Frequency of pathogenic germline variants in cancer susceptibility genes in 1336 renal cell carcinoma cases

Bryndis Yngvadottir1,2, Avgi Andreou1,3, Laia Bassaganyas1, Alexey Larionov4,5, Alex J. Cornish3, Daniel Chubb3, Charlie N. Saunders3, Philip S. Smith1, Huairen Zhang1, Yasemin Cole1, Genomics England Research Consortium6,7, Alexey Larionov, Lisa Browning8,9, Samra Turajlic6,7,10, Kevin Litchfield1,2, Richard S. Houlston3 and Eamonn R. Maher1,4

1Department of Medical Genetics, School of Clinical Medicine, University of Cambridge, Cambridge, CB2 0QQ, UK
2School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK
3Division of Genetics and Epidemiology, The Institute of Cancer Research, London, SW7 3RP, UK
4Genomics England, Queen Mary University of London, Dawson Hall, Charterhouse Square, London, EC1M 6BQ, UK
5William Harvey Research Institute, Queen Mary University of London, London, EC1M 6BQ, UK
6Department of Medical Oncology, Renal and Skin Units, The Royal Marsden NHS Foundation Trust, London, SW3 6JJ, UK
7Melanoma and Kidney Cancer Team, The Institute of Cancer Research, London, SW7 3RP, UK
8Department of Cellular Pathology, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, OX3 9DU, UK
9NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, OX4 2PG, UK
10Cancer Dynamics Laboratory, The Francis Crick Institute, London, NW1 1AT, UK
11Department of Oncology, University College London Cancer Institute, Paul O’Gorman Building, London, WC1E 6DD, UK

†These authors contributed equally to this work

Abstract
Renal cell carcinoma (RCC) occurs in a number of cancer predisposition syndromes, but the genetic architecture of susceptibility to RCC is not well defined. We investigated the frequency of pathogenic and likely pathogenic (P/LP) germline variants in cancer susceptibility genes (CSGs) within a large series of unselected RCC participants. Whole-genome sequencing data on 1336 RCC participants and 5834 controls recruited to the UK 100 000 Genomes Project, a nationwide multicentre study, was analyzed to identify rare P/LP short variants (single nucleotide variants and insertions/deletions ranging from 1 to 50 base pairs) and structural variants in 121 CSGs. Among 1336 RCC participants [mean: 61.3 years (±12 SD), range: 13–88 years; 64% male], 85 participants [6.4%; 95% CI (5.1, 7.8)] had one or more P/LP germline variant in a wider range of CSGs than previously recognized. A further 64 intragenic variants in CSGs previously associated with RCC were classified as a variant of uncertain significance (VUS) [24 ‘hot VUS’] and were considered to be of potential clinical relevance as further evaluation might result in their reclassification. Most patients with P variants in well-established CSGs known to predispose to renal cell carcinoma (RCC-CSGs) were aged ~50 years. Burden test analysis for filtered variants in CSGs demonstrated a significant excess of CHEK2 variants in European RCC participants compared with the healthy European controls (P = 0.0019). Approximately, 6% of the patients with RCC unselected for family history have a germline variant requiring additional follow-up analysis. To improve diagnostic yield, we suggest expanding the panel of RCC-CSGs tested to include CHEK2 and all SDHx subunits and raising the eligibility criteria for age-based testing.

Introduction
Kidney cancer is the sixth most commonly diagnosed cancer in the more developed regions of the world and the incidence rates have been rising (1,2). Renal cell carcinoma (RCC) comprises over 90% of kidney cancers and clear cell renal cell carcinoma (ccRCC) is the major histological subtype (~75% of patients), with papillary RCC (pRCC types 1 and type 2), chromophobe RCC (chRCC) and rarer forms accounting for the remainder of patients (15, 5, 5%) (3,4).

Risk factors for kidney cancer include obesity, smoking, hypertension and multiple autosomal dominantly inherited cancer predisposition syndromes including von Hippel–Lindau (VHL), Birt-Hogg-Dubé (BHD) syndrome, hereditary leiomyomatosis and renal cell cancer syndrome (HLRCC), PTEN hamartoma tumour syndrome, hereditary pRCC, BAP1 tumour predisposition syndrome, succinate dehydrogenase subunit genes (SDHB, SDHC and SDHD) and constitutional chromosome 3 translocations (2,5,6). Common single-nucleotide polymorphisms also influence RCC risk, affording a complex heritable basis, but one that is likely to be shaped predominantly by rare variants (7,8).

Although only 3% of RCC patients have a family history of the disease, germline pathogenic variants in cancer susceptibility genes (CSGs) have been reported to be detectable in up to 16% of a referral-based cohort of advanced RCC (9). The contribution of germline variants...
Figure 1. Frequency of P/LP variants in CSGs (RCC-CSGs and other CSGs) in RCC participants. The LoF category includes stop gained, stop lost, frameshifts and splicing variants (individually listed in Supplementary Material, Table S1A).

reported from different centres varies considerably as a consequence of which genes were tested and there were variations in patient ascertainment and selection (9–12). To provide a comprehensive understanding of the contribution of pathogenic/likely pathogenic (P/LP) variants in 121 CSGs to RCC development, we analyzed the whole-genome sequencing (WGS) data on 1336 individuals with RCC recruited into the UK’s 100 000 Genomes Project (100kGP) (13).

Results
Prevalence of P/LP variants in CSGs

The CSGs harbouring clinically relevant variants were subdivided into CSGs known to predispose to renal cell carcinoma (RCC-CSGs) and CSGs CSGs (other CSGs) not previously associated with RCC. All P/LP variants (total 88 variants) were heterozygous, 85 short [single nucleotide variants (SNVs) and insertions/deletions ranging from 1 to 50 base pairs (INDELs)] and three structural variants (SVs) (deletions). Around 68.2% (60/88) of 88 P/LP variants detected were in RCC-CSGs and 31.8% (28/88) were in the other CSGs (all autosomal dominant predisposition genes) (Fig. 1, Tables 1 and 2).

The genotype and phenotypes of the RCC participants with rare P/LP germline variants are summarized in Supplementary Material, Table S1A. Two cases of multilocus inherited neoplasia alleles syndrome (14) were detected: a male with CHEK2 and ATM variants and another male with a CHEK2 and two MSH6 P/LP variants, both with ccRCC (Supplementary Material, Table S1A).

The highest prevalence of P/LP germline variants in RCC-CSGs was in CHEK2, with 27 individuals (seven unique variants) harbouring P/LP variants within the gene [27/1336 (2%)]; 24 of these were loss of function (LoF) variants. Other genes from this group with germline variants included MITF [10/1336 (0.7%)], SDHA [7/1336 (0.5%)], VHL [7/1336 (0.5%)], FLCN [4/1336 (0.3%)], FH [3/1336 (0.2%)] and SDHB [2/1336 (0.1%)].

The highest number of P/LP germline variants in the other CSG group was in ATM, with 10 individuals (9 unique variants) harbouring P/LP variants [10/1336 (0.7%)]. In addition, P/LP germline variants were detected in FANCM [4/1336 (0.3%)], BRIP1 [3/1336 (0.2%)], MSH6 [3/1336 (0.2%)], BRCA2 [2/1336 (0.1%)], PMS2 [2/1336 (0.1%)], TP53 [2/1336 (0.1%)], MSH2 [1/1336 (0.07%)], and PALB2 [1/1336 (0.07%)]. The individual variants are summarized in Table 2.

A further 64 variants in RCC-CSGs were classified as a variant of uncertain significance (VUS) but were considered to be of potential clinical relevance as further evaluation (e.g. by detailed clinical genetic assessment, tumour immunohistochemistry or family studies) might result in the reclassification of these variants as P/LP (Supplementary Material, Table S1B). No VUSs in other CSGs were considered as clinically relevant in the context of RCC. In order to clarify the 10–90% range of potential pathogenicity for the 54 SNV VUSs, we used the quantitative Bayesian framework provided by Tavtigian et al. (15) to calculate a posterior probability and then classified them to hot/warm/tepid or cool/cold/ice cold VUS according to the Association for Clinical Genomic Science (ACGS) guidelines. In summary, there were 24 ‘hot’, 6 ‘warm’, 15 ‘tepid’ and 9 ‘cool/cold’ SNV VUSs. For the remaining 10 CNV VUSs, we used the CNV score based on the American College of Medical Genetics and
Table 1. P/LP variants identified in well-established RCC-CSGs in a cohort of 1336 RCC participants

| Gene   | HGVSca          | HGVSb          | No. of participants (%) |
|--------|-----------------|----------------|-------------------------|
| CHEK2  | ENST00000382580.6:c.1229del | ENSP0000372023.2:p.Thr410MetfsTer15 | 16 (1.2)                |
| CHEK2  | ENST00000382580.6:c.1392del | ENSP0000372023.2:p.Ser465ValfsTer15 | 4 (0.3)                 |
| CHEK2  | ENST00000382580.6:c.478A>G | ENSP0000372023.2:p.Arg160Gly | 2 (0.1)                 |
| CHEK2  | ENST00000382580.6:c.573+1G>A | ENSP0000372023.2:p.Val347TrpsfsTer7 | 2 (0.1)                 |
| CHEK2  | ENST00000382580.6:c.1031del | ENSP0000372023.2:p.Leu344TrpsfsTer7 | 1 (0.07)                |
| CHEK2  | ENST00000382580.6:c.720del | ENSP0000372023.2:p.Val1241PhefsTer7 | 1 (0.07)                |
| CHEK2  | 7.5 kb del      | ENSP0000372023.2:p.Leu154IlefsTer7 | 1 (0.07)                |
| MITF   | ENST00000482267.6:c.1273G>A | ENSP0000391803.2:p.Glu318Lys | 10 (0.7)                |
| SDHA   | ENST00000264932.11:c.91C>T | ENSP0000264932.6:p.Arg31Ter | 7 (0.5)                 |
| VHL    | ENST000003565472.4:c.227_229del | ENSP0000256474.2:p.Phe76del | 1 (0.07)                |
| VHL    | ENST000003565472.4:c.233A>G | ENSP0000256474.2:p.Asn78Ser | 1 (0.07)                |
| VHL    | ENST000003565472.4:c.461C>T | ENSP0000256474.2:p.Pro154Leu | 1 (0.07)                |
| VHL    | ENST000003565472.4:c.286C>T | ENSP0000256474.2:p.Gln96Ter | 1 (0.07)                |
| VHL    | ENST000003565472.4:c.551T>C | ENSP0000256474.2:p.Leu184Pro | 1 (0.07)                |
| VHL    | 13 kb del       | ENSP0000256474.2:p.Glu297Val | 1 (0.07)                |
| VHL    | 10 kb del       | ENSP0000372023.2:p.Arg160Gly | 1 (0.07)                |
| FLCN   | ENST00000285071.9:c.33C>A | ENSP0000285071.4:p.Cys117Ter | 1 (0.07)                |
| FLCN   | ENST00000285071.9:c.490del | ENSP0000285071.4:p.Arg164GlyfsTer13 | 1 (0.07)                |
| FLCN   | ENST00000285071.9:c.890_893del | ENSP0000285071.4:p.Glu297AlafsTer25 | 1 (0.07)                |
| FLCN   | ENST00000285071.9:c.853C>T | ENSP0000285071.4:p.Glu297AlafsTer25 | 1 (0.07)                |
| FH     | ENST00000366560.3:c.1127A>C | ENSP0000355518.3:p.Gln376Pro | 1 (0.07)                |
| FH     | ENST00000366560.3:c.411G>T | ENSP0000355518.3:p.Gly144Val | 1 (0.07)                |
| FH     | ENST00000366560.3:c.413_414del | ENSP0000355518.3:p.Leu138ArgfsTer17 | 1 (0.07)                |
| SDHB   | ENST00000375499.7:c.72+1G>C | ENSP0000364649.3:p.Trp200Cys | 1 (0.07)                |
| SDHB   | ENST00000375499.7:c.600G>T | ENSP0000364649.3:p.Trp200Cys | 1 (0.07)                |

*HGVSa: Human Genome Variation Society coding sequence name. **HGVSb: Human Genome Variation Society protein sequence name.

Genomics (ACMG)/Clinical Genome Resource CNV loss and gain guidelines (2020) (16) (Supplementary Material, Table S1B).

Candidate rare SVs

Seventy-four candidate rare germline SVs (41 deletions, 13 duplications, 14 inversions and 6 translocations) with at least one breakpoint overlapping 31 CSGs (8 RCC-CSGs and 34 other CSGs) were identified in 6.9% (86/1254) participants (Supplementary Results, Supplementary Material, Fig. S1, Supplementary Material, Table S2). We focused on deletions in RCC-CSGs, and three deletions (two in VHL and one in CHEK2) were considered to be pathogenic (included in the prevalence of P/LP before) without additional functional validation. One of the participants had a 13 kb deletion starting 6 kb upstream of VHL in the non-coding sequence, removing 5 kb of the gene, including two of the three exons (Participant A, Fig. 2A). This participant had clinical evidence of VHL disease and did not carry any other P/LP variants. Further analysis, using less stringent filtering (see Supplementary Methods), identified a second germline VHL deletion in another participant with a typical VHL phenotype (Participant B, Fig. 2A). A 7.5 kb deletion in CHEK2, which removes the fifth exon of the gene and deletes a part of the protein kinase domain (Fig. 2B), was detected in a participant with later onset ccRCC, with the initial filters applied.

Combining the results for intragenic and copy number variant analysis, an overall diagnostic yield of 6.4% [95% CI (5.1, 7.8)] was calculated (82/1336 participants with a germline P/LP short variant and 3/1254 with a P/LP SV).

Genotype-phenotype relationship

Additional non-RCC tumours

Four of the RCC participants with germline VHL P/LP variants had clinical features characteristic of VHL disease (haemangioblastomas, multiple ccRCCs and spinal cord tumours). In contrast, none of the 7 participants with FH or FLCN mutations were reported to have clinical indicators of HLRCC or BHD syndrome and none of the 10 carriers of P MITF variants had a past history of melanoma. Although 10 of 60 participants with a P/LP variant in an RCC-CSG had an additional non-RCC neoplasm (breast cancer, colorectal cancer, thyroid cancer, ovarian cancer, testicular tumour, basal cell carcinoma and haematological malignancy), none of the tumour combinations were characteristic for a recognized inherited RCC syndrome.

Seven of 28 participants with a P/LP variant in other CSGs had a past history of non-RCC cancer (bladder cancer, prostate cancer, testicular cancer and breast cancer), including a case with a germline TP53 mutation with synchronous uterine cancer, central nervous system cancer and chRCC at 45 years. Breast cancer was recorded in one of ten participants with a P/LP variant in an RCC-CSG. None of the 7 participants with mismatch repair (MMR) or POLE P/LP variants had a history of colorectal cancer and their tumours did not show a cancer MMR signature.

Gender

There was no significant difference between the frequency of P/LP variants in males (5.7% (49/854)) and females (7.5% (36/482)) (P = 0.24).
Table 2. P/LP germline variants identified in other CSGs in a cohort of 1336 RCC participants

| Gene   | HGVSca | HGVSb      | No. of participants (%) |
|--------|--------|------------|-------------------------|
| ATM    | ENST00000278616.c.1339C>T     | ENSP00000278616.4.p.Arg447Ter | 2 (0.1) |
| ATM    | ENST00000278616.c.964_968del  | ENSP00000278616.4.p.Glu322lysTer6 | 1 (0.07) |
| ATM    | ENST00000278616.c.1442T>G     | ENSP00000278616.4.p.Leu481Ter | 1 (0.07) |
| ATM    | ENST00000278616.c.1782del    | ENSP00000278616.4.p.Val595CysTer19 | 1 (0.07) |
| ATM    | ENST00000278616.c.2466 +1G>A | ENSP00000278616.4.p.Lys1151Ter | 1 (0.07) |
| ATM    | ENST00000278616.c.3451A>T    | ENSP00000278616.4.p.Pro1134LeufsTer16 | 1 (0.07) |
| ATM    | ENST00000278616.c.8147T>C | ENSP00000278616.4.p.Val2716Ala | 1 (0.07) |
| ATM    | ENST00000278616.c.652C      | ENSP00000278616.8.c.1782del | 1 (0.07) |
| ATM    | ENST00000278616.c.1972C>T  | ENSP00000278616.8.c.3253_3258del | 1 (0.07) |
| ATM    | ENST00000278616.c.3401del    | ENSP00000278616.8.c.3401del | 1 (0.07) |
| BRIP1  | ENST00000259008.6.c.2992_2995del | ENSP00000278616.8.c.2992_2995del | 1 (0.07) |
| BRIP1  | ENST00000259008.6.c.2392C>T  | ENSP00000278616.8.c.2392C>T | 1 (0.07) |
| MSH6   | ENST00000234420.9.c.3261del | ENSP00000278616.8.c.3261del | 1 (0.07) |
| MSH6   | ENST00000234420.9.c.3259_3260insT | ENSP00000278616.8.c.3259_3260insT | 1 (0.07) |
| MSH6   | ENST00000234420.9.c.3562_3563del | ENSP00000278616.8.c.3562_3563del | 1 (0.07) |
| BRCA2  | ENST00000380152.7.c.925dup    | ENSP00000278616.8.c.925dup | 1 (0.07) |
| BRCA2  | ENST00000380152.7.c.4876_4877del | ENSP00000278616.8.c.4876_4877del | 1 (0.07) |
| PMS2   | ENST00000268549.12.c.1A>G    | ENSP00000278616.8.c.1A>G | 1 (0.07) |
| PMS2   | ENST00000268549.12.c.1776del | ENSP00000278616.8.c.1776del | 1 (0.07) |
| TP53   | ENST00000269305.8.c.655C>T   | ENSP00000278616.8.c.655C>T | 1 (0.07) |
| TP53   | ENST00000269305.8.c.586C>T   | ENSP00000278616.8.c.586C>T | 1 (0.07) |
| MSH2   | ENST00000233146.6.c.942_942+2del | ENSP00000278616.8.c.942_942+2del | 1 (0.07) |
| PALB2  | ENST00000261584.8.c.3113G>A   | ENSP00000278616.8.c.3113G>A | 1 (0.07) |

*HGVS: Human Genome Variation Society coding sequence name. †HGVS: Human Genome Variation Society protein sequence name.

**Age**

RCC participants with a P/LP variant tended to be younger (mean: 58.6 years versus 61.5 years; P = 0.10; Fig. 3). This was also observed for RCC participants with a P/LP in an RCC-CSG compared with other CSGs (mean: 58.0 years versus 59.9 years; P = 0.55; Supplementary Material, Table S3). Of the 19 early onset (≤45 years) participants with a P/LP variant in an RCC-CSG, the majority were in VHL (n = 7), followed by CHEK2 (n = 3), FLCN (n = 2) and SDHB (n = 2). Mean age of RCC onset in individuals with VHL and CHEK2 P/LP variants was 25.6 years (range: 18–40 years) and 64.7 years (range: 39–84 years), respectively. Of the five early onset (≤45 years) participants with a P/LP variant in an RCC-CSG, the genes involved were ATM (n = 2), BRIP1 (n = 1), TP53 (n = 1) and PALB2 (n = 1). Applying an age cut-off of ≤46 years would have detected a P/LP variant in 1.3% (18/1336) of the entire cohort and would have identified only 23.3% (14/60) of participants with a P/LP variant in an RCC-CSG (Supplementary Material, Table S4).

**Histology**

Participants with non-ccRCCs were more commonly associated with germline P/LP variants than clear cell [8.5% (19/224) and 5.8% (53/912), respectively], but the difference was not statistically significant (P = 0.17). Of the 72 participants with P/LP germline variants and detailed histology available, 73.6% (53/72) were classified as ccRCC and 26.4% (19/72) had a non-clear tumour. For full information on histopathology, see Table 3 and Supplementary Material, Table S1A and B; but in brief, germline VHL variants were associated with ccRCC (n = 4 ccRCC, n = 3 histology not available), two chRCCs were seen in association with a germline FLCN variant (overall histologies in four participants with a P/LP FLCN variants: n = 2 chRCC, n = 1 oncotic, n = 1 histology not available) and FH variants were seen in three participants, whose RCC tumour was classified as collecting duct (n = 1), ccRCC (n = 1) and the histology of the remaining one was not available.

Most CHEK2-associated tumours were classified as ccRCCs [19 ccRCC, five non-ccRCC (n = 4 ccRCC, n = 1 oncotic) and n = 3 histology not available]. For the participants with a MITF subunit variant, four were classified as ccRCC, four as non-ccRCC (n = 2 chRCC, n = 2 papillary) and, for the remaining two, histology was not available. Of those with an SDH subunit variant, five were classified as ccRCC, with the remainder classified as pRCC (n = 1), chRCC (n = 1) and, for two, histology was not available. More specifically, seven patients shared the same variant in SDHA, four of which were classified as ccRCC, two as a non-ccRCC (n = 1 papillary, n = 1 chRCC) and, for one, histology was not available. Of the two participants with a P/LP SDHB variant, one had a ccRCC and the histology for the other was not available.

**Stage**

There was no significant difference between the frequency of germline P/LP CSG variants in non-advanced...
Figure 2. Germline deletions identified in RCC-CSGs. (A) Two VHL gene exons are deleted in two participants. Participant A has a 13 kb deletion (chr3:10 135 484–10 148 568, GRCh38) and the breakpoint locations are displayed in red on the MANE select VHL transcript (ENST00000256474). Participant B has a 10 kb deletion (chr3:10 138 433–10 148 506, GRCh38) with the breakpoints shown in blue (created with Bioconductor’s ggbio package (56), with some adaptation). (B) A 7.5 kb deletion involving CHEK2 was identified in one participant. The deletion (pink shaded area) takes out part of the protein kinase domain (created with (57)).

and advanced RCC [6.9% (45/649) versus 5.5% (29/529); (P = 0.24)].

Burden test results

Burden test analysis showed an excess of CHEK2 variants, that passed our stringent filtering as detailed in Supplementary Methods, in European RCC participants compared with the healthy European controls that reached statistical significance [Fisher’s false discovery rate (FDR) adjusted P = 0.0019] (Supplementary Material, Table S5), confirming an association of CHEK2 with the RCC phenotype. For other CSGs, an excess of variants was seen in European cases compared with controls in the following genes: ATM, AXIN2, BAP1, BLM, BMFR1A, BRIP1, CBL, CDKN1B, CDKN1C, CDKN2A, CYLD, DDB2, DIS3L2, ELANE, EPCAM, EZH2, FANCA, FANCD2, FANCE, FANC,G, FANCI, FH, HRAS, MAX, MET, MLH1, NBN, NTHL1, PMS1, POLE, POLH, PTC1, RB1, RECQL4, RBHDF2, SBDS, SDHA, SDHAF2, SDH8, SDHC, SLC25A13, STK11, TP53, VHL, WRN, XPA and XPC, but none of these genes in contrast to CHEK2 showed a statistically significant association (P-values are available in Supplementary Material, Table S5).

Discussion

The availability of WGS data has allowed us to provide a more comprehensive appraisal of the contribution of germline pathogenic variants (including SNVs, INDELs and SVs) in CSGs to RCC in an unselected patient cohort. Our analysis suggests an overall detection rate of 6.4% and most P/LP variants were detected in CSGs known to predispose to RCC [4.5% (60/1336); 95% CI (3.4, 5.7)]. P/LP variants in other CSGs (ATM, FANCM, BRIP1, MSH6, BRCa2, PMS2 and TP53) found in 2% participants could reflect a background prevalence in the population or an association with RCC that has not yet been validated. To our knowledge, ATM has not previously been implicated in RCC, but truncating BRIP1 variants have been reported in a subset of patients with inherited RCC (17,18). Further studies are required to confirm potential links. A further 4.7% [64/1336; 95% CI (3.7, 6.1)] of participants had a VUS in an RCC-CSG which was considered to be clinically relevant. Further studies into family history and tumour immunohistochemistry and a more detailed clinical assessment would be needed to evaluate the true relevance of these VUSs in order to upgrade their status to P/LP, but 24 were classified as ‘hot’ VUSs (16).
Figure 3. Onset of RCC in participants with and without a P/LP variant. Dotted red line shows the age cut-off (46 years) for offering germline testing.

Reclassification of these 24 variants to LP would increase the overall diagnostic yield to 8.2%.

Our diagnostic yield of 6.4% is lower than that reported in studies enriched for a young RCC onset and/or later stage disease. Wu et al. (10) reported a diagnostic yield of 9.5% in an RCC cohort (n=190) of young patients (<45 years) which had germline testing on a 23-gene panel and a diagnostic yield of 16.1% was reported in an RCC cohort (n=254) enriched for advanced RCC (World Health Organization stage 3/4) referred for germline testing (76 CSGs analyzed) (9). In a recent large referral-based study (n=1829), 10.3% of participants had clinically actionable P/LP variants and there were some interethnic differences of variant frequency in specific genes (FH and CHEK2) (11). However, our diagnostic yield is comparable with the 6% reported by the Pan-Cancer Atlas study of 742 cases (19) and the 6.1% reported in a referral-based analysis of 1235 RCC patients (30% with family history) using a panel of 19 genes (12). Interstudy variations likely reflect patient ascertainment and selection and the extent of genetic testing, but our results provide a good estimate of diagnostic yield by comprehensive testing in an unselected series.

In the UK, patients with suspected inherited RCC are examined for features of an inherited cancer syndrome and offered gene panel testing that includes VHL, MET, FLCN, SDHB, FH and BAP1 (20). Other countries and commercial laboratories often include additional RCC-CSGs such as TSC1, TSC2, PTEN, TP53 and SDHC/D. In our study, 44 participants had variants in either CHEK2 (n=27), MITF (n=10) or SDHA (n=7) which are not routinely tested for in the UK. While CHEK2 variation was originally identified as conferring a 2-fold increase in breast cancer risk (21), it is increasingly being recognized that variants predispose to other cancers, including colorectal (22) and prostate (23), and more recently has been linked to RCC with studies suggesting a lifetime risk of 2% (24–26).

The MITF E318K/p.Glu419Lys) variant (rs149617956) was present in 10 participants. This variant was initially linked to RCC in a study of individuals with RCC, and malignant melanoma and functional studies of this variant demonstrated MITF upregulation through loss of a SUMOylation site (27,28). Though subsequent studies have confirmed an association with melanoma (29), a recent meta-analysis failed to demonstrate a significant association with RCC (30).

SDH-associated RCC is more commonly associated with SDHB mutations with carriers having an estimated 5% lifetime risk of RCC (31,32). However, in our cohort, germline SDHA variants were more common than SDHB variants. Germline mutations in SDHx are a major cause of phaeochromocytoma and paragangliomas but inheritance patterns and risks differ between genes, and the penetrance of germline SDHA mutations is much lower than for SDHB, SDHC or SDHD (33–35).

There are a number of limitations to our study. Although participants were recruited from a large number of individual centres and were not selected for any specific characteristics, all were fit to undergo surgery. In addition, there was no centralized review of histopathology, some clinical data were not available for all participants and data on the presence of syndromic
Table 3. Characteristics of 1336 participants with RCC

| Participants with RCC | N = 1336 | % |
|-----------------------|----------|---|
| Age (years)           | Mean (range) | 61.3 (13–88) |
| Sex                   |           |   |
| Male                  | 854       | 63.9 |
| Female                | 482       | 36.1 |
| Ethnicity (PCA-based) |           |   |
| Predominantly European ancestries | 1184 | 89 |
| Predominantly South and East Asian ancestries | 53 | 4 |
| Predominantly African ancestries | 27 | 2 |
| Other                 | 72        | 5  |
| Ethnicity (self-reported) |           |   |
| White British         | 937       | 70.1 |
| Other White background | 67       | 5.0 |
| Asian (Indian, Pakistani, Bangladeshi and other Asian) | 38 | 2.8 |
| Black (Caribbean, African and other Black) | 20 | 1.5 |
| Mixed background      | 7         | 0.5 |
| Other ethnic group    | 20        | 1.5 |
| Not stated            | 175       | 13.1 |
| Not available         | 72        | 5.4 |
| Number of RCC tumours |           |   |
| 1                     | 1287      | 76.3 |
| ≥2                    | 48        | 3.6 |
| Personal history of other cancer |           |   |
| 0                     | 1090      | 81.6 |
| 1                     | 197       | 14.7 |
| 2                     | 36        | 2.7 |
| ≥3                    | 13        | 1.0 |
| Histologya            | N = 1388a |   |
| Clear cell            | 939       | 67.7 |
| Non-clear cell        | 237       | 17.0 |
| Unspecified RCC histology | 149     | 10.7 |
| Not available         | 61        | 4.4 |
| Uncertain malignancy  | <5a       | 0.1 |
| Tumour stage          | N = 1388a |   |
| 1                     | 553       | 39.9 |
| 2                     | 123       | 8.9 |
| 3                     | 420       | 30.3 |
| 4                     | 116       | 8.4 |
| Unclassified          | <5        | 0.1 |
| Not available         | 174       | 12.5 |

PCA, Principal component analysis. *Histology and Stage numbers shown are for RCC tumours overall in our cohort, as there were 48 participants with ≥2 RCC tumours.

RCC extra-renal manifestations and family history were not collected.

Our findings have implications for the application of genetic testing for germline variants in individuals with RCC. Though most centres offer testing to patients with features of an inherited cancer syndrome, there is less consensus for testing isolated non-syndromic cases. Within the UK, testing is offered to patients <40 years of age (or <50 years of age for pRCC), but internationally, it has been suggested that an age cut-off <46 years (equivalent to the 10th percentile) would maximize the sensitivity and specificity and an age cut-off of 50 has also been recommended (20,36–39). However, in our cohort, only 23.3% (14/60) of those with an RCC-CSG variant, were aged <46 years. Although there was a trend for a younger age at diagnosis in the genes most frequently tested in clinical practice (VHL, SDHB, FH and FLCN; mean: 38 years), mutations in less penetrant genes were detected, on average, in older patients (e.g. CHEK2 and SDHA; mean: 65.5 years). This makes it difficult to define an age cut-off that would efficiently enable the identification of all cases with an RCC-CSG variant without testing the majority of the cohort. In addition, we did not find statistically significant associations in our series with tumour stage or histology (e.g. ccRCC or non-clear cell). In rare cases, characteristic histopathological features may suggest an underlying inherited disorder (e.g. SDH-deficient RCC, hybrid chromophobe-oncocytic and BHD syndrome), but, in general, in the absence of family history or multicentric disease, age at diagnosis seems to be the most practical approach (with 70% general consensus) (38) for stratifying genetic testing. Based on our results, testing a further 106 participants presenting between 45 and 50 years of age would enable detection of an extra 5 participants (Supplementary Material, Table S4). Therefore, we suggest that genetic testing should be extended to <50 years of age and that the small clinical gene panels currently used in the UK to be expanded to
include CHEK2, SDHA, SDHC and SDHD. These additional CSGs also predispose to other tumour types and surveillance recommendations are available for gene carriers (35,40). In addition, for SDHA, SDHC and SDHD, functional investigations (e.g. SDHB/SDHA immunohistochemistry and metabolomics) are available that can aid SDHx variant interpretation (41,42). The associated cancer risks with MITF variants (observed in 10 participants) are not well defined and hence we suggest that further evidence is required before incorporating MITF into RCC panels.

Detection of a germline P/LP in RCC-CSG variants can enable RCC prevention strategies (e.g. renal cancer surveillance, cascade testing and awareness of non-RCC tumour risks). In some cases, it may also suggest genotype-driven therapies as exhibited with foretinib for RCC patients carrying germline MET variants (43). As the importance of knowledge of germline findings to determine management increases and the cost of genomic analysis falls, the indications for germline and somatic sequencing in RCC should be extended and play a large part in routine clinical care. However, the selection of genes that should be tested requires careful consideration of diagnostic yields, VUS likelihood and the clinical utility (e.g. availability of management guidelines) of diagnostic findings.

Materials and Methods

Participants

All subjects gave written consent; 100kGP was approved under Research Ethics Committee Ref 14/EE/1112. We studied 1336 RCC participants [64% male; mean: 61 years (±12 SD); range: 13–88 years]. Their clinical characteristics are described in Table 3. Sex reported is according to participant phenotypic sex classification at birth. Ethnicity reported is self-reported by participants and also reported based on principal component analysis performed by Genomics England (GEL). For more details, see Supplementary Methods.

Healthy unrelated parents (n = 5834) [mothers (n = 3149, mean age: 39 years) and fathers (n = 2685, mean age: 42 years)] of children recruited to the intellectual disorders disease group of the 100kGP rare disease domain served as a source of controls. All controls were of European ancestry and a cancer diagnosis was excluded based on available data in 100kGP. For more details on their selection, see Supplementary Methods.

Cancer susceptibility genes

We focused on 121 CSGs previously described in the Catalogue of Somatic Mutations in Cancer (Cosmic) (44), including 18 well-established CSGs for RCC (6,45) (Supplementary Material, Table S6). Genomic positions of canonical gene transcripts were retrieved from the Ensembl database (EnsDb.Hsapiens.v86) (46) and were referenced to build GRCh38.

Short variant analysis

Short variant (SNV and INDEL) analysis was based on variants extracted for the 1336 RCC participants from the germline aggregate multi-sample VCF (aggV2) available in the 100kGP Main Programme V10 data release. In summary, extracted variants were annotated using the variant effect predictor (VEP) (v99) (47) and additional filtering was applied to include only rare variants (maximum minor allele frequency across all populations in the Genome Aggregation Database <0.5%) (48) in our selected 121 CSGs. We then prioritized the variants to assess their potential clinical relevance. Firstly, we selected those with a ‘HIGH’ impact VEP severity rating, which includes all LoF variants: stop-gained, frameshift or splice-site disruption. Secondly, we sought out functionally important missense variants and inframe indels. These variants have a ‘MODERATE’ impact rating and missense variants were only included if they were predicted to be deleterious by SIFT (49), possibly/probably damaging by Polyphen (50) and had a CADD Phred score ≥20; inframe indels were included if they had a CADD Phred score ≥20. The variants were classified based on ACMG/Association for Molecular Pathology criteria (52) and, after further manual curation, were assigned to five distinct categories; P, LP, VUS, likely benign or benign. VUSs were further subclassified to hot/warm/tepid or cool/cold/ice cold VUS according to the ACGS guidelines (16). For more details on the filtering and classification, see Supplementary Methods. The bioinformatics workflow is visualized in Supplementary Material, Figure S2.

SV analysis

SV analysis was based on the 100kGP Main Programme V8 data release. Individual VCFs with SV calls were available for 1254 of 1336 RCC participants. Germline SVs were interrogated using an adapted version of the PCAWG-SV-merge pipeline (53). For more detailed methodology, see Supplementary Methods.

Mutational signatures in tumours

Mutational signatures were computed by 100kGP using NNLS R package (54) based on Cosmic version 2 cancer signatures (44). We examined the mutational signatures present in matched somatic RCC samples of RCC participants with germline variants in a MMR gene (MLH1, MSH2, MSH6 and PMS2) or POLE. The presence of signatures 6, 15, 20 or 26 was interpreted as indicative of MMR deficiency and the presence of signature 10 was interpreted as POLE exonuclease deficiency.

Statistical analysis

The relationship between age of RCC onset and germline P/LP variant status was evaluated using Welch’s two-sample t-test. Fisher’s exact test was applied for differences in histology, stage and sex between participants with and without P/LP variants. For the participants whose histology or stage was not available, these
cases were excluded from the statistical analysis. For participants with multiple tumours, analysis of histology and stage was based on the first diagnosed tumour. The Burden test analysis was performed using Fisher’s exact test on carrier count of short variants which passed our filters aggregated per gene in RCC participants of European ancestry compared with controls of European ancestry. Multiple testing correction was performed by FDR. Statistical analyses were performed using R studio (v3.4.4) (55). A two-sided P-value of $<0.05$ was considered to be statistically significant.

Data availability
The WGS data analyzed in this study can be accessed through a secure research environment hosted within the GEL Data Centre https://www.genomicsengland.co.uk/about-gecip/for-gecip-members/data-and-data-access/. In order to gain access, researchers will need to apply and become a member of the Genomics England Clinical Interpretation Partnerships (GeCIPs). Researchers, clinicians and students can apply to join any GeCIP domain that is relevant to their intended research projects. https://www.genomicsengland.co.uk/about-gecip/joining-research-community/.

Conflict of Interest statement. S.T. has received speaking fees from Roche, Astra Zeneca, Novartis and Ipsen. S.T. has the following patents filed: Indel mutations as a therapeutic target and predictive biomarker PCTGB2018/051892 and PCTGB2018/051893 and ccRCC Biomarkers P113326GB. E.R.M. has received speaker fees from MSD.

Supplementary Material
Supplementary Material is available at HMG online.

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1Genomics England, Queen Mary University of London, Dawson Hall, Charterhouse Square, London EC1M 6BQ, UK. 2William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, UK.

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