Pathogenic germline variants in patients with features of hereditary renal cell carcinoma: evidence for further locus heterogeneity

Philip S Smith[a], Hannah West[a], James Whitworth[a], Bruce Castle[b], Francis H Sansbury[c], Anne Y Warren[d], Emma R Woodward[e], Marc Tischkowitz[a], Eamonn R Maher[a]

a) Department of Medical Genetics, University of Cambridge and NIHR Cambridge Biomedical Research Centre, and Cancer Research UK Cambridge Centre, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK

b) Peninsula Clinical Genetics Service, Royal Devon & Exeter NHS Foundation Trust, Royal Devon & Exeter Hospital (Heavitree), Exeter, UK University of Exeter Medical School, University of Exeter, Exeter, UK

c) All Wales Medical Genomics Service, Cardiff and Vale University Health Board, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF14 4XW

d) Department of Histopathology, Cambridge University NHS Foundation Trust and Cancer Research UK Cambridge Centre, Cambridge CB2 OQQ, United Kingdom

e) Manchester Centre for Genomic Medicine and NW Laboratory Genetics Hub, Manchester University Hospitals NHS Foundation Trust, and Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Health Innovation Manchester, Manchester, UK

Correspondence

Professor Eamonn Maher, Department of Medical Genetics, University of Cambridge, Box 238, Cambridge Biomedical Campus, Cambridge, CB2 0QQ, United Kingdom

E-mail: erm1000@medschl.cam.ac.uk

Telephone: 01223 746714

Fax: 01223 746777

Abstract

Inherited renal cell carcinoma(RCC) is associated with multiple familial cancer syndromes but most individuals with features of non-syndromic inherited RCC do not harbour variants in the most commonly tested renal cancer predisposition genes (CPGs). We investigated whether undiagnosed cases might harbour mutations in CPGs that are not routinely tested for by testing 118 individuals with features suggestive of inherited RCC (family history of RCC, two or more primary RCC aged <60 years, or early onset RCC≤46 years) for the presence of pathogenic variants in a large panel of CPGs. All individuals had been pre-screened for pathogenic variants in the major RCC genes. We detected **p**athogenic or likely pathogenic (P/LP) variants of potential clinical relevance in 16.1% (19/118) of individuals, including P/LP variants in *BRIP1* (N=4), *CHEK2* (n=3), *MITF* (n=1) and *BRCA1* (n=1). Though the power to detect rare variants was limited by sample size the frequency of truncating variants in *BRIP1,* 4/118, was significantly higher than in controls (*P*=5.92E-03). These findings suggest that the application of genetic testing for larger inherited cancer gene panels in patients with indicators of a potential inherited RCC can increase the diagnostic yield for P/LP variants. However, the clinical utility of such a diagnostic strategy requires validation and further evaluation and in particular confirmation of rarer RCC genotype-phenotype associations is required.

Introduction

Renal cell carcinoma (RCC) is a group of human cancers derived from renal epithelium that comprise a variety of histological and genetic backgrounds. Worldwide RCCs account for around 2.4% of all malignancies, with a prevalence of about 4.4 per 100,000 individuals and a cumulative lifetime risk (to age 75 years) of approximately 0.5% [1]. Molecular genetic studies have identified multiple genetic causes for RCC predisposition. The best recognised cause of familial RCC is the dominantly inherited familial cancer syndrome von Hippel-Lindau (VHL) disease caused by germline mutations in the *VHL* tumour suppressor gene [2,3]. Inactivating mutations in a number of tumour suppressor genes (TSGs) including *VHL, FH, FLCN, SDHB* and *BAP1*, activating mutations in the *MET* proto-oncogene and constitutional chromosome 3 translocations are well established causes of inherited predisposition to renal cancers [4]. Though it has been suggested that 24-33% of individuals with RCC may meet referral criteria for genetic testing [5], the majority of patients who undergo routine genetic testing for germline variants in the "major inherited RCC genes" (i.e.. *VHL*, *FH, FLCN, SDHB, BAP1, MET*) do not have detectable pathogenic variants (unpublished observations).

Recently, studies in a number of different human cancer types have identified pathogenic variants in a wider range of cancer predisposition genes (CPGs) than have been traditionally associated with the cancer of interest [6,7]. In addition, germline genetic testing of a cohort of individuals with advanced RCC revealed 16% of individuals presented with a pathogenic cancer-associated germline variant, of which only about a third occurred in the widely recognised RCC-associated genes [8]. We hypothesised that applying a wider CPG testing strategy to a cohort of affected individuals with features of inherited RCC might increase the diagnostic yield of pathogenic/likely pathogenic (P/LP) variants and we proceeded to investigate a large panel of CPGs in 118 unrelated probands pre-screened for germline mutations in *VHL, MET, FLCN, SDHB, FH,* and *BAP1*.

Materials and methods

Subjects: Individuals diagnosed with RCC referred to Regional Genetics Centres for consideration of genetic testing were assessed for eligibility based on the presence of clinical features associated with inherited RCC. Individuals were recruited if they matched one or more of the following criteria: 1) At least one first or second degree relative with RCC or 2) no family history of RCC but two or more separate primary RCC before age 60 years, or 3) diagnosed with RCC at age 45 years or less. Assignment of groupings based on clinical criteria was carried out hierarchically in the order given, where, for example, a patient with bilateral RCC aged under 45 years with a family history of RCC would be categorised as familial and a patient with bilateral RCC aged under 45 years without a family history of RCC would be categorised as multiple RCC. For four individuals in whom the precise age at diagnosis of RCC was not available the age at genetic testing was used. Individuals with confirmed or likely pathogenic variants in BAP1, FH, FLCN, MET, SDHB and VHL were excluded from the study. All study participants gave written informed consent, and the study was approved by the South Birmingham Research Ethics Committee.

Molecular Genetics Studies: DNA was extracted from peripheral blood lymphocytes in a regional genetics laboratory using standard techniques. A total of 100 samples were analysed using Illumina TruSight Cancer Sequencing Panel (Illumina, San Diego, CA) on the Illumina MiSeq platform. 75 probands (18 of whom were also analysed by the Illumina TruSight Cancer Sequencing Panel) had exome sequencing data generated by Illumina TruSeq Exome library preparation on the Illumina HiSeq 4000 or Illumina NextSeq platform. In total 118 probands were analysed by panel and/or exome sequencing.

Bioinformatics; Further details of bioinformatic protocols and methodology can be found in the Supplemental Information. FASTQ files for both case and ICR1000UK exomes [9] were aligned to genome reference GRCh38 using BWA-MEM (version 0.7.15-r1140) with ALT-contig post-processing. PCR duplicates were flagged by SAMtools rmdup (version 1.4.1) and variant calling carried out using GATK unified genotyper (version 3.7-0-gcfedb67). Variants from targeted sequencing panel and exome datasets were called independently and a 'virtual' panel applied to the exome variants via vcftools, restricting the reported variants to the Illumina TruSight Cancer sequencing panel target bed intervals (with an additional 3 bp padding; supplemental notes). Full alignment and variant calling pipeline provided in supplemental notes. VCF files were filtered to remove low quality calls and sequencing artefacts using vcftools and in-house bioinformatics pipelines (supplementary table S1). Lastly, genomic regions were restricted to a total of 67 cancer-related genes sub-selected from the original cancer gene panel as utilised previously [7] which were targeted on the Illumina TruSight Cancer sequencing panel. In addition, a single variant in *MITF* (rs149617956) was also assessed in conjunction with the previously described genes due to previous associations with RCC risk [10] (Supplementary table S2 & S3).

Variants passing quality filtering were annotated with ANNOVAR to provide genomic region annotation, variant consequence, functional in-silico prediction, reference minor allele frequencies for datasets of 1000 genomes project (1KG) & Exome Aggregation Consortium (ExAC) [11], and reported ClinVar data, where available. Variants were selected by variant consequence, filtered to be rarer than 1% (minor AF < 0.01) in both 1KG and ExAC, in order to exclude common SNPs. *In silico* predictive metrics provided by ANNOVAR were used to inform potential pathogenicity but were not used as filtering cut-offs for candidate selection. ACMG guidelines [12,13] were applied to all candidate variants to determine clinical significance utilising InterVar (version 20180827). Somatic variant calling was performed jointly using both Strelka2 (version 2.9.10) and Mutect2 (version 3.7-0-gcfedb67) with annotation performed as described for germline variant calls. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Structural variant calling was performed using SvABA (version 1.1.3) [14] to identify any large indels or structural variants within the same genomic regions described for SNV calling. Full details of structural variant calling process are described in the supplementary notes.

Statistical Analysis Proportion confidence intervals were calculated using R base function binom.test at CI 95%, Odds ratios were calculated using the oddsratio.fisher function in epitools package (version 0.5-10), and two-tailed fisher's exact tests were calculated using the fisher.test function in base, using R (version 3.5.1).

Results

Clinical features

The 118 unrelated individuals with RCC eligible for inclusion were subdivided into three clinical subsets: 44 cases with a positive family history and 74 sporadic cases comprising 30 cases with multifocal or bilateral disease and 44 cases with early onset RCC only). Median age of onset across all cases was 42 years (range 10-74) and 52 years (range 29-74) in the familial cases, 48 years (range 31-72) in multifocal/bilateral cases and 33 years (range 10-46) in early onset cases). Histological subtype was available for 70 of 118 cases (59.3 %) and comprised 68.6% clear cell RCC, 27.1% papillary RCC, and 4.29% chromophobe RCC). Summary of the distribution of clinical features are given in Table 1 (full details in Supplementary Table S6).

Variant filtering

A total of 1,955 and 237 variants passed quality control filtering requirements (Supplementary Information) in the targeted sequencing and virtual panel sets, respectively. After variant filtering (Supplementary Information), a total of 159 variants were retained from the targeted sequencing and 25 variants were retained from the virtual panel sets, respectively. Variants present in both sets were merged resulting in a total of 174 variants across the targeted regions.

Analysis of the pathogenic or likely pathogenic (P/LP) variants identified in this set were divided into three categories subpanels based on the clinical associations and inheritance patterns of the affected genes:1) Category I genes (n=14) had a known association with syndromic or non-syndromic RCC predisposition 2) Category II genes (n=18) were those in which heterozygous pathogenic variants are known to be associated with predisposition to multiple tumour types and 3) Category III genes (n=35) which are associated with cancer predisposition when there are biallelic pathogenic variants or those which have been associated with a single non-RCC tumour phenotype. List of targeted genomic regions are listed in supplemental information table S2.

Of the 174 variants assessed, 16 were classified as pathogenic or likely pathogenic (P-LP) variants (three pathogenic, 13 likely pathogenic), corresponding to four nonsense variants, three frameshift deletions, one frameshift insertions, and eight nonsynonymous substitutions. The 16 variants were observed in 19 cases (16.1%; 95% CI: 9.98-23.0). P/LP variants were equally distributed by count across the inherited subtypes (9 variants in familial, six variants in early onset, and four variants in bilateral/multifocal). All 16 P/LP variants are described in Table 2 and all 19 patients harbouring the aforementioned variants in Table 3.

Detection of variants in category I: RCC predisposition genes

As expected, no P/LP variants were detected in genes that had previously been analysed before inclusion in this study (*VHL, MET, FLCN, SDHB,* or *BAP1*) and only a single P/LP variant was identified in a gene previously linked to RCC: a *MITF* nonsynonymous variant in (NM_000248.3: c.952G>A: p.E318K) was identified in an individual who presented with clear cell RCC at age 74 years and whose son was reported to have presented with clear cell RCC at age 53 years. Sequencing in the

individual's unaffected brother did not reveal the variant. Though this variant had been previously associated with predisposition to RCC and melanoma [10] there was no reported family history of melanoma.

Detection of variants in category II: multisite cancer predisposition genes

Six distinct P/LP variants in three genes in which heterozygous pathogenic variants are known to be associated with predisposition to multiple non-RCC tumour types were identified in 8/118 cases. Two category II genes, *BRIP1* and *CHEK2*, harboured germline P/LP variants in more than one proband. Four probands harboured a heterozygous truncating variants in *BRIP1* (two cases with NM_032043.3: c.1871C>A: p.Ser624*, and one each with NM_032043.3: c.1161dupA: p.Gln388Thrfs*7, and NM_032043.3: c.2392C>T: p.Arg798*) (Supplementary Table S4). The four probands consisted of two familial cases and two multifocal/bilateral cases. Age at diagnosis of RCC was 54, 64, 46, and 39 years and these patients presented with papillary, two non-specified, and clear cell RCC, respectively (Table 3; Individuals RCC-043, RCC-074, RCC-031, RCC-102). DNA from an affected family member (second-degree relative) was available for one of the familial cases (RCC-102) and the affected relative (who developed clear cell RCC at age 57 years) harboured the *BRIP1* nonsense variant (NM_032043.3: c.2698G>A: p.Arg798*) identified in the proband (see Supplementary Figure 1).

To compare the frequency of *BRIP1* truncating variants (3.39%; 4/118) in the patient cohort to controls, the ICR1000UK control set was analysed for number of truncating variants. The ICR1000UK control cohort harboured *BRIP1* truncating variants in 0.4% (4/999) of individuals (Supplementary Table S7), corresponding to an enrichment of truncating variants in our cases (*P*=5.92E-03, OR=8.70, 95% CI: 1.60 – 47.4). In addition, evaluation of rare truncating variants in *BRIP1* detected in both the ExAC non-TCGA dataset and gnomAD exome dataset [15] revealed an estimated at 0.24% (123/51,300) and 0.20% (252/124984), respectively, which results in a significant enrichment in the case set (*P*=2.19E-04, OR=14.6, 95% CI: 3.85 – 39.3 and *P*=1.09E-04, OR=17.4, 95% CI: 4.61 – 46.3). This association is still present in ExAC non-TCGA and gnomAD exome datasets after false discovery rate correction (Table 4). Finally, statistical comparison to data published by Easton et al [16] also demonstrated a statistical enrichment in this series (*P*=1.21E-04, OR=18.2, 95% CI: 4.55 – 53.1) when compared to truncating variants in *BRIP1* in breast cancer, found at a rate of 0.19% (28/14,526) (Supplementary Table S5).

A frameshift deletion in *CHEK2* (NM_007194.4: c.1263delT: p.Ser422Valfs*15) was identified in two individuals, both of whom presented with multifocal RCC in their fifth decade. The frameshift deletion is considered to be pathogenic and has previously been detected in both germline sequencing of breast [17] and prostate cancer [18,19]. An additional *CHEK2* nonsynonymous variant (NM_007194.4: c.1427C>T: p.Thr476Met) was also identified in one individual with non-specified RCC at 58 years and had a reported family history. The variant falls within the protein kinase domain of *CHEK2* and *in vitro* studies had reported loss of kinase activity and loss of DNA repair function [20,21]. A single individual with early onset papillary RCC at age 40 years was found to carry a *BRCA1* frameshift deletion in exon15 (NM_007300.3: c.4563delA: p.Lys1521Asnfs*5), which was absent in the non-cancer gnomAD data set.

A *PMS2* nonsense variant was identified in three individuals, purported to occur within the 4th amino acid (NM_001322015: c.11C>G: p.Ser4*) but on review was found only to affect non-canonical isoform 14, resulting in an intronic substitution within the canonical isoforms of *PMS2*. Furthermore, one individual identified was identified with a *PMS2* nonsynonymous variant, occurring within the canonical transcript (NM_000535: c.2066C>T: p.Thr689Ile). The *PMS2* nonsynonymous substitution occurs within exon 12 resulting a Threonine to Isoleucine substitution in a c-terminal dimerization domain. The variant occurs as a singleton in the gnomAD data set [15] and is considered to be highly deleterious by multiple in silico predictive tools.

Analysis of tumours from cases with germline BRIP1 truncating variants

Pathology blocks from RCC from two related patients with a truncating *BRIP1* variant (*BRIP1* NM_032043: c.2698G>A: p.Arg798*) available for analysis. The proband (RCC-102) presented with a 63 mm RCC at age 39 years. Histopathological review revealed that the tumour contained some sheets of cells with clear cytoplasm, in keeping with classification as a clear cell RCC. However, in many areas the tumour showed very variable morphology, with a tubulo-papillary architecture and areas where the cells had very abundant eosinophilic cytoplasm (see Figure S2). The tumour cell nuclei were predominantly WHO/ISUP grade 2, but some were interpreted as grade 3. There was no sarcomatoid or rhabdoid morphology. There was no tumour necrosis but there was a marked infiltrate of chronic inflammatory cells within the tumour, including lymphocytes and macrophages. Immunohistochemistry studies were performed and the tumour showed positive staining for CA-IX, CD10, RCC, EMA, CD15, CAM5.2, AMACR, MNF116, AE1/3 and Vimentin and there was very weak and patchy staining for E-Cadherin. SDHB and FH expression was retained. The tumour was negative for CD117, CK7, CK20, Mel-A and HMB45. This immunoprofile was in keeping with the diagnosis. In summary the tumour was categorised as a clear cell RCC WHO/ISUP Grade 3; pT1b pNX (UICC TNM 8th Edition); Leibovich score: 3. The affected relative (RCC102.1) had a >120 mmm diameter tumour with involvement of a renal vein tributary, stage pT3a with a Leibovitch score =5. Histopathological review showed typical morphological features of a clear cell RCC (see Figure S2), with WHO/ISUP Grade 2 tumour cells and no tumour necrosis. Immunohistochemistry was positive for Vimentin, RCC, CA-IX, AE1/3 and EMA (focal). SDHB and FH expression were retained. Targeted somatic gene panel se-

quencing was performed as described previously [22] to assess 68 cancer-related genes including several associated with RCC. Only a single *VHL* variant in the tumour from the affected relative (RCC102.1) was identified. The variant was consistent with clonal heterozygous inactivation of *VHL* resulting from a large deletion within exon 3. Both Strelka2 and Mutect2 called the somatic variant but were not identically. Strelka2 called a single 30bp non-frameshift deletion (NM_000551; c.492_521del; p.Gln164_Asn174delinsHis) at a variant allelic fraction of 0.31. Mutect2 called two separate but contiguous frameshift deletions (NM_000551; c.492_501del; p.Val165AlafsTer2 and NM_000551: c.503_522del: p.Ser168IlefsTer81) at variant allelic fractions of 0.31 and 0.46, respectively. No additional protein-altering somatic mutations were detected at variant allele fraction greater than 10% in either tumour.

Discussion

We analysed a cohort of 118 individuals with clinical characteristics suggestive of inherited RCC (but no known genetic cause) for germline variants in 68 cancer-related genes. This gene panel strategy was previously used to analyse a large cohort of patients with multiple primary tumours and in that study we found that there was a significant diagnostic yield of P/LP variants in CPGs for which the tumour phenotype in the relevant patient was atypical [7].

The only pathogenic variant identified in a category I gene was a previously described nonsynonymous variant in *MITF* (c.952G>A: p.E318K). The E318K variant was linked to non-syndromic RCC predisposition in a cohort of individuals presenting with both RCC and melanoma in which variant carriers demonstrated a 5-fold increased risk for melanoma, RCC, or both and functional studies demonstrated MITF upregulation and differential expression of MITF target transcripts [10,23]. Subsequently the E318K variant was confirmed to be associated with a melanoma predisposition [24,25], however the association of *MITF* E318K with RCC predisposition has been less well studied and provide limited support the association between RCC predisposition and *MITF* E318K [26,27]. In this instance, the identification of *MITF* E318K in this cohort is difficult to interpret given the limited sample sizes and the identification of only a single carrier. The category I genes also included rarer RCC cancer predisposition genes such as *CDC73, PTEN, TSC1* and *TSC2* that have been linked to syndromic forms of inherited RCC and we did not identify pathogenic variants in these genes (the cohort had been ascertained via clinical geneticists and so we would have expected syndromic cases to have been identified prior to recruitment).

Several VUS variants were identified in *TSC2* and *MET*. Three variants in *MET* (NM_000245: c.T2543C: p.V848A, NM_000245: c.G1406C: p.R469P, and NM_000245: c.A1336G: p.I446V) were present at allelic frequencies lower than 8.5E-05, with in silico predictions being variable, but none of the variants fall within the tyrosine kinase domain associated with constitutional activation of c-MET [28,29], and none had been reported as somatic events in sporadic RCC based on data from the catalogue of somatic mutations in cancer (COSMIC)[30].

Six missense variants were identified in *TSC2*, associated with tuberous sclerosis complex (MIM: 613254) which predisposes individuals to renal angiomyolipomas and cysts, as well as hybrid or oncocytic RCC in between 2-4% of cases [31,32]. Histological information was not available for these individuals to assess if they presented with histologies consistent with loss of *TSC2*. The predicted pathogenicity of these missense variants, as well as the allele rarity, is variable but two variants (NM_000548 c.G4657T: p.G1553C & NM_000548: c.G5117A: p.R1706H) occur within the Rap GTPase activating protein domain implicated in RHEB inhibition [33] and one variant (NM_000548: c.C2476A: p.L826M) arises in a Tuberin-type domain, though its direct function is not known. None of the 6 variants identified in *TSC2* had been reported as somatic events in sporadic RCC in COSMIC. All VUS variants are detailed in the supplementary data.

Previously, segregation analysis of non-syndromic familial RCC was found to be consistent with an autosomal dominant inheritance pattern with incomplete penetrance [34]. Together with recent findings that the cancer phenotype of well-established cancer predisposition genes may be wider than initially recognised [6,7,35,36] this raised the possibility that we might find pathogenic variants in category II genes in individuals with features of inherited RCC. We identified pathogenic variants in three category II genes (*BRIP1, BRCA1, CHEK2*) in 6.8% (8/118 probands), of our cohort (6.8% of familial cases, 9.1% of multi/bilateral cases and 2.3% of early onset cases). Four probands harboured truncating mutations in *BRIP1.* Pathogenic *BRIP1* variants were initially reported to predispose individuals to both breast and ovarian cancers [37,38], though more recent evidence from epidemiological studies of pathogenic *BRIP1* variants in breast cancer have found no association with breast cancer susceptibility [16,39]. We note that the potential link between RCC predisposition and pathogenic BRIP1 variants would be strengthened if any of the rare non-truncating BRIP1 variants identified in probands were to be proved to be pathogenic. Only a single additional variant in *BRIP1* (NM_032043: c.C1207T: p.R403W) was identified as at least a VUS. This variant was enriched in comparison to the gnomAD non-cancer population (p=7.0E-04), but singleton variants in lower sample sizes are more difficult to accurately assess. A recent study assessed the functional impact of several novel or rare nonsynonymous variants [40] and, though none of these variants were present in our cohort, it highlights the potential for non-truncating variants to contribute to cancer predisposition and need for thorough functional evaluation of variants of uncertain significance.

Pathogenic variants were also detected in two other DNA repair genes, *BRCA1* (n=1) and *CHEK2* (n=3). While in this study we did not demonstrate statistical enrichment of *CHEK2* P-LP variants in our cohort of individuals with features of inherited RCC, joint assessment of the frequency of P-LP variants in *CHEK2* in this case series and our cohort of individuals with multiple primary tumour–associated RCC that we analysed with a similar cancer predisposition gene panel strategy [7], demonstrated that P-LP *CHEK2* variants are overrepresented after multiple testing correction (7/192; p = 2.14E-04, FDR corrected = 1.77E-02). This is also strengthened the association described by Carlo *et al*. which reported an enrichment of germline *CHEK2* variants in patients with advanced RCC [8].

The significance of the *BRCA1* mutation in a single individual with early onset papillary RCC is difficult to interpret. Germline *BRCA1* and *BRCA2* variants have been reported previously and in a recent

study of 190 unrelated Chinese patients with RCC aged <45 years, analysis of 23 CPGs revealed four RCC patients with pathogenic *BRCA1* (n=1) or *BRCA2* (n=3) germline variants [41]. However, in BRCA1 and BRCA2 mutation carriers ascertained through a personal and/or family history of breast/ovarian cancer the risk of RCC had not been reported to be increased [42].

While some inherited RCC cases are caused by genes (e.g. *VHL, MET, BAP1*) which show high somatic alteration rates in sporadic RCC, others inherited RCC genes (e.g. *FLCN* and *SDHB*) do not display frequent somatic alteration in sporadic RCC. Neither *BRCA1*, *CHEK2,* or *BRIP1* are frequently somatically altered in sporadic RCC in the TCGA dataset (1.2%, 1%, and 1.1%, respectively) at a rate that would be indicative of common somatic driver genes [43]. However, *BAP1, BRCA1, BRCA2, BRIP1* and *CHEK2* gene products have related functions in DNA repair pathways that may make a common role in RCC predisposition more plausible. BAP1 (BRCA1 associated protein) was originally identified due to direct interactions with the RING finger domain of BRCA1 and functions as a de-ubiquitinating enzyme. BAP1 has been determined to form multiple protein complexes and known functions include removal ubiquitin groups from histone H2A lysine 119 residues to regulate gene expression [44], modulation of DNA damage repair by de-ubiquitinating BARD1 (which binds to BRCA1), indirectly modulating the efficacy of BRCA1-driven DNA repair pathways [45], and mediates the recruitment of homologous recombination proteins to DNA damage foci [46]. Given the interconnected functions and pathways associated with *CHEK2, BRIP1,* and *BRCA1*, it can be hypothesised that, germline pathogenic variants in these gene might predispose to a broader range of cancers in a manner similar to that described with *BAP1* predisposition syndrome, including RCC. Two P-LP variants were also identified in *PMS2* across four individuals though the truncating variant present in three of these individuals occurs in a non-canonical isoform, annotated as an intronic substitution. The *PMS2* variants in this study were not independently confirmed and known issues regarding *PMS2* pseudogenes [47] make interpretation more complex.

The observation that eight of the nine genes identified in this study with pathogenic or likely pathogenic variants are associated with DNA repair pathways in some capacity could suggest a potential enrichment across all DNA repair pathways but interpretation should be cautious given that cancer panels are bias towards DNA repair pathway components due to frequent alterations in somatic sequencing, and several of these genes only result in cancer presentation under autosomal recessive inheritance, which was not demonstrated here.

Epidemiological studies have reported multiple risk factors including smoking, obesity and hypertension [48,49] but these features was not reported in this study. An interesting further examination of the results described herein is the relationship between what appear to be generalised cancer predisposition genes, or at least rare causes of cancers outside of the canonical cancer spectrum, and impact of environmental factors in the resulting genotype-phenotype correlations.

In summary we found that in a cohort of patients with features associated with inherited predisposition to RCC and no detectable mutation in routinely tested RCC CPGs, extension of testing to a larger CPG panel revealed pathogenic variants in CPGs associated with multiple cancer types in a subset of

patients. This finding is consistent with previous studies of patients with early onset or advanced RCC that have been analysed by larger gene panels and with the results of patients with multiple primary tumours [7,8,43] If patients with germline mutations in DNA repair genes such as *BRCA1, BRCA2, BRIP1* and *CHEK2* were shown to benefit from specific targeted therapies there would be a clear case for incorporating a wider genetic testing protocol into clinical care. However, we suggest that before the implementation into routine clinical practice of wider CPG testing for patients with potential non-syndromic inherited RCC further studies are required a causal link between RCC predisposition and pathogenic variants in *BRIP1, BRCA1*, *BRCA2,* and *CHEK2* and to determine more accurately cancer risks in patients so that appropriate renal screening protocols for asymptomatic gene carriers can be defined.

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Conflict of Interest Statement

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TABLES

Table 1: Summary of clinical features of individuals with suspected inherited RCC where available

Clinical feature	Value
Sex, Num. (%)	
Male	71 (60.2)
Female	47 (39.8)
Age, median (range)	
All	43 (10-74)
Familial	52 (29-74)
Early onset	33 (10-46)
Bi/Multi	48 (31-74)
Case type, Num. (%)	
Familial	44 (37.2)
Early onset	44 (37.2)
Bi/Multi	30 (25.4)
Histology, Num. (%)	
clear cell RCC	48 (68.6)
papillary RCC	19 (27.1)
chromophobe RCC	3 (4.29)
non-specified RCC	48
Family history, Num. (%)	
1st degree	27 (61.4)
2nd degree	8 (18.2)
Unspecified	9 (20.5)

Table 2: 16 variants identified as pathogenic or likely pathogenic by ACMG guideline classifications assigned by InterVar

GENE	Pos (GRCh38)	rsID	CONSEQUENCE	Transcript (Canonical)	DNA	Exon	AA	Genomad AF	InterVar classification
BRCA1	chr17:43074505	N/a	frameshift deletion	NM_007300.3	c.4563delA	exon15	p.Lys1521Asnfs*5	NS	Likely pathogenic
BRIP1	chr17:61780325	rs587781321	nonsense	NM_032043.2	c.1871C>A	exon13	p.Ser624*	1.86E-05	Pathogenic
BRIP1	chr17:61799278	N/a	frameshift insertion	NM_032043.2	c.1161dupA	exon9	p.Gln388Thrfs*7	NS	Likely pathogenic
BRIP1	chr17:61716051	rs137852986	nonsense	NM_032043.2	c.2392C>T	exon17	p.Arg798*	1.40E-04	Pathogenic
CHEK2	chr22:28694066	rs142763740	nonsynonymous	NM_007194.4	c.1427C>T	exon13	p.Thr476Met	3.00E-04	Likely pathogenic
CHEK2	chr22:28695238	rs587780174	frameshift deletion	NM_007194.4	c.1263delT	exon12	p.Ser422Valfs*15	4.49E-05	Pathogenic
ERCC2	chr19:45352315	rs746618110	nonsynonymous	NM_000400.3	c.2084G>A	exon22	p.Arg695His	1.19E-05	Likely pathogenic
ERCC2	chr19:45353112	rs140522180	nonsynonymous	NM_000400.3	c.1802G>A	exon19	p.Arg601Gln	1.81E-04	Likely pathogenic
ERCC2	chr19:45364278	rs767916267	nonsynonymous	NM_000400.3	c.772C>T	exon9	p.Arg258Trp	4.00E-06	Likely pathogenic
MITF	chr3:69964940	rs149617956	nonsynonymous	NM_000248.3	c.952G>A	exon9	p.Glu318Lys	1.37E-03	Likely pathogenic
MUTYH	chr1:45331556	rs36053993	nonsynonymous	NM_012222.2	c.1178G>A	exon13	p.Gly393Asp	3.06E-03	Likely pathogenic
MUTYH	chr1:45332803	rs34612342	nonsynonymous	NM_012222.2	c.527A>G	exon7	p.Tyr176Cys	1.54E-03	Likely pathogenic
PMS2	chr7:5982932	rs1254554953	nonsynonymous	NM_000535.4	c.2066C>T	exon12	p.Thr689Ile	4.63E-06	Likely pathogenic
PMS2	chr7:6002670	rs200029834	nonsense	NM_001322015.2	c.11C>G	exon5	p.Ser4*	2.48E-04	Likely pathogenic
XPA	chr9:97687186	N/a	nonsense	NM_000380.3	c.464delT	exon4	p.Leu155*	NS	Likely pathogenic
XPC	chr3:14172946	N/a	frameshift deletion	NM_004628.4	c.219delG	exon2	p.Val75Trpfs*4	NS	Likely pathogenic

Table 3: 19 RCC samples carrying variants identified as pathogenic or likely pathogenic *by ACMG guideline classifications assigned by InterVar*

Full Id	Sex	Subtype	Histology	Age	*Gene*	*Variants*
RCC-022	F	Familial	ccRCC	46	*XPA*	*XPA:c.464delT:p.Leu155**
RCC-030	M	Early onset	pRCC	40	*BRCA1*	*BRCA1:c.4563delA:p.Lys1521Asnfs*5*
RCC-023	F	Bi/Multi	nsRCC	56	*CHEK2*	*CHEK2:c.1263delT:p.Ser422Valfs*15*
RCC-070	M	Familial	pRCC	44	*XPC*	*XPC:c.219delG:p.Val75Trpfs*4*
RCC-074	F	Familial	nsRCC	64	*BRIP1*	*BRIP1:c.1161dupA:p.Gln388Thrfs*7*
RCC-011	M	Familial	nsRCC	58	*CHEK2*	*CHEK2:c.1427C>T:p.Thr476Met*
RCC-089	F	Bi/Multi	nsRCC	40	*ERCC2*	*ERCC2:c.2084G>A:p.Arg695His*
RCC-025	F	Familial	ccRCC	N/a	*ERCC2*	*ERCC2:c.1802G>A:p.Arg601Gln*
RCC-052	F	Bi/Multi	nsRCC	61	*ERCC2*	*ERCC2:c.772C>T:p.Arg258Trp*
RCC-068	M	Familial	ccRCC	74	*MITF*	*MITF:c.952G>A:p.Glu318Lys*
RCC-059	M	Bi/Multi	nsRCC	56	*CHEK2*	*CHEK2:c.1263delT:p.Ser422Valfs*15*
					MUTYH	*MUTYH:c.1178G>A:p.Gly393Asp*
RCC-088	F	Early onset	nsRCC	45	*MUTYH*	*MUTYH:c.527A>G:p.Tyr176Cys*
RCC-099	M	Early onset	nsRCC	27	*PMS2*	*PMS2:c.2066C>T:p.Thr689Ile*
RCC-031	M	Bi/Multi	nsRCC	46	*BRIP1*	*BRIP1:c.1871C>A:p.Ser624**
RCC-001	M	Familial	nsRCC	38	*PMS2*	*PMS2:c.11C>G:p.Ser4**
RCC-043	M	Bi/Multi	pRCC	54	*BRIP1*	*BRIP1:c.1871C>A:p.Ser624**
RCC-029	F	Familial	ccRCC	47	*PMS2*	*PMS2:c.11C>G:p.Ser4**
RCC-096	F	Early onset	nsRCC	34	*PMS2*	*PMS2:c.11C>G:p.Ser4**
RCC-102	M	Familial	ccRCC	39	*BRIP1*	*BRIP1:c.2392C>T:p.Arg798**

Table 4: Statistical association of truncating variant carrier status between the case set 1958 birth control, gnomAD exomes, and ExAC non-TCGA (Fisher's exact test with FDR correction)

Gene	Cases		gnomAD		gnomAD p.value	gnomAD q.value (n=65)	ExAC non-TCGA		ExAC p.value	ExAC q.value (n=58)	ICR 1958 BC		1958-BC p.value	1958-BC q.value (n=19)
	Carrier	Non-carrier	Carrier	Non-carrier			Carrier	Non-carrier			Carrier	Non-carrier		
AIP	0	118	150	124530	1.00E+00	1.00E+00	60	53032	1.00E+00	1.00E+00	0	999	N/a	N/a
ALK	0	118	415	123175	1.00E+00	1.00E+00	24	53080	1.00E+00	1.00E+00	2	994	1.00E+00	1.00E+00
APC	0	118	51	120997	1.00E+00	1.00E+00	186	48761	1.00E+00	1.00E+00	0	999	N/a	N/a
ATM	0	118	401	124424	1.00E+00	1.00E+00	398	52703	1.00E+00	1.00E+00	4	994	1.00E+00	1.00E+00
BAP1	0	118	45	125458	1.00E+00	1.00E+00	20	53084	1.00E+00	1.00E+00	0	999	N/a	N/a
BMPR1A	0	118	7	125516	1.00E+00	1.00E+00	13	53092	1.00E+00	1.00E+00	0	999	N/a	N/a
BRCA1	1	117	328	124617	2.67E-01	1.00E+00	154	52085	2.95E-01	1.00E+00	1	998	2.00E-01	5.43E-01
BRCA2	0	118	2146	121389	2.77E-01	1.00E+00	929	52165	2.77E-01	1.00E+00	24	974	1.00E-01	4.03E-01
BRIP1	4	114	252	124984	**1.09E-04**	**7.06E-03**	174	52927	**6.90E-04**	**4.00E-02**	4	994	**5.94E-03**	1.13E-01
CDC73	0	118	7	124886	1.00E+00	1.00E+00	3	53100	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
CDH1	0	118	21	121649	1.00E+00	1.00E+00	9	48329	1.00E+00	1.00E+00	0	999	N/a	N/a
CDK4	0	118	14	125686	1.00E+00	1.00E+00	3	53102	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
CDKN2A	0	118	18	117900	1.00E+00	1.00E+00	6	53021	1.00E+00	1.00E+00	0	999	N/a	N/a
CEBPA	0	118	0	118332	N/a	N/a	0	52127	N/a	N/a	0	999	N/a	N/a
CHEK2	2	116	728	119600	1.61E-01	1.00E+00	266	51488	1.25E-01	1.00E+00	5	992	1.64E-01	5.19E-01
CYLD	0	118	10	124515	1.00E+00	1.00E+00	3	52828	1.00E+00	1.00E+00	0	999	N/a	N/a
DDB2	0	118	40	125271	1.00E+00	1.00E+00	13	53092	1.00E+00	1.00E+00	0	999	N/a	N/a
DICER1	0	118	17	125424	1.00E+00	1.00E+00	9	53096	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
EGFR	0	118	79	118688	1.00E+00	1.00E+00	26	52854	1.00E+00	1.00E+00	0	999	N/a	N/a
EPCAM	0	118	43	122455	1.00E+00	1.00E+00	30	52947	1.00E+00	1.00E+00	0	999	N/a	N/a
ERCC2	0	118	189	119333	1.00E+00	1.00E+00	70	52787	1.00E+00	1.00E+00	0	999	N/a	N/a
ERCC3	0	118	295	124460	1.00E+00	1.00E+00	113	52991	1.00E+00	1.00E+00	0	999	N/a	N/a
ERCC4	0	118	101	124884	1.00E+00	1.00E+00	46	53053	1.00E+00	1.00E+00	0	999	N/a	N/a

ERCC5	0	118	146	125186	1.00E+00	1.00E+00	41	53055	1.00E+00	1.00E+00	0	999	N/a	N/a
EXT1	0	118	7	125399	1.00E+00	1.00E+00	2	53103	1.00E+00	1.00E+00	0	999	N/a	N/a
EXT2	0	118	74	123212	1.00E+00	1.00E+00	22	52900	1.00E+00	1.00E+00	0	999	N/a	N/a
FH	0	118	16	116752	1.00E+00	1.00E+00	5	47640	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
FLCN	0	118	189	125301	1.00E+00	1.00E+00	128	52976	1.00E+00	1.00E+00	0	999	N/a	N/a
GATA2	0	118	2	114936	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
HNF1A	0	118	15	122880	1.00E+00	1.00E+00	6	53097	1.00E+00	1.00E+00	0	999	N/a	N/a
KIT	0	118	21	125545	1.00E+00	1.00E+00	4	53101	1.00E+00	1.00E+00	0	999	N/a	N/a
MAX	0	118	21	125062	1.00E+00	1.00E+00	6	53043	1.00E+00	1.00E+00	0	999	N/a	N/a
MEN1	0	118	2	122596	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
MET	0	118	26	122952	1.00E+00	1.00E+00	9	52843	1.00E+00	1.00E+00	0	999	N/a	N/a
MLH1	0	118	80	122372	1.00E+00	1.00E+00	10	53095	1.00E+00	1.00E+00	0	999	N/a	N/a
MSH2	0	118	61	122691	1.00E+00	1.00E+00	25	53053	1.00E+00	1.00E+00	0	999	N/a	N/a
MSH6	0	118	1149	123628	6.32E-01	1.00E+00	949	52149	2.80E-01	1.00E+00	7	992	1.00E+00	1.00E+00
MUTYH	0	118	533	123827	1.00E+00	1.00E+00	348	52754	1.00E+00	1.00E+00	2	996	1.00E+00	1.00E+00
NF1	0	118	97	122299	1.00E+00	1.00E+00	52	53020	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
NF2	0	118	8	120896	1.00E+00	1.00E+00	1	53104	1.00E+00	1.00E+00	0	999	N/a	N/a
PALB2	0	118	222	125060	1.00E+00	1.00E+00	83	53020	1.00E+00	1.00E+00	1	998	1.00E+00	1.00E+00
PHOX2B	0	118	0	118332	N/a	N/a	0	52127	N/a	N/a	0	999	N/a	N/a
PMS2	3	115	259	119201	**2.30E-03**	7.49E-02	454	52338	8.29E-02	1.00E+00	4	994	**2.94E-02**	2.79E-01
PRKAR1A	0	118	2	125224	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
PTCH1	0	118	40	123146	1.00E+00	1.00E+00	14	52903	1.00E+00	1.00E+00	0	999	N/a	N/a
PTEN	0	118	25	122703	1.00E+00	1.00E+00	1	53104	1.00E+00	1.00E+00	0	999	N/a	N/a
RAD51C	0	118	121	125258	1.00E+00	1.00E+00	50	53054	1.00E+00	1.00E+00	2	996	1.00E+00	1.00E+00
RAD51D	0	118	85	121475	1.00E+00	1.00E+00	31	52911	1.00E+00	1.00E+00	1	997	1.00E+00	1.00E+00
RB1	0	118	5	120731	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
RET	0	118	6	125729	1.00E+00	1.00E+00	3	53102	1.00E+00	1.00E+00	0	999	N/a	N/a
RHBDF2	0	118	36	119924	1.00E+00	1.00E+00	20	53035	1.00E+00	1.00E+00	0	999	N/a	N/a
RUNX1	0	118	13	103237	1.00E+00	1.00E+00	7	52745	1.00E+00	1.00E+00	0	999	N/a	N/a
SDHAF2	0	118	27	125597	1.00E+00	1.00E+00	15	53090	1.00E+00	1.00E+00	0	999	N/a	N/a

SDHB	0	118	27	125031	1.00E+00	1.00E+00	12	53093	1.00E+00	1.00E+00	0	999	N/a	N/a
SDHC	0	118	27	123500	1.00E+00	1.00E+00	7	53075	1.00E+00	1.00E+00	0	999	N/a	N/a
SDHD	0	118	30	88498	1.00E+00	1.00E+00	6	21717	1.00E+00	1.00E+00	0	999	N/a	N/a
SMAD4	0	118	14	114572	1.00E+00	1.00E+00	1	53104	1.00E+00	1.00E+00	0	999	N/a	N/a
SMARCB1	0	118	4	125639	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
STK11	0	118	4	80810	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
SUFU	0	118	2	124946	1.00E+00	1.00E+00	0	52127	N/a	N/a	0	999	N/a	N/a
TMEM127	0	118	15	122589	1.00E+00	1.00E+00	3	53102	1.00E+00	1.00E+00	0	999	N/a	N/a
TP53	0	118	32	119966	1.00E+00	1.00E+00	6	52658	1.00E+00	1.00E+00	0	999	N/a	N/a
TSC1	0	118	8	125513	1.00E+00	1.00E+00	3	53100	1.00E+00	1.00E+00	0	999	N/a	N/a
TSC2	0	118	36	122583	1.00E+00	1.00E+00	6	53093	1.00E+00	1.00E+00	0	999	N/a	N/a
VHL	0	118	13	106153	1.00E+00	1.00E+00	14	52727	1.00E+00	1.00E+00	0	999	N/a	N/a
XPA	1	117	155	124232	1.38E-01	1.00E+00	60	53038	1.27E-01	1.00E+00	0	995	1.06E-01	4.03E-01
XPC	1	117	132	122045	1.21E-01	1.00E+00	71	52812	1.48E-01	1.00E+00	0	995	1.06E-01	4.03E-01