Journal of Nuclear Medicine, published on March 20, 2020 as doi:10.2967/jnumed.120.245613

Genetic determinants of pheochromocytoma and paraganglioma imaging phenotypes

David Taïeb[1], Karel Pacak[2]

[1]Department of Nuclear Medicine, La Timone University Hospital, CERIMED, Aix-Marseille Univ, Marseille, France.

[2]Section on Medical Neuroendocrinology, *Eunice Kennedy Shriver* National Institute of Child Health & Human Development (NICHD), National Institutes of Health, Bethesda, Maryland, 20892 USA.

Abbreviated title: Imaging of pheochromocytoma and paraganglioma

Key Terms: pheochromocytoma, paragangliomas, PET-CT, radiopharmaceuticals, genetics, precision medicine

Total word count: 1213

Table(s): 1

Figure(s): 1

Corresponding author:

Prof. David Taïeb, MD, PhD

Department of Nuclear Medicine, La Timone University Hospital, European Center for Research in Medical Imaging, Aix-Marseille University, 264 rue Saint-Pierre, 13385 Marseille, France, Email: david.taieb@ap-hm.fr, Phone/FAX: +33 (0) 4-91-38-44-06.

Nuclear (molecular) imaging allows visualization of various pathophysiological processes on a whole body scale with subsequent detailed characterization of a specific area of interest. As a general concept, the main goal of proliferating cells is to uptake and transform available nutrients into biomass powered by the energy generation needed to produce new cells. One of the most well known cancer phenotypes is the avid uptake of glucose via (often upregulated) GLUT family glucose transporters. This feature has been attributed by *Otto H. Warburg* (Nobel Prize received in 1931) to switch from cellular respiration to aerobic glycolysis which, despite less efficacy for generating ATP, facilitates rapid cell division and new cell maintainance (*1*). The nutrient demand is determined by several cell-intrinsic factors that include the presence of specific tumor-promoting mutations, chromosomal abnormalities, phenotypic states, the tumor tissue of origin, as well as extra-cellular factors (e.g. cell microenviroment). A prime example of the relationship between genetics and imaging phenotype is given by pheochromocytoma and paraganglioma (PPGL).

Pheochromocytoma and paraganglioma (PPGL) arise from adrenal medulla which is a main hormonal component of the autonomic nervous system, or neurosecretory tissue called paraganglia involved in various sensory reflex loops, respectively. PPGL are related to genetic driver events in 70-80% of cases, considering germline and somatic mutations together (in more than 20 genes with mutually exclusive events). The major genetic events involve hypoxia-response (cluster 1) or kinase signalling and protein translation genes (cluster 2) (*2, 3*).

Most of PPGLs exihibit, even at metastatic stage, a low-growing pattern. Therefore, their nutrient demand is most likely oriented towards biosynthesis, storage and secretion of catecholamines rather than rapid cell division. This is nicely illustrated on PET imaging by the highly elevated avidity of PPGL for [^{18}F]FDOPA, allowing catecholamine building blocks

(amino acids) to enter a PPGL cell with a low-to-moderate [^{18}F]FDG uptake to maintain slightly elevated but not accelerated cell growth and proliferation. Cluster 2 PPGL *(RET, NF1, MAX, TMEM127)* which are almost always confined to the adrenal medulla and are well-differentiated usually follow this rule. In contrast, under certain circumstances referred to as « pseudohypoxic states », imaging phenotypes can profoundly differ. Since the seminal discoveries by the 2019 Nobel Laureates *(William G. Kaelin Jr., Sir Peter J. Ratcliffe and Gregg L. Semenza)*, it has been established that in presence of oxygen, VHL targets HIF-α for its subsequent proteasomal degradation. However, under specific circumstances, particulalry related to PPGL, decreased HIF-α degradation, causing enhanced stabilization, can be observed despite normoxia (so called pseudohypoxia when normally available oxygen cannot be properly utilized). Thus, HIF stabilization viewed as its prolonged activation contributes to an increase in cellular dopamine and norepinephrine content. This has been attributed to HIF-2α mediated activation of TH (tyrosine hydroxylase), the rate-limiting enzyme and inhibition of PNMT (phenylethanolamine-N-methyl-transferase) that converts norepinephrine to epinephrine (*4*). Altered genes expression, proliferation rate and other cellular characteristics as well a and the presence of dopaminergic or noradrenregic phenotypes of cluster 1 compared to cluster 2 PPGLs, especially for *VHL* or *EPAS1/HIF-2α* mutations, is reflected by very avid [^{18}F]FDOPA uptake as well as high [^{18}F]FDG uptake due to parrallel activation of GLUT family glucose transporters and glycolytic enzymes (*5*).

However, the situation becomes more complex for PPGL linked to mutations in one of the genes encoding for the SDH enzyme complex (A-D, collectively named *SDHx*). SDH is at the crossroad for the TCA cycle where it catalyzes the oxidation of succinate to fumarate and respiratory electron transfer chain (complex II) where it mainly functions as an electron

transporter. Although truncation of the succinate dehydrogenase complex is currently viewed to be the cause of PPGL and other tumors, clinical phenotypes can be different across various *SDHx*-related mutations and other unknown variables. These phenotypic differences are currently unexplained and conceptually challenging. SDH deficiency results in a partial TCA blockade with accumulation of enormous concentrations of succinate that can be detected by *in vitro* and *in vivo* metabolomic studies. In regards to [^{18}F]FDG uptake, these tumors share a marked [^{18}F] FDG avidity (*6*). For many years, this profile has been attributed to a succinate-driven pseudohypoxic state due to its inhibitor effect on prolyl hydroxylases with subsequent stabilization of HIF-α, followed by upregulation of GLUTs. Although there are conflicting results with certain molecular genetic studies that have failed to detect a glycolytic signature, this hypothesis remains appealing since there are some other mechanistic increases in enzymatic activity (e.g. for hexokinase) to be associated with this pathogenic mechanism. An alternative hypothesis relies on the extracellular effects of succinate on stroma cells. Fuxomic studies performed in mutated yeast have shown that succinate can efflux out of cells through via specific mitochondria and plasma membrane transporters. Such an abberant retrograde pathway is expected to prevent the potential detrimental effects of high levels of succinate on cytosolic metabolic processes. Interestingly, intratumoral injection of succinate in human xenografts, unlike fumarate, induces [^{18}F]FDG uptake whereas succinate has no effect on tumor metabolism *in vitro*. By contrast, succinate induces [^{18}F]FDG uptake by endothelial cells *in vitro* and can also induce muscular uptake after direct intramuscumar injection in mice (*7*). These data suggest that together with intracellular effects of succinate on HIF-α stabilization, succinate may have also extracellular effects on peri-tumoral stroma cells that contribute to the [^{18}F]FDG phenotype in these tumors. *SDH*-related PPGLs also overexpress somatostatin receptors and are therefore targetable with somatostatin analogs (SSAs)

labeled with diagnostic radionuclides (e.g. [^{68}Ga]SSA). Regarding [^{18}F]FDOPA, the phenotype largely depends on the tissue of origin with positivity for head and neck PGLs (almost always) and less sensitivity in PPGLs of sympathetic origin (*8*) (Figure 1). This is currently unexplained and in our opinion, needs further research in genetics and embryology. Until recently, paraganglia were thought to be originating from freely sympathoadrenal neural crest migratory cells. However, several new sophisticated studies have shown that adrenal medulla and extradrenal paraganglia are mainly derived from multifated Schwann Cell Precursors (SCPs), pointing to the fact that SCPs can be one of the initiating tumorigenic cell types (*9*). The acquisition of a mature catecholaminergic phenotype requires a subsequent conversion from glia-to-chromaffin gene expression process. Interestingely, SCPs can stay in their « niche » along the preganglionic nerves. Thus, the timing of development, tumor-initiating cells and SCP-to-chromaffin fate may be different across locations and genotypes. In the future, beyond a better understanding of genotype-related imaging phenotypes, new PPGL classification (e.g. related to their specific developmental characteristics and microenvironment) may allow more selective and effective treatment.

This editorial dicusses how genotype can be considered as a critical determinant of imaging phenotype in PPGL at the current time. A question often raised is « should we wait for genetic screening before imaging PPGL patients ?». In an ideal situation, one would expect that genotype could guide the choice of an optimal radiopharmaceutical. However, the genetic testing process can take time. The revised EANM/SNMMI joint recommendations provide a personalized approach and are applicable in PPGL with or without knowledge on the genetic background (*10*) (Table 1). Nuclear physicians should be aware of PPGL imaging phenotypes (location, multifocality, uptake pattern) since these may also suggest which mutation could be involved in

the disease further offering a specific imaging alghorithm during follow-up. Finally, specific genotype imaging-related phenotypes may accelerate our thinking of which radiotherapeutic strategy can be used in some patients with metastatic PPGL.

Compliance with ethical standards

NA

Conflicts of interest

The authors have nothing to disclose.

Noteworthy

- PPGL are caused by inherited genetic mutations in more than 40% of cases
- Nuclear imaging phenotype is mainly driven by genotype
- The choice of the optimal radiophamaceutical can be guided by tumor location and genetic background
- The revised EANM Practice Guideline/SNMMI Procedure Standard 2019 provides very up-to-date information for nuclear physicians encountering patients with PPGL.

References

1. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* May 22 2009;324(5930):1029-1033.

2. Crona J, Taieb D, Pacak K. New Perspectives on Pheochromocytoma and Paraganglioma: Toward a Molecular Classification. *Endocr Rev.* Dec 1 2017;38(6):489-515.

3. Fishbein L, Leshchiner I, Walter V, et al. Comprehensive Molecular Characterization of Pheochromocytoma and Paraganglioma. *Cancer Cell.* Feb 13 2017;31(2):181-193.

4. Bechmann N, Poser I, Seifert V, et al. Impact of Extrinsic and Intrinsic Hypoxia on Catecholamine Biosynthesis in Absence or Presence of Hif2alpha in Pheochromocytoma Cells. *Cancers (Basel).* Apr 28 2019;11(5).

5. Favier J, Briere JJ, Burnichon N, et al. The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS One.* 2009;4(9):e7094.

6. Taieb D, Timmers HJ, Shulkin BL, Pacak K. Renaissance of (18)F-FDG positron emission tomography in the imaging of pheochromocytoma/paraganglioma. *J Clin Endocrinol Metab.* Jul 2014;99(7):2337-2339.

7. Garrigue P, Bodin-Hullin A, Balasse L, et al. The Evolving Role of Succinate in Tumor Metabolism: An (18)F-FDG-Based Study. *J Nucl Med.* Nov 2017;58(11):1749-1755.

8. Taieb D, Pacak K. New Insights into the Nuclear Imaging Phenotypes of Cluster 1 Pheochromocytoma and Paraganglioma. *Trends Endocrinol Metab.* Nov 2017;28(11):807-817.

9. Furlan A, Dyachuk V, Kastriti ME, et al. Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla. *Science.* Jul 7 2017;357(6346).

10. Taieb D, Hicks RJ, Hindie E, et al. European Association of Nuclear Medicine Practice Guideline/Society of Nuclear Medicine and Molecular Imaging Procedure Standard 2019 for radionuclide imaging of phaeochromocytoma and paraganglioma. *Eur J Nucl Med Mol Imaging.* Sep 2019;46(10):2112-2137.

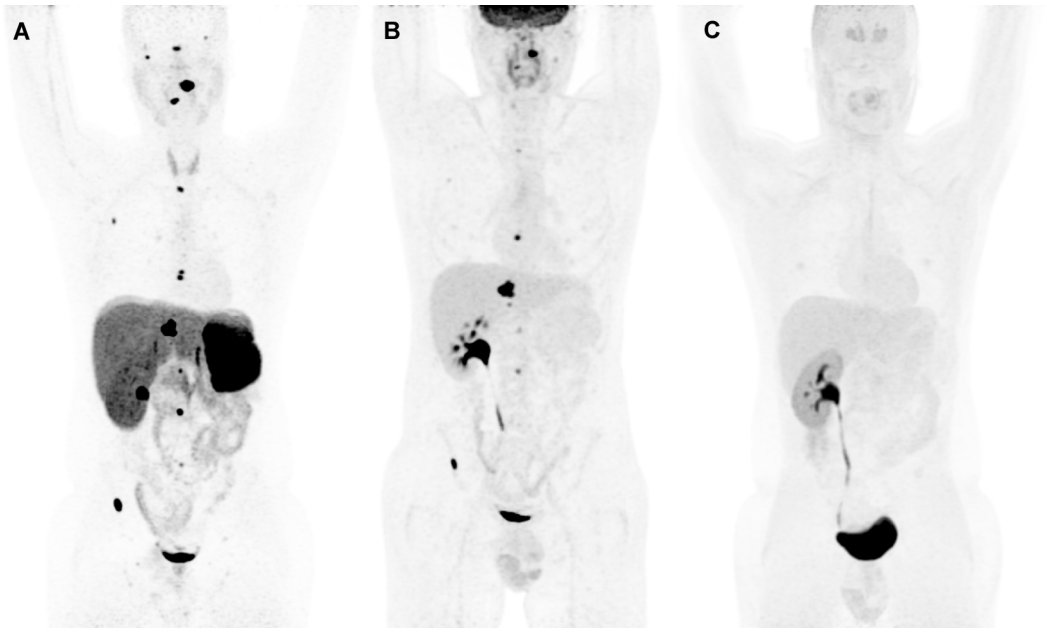

Figure 1. Typical imaging phenotype in a patient with *SDHB*-related metastatic retroperitoneal paraganglioma.

[^{68}Ga]DOTATATE (A), [^{18}F]FDG (B), [^{18}F]FDOPA (C). Previous history of surgery for a large retroperitoneal paraganglioma. [^{68}Ga]DOTATATE identified more bone metastases than [^{18}F]FDG, whereas [^{18}F]FDOPA was negative.

Table 1. Proposed clinical algorithm for nuclear imaging investigations in cases of pheochromocytomas and paragangliomas (from EANM Practice Guideline/SNMMI Procedure Standard 2019) (*10*)

	First choice	**Second choice**
PHEO (apparently sporadic)	[^{18}F]FDOPA or or [^{123}I]MIBG	[^{68}Ga]SSA
Inherited PHEO (except *SDHx*): *NF1/RET/VHL/MAX*	[^{18}F]FDOPA	[^{123}I]MIBG or [^{68}Ga]SSA
HNPGL	[^{68}Ga]SSA	[^{18}F]FDOPA
Extra-adrenal sympathetic and/or multifocality and/or metastatic disease and/or *SDHx* mutation	[^{68}Ga]SSA	[^{18}F]FDG and [^{18}F]FDOPA