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Single nucleotide polymorphisms of microRNA-machinery genes modify the risk of renal cell carcinoma

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Abstract

Purpose—MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that have been implicated in a wide diversity of basic cellular functions through post-transcriptional regulations on their target genes. Compelling evidence has shown that miRNAs are involved in cancer initiation and progression. We hypothesized that genetic variations of the miRNA-machinery genes could be associated with the risk of renal cell carcinoma (RCC).

Experimental Design—We genotyped 40 single nucleotide polymorphisms (SNPs) from 11 miRNA processing genes (*DROSHA, DGCR8, XPO5, RAN, DICER1, TARBP2, EIF2C1, AGO2, GEMIN3, GEMIN4, HIWI*) and 15 miRNA genes in 279 Caucasian patients with RCC and 278 matched controls.

Results—We found that two SNPs in the *GEMIN4* gene were significantly associated with altered RCC risks. The variant containing genotypes of the Asn929Asp and Cys1033Arg exhibited a significantly reduced risk with an odds ratio [OR] of 0.67 (95% confidence interval [CI], 0.47–0.96) and 0.68 (95% CI, 0.47–0.98), respectively. Haplotype analysis showed that a common haplotype of the *GEMIN4* was associated with a significant reduce in risk of RCC (OR, 0.66; 95% CI, 0.45–0.97). We also conducted a combined unfavorable genotype analysis including five promising SNPs showing at least a borderline significant risk association. Compared with the low-risk reference group within one unfavorable genotype, the median-risk and high-risk group exhibited a 1.55-fold (95% CI, 0.96–2.50) and a 2.49-fold (95% CI, 1.58–3.91) increased risk of RCC, respectively (*P* for trend <0.001).

Conclusion—Our results suggested that genetic polymorphisms of the miRNA-machinery genes may impact RCC susceptibility individually and jointly.

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Clinical relevance

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

Conflict of Interest Statement. The authors declare that they have no competing financial interest.

Keywords

renal cell carcinoma; microRNA-machinery gene; single nucleotide polymorphism; cancer susceptibility; molecular epidemiology

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 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 RCC. For example, loss of chromosome 3p and *VHL* gene mutations were frequently identified in conventional RCCs and *MET* mutations in papillary type RCCs (2). However, RCC is recognized as a heterogeneous disease, concerning its presentation, pathology, and clinical course. Moreover, the underlying molecular and genetic mechanisms for RCC initiation and development have largely remained unclear.

MicroRNAs (miRNA) are a class of small non-coding RNA molecules with ~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). It is predicted that approximately 30% of human genes are regulated by miRNAs. Aberrant expression of miRNAs contributes to the etiology of many common human diseases including cancer (3). Numerous recent studies have demonstrated that alteration of miRNAs play a critical role in cancer development (3,4) through regulating the expressions of proto-oncogenes or tumor suppressor genes (3–5).

MiRNA genes are first transcribed by RNA polymerase into pre-miRNAs with several hundred nucleotides. Processing of pri-miRNAs by the nuclear RNase DROSHA within the microprocessor complex also including DGCR8 produces the 70–100 nt pre-miRNAs. The pre-miRNAs is then exported into the cytoplasm by the Exportin-5/Ran-GTP complex (6) and cleaved by RNaseβ/DICER as part of the RNA-induced silencing complex (RISC) loading complex including TRBP and AGO2 (7). This complex also includes GEMIN3 and GEMIN4 and contributes to both miRNA processing and target gene silencing (8,9).

The aberrations of miRNA biogenesis pathway have been associated with several types of cancer. For example, altered expression of DICER modified the development of lung and prostate cancers (6,10,11). Several argonaute proteins of the RISC complex were associated with Wilms tumor (3). An argonaute gene, *HIWI*, which is the human orthologue of the *Drosophila* Argonaute gene *PIWI*, is linked with testicular germ-tumors (12). Taken together, these emerging lines of evidence suggest that miRNA machinery protein may play a crucial role on cancer development and progression.

Although single nucleotide polymorphisms (SNPs) have been widely implicated in cancer development and treatment response, such evidence is lacking for miRNA-related genes. Although SNPs in miRNA gene regions have been reported to be rare and unlikely to be functionally important (13), recent studies implicated that nucleotide variations within the seed sequence on miRNA genes 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 RCC predisposition. We also took a polygenic approach to assess the cumulative effects of these

SNPs. To our knowledge, this is the first study investigating the associations between miRNA-related polymorphisms and RCC susceptibility.

MATERIALS AND METHODS

Study population

Incident RCC cases were recruited from The University of Texas M. D. Anderson Cancer Center in Houston, Texas. M. D. Anderson Cancer Center staff interviewers identified RCC 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 RCC. There was 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 non-melanoma skin cancer, were identified and recruited using the random digit dialing (RDD) methods. In RDD, randomly selected phone numbers from household were used to contact potential control volunteers in the same residency of cases accordingly to the telephone directory listings. Controls must have lived in the same county or socio-economically matched surrounding counties in Texas that the case resides in for at least one year and have no prior history of cancer. The controls were frequency matched to the cases by age (±5 years), sex, ethnicity and county of residence. This population-based RCC case-control study started in 2002 and is currently on going. A total of 677 subjects were included in this analysis.

Epidemiologic data collection

For both cases and controls, after obtaining written informed consent, trained M.D. Anderson staff interviewers 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 M.D. Anderson Cancer Center.

SNP selection

Through an extensive mining of the databases of the International HapMap Project (http://www.hapmap.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and miRBase registry (http://microrna.sanger.ac.uk), we identified 40 potential functional polymorphisms: 23 SNPs in 11 genes in the miRNA biogenesis pathway, seven SNPs in seven pre-miRNAs, and ten SNPs in eight pri-miRNAs (Table 1). All SNPs have a reported minor allele frequency (MAF) of more than 0.01 in Caucasians. In the miRNA biogenesis pathway, except for two *AGO1* SNPs (rs636832 and rs595961) 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 $r^2>0.8$), only one SNP was included. All SNPs identified from the pre-miRNA regions were included if the MAF was more than 0.01 in Caucasians. For SNPs in pri-miRNAs but not in pre-miRNAs, since we identified more than 200 such SNPs with an MAF of more than 0.01 in Caucasians, we included ten SNPs from eight pri-miRNAs whose mature counterparts have been extensively implicated in cancer etiology or clinical outcome.

Genotyping

DNA was isolated form peripheral blood using QIAamp DNA extraction kit (Qiagen, Valencia, CA). SNP genotyping was performed using the SNPlex technology (Applied Biosystems,

Foster city, CA, USA), based on an oligonucleotide ligation assay combined with multiplex PCR target amplification, following the manufacturer's recommendations. All pre-PCR steps were performed on a cooled block. Reactions were carried out in the dual-384-well GeneAmp® 9700 Thermocycler (Applied Biosystems). Allelic discrimination was performed through capillary electrophoresis analysis, using an Applied Biosystems 3730xl DNA sequencer (Applied Biosystems, Foster city, CA, USA). 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 preformed using Stata 8.0 statistical software package (Stata Corp., Colleage Station, TX). Pearson's chi-square 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 (HWE) was determined using the Goodness-of-fit Chi-square test to compare the observed frequency with the expected frequency in both cases and controls. RCC risks were estimated as odds ratios (ORs) 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 less than 95% were excluded from the final analysis. The adjusted ORs and 95% CI for each haplotype were calculated using multivariate logistic regression using the most abundant haplotype as the reference group. In addition to single SNP analysis and haplotype analysis, we also analyzed the association between total number of unfavorable genotypes and RCC risk. The unfavorable genotype was defined as SNPs showing at least a borderline statistical significance in the single SNP analysis. The unfavorable genotypes were collapsed into four groups according to the quartiles (low-, medium low-, medium high-, 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 tow-sided. $P < 0.05$ was considered the threshold if significance. The nonparametric Mann-Whitney test was used to test for differences in the distribution of expression levels between genotypes implemented in SPSS version 11.0 (SPSS, Inc., Chicago, IL).

RESULTS

Subject characteristics

There were a total of 677 study subjects recruited. The population consisted of 557 Caucasian (82.0%), 90 mexian Amaricans (13.0%), 30 African Americans (4.0%). Among Caucasians, there were 279 RCC patients and 278 controls (Table 2). There was no significant age difference ($P = 0.845$) 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 nine 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 stage II, III, and IV diseases were found in 11.1%, 20.4%, and 22.9% of patients, respectively. In addition, the majority (68.8%) of patients had a high-grade disease (grade 3 or 4) (Table 2).

Main effects on RCC risk by individual polymorphisms

Because most subjects were Caucasian, we focused on this population for risk analysis. The overall RCC 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 HWE in the controls, and were excluded from further analyses. Overall, five SNPs exhibited at least borderline significant with RCC risk (Table 3). Most significant effects were observed in *GEMIN4*. For *GEMIN4* rs2740348, compared with the homozygous wild-type (GG) genotype, the GC+CC genotype exhibited a significantly reduced risk of RCC (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; $P = 0.012$; Table S2). For *GEMIN4* rs7813, the variant allele-containing genotypes exhibited a reduced RCC 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; $P = 0.023$; Table S2). We also conducted stratified analyses in 215 patients with the conventional clear cell RCC 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, 0.66; 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).

Haplotype analysis

We conducted haplotype analysis for six genes (*DGCR8, DICER1, AGO1, GEMIN4, GEMIN3, mir219-1, mir373*) in this study and found that common haplotypes of both *AGO1* and *GEMIN4* were associated with altered RCC risk (Table 4). The H3 haplotype of *AGO1* (mw, w: wild type allele, m: minor allele, in the order of rs636832, rs595961) haplotype exhibited a borderline significant decrease in risk with OR of 0.66 (95% CI, 0.41–1.08; P=0.099). In addition, the H3 (wmmwww) haplotype of *GEMIN4*, consisting of 6 non-synonymous SNPs in the order of rs910924, rs2740348, rs7813, rs3744741,rs1062923, rs4968104, was associated with a significantly decreased RCC risk with an OR of 0.66 (95% CI, 0.45–0.97; $P = 0.035$; Table 4).

Cumulative risk analysis

We further evaluated the combined effects of high-risk genotypes on RCC carcinogenesis by summing the unfavorable genotypes of four risk-conferring SNPs including *XPO5* 3'UTR (rs11077), *AGO1* (rs595961), *GEMIN4* (rs2740348), *GEMIN4* (rs7813), and *GEMIN3* (rs197412). Using the combination of AA+AC, AG+GG, GC+CC, TC+CC, TT genotypes (for rs11088, rs595961, rs2740348, rs7813, and rs197412, respectively) as the reference group, a progressively increased gene-dosage effect was observed when subjects were categorized on the basis of increasing number of unfavorable genotypes (Table 5). The groups with medium and high risk genotypes exhibited a significantly increased risk of RCC with ORs of 1.55 (95% CI, 0.95–2.50; $P = 0.075$) and 2.49 (95% CI, 1.58–3.91; $P<0.001$), respectively (P for trend < 0.001).

DISCUSSION

In this study, we found significant associations between SNPs in miRNA biogenesis pathway and the risk of RCC. Recent studies have shown that, disrupting miRNA processing through the knockdown of DROSHA, DGCR8, and DICER1, could accelerate cellular transformation and tumorigenesis (17). Thomson et al. (18) have shown that the repression of mature miRNAs is not consistent with the reductions in the primary miRNA transcripts, suggesting the existence of altered regulations of miRNA processing in human cancers. These lines of evidence are in concordance with the recent profiling of miRNAs expression, which showed the general

repression of miRNAs in a variety of tumors and cancer cell lines (18–21). Our results, taken together with these findings, indicate that genetic alterations of the miRNA biogenesis pathway might be associated with cancer development and progression.

In this study, three nonsynonymous SNPs (nsSNPs) of the *GEMIN4* (rs7813 and rs2740348) and *GEMIN3* gene (rs197412) were found to be associated with altered RCC risk. Both GEMIN3 and GEMIN4 are reported to be core components of the SMN (survival of motor neuron) complex and implicated in the etiology of spinal muscular atrophy (9). In addition, these GEMIN proteins have been identified in miRNA ribonucleoprotein particle (miRNP) with an Argonauts 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 RISC 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 heptacellular carcinoma cell line (23), suggesting that the amino acid changes caused by these SNPs might have a physiological significance on cancer development. Moreover, recently our study for bladder cancer has shown the association between an altered risk and *GEMIN4* rs7813 polymorphism (24). However, whether the associations between SNPs of *GEMIN4* and altered RCC 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 RCC risk were also observed in two genes, *XPO5* and *AGO1* genes (Table 3). In particular, the *XPO5* rs11070 exhibited an increased risk of RCC in the recessive model. XPO5 mediate 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 in high grade prostate cancer XPO5 have been shown to be up-regulated (6). AGO1 (EIF2C1), a component of RISC with AGO2 and DICER1, are 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 RCC 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 were 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 public SNP database (13). However, many of these SNP 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 (CLL) and these mutations might influence the 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 RCC risk, we could not exclude the possibility that genetic variations in miRNA genes might have a potential regulatory effect on RCC 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 gene 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 powerful elucidate the influence of these SNPs on RCC susceptibility using a pathway-based polygenic approach and identified a trend toward an increasing RCC risk with an increasing number of unfavorable genotypes that occurred in a dose-dependent manner. This finding reinforces the notion that RCC is a polygenic process and thus a combined analysis of multiple variants may have a greater ability to characterize high-risk populations. Further epidemiological and functional studies in a larger population are warranted to validate these results.

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

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TABLE 1

MiRNA-related Genes and Polymorphisms Evaluated in this Study

Gene name (Gene symbol)	SNP ID	Position	Major/minor allele	MAF*(%)
miRNA machinery pathway				
DROSHA	rs10719	3'UTR	C/T	23
	rs6877842	5'UTR	G/C	18
Digeorge syndrome critical region gene8 (DGCR8)	rs417309	3'UTR	G/A	11
	rs3757	3'UTR	G/A	27
	rs1640299	3'UTR	G/T	47
Exportin 5 (XPO5)	rs11077	3'UTR	A/C	40
Ras-related nuclear protein (RAN)	rs14035	3'UTR	C/T	12
DICER1	rs3742330	3'UTR	A/G	12
	rs13078	3'UTR	T/A	14
Tar RNA-binding protein 2 (TRBP)	rs784567	5'UTR	C/T	48
Eukaryotic translation initioation factor 2C (AGO1)	rs636832	intron	G/A	9
	rs595961	intron	A/G	15
Argonoute 2 (AGO2)	rs4961280	promoter	C/A	13
Gem-associated protein 4 (GEMIN4)	rs910924	promoter	C/T	35
	rs2740348	*Asn929Asp*	G/C	18
	rs7813	*Cys1033Arg*	T/C	14
	rs3744741	*Gln684Arg*	C/T	13
	rs1062923	*Thr731Ile*	T/C	11
	rs4968104	*Val593Glu*	T/A	22
Gem-associated protein 3 (GEMIN3)	rs197414	*Ser693Arg*	C/A	19
	rs197388	Promoter	T/A	29
	rs197412	*Thr636Ile*	T/C	10
HIWI	rs1106042	*Lys527Arg*	G/A	8
Pre-miRNAs				
mir416a	rs2910164	Pre-miRNA	G/C	24
mir196a-2	rs11614913	Pre-miRNA	C/T	44
mir423	rs6505162	Pre-miRNA	C/A	0.9
mir492	rs2289030	Pre-miRNA	C/G	8
mir604	rs2368392	Pre-miRNA	C/T	25
mir608	rs4919510	Pre-miRNA	C/G	17
mir631	rs5745925	Pre-miRNA	CT/-	7
Pri-miRNAs				
let7f-2	rs17276588	5'region	G/A	2
mir26a-1	rs7372209	5'region	C/T	27
mir30a	rs1358379	5'region	A/G	4
mir30c-1	rs16827546	5'region	C/T	4
mir100	rs1834306	5'region	C/T	44

Gene name (Gene symbol)	SNP ID	Position	Major/minor allele	MAF* (%)
mir124a-1	rs531564	5'region	C/G	12
mir219-1	rs107822	5'region	G/A	23
	rs213210	3'region	T/C	6
mir373	rs1298273	5'region	C/T	13
	rs10425222	3'region	C/A	3

* Minimum allele frequency in Caucasians

Table 2

Distribution of Selected Host Characteristics by Case-Control Status in Caucasian

Variables		Case (N=279)	Control (N=278)	*P* value*
Age (Mean ± SD)		60.29 ± 10.57	60.46 ± 10.88	0.845
Gender, N (%)				
	Male	187 (67.0)	186 (67.0)	0.976
	Female	92 (33.0)	92 (33.0)	
Smoking Status †, N (%)				
	Never	137 (49.0)	116 (42.0)	
	Former	104 (37.0)	112 (40.0)	
	Current	38 (14.0)	50 (18.0)	0.159
Pack-years (Mean ± SD)		30.27 ± 26.33	32.39 ± 31.43	0.538
Tumor histology, N (%)				
	Clear cell	198 (71.0)		
	Papillary	32 (11.5)		
	Chromophobe	9 (3.2)		
	Sarcomatoid	2 (0.7)		
	Other §	8 (2.7)		
	Clear cell and Papillary	2 (0.7)		
	Clear cell and sarcomatoid	15 (5.4)		
	Chromophobe and other	1 (0.4)		
	Sarcomatoid and other	2 (0.7)		
	Incomplete	10 (3.6)		
Tumor stage, N (%)				
	I	126 (45.2)		
	II	31 (11.1)		
	III	57 (20.4)		
	IV	64 (22.9)		
	Incomplete	1 (0.4)		
Tumor grade, N (%)				
	1	2 (0.7)		
	2	70 (25.1)		
	3	128 (45.9)		
	4	64 (22.9)		
	Incomplete	15 (5.4)		

Abbreviation: SD, standard deviation

*P values were derived from the χ^2 test for categorical variables (gender and smoking status) and t test for continuous variables (age and pack-years).

†Individuals who smoked < 100 cigarettes in lifetime are never smokers; light smokers are ever smokers who smoked ≤ 31 pack-years; and heavy smokers are ever smokers who smoked > 31 pack-years

§Included collecting duct carcinoma, medullar carcinoma, and other unclassified RCC.

Table 3

Associations of Selected SNPs with RCC risk in Caucasians

SNP	Position	Genotype	In all patients				In patients with clear cell RCC *			
			Case/Control	OR (95% CI)**	*P*	*P* for trend	Case/Control	OR (95% CI)**	*P*	*P* for trend
XPO5	3'UTR	AA/AC	222/239	Reference			173/239	Reference		
(rs11077)		CC	54/38	1.55 (0.98–2.44)	0.062	0.295	39/38	1.38 (0.84–2.27)	0.297	0.739
AGO1	Intron	AA	202/186	Reference			149/186	Reference		
(rs595961)		AG/GG	75/72	0.74 (0.51–1.07)	0.106	0.099	64/72	0.85 (0.58–1.26)	0.423	0.321
GEMIN4	Exon 2	GG	192/168	Reference			144/168	Reference		
(rs2740348)	*N929D*	GC/CC	84/110	0.67 (0.47–0.96)	0.027	0.014	68/110	0.74 (0.50–1.08)	0.115	0.073
GEMIN4	Exon2	TT	96/75	Reference			75/75	Reference		
(rs7813)	*C1033R*	TC/CC	181/203	0.68 (0.47–0.98)	0.039	0.069	138/203	0.66 (0.45–0.98)	0.039	0.103
GEMIN3	Exon11	TT	97/115	Reference			75/115	Reference		
(rs197412)	*T636I*	TC/CC	180/163	1.31 (0.93–1.85)	0.128	0.077	138/163	1.30 (0.90–1.89)	0.162	0.071

* In 215 patients with conventional clear cell RCC

** Adjusted for age, gender, and smoking status

Table 4

Haplotype Analysis for Selected Genes in Caucasians

Haplotype	Case/Control	OR (95%CI)[a]	*P* value
DGCR8 [b]			
H1 (www)	242/234	Reference	
H2 (wwm)	148/156	0.89 (0.66–1.20)	0.459
H3 (wmm)	124/130	0.92 (0.67–1.25)	0.591
H4 (mww)	38/36	1.00 (0.62–1.61)	0.996
DICER1 [c]			
H1 (ww)	403/414	Reference	
H2 (wm)	103/86	1.23 (0.89–1.70)	0.218
H3 (mw)	32/36	0.92 (0.55–1.53)	0.746
AGO1 [d]			
H1 (ww)	473/454	Reference	
H2 (wm)	51/58	0.85 (0.57–1.28)	0.439
H3 (mm)	30/42	**0.66 (0.41–1.08)**	**0.099**
GEMIN4 [e]			
H1 (wwwwww)	118/104	Reference	
H2 (mwmwmm)	125/130	0.82 (0.57–1.18)	0.29
H3 (wmmwww)	89/119	**0.66 (0.45–0.97)**	**0.035**
H4 (wwwmww)	72/72	0.95 (0.63–1.44)	0.815
H5 (wwwwmw)	84/80	0.92 (0.61–1.39)	0.69
Others	13/7	1.55 (0.61–3.93)	0.358
GEMIN3 [f]			
H1 (www)	330/361	Reference	
H2 (wwm)	108/95	1.24 (0.90–1.71)	0.195
H3 (mmm)	60/55	1.31 (0.82–1.81)	0.331
H4 (wmm)	46/40	1.41 (0.81–2.04)	0.284
Others	8/3	2.13 (0.74–11.03)	0.129
mir 219-1 [g]			
H1 (ww)	410/419	Reference	
H2 (mw)	92/98	0.93 (0.67–1.30)	0.68
H3 (mm)	36/31	1.19 (0.71–1.99)	0.506
mir 373 [h]			
H1 (ww)	437/465	Reference	
H2 (mw)	76/73	1.10 (0.76–1.57)	0.618
H3 (wm)	15/14	1.10 (0.54–2.22)	0.801

[a] ORs were adjusted for age, gender, and smoking status.

[b] Order of SNPs-rs417309, rs3757, rs1640299, with w being the major allele and m being the minor allele.

[c] Order of SNPs-rs3742330, rs13078.

[d] Order of SNPs-rs636832, rs595961.

[e] Order of SNPs- rs910924, rs2740348, rs7813, rs3744741,rs1062923, rs4968104.

[f] Order of SNPs- rs197414, rs197388, rs197412.

[g] Order of SNPs- rs107822,rs213210.

[h] Order of SNPs-rs12983273, 10425222.

Table 5

Joint Effects of Unfavorable Genotypes in Case and Control Subjects in Caucasinas

Risk group (No. unfavorable genotypes)	Case	Control	OR (95% CI)*	*P* value
Low risk reference group (n= 0–1)	43	76	Reference	
Medium risk group (n= 2)	83	93	**1.55 (0.96–2.50)**	**0.075**
High risk group (n= 3–5)	150	108	**2.49 (1.58–3.91)**	**<0.001**
P for trend				**<0.001**

*Adjusted for age, gender, and smoking status.

†Unfavorable genotype: *DICER1* (rs3742330): AA, *AGO1* (rs595961): AA, *GEMIN4* (rs2740330): GG, *GEMIN4* (rs7813): TT, *GEMIN3* (rs197412): TC+CC, *GEMIN3* (rs197388) : TA+AA