Germline BRCA2 mutations drive prostate cancers with distinct evolutionary trajectories

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Germline mutations in the BRCA2 tumour suppressor are associated with both an increased lifetime risk of developing prostate cancer (PCa) and increased risk of aggressive disease. To understand this aggression, here we profile the genomes and methylomes of localized PCa from 14 carriers of deleterious germline BRCA2 mutations (BRCA2-mutant PCa). We show that BRCA2-mutant PCa harbour increased genomic instability and a mutational profile that more closely resembles metastatic than localized disease. BRCA2-mutant PCa shows genomic and epigenomic dysregulation of the MED12L/MED12 axis, which is frequently dysregulated in metastatic castration-resistant prostate cancer (mCRPC). This dysregulation is enriched in BRCA2-mutant PCa harbouring intraductal carcinoma (IDC). Microdissection and sequencing of IDC and juxtaposed adjacent non-IDC invasive carcinoma in 10 patients demonstrates a common ancestor to both histopathologies. Overall we show that localized castration-sensitive BRCA2-mutant tumours are uniquely aggressive, due to de novo aberration in genes usually associated with metastatic disease, justifying aggressive initial treatment.

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Germline mutation of the BRCA2 tumour suppressor gene substantially increases the lifetime risk of developing prostate cancer (PCAs). In BRCA2-mutation carriers, localized PCAs rapidly progresses to metastatic castrate-resistant prostate cancer (mCRPC) with 5-year cancer-specific survival rates of ~50–60% (refs 1,3,4). BRCA2-mutant tumours also exhibit an increased frequency of intraductal carcinoma (IDC), a pathology that predicts adverse outcome in both familial and sporadic PCAs. Prostate tumours arising in men with an inactivating BRCA2 germline mutation (BRCA2-mutant PCAs) are uniquely aggressive, associated with younger age of onset, have higher rates of lymph node and distant metastasis, and increased mortality relative to sporadic, non-BRCA2-mutant disease. Precision surgery or radiotherapy with curative intent inexplicably fails, with a rapid onset of incurable mCRPC in BRCA2-mutant PCAs. The molecular origins of the clinical aggressiveness of BRCA2-mutant PCAs are unknown. Understanding them may allow better treatment decision-making; for example, given their DNA repair deficient genotype, these cancers may be amenable to treatment with PARP inhibitors, based on genetic synthetic lethality.

In this study, to identify the drivers of aggression in BRCA2-mutant PCAs, we characterized genomic alterations in localized PCA tumour specimens from 14 men carrying a deleterious germline mutation BRCA2 allele using either whole-genome sequencing (WGS) and/or SNP array-based copy-number analyses (CNAs). We find that BRCA2-mutant PCAs harbour a mutational profile more akin to that of mCRPC than localized sporadic PCAs, and shows broad dysregulation of pathways associated with aggressive disease, including the MEDI2/MEDI2L axis. Micro-dissection and subclonal reconstruction shows distinct evolutionary trajectories and identifies a common precursor to IDC and adjacent invasive carcinoma. Taken together, these data demonstrate the genomic correlates underlying aggressive BRCA2-mutant PCAs.

Results
The Mutational Landscape of BRCA2-mutant PCAs. To address intra-prostatic and intra-lesion heterogeneity, we microdissected regions of invasive carcinoma (IC) and of IDC from five tumours, resulting in 19 PCA specimens (Supplementary Table 1; Supplementary Fig. 1).

We first identified single-nucleotide variants (SNVs), genomic rearrangements (GRs) and CNAs from these samples using validated pipelines (Supplementary Table 1; Supplementary Fig. 2). We compared the mutational profiles of BRCA2-mutant PCAs to a set of 200 sporadic PCAs with whole-genome sequencing. We separated this comparison cohort into patients 50 years of age or younger at the time of treatment (n = 8) and those older than 50 years (n = 192), to control for age effects. BRCA2-mutant PCAs showed elevated genomic instability, as measured by percent genome alteration (PGA), an established prognostic factor for relapse in localized disease (P = 7.53 × 10⁻⁴, two-sided t-test; Fig. 1a). Similarly, BRCA2-mutant tumours showed elevated numbers of SNVs and GRs than sporadic tumours arising in either older or younger men (Supplementary Table 2; Supplementary Fig. 3).

Overall, BRCA2-mutant PCAs shows the same C-class character as sporadic PCAs, with a large number of CNAs and a relative paucity of SNVs. Regions of the genome recurrently gained or deleted in BRCA2-mutant PCAs but not sporadic PCAs include a large amplification on chromosome 3q (Fig. 1b). Genomic changes associated with aggressive disease were more common, including gain of the MYC oncogene, which occurred in 72% (13/18) of BRCA2-mutant PCAs which was significantly higher than the 20.1% rate in sporadic PCAs of equivalent Gleason score (57/283; \( \bar{P} = 1.42 \times 10^{-3} \), two-sided proportion test). Gains in MYCN, GSK3B and MTOR were also more common in BRCA2-mutant and sporadic PCAs. By contrast, losses of the tumour suppressors TP53 and NKX3-1 occurred at similar rates in BRCA2-mutant and sporadic PCAs (Fig. 1c).

Surprisingly, BRCA2-mutant PCAs showed global hypomethylation relative to sporadic PCAs (Supplementary Fig. 9) which was retained even after controlling for the increased CNA rate of BRCA2-mutant PCAs (Supplementary Figs 10 and 17). Overall ~1% (4,979/435,377) of copy-number neutral probes showed differential methylation between BRCA2-mutant and sporadic PCAs specimens (Supplementary Data 3), and 74% (3,677/4,979) of these were hypomethylated, including 21 of the 25 most differentially methylated genes (Supplementary Fig. 11). Differentially methylated genes in BRCA2-mutant PCAs were enriched in pathways associated with VEGF signaling, neuronal development, and other neuronal processes characteristic of NED (Supplementary Fig. 12; Supplementary Table 3). Other hypomethylated NED-related genes include modulators of WNT signaling (for example, AXIN, APC, ROCK1/2), IL6 signaling (for example, IL6ST, BMP6), and mTOR signaling (for example, TSC2), and many of these genes were also altered by CNAs (Supplementary Fig. 13). The 3’ UTR of the BRCA2 locus was hypomethylated in BRCA2-mutant PCAs specimens (Supplementary Fig. 14).

MED12/MED12L amplification is associated with aggressive PCAs.
To identify other driver events that might help explain the aggressiveness of BRCA2-mutant PCAs, we investigated the amplification of a region on chromosome 3q (Fig. 1b) that is common in BRCA2-mutant PCAs (11/18; 61%), but rare in sporadic PCAs (55/809; 6.8% \( \bar{P} = 1.64 \times 10^{-9} \), two-sided proportion test). The consensus amplicon of this locus contains the WNT/β-catenin pathway modulator MED12L (Supplementary Data 4 and 7), as well as ATR, which encodes the DNA damage response factor ataxia telangiectasia-and-Rad3 related protein.
Figure 1 | The genomics of BRCA2-mutant prostate cancer. (a) Percent genome altered (PGA) and number of SNVs, GRs and CNAs per tumour sample is shown. GRs are stratified by type: orange—deletions (del), green—inversions (inv), purple—duplications (dup), yellow—translocations (ctx). Y axis values for CNAs and GRs are in log_{10} scale. Box plots compare the number of events observed in BRCA2-mutant specimens versus sporadic PCA samples split into two groups by age. Y axis values are the same as in the adjacent bar plot. Whiskers indicate the maximum and minimum values, the box outline indicates the third and first quartile and the bar indicates the mean. Multiple foci from the same patient are indicated in the same patient covariate colour, where sample type colour indicates IC in blue, IDC in yellow and a mix of IDC and IC in green. Unavailable data is indicated by grey background. (b) Per gene copy-number profiles of 18 prostate tumour specimens from 14 germline BRCA2-mutation carriers, including 4 patients with multifocal disease. Red indicates gain; blue indicates loss. Rows represent specimens and columns represent genes. Top plots show frequency of each gene per group (BRCA2, \( n = 14 \); sporadic PCA arising in individuals 50 years of age or younger, \( n = 7 \); sporadic PCA arising in individuals older than 50 years of age, \( n = 276 \)) and the q-value shown is from a two-sided proportion test comparing BRCA2-mutant and all sporadic PCA samples. Genes are ordered by genomic co-ordinates per chromosome. Multiple foci from the same patient are indicated in the same patient covariate colour, where sample type colour indicates IC in blue, IDC in yellow and a mix of IDC and IC in green. (c) Key prostate cancer driver genes that are mutated at elevated proportions in BRCA2-mutant PCA relative to sporadic PCA. Columns are patients, rows are genes, a black square indicates a CNA. Bar plots to the right show the frequency of each gene in the three groups (BRCA2, \( n = 14 \); sporadic PCA arising in individuals 50 years of age or younger, \( n = 7 \); sporadic PCA arising in individuals older than 50 years of age, \( n = 276 \)).
Figure 2 | Genomic rearrangements in BRCA2-mutant prostate cancer. (a) Survey of genome-wide somatic genomic rearrangements (GRs) in BRCA2-mutant tumour PCa from seven patients. CTX—inter-chromosomal translocation; DEL—deletion; INV—inversion. Multiple foci from the same patient are indicated in the same patient covariate colour, where sample type colour indicates microdissected invasive carcinoma (IC) in blue and intraductal carcinoma of the prostate (IDC) in yellow. Top bar plots show the frequency of specimens in BRCA2-mutant PCa (bottom) and sporadic PCa from men 50 years of age or younger (middle) or sporadic PCa from men older than 50 years of age (top). Scatterplot at top indicates FDR-corrected P values of a two-sided proportion test comparing frequency of GRs observed in BRCA2-mutant versus sporadic PCa. (b) A circos plot showing the proportions of BRCA2-mutant (blue) and sporadic PCa (red) with breakpoints in each mega-basepair bin. Bins with significantly different proportions between BRCA2-mutant and sporadic PCa (FDR-correct proportions test) are highlighted in yellow, and have the number of BRCA2-mutant samples indicated. Only chromosomes with at least one differentially recurrent GR are shown.
Importantly, the MEDI12 homolog, MEDI12 (chromosome Xq13.1), is amplified in 44% of BRCA2-mutant PCa specimens (8/18) compared with only ~0.1% of localized sporadic PCa (1/809, \(P = 3.64 \times 10^{-44}\), two-sided proportion test). Further, MEDI12L (but not MEDI12) showed frequent amplification in distant metastases from metastatic castrate resistant prostate cancer (mCRPC) patients (7/37 cases, 19%). Supporting this model, four loci within the MEDI12L gene were significantly hypomethylated in BRCA2-mutant versus sporadic PCa (Fig. 3a; Supplementary Fig. 15). Consistent with their alteration at the copy-number and methylation levels in aggressive BRCA2-mutant PCa, MEDI12/MEDI12L are also amplified and/or show elevated RNA expression levels in 18% (26/150) of mCRPC\(^9\) but only 7.5% (25/333) of sporadic, localized PCa (Supplementary Fig. 16). Consistent with their alteration at the copy-number and methylation levels in aggressive BRCA2-mutant PCa, MEDI12/MEDI12L amplifications are preferentially mutated in aggressive disease—both BRCA2-driven primary tumours and sporadic mCRPC.

MEDI12L/MEDI12 amplification was also enriched in BRCA2-mutant PCa harbouring IDC (6/8 IDC-positive specimens with MEDI12L/MEDI12 gain versus 1/6 IDC negative specimens). Importantly, these mutations are not enriched in sporadic tumours harbouring IDC. Therefore we evaluated global differences between IDC and IC. Presence of IDC in BRCA2-mutant PCa associates with CNAs and/or show elevated RNA expression levels in 18% (26/150) of mCRPC\(^9\) but only 7.5% (25/333) of sporadic, localized PCa (Supplementary Fig. 16). Consistent with their alteration at the copy-number and methylation levels in aggressive BRCA2-mutant PCa, MEDI12L/MEDI12 amplifications are preferentially mutated in aggressive disease—both BRCA2-driven primary tumours and sporadic mCRPC.

**Evolutionary trajectories of BRCA2-mutant and sporadic PCa.** Enrichment for negative prognostic factors in BRCA2-mutant PCa harbouring IDC suggested that these tumours may have distinct evolutionary trajectories. To assess the subclonal architectures of sporadic and BRCA2-mutant PCa harbouring IDC we selected six sporadic PCa and microdissected the IDC and IC components (Supplementary Fig. 16). Both components were subjected to whole-genome sequencing and CNA profiling and tumour phylogenies were reconstructed. This procedure was repeated for four BRCA2-mutant PCa cases that harboured both IDC and IC components. Reconstruction began with subclonal CNA profiling using TITAN\(^19\), followed by clustering of SNV frequencies with PhyloWGS\(^20\). In all four BRCA2-mutant PCa samples (Fig. 4a; Supplementary Data 5) the IDC and IC components arose from the same founding clone, with no evidence of multiple independent tumours\(^21\). The parental population was found in both the IDC and IC regions, providing no inference as to where the tumour may have initiated.

MYC amplifications were observed in three of these four patients, and in each occurred before divergence of IDC and IC components. By contrast, MEDI12L gain was clonal in two samples and sub-clonal in two others, in one case only occurring in the IC component.

In sporadic PCa with evidence of IDC (Fig. 4b; Supplementary Data 6) there was again no evidence of multiple independent tumours—the IDC and IC components arose from a common ancestor and there was no clear evidence as to which compartment this ancestor arose in. MYC amplification was observed in 4/6 tumours, once clonally, once in a clone only present in IDC and twice only in a clone present in IC. NKX3-1 deletion was present in the trunk of 3/6 tumours. Intriguingly, PIK3CA SNVs were observed in 2/6 patients with IDC (once clonally and once only in the IC component), as compared with 7/333 patients in the TCGA localized PCa dataset (\(P = 5.92 \times 10^{-4}\), proportion test)\(^9\).

**Discussion**

While the clinical aggressiveness of germline BRCA2-mutant PCa remains unexplained, it is distinguished from sporadic PCa by a unique mutational profile. BRCA2-mutant PCa is characterized by elevated global genomic instability, which is a well-established biomarker of biochemical failure and metastasis\(^14,15\). BRCA2-mutant PCa harboured specific mutations that are rarely, if ever, observed in sporadic localized PCa. These lead to dysregulation of
pathways associated with NED and mCRPC, despite no exposure to androgen deprivation therapy in these patients. These observations help rationalize our recent clinical association between disease outcome and the presence of IDC, in part, with increased activation of the MED12L/MED12 axis.

Surprisingly, and counter to previous expectations, the IDC and IC components share a common precursor, and share a large portion of their mutational profile. MED12L/MED12 are components of the mediator complex and important regulators of the WNT pathway, which has been implicated in cell proliferation and neuroendocrine differentiation in PCa. The MED12L/MED12 axis is implicated in metastatic sporadic PCa, with MED12 nuclear overexpression common in mCRPC and localized recurrent PCa but not in localized primary PCa or benign prostate tissue. MED12 SNVs, which occur in both localized and advanced PCa, are also implicated in prostate tumourigenesis and genome instability.

BRCA2-mutant PCa are also enriched for aberrant DNA methylation in genes involved in neurogenesis and neuron development, consistent with recent reports that autonomic nerve development contributes to PCa progression and metastasis. Overall, our data strongly support a model in which germline BRCA2-mutant PCa are genotypically-similar to mCRPC and frequently harbour genomic aberrations in key pathways (for example, WNT/APC, IL6-BMP6, mTOR, ATR, MYCN, and so on; Supplementary Data 6; Fig. 4c) leading to genomic instability, NED, castrate-resistance and metastasis, together explaining
adverse patient prognosis. It remains to be shown if the temporal change in genomic stability of BRCA2-mutation carrier PCa tumors is relevant to other solid tumors with specific predispositions for aggressive disease such as those arising in breast, colon and pancreas.

Collectively, our data provide genome-wide, nucleotide-resolution characterization of the molecular architecture of localized PCa and IDC that develops in men who harbour a mutant germline BRCA2 allele. While the population frequency is relatively low (<1%)32, the aggressive natural history of localized tumors may justify a change in clinical management to a more intense approach including upfront use of PARP inhibitors with anaplastic thyroid cancer and 10 female HapMap cell line samples was created. The final VCF file that contains high confidence somatic SNVs was used in the downstream analysis. After somatic SNV calling using SomaticSniper, identified SNVs were passed through institutional Research Ethics Board protocols (UHN #11-0024 and #11-0091; T: 96727).

In Canada, patients with a known BRCA2 germline mutation were approached to combat artefacts created due to the difference in sample preparation (FFPE versus Fresh Frozen).

To determine if the frequency differed between samples that had an IDC component and those that did not. Samples that had both IDC and IC specimens were classified to get a 'complete' profile, in which both were used. Samples without an IDC component were classified to get a 'sporadic profile'. When only one sample was available, it was taken. Only CNAs that are in the same state (amplification versus deletion) as known prognostic genes are shown. For example if a specimen has amplification of NKX3-1, it will not be shown, as only NKX3-1 deletions have prognostic value. Whole-genome sequencing data analysis. Each lane of raw sequencing reads was aligned against human reference build GRCh37 decoy with bwa (v0.7.11)34. Lane level BAMS from the same library were merged, marking duplicates using picard (v1.92). Library level BAMS from each sample were merged without marking duplicates. The Genome Analytics Toolkit (GATK v2.4.9) was used for local realignment and base quality recalibration, processing tumour/normal pairs together32. Separately tumour and normal sample level BAMS were extracted, headers were corrected using samtools (v0.1.19)39 and files were indexed with picard (v1.107). To evaluate cross-individual contamination, ContEst (v1.24530) was used for all the normal and tumour sequences37. Both the sample and lane-level analysis were performed and confirmed no significant contamination. Regarding the input VCFs, genotype information was gained from the germline SNVs generated by GATK (v2.4.9) and the VCF for population allele frequencies for each SNP in HapMap (hg19) was downloaded from https://www.broadinstitute.org/cancer/cga/context_download.

Somatic SNVs were predicted using SomaticSniper (v1.0.2)35. First, somatic SNV candidates were detected using bam-somaticsniper with the default parameters except -q option (mapping quality threshold). The -q was set to 1 instead of 0 as recommended by the developer. To filter the candidate SNVs, a pileup indel file was generated for both normal BAM and tumour BAM file using SAMtools (v0.1.6). The SomaticSniper package provides a series of Perl scripts to filter differenced possible false positives (http://gmt.genome.wustl.edu/somatic-sniper/1.0.3/documentation.html). First, standard and LOH filtering were performed using the pileup indel files and then, bam-readcount filter was also performed (bam-readcount downloaded on 10 January 2014) with a mapping quality filter -q 1 (otherwise default settings). In addition, we ran the false positive filter. Subsequently, a high confidence filter was used with the default parameters. The final VCF file that contains high confidence somatic SNVs was used in the downstream analysis.

After somatic SNV calling using SomaticSniper, identified SNVs were passed through an annotation pipeline. SNVs were functionally annotated by ANNOVAR (v2015-06-17)38, using the RefGene database (downloaded on 2014-07-15). Nonsynonymous, stop-loss, stop-gain and splice-site SNVs (based on RefGene annotations) were considered functional. If more than one mutation is found in a sample for a gene, then the mutation of the higher priority functional class was used for visualization. SNVs were filtered using tabix: 

\[ \text{SNV FILTERING} \]

(bam-readcount downloaded on 10 January 2014) with a mapping quality filter -q 1 (otherwise default settings). In addition, we ran the false positive filter. Subsequently, a high confidence filter was used with the default parameters. The final VCF file that contains high confidence somatic SNVs was used in the downstream analysis.

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Methods

Patient cohorts. Men were treated for sporadic or BRCA2-mutation PCa by radical prostatectomy at Princess Margaret Cancer Centre (University Health Network, Toronto, Canada) or were recruited by kConFab (Kathleen Cunningham Foundation Consortium for research into Familial Breast cancer) at the Peter MacCallum Cancer Centre (Melbourne, Australia). Informed consent for analysis was obtained through institutional Research Ethics Board protocols (UHN #11-0024 and #11-0091; T: 96727).

In Canada, patients with a known BRCA2 germline mutation were approached for retrospective informed consent based on the following inclusion criteria:

1. Intermediate or high risk localized Prostate cancer, as defined by NCCN guidelines, treated at the Princess Margaret Cancer Centre BRCA clinic;
2. confirmed presence of primary tumour tissue in the UHN genitourinary biobank;
3. whole blood (5 ml) was obtained at the time of consenting. Consent was for use of tumour and normal tissues and associated DNA, RNA and protein, including whole-genome and whole-transcriptome sequencing of both somatic and germline specimens.

In Australia, prostate cancer cases were identified from 1,600 families recruited into the Kathleen Cunningham Consortium for Research into Familial Breast Cancer (kConFab), the Australian and New Zealand consortium for families at high-risk of breast cancer (http://www.kconfab.org). Inclusion in kConFab requires a strong multi-case family history of breast and/or ovarian cancer, or a germline mutation in a breast cancer predisposition gene such as BRCA1 and BRCA2 (see www.kconfab.org for full recruitment criteria). Male kConFab members were eligible for inclusion in this study if:

1. they had a verified diagnosis of prostate cancer,
2. complete diagnostic and treatment notes were available and
3. family BRCA mutation status was known. Human Research Ethics Committee approval was obtained from the Peter MacCallum Cancer Centre (Melbourne, Canada) or were recruited by kConfab (Kathleen Cunningham Foundation Consortium for research into Familial Breast cancer).

Tissue collection and microdissection. Hematoxylin and Eosin-stained sections from fresh-frozen, formalin-fixed paraffin-embedded (FFPE) prostate cancer tissues were assessed by a clinical genitourinary pathologist (TvdK or DC). Regions from fresh-frozen or formalin-fixed paraffin-embedded (FFPE) prostate cancer tissue were assessed by a clinical genitourinary pathologist (TvdK or DC). Hematoxylin and Eosin-stained sections

(Continues)
TPA, MUT and OM)\(^4\). 1,000 Genomes Project (v3), Complete Genomics 69 whole genomes, duplicate gene database (v68)\(^4\). ENCODE DAC and Duke Mabpatability Consensus Excludable databases (comprising poorly mapping reads, repeat regions, and mitochondrial and ribosomal DNA)\(^2\), invalidated somatic SNVs from 68 human colorectal cancer exomes (unpublished data) using the AccusSNP platform (Roche NimbleGen), germline SNVs from 477 sporadic PCa patients with the interior of each region (X, Y, C2) and additionally 10 prostate cancer patients with higher GS\(^3\), and the Fuentes database of likely false positive variants\(^3\). SNVs were whitelisted (and retained, independently of the presence in other filters) if they were contained within the Catalogue of Somatic Mutations in Cancer (COSMIC) database (v70)\(^5\).

**SNV mutation load.** To find the total number of bases somatic SNVs can be called on, each position in the reference was classified as ‘callable’ or not. Positions were considered callable if it had a minimum coverage of 10X in 17 and 17\(\times\) in the tumour as calculated using BEDTools (v2.18.3)\(^33\). Mutation load for each sample was then calculated as the number of somatic SNVs divided by the total number of callable bases and converted to per mega-basepair of sequence. Mutation load was not calculated for samples missing a normal specimen to avoid upwards bias on estimated mutation rates.

**Genomic rearrangements.** For each patient, aligned tumour and normal BAM files were used to call structural variants with Delly (v0.5.3)\(^45\) at a minimum median mapping quality of 20 and a paired-end cut-off of five. A list of somatic variants were produced by removing germline mutations from the resulting VCF files, which were further filtered using a consolidated list of structural variants from 124 normal samples. To identify genes affected by the genomic rearrangements, bed files were generated for each sample from deleted regions, and breakpoints from inversions, inter-chromosomal translocations, and tandem duplications. The resultant bed files were examined with SnvEff (v3.5)\(^46\) and gene names were subsequently extracted for downstream analyses. Recurrent translocation events were visualized using Circos (v0.67-41)\(^7\). Input files were bed files containing paired translocation breakpoints and the number of samples the event was observed. Differentially aberrant regions between sporadic and BRCA2 patients were identified by first dividing the genome into mega-basepair bins, and tabulating the number of samples with a deletion, translocation or inversion breakpoint in each bin. Subsequently, a difference proportions test was performed for each bin and the resultant \(P\) values were corrected for multiple comparisons with FDR. Entire chromosomes containing mega-basepair bins with significantly different proportions of breakpoints were visualized using Circos (v0.67-41)\(^7\).

**Chromothripsis detection.** ShatterProof\(^48\) was used with default settings to examine the presence of chromothripsis through the analysis of copy-number aberrations, loss of heterozygosity, and genomic translocations, and duplications. Samples with a maximum ShatterProof score of at least 0.517 were defined as having chromothripsic characteristics.

**Subclonal reconstruction.** For each of the four BRCA2-mutant PCs and six sporadic PCs subjects with microdissected IDC and IC components, phylogenetic trees were constructed using the predicted SNVs and CNAs inferred by TITAN\(^39\). TITAN was run, from a range of one to five clonal clusters, on each sample and the CNAs segments associated with the run having lowest TITAN12.hg19 (v0.2.1) annotation package, resulting in 2,624 unique genes; this gene list was then filtered out 27,296 probes that are known to cross-hybridize to multiple locations in the genome and 17,079 probes that contain a SNP with an annotated minor allele frequency of greater than 5\% with a maximum distance of two nucleotides to the closest CpG site. Average intensity levels were taken for technical replicates. Probes showing differential methylation between BRCA2 and sporadic samples (age \(=\)50 years) were identified using the DMRCate package. Probes deemed to be significantly differentially methylated based on \(q < 0.05\), \(\log FC\) of \(B > 0.1\), and CNA status. The fraction of patients having a CNA event (whether it is a gain or loss) is calculated and only probes with fraction CNA \(=\)0.1 were kept. Next, probes were filtered to their respective gene via g:profiler\(^52\), as an output from g:profiler\(^25\), were then visualized using Cytoscape (v3.3.0)\(^53\). Tumour purities for all the samples were assessed with LUMP\(^54\).

**Public data analysis.** To assess the occurrence of MET12/MED12 amplifications in sporadic and mCRPC relative to BRCA2-mutant cancers, we downloaded publicly available gene copy-number data from TCGA\(^3\) (\(n = 203\)), Baca\(^2\) (\(n = 57\)), Barbieri\(^56\) (\(n = 109\)) and Taylor\(^8\) (sporadic, \(n = 157\); mCRPC, \(n = 37\)). To assess the relationship between RNA expression and MET12/MED12 amplification, we queried cBioPortal for the presence of RNA over-expression (\(z\)-score \(>\) \(\pm 2.0\)) and amplification of one or both of these genes in two studies for which both RNA-seq and CNA data were available: Robinson et al.\(^57\) (mCRPC) and TCGA\(^8\) (localized sporadic PCa). A case was counted as positive if either RNA was overexpressed or the gene was amplified.

**Data availability.** Raw sequencing and SNP microarray data from this study is available at EGA under the study accession EGAS0000106165. Methylation data from this study is available at EGA under the accession EGAS000106165. Genomic rearrangements data accessed from GEO with the accession code GSE4043 was used to support the findings of this study. Sequencing data accessed from EGA with the accession codes EGAS00001000258 and EGAS00001000900 and from dbGaP with the accession codes phs000447.v1.p1 and phs000330.v1.p1 was used to support the findings of this study. A subset of the TCGA-PRAD dataset was also downloaded from CGHub (data now stored at the Genomic Data Commons). The authors declare that all remaining data are contained within the Article and its Supplementary Information files or available from the author upon request.

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