Single nucleotide polymorphisms and risk of recurrence of renal-cell carcinoma: a cohort study

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Summary

Background Germline genetic polymorphisms might affect the risk of recurrence in patients with localised renal-cell carcinoma. We investigated the association between genetic polymorphisms and recurrence of renal-cell carcinoma.

Methods We analysed germline DNA samples extracted from patients with localised renal-cell carcinoma treated at the Dana-Farber/Harvard Cancer Center (Boston, MA, USA). We selected a discovery cohort from a prospective database at the Dana-Farber/Harvard Cancer Center and selected a validation cohort from department records at the Brigham and Women's Hospital (Boston, MA, USA). We validated the findings from the discovery cohort in the validation cohort. We genotyped 70 genes involved in the pathogenesis of renal-cell carcinoma (including the VHL/HIF/VEGF and PI3K/AKT/mTOR pathways, and genes involved in immune regulation and metabolism) for single nucleotide polymorphisms. We assessed the association between genotype and recurrence-free survival, adjusted for baseline characteristics, with the Cox proportional hazards model, the Kaplan-Meier method, and the log-rank test. We used a false discovery rate q value to adjust for multiple comparisons.

Findings We included 554 patients (403 in the discovery cohort and 151 in the validation cohort). We successfully genotyped 290 single nucleotide polymorphisms in the discovery cohort, but excluded five because they did not have a variant group for comparison. The polymorphism rs11762213, which causes a synonymous aminoacid change in *MET* (144G→A, located in exon 2), was associated with recurrence-free survival. Patients with one or two copies of the minor (risk) allele had an increased risk of recurrence or death (hazard ratio [HR] 1·86, 95% CI 1·17–2·95; p=0·0084) in multivariate analysis. Median recurrence-free survival for carriers of the risk allele was 19 months (95% CI 9–not reached) versus 50 months (95% CI 37–75) for patients without the risk allele. In the validation cohort the HR was 2·45 (95% CI 1·01–5·95; p=0·048).

Interpretation Patients with localised renal-cell carcinoma and the *MET* polymorphism rs11762213 might have an increased risk of recurrence after nephrectomy. If these results are further validated in a similar population, they could be incorporated into future prognostic instruments, potentially aiding the design of adjuvant clinical trials of *MET* inhibitors and management of renal-cell carcinoma.

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Introduction

Localised renal-cell carcinoma often recurs after treatment, usually leading to incurable disease.[1] The risk of recurrence is highly associated with clinical and pathological factors, such as TNM stage, performance status, and Fuhrman grade. However, outcomes for patients with similar clinical and pathological features still differ significantly. Improved predictors of recurrence of renal-cell carcinoma are needed.[2,3]

Although several molecular markers of disease progression have been proposed, no biomarkers of recurrence risk have been well established. Germline DNA polymorphisms are particularly attractive biomarkers since they are present at the time of diagnosis and are not affected by the state of the disease or the timing of diagnosis. Single nucleotide polymorphisms (SNPs) are inherited germline DNA sequence variants. These variants occur throughout the entire genome, in both coding and non-coding regions, and can modify biological pathways.[4]

A genome-wide association study identified SNPs in *EPAS1* (also called *HIF2a*) and a complex genetic architecture that were associated with risk for renal-cell carcinoma.[5–8] A follow-up study reported that a variant in 11q13.3 remodulates the binding and function of hypoxia inducible factor (HIF) at a previously undiscovered transcriptional enhancer of *CCND1* (which codes for cyclin D1). The protective haplotype impairs binding of HIF-2, resulting in an allelic imbalance of cyclin D1 expression.[9] However, to our knowledge, no large studies have assessed SNPs and renal-cell carcinoma recurrence and survival after resection. Positive associations exist between some germline polymorphisms and outcome for prostate cancer, breast cancer, lymphoid neoplasm, and nasopharyngeal cancer (after initial treatment).[10–13] We assessed the association between SNPs in genes

implicated in renal-cell carcinoma and the likelihood of recurrence of renal-cell carcinoma after resection.

Methods

Participants

We enrolled patients into a discovery cohort and a validation cohort. We enrolled patients into the discovery cohort from the Clinical Research Information System database of the Dana-Farber/Harvard Cancer Center Kidney Cancer Program (Boston, MA, USA). This database—started in 2001—is designed to prospectively study patients diagnosed with localised renal-cell carcinoma undergoing treatment. All patients had histologically proven renal-cell carcinoma and were reviewed by a genitourinary pathologist at Dana-Farber/Harvard Cancer Center. The database included patients' baseline clinical and pathological data and information about disease outcome, including date of recurrence, and date of death or last follow-up. Date of recurrence was classed as the date of first diagnosis of local or distant recurrence, or diagnosis of contralateral renal-cell carcinoma by the treating physician recorded in patient charts. Patients were followed up prospectively in a routine schedule devised by the treating physician. We only included patients of European–American ancestry to ensure that participants had similar genetic backgrounds. Ancestry information was gathered from patients' charts. The first patient was enrolled on Jan 18, 2002, and last patient enrolled on May 6, 2010. All patients provided written informed consent. Study of this cohort was approved by the Dana-Farber/Harvard Cancer Center institutional review board.

We also enrolled an independent validation cohort of 151 patients with localised renal-cell carcinoma from the Brigham and Women's Hospital (Boston, MA, USA) pathology department. Patients' clinical information was ascertained from medical records. Enrolment for this cohort was from Aug 28, 1991, to June 10, 2010. Study of this cohort was approved by the independent institutional review board under a different protocol.

	Discovery cohort (n=403)	Validation cohort (n=151)
Follow-up (months)	43 (26–64)	66 (23–106)
Age (years)	58·0 (49·6–65·3)	61·2 (52·0–72·2)
Tumour size (cm)	6·0 (3·5–9·0)	6·0 (4·5–8·3)
Missing data	19	0
Sex		
Women	134 (33%)	81 (54%)
Men	269 (67%)	70 (46%)
ECOG PS		
0	321 (81%)	86 (57%)
≥1	77 (19%)	64 (43%)
Missing data	5	1
Clinical stage		
I or II	236 (62%)	109 (72%)
III or IV	147 (38%)	42 (28%)
Missing data	20	0
Fuhrman grade		
1–2	190 (50%)	90 (60%)
3–4	188 (50%)	59 (40%)
Missing data	25	2
Histology		
Clear cell	310 (78%)	111 (75%)
Non-clear cell	86 (22%)	37 (25%)
Papillary	43 (50%)	19 (51%)
Chromophobe	31 (36%)	18 (49%)
Mixed	8 (9%)	0 (0%)
Other	4 (5%)	0 (0%)
Missing data	7	3

Data are median (IQR) or n (%). ECOG PS=Eastern Cooperative Oncology Group performance status.

Table 1: **Patient characteristics**

For the **HapMap database** see http://www.broadinstitute.org/mpg/snap/

Procedures

In the discovery cohort we analysed several signalling pathways and key processes that have an important role in the pathogenesis and progression of renal-cell carcinoma. 70 genes were selected, including genes from the VHL/HIF/VEGF and PI3K/AKT/mTOR pathways, and genes involved in immune regulation, metabolism, and detoxification.[14–19]

SNPs were selected from several genes, including regions 50 kb upstream and 50 kb downstream of each gene with the goal of capturing common genetic variation. We used the European–American ancestry population (CEU) of the HapMap database (release 21) for the selection of SNPs. A minimum allele frequency of at least 5% and a minimum pairwise correlation index (r^2) of 80% were needed for each polymorphism to ensure an adequate coverage of the gene sequence and statistical power.[20] SNPs from previously published studies that were significantly associated with risk of developing renal-cell carcinoma were also selected for genotyping. All eligible non-synonymous coding SNPs in the 70 selected genes were included in the analysis. In the validation cohort, only the top 12 SNPs showing potential association with recurrence-free survival ($p<0·05$) in the discovery cohort were assessed.

For the discovery cohort, we extracted DNA samples from peripheral blood mononuclear cell by QIAamp DNA Blood mini kit (Qiagen, Valencia, CA, USA). For the validation cohort, we extracted DNA from the normal kidney parenchyma tissue in formalin-fixed paraffin-embedded blocks with DNeasy 96 Blood & Tissue Kit (Qiagen). Genotyping was done with the Sequenom iPlex Gold platform (Sequenom, San Diego, CA, USA) with MALDI-TOF. The staff who did the genotyping were masked to the patient's diagnosis. All SNP assays were combined into a 12 multiplex pool design, and all reactions were done in a 384 well format. Roughly 5% of duplicate samples were randomly selected and

interspersed among plates for quality control. The concordance of the duplicates was 100%.

We only analysed SNPs that had passed quality checks. We usually excluded those with a genotyping success rate of less than 85% or with a significant deviation from Hardy-Weinberg equilibrium. In the discovery cohort, 290 of 368 SNPs were successfully genotyped with an average rate of 97%.

The primary analysis endpoint was recurrence-free survival, defined as time from curative surgery to recurrence or death or censored at the last date at which the patient was known to be alive. Other endpoints were recurrence-free interval, defined as time from surgery to recurrence or censored at death before recurrence or last follow-up, and time from surgery to all-cause death or overall survival.

Statistical analysis

For the discovery cohort, we treated each SNP as a categorical variable—either a common homozygote, a rare homozygote, or a heterozygote. Rare homozygotes were combined with heterozygotes if the rare homozygote count was very low (using a cutoff of ten or frequency of 2·5% for a variant group) to ensure that the analysis (regression model) could properly estimate the coefficient for the association. We tested the association between recurrence-free survival and genotype with the log-rank test. We used the false-discovery rate measure q value with a cutoff less than 0·1 to adjust for multiple comparisons.[21] The q value represents the expected proportion of false-positive results when testing for significance. We used a false discovery rate threshold of 0·1 on the basis of the REMARK guidelines,[22] which state that a very stringent criterion needs to be used if any biomarkers are to hold up in future studies.

Once we identified a polymorphism on the basis of log-rank test and false-discovery rate, we estimated the distribution of recurrence-free survival stratified by allelic status by the Kaplan-Meier method. We tested the association between the polymorphism and endpoints

	Discovery cohort				Validation cohort			
	Coefficient estimate (SE)	z	HR (95% CI)	p value	Coefficient estimate (SE)	z	HR (95% CI)	p value
ECOG PS (≥1 *vs* 0*)	0·95 (0·16)	5·80	2·60 (1·88–3·59)	<0·0001	0·47 (0·30)	1·57	1·60 (0·89–2·88)	0·12
Tumour size (cm)	0·15 (0·02)	9·07	1·17 (1·13–1·21)	<0·0001	0·03 (0·05)	0·55	1·03 (0·93–1·13)	0·59
Clinical stage (III or IV *vs* I or II*)	1·43 (0·16)	9·06	4·20 (3·08–5·72)	<0·0001	1·34 (0·30)	4·45	3·83 (2·12–6·93)	<0·0001
Fuhrman grade (3 or 4 *vs* 1 or 2*)	1·23 (0·17)	7·32	3·43 (2·47–4·77)	<0·0001	0·73 (0·31)	2·39	2·08 (1·14–3·80)	0·017
Histology (clear cell *vs* non-clear cell*)	0·61 (0·20)	3·00	1·84 (1·24–2·74)	0·0027	0·21 (0·36)	0·58	1·23 (0·61–2·49)	0·56

ECOG PS=Eastern Cooperative Oncology Group performance status. HR=hazard ratio. *Reference group.

Table 2: **Recurrence-free survival by baseline characteristics**

	Gene	Number assessable*	Minor allele frequency (%)	Homozygous (%)	Heterozygous (%)	Wild-type (%)	p value for HWE	p value for recurrence-free survival†	q value
Discovery cohort									
rs11762213 (G→A)	*MET*	393	5·3%	1·3%	8·1%	90·6%	0·0028	$9{\cdot}40\times10^{-5}$	0·027
rs3820546 (A→G)	*SLC2A1*	387	46·8%	23·8%	46·0%	30·2%	0·15	0·0019	0·27
rs38846 (T→C)	*MET*	389	18·6%	3·9%	29·6%	66·6%	0·62	0·0093	0·73
rs1531290 (A→G)	*KDR*	397	46·3%	22·4%	47·9%	29·7%	0·48	0·01	0·73
rs2236416 (A→G)	*MMP9*	398	13·8%	2·5%	22·6%	74·9%	0·29	0·023	0·89
rs38845 (G→A)	*MET*	390	45·5%	19·7%	51·5%	28·7%	0·48	0·029	0·89
rs1326889 (T→C)	*AGT*	362	48·3%	25·4%	45·9%	28·7%	0·12	0·031	0·89
rs3093662 (A→G)	*TNF*	386	8·2%	0·5%	15·3%	84·2%	1·00	0·032	0·89
rs361525 (G→A)	*TNF*	398	4·8%	0·3%	9·0%	90·7%	0·60	0·033	0·89
rs10267099 (A→G)	*ABCB1*	347	23·3%	6·3%	34·0%	59·7%	0·37	0·034	0·89
rs779805 (A→G)	*VHL*	399	32·3%	9·8%	45·1%	45·1%	0·57	0·035	0·89
rs10271561 (T→C)	*MET*	391	10·4%	0·8%	19·2%	80·1%	0·78	0·037	0·89
Validation cohort									
rs11762213 (G→A)	*MET*	148	5·4%	0%	10·8%	89·2%	1·00	0·042	..
rs3820546 (T→C)	*SLC2A1*	148	47·3%	21·6%	51·4%	27·0%	0·87	0·064	..

p value for HWE represents the exact test for HWE. Data are for the top 12 single nucleotide polymorphisms associated with recurrence-free survival in the discovery cohort and the top two in the validation cohort. HWE=Hardy-Weinberg equilibrium. *Patients whose genotyping had failed were excluded from the analysis. †For test of association between recurrence-free survival and single nucleotide polymorphism.

Table 3: **Single nucleotide polymorphisms associated with recurrence-free survival**

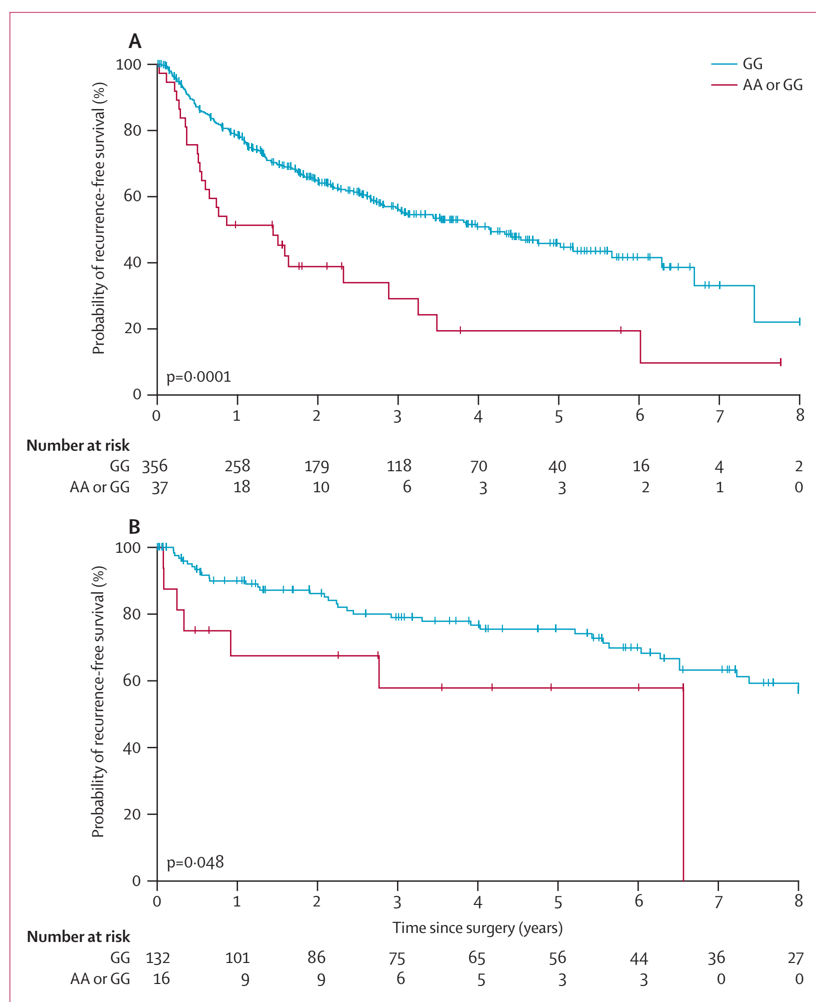

Figure: **Kaplan-Meier curves of recurrence-free survival for patients with single nucleotide polymorphisms in *MET* (rs11762213)**
In the discovery cohort (A) and the validation cohort (B).

with Cox regression models adjusted for clinical and pathological factors including Eastern Cooperative Oncology Group (ECOG) performance status, clinical stage, tumour size, tumour Fuhrman grade, and histology (clear cell *vs* non clear cell).[23] We also used the Cox model to estimate hazard ratios (HRs) and 95% CI between the SNP variants. We tested associations between the SNP variants and clinical and pathological factors with the χ^2 test for categorical variables and the Wilcoxon (for two groups of polymorphisms; eg, homozygote plus heterozygote *vs* wild type) or Kruskal-Wallis (for three groups of polymorphisms; eg, homozygote *vs* heterozygote *vs* wild type) tests for continuous variables.

See Online for appendix

All reported p values were two-sided. We calculated q values with R on the basis of the measured distribution of p values from the log-rank test for individual SNPs. The analyses were done with SAS (version 9.2) and R (version 2.10.1).

For the **R package** see http://bioconductor.org/packages/devel/bioc/html/qvalue.html

Role of the funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
We analysed 554 patients; 403 patients in the discovery cohort and 151 in the validation cohort. Most patients in the discovery cohort were men, had an ECOG performance status of 0, clinical stage I or II disease, and clear-cell histology, with a median follow-up of 43 months (IQR 26–64) since surgery (table 1). By comparison, the validation cohort had more women and more patients with an ECOG performance status of 1 or more; median follow-up was 66 months (IQR 23–106). Other clinical characteristics were much the same in each cohort.

184 (46%) of 403 patients had recurrence or died in the discovery cohort; 167 patients had recurrence and 104 patients died during the follow-up period. 44 (29%) of 151 patients had recurrence or died in the validation cohort; 22 patients had recurrence and 35 died.

Consistent with previous reports of patients with localised renal-cell carcinoma, patients with advanced stage and high tumour grade had a high risk of recurrence in both cohorts (table 2).[3]

We successfully genotyped 290 SNPs in the discovery cohort but excluded five from the analysis because they had only a single variant group in our American–European population. 12 SNPs were associated with recurrence-free survival (log-rank test $p<0·05$; table 3). One synonymous coding polymorphism (rs11762213; 144G→A) located in exon 2 of *MET* was significantly associated with recurrence-free survival after adjusting for false discovery with the q value (univariate log-rank $p<0·0001$, $q=0·027$; figure). Table 3 shows the distribution of the rs11762213 variants; the minor allele frequency of the polymorphism rs11762213G→A was 5·3%, consistent with the frequency in the CEU population of the HapMap database. The baseline clinical and pathological characteristics of patients with different variants of this polymorphism did not differ significantly (appendix).

We further analysed the association between rs11762213 polymorphisms and outcomes in the discovery cohort with a multivariable Cox regression model, adjusting for ECOG performance status, clinical stage, tumour size, Fuhrman grade, and histology (table 4). Patients with one or two copies of the risk allele A (AG or AA genotypes) had a significantly increased risk of recurrence or death compared with patients with the GG genotype (table 4), with a median recurrence-free survival of 19 months (95% CI 9–not reached) versus 50 months (95% CI 37–75). For complete results from the multivariable model, see appendix.

	Discovery cohort (n=403)						Validation cohort (n=151)					
	n (events*)	5-year event rate (%; 95% CI)	Univariate analysis		Multivariable analysis†		n (events*)	5-year event rate (%; 95% CI)	Univariate analysis		Multivariable analysis†	
			HR (95% CI)	p value	HR (95% CI)	p value			HR (95% CI)	p value	HR (95% CI)	p value
Recurrence-free survival												
Overall	393 (184)	43% (37–50)	..	..	..	..	148 (44)	74% (66–82)	..	..	..	..
GG‡	356 (157)	46% (40–53)	..	..	..	..	132 (37)	75% (68–84)	..	..	..	..
AA or AG	37 (27)	19% (9–44)	2·22 (1·47–3·35)	0·0001	1·86 (1·17–2·95)	0·0084	16 (7)	58% (36–92)	2·29 (1·01–5·18)	0·048	2·45 (1·01–5·95)	0·048
Recurrence-free interval												
Overall	393 (167)	48% (42–55)	..	..	..	..	148 (22)	85% (79–92)	..	..	..	..
GG‡	356 (143)	50% (44–57)	..	..	..	..	132 (17)	87% (81–94)	..	..	..	..
AA or AG	37 (24)	25% (12–52)	2·18 (1·41–3·37)	0·00043	1·69 (1·03–2·76)	0·039	16 (5)	68% (48–96)	2·93 (1·08–7·99)	0·035	2·99 (0·97–9·20)	0·056
Overall survival												
Overall	393 (104)	61% (54–68)	..	..	..	..	148 (35)	80% (73–88)	..	..	..	..
GG‡	356 (88)	62% (55–70)	..	..	..	..	132 (29)	82% (75–90)	..	..	..	..
AA or AG	37 (16)	52% (35–75)	1·68 (0·98–2·87)	0·061	1·24 (0·67–2·27)	0·5	16 (6)	62% (40–96)	3·06 (1·24–7·56)	0·015	3·52 (1·32–9·38)	0·012

Ten patients in the discovery cohort and three in the validation cohort had failed genotyping results so were excluded from the analysis. HR=hazard ratio. *Recurrence or death (for recurrence-free survival), recurrence (for recurrence-free interval), death (for overall survival). †Adjusted for Eastern Cooperative Oncology Group performance status, clinical stage, tumour size, Fuhrman grade, and histology (clear cell *vs* all others). ‡Reference group.

Table 4: **Association between outcomes and variants of the *MET* polymorphism rs11762213**

5-year recurrence-free survival was estimated to be 19% (95% CI 9–44) for patients with AA or AG variants and 46% (95% CI 40–53) for patients with GG. The association between recurrence-free interval and the polymorphism was much the same (table 4), with allele A being associated with an increased risk of recurrence. The association of overall survival with the polymorphism was not statistically significant (table 4).

In the validation cohort, patients with risk allele A had an increased risk of recurrence or death (table 4; p=0·048). The p value was only just significant, with a wide 95% CI, which was probably a result of the small sample size and a low count in the variant comparison group (16 for AA or AG *vs* 132 for GG). The results for recurrence-free interval were consistent with those from the discovery cohort (appendix). By contrast, the association between the genotype variants with one or two copies of the A allele and overall survival was significant in the validation cohort. We used integrated discrimination improvement[24] to estimate the effect of including the rs11762213 polymorphism as a risk factor for prediction of recurrence. Mean predicted probability of recurrence improved by 4% (95% CI 1 to 8) at 3 years and 13% (95% CI –3% to 30%) at 8 years compared with the model that did not include rs11762213 as a covariate (appendix).

Discussion

Patients with one or two copies of the minor risk allele A of the SNP rs11762213 in exon 2 of *MET* (144G→A) had a higher risk of recurrence of renal-cell carcinoma than did patients without this high risk allele. In our multivariable analysis, the A allele was also statistically significantly associated with overall survival in the validation cohort, but not in the discovery cohort, which might be because of the longer follow-up in the validation cohort, or possibly the use of targeted treatment in the discovery cohort, which is more recent than the validation cohort. To our knowledge, this study is the first to report an association between a germline single nucleotide polymorphism and an increased risk of recurrence in patients with localised renal-cell carcinoma (panel).

MET is an intriguing biomarker candidate gene. *MET* is the transmembrane tyrosine kinase receptor for the hepatocyte growth factor. Activating *MET* mutations in the tyrosine kinase domain have been identified in patients with type 1 hereditary papillary renal-cell carcinoma.[25] *MET* expression and signalling is increased in tumour tissues of several cancers, including sporadic renal-cell carcinoma.[26] *MET* also plays a part in the pathogenesis of renal-cell carcinoma, usually cooperating with VEGFR to promote tumour growth.[27] Additionally, MET protein might act as a sensor of adverse micro-environments (ie, hypoxia), driving cell invasion and metastasis through activation of genes involved in blood coagulation.[28]

Fine mapping will be necessary to identify the true causal allele, since rs11762213 might simply be correlated with other alleles. The true causal polymorphism in linkage disequilibrium with rs11762213 could have direct downstream effects. If the causal allele is synonymous or non-coding, it will not directly alter the downstream protein product. However, synonymous or non-coding single nucleotide polymorphims can affect protein abundance, structure, or function through various means: (1) changing mRNA structure and stability; (2) changing

Panel: Research in context

Systematic review

We searched PubMed with the terms “renal cell carcinoma”, “polymorphisms”, and “SNP” for germline studies of renal-cell carcinoma and other malignancies published between Jan 1, 1966, and Sept 30, 2012. We reviewed studies that assessed survival, recurrence-free survival, or cancer-specific survival in patients who underwent nephrectomy. We did not find any studies of correlation between germline genetic polymorphisms and renal-cell carcinoma.

Interpretation

Our findings show that a SNP in *MET* (rs11762213, located in an exon) is independently associated with recurrence-free survival in the discovery and the validation cohorts. Our study is the first to show a positive association between germline genetic polymorphisms in *MET* and recurrence-free survival.

kinetics of transcription or translation; or (3) alternative splicing.[29] Additionally, functional analysis should be done to confirm that *MET* is a target gene driving disease aggressiveness and to determine exactly how genotype affects the risk of recurrence or death. If inherited variation within *MET* does increase the risk of recurrence of renal-cell carcinoma, use of *MET* inhibitors in high-risk patients could be beneficial. Drugs targeting *MET* in many advanced solid tumours—including metastatic renal-cell carcinoma—are being tested in clinical trials.[26]

Our results are strengthened by several facts. First, we assessed a well-defined cohort of patients with European–American ancestry, making this the largest study of its kind. Second, all patients in the discovery cohort were followed up prospectively after treatment, and their clinical and pathological characteristics are similar to other cohorts of patients with renal-cell carcinoma. Third, we included a broad range of relevant genes on the basis of previously published work. Fourth, we adjusted for multiple comparisons, reducing the chance of false-positive results. Fifth, our results were still significant after adjusting for clinical and pathological covariates. Finally, we reproduced our findings in an independent, albeit smaller, cohort of patients with localised renal-cell carcinoma with DNA extracted from normal tissue of formalin-fixed paraffin-embedded samples. Such tissue has been shown to provide excellent quality and quantity DNA for genome-wide genotyping of germline DNA, sufficient for both linkage and association analyses.[30]

Of note, we recorded a deviation from Hardy-Weinberg equilibrium for rs11762213 in our discovery cohort caused by a higher than expected minor homozygote rate, even though the minor homozygote rate was only 1%. This difference has several potential causes including selection bias, since we only included patients with renal-cell carcinoma with no control group. The deviation from the Hardy-Weinberg equilibrium is a limitation, and our results should be interpreted with caution—direct comparisons with other populations might not be appropriate. Additionally, it is important to note that the frequency of the A allele in our cohort was much the same as the expected frequency in the HapMap CEU database (5%) and in the CEU cohort in the 1000 genome project (4·85%).

Most other studies of germline polymorphisms and renal-cell carcinoma assessed associations between SNPs and the risk of developing cancer, rather than recurrence.[5–7] Most of the previously reported polymorphisms were included in our analysis but were not associated with risk of recurrence. We are not aware of previous germline studies of *MET* polymorphisms and cancer risk or prognosis.

Our study should be replicated in other populations and larger cohorts to further validate our findings. If validated as a risk factor, the polymorphism could be incorporated into future prognostic instruments, potentially aiding in the design of adjuvant clinical trials with *MET* inhibitors, and clinical management. Additionally, functional studies should be done to identify the biological mechanisms involved in this association.

Contributors

TKC designed the study. FABS, TKC, MEL, MMP, and MLF collected data. TKC, FABS, KPG, MBA, JER, MSH, DFM, G-SML, MEL, SS, PWK, MMP, and MLF analysed and interpreted data. TKC, FABS, and KPG designed the tables and figure. TKC, FABS, KPG, MBA, JER, MSH, DFM, G-SML, MEL, SS, PWK, MMP, and MLF wrote the manuscript.

Conflicts of interest

TKC has received research funding from Pfizer and has served on advisory boards for Novartis, Pfizer, Aveo, and GlaxoSmithKline. MBA has served on advisory boards for Genentech, Bristol-Myers Squibb, Novartis, Prometheus, Amgen, Merck, and Aveo. JER has served on advisory boards or has been a consultant for Genentech, Abbott, and Novartis and has taken part in a speakers bureau for Novartis. He has also received research funding from GlaxoSmithKline, Sanofi-Aventis, and Novartis. The other authors declare that they have no conflicts of interest.

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