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ATRX driver mutation in a composite malignant pheochromocytoma

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Pheochromocytomas (PCCs) and paragangliomas (PGLs) are tumors arising from the adrenal medulla and sympathetic/parasympathetic paraganglia, respectively. Approximately 40% of PCCs/PGLs are due to germline mutations in one of 16 susceptibility genes, and a further 30% are due to somatic alterations in 5 main genes. Recently, somatic *ATRX* mutations have been found in succinate dehydrogenase (SDH)-associated hereditary PCCs/PGLs. In the present study we applied whole-exome sequencing to the germline and tumor DNA of a patient with metastatic composite PCC and no alterations in known PCC/PGL susceptibility genes. A somatic loss-of-function mutation affecting *ATRX* was identified in tumor DNA. Transcriptional profiling analysis classified the tumor within cluster 2 of PCCs/PGLs (without SDH gene mutations) and identified downregulation of genes involved in neuronal development and homeostasis (*NLGN4*, *CD99* and *CSF2RA*) as well as upregulation of *Drosha*, an important gene involved in miRNA and rRNA processing. CpG island methylator phenotype typical of SDH gene-mutated tumors was ruled out, and SNP array data revealed a unique profile of gains and losses. Finally, we demonstrated the presence of alternative lengthening of telomeres in the tumor, probably associated with the failure of ATRX functions. In conclusion, somatic variants affecting *ATRX* may play a driver role in sporadic PCC/PGL.

Keywords ALT, *ATRX*, exome sequencing, pheochromocytoma

Introduction

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are neural crest-derived catecholamine-secreting tumors arising from the adrenal medulla and sympathetic/parasympathetic paraganglia, respectively. While initially it was thought that only 10% of cases were caused by germline mutations, the discovery of mutations in several additional susceptibility genes during the last fifteen years has brought the percentage of hereditary cases up to approximately 40%. These mutations affect 16 genes: *VHL*, *RET*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MEN1*, *KIF1β*, *EGLN1*, *EGLN2*, *TMEM127*, *MAX*, *FH* and *MDH2* (1–3). On the other hand, the presence of somatic events in PCC/PGL has been largely uncharacterized and underestimated because of the limited number of studies based on tumoral DNA. It is now widely accepted that some of the genes involved in hereditary PCC/PGL also play a role in sporadic disease (especially *NF1*, *VHL* and *RET*), while mutations in *EPAS1*, *HRAS*, *ATRX*, *BRAF* and *TP53* occur in non-hereditary tumors (1,4,5). Thus, somatic mutations can be detected in an additional 25–30% of tumors (1). Although patients with PCC/PGL rarely develop distant metastases, those that do usually carry germline *SDHB* mutations and have 5-year overall survival less than 60% (6).

We used whole-exome sequencing (WES) of germline and tumor DNA to investigate the genetic etiology of a very large malignant composite adrenal tumor diagnosed in a patient with no family history of the disease.

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Materials and methods

Patient

A 66-year-old man was diagnosed by computed tomography scan with a 20 cm left composite adrenal tumor with PCC (90%) and ganglioneuroma and ganglioneuroblastoma (10%) components (Figure 1A and B). On hospital admission, the patient reported anemia and loss of weigh. He had no personal or family history of neural-crest tumors. During clinical follow-up the patient developed several hepatic metastasis of the PCC. Mutations in the major susceptibility genes were first ruled out by Sanger sequencing. The *Instituto de Salud Carlos III* (ISCIII) ethics committee approved the study, and the patients or their relatives provided written informed consent.

DNA extraction and whole-exome sequencing analysis

DNA was extracted from blood using a standard method (7) and from frozen tissue using the DNeasy kit (Qiagen Inc.), following the manufacturer's instructions. Whole-exome sequencing was carried out in the primary tumor (PCC) and germline DNA from the index patient at the National Centre for Genomic Analysis (CNAG). Briefly, the Covaris S2 System (Covaris) was used for DNA fragmentation and exome capture was performed using the SureSelect XT HumanAllExon 50 Mb kit (Agilent Technologies). Exome sequencing at a mean coverage >50× was performed by 75-bp paired-end technology using a HiSeq2000 (Illumina). The GEM and BFAST programs were used to align the reads against the whole human genome (hg19 assembly). To identify single nucleotide substitutions and small insertions and deletions (INDELs), the SAMtools program was used (http://samtools.sourceforge.net). Variants were filtered to rule out those in genome regions with low mappability, those with low depth readings or the alternative allele present in <20% of reads, and those with alternative alleles present only in forward or reverse reads. Single nucleotide substitutions and INDELs were selected and consecutively filtered by excluding (i) variants present in the dbSNP database, the 5400 NHLBI exomes database or in internal exomes; (ii) variants in intergenic, intronic or UTR regions; (iii) variants with <10 reads and <20 genotype quality score;

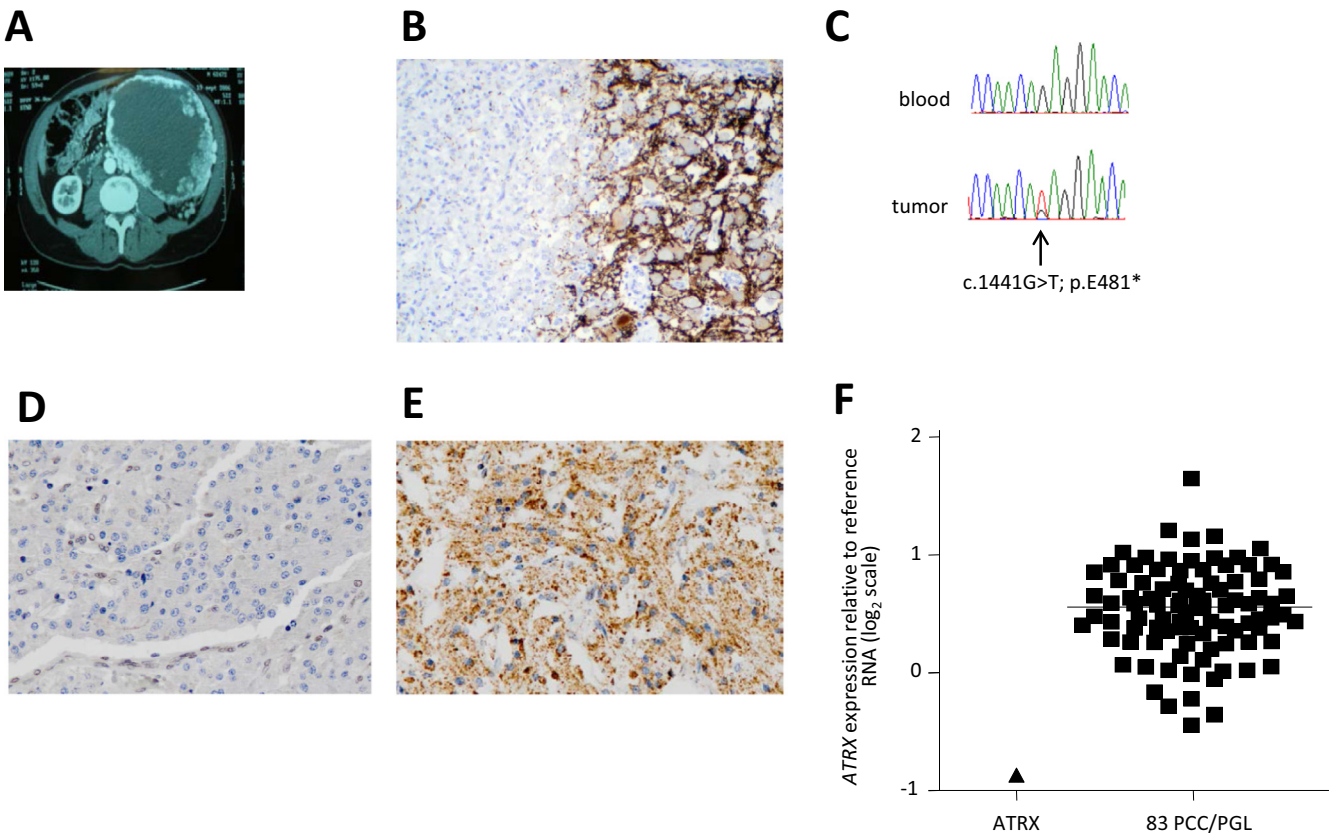

Figure 1 (A) Computerized axial tomography image showing a solid tumor of size 20 × 17 × 15 cm within the left abdominal cavity of the left kidney. (B) Immunohistochemical staining for S100 protein, revealing both the pheochromocytoma (negative staining) and the ganglioneuroma (positive staining) component of the composite tumor. (C) Sequence including the *ATRX* mutation (c.1441G>T) in the tumoral DNA from the patient compared to the wild-type sequence from blood. (D) Negative ATRX immunohistochemical staining in tumoral cells from the *ATRX*- mutated tumor, compared to normal stromal or endothelial cells. (E) Positive SDHB immunohistochemical staining of the *ATRX*-mutated tumor. (F) mRNA expression of *ATRX* (median value of A_24_P348660 and A_23_P136874 probes from the Agilent Whole Human Genome platform 4 × 44) in the tumor carrying the c.1441G>T mutation and in 83 PCCs/PGLs (GEO accession: GSE19422) used as controls. The horizontal black line represents the median.