José Viana Lima Junior

AVALIAÇÃO E CORRELAÇÃO CLÍNICA, LABORATORIAL, RADIOLÓGICA, ANÁTOMO-PATOLÓGICA E GENÉTICA DE PACIENTES COM FEOCROMOCITOMAS/PARAGANGLIOMAS ESPORÁDICOS E FAMILIAIS ACOMPANHADOS NA UNIFESP/EPM

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina para obtenção do título de Doutor em Ciências.

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Orientador: Prof. Dr. Cláudio Elias Kater

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Lima-Junior, José Viana

Avaliação e Correlação Clínica, Laboratorial, Radiológica, Anátomo-Patológica e Genética de pacientes com feocromocitomas/paragangliomas esporádicos e familiais acompanhados na UNIFESP/EPM/ José Viana Lima Junior-São Paulo,2023

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Titulo em inglês- Clinical, Laboratory, Radiological, Anatomical-Pathological and Genetic Assessment and Correlation of patients with sporadic and familial pheochromocytomas/paragangliomas followed at UNIFESP/EPM

1.Feocromocitoma 2.Paraganglioma 3. Metanefrinas

4. Painel Genético 5. Ressonância Magnética 6. MIBG

UNIVERSIDADE FEDERAL DE SÃO PAULO

ESCOLA PAULISTA DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM ENDOCRINOLOGIA E METABOLOGIA

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Lele, Manu e Delma,

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Sumário

Lista de abreviaturas, siglas e símbolos

VIP – peptídeo vasoativo intestinal

PTHrp- proteína relacionada ao paratormônio

CRH- hormônio liberador de corticotrofina

ACTH- hormônio adrenocorticotrófico

IL-6- Interleucina 6

HAS- Hipertensão Arterial Sistêmica

A - Adrenalina

NA- Noradrenalina

DA- dopamina

DM- Diabetes Mellitus

PTH- paratormônio

PGL- paraganglioma

UH- unidades Hounsfield

VP- Valor preditivo

VPP- valor preditivo positivo

GIST- *Gastrointestinal stromal tumors*

SNC- Sistema Nervoso Central

Feo- Feocromocitoma

F/PGL- Feocromocitoma/paraganglioma

MLPA- amplificação multiplex de sondas dependente de ligação

VMA- ácido vanililmandélico

TNE- Tumor Neuroendócrino

RM- Ressonância Magnética

TC- Tomografia Computadorizada

US- Ultrassonografia

MIBG- Metaiodobenzilguanidina

PETCT- tomografia por emissão de pósitrons - tomografia computadorizada

CGA – campo de grande aumento

ECA- enzima conversora de angiotensina

PA- Pressão arterial

UTI- unidade de terapia intensiva

QT- Quimioterapia

CVD- ciclofosfamida/ vincristina/ dacarbazina

CMT- carcinoma medular de tiroide

HPP- hiperparatiroidismo primário

IMC- Índice de massa corpórea

ATM- ATM serine/ threonine kinase

ATR- ATR serine/ threonine kinase

CDKN2A- cyclin-dependent kinase inhibitor 2A

EGLN1- egl-9 family hypoxia-inducible factor

FH- fumarate hydratase

Hras-Harvey rat sarcoma viral oncogene homolog

KIF1β- kinesin family member 1B

KMT2D- lysine (K)- specific methyltransferase

MAX- myc associated factor X

MDH2- malate dehydrogenase 2

MERTK- MER proto-oncogene tyrosine kinase

MET- MET proto-oncogene, receptor tyrosine kinase

NF1- neurofibromin 1

RET- ret proto-oncogene

SDHA- succinate dehydrogenase complex, subunit A, flavoprotein (Fp)

SDHAF2- succinate dehydrogenase complex assembly factor 2

SDHB- succinate dehydrogenase complex, subunit B, iron sulfur (1p)

SDHC- succinate dehydrogenase complex, subunit C, integral protein, 15 KDa

SDHD- succinate dehydrogenase complex, subunit D, integral membrane protein

TMEM127- transmembrane protein 127

TP53- tumor protein p53

VHL- von Hippel Lindau tumor supressor, E3 ubiquitin protein ligase

PPGL- Pheochromocytoma and Paraganglioma

Abstracts of Manuscripts / Resumos dos Manuscritos:

<u>Manuscript 1:</u> "The Pheochromocytoma/Paraganglioma Syndrome: An Overview on Mechanisms, Diagnosis and Management"

Pheochromocytomas/paragangliomas (PPGL) are rare, metastatic, and potentially fatal neuroendocrine tumors, often neglected because they present symptoms similar to other prevailing clinical conditions such panic syndrome, thyrotoxicosis, anxiety, hypoglycemia, etc., delaying diagnosis and treatment. The rate of diagnosis of PPGL has been increasing with the improvement in the measurement of catecholamine metabolites and the expanding availability of imaging procedures. Its essential genetic nature has been extensively investigated, comprising more than 20 genes currently related to PPGL and more new genes will probably be revealed. This overview will shed some light on the clinical, laboratory, topographical, genetic diagnosis, and management of PPGL.

Keywords: Pheochromocytoma; Paraganglioma; Metanephrine

<u>Manuscrito 1:</u> "Síndrome Feocromocitoma/Paraganglioma: Um Panorama dos Mecanismos, Diagnóstico e Manuseio"

Os feocromocitomas/paragangliomas (PPGL) são tumores neuroendócrinos raros, metastáticos e potencialmente fatais, muitas vezes negligenciados por apresentarem sintomas semelhantes a outras condições clínicas prevalentes, como síndrome do pânico, tirotoxicose, ansiedade, hipoglicemia, etc., atrasando o diagnóstico e o tratamento. A taxa de diagnóstico de PPGL tem aumentado com a melhoria na dosagem dos metabólitos das catecolaminas e a crescente disponibilidade de procedimentos de imagem. Sua natureza genética essencial tem sido extensivamente investigada, compreendendo mais de 20 genes atualmente relacionados ao PPGL e provavelmente mais novos genes serão

descobertos. Esta visão geral lançará alguma luz sobre o diagnóstico clínico, laboratorial, topográfico, genético e o manejo do PPGL.

Palavras-chave: Feocromocitoma; Paraganglioma; Metanefrina

<u>Manuscript 2:</u> "Germline genetic variants in pheochromocytoma/ paraganglioma: Single-center experience"

Pheochromocytoma/paraganglioma (PPGL) are rare neuroendocrine tumors carrying 25-40% pathogenic germline gene variants (PGV). We evaluated clinical, laboratory, and germline molecular profile of 115 patients with pathologic (fourteen patients were relatives from 8 different families recruited for genetic survey) confirmed PPGL followed in our institution. Patients with classic MEN2A/MEN2B phenotypes and at-risk relatives underwent direct analysis of *RET* proto-oncogene, and the remained had samples submitted to complete Next Generation Sequencing (NGS) aiming 23 PPGL-related genes: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53,* and *VHL.* We also developed a clinical judgment score (CJS) to determine the probability of patients having a potentially hereditary disease. The resulting genetic landscape showed that 67 patients (58.3%) had variants in at least one gene: 34 (50.7%) had exclusively pathogenic or likely pathogenic variants, 13 (19.4%) pathogenic or likely pathogenic variants and VUS and 20 (29.8%) carried only VUS. PGV were found in *RET* (N=18; 38.3%), *VHL* (N=10; 21.3%), *SDHB* and *NF1* (N=8; 17% each), and *MAX*, *SDHD*, *TMEM127,* and *TP53* (N=1; 2.1% each). Direct genetic testing disclosed 91.3% sensitivity, 81.2% specificity, and 76.4% and 93.3% positive (PPV) and negative (NPV) predictive values, respectively. The CJS to identify patients who would not benefit from genetic testing had 75% sensitivity, 96.4% specificity, and 60% and 98.2% PPV and NPV, respectively. In summary, the landscape of PPGL germline gene variants from 115 Brazilian patients resulted in slightly higher prevalent pathogenic and likely pathogenic variants, especially in the *RET* gene. We suggest a CJS to identify PPGL patients who would not require initial genetic evaluation, improving test specificity and reducing costs.

Key words: *SDHB, VHL, RET, NF1, TMEM127*, *MAX, SDHD*

<u>Manuscrito 2:</u> "Variantes genéticas germinativas em feocromocitoma/paraganglioma: Experiência de um centro isolado"

Feocromocitoma/paraganglioma (PPGL) são tumores neuroendócrinos raros sendo que em 25-40% dos casos possuem variantes genéticas patogênicas germinativas (VPG). Avaliamos o perfil molecular germinativo, clínico, laboratorial de 115 pacientes com diagnóstico patológico (quatorze pacientes eram parentes de 8 famílias diferentes recrutadas para pesquisa genética) confirmados com PPGL acompanhados em nossa instituição. Pacientes com fenótipos clássicos MEN2A/MEN2B e parentes em risco foram submetidos à análise direta do proto-oncogene RET, e os restantes tiveram amostras submetidas ao sequenciamento de próxima geração (NGS) completo visando 23 genes relacionados ao PPGL: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 e VHL*. . Também desenvolvemos um escore de julgamento clínico (CJS) para determinar a probabilidade de os pacientes terem uma doença potencialmente hereditária. O panorama genético resultante mostrou que 67 pacientes (58,3%) tinham variantes em pelo menos um gene: 34 (50,7%) tinham variantes exclusivamente patogênicas ou prováveis patogênicas, 13 (19,4%) variantes patogênicas ou provavelmente patogênicas e VUS e 20 (29,8%) carregavam apenas VUS. VPG foram encontrados em RET (N=18; 38,3%), VHL (N=10; 21,3%), SDHB e NF1 (N=8; 17% cada) e MAX, SDHD, TMEM127 e TP53 (N=1 ; 2,1% cada). O teste genético direto revelou sensibilidade de 91,3%, especificidade de 81,2% e valores preditivos positivos (VPP) e negativos (VPN) de 76,4% e 93,3%, respectivamente. O CJS para identificar pacientes que não se beneficiariam com testes genéticos apresentou sensibilidade de 75%, especificidade de 96,4% e VPP e VPN de 60% e 98,2%, respectivamente. Em resumo, o panorama das variantes do gene da linha germinativa PPGL de 115 pacientes brasileiros resultou em variantes patogênicas e provavelmente patogênicas com prevalência ligeiramente maior, especialmente no gene *RET.* Sugerimos que o CJS seja realizado para identificar pacientes com PPGL que não necessitariam de avaliação genética inicial, melhorando a especificidade do teste e reduzindo custos.

Palavras-chave: *SDHB, VHL, RET, NF1, TMEM127, MAX, SDHD*

<u>Manuscript 3:</u> "Portrait of a large series of patients with pheochromocytoma/ paraganglioma studied in a reference center in São Paulo"

Pheochromocytomas (Pheo) and paragangliomas (PGL) are catecholamine-secreting tumors. Confirmation of functional PPGL strongly relies on the finding of elevated plasma and/or 24-h urinary metanephrines (MN). We present the clinical, hormonal, and imaging aspects of 116 patients studied in our endocrine reference center in São Paulo, Brazil, in whom a diagnosis of PPGL (94 Pheo, 22 PGL) was confirmed. Systemic arterial hypertension (SAH) was present in 81% patients, 42.6% had stage 3 SAH; 9.5% were prehypertensive, and 9.5% were normotensive. SAH was accompanied by paroxysms in 58.5% PPGL patients and was exclusively sustained in the other 41.5%. Resistant SAH was observed in 27.7%, mostly associated with paroxysmal and combined SAH. Orthostatic hypotension was present in 64.7% patients, six of whom did not have hypertension. To identify functioning lesions, we used a cut-off value of 885 mcg/24-h for total urinary MN (100% sensitivity [S] and 92.5% specificity [E]) and a cut-off of 1.5 nmol/L for total plasma MN (100% S and 97.3% E). Magnetic resonance imaging (MRI) was the major imaging procedure. It identified 18 bilateral Pheo (19.2%), 53 (56.4%) Pheo on the right side and 23 (24.5%) on the left side. Thirteen (56.5%) PGL were retroperitoneal and 10 (43.5%) were cervical. Tumor size was positively correlated with total urinary MN excretion. The concordance between MRI and ^{131}I-mIBG scintigraphy, performed in 57 PPGL patients (52.3%), was almost 100%.

Key words: Hypertension; paroxysms; orthostatic hypotension; pheochromocytoma; paraganglioma; adrenal incidentaloma; metanephrines; adrenal imaging

<u>Manuscrito 3:</u> "Retrato de uma grande série de pacientes com feocromocitoma/ paraganglioma estudados em um centro de referência em São Paulo"

Feocromocitomas (Pheo) e paragangliomas (PGL) são tumores secretores de catecolaminas. A confirmação do PPGL funcional depende fortemente do achado de metanefrinas (MN) plasmáticas e/ou urinárias elevadas em 24 horas. Apresentamos os

aspectos clínicos, hormonais e de imagem de 116 pacientes estudados em nosso centro de referência endócrina em São Paulo, Brasil, nos quais foi confirmado o diagnóstico de PPGL (94 Pheo, 22 PGL). A hipertensão arterial sistêmica (HAS) esteve presente em 81% dos pacientes, 42,6% tinham HAS estágio 3; 9,5% eram pré-hipertensos e 9,5% eram normotensos. A HAS foi acompanhada de paroxismos em 58,5% dos pacientes com PPGL e foi sustentada exclusivamente nos outros 41,5%. HAS resistente foi observada em 27,7%, principalmente associada à HAS paroxística e combinada. A hipotensão ortostática esteve presente em 64,7% dos pacientes, seis dos quais não apresentavam hipertensão. Para identificar lesões funcionantes, utilizamos um valor de corte de 885 mcg/24 horas para MN urinário total (100% de sensibilidade [S] e 92,5% de especificidade [E]) e um ponto de corte de 1,5 nmol/L para lesões totais. MN plasmático (100% S e 97,3% E). A ressonância magnética (RM) foi o principal procedimento de imagem. Foram identificados 18 Pheo bilaterais (19,2%), 53 (56,4%) Pheo no lado direito e 23 (24,5%) no lado esquerdo. Treze (56,5%) PGL eram retroperitoneais e 10 (43,5%) eram cervicais. O tamanho do tumor foi positivamente correlacionado com a excreção urinária total de MN. A concordância entre a ressonância magnética e a cintilografia com 131I-mIBG, realizada em 57 pacientes com PPGL (52,3%), foi de quase 100%.

Palavras-Chave: Hipertensão; paroxismos; hipotensão ortostática; feocromocitoma; paraganglioma; incidentaloma adrenal; metanefrinas; imagem adrenal.

Apresentação/ Introdução da Tese

Essa tese de doutorado tem como tema a **"Avaliação e Correlação Clínica, Laboratorial, Radiológica, Anátomo-Patológica e Genética de Pacientes com Feocromocitomas/ Paragangliomas Esporádicos e Familiais Acompanhados na UNIFESP/EPM"**.

Nesta tese, que visa a obtenção do Título de Doutor em Ciências, apresentamos, de acordo com as recomendações do Programa de Pós-graduação em Endocrinologia Clínica da Universidade Federal, três artigos científicos que têm como base a discussão da importância do diagnóstico clínico, laboratorial, topográfico. Um quarto artigo com ênfase na terapia dos feocromocitomas/ paragangliomas está em confecção. Adicionalmente, apresentamos os vários desafios diagnósticos e propusemos soluções possíveis no sistema de saúde.

Os vários artigos apresentados nesta tese têm por objetivo discutir uma doença rara, mas letal, pouco diagnosticada e, muitas vezes, esquecida por muitos médicos. Trata-se de uma coorte de pacientes portadores de feocromocitoma/paraganglioma (FEO/PGL) estudados na Escola Paulista de Medicina da Universidade Federal de São Paulo – EPM/UNIFESP, no período de 2000 a 2019.

Muitos avanços ocorreram ao longo das últimas décadas no que diz respeito aos diagnósticos clínico, laboratorial, radiológico, genético, anatomo-patológico e terapêutico. Uma doença diagnosticada principalmente em necropsias no passado, com a melhora proporcionada pelos avanços nos exames bioquímicos e radiológicos, permitiu um aumento dos diagnósticos, possibilitando tratamento mais precoce e evitando complicações cardiovasculares e mortes.

Com relação a parte genética, o avanço foi ainda mais supreendente. Em 1993, apenas 3 genes (*NF1, RET, VHL*) tinham relação com feocromocitoma e paraganglioma e atualmente temos mais de 20 genes relacionados *(NF1, RET, VHL, SDHD, SDHB, SDHC, SDHAF2, EGLN1, TMEM127, SDHA, MAX, FH, MDH2, EGLN2, EPAS1, HRAS, IDH1, H3F3, FGFR1, DNMT3, SLC25A11, MAML3, RET fusion),* com variantes germinativas ou somáticas, possibilitando estudar novos modelos de doença e novos ensaios clínicos

terapêuticos. A clássica “regra dos 10%” se tornou obsoleta e não deve mais ser seguida como guia.

No primeiro artigo: “**The Pheochromocytoma/Paraganglioma Syndrome: An Overview on Mechanisms, Diagnosis and Management”** (Lima-Junior JV & Kater CE. Int Braz J Urol. 2023;49(3):307-319. doi: 10.1590/S1677-5538.IBJU.2023.0038)**,** fizemos uma mini-revisão sobre mecanismos, diagnóstico e manejo do FEO/PGL, dando destaque aos aspectos genéticos e diagnósticos hormonais. Destacamos os clusters genéticos e seus mecanismos de ação e a avaliação hormonal dos feocromocitomas/paragangliomas funcionantes e propusemos um fluxograma de investigação de FEO/PGL funcionantes.

No segundo artigo, “**Germline genetic variants in pheochromocytoma/ paraganglioma: Single-center experience”** (Lima-Junior JV, Scalissi NM, Oliveira KC, Lindsey SC, Olivari C, Ferreira EM & Kater CE. Endocr Oncol. 2023;3:e220091. doi: 10.1530/EO-22-0091), avaliamos a nossa coorte de pacientes do ponto de vista genético, analisados por um painel germinativo por *Next Generation Sequence* (NGS) incluindo 23 genes (*ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53, e VHL)* relacionados com FEO/PGL. Utilizamos características clínicas, radiológicas e laboratoriais para dividir o grupo de pacientes em 2 braços (“aparentemente esporádicos” e “aparentemente hereditários”) e criamos um *score* para indicar a realização do teste genético. A criação desse *score* teve por objetivo identificar de forma mais precisa os melhores candidatos para realização do teste genético. Deixamos claro que todo portador de FEO/PGL deve fazer o teste genético; entretanto, pensando no sistema de saúde do nosso País, reconhecemos a dificuldade em conseguir o teste genético para todos os pacientes. Assim, a utilização do *score* permitiria auxiliar na decisão da realização ou não do painel genético, ressaltando que constantes reavaliações devem ser feitas, uma vez que esse *score* é dinâmico, podendo mudar no decorrer do tempo e, consequentemente, sugerir a mudança de conduta na realização do exame.

Adicionalmente, comparamos os dados do nosso grupo de pacientes com os de uma coorte descaracterizada de pacientes portadores de FEO/PGL do grupo Fleury e identificamos um número maior de casos de variantes patogênicas e provavelmente patogênicas em nossa coorte, com um discreto predomínio do gene *RET.*

No terceiro artigo, "**Portrait of a large series of patients with pheochromocytoma/ paraganglioma studied in a reference center in São Paulo"** (submetido ao *Journal of the Endocrine Society* em 28 de setembro de 2023 - documento)**,** descrevemos as características clínicas, hormonais e topográficas da nossa coorte de pacientes. A hipotensão ortostática esteve muito presente, assim como a hipertensão arterial sistêmica associada ou não aos paroxismos. Observamos que em até 25% dos casos, o diagnóstico inicial era de um incidentaloma adrenal. Os pacientes com PGL apresentaram um quadro de HAS mais grave do que os de FEO. Comparamos, também, os resultados de metanefrinas plasmáticas e urinárias com dois grupos descaracterizados de portadores de FEO/PGL (grupo controle positivo) e de não-portadores de FEO/PGL (grupo controle negativo) e dessa forma criamos uma curva ROC na qual definimos valores de corte (cut-offs) de metanefrinas plasmáticas e urinárias totais para identificação de FEO/PGL funcionantes, permitindo, dessa forma, revisitar nosso fluxograma proposto no primeiro artigo.

Um quarto artigo: **"Pharmacological and surgical management of patients with pheochromocytoma/paraganglioma. A large series studied in an Endocrine reference center in São Paulo, Brazil"** (Lima-Junior JV, Lindsey SC , Scalissi NS & Kater CE, título provisório; artigo a ser submetido possivelmente para a revista *Journal of Surgical Oncology*) ainda em confecção; tem por objetivo focar no tratamento pré, intra e pós-operatório desses pacientes, e na sua evolução no que diz respeito a sintomas, HAS, hipotensão ortostática e redução do número de antihipertensivos.

Objetivos do Projeto:

2.1– Objetivos Gerais

Avaliação e correlação de parâmetros clínicos, laboratoriais, radiológicos, anátomo-patológicos e terapêuticos de uma extensa coorte Brasileira de pacientes com feocromocitoma e/ou paraganglioma aparentemente esporádicos ou genéticos acompanhados na Disciplina de Endocrinologia da EPM/UNIFESP.

2.2 – Objetivos Específicos

2.2.1. Estudo molecular, nessa mesma coorte, dos vários genes relacionados com feocromocitoma/ paraganglioma (esporádicos e familiais) em sangue periférico por sequenciamento de próxima geração (NGS) e nos casos típicos de NEM2A por PCR por Sanger. Os seguintes genes foram estudados, numa análise criteriosa em busca de variantes genéticas patogênicas: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 e VHL.*

2.2.2. Proposta de um fluxograma para diagnóstico hormonal de feocromocitoma/ paraganglioma funcionante.

Article 1:

“The Pheochromocytoma/Paraganglioma Syndrome: An Overview on Mechanisms, Diagnosis and Management”

Lima-Junior JV & Kater CE.

Int Braz J Urol. 2023;49(3):307-319. doi: 10.1590/S1677-5538.IBJU.2023.0038),

Abstract:

Pheochromocytomas/paragangliomas (PPGL) are rare, metastatic, and potentially fatal neuroendocrine tumors, often neglected because they present symptoms similar to other prevailing clinical conditions such panic syndrome, thyrotoxicosis, anxiety, hypoglycemia, etc., delaying diagnosis and treatment. The rate of diagnosis of PPGL has been increasing with the improvement in the measurement of catecholamine metabolites and the expanding availability of imaging procedures. Its essential genetic nature has been extensively investigated, comprising more than 20 genes currently related to PPGL and more new genes will probably be revealed. This overview will shed some light on the clinical, laboratory, topographical, genetic diagnosis, and management of PPGL.

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The Pheochromocytoma/Paraganglioma syndrome: an overview on mechanisms, diagnosis and management

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ABSTRACT

Pheochromocytomas/paragangliomas (PPGL) are rare, metastatic, and potentially fatal neuroendocrine tumors, often neglected because they present symptoms similar to other prevailing clinical conditions such panic syndrome, thyrotoxicosis, anxiety, hypoglycemia, etc., delaying diagnosis and treatment. The rate of diagnosis of PPGL has been increasing with the improvement in the measurement of catecholamine metabolites and the expanding availability of imaging procedures. Its essential genetic nature has been extensively investigated, comprising more than 20 genes currently related to PPGL and more new genes will probably be revealed. This overview will shed some light on the clinical, laboratory, topographical, genetic diagnosis, and management of PPGL.

ARTICLE INFO

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INTRODUCTION

Pheochromocytomas/paragangliomas (PPGL) are rare neuroendocrine tumors capable of producing, storing, and secreting catecholamines and other substances, such as VIP, PTH- and calcitonin-related peptides, opioids, CRH, ACTH, histamine, chromogranin, interleukin-6, etc (1-3).

PPGL is a serious, potentially metastatic, and fatal disease that often goes unnoticed by unexperienced doctors. Approximately 85-90% of PPGL are localized in the adrenals and 1015% are extra-adrenal, being called paragangliomas (PGL); the latter may be found from the base of the skull to the testicles but are mostly found within the abdomen (4-7).

In this mini-review article we survey on clinical, laboratory, topographical, genetic, and therapeutic aspects of PPGL, a condition that has been showing an increase in incidence with the improvement of methods to measure catecholamine metabolites and imaging techniques.

EPIDEMIOLOGY

The prevalence of PPGL among the hypertensive population is 1:500-1,000, but 75% of the cases are diagnosed *postmortem,* and in 55% of them PPGL directly contributed to death. In autopsy studies, the prevalence of PPGL ranges from 250 to 1,300 cases per million. Thus, clinical suspicion of PPGL still draws little attention (5, 6, 8).

The incidence of PPGL has been increasing over time, despite a fall in the number of necropsies, and this is due to the increase demand in the number of imaging exams and improved methods for measuring catecholamine metabolites (6).

CLINICAL PICTURE AND INVESTIGATION

The symptomatology of patients with PPGL is variable. Systemic arterial hypertension (SAH) is the most frequent clinical manifestation of the disease, being present in 90% of cases. However, paroxysms (headache, palpitation, and sweating) are the most characteristic findings, resulting from release of catecholamines by the tumor and consequent stimulation of adrenergic receptors. They are often accompanied by increased blood pressure, tremor, pallor, chest or abdominal pain, and less commonly, facial flushing. Paroxysms do not occur in all patients. In some series, one or more components of the classical triad were present in more than 90% of patients. (4, 7-11)

The frequency of paroxysms is unpredictable and varies from 30 times a day to a single episode every 2-3 months. Near 75% of patients have one or more spells per week. Duration ranges from a few minutes (usually 15 to 60 min.) to days. They may arise spontaneously or be precipitated by activities that compress the tumor or elicit an increase in catecholamine secretion, such as exercises, pressure on the abdomen, urination, defecation, the act of smoking, and drugs like beta-blockers, anesthetic agents, radiologic contrasts, glucagon, metoclopramide, and tricyclic antidepressants (1-3, 6-13).

There are clinical scores based on signs and symptoms that have high diagnostic predictability. Among the signs and symptoms are hyperhidrosis, palpitation, pallor, tremor, nausea, heart rate >85 bpm plus body mass index (BMI) (14).

SAH may be paroxysmal, but more commonly are persistent (in ~60% of cases). It tends to be severe and/or refractory to antihypertensive medications and present with ample fluctuations. Sudden elevation of blood pressure (associated or not with other symptoms) may occur during abdominal manipulation, labor, intubation, anesthetic induction, surgery, or other invasive procedures. Norepinephrine (NE)-secreting tumors are usually associated with constant SAH, whereas those that secrete substantial

amounts of epinephrine (E) in addition to NE are associated with episodic SAH. Conversely, when tumors secrete solely E, they provoke hypotension instead of hypertension; in this situation, the clinical feature may be of a cardiogenic shock. Orthostatic hypotension may be present in 40% of patients (12-14).

Cardiac abnormalities such as left ventricular hypertrophy occur quite commonly in patients with SAH, and myocarditis or dilated cardiomyopathy may result from circulating excess catecholamines. Palpitations and arrhythmias are common and occasionally fatal (12, 15).

Pre-diabetes is present in 50% of cases and diabetes mellitus (DM) in 10-20%. They are secondary to suppression of insulin secretion and increased hepatic glucose output, induced by excess catecholamines. Hypercalcemia may also occur due to concomitance of hyperparathyroidism or tumor production of PTH-related protein (PTHrp).

Atypical manifestations such as ACTHdependent Cushing's syndrome, acute abdomen, cardiovascular (shock, myocarditis, cardiac arrhythmias, acute pulmonary edema, heart failure, Takotsubo syndrome) and neurological events (altered mental status, seizures, stroke, and focal neurological manifestations), weight loss, fever of indeterminate origin, aqueous diarrhea, or constipation simulating pseudo-obstruction and paralytic ileus may also be found. Fever of mild to severe intensity (reaching up to 41^{0}C) is not uncommon and has been attributed to IL-6 secretion (11-13).

INVESTIGATION

Candidate subjects for a PPGL screening are: 1) young hypertensive patients under 30 years of age; 2) hypertensive patients refractory to treatment with 3 classes of antihypertensive drugs in effective doses; 3) hypertensive patients with paroxysms (headache, palpitation and sweating), seizures, unexplained shock, mucous neuromas, orthostatic hypotension, weight loss, presence of type I neurofibromatosis, family history of PPGL, medullary thyroid carcinoma, von Hippel-Lindau syndrome and familial PGL syndrome; 4) adrenal incidentalomas, especially in cases where pre-contrast attenuation values on computed tomography (CT) are ≥10 HU (Hounsfield units) and contrast washout <60%; 5) marked blood pressure lability; 6) episodes of shock or severe blood pressure responses during anesthesia induction, surgeries, invasive procedures, labor and use of β-blockers; 7) Takotsubo syndrome; 8) new-onset diabetes mellitus in a young lean hypertensive patient (12, 14).

GENETICS

Approximately 25% of PPGL are genetic, and 50% of such patients have a pathogenic germline variant (PV). The following genes have already been associated with PPGL: ATM, DLST, EGLN1, EGLN2, FH, EPAS1 (HIF2A), HRAS, KIF1B,

MAX, MDH2, MEN1, MERTK, MET, NF1, RET, SLC25A11, SDHA, SDHAF2,

SDHB, SDHC, SDHD, TMEM127, TP53 and VHL (10-13, 16).

Hereditary PPGL are classified according to their transcription signature and are divided into three clusters as shown in Table-1.

Next (and in Table-2) we describe briefly the main syndromic features that are associated with specific PPGL syndromes: **von Hippel-Lindau (VHL) Syndrome**

PPGL occurs in 10 to 30% of patients with VHL. The VHL syndrome is classified as: type 1, in which PPGL does not manifest, and type 2, which is subdivided into 3 subtypes: 2A (encompassing PPGL plus retinal and CNS hemangioblastomas, and low risk for renal carcinoma), 2B (PPGL plus retinal and CNS hemangioblastomas and kidney and pancreatic tumors), and 2C (PPGL only).

PV occur in the *VHL* gene, which is a tumor suppressor located on chromosome 3p25, responsible for regulating hypoxia-induced genes by ubiquitination and subsequent degradation of HIF2α. VHL disease has a penetration >90% at 65 years of age and *missense* PV are likely associated with the development of PPGL, whereas truncated or large variants are associated with the presence of hemangioblastomas and renal cell carcinoma (17-22).

Paragangliomas

PV of succinate dehydrogenase (SDH) subunits D, B, C, A, and A2F are associated with PGL. These subunits are related to signals responsive to

Table 1 - Transcriptional signature characteristics of hereditary PPGL.

Transcriptional signature		
Cluster 1 group (10-15%)	Cluster 2 group (50-60%)	Cluster 3 group (5-10%)
Cellular response to hypoxia	Proteins that activate kinase signaling	Via Wnt
Extra-adrenal syndrome + von Hippel-Lindau	Adrenal	Adrenal + Extra-adrenal
Germline / Somatic	Germline / Somatic	Somatic
Normetanephrine / 3-Methoxytyramine (3-MT)	Normetanephrine + metanephrine or metanephrine only	Normetanephrine metanephrine / Chromogranin A
SDHD, SDHC, SDHB, SDHA, SDHA2F, VHL, HIF, FH, EGLN1 (PHD2), EGLN2 (PHD1), KIF 1β, EPAS1/2 (HIF2A), MDH2	RET, NF1, MAX, TMEM127, HRAS	CSDE1, MAML3

Table 2 - Main syndromic features associated with specific hereditary PPGL.

Gene	Syndrome	Tumor location	Rate of PPGL metastases	Association with other tumors
NF1	Neurofibromatosis type 1	Mostly adrenal (bilateral)	12%	Neurofibromas, malignant tumors of the peripheral nerve sheath, optic gliomas and leukemias
RET	Multiple endocrine neoplasia type 2	Adrenal (bilateral)	<5%	Medullary thyroid carcinoma, parathyroid adenomas/ hyperplasia
VHL	von Hippel Lindau	Mostly adrenal (bilateral)	5-8 %	Renal clear cell (RCC) carcinoma, neuroendocrine tumors of the pancreas (mostly non-functioning), CNS hemangioblastomas, endolymphatic sac tumors, pituitary adenomas
SDHA	Hereditary PGL syndrome	Any	30-60%	RCC carcinoma, gastro-intestinal stromal tumors (GIST) and pituitary adenomas
SDHB	Hereditary PGL syndrome	Any, mostly extra-adrenal	35-75%	RCC carcinoma, GIST and pituitary adenomas
SDHC	Hereditary PGL syndrome	Head and neck, can be thoracic	Low	RCC carcinoma, GIST and pituitary adenomas
SDHD	Hereditary PGL syndrome	Any, mostly head and neck	15-29%	RCC carcinoma, GIST and pituitary adenomas
SDHAF 2 (SDH5)	Hereditary PGL syndrome	Head and neck (multifocal)	Not Known	RCC carcinoma, GIST and pituitary adenoma
TMEM 127	Familial PGL syndrome	Any, mostly adrenal	Low	RCC carcinoma
MAX	Familial PGL syndrome	Mostly adrenal (bilateral)	Intermediate to high	Pituitary adenomas
EPAS1	Familial PGL syndrome, polycythemia	Any	Unknown	Somatostatinoma

FH	Hereditary leiomatosis, RCC carcinoma	Any	Possibly high	Cutaneous and uterine leiomyomas, renal papillary carcinoma
MDH2		Any	Unknown	

oxygen level so that PV in the respective genes would lead to a chronic state of hypoxia and, therefore, cell proliferation. PGL are classified as follows:

PGL1: results from PV in SDHD, located on chromosome 11q23, with a maternal *imprint* mechanism, which results in the PV almost always being transmitted by the father and a PV frequency of 3 to 5%, penetrance of 31 to 50% and frequency of metastases less than 5%; these PGL are usually located in the head, neck, and adrenals bilaterally, and may or may not be functioning.

In 75% of cases, the disease manifests around the age of 40 years. Renal carcinomas are found in 8% and pituitary adenomas have been reported in a few cases.

PGL2: results from PV of the *SDHA2F* gene. Initially described in 2009, this PV is rarely found in PGL. Located on chromosome 11q13 and, as in cases that present PV in *SDHD*, transmission is also by maternal *imprint* and almost always results from paternal transmission. PGL usually appear around 22 years of age and are often multifocal, although non metastatic.

PGL3: results from PV of the *SDHC* gene, located on chromosome 1q21, with autosomal dominant transmission, and PV frequency below 0.1%, unknown penetrance and indeterminate frequency of metastases; tumors in PGL3 localize in the head and neck and are not functioning.

PGL4: results from PV in the *SDHB* gene, located on chromosome 1p36.3, with autosomal dominant

inheritance, and frequency PV ranging from 2 to 7%, penetrance of 50 to

70% and frequency of metastases from 34 to 70%; these PGL are usually located in the thorax, abdomen and adrenal bilaterally and are always functioning. Renal carcinomas occur in 14% and GIST in 2% of cases.

PGL5: results from PV of the *SDHA* gene that rarely cause PGL; corresponds to 3% of cases and has low penetrance. GIST and pituitary adenomas may be present. (10-13, 23, 24).

Neurofibromatosis (NF)

PPGL may be associated with type 1 NF, whose diagnosis is clinical and generally does not pose diagnostic problems. The *NF-1* gene localize on chromosome 17q11.2 and is responsible for encoding a protein called neurofibromine; its inheritance is autosomal dominant. In NF-1, PV inactivate the gene and occur in 1 to 5% of the cases, when PPGL is not accompanied by hypertension and in up to 50% of those with hypertension. PPGL associated to PV in NF-1 is similar to sporadic ones, occurring in older patients; less frequently they are bilateral and extra-adrenal. PPGL was present in 3 to 13% of individuals who underwent autopsy (10-13, 23, 24-26).

Multiple Endocrine Neoplasia (MEN) In MEN 2A (medullary thyroid carcinoma [MTC], PPGL, and primary hyperparathyroidism) and 2B (MTC, PPGL, and mucous neuromas/intestinal ganglioneuromas and marfanoid habit), PPGL may be present in 50% of cases. PV in the *RET* proto-oncogene (*Rearranged During Transfection*, localized on chromosome 10q11.2) is of *missense* germline. This gene encodes a tyrosinekinase receptor that is expressed in various tissues derived from the neural crest, including the CNS and peripheral nervous system, and

neuroendocrine tissues. *RET* PVs causing *MEN* 2A are mostly located in codons 609, 611, 618, 620 (exon 10) and 634 (exon 11). Although the most affected exons are 10, 11 and 16, PV in exons 13, 14 and 15 have also been reported. In MEN 2A codon 634 is the most affected. PV in codon 918 in exon 16 (methionine for threonine, M918T) are associated with 95% of cases of MEN 2B (27, 28).

TMEM127

The *TMEM127* gene, described by Dahia et al. in 2010, is positioned on chromosome 2q11; it is a tumor suppressor that, like the NF-1 gene, promotes gene inactivation (20). In a cohort of 103 samples, PV was present in 30% of cases and in 3% of apparently sporadic PPGL (23, 24).

Laboratory Diagnosis

Laboratory diagnosis of PPGL is usually accomplished by measuring blood and urine metanephrines. The current gold standard is a plasma metanephrine (MN) measurement that achieves a sensitivity of 99% for sporadic and hereditary functioning PPGL and a specificity of 99% for hereditary (and 89% for sporadic), superior to any combination of tests. Normal plasma MN virtually excludes functioning PPGL. Preferably, plasma MN and/or urinary MN should be the tests of choice for the diagnosis of PPGL.

Chromogranin A (ChrA), an acid glycopeptide co-secreted by PPGL, can be measured during laboratory investigation; it has a diagnostic sensitivity of 83-86% and specificity of 76-98%. ChrA is not influenced by antihypertensive drugs and exhibits an increase in positive predictive value (PPV) when combined with plasma MN. ChrA may be elevated in cervical PGL that do not have elevated plasma and/or urinary MN, thus functioning as a tumor marker in this situation. However, ChrA may be increased in the following conditions: renal failure (creatinine clearance

<80mL/min), use of proton pump inhibitors, liver failure, and atrophic gastritis. Also, ChrA has low specificity since other neuroendocrine tumors (NET) can also produce it.

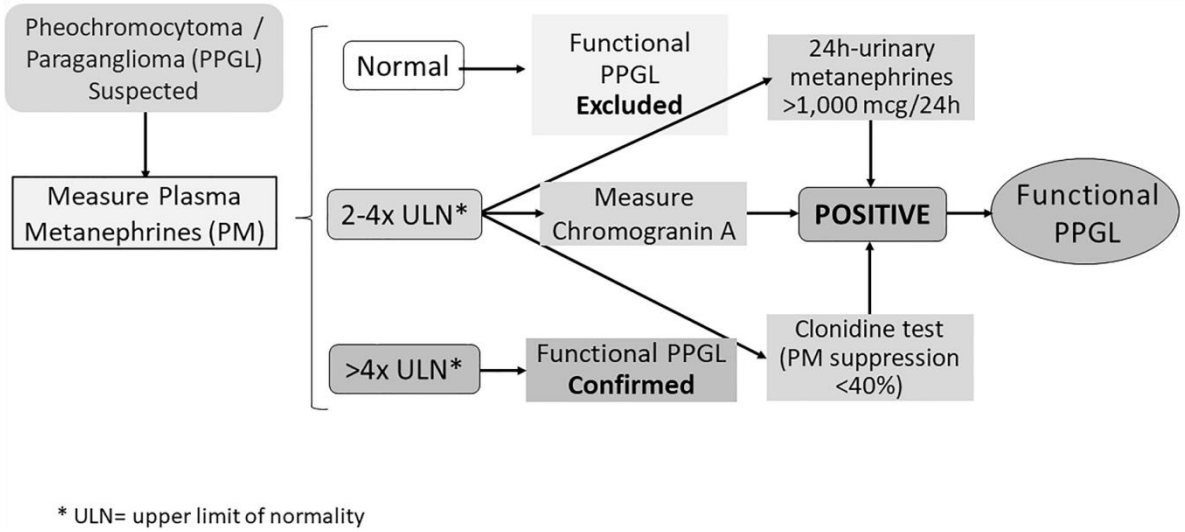
When plasma MN concentration is only 2-4 times above normal values, a clonidine test can be performed using plasma MN measurements at baseline and 3 hours after oral administration of 0.3 mg clonidine. Suppression below 40% suggests PPGL. Vanillylmandelic acid, urinary and plasma catecholamines, and the classic glucagon and clonidine tests using plasma catecholamine measurements are no longer used (7, 8, 12, 13, 29-33).

In Figure-1, we described a laboratory flowchart for the diagnosis of functioning PPGL.

Imaging / Localization Diagnosis

Localization of PPGL can be achieved by the following procedures (all employing specif-

Figure 1 - Flowchart for the diagnosis of functioning PPGL.



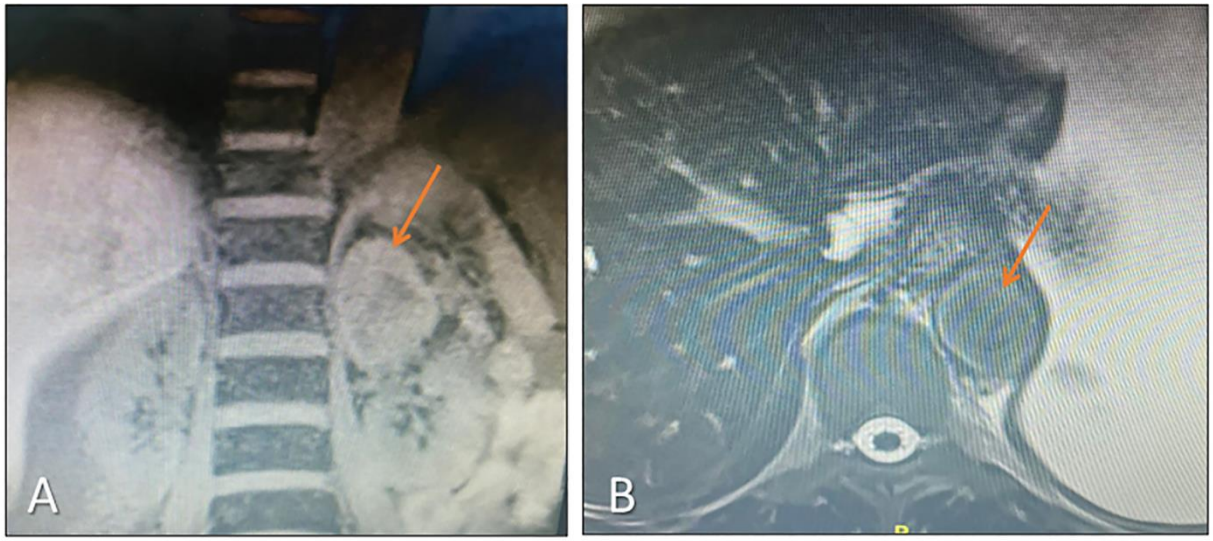
ic protocols for the adrenals): (1) magnetic resonance imaging (MRI) of the upper abdomen or whole body (when PGL is suspected), (2) computed tomography (CT) of the upper abdomen, (3) full body scintigraphy with ^{123}I/^{131}I-mIBG (metaiodo-benzylguanidine), (4) PET-CT with ^{18}FDG, and (5) PET-CT with ^{68}Ga DOTATATE, DOTATOC or DOTANOC.

Use of MRI for the diagnosis of PPGL has the following advantages: (1) high sensitivity (93100%) in detecting adrenal disease, (2) presence of a “hypersignal” in T2 sequence compared to the liver, in at least 75% of PPGL, (3) better sensitivity to localize intracardiac PGL, (4) possibility of visualization and confirmation of bone metastases suggested by mIBG scintigraphy, and (5) can be performed in pregnant women (second trimester on) (without contrast) and in children and carriers of germline variants, since there is no exposure to ionizing radiation. In Figure-2, we described the MRI with sporadic pheochromocytoma on the left adrenal with some typical features.

CT has a sensitivity of 93-100%, but low specificity (70%). Sensitivity is lower for small adrenal PPGL and for adrenal medullary hyperplasia. It is also less sensitive in the detection of PGL, small metastases and early recurrence of tumors in the adrenal surgical bed. CT is currently recommended as the first choice for topographic diagnosis of PPGL (11, 25, 30, 34, 36-38).

Figure 2 - Left adrenal pheochromocytoma in a 63 yo patient. A: 6.3 cm lesion showing a cleavage plan with necrosis (MRI, coronal section). B: Chemical shift does not show loss of signal in the out-of-phase sequence (MRI, axial section).

^{131}I-mIBG scintigraphy has diagnostic sensitivity and specificity of 77-90% and 95-100%, respectively. When ^{123}I is used instead, sensitivity reaches higher values: 83-100%, without loss of specificity. Its use should be considered in cases of adrenal Pheo that are suggestive

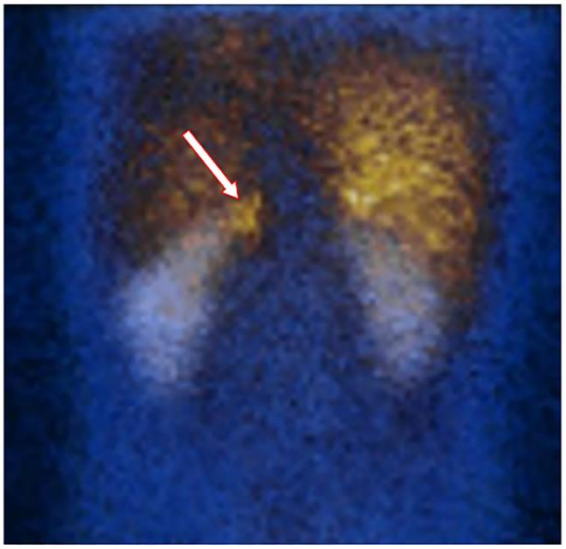


benignity. False negative results occur in 15% (approximately 60% of PGL are not avid for mIBG), and false positives can also occur, since 50% of normal adrenals have physiological uptake. The following are indications for pre-surgery mIBG: diagnostic confirmation, inconclusive biochemical results, familial disease, extra-adrenal tumors, and the possibility of treatment with therapeutic mIBG in metastatic PPGL. Post-surgical mIBG are indicated to search for disease recurrence and metastases (1, 5, 7-9).

^{18}FDG PET-CT is recommended for aggressive metastatic PPGL, lesions greater than 8 cm and those with PV in the *SDHB* gene. Sensitivity ranges from 74-100%. In Figure-3, we described a Coronal ^{123}I-MIBG scintigraphy showing increased focal radiotracer uptake in the left adrenal in a young male with a pheochromocytoma and MEN2B.

^{68}Ga PET-CT DOTATATE, DOTATOC or DOTANOC have high sensitivity and specificity for neuroendocrine tumors as well as for tumor dedifferentiation; its recommendations parallel those

Figure 3 - Coronal ^{123}I-MIBG scintigraphy (posterior image) showing increased focal radiotracer uptake in the left adrenal (arrow) in a young male with a pheochromocytoma and MEN2B.



of ^{18}FDG PET-CT (11,25,32,34). The histological concept of malignancy in PPGL is rather complex, since histological features of malignancy can be identified in “benign” PPGL, and histological absence of malignancy may be found in “malignant” tumors. Thus, malignancy is defined when there is evidence of distant metastasis; however, large Pheo (>8 cm), PGL with increased production of dopamine/methoxy-tyramine (dopamine metabolite) also suggest “malignancy”. Since 2018, the World Health Organization (WHO) has recommended the terms metastatic and non-metastatic, instead of malignant and benign PPGL (7,1 1, 12, 25, 32, 35-37).

The most used histological classification to aid in establishing malignancy potential is the PASS score (Pheochromocytoma of the Adrenal Gland Scaled Score) which considers the following items (Table-3):

Non-metastatic PPGL have a score ≤3 and those potentially more aggressive ≥4 points. To date, there is no stratification model that combines histological and genetic data.

In the GAPP system, histological classification is based on the scoring system composed of 6 parameters: histologic pattern, cellularity, necrosis, capsular/ vascular invasion, in association with immunohistochemistry (Ki-67) and hormonal secretion (production of noradrenaline or normetanephrine or associated with dopamine/methoxy-tyramine has 1 point), totaling 10 points. According to the GAPP system, patients are classified into 3 classes: 1) well differentiated: 0-2 points; 2) moderately differentiated: 3-6 points; and 3) poorly differentiated: 7-10 points (11,12,35).

Tumor immunohistochemistry for the succinate dehydrogenases, especially the investigation of *SDHB* is indicated, as the loss of its expression suggests a germline PV in the *SDHB* gene and implies greater aggressiveness; this analysis is part of COOPS (Composite

Pheochromocytoma/Paraganglioma Prognostic Score) system, in which necrosis (focal or confluent), loss of S100 expression, vascular invasion, loss of *SDHB* expression and size greater than 7 cm are evaluated. Scores greater ≥3 have a higher risk of metastasis (12, 35).

The 8th edition of the AJCC (American Joint Committee on Cancer) staging system includes a special chapter for PPGL, but not for parasympathetic PGL, as metastatic behavior is less than 5%. Pheo smaller than 5 cm in their longest axis and without vascular invasion are classified as T1;

Table 3 - The "Pheochromocytoma of the Adrenal Gland Scaled Score" or PASS score.

ITEMS CONSIDERED	PASS Score
Diffuse growth pattern or in "large nests"	2
Focal or diffuse necrosis	2
High cellularity	2
Cellular monotony	2
Tumor with spiculated cells	2
Mitotic index >3/10 large increase field	2
Atypical mitoses	2
Vascular invasion	1
Capsular invasion	1
Extension to adjacent adipose tissue	1
Intense nuclear pleomorphism	1
Nuclear hypercromasia	1

those ≥5 cm or sympathetic PGL of any size and without extra-adrenal invasion are classified as T2. PPGL of any size with invasion of surrounding tissues such as liver, pancreas, spleen and kidneys are classified as T3. Regarding lymph node involvement: Nx (without knowledge of involvement), N0 (without involvement of lymph nodes) and N1 (with involvement of regional lymph nodes). Regarding distant metastases, M0 (no distant metastases), M1a (distant metastases to bone only), M1b (distant metastases to distant lymph nodes/liver or lung) and M1c (distant metastases to bone and multiple other organs).

Classification is as follows: Stage I: T1N0M0 / Stage II: T2N0M0 / Stage III: T1N1M0, T2N1M0, T3 any N and M0 / Stage IV: any T, any N and M1 (12, 35).

Clinical treatment

Treatment of PPGL is surgical whenever possible since there is a possibility of reversal of SAH. In addition, complications of an untreated PPGL can be fatal and there is a chance of metastases in 15-17% of cases.

Preoperative clinical therapy for a minimum of 7-30 days (15 days, on average) is mandatory, aiming to prevent an intraoperative hypertensive crises and cardiac arrhythmias, and to avoid hypotension after tumor removal. The best drugs for this purpose are α-blockers, such as prazosin, doxazosin, and terazosin; phenoxybenzamine has been less accepted in Brazil, as it has a longer biological half-life (and should be withdrawn 48h preoperatively, leaving the patient a period of 2 days rather unprotected) and may produce reflex tachycardia after its withdrawal.

Prazosin and doxazosin are the most widely used drugs, in doses ranging from 1 to 16-20 mg per day. On average, 12 mg prazosin and 10 mg of doxazosin warrant good blood pressure control and prevention of paroxysms. Additionally, calcium channel blockers (amlodipine, diltiazen, verapamil and nifedipine) and angiotensin-converting enzyme (ACE) inhibitors may also be used. The use of β-blockers should be kept for when tachycardia and tachyarrhythmias are present, but always after effective control of hypertension with α-blockade; on average, β-blockers may be used after 3 days of the introduction of α-blockade.

α-Methyl-paratyrosine blocks the synthesis of catecholamines by inhibiting tyrosine hydroxylase, a key enzyme in the hormonal synthesis process; it can reduce catecholamine excretion by 35-80%. In general, it is recommended to treat SAH in patients with unresectable tumors or in those with metastases and in the preoperative period when there is no effective control with α-adrenergic blockers. Initial dose is 250mg 4x per day, a dose that can be adjusted every 3-4 days according to blood pressure response and possible side effects (sedation, psychiatric disorders, extrapyramidal symptoms, urolithiasis). The largest recommended dose is 4g/day (2, 8, 12, 15, 32).

SURGICAL TREATMENT

Only experienced surgeons and anesthesiologists should be responsible for the PPGL surgical procedure. The laparoscopic approach is preferred for tumor access, except for cases of suspected metastases and tumor size larger than 7 cm, conditions in which the classic open access is mandatory. Ideally, the entire immediate postoperative (post-op) period should be done in an intensive care unit (ICU), because even with adequate preparation there is a risk of arrhythmias and blood pressure instability, with the possibility of hypertensive crises and hypotension in the post-op period. There is still also a risk of hypoglycemia in the post-op, and installation of a 10% IV glucose solution is recommended for a period of 48h, with capillary glucose controls. The patient may remain hypertensive for a period of 2 weeks, after which a new 24h-plasma and/ or urinary MN measurement is recommended.

For PPGL patients with metastases, the target is to achieve tumor reduction and to control hypertension. Large PPGL can be reduced through surgery to obtain symptom relief and control of blood pressure levels; however, rarely will this surgery be curative, as there are often distant metastases, especially in bones (70%). Exceptionally, when metastases are restricted to the liver but are not surgically removable, transplantation will be an option. Tumor reduction can also be achieved by other interventional techniques such as transcatheter selective embolization or chemoembolization.

Thermal perfusion of the liver with cytotoxic drugs is used in some centers in cases of hepatic metastases.

Alternatives for surgical resection in cases of metastatic PPGL include radiotherapy (effective for bone pain), cryoablation, and radiofrequency thermal ablation (2, 8, 12, 15, 32).

Treatment with 131I-mIBG

The use of radiolabeled mIBG in metastatic PPGL therapy should be considered, as mIBG may cross the cell membrane and be stored in cytoplasmic granules via VMS transporters (VMAT1 and 2). Since 1984, several patients with PPGL have been treated using different therapeutic protocols. Such patients are selected by demonstrating significant uptake of the radioisotope during a scintigraphy with $^{123}I/^{131}I$ mIBG.

The only impediment to this treatment is the total dose of radiation delivered to vital organs, such as bone marrow. Approximately 60% of metastases are avid for ^{131}I-mIBG. Recently, quantitative determination of VMAT 1 and 2 expression in surgical specimens proved useful in selecting patients suitable for treatment with ^{131}ImIBG (12, 36, 37).

A review of 116 patients treated with 100 to 300 mCi of ^{131}I-mIBG per session (mean of 3 doses at intervals of 3-14 months) showed tumor shrinkage in 30% of patients, disease stabilization in 57% and progression in 13%. A positive hormonal response ranged from 15 to 45%. (12, 24, 36-38).

In general, patients with limited disease have an increased chance of tumor response. Similarly, soft tissue metastases respond better than bone metastases. Hormonal and symptomatic responses to ^{131}I-mIBG are independent of tumor size response (36-38).

Major side effects include transient leukopenia and thrombocytopenia. Myelosuppression, infections, and liver failure are rare occurrences in patients with spread liver metastases (11, 12, 36-38).

Treatment with radioactive somatostatin analogues

Due to the expression of somatostatin receptors in metastatic PPGL, the use of radiopharmaceuticals (RP) based on somatostatin analogues has been tested.

Several RP with different physical properties is employed, including octreotide-^{111}In-DOTA /pentreotide-^{111}In-DOTA, octreotide-^{90}Y-DOTA-, octreotate-^{177}Lu-DOTA, plus lanreotide-^{111}In-DOTA and lanreotide-^{90}Y-DOTA (25, 28, 32).
Patients who will benefit from treatment are those who have an increased tumor uptake on scintigraphy (currently ^{68}Ga PET-CT with DOTATATE, DOTATOC or DOTANOC).

Stabilization or decrease in hormonal secretion and tumor growth have been reported in 20-25% of cases. Main side effects include leukopenia and thrombocytopenia.

Treatment with unlabeled octreotide is generally unsuccessful and only in some patients a transient response was observed because they express a low density of subtype 2 somatostatin receptors (SST2) (7, 11, 12).

Chemotherapy

Chemotherapy (QT) is an option when the tumor is inoperable and/or there is extensive residual disease. The combination of cyclophosphamide, vincristine and dacarbazine (CVD) may provide partial remission and transient symptomatic relief in up to 50% of patients with metastatic PPGL, although short-lived (1, 7, 9, 11, 12, 36, 37).

Other QT options are etoposide and cisplatin, anthracycline plus CVD and arabinoside cytokine. Some authors suggest a combination of lomustine and 5-fluorouracil or capecitabine for tumors with slow progression, whereas for rapidly progressive tumors, the best option would be the association of etoposide with a drug based and platinum (7, 9, 11, 12, 36, 37).

New and emerging therapies

New antineoplastic therapies are being tested in patients with metastatic PPGL. The combination of temozolomide and thalidomide provided biochemical and radiological responses in 40 and 33% of the cases, respectively; however, lymphopenia accompanied by opportunistic infections occurred in most patients.

Other therapeutic options include 17-alilamine protein inhibitors (17-demethoxy-geldanamycin), mTOR inhibitors (everolimus), tyrosine-kinase inhibitors with anti-VEGF activity, antiangiogenic factors, gene therapy, etc. Lutetium-octreotate has relatively few side effects and can complement the effect of 131ImIBG for small lesions or micro-metastases (7, 9, 11, 12, 36, 37).

Follow-up

Patients with PPGL should undergo annual reevaluations by measuring urinary or plasma MN and chromogranin A. Follow-up is for life. When there is clinical and laboratory recurrence, with no radiological evidence, a new full body scintigraphy should be performed with ^{123}I/^{131}I-mIBG, or ^{68}Ga PET-CT with DOTATE, DOTATOC or DOTANOC or ^{18}FDG-PET-CT (11-13). There are specific follow-up protocols for some genetic syndromes such as MEN2A and 2B, Von Hippel Lindau syndrome and familial paraganglioma syndrome (10, 12, 22, 25-28).

CONCLUSIONS

The PPGL syndrome is a rare condition, but the improvement of catecholamine metabolite assays and topographic/functional location procedures, have helped to demonstrate its actual higher incidence. Recognizing and treating hypertensive patients with PPGL is extremely important, avoiding serious cardiovascular complications, metastases and death.

The importance of genetics in PPGL is essential nowadays, with more PPGL-related genes being discovered, which allows better treatment strategies, monitoring of genetic syndromes related to PPGL, and familial counselling.

The treatment and follow-up of PPGL should be carried out by a multidisciplinary team with experience in this disease, composed of endocrinologists, radiologists, radiointerventional physicians, nuclear physicians, anesthesiologists, geneticists, urologists/oncological surgeons, head and neck surgeons/ neurosurgeons (for head and neck PGL), thoracic surgeons (for thoracic PGL), intensive care physicians, pathologists, clinical oncologists, radiotherapist physicians and psychologists.

CONFLICT OF INTEREST

None declared.

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Article 2:

“Germline genetic variants in pheochromocytoma/ paraganglioma: Single-center experience”

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Abstract

Pheochromocytoma/paraganglioma (PPGL) are rare neuroendocrine tumors carrying 25-40% pathogenic germline gene variants (PGV). We evaluated clinical, laboratory, and germline molecular profile of 115 patients with pathologic (fourteen patients were relatives from 8 different families recruited for genetic survey) confirmed PPGL followed in our institution. Patients with classic MEN2A/MEN2B phenotypes and at-risk relatives underwent direct analysis of *RET* proto-oncogene, and the remained had samples submitted to complete Next Generation Sequencing (NGS) aiming 23 PPGL-related genes: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD,*

TMEM127, TP53, and *VHL.* . We also developed a clinical judgment score (CJS) to determine the probability of patients having a potentially hereditary disease. The resulting genetic landscape showed that 67 patients (58.3%) had variants in at least one gene: 34 (50.7%) had exclusively pathogenic or likely pathogenic variants, 13 (19.4%) pathogenic or likely pathogenic variants and VUS and 20 (29.8%) carried only VUS. PGV were found in *RET* (N=18; 38.3%), *VHL* (N=10; 21.3%), *SDHB* and *NF1* (N=8; 17% each), and *MAX*, *SDHD*, *TMEM127,* and *TP53* (N=1; 2.1% each). Direct genetic testing disclosed 91.3% sensitivity, 81.2% specificity, and 76.4% and 93.3% positive (PPV) and negative (NPV) predictive values, respectively. The CJS to identify patients who would not benefit from genetic testing had 75% sensitivity, 96.4% specificity, and 60% and 98.2% PPV and NPV, respectively. In summary, the landscape of PPGL germline gene variants from 115 Brazilian patients resulted in slightly higher prevalent pathogenic and likely pathogenic variants, especially in the *RET* gene. We suggest a CJS to identify PPGL patients who would not require initial genetic evaluation, improving test specificity and reducing costs.

RESEARCH

Germline genetic variants in pheochromocytoma/paraganglioma: single-center experience

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Abstract

Pheochromocytoma and paragangliomas (PPGLs) are rare neuroendocrine tumors carrying 25–40% pathogenic germline gene variants (PGVs). We evaluated clinical, laboratory, and germline molecular profile of 115 patients with pathologic (14 patients were relatives from 8 different families recruited for genetic survey) confirmed PPGL followed in our institution. Patients with classic MEN2A/MEN2B phenotypes and at-risk relatives underwent direct analysis of *RET* proto-oncogene, and the remaining had samples submitted to complete next-generation sequencing aiming 23 PPGL-related genes: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53,* and *VHL*. We also developed a clinical judgment score (CJS) to determine the probability of patients having a potentially hereditary disease. The resulting genetic landscape showed that 67 patients (58.3%) had variants in at least one gene: 34 (50.7%) had exclusively pathogenic or likely pathogenic variants, 13 (19.4%) had pathogenic or likely pathogenic variants and variant of undetermined significance (VUS), and 20 (29.8%) carried only VUS. PGVs were found in *RET* (*n* = 18; 38.3%), *VHL* (*n* = 10; 21.3%), *SDHB* and *NF1* (*n* = 8; 17% each), and *MAX*, *SDHD*, *TMEM127,* and *TP53* (*n* = 1; 2.1% each). Direct genetic testing disclosed 91.3% sensitivity, 81.2% specificity, and 76.4% and 93.3% positive predictive value (PPV) and negative predictive values (NPV), respectively. The CJS to identify patients who would not benefit from genetic testing had 75% sensitivity, 96.4% specificity, and 60% and 98.2% PPV and NPV, respectively. In summary, the landscape of PPGL germline gene variants from 115 Brazilian patients resulted in slightly higher prevalent pathogenic and likely pathogenic variants, especially in the *RET* gene. We suggest a CJS to identify PPGL patients who would not require initial genetic evaluation, improving test specificity and reducing costs.

Key Words

- SDHB
- VHL
- RET
- NF1
- TMEM127
- MAX
- SDHD

Introduction

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that produce, store, and secrete catecholamines (Sutton *et al.* 1981, Karagiannis *et al.* 2007, Neumann *et al.* 2019, Al Subhi *et al.* 2022). The outdated general 'rule of 10%', which was formerly applied for genetic behavior for PPGL (Sutton *et al.* 1981, Bravo & Tagle 2003) also used to include inheritance. However, this rule no longer stands. Regarding genetic behavior, 25–40% of PPGL carriers have germline variants (Lenders *et al.* 2014, Garcia-Carbonero *et al.* 2021); for paragangliomas (PGLs) alone, there is a 50% chance of finding a pathogenic variant (PV) (Antonio *et al.* 2020), a rate greater than that for any other human neoplasm (Toledo *et al.* 2017).

Currently, nearly 70% of PPGLs result from germline or somatic PVs in a single driver gene. To date, there are more than 20 genes related to PPGLs, and more new genes are being discovered (7–10).

The genomic characteristics of PPGLs allow us to allocate them into three clusters according to their transcriptional signature (Buffet *et al.* 2017, Toledo *et al.* 2017, Jiang *et al.* 2020, Garcia-Carbonero *et al.* 2021, Cascon *et al.* 2023, Giminez-Roqueplo *et al.* 2023).

Cluster 1 includes genes related to the Krebs cycle. Characteristic genes are *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *FH*, *MDH2*, *GOT2*, *IDH1*, *SCLC25A11*, *EPAS1*, and *VHL*. The presence of germline or somatic PVs in these genes leads directly or indirectly to deregulaton in *HIF1a* and *HIF2a*, generating pseudohypoxia and increasing angiogenesis and cell proliferation (Buffet *et al.* 2017, Toledo *et al.* 2017, Jiang *et al.* 2020, Garcia-Carbonero *et al.* 2021).

Cluster 2 has signature activation of MAP kinase signaling pathways. The following genes stand out: *NF1, RET, HRAS,* and *TMEM127* (9–11).

Cluster 3 (identified by The Cancer Genome Atlas) has WnT pathways based on a transcriptional signature, which also increases the risk of metastatic PPGLs.

The aim of this study was to evaluate the germline molecular profile of a cohort of patients with PPGL from a single center in Sao Paulo, SP, Brazil.

Material and methods

Study design and participants

This prospective and retrospective study included 115 PPGL patients followed at the Endocrine Division, Universidade Federal de Sao Paulo (Unifesp), Brazil, who were evaluated due to the presence of (i) adrenal/extra- adrenal tumors and/or (ii) secondary arterial hypertension and/or (iii) because they had first-degree relatives with PPGLs. Fourteen patients were relatives from eight different families recruited for genetic survey.

Clinical, laboratory, and genetic data were collected between February 2000 and December 2019. The inclusion criterion was pathologic confirmation of PPGL.

The study was approved by the Ethics and Research Committee of Unifesp. All patients or their legal responsible signed an informed written consent form. Patients were distributed into two groups, named as 'potentially hereditary' (group 1) and as 'apparently sporadic' (group 2). The characteristics that defined the potentially hereditary cases are shown in Table 1. Patients presenting at least one of the characteristics suggestive of hereditary behavior entered group 1 (n = 91 patients) and those who did not present any of those characteristics comprised group 2 (n = 24 patients).

Additionally, we developed a clinical judgment score (CJS) (Table 2) to determine the probability of a patient having a potentially hereditary disease.

We accessed the Fleury laboratory database of uncharacterized patients who underwent a genetic panel by next-generation sequencing (NGS) for PPGL from January 2018 through July 2022 and collected data from 105 unrelated patients. The Fleury Group operates in the four main regions of Brazil – Northeast, Midwest, Southeast, and South. Patients underwent the exam because they had a PPGL.

Data collection

The following data were collected from each patient: age; sex; family history of PPGL; presence of syndromic presentation; presence of comorbidities; use of

Table 1 Clinical, laboratory, and imaging features suggestive of germline pathogenic variants.

	Potentially hereditary
Associated diseases	Medullary thyroid carcinoma, primary hyperparathyroidism, neurofibromatosis, middle ear tumors, central nervous system hemangioblastoma, pancreatic neuroendocrine tumor, gastrointestinal stromal tumor (GIST), clear cell renal carcinoma, pituitary adenoma, Hirschsprung's disease
Clinical, laboratory, and imaging features	Skin pigmentation (*cafe-au-lait* spots), precocious puberty, marfanoid habitus, mucosal neuromas, retinal angiomas, renal and/or pancreatic cysts, polycythemia, intestinal ganglioneuromatosis, megaesophagus, megacolon, and amyloidotic cutaneous lichen

Table 2 Clinical judgment score.

Clinical score	**Points**
<20 years old	Mandatory genetic test
Any paraganglioma (PGL), regardless of age	
Classic clinical picture of NF1, VHL, MEN2A, MEN2B, or familial PGL syndrome	
20–45 years of age	3
Family history of PPGL	3
Presence of bilateral masses	2
Presence of metastasis	1
Total	9

antihypertensive medications; measurements of urinary and plasma metanephrines and catecholamines, vanillylmandelic acid (VMA), and chromogranin A; imaging findings of the abdomen, chest, and neck; pathologic results; and genetic testing (germline genetic panel by NGS for PPGL or Sanger genetic screening of the *RET* proto-oncogene, if the case had a typical MEN2A or 2B presentation). Somatic assessment was not performed at initial assessment and will not be discussed in this paper. The results of the genetic tests were classified as pathogenic, likely pathogenic, or variant of undetermined significance (VUS) according to the American College of Medical Genetics (ACMG) (Richards *et al.* 2015). Benign findings were not reported.

Genetic testing

Patients with the classic MEN2A or MEN2B phenotypes and their at-risk relatives underwent direct analysis of the *RET* proto-oncogene at the Laboratory of Molecular and Translational Endocrinology at Unifesp. DNA extraction and PCR amplification of genomic DNA were prepared from blood leukocytes according to standard protocols. Oligonucleotide primers for the amplification of different *RET* exons were designed at intronic sequences flanking exons 8, 10, 11, 13, 14, 15, and 16. PCRs were performed in a final volume of 25 µL containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate, 1 U Taq polymerase, and 1 mM specific primers and using 100 or 200 ng of genomic DNA as input. Genomic DNA was denatured for 3 min at 94°C before 35 cycles of 1 min each at 94°C, at 65°C, and at 72°C, followed by 5 min at 72°C in a thermocycler (Corbett Research, Qiagen Ltd, Sydney, Australia). Following PCR, the amplicon sizes were analyzed on a 1.8% agarose gel, and the products were visualized by staining with GelREd® (Uniscience Ltd, Osasco, SP, Brazil). PCR products were purified with a commercial kit (Life Technologies, Inc.)

and sequenced using an automated system employing fluorescent dye terminators (ABI Prism 3100, Applied Biosystems) (Lindsey *et al.* 2012).

Variant analyses were performed based on the medullary thyroid carcinoma guidelines by the American Thyroid Association (Wells *et al.* 2015, Thomas *et al.* 2019) and scored according to the evidence evaluated (prediction, literature, and information deposited in Arup, ClinVar, PubMed, and Varsome).

Patients who did not have the classic MEN2A or MEN2B phenotypes had their samples submitted to a genetic panel by NGS at Fleury Group laboratory with the complete sequencing of all coding region and adjacent flanking regions of 23 genes related to PPGL. The analysis included identification of pathogenic genetic variants and copy number variations by NGS.

DNA was extracted from peripheral blood leukocytes using the QIAsymphony DNA Mini Kit according to the manufacturer's recommendations: 200 ng of DNA were used for library preparation following the protocol for Sophia Genetics' Custom Bundle Solution. Capture-based target enrichment was performed on pools of up to eight samples using a custom panel for analysis of full-coding sequencing and flanking splice sites of 23 PPGL-related genes.

Sequencing was performed using a NextSeq® 500/550 Mid Output Kit v2 (300 cycles) on a NextSeq® platform (Illumina). The mapping of reads to the human reference genome GRCh37/hg19 and the calling of variants were performed by a custom bioinformatics pipeline, and the annotation and interpretation of variants were performed on the Sophia DDM® platform (Sophia Genetics, Boston, USA; https://www.sophiagenetics.com/).

Average coverage for targeted region was 722.5× of depth (minimum of 357× and maximum of 1788×). For the majority of the samples, we had ≥99.98% of the target region with minimum of 50× of depth of coverage (minimum 99.3% and maximum of 100%).

We used the scores from the association of the ACMG (American College of Medical Genetics and Genomics) and the ACGS (American Clinical Genomic Science) Best Practice Guidelines for Variant Classification 2019 for the analyses.

We used Emedgene® software with all information relevant to the criteria and scored the reads according to the evidence evaluated (prediction, literature, and information deposited in PubMed, Arup, ClinVar, and Varsome).

The 23 genes analyzed were *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK,*

MET, NF1, PIK3CA, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53, and *VHL*.

The genetic analysis of 47 of the 105 controls (44.7%) was performed by the same platform Sophia DDM®, whereas the remaining 58 (55.3%) were investigated using the platform Twist Bioscience®; the average coverage for targeted region was 752.5× of depth (minimum of 411.75× and maximum of 1065×). For the latter 58 samples, we had 100% of the target region with minimum of 50× of depth of coverage (minimum 99.6% and maximum of 100%).

Hormonal testing

Hormonal evaluation for the biochemical diagnosis of PPGL was performed with 24-h urinary metanephrines, 24-h urinary catecholamines, 24-h urinary VMA, plasma metanephrines, and plasma catecholamines. As of 2014, and in accordance with the Endocrine Society's PPGL Guideline (Lenders *et al.* 2014), we only performed tests for plasma and/or 24-h urinary fractionated metanephrines and VMA, whereas urinary and plasma catecholamines were discontinued.

Statistical testing

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (Released 2019; IBM Corp, Armonk, NY, USA). Descriptive analysis included absolute (*n*) and relative (%) frequencies and bar graphs of qualitative variables and summary measures (mean, standard deviation, median, minimum, and maximum). Inferential analysis included the association test using the chi-square or Fisher's exact test and the logistic regression-stepwise forward method and receiver operating characteristic (ROC) curve analysis to evaluate possible cutoff points for quantitative variables, complemented by the calculation of sensitivity and specificity. A significance level of 5% was adopted for all tests.

Results

Patients, age, clinical presentation, time of presentation

The median age of the patients was 44.5 years (range 14–79); 74 patients were women and 41 were men.

Ninety-one patients (79.1%) satisfied the criteria that suggested PPGL was potentially hereditary (group 1) and 24 (20.9%) did not (group 2). In groups 1 and 2, 63.4% and 66.6% of the patients were women.

The association of systemic arterial hypertension (SAH) + paroxysms and orthostatic hypotension was present in 16 (34%) of genetically confirmed cases, followed by SAH + paroxysms in 3 (6.4%), orthostatic hypotension in 4 (8.5%), isolated SAH in 6 (12.8%), SAH + orthostatic hypotension in 8 (17%), isolated paroxysms in 3 (6.4%), and orthostatic hypotension + paroxysms in 1 (2.1%). Six (12.8%) of the patients were normotensive.

Distributions of germline variants

The genetic landscape of the 115 PPGL patients submitted to the genomic study showed that 67 (58.3%) had a germline variant in at least 1 of the 23 PPGL-related genes studied: 34/67 (50.7%) had exclusively pathogenic or likely PVs, 13 (19.4%) pathogenic or likely PVs and VUS, and 20 (29.8%) carried only VUS. This group included germline variants in the following genes: *RET* (18; 38.3%), *VHL* (10; 21.3%), *SDHB,* and *NF1* (8; 17% each*)* and *MAX*, *SDHD*, *TMEM127,* and *TP53* (1; 2.1% each). The VUS group (n = 42 germline variants (GVs)) included GVs in the following genes: *ATM* (9; 21.4%), *KMT2D* (6; 14.2%), *MERTK* (5; 11.9%), *SDHC*, *MET*, *RET*, and *FH* (3; 7.1% each), *SDHA* and *NF1* (2; 4.7% each), and *ATR*, *CDKN2A*, *SDHAF2*, *KIF1B*, *MDH2*, and *MAX* (1; 2.4% each).

Since 14 subjects from 115 PPGL patients were relatives from eight different families, we recalculated germline variants distribution exclusively from the 101 PPGL index cases. This information is presented in Figs. 1 and 2.

A table with VUS cases is provided as a supplementary material (see section on supplementary materials given at the end of this article). We did not perform segregation or functional studies of VUS cases, but all patients who were followed up in the Adrenal Unit at Unifesp were reassessed regularly.

In the sample of 105 PPGL individuals from the Fleury Group lab, PVs were present in 22 (21%) individuals and VUS in 35 (33.3%), whereas 56 (53.3%) subjects did not have any variants (Fig. 1). The PV distribution was as follows: 5 (22.7%) in each *SDHB*, *VHL*, and *RET*, followed by 2 (9%) in each *SDHD* and *MERTK*, and 1 (5.7%) in each *ATM*, *NF1*, *SDHA*, and *TMEM127*. The VUS distribution was as follows: 8 (22.8%) *ATM*, 5 (14.3%) *KMT2D*, 4 (11.4%)in each *MERTK* and *SDHA*, 2 (5.7%) in each *NF1, FH*, *RET*, and *CDKN2A*, and 1 (2.8%) in each *ATR, KIF1B, SDHC, TMEM127, SDHB,* and *MET*.

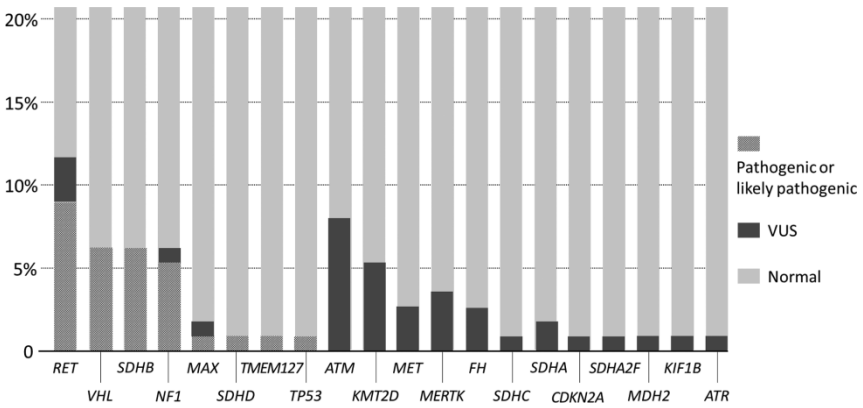

Figure 1

Genetic distribution of 101 PPGL patients (excluding 14 relatives) and 105 PPGL controls according to ACMG criteria.

Regarding age distribution, none of the patients were in the first decade of life. For patients in the second decade of life, 50% had PV in the *RET* gene, 25% in *VHL,* and 25% in *SDHB* genes. For patients in the third decade of life, the incidence of PV was highest for the *VHL* and *SDHB* genes (28.6% each), followed by *RET*, *MAX*, and *NF1* genes (14.2% each). For patients in the fourth decade of life, the incidence of PV was highest for the *RET* gene (36.3%), followed by *VHL* and *SDHB* (18.2% each) and the *SDHD*, *TMEM127*, and *NF1* genes (9.1% each). For patients in the fifth decade of life, PV in the *RET* gene predominated (61.5%), followed by *VHL* and *NF1* (15.4%, each) and the *SDHB* gene (7.7%). For patients in the sixth decade of life, PV in the *NF1* gene predominated (66.6%), followed by the *RET* and *VHL* genes (16.7% each). For patients in the seventh decade of life, 50% had PV in *SDHB* and 50% in *VHL* genes.

Table 3 shows the general characteristics of the PPGL

cohort and Table 4 shows the genetic (B) characteristics of the PPGL cohort.

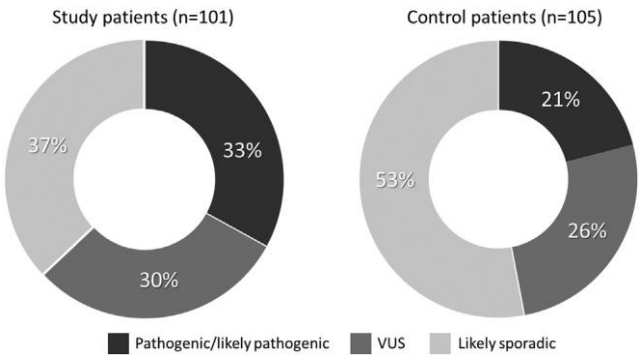

Figure 2

Distribution of 72 germline variants in 101 PPGL patients (excluding 14 relatives).

Topographic distribution and metastases

In the confirmed hereditary patients, 34% had bilateral PPGL, 34% had right adrenal PPGL, 15% had retroperitoneal PGL, 8.5% had left adrenal PPGL and 8.5% had neck PGL. Among the bilateral PPGL cases, we identified germline PVs in *RET* (56.2%), *VHL* (37.5%), and *NF1* (6.3%) genes (Table 3).

Six patients (12.7%) with germline PV had metastatic PPGL: four had PV in *SDHB*, one in *VHL*, and one in *NF1* genes. The most frequent sites of metastasis were lymph nodes (n = 5), bone (n = 5), liver (n = 4), blood vessels (n = 4), and lungs (n = 3). Three patients with metastatic PPGL (two with PV in *SDHB* and one with PV in *NF1*) died because of tumor progression. Table 3 also shows individual imaging procedures for PPGL diagnosis and time of follow-up for each patient.

Clinical judgment score

Using the criteria for direct genetic testing, we found a sensitivity of 91.3%, specificity of 81.2%, positive predictive value (PPV) of 76.4%, negative predictive value (NPV) of 93.3%, and accuracy of 85.2%.

When the CJS was used to identify patients who did not benefit from the genetic test using a cutoff of ≥4 points, we found a sensitivity of 75%, specificity of 96.4%, PPV of 60%, NPV of 98.2%, and accuracy of 95%.

This clinical score developed from our cohort of patients was useful to exclude those who would not benefit from genetic testing.

The following characteristics were statistically significant: age, bilateral masses, classic PPGL-related disease phenotypes, and family history of PPGL. Metastasis and topography (adrenal or extra-adrenal) were not statistically significant. On logistic regression, the association with the greatest statistical power (to

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Table 3 General characteristics of the genetic PPGL cohort.

Family	Positive cases		Age at diagnosis (years)	PPGL[a] (with metastasis[g])	Hormonal profile[b]	Imaging[c]	Years of follow-up
	With PPGL	Without PPGL					
01	1	–	22	Unilateral Pheo	NMN + MN	CT	14
	3		45	Bilateral Pheo		MRI + MIBG	
			19				
			14				
02	3	–	44	Unilateral Pheo	NMN + MN	MRI + MIBG	12
			40				
			32				
	2		44	Bilateral Pheo			
			52				
03	2	–	49	Unilateral Pheo	NMN + MN	CT + MIBG	14
			18				
04	1	–	50	Unilateral Pheo	NMN + MN	MRI + MIBG	10
05	1	–	35	Unilateral Pheo	NMN + MN	MRI + MIBG	10
06	1	–	17	Unilateral Pheo	MN	MRI + MIBG	10
07	1	1	42	Bilateral Pheo	NMN + MN	MRI + MIBG	11
08	1	–	45	Unilateral Pheo	MN	MRI + MIBG	12
09	1	–	34	Bilateral Pheo	MN	MRI + MIBG	3[f]
10	1	1	37	Bilateral Pheo	NMN + MN	MRI + MIBG	5
11	3	–	45	Bilateral Pheo	NMN	MRI + MIBG	15
			40				
			18				
12	1	5	38	Unilateral Pheo	Nonfunct.[f]	MRI + MIBG	4
13	1	–	21	Abdominal PGL [#]	NMN	MRI + MIBG	17
	1		17				13
14	1	–	48	Unilateral Pheo	NMN	MRI + MIBG	14
15	1	–	23	Unilateral Pheo	NMN	MRI + MIBG	15
16	1	1	33	Bilateral Pheo	NMN	MRI + MIBG	13
17	1	1	38	Bilateral Pheo	NMN	MRI + MIBG	7
18	1	2	62	Neck PGL[#]	Nonfunct.	MRI + MIBG	15
19	1	–	50	Unilateral Pheo	NMN	MRI + MIBG	14[f]
20	1	8	17	Abdominal PGL[#]	NMN	MRI + MIBG	5
	1		39	Neck PGL	Nonfunct.		7
21	1	–	23	Abdominal PGL [#]	NMN	MRI + MIBG	12
22	1	–	28	Abdominal PGL	NMN	MRI + MIBG	12
23	1	–	32	Abdominal PGL	NMN	MRI + MIBG	13
24	1	–	17	Neck PGL	Nonfunct.	MRI + MIBG	4
25	1	–	59	Unilateral Pheo	NMN + MN	MRI + MIBG	14
26	1	–	33	Unilateral Pheo [#]	NMN + MN	MRI + MIBG	24[f]
27	1	–	52	Unilateral Pheo	Nonfunct.	MRI + MIBG	13
28	1	–	31	Unilateral Pheo	NMN + MN	MRI + MIBG	13
	1		53	Bilateral Pheo	Nonfunct.		
29	2	–	49	Unilateral Pheo	NMN + MN	MRI + MIBG	11[f]/15
			29				

(*Continued*)

Table 3 Continued.

Family	Positive cases		Age at diagnosis (years)	Hormonal profile[b]	PPGL[a] (with metastasis[g])	Imaging[c]	Years of follow-up
	With PPGL	Without PPGL					
30	1	–		NMN + MN	Unilateral Pheo	MRI + MIBG	15
		1	60		Neck PGL	MRI + MIBG	
31	1	–		Nonfunct.	Abdominal PGL	MRI	8
		1	39	NMN + MN	Unilateral Pheo		
32	1			NMN + MN		CT + MIBG	12
		1	22				
			32		Unilateral = 21		21
				NMN+MN= 23		MRI + MIBG= 41	
					Bilateral = 15	CT + MIBG=4	

[a]PPGL, pheochromocytoma (= Pheo; adrenal)/paraganglioma (= PGL; extra-adrenal).

[b]Hormonal profile: MN, metanephrines; NMN, normetanephrines.

[c]Imaging: CT, computerized tomography, MIBG, metalodo-benzyl-guanidine; MRI, magnetic resonance imaging.

[d]Index case and one relative (without PPGL) with PV in the *TMEM127* and *TP53* genes and one relative with PV in the TMEM127 gene only.

[e]*MAX, SDHD,* and *TMEM127/TP53.*

determine whether to perform the genetic test) was age ≤45 (P = 0.016 with odds ratio (OR) of 5.9 (1.4–25)), classic PPGL-related disease phenotypes (P < 0.001 with OR of 189 (34.4–1,038.3)), and paraganglioma (P < 0.001 with OR of 8.3 (1.0–39.3)). Family history, bilateral masses, and metastasis were not statistically significant in the logistic regression model.

The age cutoff of 45.5 years disclosed a sensitivity of 70.2% and a specificity of 54.4% (area under the curve of 0.694; P < 0.001) for the diagnosis of potentially genetic PPGL, as per ROC curve analysis.

Correlation of germline variants and metanephrines

Hormone production (urine and/or plasma metanephrines and normetanephrines) correlated with germline PV, as shown in Table 3. Note that cases with PVs in the *RET, NF1, MAX,* and *TMEM127* genes had a predominant adrenergic profile, whereas cases with PVs in *VHL* and *SDHB* had an exclusive noradrenergic profile.Discussion

The incidence of certain affected genes in PPGL is higher according to the population studied. The Chinese population, for instance, has fewer germline variants than Europeans. *SDHx* genes predominate among the European population (Bausch *et al.* 2017, Jiang *et al.* 2020).

Our study evaluated the profile of germline PPGL gene variants from 115 patients from a single reference center in São Paulo, Brazil. This population sample originated in part from an outpatient clinic that is specialized in MEN; this may have produced some reference bias, resulting in a higher PV frequency of the *RET* gene; on the other hand, it is also possible that the prevalence of *RET* gene PV in the Brazilian population is actually higher, as was also observed with the control sample from the Fleury Group databases, which encompasses patients from the four major regions of Brazil. Some reports have demonstrated that the *RET* PV profile may vary according to geographical area (Pena *et al.* 2009, Pena *et al.* 2011, Maia *et al.* 2014, Bausch *et al.* 2017, Cunha *et al.* 2017, Maciel *et al.* 2019, 2021). Further studies should be carried out to clarify this issue. Neumann *et al.* highlighted a greater incidence of PV in the *VHL* gene in the first decade of life, which decreases in the third decade of life but remains one of the most important genes found. From the second decade of life onward, the incidence of PV in the genes of the *SDHx*

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Table 4 Genetic characteristics of the PPGL cohort.

Family	Patients with PPGL	Gene	Familial syndromes	Other tumors	Nucleotide (NT)	Protein
01	4	*RET*	MEN2A	MTC / PHP	c.1900T>G	p. Cys634Gly
02	5	*RET*	MEN2A	MTC / PHP	c.1900T>G	p. Cys634Gly
03	2	*RET*	MEN2A	MTC	c.1901G>A	p. Cys634Tyr
04	1	*RET*	MEN2A	MTC	c.1901G>A	p. Cys634Gly
05	1	*RET*	MEN2A	MTC	c.1900T>C	p. Cys634Arg
06	1	*RET*	MEN2B	MTC	c.2753T>C	p. Met918Thr
07	1	*RET*	MEN2A	MTC	c.1859G>C	p. Cys620Ser
08	1	*RET*	MEN2B	MTC	c.2753T>C	p. Met918Thr
09	1	*RET*	MEN2B	MTC	c.2753T>C	p. Met918Thr
10	1	*RET*	MEN2A	MTC/PHP	c.1900T>C	p. Cys634Arg
11	3	*VHL*	VHL2A	Pancreatic NET (1)/retinal angioma (2)/cerebellum HB (2)	c.496G>T	p. Val166Phe
12	1	*VHL*	VHL2C		c.256C>T	p. Pro86Ser
13	2	*VHL*	VHL2C		c.467A>G	p. Tyr156Cys
14	1	*VHL*	VHL2B	Pancreatic NET/CCRC	c.233A>G	p. Asn78Ser
15	1	*VHL*	VHL2C		c.239G>T	p. Ser80Ile
16	1	*VHL*	VHL2B	Pancreatic NET+retinal angioma+cerebellum HB+CCRC	c.499C>T	p. Arg167Trp
17	1	*VHL*	VHL2A	Cervical HB	c.293A>G	p. Tyr98Cys
18	1	*SDHB*	PGL4	PHP	c.293G>A	p. Cys98Tyr
19	1	*SDHB*	PGL4		c.591del	p. Ser198Alafs*22
20	2	*SDHB*	PGL4	GIST (1)	c.137G>T	p. Arg46Leu
21	1	*SDHB*	PGL4		Del involving exon 1, identified by CNV	
22	1	*SDHB*	PGL4		c.3G>A	p. Met1?
23	1	*SDHB*	PGL4		Del involving exon 1, identified by CNV	
24	1	*SDHB*	PGL4		c.724C>A	p. Arg242Ser
25	1	*NF1*	NF1		c.1260+1G>A	p. (?)
26	1	*NF1*	NF1		c.5487_5490 dup	p. Gly1831Profs*11
27	1	*NF1*	NF1		c.4537C>T	p. Arg1513*
28	2	*NF1*	NF1		c.1527+4_1527+7 del (Del of 4 NT at position +4 (intron 13)	Affects a NT from the splicing donor site that possibly alters the processing of messenger RNA by skipping exon 13
29	2	*NF1*	NF1		c.6999 +2 T>G	p. (?)
30	1	*NF1*	NF1		c.7248_7256del	p. Tyr2417_Ala2419del
31	1	*SDHD*	PGL1		c.196A>G	p. Met66Val
32	1	*MAX*			Del involving exons 1 through 4*,	identified by CNV
33**	1	*TMEM127 / TP53*	-/Li-Fraumeni	Breast Ca+pancreatic Ca	c.117_120 delc.1010_G>A	p. Ile41Argfs*39 p. Arg337His
Total	47	*RET= 18* *VHL= 10* *SDHB= 8* *NF1= 8* *Other§ = 3*	MEN2A=15 MEN2B= 3 VHL2A=4 VHL2B=2 VHL2C=4 PGL4=8 PGL1=1			

(1), one affected patient; (2), two affected patients.

CCRC, clear cell renal carcinoma; HB, hemangioblastoma; MTC, medullary thyroid carcinoma; PHP, primary hyperparathyroidism.

complex began to increase, assuming a greater proportion in cases of PGL and metastasis. The incidence of *RET* gene variants is important around the third decade of life but is less than those of the *VHL* and *SDHx* genes, highlighting the characteristic of bilateral disease and virtual absence of metastases. *NF1* gene variants stand out in the fourth and fifth decades of life. (Neumann *et al.* 2019).

The distribution in our population sample was different: *RET* was the most prevalent gene with PV in the second, fourth, and fifth decades of life, whereas *NF1* was the most prevalent gene with PV in the sixth decade of life. The *SDHB* and *VHL* genes were present in all decades of life.

The pathogenic or likely PVs found in this study were comparable to those reported in the medical literature. Rare variants, however, were found in the *SDHB*, *VHL*, and *NF1* genes, such as *SDHB*: c.137G>T in exon 2, c.293G>A in exon 4, and c.724C>A in exon 7; *VHL*: c.467A>G and c.496G>T, both in exon 3; and *NF1*: c.5487_5490dup resulting in frameshift in exon 37, c.1527+4_1527+7del in intron 13, and c.6999+2T>G in intron 46. We also found a unique association of PV in the *TMEM127* and *TP53* genes (R337H) in a 47-year-old patient who developed bilateral metastatic breast cancer, meningioma of the CNS, and pancreatic cancer; her 20-year-old daughter is a carrier of the same variants who did not develop PPGL thus far but faced bilateral breast cancer and meningioma of the CNS and her 5-year-old granddaughter carries only the *TMEM127* gene variant and currently has no clinical or laboratory evidence of PPGL.

The patient with PV c.5487_5490dup in the *NF1* gene developed bone, liver, and lung metastases at 22 years after PPGL resection and died after developing severe febrile neutropenia following therapy with ^{131}I-mIBG; to our knowledge, this is the first NF1-associated PPGL case reported in which metastasis developed only 20 years later. There is a strong genetic determinism in PPGL. Clinical features at the initial diagnosis of PPGL may suggest germline variants, allowing for grouping of such cases as potentially hereditary, especially in the presence of typical phenotypic conditions, as mentioned before.

periodically. Although our CJS had excellent specificity, NPV, and accuracy, it must be validated prospectively. On the other hand, the association of the presence of genetic variants with age <45 years, the presence of PPGL- related genetic syndromes, and topography (adrenal/ extra-adrenal) strongly indicates that patients with such characteristics should undergo genetic testing.

The clinical characteristics employed to direct NGS research in our patients were sufficient to guarantee helpful coverage for the main related genes, achieving good sensitivity with acceptable specificity and useful NPV.

As per the ACMG, 33% of our cohort of 101 index cases was classified as pathogenic/likely pathogenic, 30% as VUS, and 37% as probably sporadic. Therefore, 63% carry some germline variant, which is slightly higher than other published series. All VUS cases are periodically reassessed to determine whether they will change category; thus far, however, all such cases in this study remain VUS, a percentage that agrees with the literature.

Although *ATM* gene variants were significantly in our cohort, there is no current evidence between *ATM* variants and the presence of PPGL, making this finding of unknown clinical significance.

When compared to the control group (patients from the Fleury Lab database), we noticed a higher rate of PV. We did not have access to clinical or imaging characteristics of the Fleury Group of patients, once they were uncharacterized, but it is possible that they were not properly selected for genetic testing; conceivably, the use of the CJS would have reduced the number of sporadic cases. We believe that use of the CJS would make genetic testing more judicious and cost-effective for public and private health systems.

In summary, we describe the landscape of PPGL germline gene variants from a large sample of Brazilian patients in which pathogenic and likely PVs were highly prevalent, especially in the *RET* gene. We also suggest a CJS to identify PPGL patients who would not require initial genetic evaluation, improving test specificity and reducing costs.

It is generally agreed that all PPGLs must be submitted to genetic evaluation (Jiang *et al.* 2020, Garcia-Carbonero *et al.* 2021, Cascon *et al.* 2023, Gimenez-Roqueplo *et al.* 2023); however, when the availability of genetic NGS panels is greatly limited, as in Brazil, we suggest the use of a CJS to define which individuals should be initially exempt from genetic testing while maintaining a permanent follow-up, with the need for genetic testing being reassessed

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EO-22-0091.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

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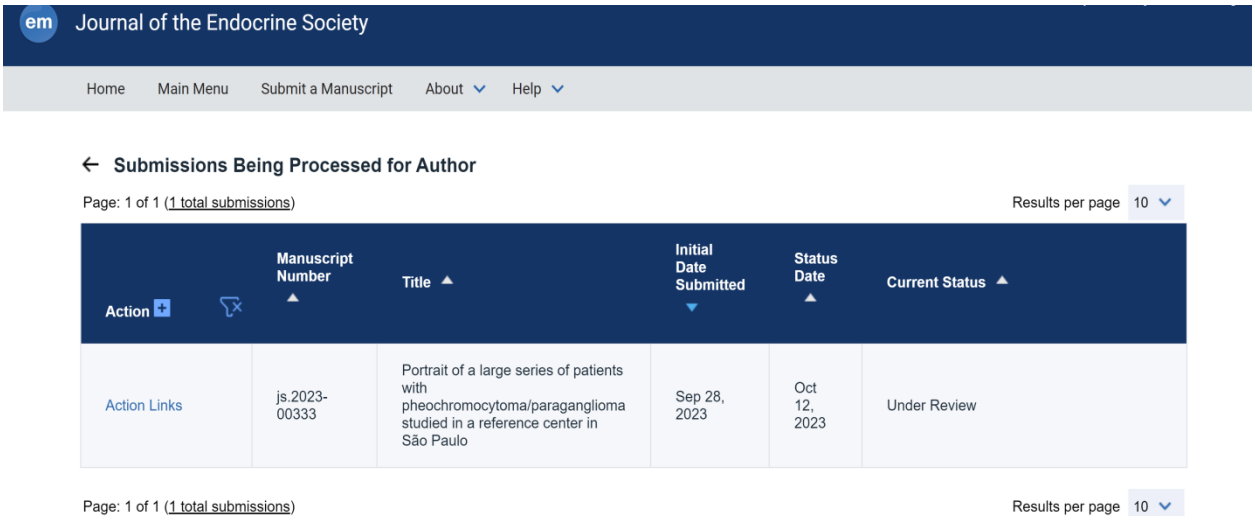

Article 3:

Portrait of a large series of patients with pheochromocytoma/ paraganglioma studied in a reference center in São Paulo

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Abstract

Pheochromocytomas (Pheo) and paragangliomas (PGL) (PPGL) are catecholamine-secreting tumours, whose functionality is confirmed by elevated plasma (Pl) and/or 24-h urinary (Ur) metanephrines (MN). We present clinical, hormonal, and imaging aspects of 116 patients (94 Pheo; 22 PGL; 25% discovered incidentally) from our service in São Paulo, Brazil. Systemic arterial hypertension (SAH) was present in 81% (43% on stage 3), whereas 9.5% were prehypertensive and 9.5%, normotensive. SAH plus paroxysms occurred in 31 (32.9%) patients, being exclusively sustained in the remaining; 26 (28%) had resistant SAH. Orthostatic hypotension was seen in 65% of patients. Pl/Ur MN and normetanephrine (NMN) were compared to those of a positive (56 functioning PPGL) and a negative control group (654 subjects with normal MN/NMN). Total and fractionated Ur MN were elevated in 94% PPGL patients. Cut-off values of 885 mcg/24-h for Ur MN, and of 1.5 nmol/L for Pl MN identified functioning lesions with 100%/100% sensitivity and 93%/97% specificity, respectively. Magnetic resonance imaging (MRI) detected 56% right-side Pheo, 25% on the left, and 19% bilateral; PGL were 57% retroperitoneal and 43%, cervical. Right-side Pheo were larger (5.8 cm) than left-side ones (3.7 cm), but retroperitoneal (6.5 cm) and neck PGL (6.9 cm) were similar. Tumour size positively correlated with total Ur MN. Concordance between MRI and ^{131}I-mIBG scintigraphy (in 57 patients), was near 100%. Summary: in this large cohort of PPGL patients we highlighted relevant aspects of SAH, the frequently overlooked manifestation of orthostatic hypotension, common incidental presentation, significant tumour size/hormonal production correlation and good agreement between MRI and ^{131}I-mIBG scintigraphy.

Introduction/Background

Pheochromocytomas (Pheo) and paragangliomas (PGL) are uncommon catecholamine-secreting tumors of the adrenal medulla and extra-adrenal chromaffin tissue, respectively. PGL usually develops from the sympathetic paraganglia of the chest and abdomen. Both are derived from ectodermal neural crest cells and are grouped under the term Pheo/PGL (PPGL) syndrome [1]. Recently, the WHO recommended the single name paraganglioma to designate both adrenal and extra-adrenal pheochromocytomas [2,3].

The incidence of PPGL is between 500 and 1,600 cases per year, with an equal sex distribution and the highest frequency in the 4^{th} and 5^{th} decades of life. PPGL has a prevalence of 0.1 to 0.6% among the hypertensive population [4-6].

PPGL manifests clinically as sustained arterial hypertension that is usually accompanied by paroxysms (headache, palpitation, and sweating) and refractoriness to antihypertensive medications. Silent PPGL may be found as adrenal incidentalomas. Hereditary syndromes are common, whereas sporadic disease was the rule in the past [6-10].

Laboratory confirmation is based on elevated levels of plasma and/or urinary fractionated metanephrines. Plasma and urinary catecholamines can also be used, but are less sensitive, as is urinary vanillylmandelic acid (VMA), which was used in the past [11-16]. The imaging location of unilateral or bilateral Pheo and cervical (head and neck), thoracic and pelvic PGL can be found by both CT and MRI, which may be functionally complemented by scintigraphy with ^{131}I-mIBG (meta-iodobenzylguanidine) and ^{68}Ga-DOTATATE (DOTA-octreotate) [6,8,13,17-20].

This article will focus on the major clinical, hormonal, and imaging aspects of a large series of PPGL patients studied in a single endocrine reference center in São Paulo, state of São Paulo, Brazil.

Patients and methods

Study population. From 2001 to 2019, we prospectively studied 137 patients ranging in age from 12 to 82 years in whom a diagnosis of Pheo and/or PGL was clinically considered and later confirmed by (i) hormonal assessment, (ii) specific imaging procedures, (iii) genetic evaluation for germline variants, (iv) surgery, and (v) pathological examination. Twenty-one patients were later excluded: 18 declined further participation, and three were under 14 years, the limit age per our protocol. The remaining 116 were 75 females (64.7%) and 41 males, with a median age of 45 years (ranging from 14 to 79 years).

All patients were referred to the Adrenal and Hypertension Outpatient Clinic of the Division of Endocrinology and Metabolism, Department of Medicine at EPM/UNIFESP for 1) evaluation of a possible secondary form of hypertension; 2) the presence of a suspicious adrenal or extra-adrenal mass; and 3) the existence of first-degree relatives with PPGL.

Control groups. To analyse and compare values of plasma and urinary metanephrines, we used two control groups, both extracted from an uncharacterized database registry of the Fleury Group, São Paulo, Brazil, after formal authorization: one consisted of data from patients with elevated plasma and/or urinary metanephrines in whom a diagnosis of PPGL was unequivocally established (positive control group); the other comprised data from a large cohort of subjects who had been evaluated for different disorders and included a panel of plasma and/or urinary metanephrines that were all normal. None of those patients were later diagnosed with a PPGL (negative control group).

Positive controls were 56 subjects: 33 females (58.9%) and 23 males ranging in age from 26 to 82 years (median of 54). Fifty of them had Pheo and six had PGL. They were all “functioning” PPGL (hormone-secreting) by definition.

The negative controls were 654 subjects: 412 females (63%) and 242 males ranging in age from 16 to 82 years (median of 51). All had plasma and urinary

metanephrines within the Fleury laboratory's reference ranges. "Nonfunctioning" PPGL could not be excluded in them.

All 116 patients from this study cohort (and/or their parents or liable) signed a written informed consent form for all investigational procedures and therapeutic decisions, which were previously approved by the Committee of Ethics in Clinical Research of the Institution. Patients from the positive and negative control groups were individually investigated by their respective physicians. Authorization to use the biochemical data employed in this manuscript, plus sex, age and information that led to the final diagnosis, was provided by the responsible Board of Directors of Fleury Group without personal patient identification.

Study design. The following data were obtained from all patients at the time of admission: sex and age, height and weight (and body mass index, BMI), systolic and diastolic blood pressures and heart rate (HR), specific manifestations of the disease (such as sustained hypertension and/or paroxysms, tachyarrhythmia, orthostatic hypotension, cardiogenic shock), dysglycaemia (prediabetes or diabetes mellitus), presence of comorbidities, family history of PPGL and specific conditions suggesting the presence of germline pathogenic variants (PVs, see below), and current or past use of antihypertensive medications. We used the 2020 Brazilian Guidelines of Hypertension to grade the patient's arterial hypertension [21].

Conditions suggesting the presence of germline PVs were as presented elsewhere [22-24].

Laboratory evaluation

Urinary and blood collections. All patients clinically suspected underwent hormonal evaluation directed towards the hormonal detection of PPGL. Up to 2014, we employed 24-h urinary collections to measure 1) catecholamine excretion: norepinephrine [NE], epinephrine [E], and dopamine [Dp], 2) fractionated metanephrines: normetanephrine [NMN], metanephrine [MN], and 3-methoxy-tyramine [3MT], and 3) vanillylmandelic acid

(VMA). In addition, we simultaneously withdrew blood to measure plasma catecholamines (NE, E, and Dp). From 2014 on, as per *the Endocrine Society's PPGL Guideline* [8], we started measuring plasma NMN and MN in addition to 24-h urinary metanephrines and discontinued urinary and plasma catecholamines.

In several patients, we also measured plasma chromogranin A by immunoassay or targeted proteomics, and when indicated, we performed a clonidine test (0.3 mg PO with blood collected before and after 3 h) and measured plasma catecholamines or metanephrines.

Hormonal measurements: We measured plasma catecholamines, 24-h urinary catecholamines and metanephrines, and VMA by high-performance liquid chromatography (HPLC) using the *Chromsystem Commercial Kit*. [Chromsystems Instruments & Chemicals, GmbH]

Plasma metanephrines were determined by HPLC coupled to tandem mass spectrometry (LC-MS/MS) through an in-house method developed and validated at the Fleury Group in 2010 [unpublished], in consonance with other protocol [25].

Radiological/Imaging evaluation

We performed the following imaging procedures, as indicated and available: 1) abdominal (adrenal) and pelvic computerized tomography (CT) and/or magnetic resonance imaging (MRI); 2) cervical and/or chest MRI for patients with a suspected head/neck or thoracic tumor; 3) ^{131}I-mIBG full body scintigraphy; and 4) ^{18}FDG and/or ^{68}Ga-DOTATATE or DOTATOC PET-CT.

We analyzed the following CT and MRI data: topography, size, and characteristics of the lesion.

We performed whole-body ^{131}I-mIBG scintigraphy if the image was uncertain, dubious, or suggestive of the presence of metastases (on clinical or hormonal grounds) and in cases of confirmed metastasis to evaluate the possibility of radiopharmaceutical therapy.

We analyzed the following data: moderate/strong uptake in adrenal and/or extra-adrenal topographies and uptake in target organs of metastasis (bones, liver, lungs, and lymph nodes).

Not all biochemical and imaging diagnostic procedures were necessary for each patient. We requested specific hormonal and topographic tests only for diagnostic purposes, mainly 24-h urinary NMN/MN and adrenal MRI or CT and/or ^{131}I-mIBG and ^{18}FDG PET-CT, whenever necessary and available.

Genomic evaluation

Genetic analysis was performed in 115 patients to determine the familial (hereditary) or sporadic nature of their respective diseases using a panel that had the following 23 PPGL-related genes: *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF-1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53, VHL,* and *PIK3CA.* The results of the genomic studies are reported elsewhere [22].

Statistical analysis

All patient variables were deposited in a computer program database (Microsoft Excel®) and updated at each follow-up visit. For statistical purposes, all nondetectable values were arbitrarily considered equal to the limit of sensitivity for the assay divided by the square root of 2 [26]. We performed parametric and nonparametric statistical tests according to the nature of the variable, which were tested for normality by the Kolmogorov–Smirnov test. Descriptive analysis included absolute (n) and relative (%) frequencies and bar graphs of qualitative variables and summary measures (mean, standard deviation, median, minimum, and maximum). Inferential analysis included the association test using Chi-square or Fisher's exact test and the logistic regression-stepwise forward method and ROC curve analysis to evaluate possible cutoff points for quantitative variables, complemented by calculation of sensitivity and specificity. Cut-off

points were established by ROC curve analysis as recommended [27]. P<0.05 was significant.

Results

Among the 116 PPGL patients in the present series, 102 were index cases (probands), and 14 were relatives from 8 families.

Anthropometry and clinical presentation (Table 1)

Table 1 depicts anthropometric and clinical features of the 116 PPGL patients, separated into Pheo (n= 94) and PGL (n= 22). Among the 94 Pheo patients, one patient also had a neck PGL (thus, in table 3 and figure 3, total PGL are 23). The sex ratio was approximately 2 F:1 M. PGL patients were younger (35.5 vs. 45 years, p<0.01) and comprised significantly more subjects ≤20 years of age than Pheo (18.2% vs. 6.4%; p<0.01). Median BMI and heart rate were similar between groups.

Patients were referred for investigation mainly owing to systemic arterial hypertension (SAH; 81%) (see below), but also due to a previous positive genetic screening (26.7%), the presence of an adrenal incidentaloma (23.4%), and other reasons (11.2%).

Virtually all Pheo (98.9%) (including the one combined with a nonfunctional neck PGL) and 59.1% of PGL were functioning lesions, in the sense that they produced excessive catecholamines that resulted in clinical manifestations. At presentation, 64.7% of the patients had two clinical manifestations, 25% had only one, and 13.8% had three or more. The most common clinical manifestation was SAH in its various forms.

Blood pressure

Table 1 and Figure 1 show the results related to hypertension. SAH was present in 94 of the 116 PPGL patients (81%), whereas 11 (9.5%) were prehypertensive and 11 (9.5%) had normal blood pressure. Among the 94 hypertensive patients, 28 (29.8%) were in stage 1, 25 (26.6%) were in stage 2, and 41 (43.6%) were in stage 3. Stage 3 SAH prevailed over stages 1 and 2 in the whole PPGL group, especially among the PGL

patients. Overall, systolic and diastolic blood pressure (BP) were higher in hypertensive PGL than in Pheo, albeit not significantly.

High BP was found alone in 29 (37.2%) hypertensive PPGL patients (29 Pheo) and was accompanied by paroxysms in the remaining 31 (32.9%) (29 Pheo). Hypertension that was difficult to control (resistant SAH) was present in 26 (27.7%) PPGL patients (20 Pheo) and was mostly found as paroxysmal and combined hypertension. Seventy-five (64.7%) patients (65 Pheo) had orthostatic hypotension, six of whom (8%, all Pheo) did not have hypertension of any form.

The estimated median time for presentation of SAH (with or without paroxysms) was 24 mo. (ranging from 1 to 480 mo.).

PPGL-associated comorbidities

Dysglycaemia was the most frequently associated comorbidity, present in 18.1% of cases. These other comorbidities were mostly associated with genetic disease: medullary thyroid carcinoma (15%), type 1 neurofibromatosis (7.8%), primary hyperparathyroidism (4.3%), retinal angioma and central nervous system (CNS) haemangioblastomas (3.4%), and clear cell renal carcinoma (3.4%).

Metastases

Recurrence of PPGL and metastasis occurred in 11 (9.5%) patients (table 1): seven PGL (six with germline PVs in the *SDHB* gene and one in the *VHL* gene) and four Pheo (one with a PV in the *NF1* gene). Metastases involved lymph nodes in all, blood vessels in eight, bone (spine and pelvis) and liver in five each, lungs in three, and CNS (head and neck PGL) in two.
Two patients relapsed after 12 mo., five after 36 mo., and one each after 5, 28, and 33 years. The last two patients have already been discharged from their respective medical services after 10 years of uncomplicated follow-up. Nevertheless, paroxysmal

hypertension and a wasting syndrome manifested in them after another 18 and 23 years, respectively.

Hormonal presentation

Urinary and plasma metanephrines (table 2, figure 2)

With the large negative control population (n= 654) as reference, we defined our own reference ranges for total and fractionated 24-h urinary and plasma MN as follows (results in mean ±SE, and [range]): 24-h urinary NMN: 214.8 ±4.0 mcg/24 h [15-784]; MN: 81.9 ±1.6 mcg/24 h [3.5-326], and total metanephrines: 296.7 ±4.7 mcg/24 h [33-878]. Plasma NMN: 0.45 ±0.01 nmol/L [0.14-1.0] and MN: 0.18 ±0.01 nmol/L [0.14-0.6), and total metanephrines: 0.63 ±0.01 nmol/L [0.28-1.5].

As shown in table 2, 88 PPGL patients from the present study and, especially, 56 from the positive control group had significantly higher levels of NMN, MN, and total MN than the negative controls. The individual values of 24-h urinary MN from these subjects are shown in figure 2.

A cut-off value of 885 mcg/24 h for total urinary MN, determined by ROC curve analysis, had 100% sensitivity and 92.5% specificity at separating functioning from nonfunctioning PPGL (study cases and positive controls vs. negative controls). In fact, 67.4% of the 24-h urinary total MN values were above 885 mcg/24 h in the subgroup of functioning PPGL from this study and the positive control groups, compared to none in the negative controls (figure 2).

Table 2 also shows that plasma metanephrines (NMN and MN) from the study group (n= 20) and the positive control group (n= 56) were significantly higher than those from the negative control group ($p<0.01$). A cut-off value of 1.5 nmol/L for total plasma MN determined by ROC curve analysis had 100% sensitivity and 97.3% specificity to separate functioning from nonfunctioning PPGL lesions (study cases and positive controls vs. negative controls).

Urinary and plasma catecholamines (figure 3)

The following results were obtained for 24-h urinary catecholamines from 13 patients with Pheo and two with PGL: epinephrine: 906 ±216 mcg/24 h (800; range: 70-2,500); norepinephrine: 1,275 ±263 mcg/24 h (900; range: 100-3,900); and dopamine: 360 ±123 mcg/24 h (250; range: 70-2,000). Figure 3 depicts all the individual values.

None of the PPGL patients had detectable values for plasma epinephrine, whereas plasma norepinephrine was 2,373 ±221 pg/mL (2,500; range: 900-3,500) and dopamine was 215 ±42 (180; range: 28-500). Individual values are shown in figure 3.

Clonidine test and chromogranin A

Clonidine tests were performed in seven PPGL patients (six Pheo) and were positive in all.

Plasma chromogranin A was above 800 ng/mL (reference values <93 ng/mL) in three patients with metastatic PPGL.

Topographic distribution and tumor size (table 3, figure 4)

Most PPGL patients (93.9%) were initially imaged by MRI instead of CT (given the ease of performing it in our service). Imaging features from all patients are shown in table 3: 53 Pheo (56.4%) were located on the right side, 23 (24.5%) on the left, and 18 (19.2%) were bilateral. Among the 23 PGL, 13 (56.5%) were retroperitoneal and 10 (43.5%) were cervical.

Table 3 also shows tumor sizes (larger diameter). Note that the Pheo located on the right side were significantly larger than those on the left (including the bilateral ones) (median of 5.8 vs. 3.7 cm, respectively) ($p<0.01$). Retroperitoneal and neck PGL had similar sizes (medians of 6.5 and 6.9 cm, respectively). The individual sizes of all PPGL lesions are depicted in figure 4. Note that 70% of all 117 PPGL lesions were larger than 3 cm in diameter (and 61% were larger than 4 cm).

High signal intensity on the T2-weighted sequence was observed in 108 of the 109 patients (99.1%) who underwent MRI. Among the 11 PPGL patients who underwent CT imaging, all typically had pre-contrast attenuation values above 10 HU, above 20 HU in the portal phase, and a contrast *washout* <50%.

^{131}I-mIBG whole-body scintigraphy

As shown in table 3, 91 PPGL patients underwent ^{131}I-mIBG scintigraphy: 77 in the Pheo group (40 right-side, 16 bilateral) and 14 in the PGL group (nine retroperitoneal).

Fifty-seven PPGL patients (52.3%) underwent both MRI and ^{131}I-mIBG scintigraphy: 29 (50.9%) had right-side and 17 (29.8%) left-side Pheo (including one with a neck PGL), six (10.5%) had retroperitoneal and three (5.3%) neck PGL, and two (3.5%) had bilateral Pheo. The concordance between both procedures was 98.2%. The only discordance occurred in the patient with adrenal Pheo and neck PGL, in whom uptake was observed only in the left adrenal Pheo. ^{131}I-mIBG scintigraphy was done in 30 (63.8%) of the 47 patients carrying PVs: uptake was observed in 29 of them [22].

In eight PPGL patients (two Pheo with an *SDHB* gene PV), metastases were present or developed during follow-up. Lesions were identified in the spine and pelvis by ^{68}Ga-DOTATATE PET-CT.

Metanephrines and tumor size (figure 5)

A significant positive correlation was observed between tumor size (larger diameter, in cm) and total urinary MN (in mcg/24 h) for 91 functioning PPGL (74 Pheo and 17 PGL): r= 0.27; p<0.01 (figure 5). Nonfunctioning PPGL, especially head and neck PGL, presented 24-h urinary MN within the normal range.

Discussion

PPGL is a rare neuroendocrine tumor that is potentially metastatic and fatal if this diagnosis is not promptly suspected and treatment is not started soon. Its incidence has

been increasing recently due to improvements in the measurement of catecholamine metabolites and a greater demand for imaging tests. Unfortunately, there is still a significant diagnostic delay, some diagnosis only coming postmortem [2,3].

This diagnostic delay was confirmed in our population sample: most patients took two or more years to seek medical assistance, and one of them took almost 40 years to be diagnosed. Hence, major complications related to hypertension are to be expected.

PPGL is one of the main diagnostic possibilities in the investigation of secondary and/or resistant forms of SAH, regardless of the presence of paroxysms. Clinical scores are available to help define an individual's risk of having PPGL. Features such as hyperhidrosis, palpitations, pallor, tremors, and nausea are 30-90% more prevalent in the PPGL population than in other hypertensive patients. These characteristics plus a heart rate above 85 bpm and BMI <25 kg/m^2 guarantee scores close to the maximum of 7 points, a score that makes PPGL 5.8 times more likely than lower scores do [14].

The main manifestation in our patients that led to the suspicion of PPGL was sustained SAH. Other common ones were paroxysms, genetic screening, difficult-to-control SAH and adrenal incidentalomas, consistent with the medical literature [7,13,19]. When first investigated, most patients were already taking two or more classes of antihypertensive drugs. Orthostatic hypotension was a mostly relevant finding, often associated with SAH but also found in normotensive patients who were initially referred for genetic screening or evaluation of adrenal incidentaloma. This finding is important in the initial evaluation, as it is often present whether the individual has SAH or not.

Normotensive and prehypertensive patients (almost 20% of our sample) were referred for evaluation of PPGL due to a positive family history or presence of adrenal incidentaloma (22%), as has been reported [19,22-24,28,29]. These data also help to dismiss the outdated "rule of 10%" for PPGL.

Investigation of PPGL is recommended for recent-onset diabetes mellitus in a young, lean, hypertensive patient since catecholamine excess favours hepatic gluconeogenesis and less degranulation of insulin by pancreatic beta cells [13]. Eighteen

percent of our patients had dysglycaemia, raising the question whether diagnosis and treatment of PPGL could change some subjects' DM history, even leading to a "cure".

The landscape of PPGL germline gene variants from 115 of our patients had many pathogenic and likely pathogenic variants, most often in the *RET* gene, followed by the *VHL* and *SDHB* genes [22].

Total plasma MN are considered the gold standard for the diagnosis of functioning PPGL, with slight superiority over 24-h total urinary MN. In the past, the combination of urinary MN with plasma/urinary catecholamines was recommended because of its lowest false negative rate; however, plasma MN alone is better than any of the combined tests. Negative plasma MN virtually excludes functional PPGL, but in its absence, 24-h urinary MN can replace them. Thus, the current laboratory diagnosis of PPGL must include plasma and/or 24-h urinary MN [6-9,13,15,30].

Both our PPGL study patients and the positive control group presented very high values of 24-h urinary and plasma MN compared to our large reference negative control group, even when nonfunctioning PPGL (especially those located in the neck) were included. The cut-off value of 885 mcg/24 h had a sensitivity and specificity of 100% and 92.5%, respectively, for total urinary MN. Thus, 24-h urinary MN alone is likely a sensitive and good marker of PPGL functionality, useful for screening patients for the disease.

Plasma MN values twice above the upper limit of normal are diagnostic of PPGL, whereas values between 1X and 2X are considered suspect, requiring diagnostic complementation with 24-h urinary MN and/or chromogranin A measurement and/or a suppression test with clonidine. Based on our findings and others, we suggest a simple and direct algorithm (figure 6) to diagnose functioning PPGL. The measurement of 3-methoxy-tyramine can help in the diagnosis of PPGL, especially in dopamine-producing, metastatic, and neck PGL [13].

The topographic diagnosis of PPGL should initially be made with CT of the adrenals and pelvis, whereas MRI would be reserved for specific cases such as carriers of PV in PPGL-related genes, intracardiac and head/neck PGL, pregnant women,

children, tumor recurrence and metastatic PPGL. Both imaging procedures have good sensitivity and specificity for the diagnosis of PPGL. The characteristics of PPGL on CT are attenuation values in the precontrast phase greater than 10 HU, usually above 20, with a contrast washout <60% and high signal intensity (“light-bulb” bright) on the T2-sequence and out-of-phase sequence of MRI, with no loss of signal [8,9,13].

The ready availability of MRI over CT in our group prompted us to study 109 of our 116 patients with MRI at first, which was able to identify the high signal intensity on T2 in 108 of them; seven of the patients who underwent adrenal/pelvis CT had characteristics of a nonadenomatous lesion. The tumor diameter in our patients was mostly in the range of 5 to 6 cm. We identified three micropheochromocytomas (the smallest measuring 3 mm in diameter) whose images disclosed only adrenal thickening, but their adrenergic clinical picture was exuberant. The largest Pheo in our series was 24 cm in diameter and was initially suspected to be a primary adrenal carcinoma.

We found a significant positive correlation between tumor size and total 24-h urinary MN. Functioning PPGL with a diameter greater than 5 cm tend to produce total urinary MN above1,000 mcg/24 h.

Whole-body scintigraphy with ^{131}I-mIBG was employed in 57 of our patients for the following reasons: to search for PGL foci (mainly in hereditary cases) and to investigate metastases, dubious MRI or CT images and the possibility of treatment with radiopharmaceuticals. In all, scintigraphy had excellent agreement with MRI, in addition to accurately mapping hereditary cases and metastases. One patient with a PV in the *NF1* gene underwent an exam that was able to identify metastases following tumor recurrence, which made treatment with ^{131}I-mIBG possible.

In advanced and aggressive metastatic PPGL cases and in those with PV in the *SDHB* gene, ^{131}I-mIBG scintigraphy was not able to identify all foci of metastasis, unlike ^{68}Ga-DOTATATE PET-CT and ^{18}FDG PET-CT. Therefore, in these situations, we suggest initially performing ^{68}Ga-DOTATATE PET-CT and later performing ^{18}FDG PET-CT.

In summary, we present the clinical, hormonal, and imaging picture of a representative cohort of PPGL patients studied in a single reference center in São Paulo, Brazil, whose data agree with other series reported worldwide. Nearly 25% of the Pheo were discovered incidentally. Sustained SAH was present in more than 80% of cases (especially PGL patients), mostly severe and treatment-resistant and mostly accompanied by paroxysms. Two-thirds of patients had orthostatic hypotension, an important but generally overlooked diagnostic clue. Diagnostic confirmation of a functioning PPGL strongly relies on the finding of elevated plasma and/or 24-urinary metanephrines, complemented by chromogranin A and/or a clonidine test, as necessary. MRI was particularly valuable to identify PPGL and to investigate patients with germline variants: the right adrenal gland was more frequently affected than the left, and bilateral masses were present in 20% of cases. The average mass diameter was 5-6 cm, and the larger the mass, the more marked its total urinary metanephrine excretion. mIBG was almost 100% concordant with MRI. Although uncommon, the diagnosis of PPGL should always be considered in young, lean, treatment-resistant hypertensive patients with new-onset diabetes mellitus.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

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Legends for the Tables and Figures

Table 1. Anthropometric and clinical data of 116 PPGL patients, divided into pheochromocytoma (Pheo) and paraganglioma (PGL). Data are mean ±SEM, median and range, or n (%).

Table 2. Urine and plasma total and fractionated metanephrines from 88 PPGL study patients and the positive and negative control groups. Their laboratory upper reference values are written (see text for explanation). Data are mean ±SEM, median and range, or n (%).

Table 3. Imaging data from all 117 PPGL lesions (116 patients*), divided into pheochromocytoma (Pheo) and paraganglioma (PGL). Data are mean ±SEM, median and range, or n (%).

Figure 1. Individual paired values of systolic and diastolic blood pressure from the 116 PPGL patients studied. Background shaded grades define areas of normal blood pressure (Nl BP), prehypertension (pre-Htn), and stages 1 to 3 hypertension. Solid circles denote pheochromocytomas; open lozenges denote PGL.

Figure 2. Individual values of total and fractionated 24-h urinary metanephrines from 89 patients with PPGL and 56 positive controls (see text for explanation). Shaded areas represent the normal ranges (minimum and maximum values from the negative control group). Note the semilogarithmic scale; horizontal bars denote means. Boxed numbers atop each series represent the percentage of values above the upper limit of normal (ULN).

Figure 3. Individual values of fractionated plasma and 24-h urinary catecholamines (epinephrine, norepinephrine, and dopamine) from 15 patients with PPGL. Shaded areas represent reference laboratory values. Note the semilogarithmic scale; horizontal bars denote means.

Figure 4. Individual location and size of lesions from 116 PPGL patients. Size is the largest diameter (in cm) obtained by CT or MRI. Horizontal bars denote means. Boxed numbers atop each series represent percentages of total PPGL.

Figure 5. Correlation between lesion size (largest diameter, in cm) and 24-h urinary excretion of total metanephrines from 89 patients with PPGL. Represented are either unilateral pheochromocytomas or the larger lesion of the bilateral ones.

Figure 6. Suggested algorithm for laboratory investigation of functioning pheochromocytoma/paraganglioma (PPGL). [1] Measured by HPLC coupled with tandem mass spectrometry (LC-MS/MS); [2] ULN= upper limit of normal; [3] Measured by HPLC or LC-MS/MS.

Table 1. Anthropometric and clinical data from all 116 PPGL patients, divided into pheochromocytoma (Pheo) and paraganglioma (PGL). Data are mean ±SEM, median and range, and absolute and percent valu

	Pheochromocytoma	Paraganglioma	All PPGL	p
N=	94*	22	116	
F (%) / M	60 (64%) / 34	15 (68%) / 7	75 (65%) / 41	NS
Age, y (range)	45 (14-72)	35.5 (14-79)	45 (14-79)	<0.001
Age ≤ 20y (%)	6 (6.4%)	4 (18.2%)	10 (8.6%)	<0.001
BMI (kg/m^2)	25.0 ± 0.4	25.5 ± 1.0	25.1 ± 0.4	NS
median [range]	24.5 [17.7-42.0]	25.3 [16.5-36.6]	24.6 [16.5-42.0]	
Functioning PPGL	93 (98.9%)	13 (59.1%)	106 (91.3%)	<0.05
Metastatic	4 (4.3%)	7 (31.8%)	11 (9.5%)	<0.001
Heart rate (bpm)	85.0 ± 1.0	86.7 ± 2.8	85.3 ± 1.0	NS
median [range]	88 [64-100]	88 [68-120]	88 [64-120]	
Normal BP	9 (9.6%)	2 (9.1%)	11 (9.5%)	
Pre-hypertension	7 (7.5%)	4 (18.2%)	11 (9.5%)	NS
Hypertension	78 (83%)	16 (72.7%)	94 (81%)	
Paroxysms only	6 (6.4%)	1 (4.6%)	7 (6.0%)	
Post. hypotension	65 (69.1%)	10 (45.5%)	75 (64.7%)	<0.05
With Htn (%)	59 (90.8%)	10 (100%)	69 (92%)	
Without Htn (%)	6 (9.2%)	0 (0%)	6 (8%)	
Only hypertensives:				
– Stage 1 Htn	25 (32.1%)	3 (18.8%)	28 (29.8%)	
– Stage 2 Htn	21 (26.9%)	4 (25%)	25 (26.6%)	NS
– Stage 3 Htn	32 (41%)	9 (56.3%)	41 (43.6%)	
SBP (mmHg)	159.9 ± 3.2	173.4 ± 8.0	169.1 ± 2.8	
median [range]	160 [90-270]	170 [130-250]	170 [130-270]	
DBP (mmHg)	99.6 ± 1.7	110.0 ± 5.5	101.4 ± 1.7	
median [range]	95 [70-150]	100 [80-150]	100 [70-150]	
Sustained BP only	29 (37.2%)	8 (50.0%)	37 (39.4%)	
Sustained + Paroxysms	29 (37.2%)	2 (12.5%)	31 (32.9%)	NS
Difficult control	20 (25.6%)	6 (37.5%)	26 (27.7%)	

* includes one pt. with both a right pheo and a neck PGL

Table 2. Urine and plasma total and fractionated metanephrines in PPGL study patients, and positive and negative control groups, plus laboratory upper reference values.

	Pheochromocytoma	Paraganglioma	All PPGL	Positive Controls (Pheo)	Positive Controls (PGL)	Positive Controls (All PPGL)	Negative Controls	Reference Values
24h-Urine data (µg/24h)	*N= 71*	*N= 17*	*N= 88*	*N= 50*	*N= 6*	*N= 56*	*N= 654*	
NMN (mean ± SE)	1,354 ± 158	1,748 ± 554	1,430 ± 165	4,860 ± 2,026	437 ± 65	4,387 ± 1,817	214.8 ± 4.0	< 800
median [range]	971 [21-5,800]	600 [48-7,543]	892 [21-7,543]	697 [150-72,768]	425 [217-716]	639 [150-72,768]	201 [15-784]	
MN (mean ± SE)	1,123 ± 173*	170 ± 66**	991 ± 154***	911 ± 350	82 ± 22	822 ± 315	81.9 ± 1.6	< 400
median [range]	615 [5-6,572]	100 [15-800]	499 [5-6,572]	147 [37-15,453]	85 [4-170]	145 [4-15,453]	76 [3.5-326]	
Total MN (mean ± SE)	2,430 ± 239	1,859 ± 540	2,319 ± 219	5,771 ± 2,048	519 ± 87	5,208 ± 1,840	296.7 ± 4.7	< 1.200
median [range]	1,950 [28-10,300]	675 [112-7,543]	1,882 [28-10,300]	859 [258-72,835]	497 [221-886]	822 [221-72,835]	286 [33-878]	
Plasma data (nmol/L)	*N= 13*	*N= 7*	*N= 20*	*N= 50*	*N= 6*	*N= 56*	*N= 654*	
NMN (mean ± SE)	31.9 ± 7.6	6.5 ± 4.8	23.0 ± 5.8	19.1 ± 10.4	1.5 ± 0.2	17.2 ± 9.3	0.45 ± 0.01	< 0.9
median [range]	25 [0.6-80.2]	2 [0.6-35]	8.6 [0.6-80.2]	2.3 [1-502]	1.5 [1-2.3]	2 [1-502]	0.4 [0.14-1.0]	
MN (mean ± SE)	13.1 ± 4.8	0.6 ± 0.2	9.0 ± 3.4	1.5 ± 0.5	0.2 ± 0.0	1.4 ± 0.4	0.18 ± 0.001	< 0.5
median [range]	3.3 [0.2-45.0]	0.7 [0.2-1.1]	1.0 [0.2-45.0]	0.3 [0.1-17.2]	0.2 [0.1-0.3]	0.3 [0.1-17.2]	0.14 [0.14-0.6]	

*N= 68; **N= 11; ***N= 79

Table 3. Imaging data from all 117 PPGL lesions (116 patients*), divided into pheochromo-cytoma and paraganglioma. Data are mean±SEM, median and range, and absolute and percent values.

	Pheochromocytoma	**Paraganglioma**	**All PPGL lesions**
N=	94*	23*	117
Imaging data			
MRI / CT	87 / 10	23 / 1	110 / 11
Incidentaloma	22 / 94 (23.4%)	0 / 23	22 / 117 (18.8%)
Unilateral Pheo (R/L)	53 (56.4%) / 23 (24.5%)	----	76 (65.5%)
Bilateral	18 (19.2%)	----	18 (19.2%)
Size# R/L (cm)	5.8±0.4 / 4.5±0.5	----	5.3 ± 3.3
Median [range]	5.8 [1-24] / 3.7 [0.6-12]	----	5.0 [6-24]
Retroper/Neck*	----	13 (56.5%) / 10 (43.5%)	23 (19.6%)
Size Retr/Neck (cm)	----	7.2 ± 1.0 / 6.3 ± 0.7	6.8 ± 6.2
Median [range]	----	6.5 [2.2-14] / 6.9 [3-9]	6.8 [2.2-14]
Functional imaging			
^{131}I-mIBG	77	14	91
Unilateral Pheo (R/L)	40 (52.0%) / 21 (27.3%)	----	61 (67.0%)
Bilateral	16 (20.7%)	----	16 (17.6%)
Retroper/Neck*	----	9 (64.3%) / 5 (35.7%)	14 (15.4%)

***** includes one pt. with both a right pheo and a neck PGL

includes bilateral lesions (all right side= 71; all left side= 41)

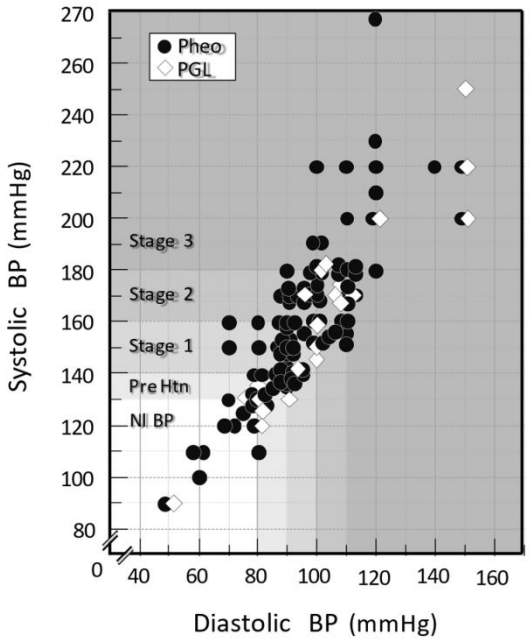

Figure 1.

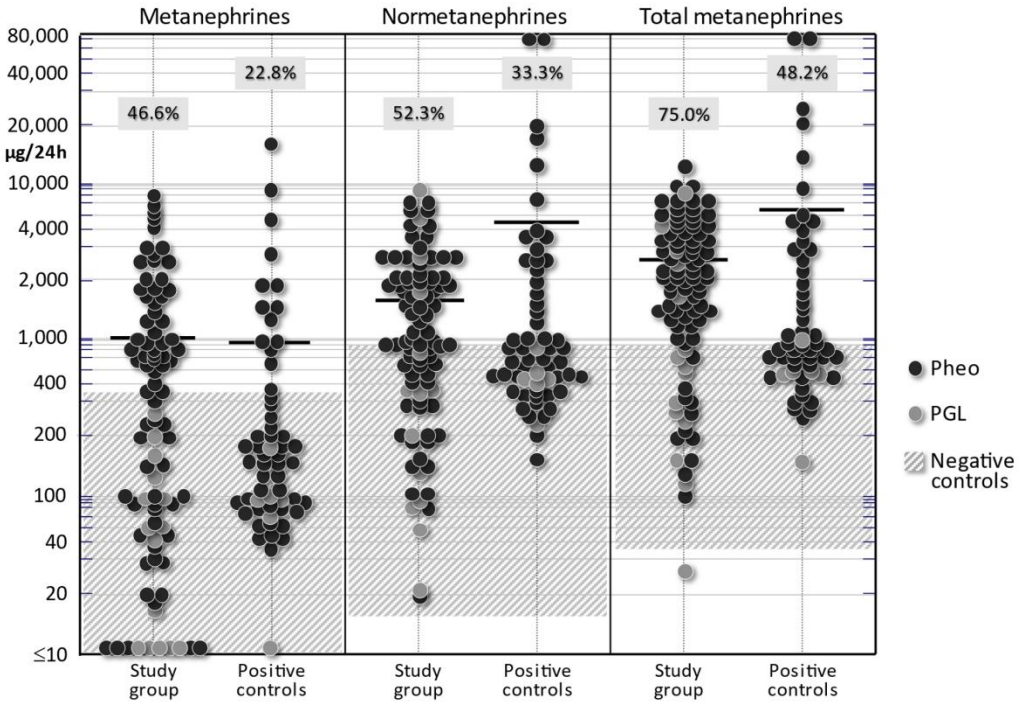

Figure 2.

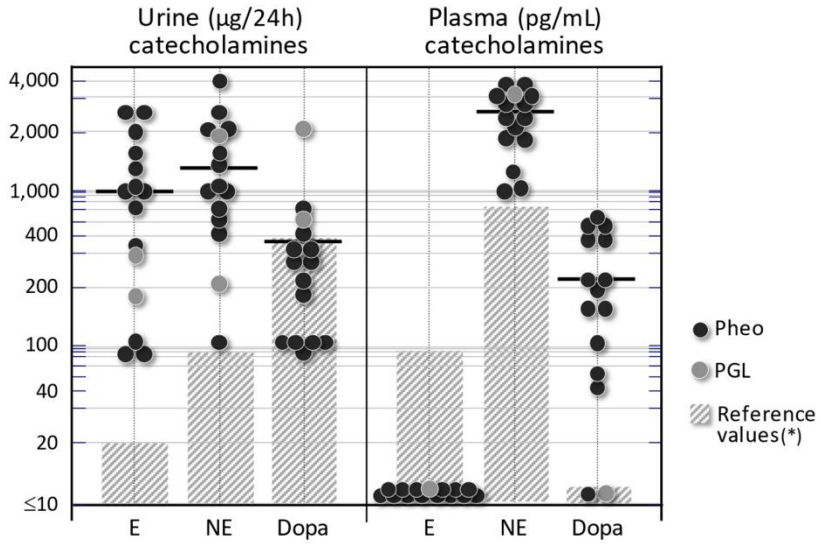

Figure 3.

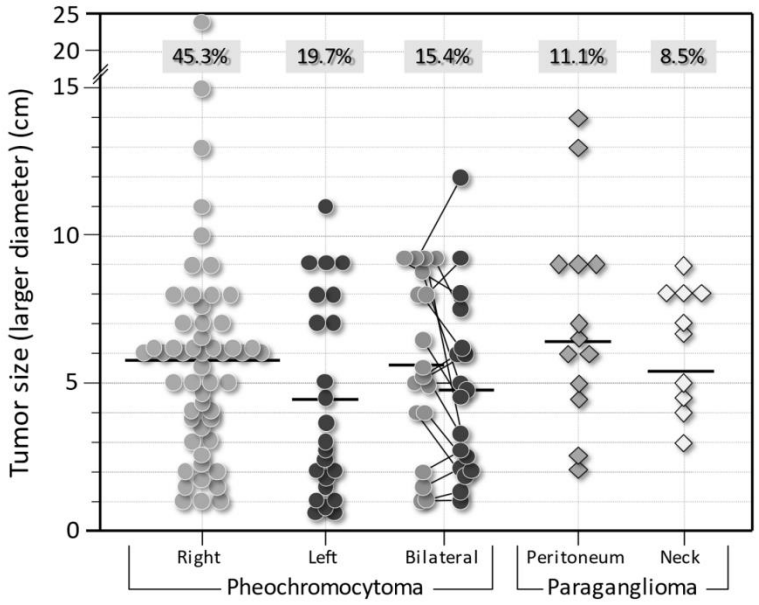

Figure 4.

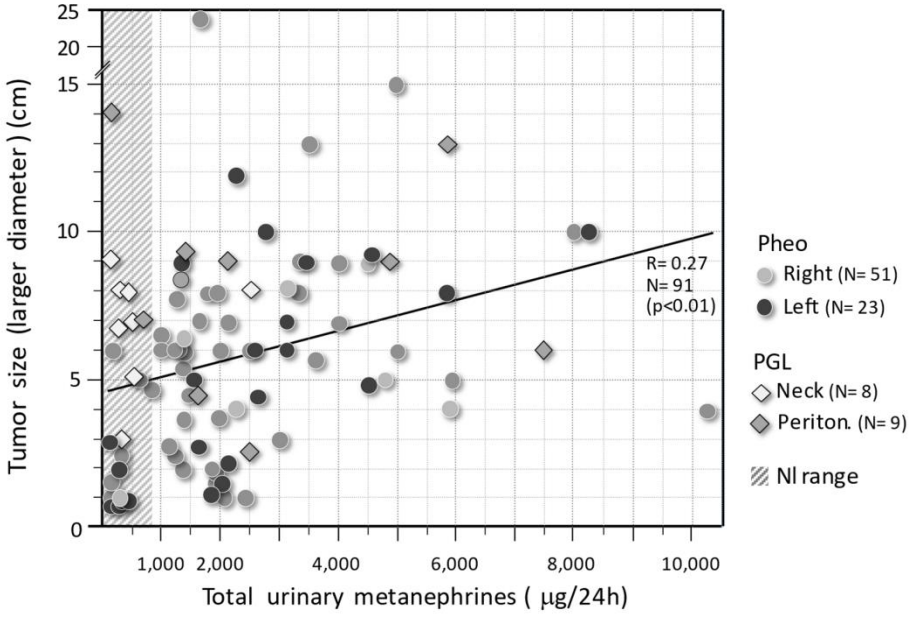

Figure 5.

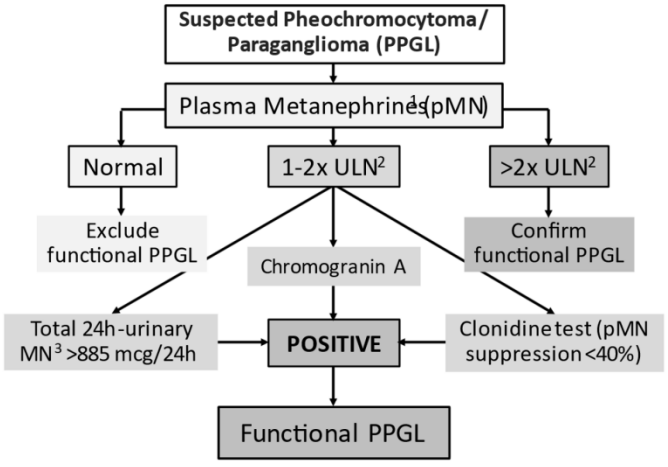

Figure 6.

Conclusões e Considerações Finais

Com os nossos 3 manuscritos, podemos evidenciar que nessa extensa coorte de pacientes brasileiros portadores de feocromocitoma/ paraganglioma acompanhados no período de 2000-2019, os parâmetros clínicos, laboratoriais, radiológicos e anátomo-patológicos foram, em geral, concordantes com os dados da literatura mundial.

O atraso no diagnóstico foi evidenciado nessa coorte podendo chegar, em alguns casos, a até 40 anos. A hipotensão ortostática esteve presente em mais da metade dos nossos casos, devendo ser obrigatoriamente investigada em todos os portadores de feocromocitoma/ paraganglioma. Adicionalmente, 25% dos casos chegaram inicialmente como incidentalomas adrenais, sendo que os achados radiológicos eram de lesões não típicas de adenomas.

No nosso primeiro manuscrito, propusemos um fluxograma laboratorial para o diagnóstico de funcionalidade, com base nas dosagens de metanefrinas plasmáticas e/ou urinárias totais, e o adaptamos no nosso terceiro manuscrito, a partir dos exames desses pacientes e do extenso banco de dados de amostras de um grande laboratório no Brasil.

As análises genéticas de sangue periférico demonstraram que variantes germinativas são muito frequentes e que devem ser avaliadas em todos os portadores da doença, e em situações em que possa existir a dificuldade na realização do teste genético, por exemplo o custo do exame, sugerimos a realização de um *score* clínico, reforçando que ele é dinâmico e que no decorrer do tempo, a depender de novos achados clínicos, ele pode ser modificado. Dessa forma, os pacientes devem ser seguidos por um período indeterminado. A validação desse *score* deverá ser realizada posteriormente com uma amostra maior de indivíduos.

O diagnóstico clínico, laboratorial e genético de feocromocitoma/paraganglioma, chamado de “o grande camaleão da medicina”, continua um desafio, mas com os avanços nos métodos, nas dosagens dos metabólitos das catecolaminas, exames de imagem e de medicina nuclear, e análise genética por NGS, mais diagnósticos serão realizados em menor tempo, permitindo o melhor tratamento e seguimento para os pacientes.

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Nota à população

Essa tese de doutorado teve como objetivo estudar pacientes portadores de feocromocitoma/paraganglioma que são acompanhados no ambulatório de adrenal e hipertensão arterial da Escola Paulista de Medicina/ Universidade Federal de São Paulo, uma doença rara e genética, potencialmente metastática e fatal.

Tivemos a oportunidade de verificar que muitos tiveram positividade para alterações genéticas que favorecem o aparecimento do tumor, assim como de outros tumores relacionados e a depender do achado genético, maior agressividade.

Este trabalho resultou em dados que foram publicados em revista internacional e apresentados em congressos nacionais e internacionais.

Como conclusão dessa tese, verificamos que os sinais e sintomas dessa doença podem se confundir com outras doenças, gerando atrasos diagnósticos. Alterações genéticas são muito frequentes e a sua investigação nunca deve ser esquecida.

Anexo 1

TERMO DE CONSENTIMENTO
Universidade Federal de São Paulo / Escola Paulista de Medicina
Disciplina de Endocrinologia
Termo de Consentimento Livre e Informado

Estas informações estão sendo fornecidas de maneira clara e simples para sua participação voluntária neste estudo. Portanto, leia este termo com atenção e pergunte aos pesquisadores responsáveis sobre quaisquer dúvidas, sempre que considerar necessário. Após a leitura, caso concorde voluntariamente em participar, assine-o.

O Sr(a)_____

está sendo convidado a participar de um projeto de pesquisa da Disciplina de Endocrinologia da Escola Paulista de Medicina / UNIFESP envolvendo pacientes portadores de feocromocitoma/paraganglioma, caso não queira participar da pesquisa não há problema e o seu tratamento continuará sendo realizado em nossa instituição sem nenhum prejuízo.

O objetivo desta pesquisa é identificar alterações genéticas (alterações no DNA) em pacientes com feocromocitoma esporádicos (= não familiares) acompanhados em nossa instituição, em comparação com tumores extra-adrenais e síndromes genéticas já estabelecidas na literatura médica.

As glândulas adrenais estão localizadas em cima dos rins e podem ser sítios de tumores produtores de hormônios dentre eles o feocromocitoma que é capaz de produzir hormônios como noradrenalina, adrenalina e dopamina e podem causar hipertensão

arterial sistêmica, arritmias cardíacas, podem ser malignos e geralmente fatais se não descobertos e tratados adequadamente. Alterações dos genes (=DNA) ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53, VHL RET, SDHD, SDHB, VHL e TMEM127 podem levar o aparecimento de feocromocitomas adrenais e extra-adrenais (fora das adrenais, também chamados de paragangliomas). O proto-oncogene RET será pesquisado em nossa instituição (UNIFESP-EPM), a mutação desse proto-oncogene pode também levar a aparecimento de tumores em outras glândulas como a tiroide (dando o câncer medular de tiroide) e paratiroides (dando o adenoma ou mais frequentemente hiperplasia das paratiroides).

Os genes *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 e VHL* serão analisados em parceria com laboratório Fleury Medicina e Saúde.

Tais genes serão estudados em sangue periférico (sendo necessária apenas a coleta de sangue do braço, como um exame de sangue comum). A quantidade sangue periférico necessária e de **5 mL**.

Alem da avaliação genética serão também avaliados dados clínicos (historia clinica= sintomas que levaram a pesquisar a doença, exame físico, historia familiar de feocromocitoma, epidemiológicos), dados hormonais (avaliação dos hormônios quanto aos valores encontrados tentando determinar o quão sensível e especifico o hormônio é para o diagnostico da doença), dados de exames radiológicos e de medicina nuclear (avaliação de quão sensível, especifico e acurado é o exame para definição diagnóstica do tumor) e dados anátomo-patológico (verificar características que ajudem a definir o diagnóstico e encontrar características que podem definir quanto a benignidade e malignidade do tumor).

A doença já traz consigo um grande risco de morrer. O não tratamento da doença assim como a sua não investigação acarretará riscos de morte ao doente, pois como falado acima na grande maioria das vezes levam a hipertensão arterial, arritmias

cardíacas fatais e risco de malignidade. Dados da literatura médica mostram que a grande maioria dos diagnósticos dessa doença são feitos na necropsia do individuo portador do feocromocitoma sendo que a doença contribuiu em 55% para causa da morte.

Com relação ao nosso projeto de pesquisa **OS RISCOS SÃO MÍNIMOS**, pois serão coletados dados de história clínica, laboratório, radiológico, medicina nuclear, anátomo-patológico e pesquisa genética dos genes já citados. Todos os exames solicitados são obrigatoriamente feitos para diagnóstico em qualquer paciente com feocromocitoma, independente do projeto proposto por nós. **O desconforto do projeto seria a punção venosa para coleta de sangue para pesquisa genética, podendo ficar, raramente, equimoses e hematomas e caso isso ocorra serão fornecidas orientação médica para o tratamento das equimose e hematomas além de medicação (Hirudoid). O SIGILO DE SUAS INFORMAÇÕES SERÁ GARANTIDO PELOS PESQUISADORES E NÃO SERÃO UTILIZADOS PARA OUTROS FINS QUE NÃO SEJAM PARA A PESQUISA.**

As vantagens em participar desta pesquisa são: ajudar os pesquisadores a determinar a frequência de alterações genéticas em pacientes com feocromocitoma esporádico em nossa instituição, permitindo, assim, detectar precocemente essas alterações e planejar o plano terapêutico da melhor forma para o paciente, alem de tentar entender o perfil clinico, laboratorial, radiológico, de medicina nuclear e anátomo-patológico dessa doença que é considerada rara, entretanto dados da literatura indicam que é subdiagnosticada.

Todos indivíduos que participaram do projeto de pesquisa serão reconvocados para informar o resultado da pesquisa genética e oferecido orientação genética em nossa instituição. Os que apresentaram alterações nos genes serão rastreados para pesquisa de outros tumores que podem vir associados ao gene alterado (Por exemplo: se vier com o proto-oncogene RET positivo, obrigatoriamente será rastreado câncer medular de tiroide e adenoma e/ou hiperplasia das paratiroides). Os familiares de primeiro grau (pais,

irmãos, filhos, primos de primeiro grau) serão convocados pela nossa equipe e será oferecida a pesquisa genética e avaliação clínica. A pesquisa clínica, laboratorial, radiológica e de medicina nuclear e tratamento (caso tenham o tumor) será oferecida independente de querer participar do projeto, se não quiserem participar da pesquisa em nenhum momento a investigação clínica e tratamento serão prejudicados. A avaliação genética só será feita apenas se o individuo quiser participar do projeto e após ler o termo de consentimento, concordar e assinar o termo. Todos os indivíduos com feocromocitoma independentes de participarem ou não do projeto terão acompanhamento por tempo indeterminado em nossa instituição, pois é descrito na literatura que 10% dos casos podem recidivar, mesmo anos depois de tratamento.

É garantida a total liberdade de saída do estudo em qualquer momento que desejar sem qualquer prejuízo à continuidade de seu tratamento no Hospital São Paulo. Assim como a retirada do termo de consentimento.

O voluntário tem o direito de ser mantido atualizado sobre os resultados parciais das pesquisas, quando em estudos abertos, ou de resultados que sejam do conhecimento dos pesquisadores. Não há despesas pessoais para o voluntário em qualquer fase do estudo, incluindo exames e consultas. Também não há compensação financeira relacionada à sua participação. Se existir qualquer despesa adicional relacionada à pesquisa genética, ela será absorvida pelo orçamento da pesquisa. **O (A) SR(A) TERÁ GARANTIDO O DIREITO A TRATAMENTO IMEDIATO E GRATUITO NA INSTITUIÇÃO. E SE OCORRER QUALQUER DANO DECORRENTE DA PESQUISA, O (A) SR (SRA) TERÁ DIREITO À INDENIZAÇÃO DETERMINADA POR LEI.**

Em qualquer etapa do estudo, você terá acesso aos pesquisadores responsáveis pelo estudo para esclarecimento de eventuais dúvidas. O investigador principal é Dr. José Viana Lima Junior, médico endocrinologista e pós-graduando da disciplina de endocrinologia do departamento de medicina da EPM/Unifesp e o Dr. Claudio Elias Kater-professor titular pela disciplina de Endocrinologia do Departamento de Medicina da Unifesp-EPM que podem ser encontrados nos seguintes endereços (R. Pedro de

Toledo 13° andar), ou no Ambulatório de Adrenal (Rua Estado de Israel, 639, Vila Clementino/São Paulo) – telefone (11) 5576-4982ou através dos celulares (11) 982637253 ou **(11) 993383000 OU NOS EMAILS: jovilijr2000@yahoo.com.br ou claudio.kater@gmail.com** .Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Botucatu, 572 – 1º andar – cj 14, 5571-1062, FAX: 5539-7162 – E-mail: cepunifesp@epm.br.

Os dados coletados na pesquisa são sigilosos e serão analisados em conjunto com os de outros pacientes e seu nome não será divulgado ou identificado em nenhum momento da pesquisa.

É compromisso do pesquisador utilizar os dados e o material coletado somente para esta pesquisa. **O MATERIAL DE DNA SERÁ GUARDADO NO FINAL DA PESQUISA CASO HAJA NECESSIDADE DE REPETIÇÃO DO EXAME OU CASO SEJA NECESSÁRIO REAVALIAÇÃO CASO NOVOS GENES QUE TENHAM RELAÇÃO COM FEOCROMOCITOMA/PARAGANGLIOMA SEJAM DESCOBERTOS.**

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo “Avaliação e correlação clínica, laboratorial, radiológica, anátomo-patológica e genética de pacientes com feocromocitoma/ paragangliomas esporádicos e familiais acompanhados na UNIFESP/EPM”.

Eu,

_____

, discuti com os pesquisadores: Dr. José Viana Lima Junior e Dr. Claudio Elias Kater sobre minha decisão em participar desse estudo. Ficou claro para mim qual é o propósito do estudo, quais os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas e que tenho garantia do acesso a

tratamento hospitalar quando necessário e, desta forma, concordo voluntariamente em participar deste estudo e assino 2 vias desse documento, sendo que uma ficara comigo e outro com o pesquisador responsável.

_____

PARTICIPANTE

_____

RESPONSÁVEL

PESQUISADOR

SÃO PAULO, ___DE _____DE 20___

Rua Estado de Israel, 639, CEP 04021-001-São Paulo/Brasil

Tel: (11) 5576-4982

Endereço do Conselho de Ética e Pesquisa (CEP)

Rua PROFESSOR FRANCISCO DE CASTRO, 55, CEP 04020-050- São Paulo/Brasil

Tel: (11) 5571-1062 ou (11) 5539-7162- HORÁRIO DE ATENDIMENTO TELEFÔNICO E PRESENCIAL: SEGUNDAS, TERÇAS, QUINTAS E SEXTAS, DAS 09:00ÀS 13:00 HS.

Anexo 2

TERMO DE ASSENTIMENTO
Universidade Federal de São Paulo / Escola Paulista de Medicina
Disciplina de Endocrinologia

Meu nome é José Viana Lima Junior, sou médico endocrinologista e pós-graduando da Disciplina de Endocrinologia (Unidade de Adrenal e Hipertensão) do Departamento de Medicina da Unifesp/EPM, sob orientação do Prof. Dr. Claudio E. Kater. Somos os pesquisadores do seguinte projeto "Avaliação e correlação clínica, laboratorial, radiológica, anátomo-patológica e genética de pacientes com feocromocitoma/paraganglioma esporádicos e familiais acompanhados na Unifesp/EPM". Conversamos com seus pais ou seu responsável sobre o projeto e o consentimento deles também será necessário para participar da pesquisa. Você e seus pais podem pedir a opinião de outros familiares ou amigos sobre a pesquisa antes de tomarem a decisão de participar da mesma.

O objetivo desta pesquisa é identificar alterações nos genes (alterações no DNA) de pacientes com feocromocitoma esporádico acompanhados em nosso Hospital em comparação com tumores extra-adrenais (fora das glândulas adrenais) e outras síndromes genéticas já estabelecidas. Os genes analisados serão - *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 e VHL*. O proto-oncogene *RET* (um dos genes que será estudado e cuja alteração pode resultar no feocromocitoma) será pesquisado em nossa instituição. Tais genes serão estudados no sangue periférico, sendo necessária apenas coleta de sangue do braço (como um exame de sangue comum) - coletaremos 10 mL de sangue. Os riscos da coleta de sangue são mínimos, podendo ficar um hematoma ocasionalmente no local da punção que em alguns dias

será reabsorvido naturalmente pelo seu corpo. O único desconforto será a coleta de sangue.

As vantagens em participar desta pesquisa são: ajudar os pesquisadores a encontrarem a frequência de alterações genéticas em pacientes com feocromocitoma esporádico em nossa instituição e assim detectar precocemente essas alterações e planejar o plano terapêutico de forma melhor para o paciente.

É garantida a total liberdade de saída do estudo em qualquer momento que desejar sem qualquer prejuízo à continuidade de seu tratamento no Hospital São Paulo. Você não precisa participar desta pesquisa se não quiser. É você que decide. Se decidir não participar é seu direito e nada mudará no seu tratamento de saúde. Até mesmo se você disser “sim” agora, poderá mudar de idéia depois, sem nenhum problema. Se qualquer coisa mudar e nós quisermos que você participe da pesquisa até mesmo se você quiser parar, nós falaremos primeiro com você.

Os dados coletados na pesquisa são sigilosos e serão analisados em conjunto com de outros pacientes e seu nome não será divulgado ou identificado em nenhum momento da pesquisa.

Você tem direito de ser mantido atualizado sobre os resultados parciais da pesquisa desde que estes sejam de conhecimento do pesquisador.

Não existem despesas pessoais para o participante do estudo incluindo exames e consultas, assim como também não há compensação financeira relacionada a sua participação na pesquisa.

Em caso de dano pessoal, diretamente causado pelos procedimentos ou tratamentos propostos neste estudo, o participante tem direito a tratamento médico na Instituição.

É compromisso do pesquisador utilizar os dados e o material coletado somente para esta pesquisa.

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo Avaliação e correlação clínica,

laboratorial, radiológica, anátomo-patológica e genética de pacientes com feocromocitoma/paragangliomas esporádicos e familiais acompanhados na UNIFESP/EPM.

Eu,

_____

, discuti com o pesquisador José Viana Lima Junior e Dr Cláudio Elias Kater sobre minha decisão em participar desse estudo. Ficou claro para mim qual é o propósito do estudo, quais os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas e que tenho garantia do acesso a tratamento hospitalar quando necessário, desta forma, concordo voluntariamente em participar deste estudo.

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PACIENTE RESPONSÁVEL

_____

PESQUISADOR

Data:

Rua Estado de Israel, 639, CEP 04021-001-São Paulo/Brasil Tel: (11) 5576-4982

Endereço do Conselho de Ética e Pesquisa (CEP)

Rua Botucatu, 572, 1º. andar, conj. 14 CEP 04023-062-São Paulo/Brasil

Anexo 3

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação e Correlação Clínica, Laboratorial, Radiológica, Anátomo-Patológica e Genética de pacientes com feocromocitomas/paragangliomas esporádicos e familiais acompanhados na UNIFESP/EPM **Pesquisador:** jose viana lima junior

Área Temática: Genética Humana:

(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 3

CAAE: 11936719.0.0000.5505

Instituição Proponente: Escola Paulista de Medicina

Patrocinador Principal: Fleury S.A

DADOS DO PARECER

Número do Parecer: 3.436.233

Apresentação do Projeto:

-Projeto CEP/UNIFESP n:0414/2019 (parecer final)

-Trata-se de projeto de doutorado de José Viana Lima Junior. Orientador: Prof. Dr. Claudio Elias Kater;

Colaboradores: Janete Cerutti (UNIFESP/EPM); Susan Lindsey (UNIFESP/EPM); Kelly C. Oliveira

(UNIFESP/EPM); Cássio Andreoni (Urologia – UNIFESP/EPM); Suzan M. Goldman (Diagnóstico por

Imagem – UNIFESP/EPM); Maria Teresa Seixas Alves (patologia UNIFESP/EPM); Rosana Delcelo

(patologia UNIFESP/EPM); Nilza Maria Scalissi (Endocrinologia - Sta. Casa-SP) ; Rosa Paula Mello Biscolla

(Fleury e UNIFESP/EPM); Maria Izabel Chiamolera (Fleury e UNIFESP/EPM); Wagner Antônio da Rosa

Baratela (Fleury); Elisa Napolitano E. Ferreira (Fleury). Projeto vinculado ao Departamento de Medicina, Campus São Paulo, Escola Paulista de Medicina, UNIFESP.

-Patrocinador Principal: Fleury S.A

APRESENTAÇÃO: Feocromocitomas são tumores neuroendócrinos capazes de produzir, armazenar e secretar catecolaminas e outras substâncias (VIP, PTHrp, opióides, peptídeo relacionado com a calcitonina, CRH, ACTH, histamina, cromogranina, IL-6, dentre outros). (1, 3,7,8A). Aproximadamente 90% estão localizados nas adrenais e 10% extra-adrenais, sendo chamados, nesse caso, de paragangliomas, que podem estar localizados desde a base do crânio até os testículos e a grande maioria em região abdominal. (11,19)

-HIPÓTESE: Na literatura, atualmente é recomendado que todo paciente com feocromocitoma/paraganglioma tenha uma avaliação genética, pois até 50% desses pacientes possuem de hereditariedade e outro grupo que não apresente tais características, sendo considerados inicialmente como esporádico e se realmente vale a pena oferecer o teste genético para todos os pacientes com feocromocitoma/paraganglioma através do estudo dessa população.

Objetivo da Pesquisa:

-OBJETIVO PRIMÁRIO: 1) Avaliação e correlação de parâmetros clínicos, laboratoriais, radiológicos e anátomo-patológicos de pacientes com feocromocitoma e/ou paraganglioma aparentemente esporádicos e genéticos acompanhados na Disciplina de Endocrinologia da UNIFESP/EPM; 2) Estudo molecular dos seguintes genes relacionados com eocromocitomas/paragangliomas (esporádicos e familiais) em sangue periférico por sequenciamento de próxima geração (NGS) e nos casos típicos de NEM2A por PCR por Sanger, numa extensa coorte brasileira. Os genes estudados serão: *ATM, ATR, CDKN2A, EGLN1, FH,HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD,TMEM127, TP53 e VHL.* Análise criteriosa em busca de variantes genéticas patogênicas. -OBJETIVO SECUNDÁRIO: 1) Avaliar se oferecer o teste genético para essa população é de fato mandatório. 2) Identificação hormanal e topográfica do feocromocitoma/paraganglioma

Avaliação dos Riscos e Benefícios:

Em relação aos riscos e benefícios, o pesquisador declara:

Riscos: : O risco é mínimo, e está relacionado à punção venosa: podem surgir hematomas ou equimoses no local da punção. Caso isso aconteça, será oferecida pomada de Hirudoid para uso no local afetado. Benefícios: Através desse estudo, conseguiremos entender melhor do ponto de vista clínico,laboratorial, radiológico, anátomo-patológico e genético de uma doença rara e potencialmente fatal e maligna numa coorte brasileira

Comentários e Considerações sobre a Pesquisa:

TIPO DE ESTUDO: Estudo retrospectivo e prospectivo. O estudo tem por objetivo avaliar e correlacionar com quadro clínico, laboratorial, radiológica, anátomo-Patológica e genética de pacientes com feocromocitomas /paragangliomas acompanhados na UNIFESP/EPM. Serão divididos em 2 grupos (braços): o primeiro grupo consta de pacientes feocromocitomas/parganglíomas aparentemente esporádicos e o segundo grupo consta de pacientes feocromocitomas/parganglíomas aparentemente genéticos.

LOCAL: Disciplina de Endocrinologia da UNIFESP/EPM; laboratório Fleury Medicina e Saúde;

PARTICIPANTES: pacientes atendidos na UNIFESP/EPM, no período de 2000 a 2019, num total de 120 pacientes que sejam separados em 2 grupos como aparentemente esporádicos e aparentemente genéticos. -Critério de Inclusão: Para o grupo aparentemente genéticos serão utilizados os seguintes critérios de inclusão: Pacientes com 13 anos ou mais de idade com diagnóstico de feocromocitoma/ paraganglioma com características genéticas (manchas café com leite, neurofibromas, puberdade precoce, cistos renais, cistos pancreáticos, tumores pancreáticos, carcinoma renal de células claras, adenomas de pituitária, angiomas de retina, hemangioblastoma de SNC, tumores de orelha média, carcinoma medular de tiroide, hiperparatiroidismo primário, hábitos marfanóide , neuromas de língua, ganglioneuromatose intestinal, megaesôfago, megacólon, liquen cutâneo amiloidótico, doença de Hirschsprung, GIST e policitemia) que estejam de acordo com os termos de consentimento e de assentimento.
Para o grupo aparentemente esporádico: pacientes com 13 anos ou mais de idade com diagnóstico de feocromocitoma/ paraganglioma sem características genéticas (manchas café com leite, neurofibromas, puberdade precoce, cistos renais, cistos pancreáticos, tumores pancreáticos, carcinoma renal de células claras, adenomas de pituitária, angiomas de retina, hemangioblastoma de SNC, tumores de orelha média,

carcinoma medular de tiroide,hiperparatiroidismo primário, hábitos marfanóide , neuromas de língua, ganglioneuromatose intestinal, megaesôfago, megacólon, liquen cutâneo amiloidótico, doença de Hirschsprung, GIST e policitemia) que estejam de acordo com os termos de consentimento e de assentimento.
-Critério de Exclusão: Pacientes com idade inferior a 13 anos e aqueles que não quiserem participar e assinar o TCLE.

PROCEDIMENTOS: Os dados são coletados no momento da consulta médica e o termo de consentimento informado para coleta de sangue.

-O proto-oncogene RET será estudado na Disciplina de Endocrinologia da UNIFESP/EPM (em colaboração com a Dra. Janete Cerutti) pela técnica de sequenciamento por PCR em Sanger e os genes *ATM, ATR, CDKN2A, EGLN1, FH, HRAS, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 e VHL* por sequenciamento de próxima geração (NGS) em colaboração com laboratório Fleury Medicina e Saúde.

-Os dados clínicos, laboratoriais, radiológicos, cirúrgicos, anátomo-patológicos e genético-moleculares são anotados em fichas de papel e colocados em um banco de dados em programa de computador (Microsoft Excel) e atualizados em cada visita do paciente. Não necessariamente serão realizados todos os exames diagnósticos bioquímicos, e topográficos listados acima. Todos são importantes, mas não necessariamente precisam ser realizados para diagnóstico da doença, em geral, dos exames bioquímicos focaremos em metanefrinas plasmáticas e/ou metanefrinas urinárias de 24 horas o que é preconizado pela Endocrine Society Guideline de 2014 e dos exames topográficos, focaremos em TC ou RM de adrenais e/ou MIBG e quando houver disponibilidade Octreoscan/PETCT com gálio68DOTA/PETCT com FDG . (mais informações, ver projeto detalhado).

Considerações sobre os Termos de apresentação obrigatória:

1- Foram apresentados os principais documentos: folha de rosto; projeto completo; cópia do cadastroCEP/UNIFESP, orçamento financeiro apresentados adequadamente.

2- TCLE a ser aplicado aos participantes.

3- outros documentos importantes anexados na Plataforma Brasil:

a)-autorização da coep n: 46/2019 (Pasta: Declaração de Instituição e Infraestrutura- Submissão 4;

Documento: carta_coep.JPG)

b)- acordo de cooperação Técnica entre Escola Paulista de Medicina, UNIFESP e Fleury s/a (Pasta: Declaração do Patrocinador- Submissão 4; Documento: de Fleury_unifesp1.pdf a Fleury_unifesp20.pdf) 4- Orçamento Financeiro: R$ 100.000,00. Os exames de análises clinica, radiológico,de medicina nuclear e anatomia-patológica serão realizados obrigatoriamente no hospital, não necessariamente todos, mas o conjunto de exames que seja capaz de diagnosticar a doença, levando ao tratamento adequado. O exame genético do protooncogene RET será realizado em parte no laboratório de genética da responsabilidade da dra Janete Cerutti e o painel genético para feocromocitoma/paraganglioma será realizado em parceria do laboratório Fleury, conforme carta de aprovação anexada.

5. Resposta as pendências
6.Cronograma atualizado
7. TCLE
8-Termo de confidencialidade
9. PB-Informações
10. Resposta pendencias

11. PB Informações básicas do projeto
12. Termo de consentimento livre e esclarecido corrigido
13. Projeto tese braco 1
14. Projeto tese braco 2

Recomendações:

Sem recomendações

Conclusões ou Pendências e Lista de Inadequações:

Respostas ao parecer nº 3378449 de 07 de Junho de 2019. PROJETO APROVADO.
Respostas as pendências aprovadas
PENDÊNCIA 4 - Adequar, no formulário de submissão da Plataforma Brasil, o campo "Riscos": Conforme orientação da CONEP, lembramos que qualquer pesquisa com seres humanos pode causar algum risco, por mínimo que seja. No que diz respeito a esta pesquisa, por exemplo, devem ser informados os riscos da coleta de sangue e de quebra de sigilo dos dados do participante.
RESPOSTA: O risco é mínimo, e está relacionado à punção venosa: podem surgir hematomas ou equimoses no local da punção. Caso isso aconteça, será oferecida pomada de Hirudoid para uso no local afetado.

PENDÊNCIA PARCIALMENTE ATENDIDA, já que o campo riscos não foi modificado nas informações da plataforma Brasil ("Riscos: Para o estudo não há riscos, pois todos os pacientes serão tratados mesmo aqueles que não quiserem participar do estudo."
Por favor, realizar a alteração
Resposta: Alteração feita no campo de riscos.

PENDÊNCIA ATENDIDA

Pendência 7.6 – todas as páginas devem ser numeradas (ex: 1/4, 2/4, etc.), mesmo que seja uma só (1/1). Ressaltamos que as páginas deverão ser rubricadas pelo pesquisador e pelo participante da pesquisa no momento da aplicação do TCLE.

Resposta: Modificações feitas, conforme recomendações.

ANÁLISE CEP UNIFESP: No arquivo enviado, as páginas não se encontram numeradas.

PENDÊNCIA NÃO ATENDIDA

Resposta: As páginas dos projetos (braços 1 e 2) estão enumeradas. No total de 29 páginas.

PENDÊNCIA ATENDIDA

Considerações Finais a critério do CEP:

O CEP informa que a partir desta data de aprovação, é necessário o envio de relatórios parciais (semestralmente), e o relatório final, quando do término do estudo, por meio de notificação pela Plataforma Brasil.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1274517.pdf	18/06/2019 08:16:57	
TCLE / Termos de Assentimento / Justificativa de	termo_de_consentimento_livre_e_esclarecido_corrigido.doc	18/06/2019 08:16:18	jose viana lima junior

Ausência			
Recurso Anexado pelo Pesquisador	Respostas_pendencias_11_06_2019.docx	11/06/2019 09:59:50	jose viana lima junior
Brochura Pesquisa	Projeto_tese_braco_2.docx	11/06/2019 09:30:17	jose viana lima junior
Brochura Pesquisa	Projeto_tese_braco_1.docx	11/06/2019 09:26:19	jose viana lima junior
Outros	Termo_de_confidencialidade_sigilo_assinado.jpg	31/05/2019 21:41:46	jose viana lima junior

Página 06 de

Cronograma	Cronograma_Tese.docx	31/05/2019 21:37:42	jose viana lima junior	Aceito
Folha de Rosto	Folha_de_rosto_corrigida_JV.pdf	15/04/2019 22:19:40	jose viana lima junior	Aceito
Declaração de Instituição e	carta_coep.JPG	25/02/2019 20:15:41	jose viana lima junior	Aceito

Infraestrutura				
Declaração de Instituição e Infraestrutura	Comite_eticapesquisapdf.pdf	17/02/2019 10:10:23	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp20.pdf	22/01/2019 10:12:42	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp19.pdf	22/01/2019 10:12:32	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp18.pdf	22/01/2019 10:12:22	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp17.pdf	22/01/2019 10:12:10	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp16.pdf	22/01/2019 10:11:57	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp15.pdf	22/01/2019 10:11:40	jose viana lima junior	Aceito

Declaração do Patrocinador	Fleury_unifesp14.pdf	22/01/2019 10:11:28	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp13.pdf	22/01/2019 10:11:17	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp12.pdf	22/01/2019 10:11:05	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp11.pdf	22/01/2019 10:10:48	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp10.pdf	22/01/2019 10:06:25	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp9.pdf	22/01/2019 10:06:08	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp8.pdf	22/01/2019 10:05:50	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp7.pdf	22/01/2019 10:05:30	jose viana lima junior	Aceito

Declaração do Patrocinador	Fleury_unifesp6.pdf	22/01/2019 10:05:14	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp5.pdf	22/01/2019 10:05:03	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp4.pdf	22/01/2019 10:04:52	jose viana lima junior	Aceito
Declaração do Patrocinador	Fleury_unifesp3.pdf	22/01/2019 10:04:34	jose viana lima junior	Aceito
Declaração do	Fleury_unifesp2.pdf	22/01/2019	jose viana lima	Aceito

Patrocinador	Fleury_unifesp2.pdf	10:04:17	junior	Aceito
Declaração do Patrocinador	Fleury_unifesp1.pdf	22/01/2019 10:03:56	jose viana lima junior	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_tese2.doc	22/01/2019 10:01:28	jose viana lima junior	Aceito
Projeto Detalhado / Brochura	Projeto_tese1.doc	22/01/2019 10:01:16	jose viana lima junior	Aceito

Investigador				
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_assentimento.doc	20/01/2019 22:54:45	jose viana lima junior	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO PAULO, 03 de Julho de 2019

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Assinado por:

Miguel Roberto Jorge

(Coordenador(a))

Colaboração em artigo publicado ou enviado para publicação

Spectrum and Prevalence of *FP/TMEM127* Gene Mutations in Pheochromocytomas and Paragangliomas

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Luigi Mori, PhD
Mara Giacchè, PhD
Antonella Mendola, PhD
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Context Pheochromocytomas and paragangliomas are genetically heterogeneous neural crest–derived neoplasms. We recently identified germline mutations of the novel transmembrane-encoding gene *FP/TMEM127* in familial and sporadic pheochromocytomas consistent with a tumor suppressor effect.

Objectives To examine the prevalence and spectrum of *FP/TMEM127* mutations in pheochromocytomas and paragangliomas and to test the effect of mutations in vitro.

Design, Setting, and Participants We sequenced the *FP/TMEM127* gene in 990 individuals with pheochromocytomas and/or paragangliomas, including 898 previously unreported cases without mutations in other susceptibility genes from 8 independent worldwide referral centers between January 2009 and June 2010. A multiplex polymerase chain reaction–based method was developed to screen for large gene deletions in 545 of these samples. Confocal microscopy of 5 transfected mutant proteins was used to determine their subcellular localization.

Main Outcome Measures The frequency and type of *FP/TMEM127* mutation or deletion was assessed and correlated with clinical variables; the subcellular localization of 5 overexpressed mutants was compared with wild-type FP/TMEM127 protein.

Results We identified 19 potentially pathogenic *FP/TMEM127* germline mutations in 20 independent families, but no large deletions were detected. All mutation carriers had adrenal tumors, including 7 bilateral ($P=2.7\times10^{-4}$) and/or with familial disease (5 of 20 samples; $P=.005$). The median age at disease onset in the *FP/TMEM127* mutation group was similar to that of patients without a mutation (41.5 vs 45 years, respectively; $P=.54$). The most common presentation was that of a single benign adrenal tumor in patients older than 40 years. Malignancy was seen in 1 mutation carrier (5%). Expression of 5 novel *FP/TMEM127* mutations in cell lines revealed diffuse localization of the mutant proteins in contrast with the discrete multiorganelle distribution of wild-type TMEM127.

Conclusions Germline mutations of *FP/TMEM127* were associated with pheochromocytoma but not paraganglioma and occured in an age group frequently excluded from genetic screening algorithms. Disease-associated mutations disrupt intracellular distribution of the FP/TMEM127 protein.

JAMA. 2010;304(23):2611-2619 www.jama.com

PHEOCHROMOCYTOMAS AND paragangliomas are chromaffin cell tumors of neural crest origin that arise from the adrenal medulla or extra-adrenal sympathetic paraganglia, respectively, and are frequently catecholamine secreting.[1] These tumors are usually benign and can occur as a single entity or as part of various hereditary tumor syndromes. Genetically, pheochromocytomas and paragangliomas are heterogeneous, with at least one-third of cases resulting from germline but not somatic mutations in 1 of several independent genes: *RET*, *VHL*, *NF1*, and succinate dehydrogenase (*SDH*) subunit B, C, and D genes.[2-5] More recently, other candidate susceptibil-

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Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. Li Yao [1], Francesca Schiavi, Alberto Cascon, Yuejuan Qin, Lucia Inglada-Pérez, Elizabeth E King, Rodrigo A Toledo, Tonino Ercolino, Elena Rapizzi, Christopher J Ricketts, Luigi Mori, Mara Giacchè, Antonella Mendola, Elisa Taschin, Francesca Boaretto, Paola Loli, Maurizio Iacobone, Gian-Paolo Rossi, Bernadette Biondi, José Viana Lima-Junior, Claudio E Kater, Marie Bex, Miikka Vikkula, Ashley B Grossman, Stephen B Gruber, Marta Barontini, Alexandre

Persu, Maurizio Castellano, Sergio P A Toledo, Eamonn R Maher, Massimo Mannelli, Giuseppe Opocher, Mercedes Robledo, Patricia L M Dahia.

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Genotype-Specific Neoplastic Risk Profiles in Patients with von Hippel-Lindau (VHL) Disease: a multicenter observational study by Professor Hartmut Neumann The Lancet Oncology

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***Journal of the Endocrine Society*, Volume 4, Issue Supplement_1, April-May 2020, SUN-939,** https://doi.org/10.1210/jendso/bvaa046.734

SUN-939 Case Report: Malignant Pheochromocytoma

Published: 08 May 2020

Jose Viana Lima; Rosa Paula Mello Biscolla; Maria Izabel Chiamolera; Marco Antonio Conde Oliveira

Abstract

Introduction: The concept of malignancy for pheochromocytoma is complex and the best definition is the presence of metastases, according to WHO. Anatomopathological scoring systems are not effective in predicting metastases.

Malignancy should be considered when tumors larger than 8cm (> 80g), paragangliomas (especially retroperitoneal), dopamine / methoxythyramine increase, Ki67> 6% and SDHB mutation. At 5 years, survival ranges from 50-69%. Metastases may appear 20-40 years after initial treatment of pheochromocytoma. We describe a case that metastasis was identified 33 years after pheochromocytoma excision. Case report: A 57-year-old female patient with a postoperative history of 33 years of right adrenal pheochromocytoma was discharged from the endocrinologist after 10 years of follow-up. At diagnosis 33 years ago, she had symptoms of hypertension with paroxysms and weight loss that disappeared after tumor removal. 2 years investigating weight loss with general practitioner without another celebratory. On physical examination, orthostatic hypotension was highlighted. Plasma methanephrine 0.8 nmol / L (VR <0.5) and plasma normetanephrine 1.8 nmol / L (VR <0.9), chromogranin A 5.7 nmol / L (VR <3 nmol / L) and clonidine test with 36.6% suppression of metanephrines, suggesting tumor recurrence. MRI localized recurrence of the adrenals and MIBG scintigraphy with I131 that showed, respectively, in the topography next to the paracaval and retroportal right

diaphragmatic crura, isointense T1 and slightly hyperintense T2 at 1.8 cm and radiopharmaceutical hypercaptation in right adrenal topography. Genetic panel by NGS did not identify germline mutation in 22 pheochromocytoma-related genes. FDGPETCT was consistent with MRI and MIBG images. Gallium PETCT68 DOTATOC detected the lesions already described, inaddition to a lytic lesion in the left femoral intertrochanteric medulla. Anatomopathological approached abdominal lesion confirming pheochromocytoma metastasis in lymph node conglomerate. Currently has a negative methanephrine plasma, however chromogranin A 142 ng / mL (VR <93), and was chosen by the observant approach. Conclusion: The case of the patient illustrates that pheochromocytoma should be followed indefinitely, as metastases may appear many years later and may present different aggressiveness potentials.

***Journal of the Endocrine Society*, Volume 4, Issue Supplement_1, April-May 2020, MON-918,** https://doi.org/10.1210/jendso/bvaa046.1150

MON-918 Familial Paraganglioma: Familiar Case Report

Published: 08 May 2020

Gustavo Piech Ricardo; Murilo Marques Naldi; Camila Ricci Calasans; Claudia Veiga Chang; Renata da Cunha Scalco Tirapeli; Cristina Bellotti Formiga Bueno; Nilza Scalissi; **Jose Viana Lima**

Abstract

Introduction: Pheochromocytomas and Paragangliomas (PGL) are rare tumors originating from chromaffin cells. They may be sporadic or associated with familial inherited genetic syndromes around 50-80%. There are several PGL syndromes, the most common being PGL 1 (SDHD mutations), PGL 2 (SDHAF), PGL 3 (SDHC), PGL 4 (SDHB), PGL 5 (SDHA), PGL 6 (SLC25A11) and PGL 7 (DLST). SDHB mutations generate a higher probability of malignant PGL, as well as risk of renal, GIST and pituitary neoplasms. We report the case of a patient with a positive family history for the autosomal

dominant SDHB mutation.Clinical cases: FZR, male, 19 years old, history of headache, sweating, palpitations, and sudden onset tremors associated with hypertensive peaks. Physical examination: Blood Pressure 140x90mmHg lying down, 110x70 standing up. Performed examinations, of which altered, showed: Plasma metanephrines: 82 pg/mL (RV <65), Plasma normetanephrines: 1.488pg/mL (VR <196), Urinary Catecholamines: 1.784mcg/24h (RV: 80-500), Abdomen Resonance showed an expansive, solid, heterogeneous abdomen mass in posterior contact with the left psoas muscle, medial with the aorta, and lateral with jejunum loops, measuring 7x3.5 cm. MIBG scintigraphy: abnormal uptake in left kidney. Family history: uncle diagnosed with cervical paraganglioma with cervical lymph node metastasis, gastric GIST and PCR genetic sequencing identifying mutation in SDHB (Q.137 G > T in exon

2). Asymptomatic second cousin with positive genetic analysis for the same mutation and another deceased first cousin diagnosed with pheochromocytoma with bone metastasis. He underwent tumor resection that identified retroperitoneal paraganglioma with 10% KI67, Protein S-100, Chromogranin-A and Synaptophysin positive. Carried out PCR genetic analysis that identified the same Q.137 G > T mutation in exon 2 of the SDHB gene in heterozygosis.Twenty-six relatives were called for mutation research, of which 5 positive for the SDHB mutation, until now, including the patient's mother and twin brother, both already investigating related diseases.We await new family members and, subsequently, the result of the mutation analysis to continue the clinical and laboratory follow-up of this family.Conclusion: Although rare, this condition should be remembered as a differential diagnosis of diseases with such clinical symptoms and, once characterized, investigate possible associations with genetic syndromes.

***Journal of the Endocrine Society*, Volume 5, Issue Supplement_1, April-May 2021, Page A1002,** https://doi.org/10.1210/jendso/bvab048.2050

Non-Functioning Pheochromocytoma in a Patient With Von Hippel Lindau Syndrome (VHL): Case Report

Published: 03 May 2021

Gustavo Piech Ricardo; Nilza Scalissi; Cristina Bellotti Formiga Bueno; Renata Da Cunha Scalco; **Jose Viana Lima**

Abstract

Background: We report the case of a patient with VHL Syndrome with an adrenal lesion compatible with nonfunctioning pheochromocytoma and it's diagnostic management. **Case Report**: JMN, female, 32 years old, referred for screening for VHL syndrome, after diagnosis in a sister, who has clear cell renal carcinoma (ccRCC), cerebellar hemangioblastoma and genetic analysis, by next generation sequencing (NGS), which identified the allelic variant germline pathogenic c. 256C> T in heterozygosis in exon 1 of the VHL gene. The patient is asymptomatic and her physical examination is normal. Optic fundus examination with lesion suggestive of right hemangioblastoma. Family history: mother who died at 59 with a diagnosis of ccRCC, without genetic investigation. Maternal aunt diagnosed with VHL and involvement of the cerebellum, kidney and pancreas. Two brothers with genetic and clinical diagnosis of VHL syndrome, presenting pheochromocytoma and renal carcinoma. Laboratory tests: plasma metanephrine: 0.3 nmol/L (RV T in heterozygosity in exon 1 of the VHL gene. Topographic examinations: magnetic resonance imaging (MRI) of adrenals with nodule in the left adrenal gland, hypersignal in T2, measuring 2.9 x 2.3 cm, suggestive of pheochromocytoma and whole body scintigraphy with metaiodobenzylguanidine (MIBG) positive in the left adrenal gland. She underwent resection of the tumor in the left adrenal, without complications. Anatomopathology compatible with pheochromocytoma with immunohistochemistry for ki67 < 3%. Currently, the patient is clinically stable and with periodic follow-up, as well as family members, performing screening for diseases associated with VHL. **Conclusion**: VHL syndrome is one of the possible causes of non-functioning pheochromocytomas and paragangliomas, and adrenal lesions with negative

metanephrine levels do not exclude them; thus highlighting the importance of exams such as CT or MRI and functional topographic studies (whole body scintigraphy with MIBG and / or PETCT with Galio68DOTA) for their diagnosis.

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Simultaneous Occurrence of Germline Pathogenic Allele Variants of TMEM127 *and* TP53 *in a Brazilian Family With Li-Fraumeni Syndrome*

Published: 03 May 2021

Jose Viana Lima; Nilza Maria Scalissi; Gustavo Piech Ricardo; Mr Gustavo Piech; Rosa Paula Mello Biscolla; Maria Izabel Chiamolera; Caroline Olivati; Wagner Baratela; Elisa Napolitano Ferreira; **Claudio E Kater**

Abstract

Background: We will describe a Brazilian family whose index case had pheochromocytoma and in the evaluation of the genetic panel by Next Generation Sequence (NGS), the germline pathogenic variants in the *TMEM127* and *TP53* genes were identified. **Clinical Case:** A 32-year-old female patient with a clinical picture of paroxysms and difficult to control arterial hypertension, with a personal history of stroke and acute myocardial infarction. She had a 6.5 cm tumor in the right adrenal and urine metanephrine levels of 5.5 mg / g creatinine (VR <1 mg / g creatinine) compatible with pheochromocytoma. She underwent laparoscopic right adrenalectomy. There was a reversal of arterial hypertension and paroxysms. 10 years after adrenalectomy, she was diagnosed with bilateral breast cancer, she underwent radical total mastectomy and 2 years ago there was a recurrence of breast cancer and currently undergoing chemotherapy. Germinative genetic panel carried out by NGS had identified pathogenic variants c.1010G> A, p. (Arg337His) in heterozygosity in the *TP53* gene and c.117_120del p. (Ile41Argfs * 39) in heterozygosis in the *TMEM127* gene. Her 28-year-old daughter diagnosed bilateral breast cancer and meningeoma in the central nervous

system and she had the same pathogenic variants germlines. Thus far, there is no clinical, laboratory or radiological picture of pheochromocytoma. Her 11-year-old granddaughter has only the pathogenic allele variant c.117_120del p. (Ile41Argfs * 39) in heterozygosity in the TMEM127 gene and thus far she has no clinical, laboratory and radiological picture of pheochromocytoma. **Conclusion**: This is the first case report of the simultaneous occurrence of pathogenic germline variants in the *TMEM127* and *TP53* genes. Reference: 11) Toledo RA et al Consensus Statement on next-generation-sequencing-based diagnostic testing of hereditary pheocromocytomas and paragangliomas. Nature Reviews Endocrinology 13, 233-247 (2017).

Severe Cushing Syndrome Due to Ectopic ACTH Secretion by Pheochromocytoma

Rafael Buck Giorgi and others

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Published: 03 May 2021

Rafael Buck Giorgi; Rayssa Fabiana Chamma; Rayssa F Chamma; Ituo Filho; Giane Cristina Garcia; Patricia Wanderley; **Jose Viana Lima**; Lilian Sollitari; Cristiano Roberto Barcellos; João Carlos Ramos Dias

Abstract

Introduction: Ectopic ACTH production is a very unusual cause of Cushing Syndrome (CS). When it occurs, lung cancer is the main cause. Very rarely, this ectopic source of ACTH can arise from a Pheochromocytoma (Pheo). A recent literature review identified less than 100 cases described. We present a case of 28 years old woman who was referred for adrenalectomy for CS with notorious adrenal mass. However, during the investigation, ectopic ACTH due a Pheo was identified. ***Case Report:*** A 28-year-old woman required emergency care for ecchymosis and asymptomatic hypertension (BP: 230x130mmHg). Hyperpigmentation of the skin was evident on physical examination.

Severe hypokalemia (K: 2.5mEq/liter) was detected. She denied taking any medication and was unaware of any previous illness. She always had normal BP measurements as well as laboratory tests. No family history of adrenal disease or secondary hypertension causes. During hospitalization, the hypothesis of CS was made and confirmed after: cortisol after 1mg dexamethasone: 44.5mcg/dl (<1.8mcg/dl) and 24h urinary free cortisol: 6228 mcg/dl (28-213mcg/dl). Concomitantly, a CT scan of the abdomen exhibited a left adrenal mass (3.1x2.8x3.5cm) of uncertain etiology and ACTH: 352pg/ml (<46pg/ml). Additionally, the patient presented hyperglycemia during hospitalization. After confirmation of the ACTH dependent CS, pituitary MRI was performed with normal results and a chest CT scan ruled out lung masses. As there was still no etiological confirmation and due to clinical deterioration, it was decided to start Ketoconazole 200mg/day, rising until 600mg and spironolactone with doses up to 250mg/day with a significant improvement in hypokalemia, decreased cortisol levels and optimal BP control. Due to the extremely high levels of ACTH and indeterminate adrenal mass, the hypothesis of ACTH ectopic due Pheo was postulated. Patient underwent abdomen MRI with left adrenal mass with hypersignal at T2 and urinary metanephrines levels: 6132mcg/24h (<289mcg/24h), urinary normetanephrines: 1808mcg/24h (<732mcg/24h). Once the diagnosis was elucidated, she received preoperative preparation with alpha blocker (Doxazosin) and underwent adrenalectomy without complications. After discharge, she received prednisone 10mg/day. The patient presented normalization of BP levels, as well as glycemic control with a slight improvement in skin hyperpigmentation. The pathology department confirmed Pheo and an ACTH expression was observed in immunohistochemistry. A genetic panel for Pheo is running with no results so far. ***Conclusion:*** Despite an extremely rare cause of CS, the ectopic production of ACTH by a Pheo has extremely high mortality rates, especially when not diagnosed or managed correctly. The clinicians must always remain alert and suspect this syndrome when the patient has a confirmed ACTH dependent CS associated with adrenal masses.

***Journal of the Endocrine Society*, Volume 6, Issue Supplement_1, November-December 2022, Page A51,** https://doi.org/10.1210/jendso/bvac150.106

ODP023 Adrenal medullary hyperplasia as a differential diagnosis of paroxysmal hypertension: case report

Published: 01 November 2022

Paula de Abreu Toniolo; Nilza Maria Scalissi; Cristina Bellotti Formiga Bueno; Renata da Cunha Scalco; Fabiana Toledo Bueno Pereira; Suzan Menasce Goldman; Bruno Vaz Kerges Bueno; Pedro Ivo Ravizzini; Mônica Maria Ágata Stiepcich; **José Viana Lima** Júnior

Abstract

Background. Sporadic Adrenal Medullary hyperplasia (AMH) has been little described in the literature, being most often diagnosed during a genetic syndrome screening or the evaluation of an adrenal incidentaloma.

Case report. A 51-year-old female patient presented with progressive attacks of tachycardia, headache, nausea and mild paroxysmal hypertension, which started in December 2020. She had a history of smoking and she also presented Takotsubo syndrome in 2018. She denied other comorbidities. In March 2021, she was submitted to laboratorial testing which demonstrated indeterminate plasma fractionated metanephrines by HPLC/MS: metanephrines 0.3 nmol/L (n< 0.5) and normetanephrines 1.2 nmol/L (n< 0.9) and normal serum chromogranin A level: 82 ng/mL (n<93) by Directed Proteomic. Urinary metanephrines were collected in a spot sample after an adrenergic crisis episode, confirming the biochemical diagnosis: metanephrines 255 mcg/g creatinine (n 30-165), normetanephrine 824 mcg/g creatinine (n 105-375), total 1119 mcg/g creatinine (n 150- 510) by HPLC. She was submitted to an abdominal MRI, which showed bilateral adrenal thickening with medullary predominance with hyperintense sign on T2-weighted images. It was also performed a mIBG I 131 spect and a PET-CT DOTATATO-Galium 68 without abnormal uptakes. A genetic panel by NextGeneration Sequence (NGS) was also carried out for pheochromocytoma/paraganglioma

(PHEO/PGL) without pathological findings. The patient underwent laparoscopic left adrenalectomy after the use of alpha and beta blockade for one month, with pathological findings suggestive of adrenal medullary hyperplasia. However, she remained symptomatic after surgery. Once again, new urinary metanephrines in a spot sample were collected after an adrenergic crisis: metanephrines 188 mcg/g creatinine (n 30-165), normetanephrines 1229 mcg/g creatinine (n 105-375), total 1417 mcg/g creatinine (n 150-510) by HPLC, which demonstrated remaining cathecolamine excess. She underwent a contralateral laparoscopic adrenalectomy without surgical complications. An anatomopathological analysis was performed in both adrenals, which confirmed bilateral adrenal medullary hyperplasia without associated PHEO. The patient showed improvement in adrenergic symptoms and normalization of plasma fractionated metanephrines levels postoperatively, currently using hydrocortisone and fludrocortisone for the treatment of adrenal insufficiency.

Conclusion. Adrenal Medullary hyperplasia is a rare condition, which usually presents in a milder form and is often associated with pheochromocytoma or genetic syndromes. However it should be considered in symptomatic patients without a personal or family history of PHEO/PGL, allowing an early diagnosis and treatment.

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Published: 05 October 2023

THU523 - A Rare Case Of Functional Cervical Paraganglioma: Case Report

Vitória Dupas Oliveira; Guilherme Cavazzani Vaccarezza; Alysson Emannuel Neves Rodrigues Vieira; Nilza Maria Scalissi; Américo Rubens Leite dos Santos; Paulo Roberto Lazarini; Alexandre Baba Suehara; Caroline Olivati; **Jose Viana Lima**

Abstract

INTRODUCTION: Head and neck paragangliomas (HNPGL) are rare tumors arising from cells associated with parasympathetic ganglia. Catecholamine hypersecretion is founded in less than 5% of the patients. Evidence is growing that about 50% of the HNPGL are associated with hereditary syndromes. CASE REPORT: Male patient, 34 years old, previously healthy, presented with a 1-year history of jaw pain and hypoacusis, associated with progressive symptoms of dysphagia, facial paralysis, dysphonia and hoarseness. He also had a 2-month history of hypertension and palpitations. On physical examination, the patient had deficits in cranial nerves (CN) VII, VIII, IX, X, XI and XII and blood pressure of 150x90mmHg. Head and neck MRI showed an expansive lesion occupying the right jugular foramen extending above the cistern at the cerebellopontine angle and below the carotid space in the ipsilateral infratemporal fossa, reaching 50% of the internal carotid artery and extending into the right internal auditory canal, suggestive of glomus jugulare paraganglioma. Laboratory examination showed normetanephrines 9.1 mmol/L (VR < 0.9), confirming catecholamine-secreting paraganglioma. Next Generation Sequence (NGS) genetic testing evaluating 24 genes (*ATM, DLST, EGLN1, EGLN2, FH, EPAS1 (HIF2A), HRAS, KIF1B , MAX, MDH2, MEN1 , MERTK , MET, NF1, RET, SLC25A11, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53 and VHL)* was positive for the pathogenic variant c.166_170del:p.(Pro56Tyfs*5) of the SHDB gene. Patient with no family history of paraganglioma. Alpha-adrenergic blockade was started with doxazosin and, after two weeks, beta-adrenergic blockade with propranolol. After 3 months, he underwent preoperative embolization 48 hours before surgery with total tumor resection. Patient did not require antihypertensive drugs after surgery. CONCLUSIONS: We reported a case of

a young patient diagnosed with catecholamine secreting HNPGL that carries pathogenic variant of *SHDB*.

Presentation: Thursday, June 15, 2023.