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Whole exome sequencing identifies somatic *ATRX* mutations in pheochromocytomas and paragangliomas

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Author Contributions

Conceived and designed study: LF and KLN. Performed experiments: LF, SK, KD, KM, VL.
Performed bioinformatics: BW, DD and RD. Analyzed data: LF, SK, BW, DD, KD, SM, NC, RG, TE, KM, VL, DF, RD, DLC and KLN. All authors wrote and contributed to the final manuscript.

Competing Financial Interest

The authors report no conflicts of interest.

Accession Codes

Sequence data for the PCC/PGLs have been deposited in GenBank/EMBL/DDBJ SRA under the accession code SRP050479.

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Abstract

Pheochromocytomas and paragangliomas (PCC/PGL) are the solid tumor type most commonly associated with an inherited susceptibility syndrome. However, very little is known about the somatic genetic changes leading to tumorigenesis or malignant transformation. Here we perform whole exome sequencing on a discovery set of 21 PCC/PGL and identify somatic *ATRX* mutations in two *SDHB*-associated tumors. Targeted sequencing of a separate validation set of 103 PCC/PGL identifies somatic *ATRX* mutations in 12.6% of PCC/PGL. PCC/PGLs with somatic *ATRX* mutations are associated with alternative lengthening of telomeres and clinically aggressive behavior. This finding suggests that loss of ATRX, a SWI/SNF chromatin remodeling protein, is important in the development of clinically aggressive pheochromocytomas and paragangliomas.

Introduction

Pheochromocytomas and paragangliomas (PCC/PGL) are tumors of the autonomic nervous system arising from the adrenal medulla and extra-adrenal ganglia, respectively. PCC/PGLs are more commonly associated with inherited susceptibility gene mutations than any other solid tumor. In fact, up to 40% of PCC/PGL have a germline mutation in one of twelve genes which are known to be associated with an increased risk of PCC/PGL, including *VHL*, *RET*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127*, *MAX*, *EPAS1* and *FH*.[1] However, very little is known about the somatic genetic or genomic changes leading to tumorigenesis or malignant transformation. Most studies have identified somatic mutation rates less than 5% for candidate genes tested in PCC/PGL.[2–6] Somatic *HRAS* mutations have been identified in 10% of sporadic PCC/PGL without a known inherited mutation.[7] Somatic mutations in known susceptibility genes such as *NF1*, *VHL* and *RET* are seen at rates between 5–40% depending on the gene and the study, and these somatic mutations are seen almost exclusively in sporadic PCC/PGL as well.[8–11] Somatic mutations in the *SDHx* genes are rarely, if ever, observed.[9, 12–14]

Most patients with PCC/PGL have clinically benign disease but a fraction have clinically aggressive tumors. Patients with benign PCC/PGL usually have a single tumor, but some have more aggressive disease and develop multiple primary tumors throughout their lifetime. Approximately one-fourth of patients with PCC/PGL have malignant disease defined by the presence of distant metastases,[15] and when metastatic, PCC/PGLs are associated with a 50% five year survival rate.[16] Another group of patients have PCC/PGL with extensive local invasion or recurrence at the primary site, and while this group does not meet criteria of malignancy due to the absence of distant metastases, they share a similar burden of excessive catecholamine secretion which is not easily surgically cured. Systemic treatments used for both malignant and clinical aggressive tumors have limited efficacy.[17] No reliable predictors of aggressive disease exist other than a germline mutation in the *Succinate dehydrogenase (SDH) B* gene, which confers a higher risk of malignancy (31–75%) than mutations in other susceptibility genes (usually <5%).[18] Only half of patients with metastatic disease carry inherited *SDHB* mutations,[19] meaning many of the patients who develop malignant PCC/PGL had no known predictors of malignant potential. Thus, it is critically important to both identify molecular predictors of malignancy and new targets for therapeutic intervention.

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Here we aim to identify driver mutations for clinically aggressive PCC/PGL by performing whole exome sequencing on a discovery set of twenty-one fresh frozen tumor/matched germline DNA samples. We identify two *SDHB*-associated tumors with somatic *ATRX* mutations. Using a separate validation set of DNA from 103 formalin fixed paraffin embedded PCC/PGLs, we find somatic *ATRX* mutations in 12.6% of tumors. We are the first to report *ATRX* mutations in PCC/PGL and suggest that ATRX loss is important for tumorigenesis in a subset of PCC/PGL.

Results

Low number of somatic mutations in PCC/PGL

We performed whole exome sequencing (WES) on a discovery set of twenty-one fresh frozen tumor/matched germline DNA samples. Using Agilent SureSelect All Exon v3 technology for exome capture, the mean depth of coverage was 84x in tumor DNA and 85x in germline DNA. To identify somatic mutations, we removed any variant seen in the matched germline sample and followed a strict filtering algorithm for variant calling including removing variants with MAF>0.1% in 1000 Genomes and/or ESP6500, with read depth <20 or segmental duplication >80%. After removing synonymous variants, there was a mean of 17.38±6.27 somatic mutations per tumor (Supplementary Table 1). This relatively low number of mutations in PCC/PGL is consistent with that seen in related tumor types, such as neuroblastomas (~12–18/tumor) and pancreatic neuroendocrine tumors (PNET; ~16/tumor).[20, 21]

We examined for somatic mutations in the known susceptibility genes. Consistent with the literature, we found that three of seven sporadic tumors had somatic mutations in *NF1*. No somatic mutations were seen in other known common or rare susceptibility genes (*SDHx*, *TMEM127*, *RET*, *VHL*, *MAX*, *FH*, *EPAS1*, *KIF1B*, *EGLN1* or *MEN1*) in any tumors in the discovery set.

Interestingly, two of seven *SDHB*-associated tumors had deleterious variants in *ATRX* (PP119F1 p.W2275X and PP098F2 p.R2197H in the conserved helicase domain at an amino acid residue also reported to be altered in neuroblastoma[21]) (Supplementary Table 2). ATRX protein was absent in the tumor cells by immunohistochemistry (Fig. 1).

Somatic ATRX mutations are seen in PCC/PGL

To determine the frequency of somatic *ATRX* mutations in PCC/PGL, we sequenced the *ATRX* coding region in two validation sets of formalin fixed paraffin embedded PCC/PGL using amplicon sequencing to a mean depth of coverage of 1430x. Validation set 1 contains PCC/PGL from 52 patients with known inherited mutation status; validation set 2 contains tumors from 51 patients without clinical genetic testing (Supplementary Tables 3 and 4). We followed a strict filtering algorithm for variant calling including removing variants with MAF>0.1% in 1000 Genomes and/or ESP6500, with read depth <50 or segmental duplication >80%. *ATRX* variants were called mutations if the variant was a stop gain variant, insertion or deletion, or missense variant located within a known functional domain and called deleterious in three *in silico* prediction callers (SIFT[22], POLYPHEN2[23] and

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MutationTaster[24]). In validation sets 1 and 2, we found 11.5% of tumors (6/52) and 13.7% of tumors (7/51), respectively, had somatic mutations in *ATRX* (Table 1). The overall somatic mutation rate was 12.6%. Two tumors had two nonsynonymous alterations which passed the filtering algorithm as described in the methods section. Both tumors are from male patients, and because *ATRX* is on the X chromosome, this finding implies the mutations are *in cis*. In both tumors, the two mutations are both within the helicase domain of *ATRX*, and we hypothesize that the combination of the nonsynonymous variants disrupts the function of this domain. In validation set 1 with clinical annotation, five of six tumors with somatic *ATRX* variants were clinically aggressive (Table 1 and Supplementary Table 3). Tumors without mutations were tested for intragenic deletions by MLPA and none were found . These data confirm that *ATRX* is a frequently somatically altered gene in PCC/PGL.

ATRX heterodimerizes with a protein partner DAXX as part of the chromatin remodeling complex. Mutations in both genes are seen in pancreatic neuroendocrine tumors; however, rare to no *DAXX* mutations are reported in neuroblastomas and gliomas.[20, 21, 25–27] We sequenced *DAXX* in PCC/PGL to determine if somatic mutations were present. No somatic *DAXX* mutations were observed in our WES of the discovery set of 21 PCC/PGL or in an independent validation set of 58 PCC/PGL by Sanger sequencing.

ALT is seen in a subset of PCC/PGL

The majority of cancers with somatic *ATRX* mutations are associated with alternative lengthening of telomeres (ALT) as a mechanism for preventing senescence.[28, 29] To determine if PCC/PGL with somatic *ATRX* variants showed ALT, we performed telomere FISH on 22 FFPE PCC/PGL, six of which had somatic *ATRX* mutations (Fig. 1; Supplementary Tables 2–4). Four of the six tumors with somatic *ATRX* mutations showed ALT, and all four also had inherited *SDHB* mutations. Two tumors with somatic *ATRX* variants did not demonstrate ALT by this assay, suggesting the somatic mutation disrupts an alternative function of ATRX. In total, 27% of PCC/PGL (six of the 22, four with *ATRX* mutations and two without) showed ALT and all were from tumors with clinically aggressive features. Two tumors with ALT had no identified somatic *ATRX* mutation, indicating additional genomic defects may account for elongated telomeres in some tumors.

Discussion

We found that 12.6% of PCC/PGL examined had somatic *ATRX* mutations, of which 30% have truncating mutations and 69% have missense mutations classified as deleterious based on strict criteria of being located in a known functional domain and deleterious in three *in silico* prediction callers. *ATRX* mutations are known to play a role in human disease. Germline mutations lead to X-linked alpha thalassemia mental retardation syndrome; somatic mutations are implicated as drivers in several non-epithelial cancer types including pancreatic neuroendocrine tumors (PNETs), neuroblastomas and gliomas.[20, 21, 25, 26, 30] *ATRX* is a large gene of ~300kb on the X chromosome and a member of the SWI/SNF family of chromatin remodelers, which play a role in telomere maintenance and chromosome integrity.[31] ATRX has several binding partners involved in epigenetic

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regulation including DAXX, which acts as a chaperone to bring histone H3.3 to telomeric and pericentric regions.[32, 33]

In non-functional PNETs somatic *ATRX* and *DAXX* mutations are common (43%) and mutually exclusive with 25% having *DAXX* and 17.6% having *ATRX* mutations.[20] We identified no somatic *DAXX* mutations in PCC/PGLs. However, not all neuroendocrine related tumors carry *DAXX* mutations. Neuroblastomas, which have somatic *ATRX* mutations or intragenic deletions in 9–33% of tumors, have not been demonstrated to have any *DAXX* mutations.[21, 25] Furthermore, gliomas have somatic *ATRX* mutations in 30–58% of tumors, yet *DAXX* mutations are rarely, if ever, observed.[26, 27]

Preventing senescence is a hallmark of cancer cells, and most cancers have increased expression of telomerase to maintain the telomeres. However, at least 10–15% of cancers have an alternative mechanism for lengthening of telomeres (ALT) thought to involve homologous recombination. We performed telomere FISH assay for ALT on six PCC/PGLs with somatic *ATRX* mutations and on 16 PCC/PGLs without somatic *ATRX* mutations. Four of the six (67%) samples with somatic *ATRX* mutations showed ALT and only two of sixteen (12.5%) samples without *ATRX* mutations showed ALT. Although most human cancers and cell lines with *ATRX* mutations have associated ALT, not all of them have demonstrated ALT as reported in multiple published studies,[27, 29, 34] suggesting that some mutations may disrupt alternative functions of ATRX. Currently, ATRX function is not completely understood and cellular roles beyond ALT have been described. ATRX promotes alpha globin gene expression by reducing macroH2A deposition at the alpha globin locus,[35] and promotes transcriptional repression of TERRA, the telomere RNA transcript.[36] Thus, it is possible that some *ATRX* mutations disrupt a function of the ATRX protein other than ALT.

Other epigenetic alterations are known to exist in PCC/PGL. Studies examining the methylome suggest that PCC/PGL from patients with germline *SDHx* and *VHL* mutations are hypermethylated compared to tumors with *NF1* or *RET* mutations, and the highest rates of methylation are in *SDHB*-associated tumors.[37, 38] Mutations in *SDHx* genes lead to elevated levels of succinate, postulated to cause hypermethylation by inhibiting 2-oxoglutarate-dependent histone and DNA demethylase enzymes.[38, 39] Perhaps further epigenetic dysregulation in these tumors is occurring through additional somatic mutations in genes such as *ATRX*, as seen in gliomas with co-occurrence of *ATRX* mutations and *IDH* mutations leading to increased alpha-ketoglutarate, postulated to cause hypermethylation through the same mechanism as succinate.[26]

In PNETs, initial analysis found that tumors with somatic *ATRX* (or heterodimerizing partner *DAXX*) mutations were associated with better prognosis.[20] However, a recent study, analyzing two larger cohorts found that tumors with somatic *ATRX* (or *DAXX*) mutations (implied by negative immunohistochemical staining) were associated with worse prognosis than tumors without a mutation.[40] Our sample set of PCC/PGL with *ATRX* mutations is too small to draw definitive conclusions regarding prognosis, especially given the long latency in this disease. Nevertheless, it is interesting to note that of the tumors with known clinical

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genetic testing, most tumors with somatic *ATRX* mutations also have inherited *SDHx* mutations.

This study is the first to our knowledge to use whole exome sequencing in matched tumor and germline pairs to identify somatic alterations in PCC/PGL. We identified somatic *ATRX* mutations in 12.6% of PCC/PGL. Although our sample set of PCC/PGL with *ATRX* variants is too small to identify statistically significant associations, many had clinically aggressive features, inherited *SDHx* mutations and ALT, suggesting an interaction between the somatic and inherited genomes in solid cancers, which needs to be investigated further. Future work needs to be done to clarify the role of *ATRX* mutations on epigenetic regulation in PCC/PGL and to identify other somatic alterations involved in PCC/PGL tumorigenesis and malignant transformation.

Methods

Samples

This study was performed under an IRB approved protocol at the University of Pennsylvania with patients providing signed informed consent. A discovery set of twenty-one PCC/PGLs were selected to represent clinically benign and clinically aggressive tumors. The clinical data from all subjects is described in Supplemental Table 1. Fresh frozen PCC/PGLs were sectioned and stained with H&E to ensure sections of over 70% tumor are used for DNA extraction. DNA was extracted from tumor tissue using Qiagen AllPrep kits. Germline DNA from blood or saliva was extracted using standard protocols in the laboratory. All DNA was quantitated with a Qubit. To ensure high quality genomic DNA, the A260/280 ratio was measured on a Nanodrop and DNA was run on a 1% agarose gel.

Two separate validation sets of DNA from formalin fixed paraffin embedded (FFPE) PCC/PGL were used. The tumor samples in the validation set were collected from subjects with PCC/PGL who had surgery at the University of Pennsylvania or the University of Michigan. The University of Michigan samples were from the Cancer Genetics Registry (CGN, HUM00043430) and Michigan Endocrine Oncology Repository (MEOR, HUM00024461). Validation set 1 includes samples from 52 patients with known clinical germline testing results. Validation set 2 was derived from 51 patients who did not have clinical genetic testing. In validation set 1, 31 of 52 subjects had known inherited mutations in PCC/PGL susceptibility genes (9 *SDHB*, 8 *SDHD*, 4 *SDHC*, 2 *RET*, 6 *NF1*, and 2 *VHL*), whereas 21 other subjects who had clinical genetic testing had no identified germline mutation (considered sporadic tumors).

Massively parallel sequencing (MPS)

We performed whole exome sequencing (WES) of fresh frozen tumor and matched germline DNA using Illumina TruSeq Sample Prep kits for library preparation and Agilent SureSelect Human All Exon v3 kit for exome enrichment. In brief, for each matched germline and tumor sample, 1 ug of DNA was sheared with a Covaris sonicator to fragments of 200 base pairs. Samples were A-tailed and ligated with adaptor-embedded indexes to allow for multiplexing. DNA quality and fragment size were measured with an Agilent 2100

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Bioanalyzer and concentration measured with a Qubit. Samples were pooled and sequenced on the Illumina HiSeq2000 in the Penn Functional Genomics Core (FGC), with an average of six germline exomes and three tumor exomes per lane (each lane producing 200 million clusters of 100 bp paired end reads).

Alignment and variant calling

Raw sequencing data were aligned and mapped to the reference genome hg19 using the Burrows-Wheeler Aligner (BWA).[41] Duplicate reads were removed as well as reads which map to more than one location. Genome Analysis Toolkit (GATK) was used for single nucleotide variant (SNV) calling and for identifying small insertions and deletions in tumor and matched germline WES data.[42] Variants found in common between the matched germline and tumor DNA were removed to keep only variants unique to the tumor (somatic variants). During the analysis stage, phred scores and mapping quality scores were set to 30 allowing for 99.9% base calling accuracy and read mapping accuracy, respectively. Variants with read depth less than 20 were removed.

Annotation

Variants were annotated using ANNOVAR to call variants as frameshift indel, non-frameshift indel, stopgain, stoploss, synonymous, non-synonymous and splicing (intronic and exonic).[43] ANNOVAR also annotates SNVs using SIFT, Polyphen2 and MutationTaster to make predictions about pathogenicity and PhyloP and +GERP to make predictions about conservation.[22–24, 43–45] Variants were removed if the alternate allele frequency was less than 5%, if the segmental duplication was above 80% or the minor allele frequency (MAF) greater than 0.1% in EVS6500 and/or 1000 Genomes. Synonymous variants were considered benign and removed.

Amplicon sequencing of ATRX

DNA from FFPE PCC/PGL from the validation sets were used to test for somatic *ATRX* variants by multiplex amplicon sequencing. Primers were designed across the coding region of *ATRX* and modified with adaptors to allow for subsequent sequencing on the Illumina Miseq. One hundred and seven primer sets spanned the coding region and were divided into seven PCR primer pools. Each tumor sample was PCR amplified with all seven primer pools. Standard PCR protocol was used with the following parameters: five cycles at each of the following annealing temperature 68°C, 63°C, 60°C, 58°C followed by 15 cycles at annealing temperature of 55°C. The seven reactions per sample were combined and purified using AMPure XP beads (Agencourt). Samples were diluted 1:20. Two microliters of the purified and diluted samples were used as template for a second round of PCR using indexed primers from Illumina TruSeq Custom Amplicon Index Kit (Dual index) (FC-130-1003) to allow for multiplexing. Standard PCR conditions were used for the second round of PCR including five cycles at each of the following annealing temperatures of 68°C and then 62°C. PCR products were again purified with AMPure XP beads (Agencourt) and quantified using a Qubit. Samples were multiplexed equimolar and run on the Illumina MiSeq. Raw sequencing data was analyzed using the pipeline described in Daber et al 2013.[46] Only variants with MAF <0.1% in both EVS6500 and 1000 genomes and a read depth of at least

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50 were considered for analysis. Only nonsense, frameshift, splice site and missense variants located in a functional domain of the protein and considered damaging in at least three *in silico* pathogenicity predication programs (described above) were called somatic mutations.

Sanger sequencing of DAXX

DNA from FFPE PCC/PGL from the validation sets were used to test for somatic *DAXX* variants by Sanger sequencing. Primers were designed across the entire coding region of *DAXX*. Primer sequences are provided in Supplementary Table 5. Standard PCR protocol was used with 35 cycles at primer specific annealing temperatures given in Supplementary Table 5. PCR products were cleaned up using exonuclease reactions and sequenced using BigDye Terminator v3.1 (Applied Biosystems) and run on an ABI Prism 3130xl Genetic Analyzer.

Immunohistochemistry

Immunohistochemistry (IHC) was done to confirm the effect of the mutations on the ATRX protein in the FFPE tumor tissue. Slides were deparaffinized in xylene and rehydrated in a graded ethanol series. Heat-induced antigen retrieval was performed in a steamer using citrate buffer (pH 6.0) (Vector Laboratories) for 30 min followed by 10 min of cooling. Endogenous peroxidase was blocked for 10 min with dual endogenous enzyme-blocking reagent (Dako). Serial sections were then incubated with primary antibody; anti-ATRX (1:400) (Sigma-Aldrich; HPA001906) and anti-DAXX (1:75) (Sigma-Aldrich; HPA008736) for 1 hour at room temperature. The sections were then incubated for 30 min with secondary antibody (Leica Microsystems) followed by detection with 3,3′-Diaminobenzidine (Sigma-Aldrich) for 8 min. Sections were washed with phosphate-buffered saline with 0.1% Tween-20. Finally, sections were counterstained with hematoxylin, subsequently rehydrated in a graded ethanol series and mounted. Only nuclear labeling of either protein in tumor cells was considered positive. Endothelial cells on each section served as internal controls.

Telomere FISH

Mutations in *ATRX* have been associated with alternative lengthening of telomeres (ALT).[28] To examine the ALT phenotype, we performed telomere specific FISH per protocol by Heaphy et al[28] with some modifications. Slides were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was performed by boiling slides in 100mM Tris/50mM EDTA for 10 mins and allowed to cool for five mins at room temperature. Slides were then treated with 0.5mg/mL of protease type VIII (Sigma) treatment for 1 min at room temperature. PNA wash buffer was prepared (70% formamide, 24% ddH_2O, 10nM Tris pH 7.5 and 0.5% blocking solution (Roche)). Slides were probed using a PNA FISH probe (1:100 in PNA wash buffer) (Panagene; F1002-5 TelC-Cy3) by denaturing at 83°C for 4 min and incubating at room temperature for two hours. Slides were washed twice in PNA wash buffer for 30 mins and counterstained with DAPI (1:750) (Invitrogen). After hydration in a graded ethanol series, slides were mounted with Vectashield antifade (Vectalabs) and imaged on a Nikon 80i/ fluorescent microscope using a Qimaging retiga SRV camera. Positive cells were counted across ten 20x fields per section.

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Multiplex ligation-dependent probe amplification (MLPA)

MLPA was performed according to manufacturer conditions using a commercial kit (MRC Holland; P013-100R) and analyzed using Coffalyser.Net (MRC Holland; version 140429.1057).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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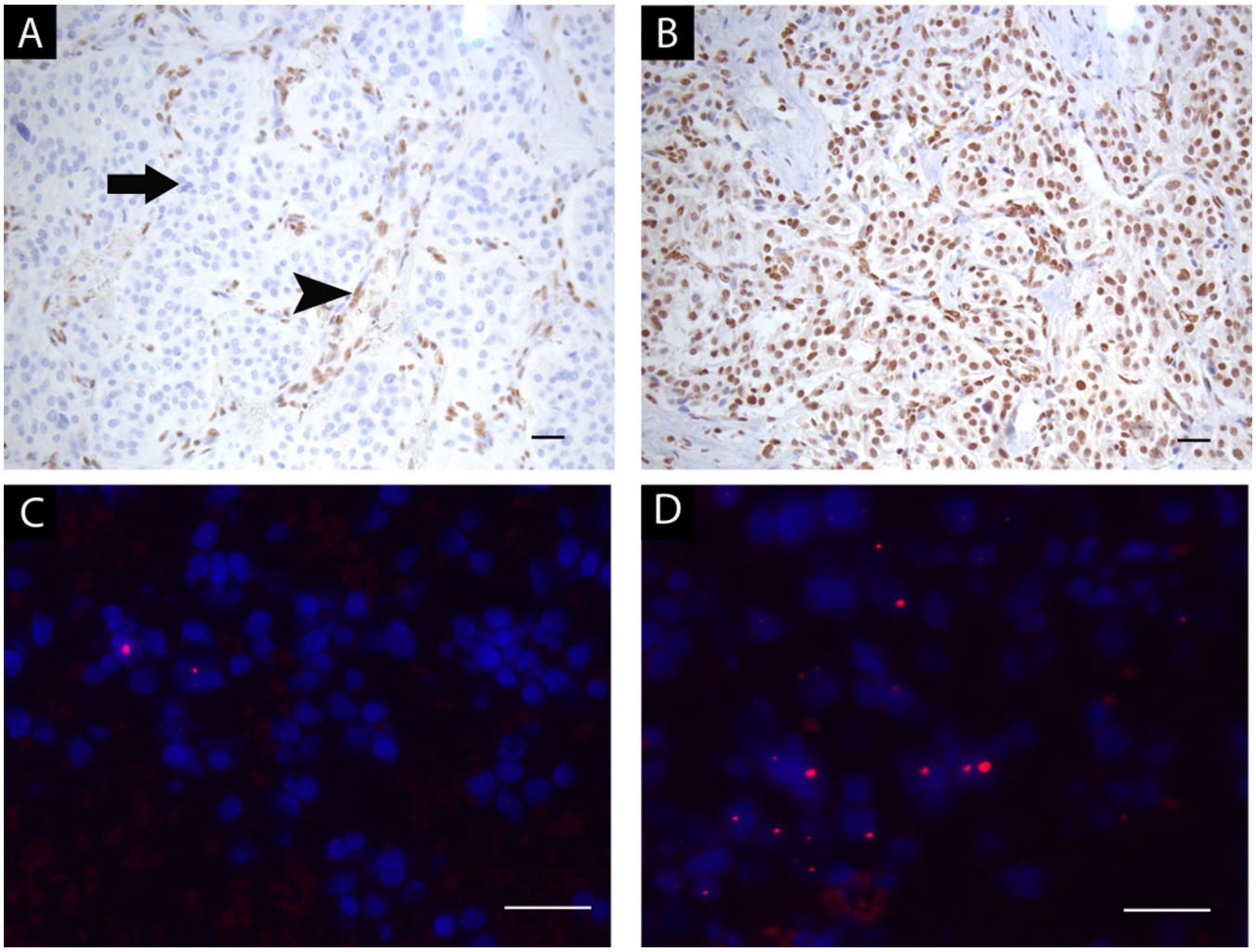

Figure 1. Immunohistochemistry and immunofluorescence in PCC/PGL with somatic *ATRX* mutations

FFPE tumor PP119Z1 shows absence of ATRX in tumor cells (arrow; panel A) with the presence in endothelial cells (arrow head) as an internal control; DAXX staining was positive (panel B). Telomere FISH shows alternative lengthening of telomeres in two *SDHB*-associated PCC/PGLs with somatic *ATRX* mutations from the discovery and validation sets, PP119Z1 (panel C) and PP142Z1 (panel D), respectively. Scale bars, 30 μm.

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Table 1

Somatic Mutations in *ATRX*

Sample ID	Consequence	cDNA change	Protein Change	Inherited mutation	Clinical information	Associated ALT by Telomere FISH
Discovery set						
PP 098 F2	nonsynonymous SNV	c.G6590A	p.R2197H	*SDHB*	EA PGL (one of multiple primary tumors including PCC and HNPGL)	yes
PP 119 F1	stop gain	c.G6825A	p.W2275X	*SDHB*	Brain metastasis from primary EA PGL	yes
Validation set 1						
PP 024 Z1	nonsynonymous SNV	c.C6253T	p.R2085C	*VHL*	PCC recurrence in surgical bed	NT
	nonsynonymous SNV	c.G5851A	p.D1951N			
PP 088 Z1	nonframeshift deletion	c.3786_3788del	p.1262_1263del	*NF1*	Adrenal PCC	NT
PP 142 Z1	frameshift deletion	c.915_918del	p.305_306del	*SDHB*	Locally invasive EA PGL	yes
PPM 007 Z1	nonsynonymous SNV	c.G4535A	p.R1512H	*SDHD*	HNPGL with metastatic disease	no
PPM 013 Z1	nonsynonymous SNV	c.C5657T	p.P1886L	*SDHB*	EA PGL	yes
PPM 014 Z1	nonsynonymous SNV	c.G4535A	p.R1512H	*SDHD*	Multiple primary tumors (adrenal PCC, EA PGL and HNPGL)	no
Validation set 2						
PPA 1005 Z1	nonsynonymous SNV	c.G6224A	p.G2075E	Not known	Not known	NT
PPA 1008 Z1	frameshift insertion	c.6793_6794insAGAAAAGCAA	p.E2265fs	Not known	Adrenal PCC	NT
PPA 1030 Z1	nonsynonymous SNV	c.G4535A	p.R1512H	Not known	Adrenal PCC	NT
PPA 1032 Z1	nonsynonymous SNV	c.G4535A	p.R1512H	Not known	Not known	NT
PPA 1033 Z1	nonsynonymous SNV	c.G5857A	p.E1953K	Not known	Not known	NT
PPA 1036 Z1	nonsynonymous SNV	c.A5788G	p.K1930E	Not known	Adrenal PCC	NT
	nonsynonymous SNV	c.G4535A	p.R1512H			
PPA 1050 Z1	nonframeshift deletion	c.3786_3788del	p.1262_1263del	Not known	Adrenal PCC	NT

PCC-pheochromocytoma; EA PGL- extra adrenal paraganglioma; HNPGL- head and neck paraganglioma; ALT –alternative lengthening of telomeres; NT–not tested