear.

MicroRNAs (miRNA) are a class of small noncoding RNA molecules ~20 nucleotides (nt) in length. MiRNAs regulate gene expression in animals and plants through binding to the 3’ untranslated region (UTR) of the mRNAs of their target genes and leading to mRNA cleavage or translation repression (3). Numerous recent studies have shown that alteration of miRNAs plays a critical role in cancer development (3, 4) and progression (5). The pre-miRNAs are then exported into the cytoplasm by the nuclear RNase DROSHA within the microprocessor complex also including DGCR8 produces the 70- to 100-nt pre-miRNAs. The pre-miRNAs are then exported into the cytoplasm by the Exportin-5/Ran-GTP complex (6) and cleaved by DICER aspart 18. The pre-miRNA is then processed by Dicer to shorter 21-23 nt mature miRNAs, which are then incorporated into the RNA-induced silencing complex (RISC) for target mRNA degradation (7). The RNA-induced silencing complex is made up of a mature miRNA (as a passenger strand), an Ago protein (Dicer-generated), and a group of proteins (e.g. TARBP2 and AGO2) which together form the RNA-induced silencing complex's loading complex (6) and cleaved by DICER aspart 18. The pre-miRNA is then processed by Dicer to shorter 21-23 nt mature miRNAs, which are then incorporated into the RNA-induced silencing complex (RISC) for target mRNA degradation (7).

miRNAs. Aberrant expression of miRNAs contributes to the etiology of many common human diseases including cancer (3). Numerous recent studies have shown that alteration of miRNAs plays a critical role in cancer development (3, 4) and progression (5). The pre-miRNAs are then exported into the cytoplasm by the nuclear RNase DROSHA within the microprocessor complex also including DGCR8 produces the 70- to 100-nt pre-miRNAs. The pre-miRNAs are then exported into the cytoplasm by the Exportin-5/Ran-GTP complex (6) and cleaved by DICER aspart 18. The pre-miRNA is then processed by Dicer to shorter 21-23 nt mature miRNAs, which are then incorporated into the RNA-induced silencing complex (RISC) for target mRNA degradation (7). The RNA-induced silencing complex is made up of a mature miRNA (as a passenger strand), an Ago protein (Dicer-generated), and a group of proteins (e.g. TARBP2 and AGO2) which together form the RNA-induced silencing complex's loading complex (6) and cleaved by DICER aspart 18. The pre-miRNA is then processed by Dicer to shorter 21-23 nt mature miRNAs, which are then incorporated into the RNA-induced silencing complex (RISC) for target mRNA degradation (7).
Translational Relevance

This study suggests that common polymorphisms in microRNA (miRNA)-machinery genes might modify renal cell carcinoma risk individually and jointly. These findings support the hypothesis that dysregulated miRNA processing pathway might influence renal cell carcinoma tumorigenesis. Although the results presented in this study have a limited value at this time, they could help us to assess individual susceptibility to renal cell carcinoma and could be useful information to build a comprehensive risk assessment model for renal cell carcinoma in the future. In addition, these results will contribute to the elucidation of how disruption of miRNA biogenesis pathway could lead to cancer initiation and development.

expression of DICER modified the development of lung and prostate cancers (6, 10, 11). Several argonaute proteins of the RNA-induced silencing complex were associated with Wilms’ tumor (3). An argonaute gene, HIWI, which is the human orthologue of the Drosophila Argonaute gene PIWI, is linked to testicular germ-tumors (12). Taken together, these emerging lines of evidence suggest that miRNA machinery proteins may play crucial roles in cancer development and progression.

Although single nucleotide polymorphisms (SNP) have been widely implicated in cancer development and treatment response, such evidence is lacking for miRNA-related genes. Although SNPs in miRNA regions have been reported to be rare and unlikely to be functionally important (13), recent studies have implicated that nucleotide variations within the seed sequence on miRNA might affect miRNA processing and lead to reduced miRNA expression (14, 15). Therefore, it is possible that SNPs in miRNA machinery genes and miRNA-containing genomic regions play an important role in cancer development.

In this case-control study, we evaluated the effects of 40 selected potentially functional SNPs and their haplotypes in miRNA machinery genes as well as in pri- and pre-miRNAs on renal cell carcinoma predisposition. We also took a polygenic approach to assess the cumulative effects of these SNPs. To our knowledge, this is the first study investigating associations between miRNA-related polymorphisms and renal cell carcinoma susceptibility.

Materials and Methods

Study population. Incident renal cell carcinoma cases were recruited from The University of Texas M.D. Anderson Cancer Center in Houston, Texas, where staff interviewers identified renal cell carcinoma cases through a daily review of computerized appointment schedules for the Departments of Urology and Genitourinary Medical Oncology. All cases were individuals with newly diagnosed, histologically confirmed renal cell carcinoma. There were no age, gender, ethnicity, or cancer stage restrictions on recruitment. To be eligible, the cases must be residents of Texas. Healthy control subjects without a history of cancer, except nonmelanoma skin cancer, were identified and recruited using the random digit dialing method. In random digit dialing, randomly selected phone numbers from households were used to contact potential control volunteers in the same residency of the cases according to the telephone directory listings. Controls must have lived in the same county or socioeconomically matched surrounding counties in Texas where the case resides for at least 1 y and have no prior history of cancer. The controls were frequency matched to the cases by age (±5 y), sex, ethnicity, and county of residence. This population-based renal cell carcinoma case-control study started in 2002 and is currently ongoing. A total of 677 subjects were included in this analysis.

Epidemiologic data collection. For both cases and controls, after obtaining written informed consent, trained staff interviewers of the University of Texas M.D. Anderson Cancer Center administered a 45-min risk factor questionnaire to study participants. Data were collected on demographic characteristics (age, gender, ethnicity, etc.), occupation history, tobacco use history, medical history, and family history of cancer. At the end of the interview, a 40-mL blood sample was drawn into coded heparinized tubes and delivered to laboratory for molecular analysis. The study was approved by the Institutional Review Boards of the University of Texas M.D. Anderson Cancer Center.

SNP selection. Through an extensive mining of the databases of the International HapMap Project, dbSNP, and miRBase registry, we identified 40 potential functional polymorphisms: 23 SNPs in 11 genes in the miRNA biogenesis pathway, 7 SNPs in 7 pre-miRNAs, and 10 SNPs in 8 pri-miRNAs (Table 1). All SNPs have a reported minor allele frequency of >0.01 in Caucasians. In the miRNA biogenesis pathway, except for two AGO1 SNPs (rs6366832 and n5959561) located in introns, all other polymorphisms reside in functional regions, including exons, UTRs, and promoters (within 2 kb of the genes). In the case of multiple potentially functional SNPs within the same haplotype block (defined by the linkage coefficient r² > 0.8), only one SNP was included. All SNPs identified from the pre-miRNA regions were included if the minor allele frequency was >0.01 in Caucasians. For SNPs in pri-miRNAs but not in pre-miRNAs, because we identified >200 such SNPs with a minor allele frequency of >0.01 in Caucasians, we included 10 SNPs from eight pri-miRNAs whose mature counterparts have been extensively implicated in cancer etiology or clinical outcome.

Genotyping. DNA was isolated from peripheral blood using QIAamp DNA extraction kit (Qiagen). SNP genotyping was done using the SNPlex technology (Applied Biosystems), based on an oligonucleotide ligation assay combined with multiplex PCR target amplification, following the manufacturer's recommendations. All pre-PCR steps were done on a cooled block. Reactions were carried out in the dual-384-well GeneAmp 9700 Thermocycler (Applied Biosystems). Allelic discrimination was done through capillary electrophoresis analysis, using a 3730xl DNA sequencer (Applied Biosystems). Obtained data were analyzed using GeneMapper v3.7 (Applied Biosystems). Internal quality controls and negative controls were used to ensure genotyping accuracy, and 5% of all samples were randomly selected and genotyped in duplicate with 100% concordance.

Statistical analysis. Statistical analyses were carried out using Stata 8.0 statistical software package (Stata Corp.). Pearson’s χ² test was used to test the differences of categorical variables such as gender and smoking status between cases and controls. Student’s t test was used to test for differences in continuous variables. The Hardy-Weinberg Equilibrium was determined using the goodness-of-fit χ² test to compare the observed frequency with the expected frequency in both cases and controls. Renal cell carcinoma risks were estimated as odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional multivariate logistic regression adjusted for age, gender, and smoking status (never and ever smoking). Haplotypes were inferred using the PHASE software version 2.1.1 (16). Haplotypes with a probability of <95% were excluded from the final analysis. The adjusted OR and 95% CI for each haplotype were calculated using multivariate logistic regression using the most abundant haplotype as the reference
In addition to single SNP analysis and haplotype analysis, we also analyzed the association between total number of unfavorable genotypes and renal cell carcinoma risk. An unfavorable genotype was defined as a SNP showing at least a borderline statistical significance in the single SNP analysis. The unfavorable genotypes were collapsed into three groups according to the tertiles (low-, medium, and high-risk) of the number of unfavorable genotypes in controls. Using the low-risk group as the reference group, we calculated the ORs and 95% CIs for the other subgroups using multivariate logistic regression adjusted for age, gender, and smoking status. All P values were two-sided, with P < 0.05 considered the threshold of significance.

Results

Subject characteristics. There were a total of 677 study subjects recruited. The population consisted of 557 Caucasians (82.0%), 90 Mexican Americans (13.0%), and 30 African Americans (4.0%). Among Caucasians, there were 279 renal cell carcinoma patients and 278 controls (Table 2). There was no significant difference in age (P = 0.845) and gender (P = 0.976). No significant difference was observed between cases and controls with regard to cigarette consumption (P = 0.538). The majority of patients (71.0%) only had the conventional clear cell carcinoma. Papillary carcinoma was present in 32 (11.5%) patients, and 9 patients (3.2%) had chromophobe carcinoma. In addition, there were 17 (6.1%) clear cell carcinoma patients who also had either papillary or chromophobe carcinoma. Approximately 45% of patients were in stage I whereas 11.1%, 20.4%, and 22.9% of patients were found to have stage II, III, and IV diseases, respectively. In addition, the majority (68.8%) of patients had a high-grade disease (grade 3 or 4; Table 2).

Main effects on renal cell carcinoma risk by individual polymorphisms. Because most subjects were Caucasian, we focused on this population for risk analysis. The overall renal cell carcinoma patients and 278 controls (Table 2). There was no significant difference in age (P = 0.845) and gender (P = 0.976). No significant difference was observed between cases and controls with regard to cigarette consumption (P = 0.538). The majority of patients (71.0%) only had the conventional clear cell carcinoma. Papillary carcinoma was present in 32 (11.5%) patients, and 9 patients (3.2%) had chromophobe carcinoma. In addition, there were 17 (6.1%) clear cell carcinoma patients who also had either papillary or chromophobe carcinoma. Approximately 45% of patients were in stage I whereas 11.1%, 20.4%, and 22.9% of patients were found to have stage II, III, and IV diseases, respectively. In addition, the majority (68.8%) of patients had a high-grade disease (grade 3 or 4; Table 2).

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Table 1. miRNA-related genes and polymorphisms evaluated in this study

<table>
<thead>
<tr>
<th>Gene name (gene symbol)</th>
<th>SNP ID</th>
<th>Position</th>
<th>Major/minor allele</th>
<th>MAF* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DROSHA</td>
<td>rs10719</td>
<td>3'UTR</td>
<td>C/T</td>
<td>23</td>
</tr>
<tr>
<td>rs6877842</td>
<td>3'UTR</td>
<td>G/C</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>rs417309</td>
<td>3'UTR</td>
<td>G/A</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>rs3757</td>
<td>3'UTR</td>
<td>G/A</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>rs1640299</td>
<td>3'UTR</td>
<td>G/T</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Exportin 5 (XPO5)</td>
<td>rs11077</td>
<td>3'UTR</td>
<td>A/C</td>
<td>40</td>
</tr>
<tr>
<td>ras-related nuclear protein (RAW)</td>
<td>rs14035</td>
<td>3'UTR</td>
<td>C/T</td>
<td>12</td>
</tr>
<tr>
<td>DICER1</td>
<td>rs3742330</td>
<td>3'UTR</td>
<td>A/G</td>
<td>12</td>
</tr>
<tr>
<td>rs13078</td>
<td>3'UTR</td>
<td>T/A</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Tar RNA-binding protein 2 (TARB2)</td>
<td>rs784567</td>
<td>5'UTR</td>
<td>C/T</td>
<td>48</td>
</tr>
<tr>
<td>Euukaryotic translation initiation factor 2C (AGO1)</td>
<td>rs636832</td>
<td>Introns</td>
<td>G/A</td>
<td>9</td>
</tr>
<tr>
<td>Argonoute 2 (AGO2)</td>
<td>rs4961280</td>
<td>Promoter</td>
<td>C/A</td>
<td>13</td>
</tr>
<tr>
<td>Gem-associated protein 4 (GEMIN4)</td>
<td>rs910924</td>
<td>Promoter</td>
<td>C/T</td>
<td>35</td>
</tr>
<tr>
<td>rs2740348</td>
<td>Asn929Asp</td>
<td>G/C</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>rs7813</td>
<td>Cys1033Arg</td>
<td>C/T</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>rs3744741</td>
<td>Gin684Arg</td>
<td>C/T</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>rs1062923</td>
<td>Thr731Ile</td>
<td>T/C</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>rs4968104</td>
<td>Val593Glu</td>
<td>T/A</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Gem-associated protein 3 (GEMIN3)</td>
<td>rs197414</td>
<td>Ser693Arg</td>
<td>C/A</td>
<td>19</td>
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<tr>
<td>rs197388</td>
<td>Promoter</td>
<td>T/A</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>rs197412</td>
<td>Thr636Ile</td>
<td>T/C</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>HIWI</td>
<td>rs1106042</td>
<td>Lys527Arg</td>
<td>G/A</td>
<td>8</td>
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<tr>
<td>Pre-miRNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mir416a</td>
<td>rs2910164</td>
<td>Pre-miRNA</td>
<td>G/C</td>
<td>24</td>
</tr>
<tr>
<td>mir196a-2</td>
<td>rs11614913</td>
<td>Pre-miRNA</td>
<td>C/T</td>
<td>44</td>
</tr>
<tr>
<td>mir423</td>
<td>rs6505162</td>
<td>Pre-miRNA</td>
<td>C/A</td>
<td>43</td>
</tr>
<tr>
<td>mir492</td>
<td>rs2289300</td>
<td>Pre-miRNA</td>
<td>C/G</td>
<td>8</td>
</tr>
<tr>
<td>mir604</td>
<td>rs2363992</td>
<td>Pre-miRNA</td>
<td>C/T</td>
<td>17</td>
</tr>
<tr>
<td>mir631</td>
<td>rs4919510</td>
<td>Pre-miRNA</td>
<td>C/G</td>
<td>8</td>
</tr>
<tr>
<td>Pri-miRNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>let7f-2</td>
<td>rs17276588</td>
<td>5'Region</td>
<td>G/A</td>
<td>2</td>
</tr>
<tr>
<td>mir26a-1</td>
<td>rs7372209</td>
<td>5'Region</td>
<td>C/T</td>
<td>44</td>
</tr>
<tr>
<td>mir30a</td>
<td>rs1358379</td>
<td>5'Region</td>
<td>A/G</td>
<td>4</td>
</tr>
<tr>
<td>mir30c-1</td>
<td>rs16827546</td>
<td>5'Region</td>
<td>C/T</td>
<td>4</td>
</tr>
<tr>
<td>mir100</td>
<td>rs1834306</td>
<td>5'Region</td>
<td>C/T</td>
<td>44</td>
</tr>
<tr>
<td>mir124a-1</td>
<td>rs531564</td>
<td>5'Region</td>
<td>C/G</td>
<td>12</td>
</tr>
<tr>
<td>mir219-1</td>
<td>rs107822</td>
<td>5'Region</td>
<td>G/A</td>
<td>23</td>
</tr>
<tr>
<td>mir373</td>
<td>rs1298273</td>
<td>5'Region</td>
<td>C/T</td>
<td>13</td>
</tr>
<tr>
<td>rs10425222</td>
<td>3'Region</td>
<td>C/A</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*Minimum allele frequency in Caucasians.

Cell line. In addition to single SNP analysis and haplotype analysis, we also analyzed the association between total number of unfavorable genotypes and renal cell carcinoma risk. An unfavorable genotype was defined as a SNP showing at least a borderline statistical significance in the single SNP analysis. The unfavorable genotypes were collapsed into three groups according to the tertiles (low-, medium, and high-risk) of the number of unfavorable genotypes in controls. Using the low-risk group as the reference group, we calculated the ORs and 95% CIs for the other subgroups using multivariate logistic regression adjusted for age, gender, and smoking status. All P values were two-sided, with P < 0.05 considered the threshold of significance.
cell carcinoma risks associated with the individual polymorphisms are listed in Table S1. Three SNPs (DROSHA rs10719, mir196a-2 rs11614913, and let7f-2 rs17276588) showed a significant deviation from Hardy-Weinberg Equilibrium in the controls, and were excluded from further analyses. Overall, five SNPs exhibited at least borderline significance with renal cell carcinoma risk (Table 3). Most significant effects were observed in GEMIN4. For GEMIN4 rs2740348, compared

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Genotype</th>
<th>In all patients</th>
<th>In patients with clear cell renal cell carcinoma*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Case/Control</td>
<td>OR (95% CI)  1</td>
</tr>
<tr>
<td><strong>XPO5</strong> (rs11077)</td>
<td>3’UTR</td>
<td>AA/AC</td>
<td>222/239</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>54/38</td>
<td>1.55 (0.98-2.44)</td>
</tr>
<tr>
<td><strong>AGO1</strong> (rs595961)</td>
<td>Intron</td>
<td>AA</td>
<td>202/186</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG/GG</td>
<td>75/72</td>
<td>0.74 (0.51-1.07)</td>
</tr>
<tr>
<td><strong>GEMIN4</strong> (rs2740348)</td>
<td>Exon 2</td>
<td>GG</td>
<td>192/168</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC/CC</td>
<td>84/110</td>
<td>0.67 (0.47-0.96)</td>
</tr>
<tr>
<td><strong>GEMIN4</strong> (rs7813)</td>
<td>Exon 2</td>
<td>TT</td>
<td>96/75</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC/CC</td>
<td>181/203</td>
<td>0.68 (0.47-0.98)</td>
</tr>
<tr>
<td><strong>GEMIN3</strong> (rs197412)</td>
<td>Exon11</td>
<td>TT</td>
<td>97/115</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC/CC</td>
<td>180/163</td>
<td>1.31 (0.93-1.85)</td>
</tr>
</tbody>
</table>

*In 215 patients with conventional clear cell renal cell carcinoma.

1 Adjusted for age, gender, and smoking status.
with the homozygous wild-type (GG) genotype, the GC+CC genotype exhibited a significantly reduced risk of renal cell carcinoma (OR, 0.67; 95% CI, 0.47-0.96; \( P = 0.027 \)). In stratified analysis, this risk remained significant in male subjects (OR, 0.62; 95% CI, 0.40-0.95; \( P = 0.021 \)) and ever smokers (OR, 0.53; 95% CI, 0.32-0.87; \( P = 0.012 \); Supplementary Table S2). For GEMIN4 rs7813, the variant allele-containing genotypes exhibited a reduced renal cell carcinoma risk (OR, 0.68; 95% CI, 0.47-0.96; \( P = 0.039 \)). The risk remained significant in male subjects (OR, 0.55; 95% CI, 0.35-0.86; \( P = 0.009 \)). In male subjects, the AG+GG genotypes of AGO1 rs595961 had a significant protective effect compared with the AA genotype (OR, 0.59; 95% CI, 0.38-0.93; \( P = 0.023 \); Supplementary Table S2). We also conducted stratified analyses in 215 patients with the conventional clear cell renal cell carcinoma histology (Table 3). We found that the protective effect conferred by the variant-containing genotypes of GEMIN4 rs7813 remained significant in clear cell patients (OR, 0.60; 95% CI, 0.45-0.98; \( P = 0.039 \)). For the other four SNPs that showed at least a borderline significance in the main analysis, although their risk associations did not reach statistical significance, possibly due to the reduced patient size, they all exhibited the same direction of risk alteration as that in the main analysis (Table 3).

### Discussion

In this study, we found significant associations between SNPs in the miRNA biogenesis pathway and the risk of renal cell carcinoma. Recent studies have shown that disrupting miRNA processing through the knockdown of DROSHA, DGC8, and DICER1, could accelerate cellular transformation and tumori-

### Table 4. Haplotype analysis for selected genes in Caucasians

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Cases/controls</th>
<th>OR (95%CI)*</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DGCR8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (www)</td>
<td>242/234</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2 (wmm)</td>
<td>148/156</td>
<td>0.89 (0.66-1.20)</td>
<td>0.459</td>
</tr>
<tr>
<td>H3 (wmm)</td>
<td>124/130</td>
<td>0.92 (0.67-1.25)</td>
<td>0.591</td>
</tr>
<tr>
<td>H4 (www)</td>
<td>38/36</td>
<td>1.00 (0.62-1.61)</td>
<td>0.996</td>
</tr>
<tr>
<td><strong>DGCR4</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>H1 (ww)</td>
<td>403/414</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2 (wm)</td>
<td>103/86</td>
<td>1.23 (0.89-1.70)</td>
<td>0.218</td>
</tr>
<tr>
<td>H3 (mw)</td>
<td>32/36</td>
<td>0.92 (0.55-1.53)</td>
<td>0.746</td>
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<tr>
<td><strong>GEMIN4</strong></td>
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<tr>
<td>H1 (wwww)</td>
<td>473/454</td>
<td>Reference</td>
<td>0.439</td>
</tr>
<tr>
<td>H2 (wmm)</td>
<td>51/58</td>
<td>0.85 (0.51-1.28)</td>
<td>0.099</td>
</tr>
<tr>
<td>H3 (mm)</td>
<td>30/42</td>
<td>0.66 (0.41-1.08)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>GEMIN3</strong></td>
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<td></td>
</tr>
<tr>
<td>H1 (wmmm)</td>
<td>118/104</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2 (wmwmw)</td>
<td>125/130</td>
<td>0.82 (0.57-1.18)</td>
<td>0.29</td>
</tr>
<tr>
<td>H3 (wwmmw)</td>
<td>89/119</td>
<td>0.66 (0.45-0.97)</td>
<td>0.035</td>
</tr>
<tr>
<td>H4 (wwwwm)</td>
<td>72/72</td>
<td>0.95 (0.63-1.44)</td>
<td>0.815</td>
</tr>
<tr>
<td>H5 (wwwwm)</td>
<td>84/80</td>
<td>0.92 (0.61-1.39)</td>
<td>0.69</td>
</tr>
<tr>
<td>Others</td>
<td>13/7</td>
<td>1.55 (0.61-3.93)</td>
<td>0.358</td>
</tr>
<tr>
<td><strong>mir 219-1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (www)</td>
<td>330/361</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2 (wmm)</td>
<td>108/95</td>
<td>1.24 (0.90-1.71)</td>
<td>0.195</td>
</tr>
<tr>
<td>H3 (mm)</td>
<td>60/55</td>
<td>1.22 (0.82-1.81)</td>
<td>0.331</td>
</tr>
<tr>
<td>H4 (wmm)</td>
<td>46/40</td>
<td>1.29 (0.81-2.04)</td>
<td>0.284</td>
</tr>
<tr>
<td>Others</td>
<td>8/3</td>
<td>2.85 (0.74-11.03)</td>
<td>0.129</td>
</tr>
<tr>
<td><strong>mir 373</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 (www)</td>
<td>437/465</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>H2 (wmm)</td>
<td>76/73</td>
<td>1.10 (0.76-1.57)</td>
<td>0.618</td>
</tr>
<tr>
<td>H3 (mm)</td>
<td>15/14</td>
<td>1.10 (0.54-2.22)</td>
<td>0.801</td>
</tr>
</tbody>
</table>

* ORs were adjusted for age, gender, and smoking status.
** Order of SNPs: rs147309, rs3757, rs1640299, with w being the major allele and m being the minor allele.
† Order of SNPs: rs3742330, rs13078.
†† Order of SNPs: rs636832, rs595961.
‡‡ Order of SNPs: rs910924, rs2740348, rs7813, rs3744741, rs1062923, rs4968104.
§§ Order of SNPs: rs197414, rs197388, rs197412.
### Order of SNPs: rs197414, rs197412.
implicated in the etiology of spinal muscular atrophy (9). In addition, these GEMIN proteins have been identified in miRNA ribonucleoprotein particle with an Argonaute family protein AGO2 (9). The additional identification of numerous miRNAs in this complex (8, 9), concordant with several other independent observations (22), strongly suggests the involvement of GEMIN proteins in the processing of miRNA precursors through their interaction with key components of the RNA-induced silencing complex. Interestingly, Wan et al. found that genetic variants of GEMIN4 (including rs2740348 and rs7813) were significantly associated with cell growth and DNA repair in the heptacellular carcinoma cell line (23), suggesting that the amino acid changes caused by these SNPs might have a physiologic significance on cancer development. Moreover, our recent study on bladder cancer has shown the association between an altered risk and GEMIN4 rs7813 polymorphism (24). However, whether the associations between the SNPs of GEMIN4 and altered renal cell carcinoma risks observed in our study are due to a similar mechanism needs to be examined with further functional assays.

In addition to the SNPs on the GEMIN genes, borderline significant associations with renal cell carcinoma risk were also observed in two genes, the XPO5 and AGO1 genes (Table 3). In particular, the XPO5 rs110707 exhibited an increased risk of renal cell carcinoma in the recessive model. XPO5 mediates the nuclear transport of pre-miRNAs and its down-regulation results in reduced miRNA levels (25). Down-regulated XPO5 have been observed in low-grade lung adenocarcinoma (11), whereas XPO5 have been shown to be up-regulated in high-grade prostate cancer (6). AGO1 (EIF2C1), a component of RNA-induced silencing complex with AGO2 and DICER1, is involved in miRNA function leading to target mRNA degradation. This gene is located at chromosome 1p35-p34 frequently lost in human malignancies (26).

The SNPs on pre- or pri-miRNA regions were evaluated in our study, but none of them had a significant influence on renal cell carcinoma risk. Diederichs and Haber explored the sequence variations in miRNA-containing genomic regions and showed that although sequence variants in miRNA precursor regions may lead to changes of secondary structures, miRNA maturation was not affected in vivo (27), suggesting that genetic variants in miRNA precursors are unlikely to have physiologic significances (27). Saunders et al. identified 65 SNPs in 474 pre-miRNAs using the public SNP database (13). Many of these SNPs, however, may not be important to population genetics because of the lack of frequency data. This observation supports predictions that genetic variants in pre-miRNA regions are rare and unlikely to be functionally important, possibly due to the constraint imposed by natural selection on the evolutionarily conserved pre-miRNA sequences (13). In contrast, several germline and somatic mutations were identified on pre- and pri-miRNA regions in patients with chronic lymphocytic leukemia and these mutations might influence cell transformation and cancer development (28). Furthermore, it was reported that polymorphisms on miRNA sequences could affect miRNA production through the influence on the function of DROSHA (29). Therefore, although we could not identify any significant association with renal cell carcinoma risk, we could not exclude the possibility that genetic variations in miRNAs might have a potential regulatory effect on renal cell carcinoma tumorigenesis because of only a limited number of SNPs examined. Further studies are warranted to assess the effects using a more comprehensive collection of miRNA SNPs.

The comprehensive list of potentially functional SNPs in most currently known miRNA biogenesis genes constructed in our study can be readily used by independent researchers for replication studies of different cancer sites. It is possible that some associations we found in this study are chance findings. Nonetheless, we sought to more powerfully elucidate the influence of these SNPs on renal cell carcinoma susceptibility using a pathway-based polygenic approach, and identified a trend toward an increasing renal cell carcinoma risk with an increasing number of unfavorable genotypes that occurred in a dose-dependent manner. This finding reinforces the notion that renal cell carcinoma is a polygenic process and thus a combined analysis of multiple variants may have a greater ability to characterize high-risk populations. Further epidemiologic and functional studies in a larger population are warranted to validate these results.

In conclusion, our study provides the first epidemiologic evidence supporting an association between miRNA-related genes and renal cell carcinoma risk. Our results imply that individual as well as combined genotypes of miRNA processing pathway genes might influence renal cell carcinoma tumorigenesis.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
References
