

Clinical Research Article

A Novel, Likely Pathogenic *MAX* Germline Variant in a Patient With Unilateral Pheochromocytoma

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Abbreviations: BP, blood pressure; *MAX* gene, MYC-associated factor X; PCC, pheochromocytoma; PGL, paraganglioma; PV, pathogenic variant; SDH, succinate dehydrogenase.

Received: 4 May 2021; First Published Online: 3 June 2021; Corrected and Typeset: 22 June 2021.

Abstract

Context: Inherited MYC-associated factor X (*MAX*) gene pathogenic variants (PVs) increase risk for pheochromocytomas (PCCs) and/or paragangliomas (PGLs) in adults and children. There is little clinical experience with such mutations.

Objective: This report highlights an important approach.

Methods: Clinical assessment, including blood chemistry, imaging studies, and genetic testing were performed.

Results: A 38-year-old Hispanic woman was diagnosed with PCC in 2015, treated with adrenalectomy, and referred to endocrinology clinic. Notably, she presented to her primary care physician 3 years earlier complaining of left flank pain, intermittent diaphoresis, and holocranial severe headache. We confirmed severe hypertension (180/100 mm Hg) over multiple antihypertensive regimens. Biochemical and radiological studies workup revealed high plasma metanephrine of 255 pg/mL (normal range, < 65 pg/mL) and plasma normetanephrine of 240 pg/mL (normal range, < 196 pg/mL). A noncontrast computed

tomography scan of the abdomen revealed a 4.2 × 4.3 × 4.9-cm, round-shaped and heterogenous contrast enhancement of the left adrenal gland, and a 2-mm nonobstructive left kidney stone. A presumptive diagnosis of secondary hypertension was made. After pharmacological therapy, laparoscopic left adrenalectomy was performed and confirmed the diagnosis of pheochromocytoma. Based on her age, family history, and a high suspicion for genetic etiology, genetic testing was performed that revealed the presence of a novel likely pathogenic variant involving a splice consensus sequence in the *MAX* gene, designated *c0.64-2A > G*.

Conclusion: The phenotype of *MAX* PV-related disease and paraganglioma are highlighted. The novel *c0.64-2A > G* mutation is reported here and should be considered in the diagnostic workup of similar cases.

Key Words: *MAX* gene, pheochromocytoma, paraganglioma, adrenal, hypertension

Pheochromocytomas (PCCs) are catecholamine-secreting tumors derived from the neural crest that arise from the adrenal medulla, whereas paragangliomas (PGLs) arise from extra-adrenal sympathetic paraganglia or from parasympathetic paraganglia [1, 2]. Though these neuroendocrine tumors are rare, with an estimated prevalence of 0.1% to 0.6% cases per 1 million people, many unselected PCCs/PGLs are associated with inherited predisposition and genetic testing should be conducted in all PCCs or PGLs [3]. Nearly 40% of PCCs and PGLs cases harbor a germline pathogenic variant (PV) [4, 5], and another 46% are attributed to one of the more than 20 somatic PVs in known susceptible genes including von Hippel-Lindau (*VHL*), neurofibromatosis 1 (*NF1*), rearranged during transfection (*RET*) oncogene, succinate dehydrogenase complex type A (*SDHA*), type B (*SDHB*), or type C (*SDHC*), and fumarate hydratase (*FH*) [6, 7].

Hereditary PCCs are associated with several familial syndromic disorders: *VHL* syndrome, multiple endocrine neoplasia type 2 (MEN2) and neurofibromatosis 1; while hereditary PGLs are associated with *SDH* types A-D (*SDH A-D*) PVs [8-11]. Over the last few years, loss-of-function PVs in the *MYC*-associated factor X (*MAX*) gene were found to confer genetic susceptibility to hereditary PCCs and/or PGLs [12]. We describe herein the case of a 38-year-old woman with a left PCC associated with a novel *MAX* PV.

Case Presentation

A 38-year-old Hispanic woman was diagnosed with PCC in May 2015, and was referred to our endocrinology outpatient clinic after adrenalectomy for follow-up. Three years earlier, she presented to her primary care physician complaining of intermittent diaphoresis and holocranial severe headache associated with blood pressure (BP) of 180/100 mm Hg. A diagnosis of essential hypertension

was made and losartan was started for 6 months, which did not relieve symptoms or achieve BP control. She was switched to a combination of candesartan, prazosin, and metoprolol. This new combination relieved her symptoms and controlled her BP. In June 2014, the patient presented to the emergency department with left flank pain and a noncontrast computed tomography scan of the abdomen was performed, revealing a 2-mm nonobstructive left kidney stone and an incidental 4-cm left adrenal lesion. Her BP at the time was 190/100 mm Hg. A subsequent contrast-enhanced computed tomography scan of the abdomen revealed a 4.2 × 4.3 × 4.9-cm, round-shaped and heterogenous contrast enhancement of the left adrenal gland (Fig. 1). Notable laboratory values included plasma metanephrine of 255 pg/mL (normal range, < 65 pg/mL), plasma normetanephrine of 240 pg/mL (normal range, < 196 pg/mL), renin of 197.4 μUI (range, 2.8-39.9 μUI), aldosterone of 62.9 pg/mL (range, 10-160 pg/mL), and a 24-hour urine excretion of cortisol of 244.15 μg/24h (range, 30-180 μg/24h). Pituitary profile, calcium, and renal function were normal. Renin and urine excretion of cortisol were normal on repeated testing. Increased fasting blood glucose and glycated hemoglobin were detected and treatment with metformin and glyburide was started. BP medications were not further altered following her diagnosis of PCC because her BP was well controlled and she was already on α and β blockade. Laparoscopic left adrenalectomy in January 2016 revealed a 4.2-cm tumor, and pathological examination confirmed the diagnosis of PCC (Fig. 2). BP, plasma metanephrine, and 24-hour urinary cortisol were normalized after surgery. She is currently asymptomatic and physical examination is unremarkable. Her current medications are antihyperglycemics and fibrates. There was a family history of thyroid cancer of unspecified type in a paternal aunt and a cousin, and a sister with acromegaly (Fig. 3). The patient is a nonsmoker and does not consume alcohol.

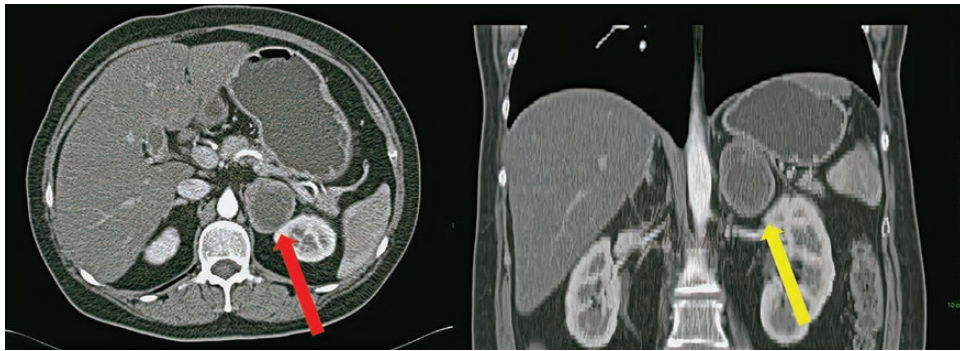


Figure 1. A, Contrast-enhanced abdominal computed tomography, axial cut: left adrenal gland measuring 4.2 × 4.3 × 4.0 cm with peripheral enhancement during the arterial phase (red arrow). B, Coronal cut: loss of anatomical architecture of the left adrenal gland due to a solid mass (yellow arrow).

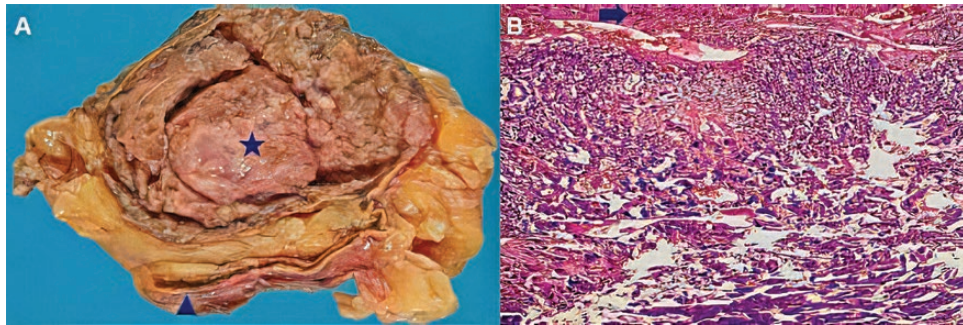


Figure 2. A, Gross macroscopy: an encapsulated but well-circumscribed tumor (star mark) that arise in the adrenal medulla which measures 4.2 × 4 cm, compress the remnant adrenal cortex (triangle mark); the cut surface is pinkish gray, with some areas of hemorrhage and fibrosis. B. Microscopy: tumor cells extend to the adrenal cortex, without a clear division, with intermingling tumor and cortical cells (black arrow).

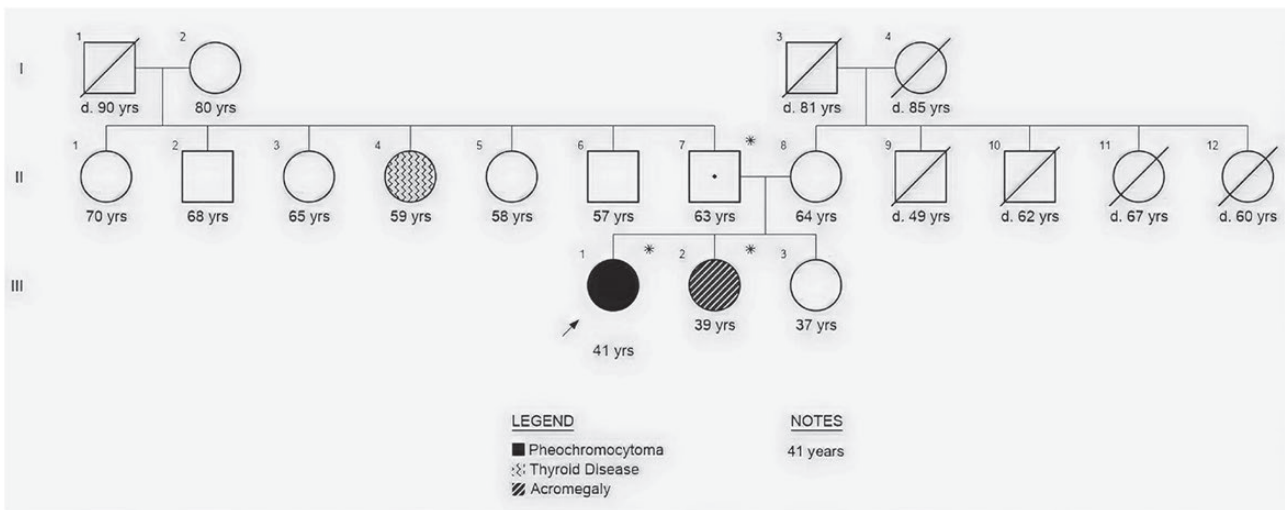


Figure 3. Patient's pedigree. Shows the familial segregation of variant *c0.64-2A > G* present in individuals II0.7 and III0.2, as well as the proband (III0.1), marked by a black arrow. Circles represent female family members; squares, males. A slash symbol represents a deceased individual.

Genetic Evaluation

The patient provided consent to the Clinical Cancer Genomics Community Research Network (ClinicalTrials.gov identifier: NCT04185935) registry protocol, and a 760-gene cancer panel was sequenced on an Illumina HiSeq Genetic Analyzer

published elsewhere [13, 14]. Full sequencing libraries were prepared using the KAPA Hyper library preparation kits and hybridized bar-coded samples to a custom Agilent Sure Select targeted-gene capture kit. The panel includes both 5' and 3' untranslated regions, as well as sequencing of 10 base pairs into all

introns. A likely pathogenic variant (*c0.64-2A > G*) was identified in the *MAX* gene. This intronic PV is predicted to disrupt the canonical splice consensus sequence at the intron-exon-3 boundary (Fig. 4). According to splice site prediction analysis (NNSplice) the wild-type acceptor site has a score of 0.99, meaning that this sequence has a 99% homology to consensus splice acceptor site. When “A” is mutated to a “G,” there is no score, predicting a total wipeout of the acceptor site (Fig. 5).

The patient was advised to have regular clinical monitoring for recurrence or new primary tumors, including physical examination, biochemical tests (urine and/or plasma metanephrines and catecholamines), and yearly thoracic and abdominal imaging studies. In addition, her father and sister had cascade genetic testing performed, revealing heterozygosity for the PV (pedigree) (Fig. 6).

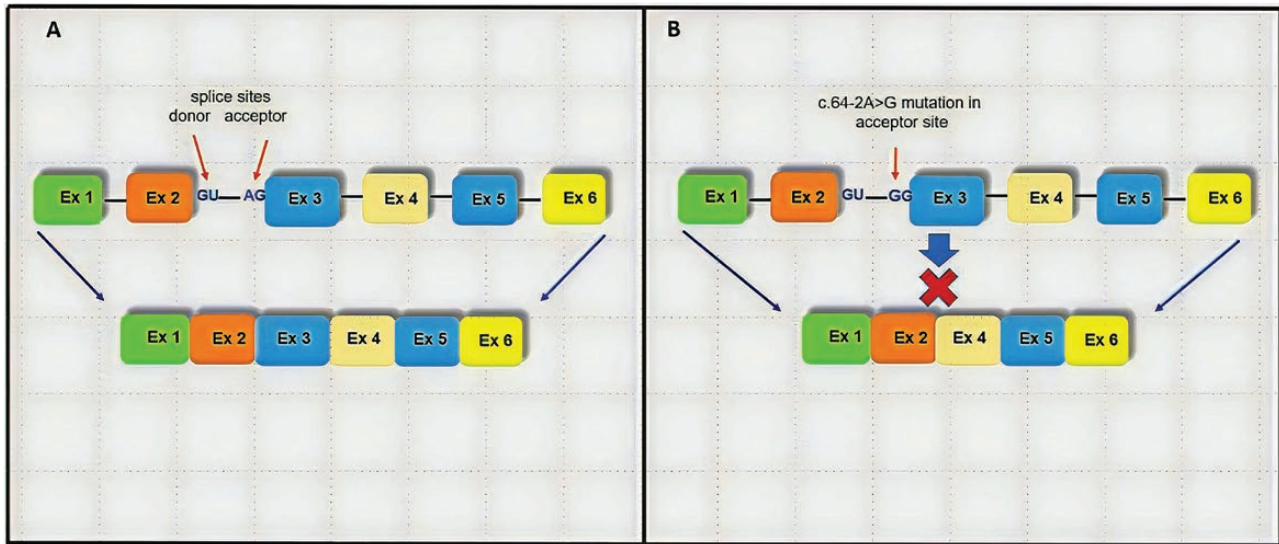


Figure 4. Exon skipping in the MYC-associated factor X (*MAX*) gene. A, Diagram of the wild transcript, showing the donor-acceptor sites. B, Putative transcript of the mutated *MAX* gene due to *c0.64-2A > G* variant, causing an exon-3 skipping in the acceptor site.

Splice site predictions for 2 sequences with donor score cutoff 0.4, acceptor score cutoff 0.40 (exón/intrón boundary shown in larger font):

Donor site predictions for *MAX*:

Start	End	Score	Exon	Intron
116	130	0.96	tagggag	gt gagttt

Acceptor site predictions for *MAX*:

Start	End	Score	Intron	Exon
54	94	0.99	ttctttctcccattctt	agg ctgacaaaacgggctcatca

Donor site predictions for *MAX*:

Start	End	Score	Exon	Intron
116	130	0.96	tagggag	gt gagttt

Acceptor site predictions for *MAX*:

Start	End	Score	Intron	Exon

Figure 5. Splice site prediction analysis. The wild-type acceptor site with “A” at the –2 position has a score of 0.99, meaning 99% homology to a consensus splice acceptor site. When the “A” is mutated to a “G,” there is no score (last line). This means the acceptor site did not reach a 0.40 (40%) cutoff for scoring.

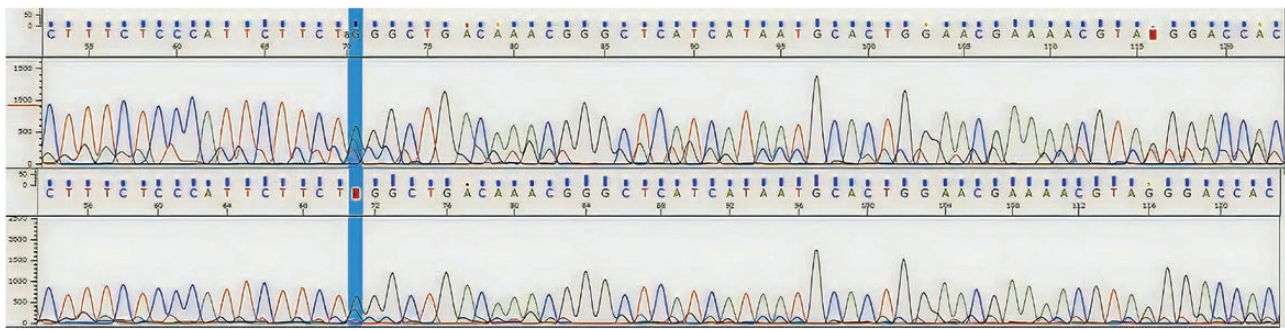


Figure 6. The point marked by R in the sequence traces shows both G and A at the nucleotide corresponding to the $-1 G > A$ likely pathogenic variant, and this is shared by the patient (top) and the patient's father (bottom). The T line is an artifact in the top trace. Top, patient; bottom, patient's father.

Discussion

Increased attention to *MAX* gene PV had been given as it was found to be a risk factor for sporadic and hereditary PCCs and/or PGLs both in adults and children [12, 15, 16]. *MAX* is a key player in the MYC-MAX-MXD1 transcription factors that regulate cell proliferation, differentiation, and apoptosis [12]. Expression of MYC affects most aspects of tumor biology including its proliferation, cell adhesion, and angiogenesis, for which its deregulation can result in MYC-dependent cell tumorigenesis [17].

MYC-MAX dimerization affects MYC's ability to transform cells through its transcriptional repression [12, 17]. A splice site PV, similar to that of our patient, with skipping of exon 4, has previously been reported [17]. Exon-intron boundaries are strongly affected by mutations in the canonical acceptor and donor sites [18]. The spliceosome recognizes the 5' and 3' splice sites, consequently any variant at this site might alter interactions between pre-messenger RNA and the intron removal machinery.

Until now, results of genotype-phenotype correlations studies have been inconsistent. It has been proposed that *MAX* PVs are associated with bilateral PCCs and a presumed paternal transmission of the disease [12]. In addition, recent cases associated with germline PV of the *MAX* gene were associated with bilateral PCCs with PGLs [19-24]. As yet, gene-expression profiling analysis in PCCs and PPGLs related to *MAX* PVs are not clearly established [25]. Our patient has no evidence of disease after 5 years of biochemical and radiological follow-up evaluations.

Comino-Méndez et al [12] described a pattern suggestive of preferential paternal transmission of the disease, as well as the absence of methylation in various CpG islands, ruling out that the expression of such genes is affected by imprinting. The *MAX* PV was detected in our patient's father, who has no personal history of any neoplasia, supporting the paternal imprinting hypothesis. The *MAX* PV was also detected in her sister, who had acromegaly.

It has been hypothesized that a fifth type of multiple endocrine neoplasia is driven by *MAX* mutations [26]. A recently reported case from Seabrook et al of 2 families with a *MAX* germline mutation was characterized by the coexistence of PCCs, ganglioneuroma, neuroblastomas, pituitary tumors, and, perhaps, parathyroid adenomas [26]. In light of these recently published reports, it is reasonable to think that our patient's sister, who had a pituitary neuroendocrine tumor and the *MAX* PV was found, should be regularly screened for the aforementioned tumors.

First-degree relatives of an individual with a known *MAX* PV should be offered molecular genetic testing. Knowledge of the genetic status improves diagnostic certainty and reduces the need for costly screening procedures in those who have not inherited the PV. The father and the sister of the patient, being heterozygotes, were both recommended to undergo screening studies to rule out lesions in the pituitary or the presence of PGLs/PCCs, with the greatest concern about the sister.

It is important to note that, despite our patient's presurgery antihypertensive regimen successfully controlling her BP, it is not a common preoperative pharmacologic preparation for PCCs worldwide. However, prazosin is the α -blocking medication we have available at our center in our country and therefore is the medication of choice here.

In summary, genetic analysis of our patient identified a novel *c0.64-2A > G* mutation and should be considered in the diagnostic workup of similar cases.

Acknowledgments

We thank the Department of Endocrinology and Metabolism, Genetic, Radiology and Pathology of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

Financial Support: This work did not receive any commercial support. The Clinical Cancer Genomics Community Research Network is supported in part by the Breast Cancer Research Foundation BCRF (BCRF-19-172); Conquer Cancer Research Professorship in Breast Cancer Disparities (J.N.W.); and the National Cancer Insti-

tute and the Office of the Director, National Institutes of Health (RC4CA153828; principal investigator: J.N.W.) The research reported in this publication and the content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Disclosures: J.N.W. is on a speaker's bureau for AstraZeneca. The other authors have nothing to disclose.

Data Availability: All data are available on reasonable request to the corresponding author. Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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