Clinical and Pathological Associations of Chromatin Modifying Tumor Suppressors in Clear Cell Renal Cell Carcinoma

A. Ari Hakimi MD

James Hsieh MD PhD

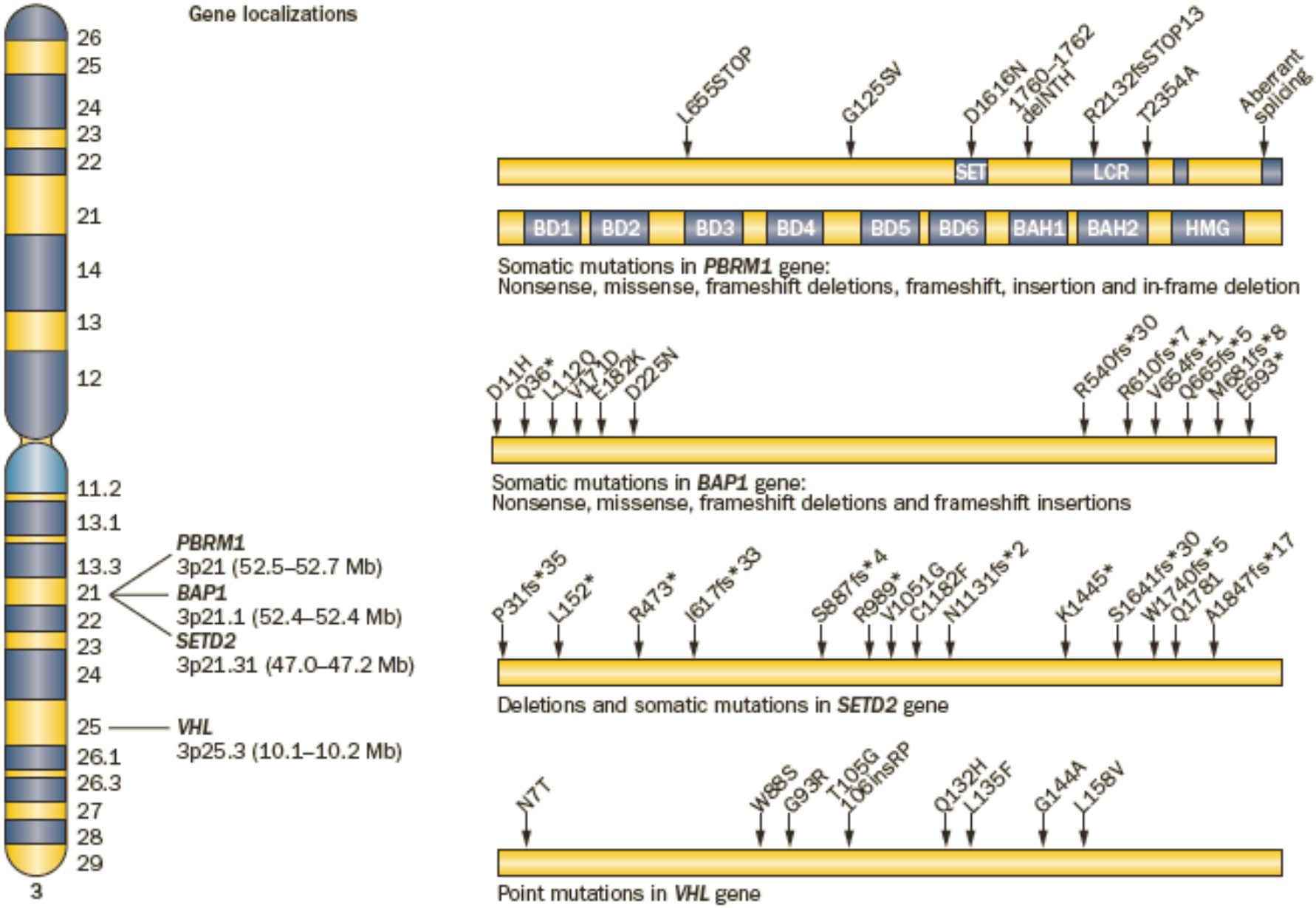
KIRC TCGA

RCC

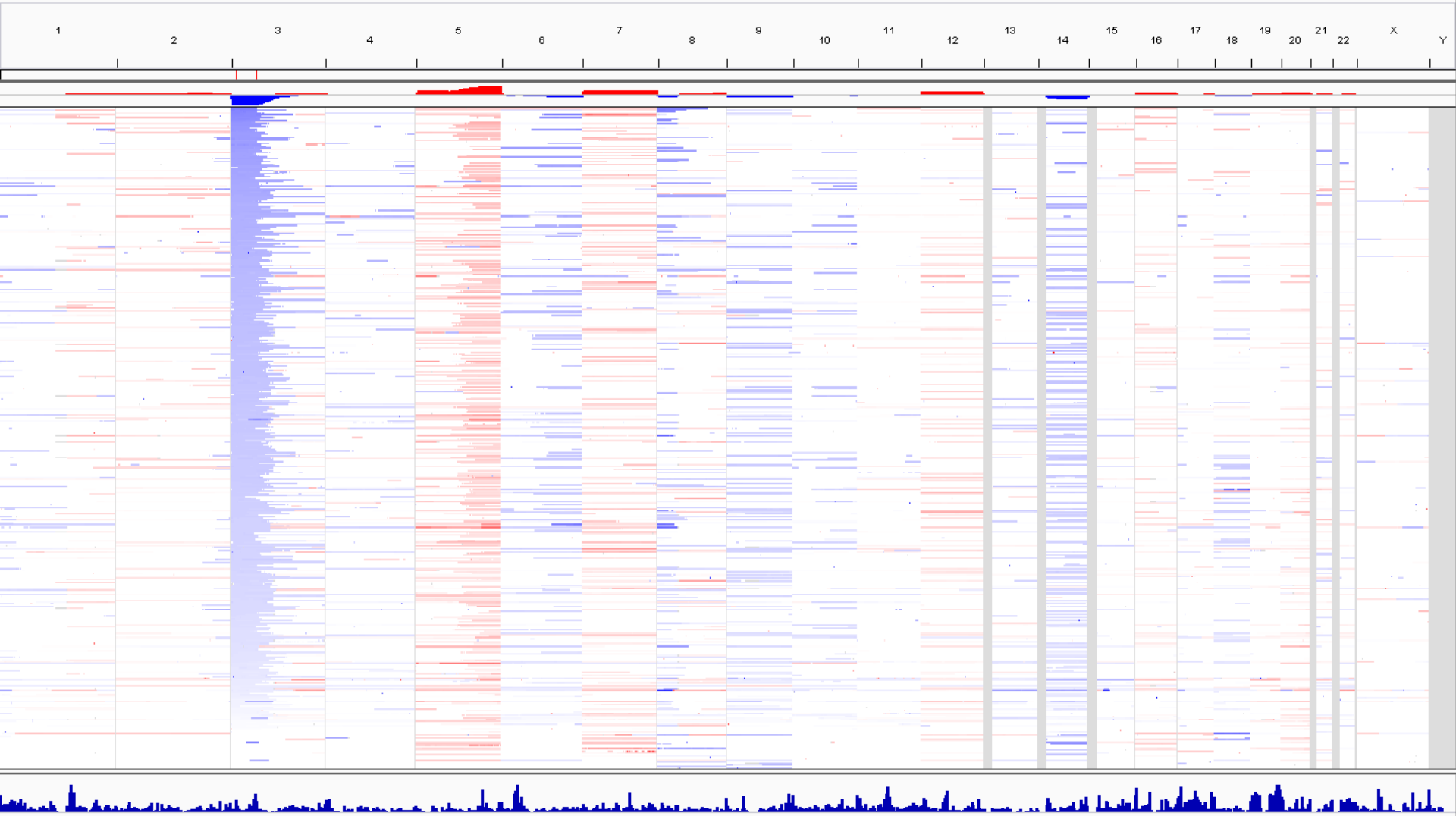
- RCC sixth leading cause of cancer death in US
- Several malignant subtypes
  - Clear cell (ccRCC) ~80%
  - Papillary
  - Chromophobe
  - Other
- Nearly 1/3rd present with metastatic disease
- Chemo/radiation resist

Novel recurrent mutations in ccRCC

- 4 prevalent mutations based on recently published studies
- *VHL*
- *PBRM1* – (Varela, Nature 2011)
- *SETD2* - (Dagliesh, Nature 2010)
- *BAP1* – (Guo, Pena-Llopis Nat Genetics 2011/12)
- All genes located on 3p(21,25)

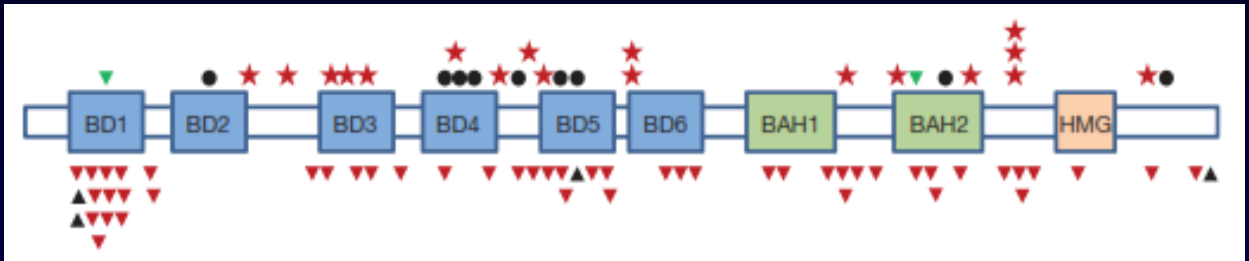

Gene localizations
26
25
24
23
22
21
14
13
12
11.2
13.1
13.3
21
22
23
24
25
26.1
26.3
27
28
29
3
PBRM1
3p21 (52.5–52.7 Mb)
BAP1
3p21.1 (52.4–52.4 Mb)
SETD2
3p21.31 (47.0–47.2 Mb)
VHL
3p25.3 (10.1–10.2 Mb)
L655STOP
G125SV
D1616N
1760–1762
delNTH
R2132fsSTOP13
T2354A
Aberrant
splicing
SET
LCR
BD1
BD2
BD3
BD4
BD5
BD6
BAH1
BAH2
HMG
Somatic mutations in PBRM1 gene:
Nonsense, missense, frameshift deletions, frameshift, insertion and in-frame deletion
D11H
Q36*
L1120
V171D
E182K
D225N
R540fs*30
R610fs*7
V654fs*1
Q665fs*5
M681fs*8
E693*
Somatic mutations in BAP1 gene:
Nonsense, missense, frameshift deletions and frameshift insertions
P31fs*35
L152*
R473*
I617fs*33
S887fs*4
R989*
V1051G
C1182F
N1131fs*2
K1445*
S1641fs*30
W1740fs*5
Q1781
A1847fs*17
Deletions and somatic mutations in SETD2 gene
N7T
W88S
G93R
T105G
106insRP
Q132H
L135F
G144A
L158V
Point mutations in VHL gene

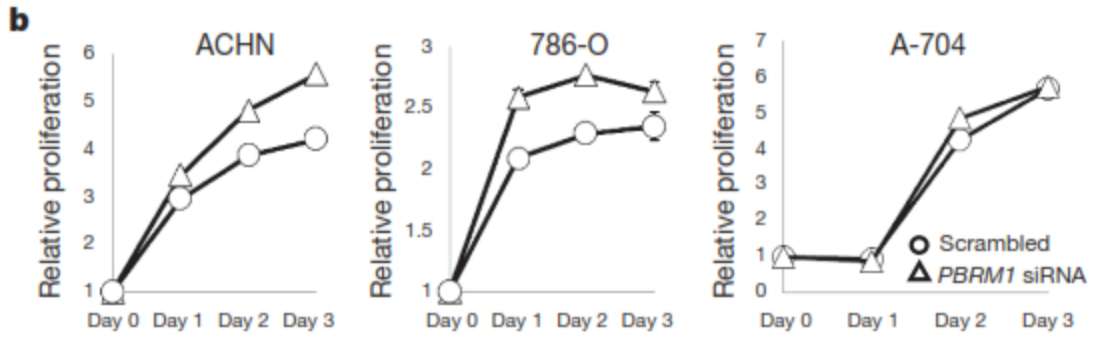
3p Loss ~90%

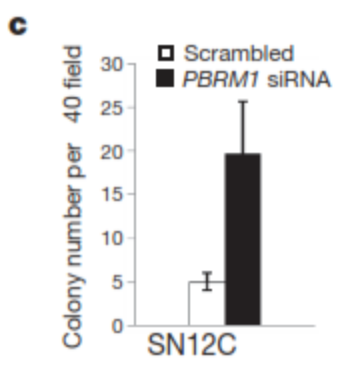


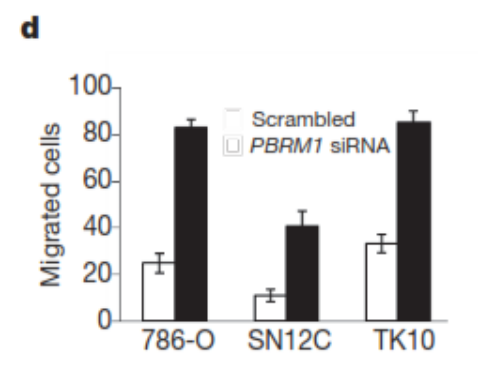
TCGA

PBRM1 Frequently Mutated in ccRCC









Varela et al., Nature 2011

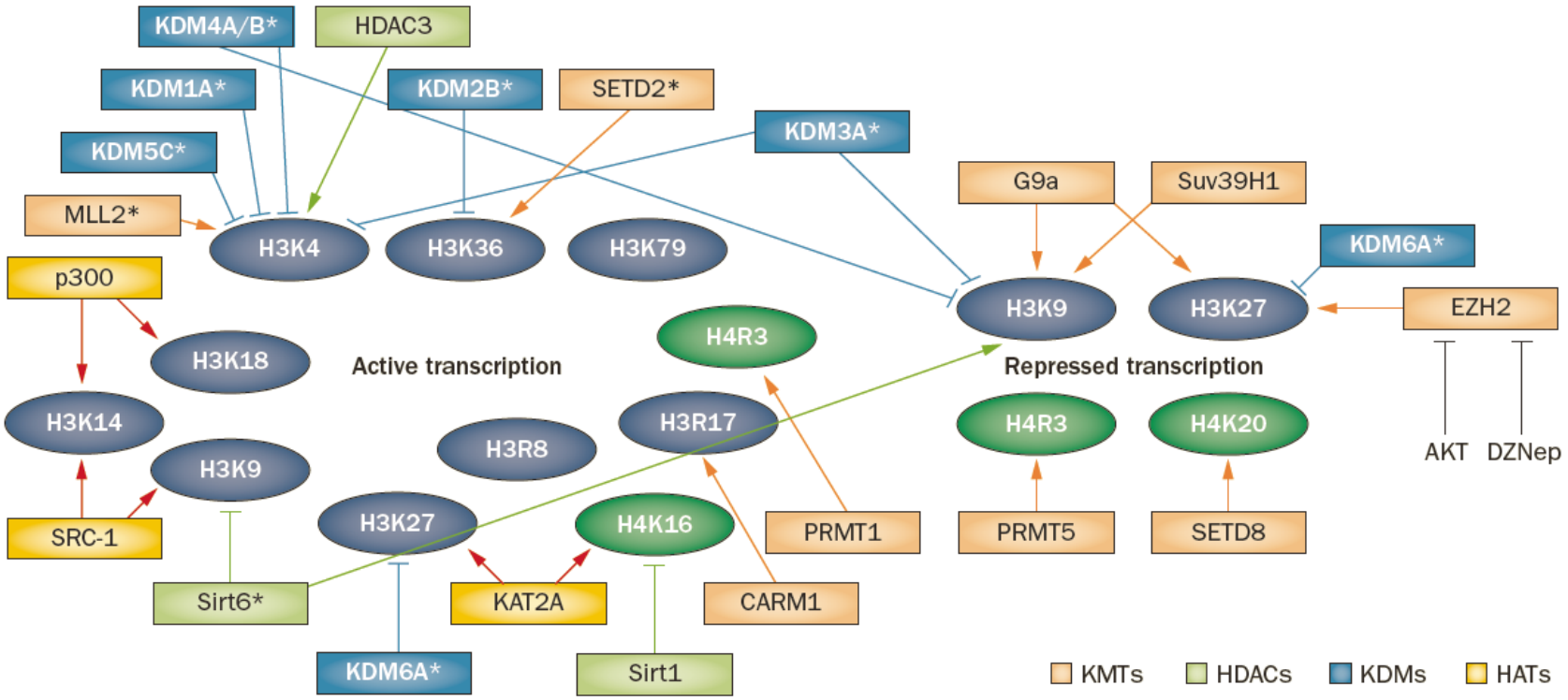
Genes	Non-silent somatic changes: Missense	Non-silent somatic changes: Nonsense, splice site or indel	Total non-silent mutations	Subjects harboring non-silent mutations	*P* value (passenger probability)
VHL	6	21	27	27	1.56×10^{-71}
PBRM1	2	19	21	20	2.83×10^{-31}
JARID1C	2	7	9	9	9.76×10^{-11}
BAP1[a]	2	7	9	8	1.45×10^{-15}
LRP1B	8	0	8	7	7.63×10^{-9}
TP53	3	3	6	6	3.34×10^{-11}
SYNE2[a]	5	1	6	6	1.07×10^{-4}
CSMD3	6	1	7	5	5.02×10^{-8}
AKAP13	5	0	5	5	3.80×10^{-5}
SPTBN4[a]	4	0	4	4	5.02×10^{-3}
SETD2	0	4	4	4	5.03×10^{-3}
RYR1[a]	4	0	4	4	2.29×10^{-2}
NAV3	4	0	4	4	1.90×10^{-4}
CARD11	5	0	5	4	1.18×10^{-5}
AHNAK[a]	7	2	9	4	9.29×10^{-9}
ZNF804A[a]	2	1	3	3	1.63×10^{-2}
TSC1[a]	0	3	3	3	1.34×10^{-2}
SHANK1[a]	2	1	3	3	9.20×10^{-3}
LRRK2[a]	3	0	3	3	4.28×10^{-4}
FMN2[a]	3	0	3	3	4.26×10^{-3}
FAM111B[a]	1	2	3	3	8.25×10^{-3}
CUL7	2	1	3	3	3.66×10^{-2}
ASB15[a]	1	2	3	3	1.27×10^{-4}

All non-silent mutations listed in this table were confirmed by genotyping or Sanger sequencing, with detailed information provided in **Supplementary Table 6.**

[a]Twelve genes previously not known to be mutated in ccRCC.

Guo et al Nat Genetics 2011

Histone Modifying/Chromatin Remodeling Genes



Nature Reviews Urology 2012

VHL Alteration as a Prognostic Marker

Table 3 | VHL alterations as potential prognostic markers

Reference	*n**	Methods used to assess mutation	Alteration‡/mutation/ hypermethylation/ LOH (%)	Associations with clinical and pathological variables
50	205	Endonuclease scanning and sequencing	91/82/8/NA	Overall prevalence of mutations not associated with clinical or pathological characteristics Late-stage metastatic lesions had more double mutations, nonsense mutations and mutations located in exon 3 than Mx or M0 lesions Nonsense mutations associated with Furhman nuclear grade and lymph-node positivity
46	62	PCR amplification and sequencing	27/27/NA/NA	Presence of mutations not associated with clinical or pathological variables
44	97	DHPLC and sequencing	90/71/20/78	LOH significantly less common in grade 4 tumors No association with other clinical and pathological variables or survival
45	151	SSCP and sequencing	45/38/7/93	*VHL* mutation or hypermethylation significantly associated with pT3 tumor stage
52	113	PCR amplification and sequencing	34/34/NA/NA	Loss-of-function mutations§ associated with significantly worse prognosis in univariate analysis Presence or absence of mutations not associated with clinical or pathological features
56	202	SSCP and direct sequencing	58/51/5/NA	In patients with stage I–III tumors who underwent radical nephrectomy, *VHL* alteration associated with improved cancer-free survival and cancer-specific survival independently of other outcome predictors *VHL* alterations not associated with survival for patients with stage IV disease Mutational subtype not associated with survival
53	185	SSCP and direct sequencing	57/52‖/11/NA	Mutations not associated with clinical or pathological features or survival
47	56	SSCP and automated sequencing	29/20/14/NA	Pro582Ser detected in six patients and correlated with M1 disease *VHL* alterations not associated with PFS or OS Patients with loss-of-function mutations showed decreased PFS and OS
48	202	SSCP and sequencing	57/51/5/90	*VHL* alterations not associated with clinical or pathological features Mutations less common in younger than older patients
55	187	SSCP and sequencing	61/61/NA/NA	Mutations not associated with clinical or pathological features
54	49	SSCP and sequencing	53/53/NA/NA	Mutations not associated with clinical or pathological features
49	102	SSCP and sequencing	54/54/NA/NA	Multiple mutations more common in grade 3 tumors than in grade 1 or 2 lesions
51	100	PCR amplification and sequencing	58/58/NA/NA	*VHL* mutations associated with low T stage, absence of distant metastases and prolonged PFS in univariate analysis

*Only included clear-cell renal-cell carcinoma cases and excluded other histological subtypes. ‡Alteration defined as mutation and/or methylation. §Loss-of-function mutations defined as 'mutations predicted to alter the open reading frame of *VHL*'. ‖Data only presented for loss-of-function mutations defined as 'a mutation that causes a change in the protein versus silent or no mutations'. Abbreviations: DHPLC, denaturing high pressure liquid chromatography; LOH, loss of heterozygosity; *n*, number of patients; NA, not assessed; M0, no distant metastases; Mx, distant metastasis could not be assessed; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; SSCP, single-strand conformational polymorphism; VHL, von Hippel–Lindau.

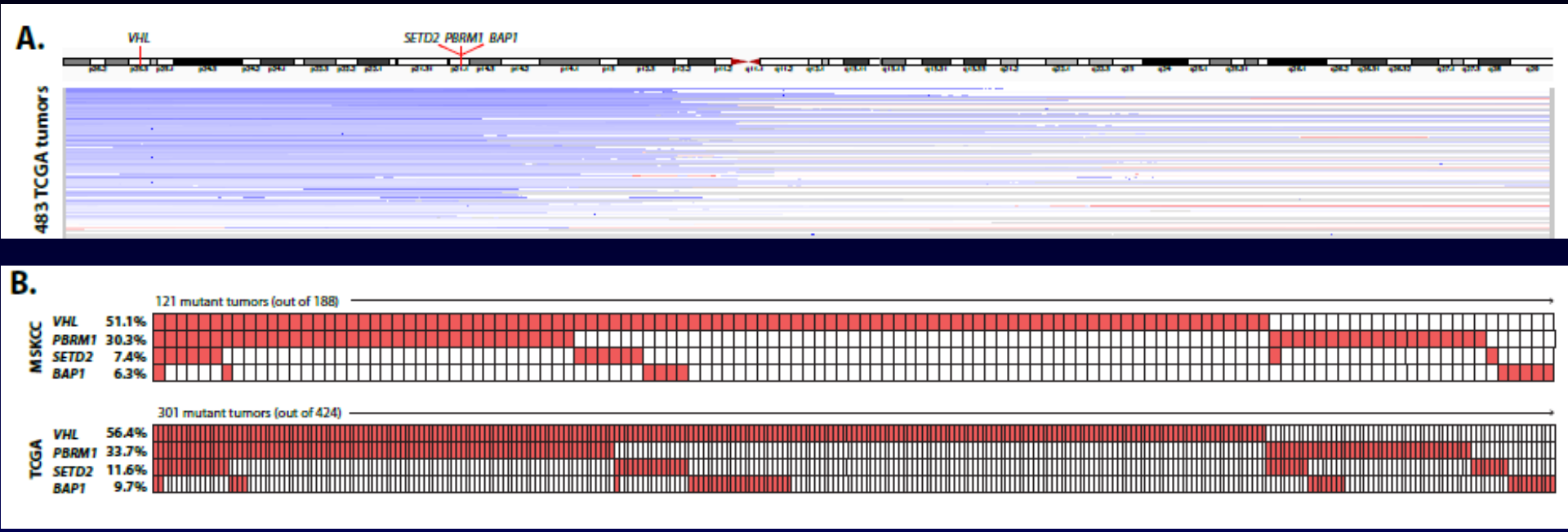
Gossage et al Nat Rev Clin Onc 2010

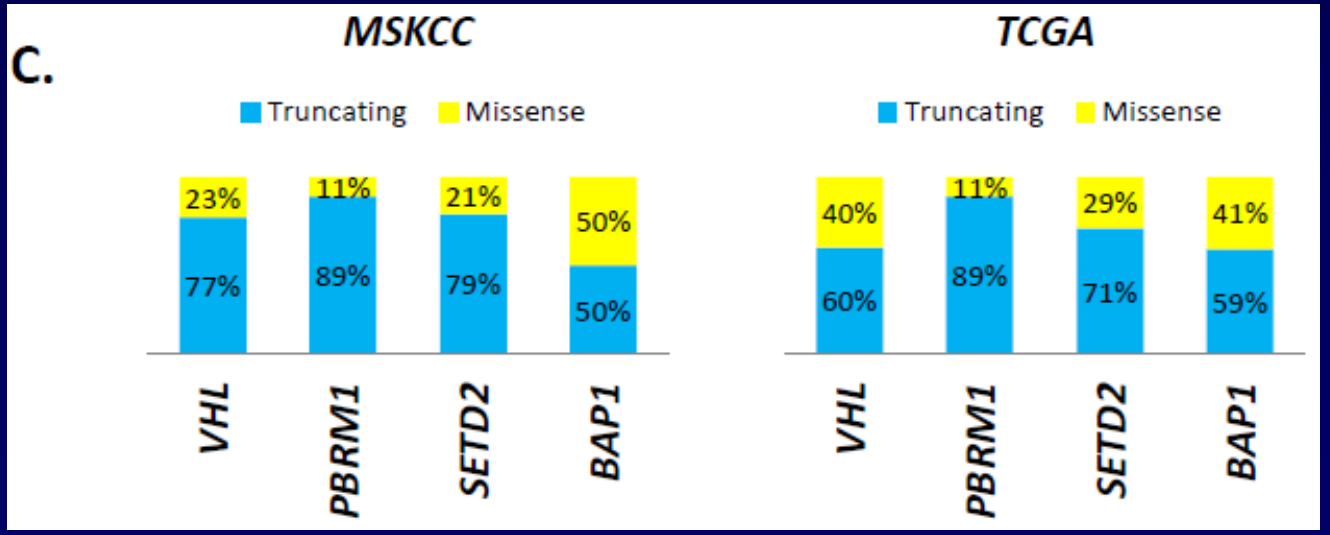
Methods

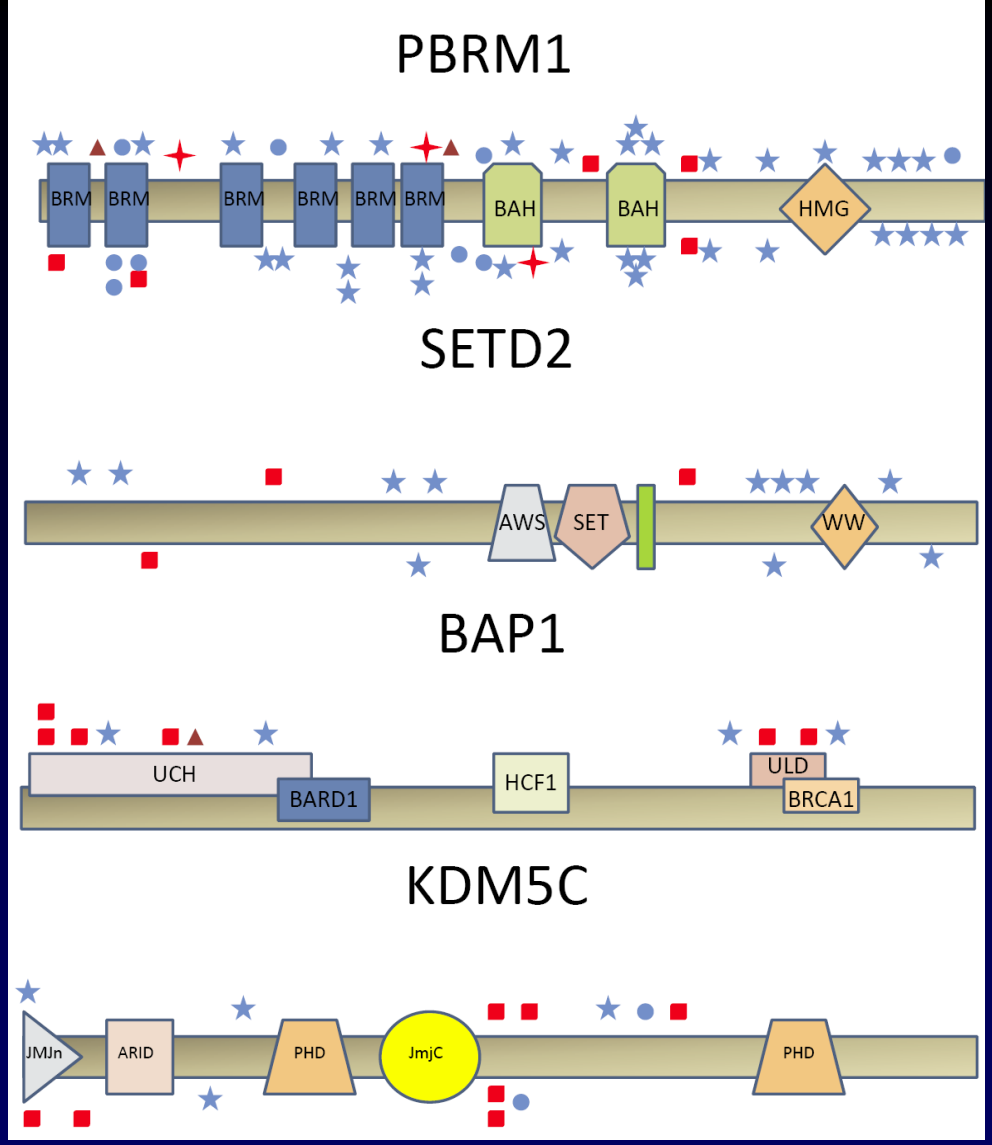
- DNA sequencing on 188 ccRCC (MSKCC - Discovery)
- Pathologic Correlations
  - Size, Grade, Stage
- Clinical Correlations
  - Survival, time to recurrence
- Validation of key findings on TCGA data (n=424)

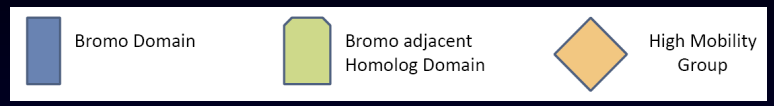
Patient and Tumor Features

Cohort	Discovery –MSK (n=188)	Validation -TCGA (n=424)
Median age (quartiles)	61 (54,69)	61 (52,70)
Gender	Male – 70% Female – 30%	Male – 65% Female – 35%
Race	White – 90% Black – 4% Other – 6%	White – 94% Black – 3% Other – 3%
Median tumor Size – cm (quartiles)	5.1 (3.4,8.5)	5.5 (4.0,8.5)
AJCC Stage I II III IV	 37.8% 5.9% 43.6% 12.8%	 47.2% 9.7% 26.4% 16.7%
Fuhrman Nuclear Grade G1 G2 G3 G4	 1% 40% 47% 12%	 2% 41% 41% 16%
Median Followup for Survivors (months)	35	46
Overall 5 Year Survival	84.3%	60.8%
Number of deaths	21	144
Number of deaths from RCC	13	103

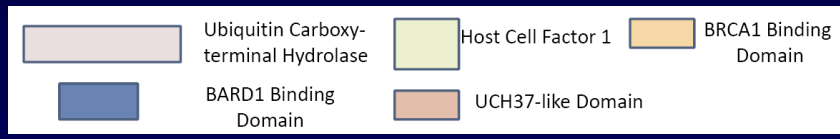

A.
VHL
SETD2 PBRM1 BAP1
483 TCGA tumors
B.
121 mutant tumors (out of 188)
MSKCC
VHL 51.1%
PBRM1 30.3%
SETD2 7.4%
BAP1 6.3%
301 mutant tumors (out of 424)
TCGA
VHL 56.4%
PBRM1 33.7%
SETD2 11.6%
BAP1 9.7%


C.
MSKCC
Truncating
Missense
23%
77%
11%
89%
21%
79%
50%
50%
VHL
PBRM1
SETD2
BAP1
TCGA
Truncating
Missense
40%
60%
11%
89%
29%
71%
41%
59%
VHL
PBRM1
SETD2
BAP1

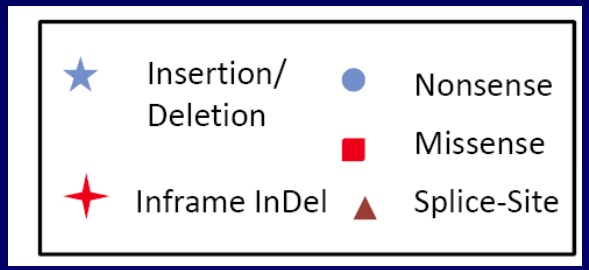

PBRM1
BRM
BRM
BRM
BRM
BRM
BRM
BAH
BAH
HMG
SETD2
AWS
SET
WW
BAP1
UCH
BARD1
HCF1
ULD
BRCA1
KDM5C
JMJn
ARID
PHD
JmjC
PHD


Bromo Domain
Bromo adjacent Homolog Domain
High Mobility Group


Associated with SET Domain
SET Domain
PostSET Domain
WW Domain


Ubiquitin Carboxy-terminal Hydrolase
Host Cell Factor 1
BRCA1 Binding Domain
BARD1 Binding Domain
UCH37-like Domain


Jumonji Protein
ARID/BRIGHT DNA binding Domain
PHD Zinc Finger
JmjC Domain


Insertion/ Deletion
Nonsense
Missense
Inframe InDel
Splice-Site

Tumor Associations

		Size >5cm	Higher T Stage	Higher Nuclear Grade	Necrosis (>5%)	LN mets (N1)	Presence of Mets on Presentation (M1)
MSKCC							
P value HR(CI)	VHL	1.000	0.107	0.458	0.748	1.000	0.825
	PBRM1	0.635	**0.011** 2.34 (1.23,4.58)	0.523	0.494	0.435	0.153
	SETD2	1.000	0.580	1.000	0.548	0.424	0.683
	BAP1	0.143	**0.041** 4.57 (1.16,30.26)	**0.029** 8.17 (1.54,150.94)	0.492	0.375	1.000
TCGA							
P value HR(CI)	VHL	0.055	0.231	0.843	0.084	0.573	0.353
	PBRM1	0.750	0.753	1.000	0.732	0.769	0.677
	SETD2	0.059	0.120	0.761	0.403	1.000	0.102
	BAP1	**0.002** 3.79 (1.67,10.23)	**0.004** 2.59 (1.35,5.11)	**0.019** 2.42 (1.19,5.34)	0.072	0.363	**0.012** 2.71 (1.29, 5.46)

nature genetics

BAP1 loss defines a new class of renal cell carcinoma

Samuel Peña-Llopis[1–3], Silvia Vega-Rubín-de-Celis[1–3], Arnold Liao[4], Nan Leng[4], Andrea Pavía-Jiménez[1–3], Shanshan Wang[1–3], Toshinari Yamasaki[1–3], Leah Zhrebker[1–3], Sharanya Sivanand[1–3], Patrick Spence[1–3], Lisa Kinch[5], Tina Hambuch[4], Suneer Jain[4], Yair Lotan[6], Vitaly Margulis[6], Arthur I Sagalowsky[6], Pia Banerji Summerour[3,7], Wareef Kabbani[8], S W Wendy Wong[9], Nick Grishin[5], Marc Laurent[4], Xian-Jin Xie[3], Christian D Haudenschild[4], Mark T Ross[9], David R Bentley[9], Payal Kapur[8] & James Brugarolas[1–3]

The molecular pathogenesis of renal cell carcinoma (RCC) is poorly understood. Whole-genome and exome sequencing followed by innovative tumorgraft analyses (to accurately determine mutant allele ratios) identified several putative two-hit tumor suppressor genes, including *BAP1*. The BAP1 protein, a nuclear deubiquitinase, is inactivated in 15% of clear cell RCCs. BAP1 cofractionates with and binds to HCF-1 in tumorgrafts. Mutations disrupting the HCF-1 binding motif impair BAP1-mediated suppression of cell proliferation but not deubiquitination of monoubiquitinated histone 2A lysine 119 (H2AK119ub1). BAP1 loss sensitizes RCC cells *in vitro* to genotoxic stress. Notably, mutations in *BAP1* and *PBRM1* anticorrelate in tumors ($P = 9 \times 10^{-6}$), and combined loss of BAP1 and PBRM1 in a few RCCs was associated with rhabdoid features ($q = 0.0007$). BAP1 and PBRM1 regulate seemingly different gene expression programs, and BAP1 loss was associated with high tumor grade ($q = 0.0005$). Our results establish the foundation for an integrated pathological and molecular genetic classification of RCC, paving the way for subtype-specific treatments exploiting genetic vulnerabilities.

BAP1 loss is associated with high tumor grade

Deep-sequencing studies were largely focused on high-grade tumors. An analysis of all 176 tumors examined showed that BAP1 loss correlated with high Fuhrman nuclear grade ($q = 0.0005$) (**Supplementary Data 1**). Because nuclear grade is associated with mTORC1 activation[15], we tested whether a correlation existed between BAP1 loss and mTORC1 activity. As determined by the phosphorylation of both S6 and 4E-BP1, BAP1 loss was correlated with mTORC1 activation

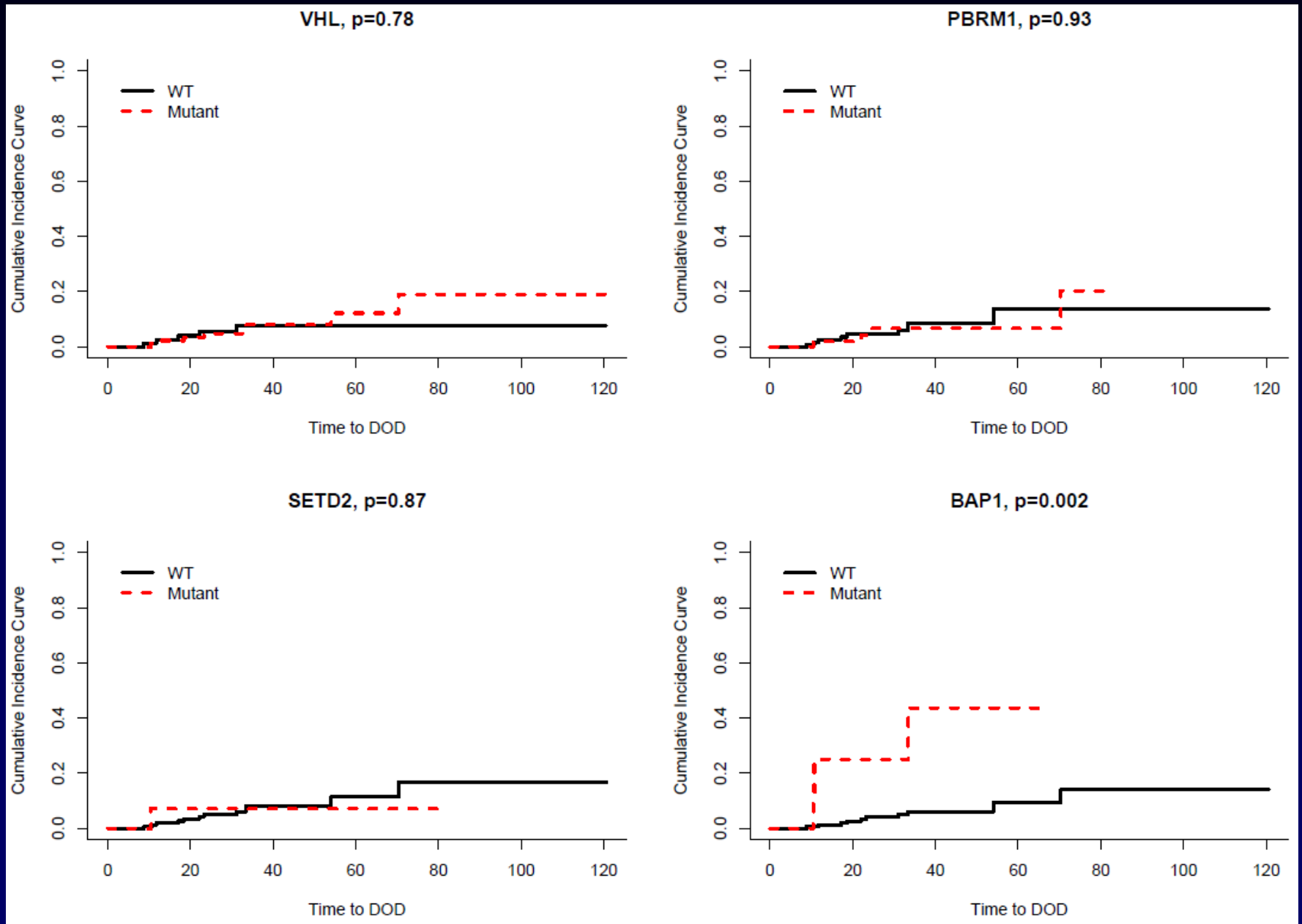
Higher Stage (pT1a to pT3a) in Tumors ≤ 4cm (Discovery)

Mutation	VHL	PBRM1	BAP1	SETD2	KDM5C	BAP1 or SETD2 or KDM5C	PBRM1 or BAP1 or SETD2 or KDM5C2
Overall Frequency (n=185)	0.49	0.29	0.06	0.08	0.08	0.19	0.42
Frequency in tumors <4cm (n=69)	0.49	0.29	0.03	0.09	0.06	0.16	0.39
T1a (n=48)	21 (44%)	8 (17%)	1 (2%)	3 (6%)	1 (2%)	4 (8%)	11 (23%)
T3a (n=21)	13 (62%)	12 (57%)	1 (5%)	3 (15%)	3 (15%)	7 (33%)	16 (76%)
Odds Ratio (CI)	2.1 (0.7 -6.9)	6.4 (1.8 - 24.7)	2.3 (0.03 - 188)	2.5 (0.3 - 20.2)	7.6 (0.6 - 418.6)	5.3 (1.2 - 28.8)	10.3 (2.83- 44.7)
	p=0.20	p=0.001	p=0.52	p=0.36	p=0.08	p=0.03	p<0.001

*Statistically significant findings are bolded (Fischer's exact)

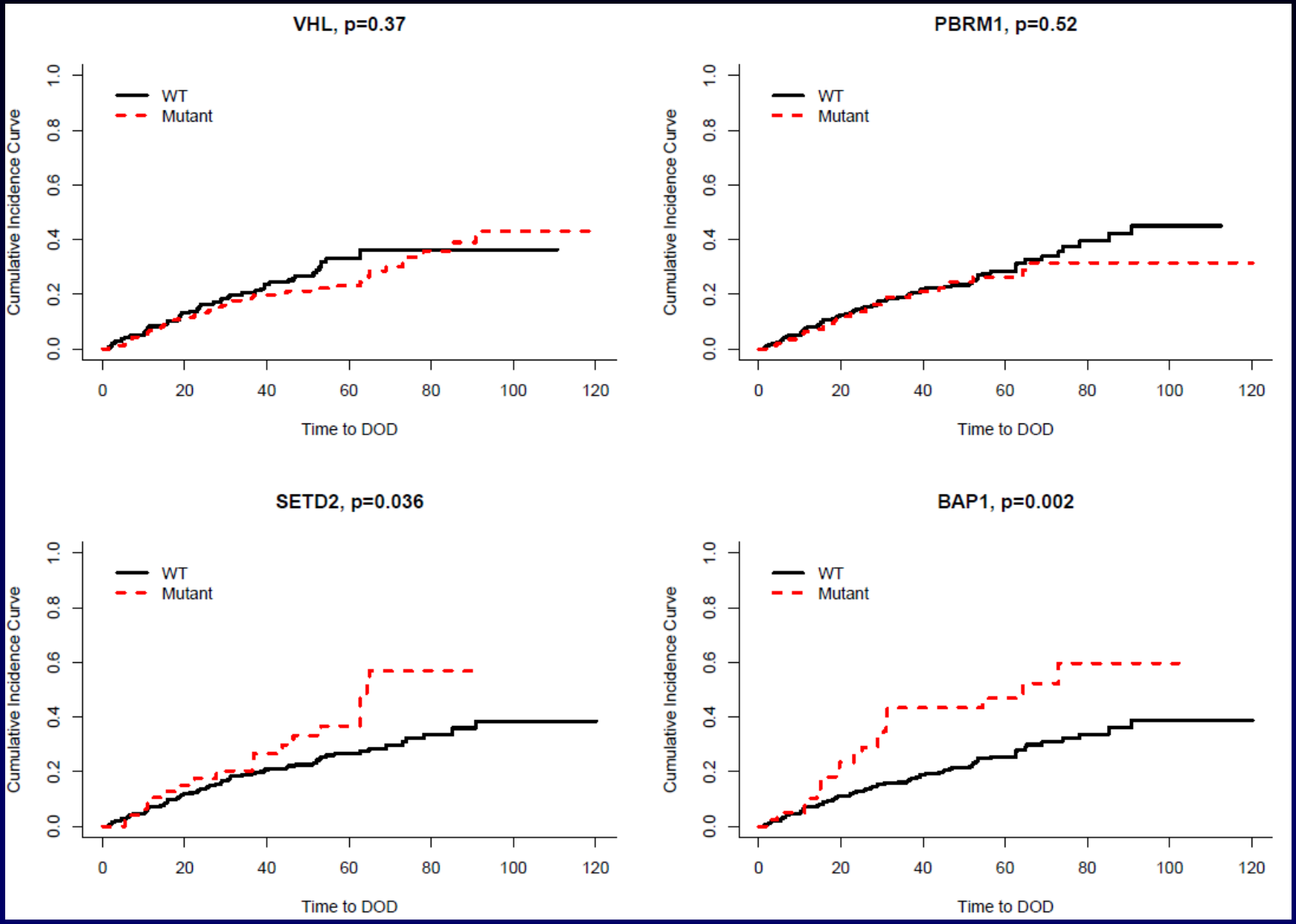
MSKCC - CSS

Figure 2

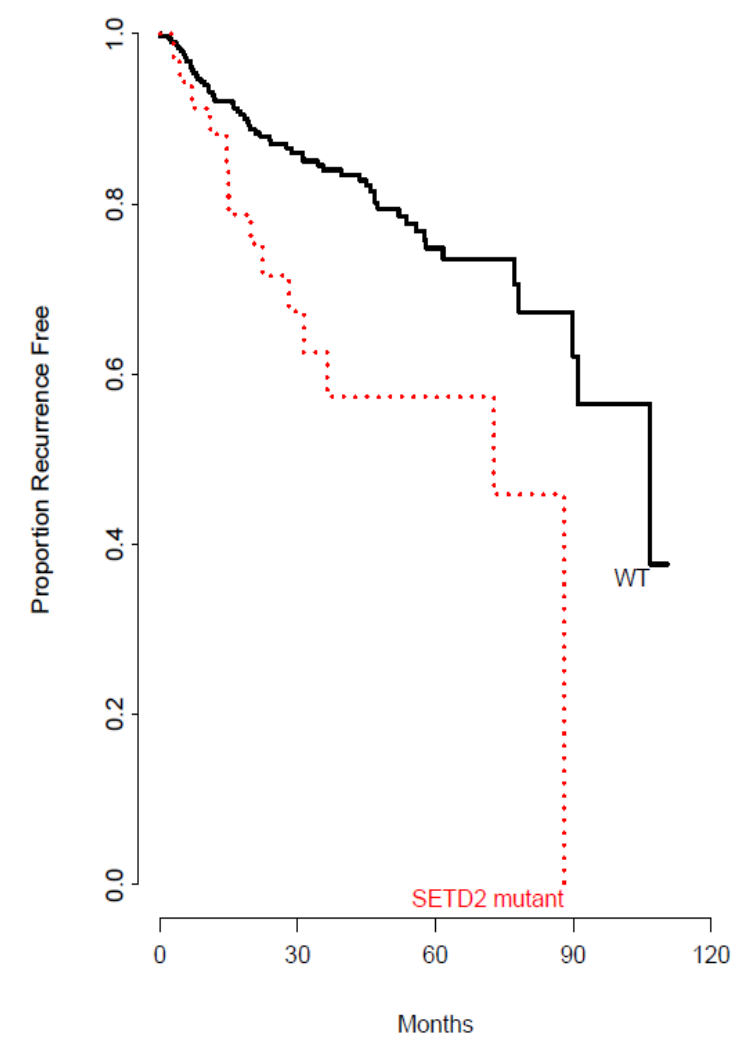


TCGA - CSS

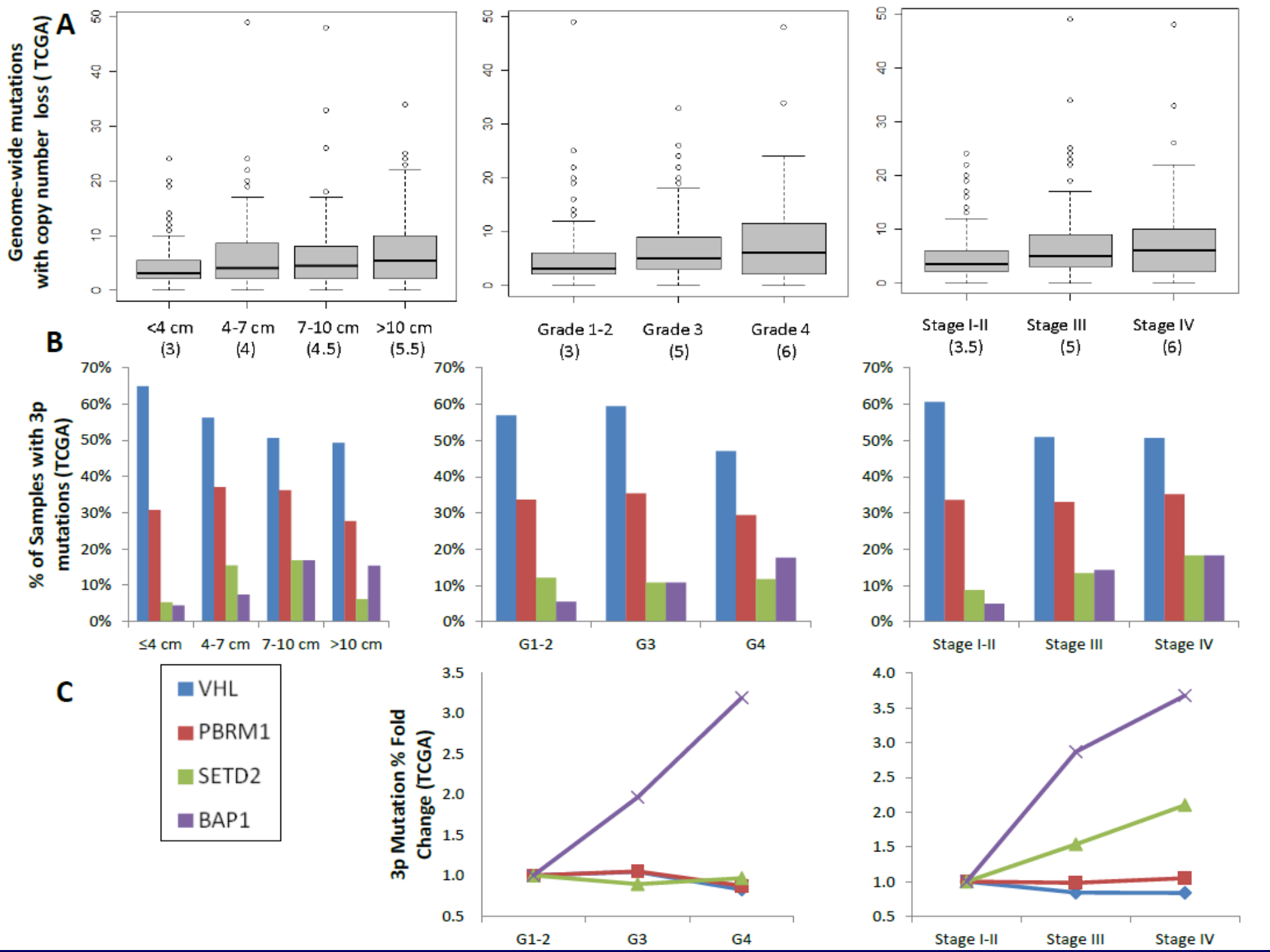
Figure 3



SETD2 – Time to Recurrence (TCGA)



p= 0.002


A
Genome-wide mutations with copy number loss (TCGA)
0
10
20
30
40
50
<4 cm (3)
4-7 cm (4)
7-10 cm (4.5)
>10 cm (5.5)
Grade 1-2 (3)
Grade 3 (5)
Grade 4 (6)
Stage I-II (3.5)
Stage III (5)
Stage IV (6)
B
% of Samples with 3p mutations (TCGA)
0%
10%
20%
30%
40%
50%
60%
70%
≤4 cm
4-7 cm
7-10 cm
>10 cm
G1-2
G3
G4
Stage I-II
Stage III
Stage IV
C
VHL
PBRM1
SETD2
BAP1
3p Mutation % Fold Change (TCGA)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
G1-2
G3
G4
Stage I-II
Stage III
Stage IV

Conclusions

- Confirms the frequency of these novel mutations in ccRCC
- Suggests they are associated with advanced stage, grade and tumor invasiveness and higher pathologic stage for smaller tumors
- BAP1 and SETD2 are associated with worse CSS
- Further studies with longer followup will be necessary to assess the clinical impact of these and possibly additional mutations.

MSKCC TKCRP

Kidney TCGA

Proteomics
Prometheus

Metabolomics
Metabolon

Cell Signaling

Cores
Genomics
Beene

Surgery
Paul Russo
Jonathan Coleman
Karim Toujier
Ari Hakimi

Computational Biology
Chris Sander
Niki Schultz
Anders Jacobson
Boris Reva
Giovanni Ciriello
William Lee

Interventional Radiology
Stephen Solomon
Jeremy Durack

Radiology
Oguz Akin
Steve Larson
Chris Karlo

HOPP
James Hsieh
Ari Hakimi
Can Pham
Y. Grigoryev
Yiyu Dong
Luis Cunha
Joe Lee
Jianing Xu
Zhenghong Dong
Smrutiben Mehta
Nina Mikkilineni

Emily Cheng
Yogesh Ganesan

Biostatistics
Mithat Gonen
Irina Ostrovnaya
Sujata Patil
H Barnes-Furberg
Vanket Sheshan
Colin Begg

Medicine
Robert Motzer
James Hsieh
Martin Voss
Ana Molina
Darren Feldman
Diogo Bastos
David Spriggs

Pathology
Victor Reuter
Yingbei Chen
Satish Tickoo
Michael Berger
Rose Brennan

Joan Massague

Scott Lowe

Robert Klein

Ken Offit

Neal Rosen

Craig Thompson