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

Ghislaine Scelo et al.

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 (rs74911261, \(P = 2.1 \times 10^{-10}\)) 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.

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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 (ccRCC) is the major histological subtype (~80% of RCC cases). Direct evidence for inherited predisposition to RCC is provided by a number of rare cancer syndromes 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 of 5,586 cases and 13,075 controls, reaching a total of 10,784 cases and 20,406 controls, all of European ancestry. Twenty-four promising loci were further tested in an independent replication set of 3,182 cases and 6,301 controls drawn from three 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 660 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 ($\lambda = 1.034$; 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 $< 5.0 \times 10^{-7}$. 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 ccRCC (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 ($rs67311347, P = 2.5 \times 10^{-8}$), 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 ($rs74911261, P = 2.1 \times 10^{-10}$) and 14q24.2 ($rs903064, P = 2.2 \times 10^{-24}$) (Table 1, Supplementary Data 1). None of SNP associations showed evidence of 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

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**Figure 1** | Overview of the study design. The six genotyping scans contributing to the meta-analysis are detailed on the left, with number of cases and controls and arrays used for the genotyping. The meta-analysis was performed after imputations on 7,437,091 SNPs. Boxes in italic bold represent genotype data newly generated for this study.
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 proliferation25. Constitutively activated β-catenin, induced by VHL inactivation, is an important pathway in ccRCC oncogenesis26. 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 challenge27,28.

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 rs98221249 ($r^2=0.97$ with rs67311347 in CEU) was weakly associated with higher expression of CTNNB1 ($P=0.03$). This gene, located 706 kb away 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 TNRFSF10B, a tumour suppressor gene encoding a mediator of apoptosis signalling29. 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 neurturin (NTN), and mediates activation of the RET tyrosine kinase receptor (Gliial 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 (P14LI) maps to KDEL2, which encodes a protein localizing to the endoplasmic reticulum, while rs1800057 (P1054R) maps to the DNA repair gene ATM. The functional prediction tools SIFT30 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/ZNF35 binding motif (Supplementary Data 3). ATM mutations in RCC are uncommon20,23, and ataxia telangiectasia patients, though at markedly elevated cancer risk, have not been reported to frequently develop RCC22, 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 KIRC20; several notable nearby genes could represent possible candidate genes, including MECom, a transcriptional regulator frequently dysregulated in ccRCC, and TERC, encoding a component of telomerase, in which mutations cause autosomal dominant dyskeratosis congenita and aplastic anaemia23,24. This risk variant is moderately correlated with variants previously associated with telomere length and risk of several malignancies, including multiple myeloma, chronic lymphocytic leukaemia, bladder cancer, glioma and colorectal cancer (rs10936599, rs12696304, rs1920116; Supplementary Table 5) and 1.46 (SE 0.10), respectively. On the basis of these estimates, the PRS did not significantly differ between cases aged <60 versus 60+ at diagnosis or across cancer stage (Supplementary Table 5).

Using Genome-Wide Complex Trait Analysis (GCTA), we estimate that the heritability and familial relative risk of RCC attributable to all common variation were 14.2% (SE = 0.023) and 1.52 (SE = 0.10), respectively. After excluding established and newly identified loci, the estimates were 12.8% (SE = 0.023) and 1.46 (SE = 0.10), respectively. On the basis of these estimates, ~90% of the heritability and familial risk remains to be elucidated.

**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

### Table 1 | Summary results for newly discovered loci associated with renal cell carcinoma.

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP*</th>
<th>Closest gene</th>
<th>Position (base pairs)</th>
<th>A/α</th>
<th>MAF†</th>
<th>Statistics</th>
<th>Discovery (10,784 cases; 20,406 controls)</th>
<th>Replication (3,182 cases; 6,301 controls)</th>
<th>Combined (13,966 cases; 26,707 controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p32.3</td>
<td>rs4381241</td>
<td>FAF1</td>
<td>50907438 T/C</td>
<td>0.44</td>
<td>OR (95% CI)</td>
<td>1.11 (1.07–1.15)</td>
<td>1.1 (1.03–1.20)</td>
<td>1.1 (1.07–1.15)</td>
<td></td>
</tr>
<tr>
<td>3p22.1</td>
<td>rs67311347</td>
<td>G/A</td>
<td>0.31</td>
<td>OR (95% CI)</td>
<td>0.89 (0.86–0.93)</td>
<td>0.94 (0.88–1.01)</td>
<td>0.9 (0.87–0.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3q26.2</td>
<td>rs10936602</td>
<td>LRRIQ4</td>
<td>169536637 T/C</td>
<td>0.27</td>
<td>OR (95% CI)</td>
<td>0.90 (0.86–0.94)</td>
<td>0.91 (0.85–0.98)</td>
<td>0.9 (0.87–0.93)</td>
<td></td>
</tr>
<tr>
<td>8p21.3</td>
<td>rs2241261</td>
<td>RHOB/T82/TFNRF510B</td>
<td>22876739 C/T</td>
<td>0.51</td>
<td>OR (95% CI)</td>
<td>1.10 (1.06–1.14)</td>
<td>1.10 (1.03–1.17)</td>
<td>1.1 (1.06–1.13)</td>
<td></td>
</tr>
<tr>
<td>10q24.33-25.1</td>
<td>rs11813268</td>
<td>OBFC1</td>
<td>105682296 C/T</td>
<td>0.16</td>
<td>OR (95% CI)</td>
<td>1.13 (1.08–1.19)</td>
<td>1.10 (1.01–1.19)</td>
<td>1.1 (1.07–1.17)</td>
<td></td>
</tr>
<tr>
<td>11q22.3</td>
<td>rs7491126</td>
<td>KDELC2</td>
<td>108357137 G/A</td>
<td>0.02</td>
<td>OR (95% CI)</td>
<td>1.42 (1.26–1.61)</td>
<td>1.38 (1.12–1.69)</td>
<td>1.4 (1.27–1.57)</td>
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<tr>
<td>14q24.2</td>
<td>rs4903064</td>
<td>DPF3</td>
<td>73279420 C/T</td>
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<td>OR (95% CI)</td>
<td>1.18 (1.13–1.23)</td>
<td>1.10 (1.21–1.39)</td>
<td>1.2 (1.16–1.25)</td>
<td></td>
</tr>
</tbody>
</table>

*SNP with lowest P value within locus. For 1q22.3, results shown for two non-synonymous SNPs in KDELC2 (rs7491126, Pro144Leu) and ATM (rs1800057, Pro1054Arg; r² = 0.83 in CEU).

†Minor allele frequency among all controls (n = 26,707). Odds ratios (OR) are shown for the minor allele, assuming a log-additive (trend) SNP effect.

Polygenic risk score analysis and explained heritability. Additional analyses were conducted by generating a polygenic 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 × 10⁻³) 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).
opportunities to discover rare variants associated with RCC. As more RCC susceptibility alleles are discovered, deciphering the biological basis of risk variants 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 IARC scans and replication: IARC Ethics Committee; for the MDA scans and replication: Institutional Review Board of the University of Texas MD Anderson Cancer Center; for the UK scan: Royal Marsden NHS Trust ethics committee; for the NCI scans: NCI Special Studies Institutional Review Board, The Vanderbilt Institutional Review Board, the Emory University Institutional Review Board, Dana-Farber/Harvard Cancer Center institutional review board, Institutional Review Board of the Harvard T.H. Chan School of Public Health, Institutional Review Board of Brigham and Women’s Hospital, Van Andel Research Institute Institutional Review Board, Spectrum Health Institutional Review Board and Fred Hutchinson Cancer Research Center Institutional Review Board; for the Mayo replication: Mayo Clinic institutional review board.

**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 2.5MArray 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.

**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. For the new IARC-2 scan, a total of 5,424 samples were genotyped on the Illumina Omni5 chip. Samples were excluded sequentially based on the following criteria: heterozygosity rate (n = 14, 0.3%), relatedness (n = 7, 0.1%), non-CEU ancestry (n = 37, 0.7%), sex discrepancy (n = 20, 0.4%), genotyping success rate < 95% (n = 14, 0.3%) and unexpected duplicates (n = 22, 0.4%). After adding the 447 previously scanned controls (OmniExpress array) from the IARC K2 study, using the above-listed criteria we excluded 22 samples (4.9%) and, due to unexpected duplicates or first-degree relatedness between the two scans, an additional 11 (2.5%) samples from the two scans, an additional 11 (2.5%) samples from this scan and three samples from the Omni5 scan. From the Omni5 scan, genotypes for 4,276,196 SNPs were obtained, of which we excluded 127,523 SNPs because of low (≤ 95%) success rate, 14,513 SNPs for departure from Hardy–Weinberg disequilibrium (HWE) (P < 10^-7) in controls, 65,300 with ambiguous strand issues, and 37,319 non-autosomal SNPs. The final Omni5 analytical data set included 4,031,541 SNPs on 2,781 cases and 2,526 controls. For the same criteria, the 951,117 SNPs obtained from the Illumina 5 M arrays, respectively, leaving a final data set of 888,491 SNPs on 414 controls. Imputation of genotypes was performed on these data sets after the exclusion of 2,485,185 SNPs from the Omni5, and 742 SNPs from the OmniExpress scans, when minor allele frequency (MAF) was < 0.05.

For the new NCI-2 scan, a total of 3,168 samples were initially genotyped by the Illumina Express array. A total of 22,775 (3%) SNPs with call rate < 90% were excluded, as were 282 samples (9%) with completion rate < 94%. After this
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 a total of 4,421 previously scanned controls (HumanOmni2.5M or in a total of 6,421 previously scanned controls) from the ATBC, CPSII, HPFS, NHS, PLCO and WHI 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 (admixture proportion for CEU <80%), both members of a pair of unexpected within-family duplicate samples, one from each of 8 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)39, IARC (IARC-1)40, the University of Texas MD Anderson Cancer Center (MDA)41 and the Institute of Cancer Research, UK (UK)42, 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 scan data set using SNPs of minor allele frequency ≥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 r²≥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 r² >0.99 with MAFs calculated from the imputed dosage data. Finally, top SNPs were technically validated through Taqman genotyping in the IARC-1 and NCI-2 scan (Supplementary Table 4). After imputation, genotypes for 7,437,091 SNPs were available for analysis.

Association testing with RCC was conducted separately for each data set assuming log-additive (trend) SNP effects using SNPTEST version 2.2 at NCI and R version 3.2.3 at IARC. The model covariates varied by data set; for the previous scans, we used the same covariates as in the initially published analyses. The covariates were as follows: sex and study for NCI-1 (no statistically significant eigenvectors present in null model); sex and four significant eigenvectors for NCI-2; age, sex and two significant eigenvectors for MDA; no covariates for the UK; and sex, and study, 19 significant eigenvectors for IARC-1 and IARC-2. Eigenvectors were considered significant if P<0.05 from the Traczy-Widoms statistics. In the IARC series, all 19 eigenvectors were significantly associated with the country of recruitment. We additionally conducted analyses restricted to cRC. The SNP association results from each data set were combined by meta-analysis using a fixed-effects model. Heterogeneity in genetic effects across discovery-stage data sets was assessed using the GCTA software44,45 and data from the NCI-1 and NCI-2 scans. Analyses assumed a disease prevalence of 1.66%, included only SNPs with MAF >0.05, and excluded all SNPs with within-family 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.

Polygenic 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 (rs1105934, rs4765623, rs718314, rs11894252, rs12105918, rs6470588, rs4381241, rs673311347, rs10936602, rs2241261, rs11813268, rs74911261 and rs4903064), as follows:

where PRS is the risk score for individual i, x_i is the number of risk alleles for the jth variant and w_j is the weight [ln(OR)] of the jth 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.

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. KIRC: Genotyping and RNASeq data for the KIRC TCGA samples (481 tumours and 71 normal renal tissues) were downloaded from The Cancer Genome Atlas database (http://cancergenome.nih.gov/, accessed on 15 November 2014) using BWA46 to demarcate positional relationships between corresponding probes/gene and SNPs. In total, 234 normal and 555 tumour tissue samples from confirmed renal cell RCC cases were used to test for allele-specific increases in gene expression for genes within a 6 Mb window. Analyses were performed using R v3.1.3. IARC: For a subset of cases from the IARC K2 and the CE studies (Supplementary Table 1) eQTL 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 lumix package47 as reported by Wozniak et al.48. 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 BWA46 to demarcate positional relationships between corresponding probes/gene and SNPs. In total, 234 normal and 555 tumour tissue samples from confirmed renal cell RCC cases were used to test for allele-specific increases in gene expression for genes within a 6 Mb window under linear trend assumption. Analyses were performed using R v3.1.3.

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)’; http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/ study.cgi?study_id=phs001271.v1.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: EGAS00001020336). The NCI-2 scan will be posted on dbGaP. TCGA data were accessed at the following url: https://gdc-portal.nci.nih.gov/projects/TCGA-KIRC.

References


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**Author contributions**


**Additional information**

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