Genome-wide association study identifies multiple risk loci for renal cell carcinoma

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Previous genome-wide association studies (GWAS) have identified six risk loci for renal cell carcinoma (RCC). We conducted a meta-analysis of two new scans of 5,198 cases and 7,331 controls together with four existing scans, totalling 10,784 cases and 20,406 controls of European ancestry. Twenty-four loci were tested in an additional 3,182 cases and 6,301 controls. We confirm the six known RCC risk loci and identify seven new loci at 1p32.3 (rs4381241, \( P = 3.1 \times 10^{-10} \)), 3p22.1 (rs67311347, \( P = 2.5 \times 10^{-8} \)), 3q26.2 (rs10936602, \( P = 8.8 \times 10^{-9} \)), 8p21.3 (rs2241261, \( P = 5.8 \times 10^{-9} \)), 10q24.33-q25.1 (rs11813268, \( P = 3.9 \times 10^{-8} \)), 11q22.3 (rs4911261, \( P = 2.1 \times 10^{-10} \)) and 14q24.2 (rs4903064, \( P = 2.2 \times 10^{-24} \)). Expression quantitative trait analyses suggest plausible candidate genes at these regions that may contribute to RCC susceptibility.
Kidney cancer is the seventh most commonly diagnosed cancer in more developed regions of the world and incidence rates have been rising. Renal cell carcinoma (RCC) comprises over 90% of kidney cancers and clear cell renal cell carcinoma (cRCC) is the major histological subtype (~80% of RCC cases). Direct evidence for inherited predisposition to RCC is provided by a number of rare cancer syndrome families with defined germline mutations in 11 genes (BAP1, FLCN, FH, MET, PTEN, SDHB, SDHC, SDHD, TSC1, TSC2 and VHL), that are associated with the development of different RCC subtypes. While identification of these genes has led to important insights into the pathogenesis of RCC, even collectively these diseases account for only a very small portion of the twofold increased risk of RCC seen in first-degree relatives of RCC patients. Support for polygenic susceptibility to RCC has come from genome-wide association studies (GWAS) that have identified single-nucleotide polymorphisms (SNPs) at six loci influencing RCC risk in populations of European ancestry at chromosome bands 2p21, 2q22.3, 8q24.21, 11q13.3, 12p11.23 and 12q24.31 (refs 9–14). Here, we present findings from a meta-analysis of six GWAS scans of RCC; two new scans of 5,198 cases and 7,331 controls were combined with four previously published scans (IARC-1, NCI-1, MDA, and UK) to support a previously suggested locus marked by rs3845536 at 1q24.1 (meta-analysis P = 0.0062).

For replication, we selected 24 SNPs marking 20 possible independent series (Fig. 1).

Results
Discovery-phase findings. For both the GWAS and replication sets, cases were restricted to invasive RCC (International Classification of Disease for Oncology second and third Edition topography code C64), including all histological subtypes, diagnosed in adults (that is, ≥18 years) (Supplementary Table 1). Comparable sample and SNP quality control exclusions were applied to the two new genotyped scans (Supplementary Online methods), which used the OmniExpress and Omni5M arrays, respectively. The discovery phase was conducted as a fixed-effect meta-analysis that included these two new scans together with four previously published scans (IARC-1, NCI-1, MDA and UK). The four previously reported scans were conducted using HumanHap 300 and 610 for IARC-1; 500 and 660w for NCI-1; 660w for MDA; and OmniExpress and HumanHap 1.2M for UK. Imputations were performed on all scans using 1,094 subjects from the 1000 Genomes Project (phase 1 release 3) as the reference panel (Supplementary Online methods). Each discovery-stage data set was analysed individually assuming log-additive (trend) SNP effects, with the exception of the two IARC scans which were pooled and analysed together (Supplementary Online methods). We then performed a fixed-effects meta-analysis of 7,437,091 SNPs that were polymorphic in at least two data sets. Quantile–quantile plots of the combined results showed little evidence for inflation of the test statistics compared to the expected distribution (λ = 1.034; Supplementary Fig. 1). For visual representation, we provide a Manhattan plot summarizing the genome-wide SNP results in Supplementary Fig. 2.

In the meta-analysis, we observed associations that surpassed the level of genome-wide significance for all six of the previously reported GWAS loci at 2p21, 2q22.3, 8q24.21, 11q13.3, 12p11.23 and 12q24.31 (Supplementary Table 2). We did not find evidence to support a previously suggested locus marked by rs3845536 at 1q24.1 (ref. 15) (meta-analysis P = 0.0062).

For replication, we selected 24 SNPs marking 20 possible new-risk regions, based on a P value <5.0 × 10−7. We also included two SNPs at the known 2p21 RCC risk locus that were potentially independent from the previously reported genome-wide significant SNPs in that region. Four additional SNPs representing four promising loci (one of which was among the 20 previously mentioned regions) were also advanced from an analysis restricted to cRCC (5,649 cases, 15,011 controls) based on the aforementioned P value criterion (Supplementary Data 1). For genotyping these markers using Taqman assays, highly correlated proxy variants were substituted for 14 SNPs for which a Taqman assay could not be optimized; two proxies per variant were selected for two SNPs in the region where the smallest P values were found. Thus, a total of 32 SNPs from 24 regions were genotyped and passed quality control metrics in three independent series totalling 3,182 cases and 6,301 controls (Fig. 1, Supplementary Table 3, Supplementary Data 1, Supplementary Online methods).

Seven new loci associated with RCC risk. In the combined analysis, SNPs at seven loci showed evidence for an association with RCC which was genome-wide significant: 1p32.3 (rs4381241, $P = 3.1 \times 10^{-10}$), 3p22.1 (rs67311347, $P = 2.5 \times 10^{-9}$), 3q26.2 (rs10936602, $P = 8.8 \times 10^{-9}$), 8p21.3 (rs2241261, $P = 5.8 \times 10^{-9}$), 10q24.33-q25.1 (rs11813268, $P = 3.9 \times 10^{-8}$), 11q22.3 (rs74911261, $P = 2.1 \times 10^{-10}$) and 14q24.2 (rs4903064, $P = 2.2 \times 10^{-24}$) (Table 1, Supplementary Data 1). None of SNP associations showed evidence for study heterogeneity. Regional LD plots for each locus are detailed in Supplementary Fig. 3. Restricting the analyses to ccRCC, no additional SNPs with genome-wide significant associations were identified (Supplementary Data 1).

We conducted further analyses of the genome-wide significant SNPs stratifying by sex and three established RCC risk factors: body mass index, smoking and hypertension (Supplementary Table 3, Supplementary Data 1).
The most notable difference in risk was observed for the 14q24 variants that had a stronger effect in women than in men [for rs4903064, odds ratios: ORs (95% confidence interval: CI) of 1.36 (1.28–1.45) and 1.13 (1.08–1.19), respectively; heterogeneity $P = 7.4 \times 10^{-5}$]. Other observed differences across strata were of smaller magnitude (Supplementary Fig. 4). No notable findings were observed in additional SNP analyses of non-clear cell histologic subtypes (papillary, chromophobe; Supplementary Data 1) and case age at onset (< 60 versus 60+) (Supplementary Data 2). For SNP rs76912165, which was not genome-wide significant overall, a trend for higher risk associated with stage 1 cases was observed (Supplementary Data 2).

We investigated whether rs6706003 and rs6755594 defined independent signals at the previously reported 2p21 locus. rs6706003 is minimally correlated with rs7579899 ($r^2 = 0.11$ in CEU) that was identified in the initial GWAS, and moderately correlated with rs12617313 ($r^2 = 0.61$), which was identified in a previous fine-mapping analysis. By comparison, the correlation of rs6755594 with both of these sites is notably weaker ($r^2 = 0.04$ and 0.08, respectively). In conditional analyses of the GWAS data adjusting for rs7579899 and rs12617313, the rs6706003 signal was substantially reduced (OR 1.07, $P = 0.05$), while the rs6755594 signal was partially attenuated (OR 1.07, $P = 4.0 \times 10^{-5}$). On the basis of these findings, there is insufficient evidence to conclude that rs6755594 marks an independent locus in this region.

**Newly identified loci and biological inferences.** To investigate plausible candidate variants and genes among the newly discovered loci for further study, we: (1) fine-mapped each locus, using 1000 Genome Phase 1, version 3 data (Supplementary Data 3); (2) screened non-coding annotation from ENCODE data using HaploReg v4.1 (ref. 18) and RegulomeDB v1.2 (ref. 19) to identify possible functional variants, primarily in cells of non-kidney origin but also in BC_kidney_01-11002 and BC_kidney_H12817N cell lines (Supplementary Data 3); and (3) performed expression quantitative trait locus (eQTL) analyses with genes located up to 3 Mb around the newly identified risk markers (or highly correlated proxies) using ccRCC and normal kidney tissue data from the Cancer Genome Atlas [Kidney Renal Clear Cell Carcinoma (KIRC) collection; 481 tumour and 71 normal tissue samples] and IARC (555 tumour and 234 normal tissue samples) (Supplementary Data 4).

The new highly significant locus marked by rs4903064 at 14q24 maps to the double PHD fingers 3 gene (DPF3), which encodes a histone acetylation and methylation reader of the BAF and PBAF chromatin remodelling complexes. This locus contains a set of correlated SNPs ($r^2 > 0.8$ in 1000G EUR) that reside within the introns of DPF3 (Supplementary Data 3), of which only rs4903064 itself is annotated as likely to disrupt transcription factor binding (RegulomeDB score < 4) (19). This variant is located within a region annotated as an enhancer in multiple tissues by the RoadMap project (22) and is predicted to alter IRX2/IRX5 binding motifs. In an eQTL analysis, we observed a consistent pattern of increased DPF3 expression associated with the rs49030604 risk allele in both the KIRC and IARC data sets ($P = 5.5 \times 10^{-8}$ and $3.8 \times 10^{-9}$, respectively, Fig. 2, Supplementary Data 4). A consistent, but statistically weaker, expression pattern in the normal kidney tissue data sets of more limited sample size was also observed ($P = 0.15$ and 0.42, respectively). It is noteworthy that 14q24 is deleted in 22–45% of ccRCC (20,21). While DPF3 mutation is rare in RCC (20), somatic alterations of BAP1 and PBRM1, components of the BAF and PBAF complexes, respectively, are commonly seen in ccRCC (22). In this regard, deregulation of this pathway is a common feature of RCC, and these data suggest that rs4903064 may play a role in RCC development through dysregulation of the DPF3 expression.

For the 1p32.3 locus marked by rs4381241, an intronic SNP within FAS-associated factor 1 (FAF1) that encodes a protein that can initiate or enhance FAS-mediated apoptosis, we identified several promising correlated variants with RegulomeDB scores, suggesting alteration of transcription factor binding (Supplementary Data 3) but did not observe a strong effect on expression (Supplementary Data 4). FAS-associated factor 1 facilitates the degradation of β-catenin, a transcriptional co-activator that stimulates expression of genes driving cell proliferation (23). Constitutively activated β-catenin, induced by VHL inactivation, is an important pathway in ccRCC oncogenesis (24). The rs4381241 risk allele is weakly correlated ($r^2 = 0.12$ in CEU) with the allele of another FAF1 variant (rs17106184) associated with reduced risk of type-2 diabetes and lower serum insulin post oral glucose challenge (25,26).

The risk variant rs67311347 maps to a region of 3p22.1 that harbours several genes. Within the KIRC tumour tissue data, the risk-associated allele of the surrogate SNP rs9821249 ($r^2 = 0.97$ with rs67311347 in CEU) was weakly associated with higher expression of CTNNB1 ($P = 0.03$). This gene, located 706 kb away from the centromeric, is a strong candidate as it encodes the RCC proto-oncogene β-catenin, although this association was not seen within the IARC data set. In both normal tissue data sets, the risk-associated allele of rs67311347 was associated with a higher expression of ZNF620 ($P = 0.03$ and 0.02). This gene encodes the Zinc finger protein 620, but the function of this protein has not been well described.

The 8p21.3 risk variants rs2241261 and rs2889 (used as proxy for rs2241260, $P = 1.6 \times 10^{-9}$, $r^2 = 0.61$ with rs2241261 in CEU; Supplementary Data 1) are located 0.9 and 1.7 kb respectively from TNFRSF10B, a tumour suppressor gene encoding a mediator of apoptosis signalling (27). In both the KIRC and IARC tumour tissue data ($P = 0.002$ and 0.03, respectively), the rs2241261 risk allele was associated with a decreased expression of GFRα2, which encodes for cell-surface receptor for glial cell line-derived neurotrophic factor (GDNF) and neuturin (NTN), and mediates activation of the RET tyrosine kinase receptor (Glial cell line-derived neurotrophic factor (Supplementary Data 4). A potential link with renal tissue function has not been described. Of the variants in strong LD with either rs2241261 or rs2889 ($r^2 > 0.8$ in 1000G EUR), only rs2889 is annotated as a strong regulatory candidate by RegulomeDB, predicted to be in a strong enhancer region and altering motifs for FOX family members of transcription factors (Supplementary Data 3).

SNPs rs74911261 and rs1800057 are located 214 kb apart on 11q22.3 and are highly correlated ($r^2 = 0.83$ in CEU) non-synonymous variants, but for separate genes; rs74911261 (P144L) maps to KDELRC2, which encodes a protein localizing to the endoplasmic reticulum, while rs1800057 (P1054R) maps to the DNA repair gene ATM. The functional prediction tools SIFT (28) and PolyPhen-2 (ref. 31) suggest that both amino acid substitutions are damaging. It is also plausible that they are correlated with regulatory variants that influence expression of nearby genes. In eQTL analyses, no consistent associations were detected. Only one of the five variants with strong LD to rs74911261 ($r^2 > 0.8$ in 1000G EUR) has a RegulomeDB score suggesting likely disruption of transcription factor binding (score < 4), rs141379009, and is located within a region annotated as an enhancer by the Roadmap project and predicted to alter a consensus Zfp105/ZNF355 binding motif (Supplementary Data 3). ATM mutations in RCC are uncommon (20,22), and ataxia telangiectasia patients, though at markedly elevated cancer risk, have not been reported to frequently develop RCC (23,24), questioning a direct role of ATM in RCC susceptibility.
For the remaining two new RCC risk loci, in silico analyses and eQTL did not indicate altered regulation of a plausible candidate gene. For each of these loci, we identified SNPs that correlate with low RegulomeDB scores for intriguing nearby candidate genes (Supplementary Data 3). The marker SNP rs10936602 maps to 3q26.2, a region amplified in 15% of ccRCC tumours in KIRC43; several notable nearby genes could represent possible candidate genes, including MECON, a transcriptional regulator frequently amplified in RCC44, and TERC, encoding a component of telomerase, in which mutations cause autosomal dominant dyskeratosis congenita and aplastic anaemia45. This risk variant is highly correlated with SNPs associated with MECON genes, including several notable nearby genes could represent possible candidate genes.

Polycyclic risk score analysis and explained heritability. Additional analyses were conducted by generating a polycyclic risk score (PRS) from 13 SNPs mapping to the six previously reported and seven newly identified susceptibility loci (Supplementary Table 5). Accepting the caveat of the winner’s curse phenomenon, whereby the strength of SNP associations may have been overestimated, subjects in the highest decile of the PRS had a threefold increased risk of RCC relative to the lowest decile (OR 3.24, 95% CI 2.86–3.67; P = 1.2 × 10⁻⁷⁶). Stratifying by histological subtypes, the PRS was most strongly associated with clear cell RCC (per unit increase: OR 3.24, 95% CI 2.91–3.62; P = 3.4 × 10⁻¹⁰⁶), with a weaker association for chromophobe RCC (OR 2.34, 95% CI 1.58–3.46; P = 3.4 × 10⁻⁵) and papillary RCC (OR 1.83, 95% CI 1.44–2.32; P = 5.3 × 10⁻⁷). The PRS did not significantly differ between cases aged <60 versus ≥ 60 at diagnosis or across cancer stage (Supplementary Table 5).

Discussion
Our meta-analysis of six GWAS scans identified seven new RCC susceptibility loci. Our findings provide further evidence for polygenic susceptibility to RCC. Future investigation of the genes targeted by the risk SNPs is likely to yield increased insight into the development of RCC. We estimate that the risk loci so far identified for RCC account for only about 10% of the familial risk of RCC. Although the power of our study to detect the major common loci (MAF > 0.2) conferring risk ≥ 1.2 was high (~80%), we had low power to detect alleles with smaller effects and/or MAF < 0.1. By implication, variants with such profiles probably represent a much larger class of susceptibility loci for RCC and hence a large number of variants remain to be discovered. In parallel, whole-exome and whole-genome sequencing of genetically enriched cases selected according to early age of onset or family history would provide new
opportunities to discover rare variants associated with RCC. As more RCC susceptibility alleles are discovered, deciphering the biological basis of risk should provide new insights into the biology of RCC that may lead to new approaches to prevention, early detection and therapeutic intervention.

**Methods**

**Informed consent and study approval.** Each participating study obtained informed consent from the study participants and approval from its Institutional Review Board; for the Mayo replication: Mayo Clinic institutional review board.

**Quality control assessment.** The quality control exclusions for the four previously published scans have been reported9–11. For the two new scans, quality control was conducted separately at each institution using comparable exclusions.

**Genome-wide SNP genotyping.** Genome-wide SNP genotyping for two new scans was coordinated by the National Cancer Institute (NCI-2; NCI, Bethesda, Maryland, USA) and the International Agency for Research on Cancer (IARC-2; IARC, Lyon, France). The NCI-2 samples, obtained from 13 studies conducted in the US and Finland (Supplementary Table 1), were genotyped at the NCI Cancer Genomics Research Laboratory (CGR, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA) using the Illumina OmniExpress array. The NCI-2 scan included controls previously genotyped by Illumina OmniExpress, or Omni 2.5M array from some of the participating studies (ATBC, CPSII, HPFS, NHS, PLCO and WHI; Supplementary Table 1). IARC-2 samples, obtained from six studies conducted in Europe and Australia (Supplementary Table 1), were genotyped at the Centre National de Genotypage, Commissariat à l’énergie atomique et aux énergies alternatives (CNG, CEa, Evry and Paris) using the Illumina Omni 5 M arrays. Additional controls (N = 447) from one study (IARC K2) were also included, which had been genotyped on the OmniExpress array at Johns Hopkins Center for Inherited Disease Research.

Figure 2 | Plots of eQTL association between rs4903064 and DPF3 expression. (a) TCGA-KIRC normal, (b) TCGA-KIRC tumour, (c) IARC normal, and (d) IARC tumour sample data sets. Box boundaries designate the twenty-fifth and seventy-fifth percentiles, black line in the centre of boxes represent the median, whiskers extend to the minimum of either the data range or 1.5 times the interquartile range and statistical outliers are plotted as points.
exclusion, the concordance rate was >99.9% for 66 pairs of blind duplicate pairs. After removing duplicates, a data set including 2,820 unique samples was advanced to further assess quality control at the subject level. In addition, we excluded 10 sex-discordant individuals and two individuals with excessively low mean heterozygosity for ChrX SNPs. For the cleaned data including genotypes for 2,808 individuals, we next pooled equal numbers of scans controlled (HumanOmni2.5M or HumanOmniExpress array) from the ATBC, CPSII, HPFS, NHS, PHCO and WHII studies (Supplementary Table 1). After merging the newly scanned data with the previously scanned controls, we obtained genotypes for 7,029 individuals. Subsequently, we excluded data for 204 non-CEU individuals (admixed proportion for CEU <0.80), both members of a pair of unexpected within-family duplicate samples, one from each of eight unexpected cross-study duplicate pairs, and one from each of eight related pairs (two parent–child pairs and six sibling pairs). The final analytic data comprised 6,808 individuals (2,417 cases, 4,391 controls) for 678,580 loci.

Statistical analysis. The statistical analysis included summary data from four previously published scans conducted at the NCI (NCI-1)3, IARC (IARC-1)5, the University of Texas MD Anderson Cancer Center (MDA)9, and the Institute of Cancer Research, UK (UK)14, as well as the two new scans from NCI (NCI-2) and IARC (IARC-2). The IARC-1 and IARC-2 data were pooled, resulting in five separate discovery-stage data sets. Imputation was performed separately for each data set using SNPs of minor allele frequency $\geq 0.01$ (0.05 for the IARC data set), with 1000 Genomes Project data (phase I release 3) used as a reference set. IMPUTE2 version 2.2.2 was used for imputation of the NCI-1, NCI-2, MDA and UK data sets, while Minimac version 3 was used for the IARC data set42,43. Imputed SNPs with sufficient accuracy as assessed by r$^2$ \geq 0.3 for both IMPUTE2 and Minimac were retained for the analysis. We further assessed the quality of imputation by randomly selecting 10% of genotyped SNPs on chromosome 1 within the IARC-1 series (which used the least-dense chip across the different scans) and removing them before running the imputation algorithm. MAFs calculated from the genotyping data correlated with r$^2$ >0.99 with MAFs calculated from the imputed dosage data.

Technical validation of imputed SNPs. To technically validate our imputation findings, we genotyped the 32 SNPs carried over for replication by Tagman assay in a subset of samples from the NCI-2 and IARC-1/2 scans (n=566 and 6,402 respectively). The concordances between imputed and directly assayed genotypes are detailed in Supplementary Table 4.

Gene expression data and eQTL analysis. IARC: For a subset of cases from the IARC K2 and the CE studies (Supplementary Table 1) we performed expression analysis of renal normal and tumour tissue samples were conducted using Illumina HumanHT-12 v4 expression BeadChips (Illumina, Inc., San Diego) for samples with RNA integrity (RIN) >5.0. Raw expression intensities of samples with signal-to-noise ratio >9.5 were processed with variance-stabilizing transformation and quantile normalization with lumi package47 as reported by Wozniak et al21. The 50 mer sequences of probes were mapped to human reference genome hg19 downloaded from UCSC Genome Browser database (http://genome.ucsc.edu/), accessed on 15 November 2014) using BWA22 to demarcate positional relationships between corresponding probes/gens and SNPs. In total, 234 normal and 555 tumour tissue samples from confirmed clear cell RCC cases were sequenced for allele-specific increases in gene expression for genotypes within a 6 Mb window. Analyses were performed using R v3.1.

Data availability. The scan IARC-2 obtained Institutional Review Board certification permitting data sharing in accordance with the US NIH Policy for Sharing of Data Obtained in NIH Supported or Conducted GWAS. Data are accessible on dbGaP (study name: ‘Pooled Genome-Wide Analysis of Kidney Cancer Risk (KDRISK)’; url: http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001271v1.p1). Similarly, the NCI-1 scan is accessible on dbGaP (phs000351.v1.p1). Data from IARC-1 and MDA scans are available from Paul Brennan and Xifeng Wu, respectively, upon reasonable request. The UK scan data will be made available on the European Genome-phenome Archive database (accession number: EGAS00001002336). The NCI-2 scan will be posted on dbGaP.

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Polycygen risk score and analyses of additional RCC phenotypes. PRS was calculated for 13 SNPs, one from each of the six previously identified loci and seven newly identified RCC risk loci (rs1059393, rs7465623, rs718314, rs11894252, rs12105918, rs6470588, rs3481241, rs673311347, rs10936602, rs2241261, rs1813268, rs7491261 and rs4903064), as follows: $\text{PRS}_i = \sum_{j=1}^{13} w_j x_{ij}$, where $\text{PRS}_i$ is the risk score for individual $i$, $x_{ij}$ is the number of risk alleles for the $j$th variant and $w_j$ is the weight [ln(OR)] of the $j$th variant. Associations with the PRS and individual SNPs selected for replication were computed for the following RCC phenotypes: papillary and chromophobe RCC histologies (through case-control analyses); age at onset ($<60$ versus $60 +$ years at diagnosis; case-only analyses) and stage (2, 3 and 4 versus 1; case-only analyses). The stage-stratified analyses were restricted to the IARC data sets, for which these data were available.
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19. The authors thank all of the participants who took part in this research and the funders and support staff who made this study possible. Funding for the genome-wide genotyping was provided by the US National Institutes of Health (NIH), National Cancer Institute (U01CA155309) for those studies coordinated by IARC and by the intramural research program of the National Cancer Institute, US NIH, for those studies coordinated by the NCI. Funding for the IARC gene expression and eQTL study was provided by the US National Institutes of Health (NIH), National Cancer Institute (U01CA155309). Additional acknowledgments can be found in Supplementary Note.

**Author contributions**

G.S., G.M.L., J.D.M., J.-F.D., K.M.B., M.P.P., N.Rot., P.Br. and S.J.C. contributed to the design and execution of the overall study. A.B., A.C., B.A.-A., C.B., E.P., F.L.C.-K., G.D., H.B., K.G.S., L.B., M.B.W., M.Y. and N.Rob. performed the experiments. A.S.P., G.Sc., J.E.E.-P., J.C., J.D.M., J.N.H., I.N.S., K.M.B., L.M.C., M.B.W., M.F., M.I., M.J.M., M.P.P., P.Br., P.L., S.J.C., V.G., X.W., Y.Y. and Z.W. contributed the design and execution of the statistical analysis. G.Sc., K.M.B., L.M.C., M.I., M.P.P., P.Br. and S.J.C. wrote the first draft of the manuscript. The remaining authors, as well as A.S.P., G.Sc., J.E.E.-P., M.I., M.P.P., P.Br., X.W. and Y.Y. conducted the epidemiological studies and contributed samples to the GWAS and/or replication studies. All authors contributed to the writing of the manuscript.

**Additional information**

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

**Competing interests:** The authors declare no competing financial interests.

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**How to cite this article:** Scelo, G. et al. Genome-wide association study identifies multiple risk loci for renal cell carcinoma. *Nat. Commun.* 8, 15724 doi: 10.1038/ncomms15724 (2017).

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
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