MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

# Succinate dehydrogenase-B fluorescence in situ hybridization in pheochromocytoma-paragangliomas

Beyza KESKIN OZTURK[1], Kadriye Ebru AKAR[1], Muhammed Hasan TOPER[2], Pelin BAGCI[2], Handan KAYA[2], Leyla CINEL[2]

[1]Department of Pathology, The Council of Forensic Medicine, Istanbul, Türkiye.
[2]Department of Pathology, School of Medicine, Marmara University, Istanbul, Türkiye.
**Corresponding Author:** Kadriye Ebru Akar
**E-mail:** kebruakar1@gmail.com

**Submitted:** 03.10.2024 **Accepted:** 19.12.2024

ABSTRACT

Objective: Preoperative identification of familial pheochromocytoma and paraganglioma (PPGL) is crucial, yet often overlooked, leading to missed diagnoses. Typically, succinate dehydrogenase-B (SDHB) and fumarate hydratase (FH) tests are applied postoperatively to confirm familial links and assess prognosis. However, routine preoperative multidisciplinary collaboration is limited, causing delayed screening requests. Consequently, routine SDHB and FH immunohistochemistry (IHC) testing is not widely practiced. This study introduces SDHB fluorescence in situ hybridization (FISH) as a diagnostic tool, akin to HER2 IHC-FISH testing in gastric carcinomas.

Materials and Methods: Succinate dehydrogenase-B and FH IHC were conducted on 43 cases. FISH analysis was performed for 28 cases with suspected familial origin or SDHB IHC loss to determine whether the protein loss was due to chromosomal changes.

Results: Complete SDHB IHC loss occurred in 8 cases, partial loss in 4, and preservation in 31. Complete FH loss occurred in 10 cases. FISH analysis revealed chromosomal breaks in 20 cases (71.4%), including those with SDHB/FH IHC loss or positive clinical history. Ten cases (35.7%) showed a "red-signal only" pattern, suggesting further genetic testing.

Conclusion: Succinate dehydrogenase-B FISH serves as a cost-effective tool for early PPGL diagnosis, complementing SDHB and FH IHC results. It can help identify cases that need genetic testing, even when IHC results are preserved.

Keywords: Pheochromocytoma, Paraganglioma, Succinate dehydrogenase-B

## 1. INTRODUCTION

Pheochromocytomas and paragangliomas (PPGLs) are rare, highly vascularized tumors originating from neural-crest-derived chromaffin cells. Pheochromocytoma refers specifically to intra-adrenal tumors, while similar tumors occurring outside the adrenal glands are called paragangliomas. Paragangliomas are further classified based on their location and catecholamine production: parasympathetic paragangliomas, found in the head and neck region, generally do not produce catecholamines, whereas sympathetic paragangliomas, located along the sympathetic trunk in the abdomen, usually produce catecholamines [1].

During the last two decades there have been breakthroughs in understanding the biology of pheochromocytomas and extra-adrenal paragangliomas. It is now known that at least 30% of these tumors are hereditary, caused by germline mutations of more than 20 genes [2-4]. These genes deregulate three main signaling pathways (hypoxia, kinase signaling, and Wnt-signaling pathways) [4]. PPGLs can occur sporadically or as part of various inherited tumor syndromes. These syndromes include; multiple endocrine neoplasia type 2 (MEN2), associated with germline mutations in the *RET* gene; von Hippel–Lindau (VHL) disease, caused by germline mutations in the *VHL* gene; neurofibromatosis type 1 (NF1), linked to germline mutations in the *NF1* gene; and familial PPGL syndrome.

The familial PPGL syndrome is the most frequent hereditary condition with manifestation of paragangliomas, and is caused by germline mutations in the *SDHA, SDHB, SDHC, SDHD* or *SDHAF2* genes [5]. It is characterised by the familial occurrence of pheochromocytomas or paragangliomas, usually at a young age, and often by multifocal disease with an increased risk of recurrence and an increased frequency of malignancy such as renal cell carcinoma and gastrointestinal stromal tumors (GIST) in the case of *SDHB* mutations [1,6]. SDHB-driven PPGLs also have high rates of metastatic behaviour [7-9], such that some studies inserted

**How to cite this article:** Keskin Ozturk B, Akar KE, Toper MH, Bagci P, Kaya H, Cinel L. Succinate dehydrogenase-B fluorescence in situ hybridization in pheochromocytoma-paragangliomas. Marmara Med J 2025;38(1): 76-83 doi: 10.5472/marumj.1629043


ISSN: 1309-9469



http://doi.org/10.5472/marumj.1629043
**Marmara Med J 2025;38(1): 76-83**

SDHB immunhistochemistry (IHC) status to GAPP scoring to generate a modified GAPP scoring method (M-GAPP) [10], and others generated a new scoring system named as COPPS [12]. A recent study identified DNA methylation anomalies in SDHB-related PPGLs that could result for the malignant behaviour [12].

Succinate dehydrogenase (SDH) is the enzyme responsible for converting succinate to fumarate in oxidative phosphorylation. Disruption of this conversion causes an increase in the amount of succinate. Succinate stabilizes the hypoxia-inducible factor (HIF). When succinate levels rise, HIF cannot function properly, disrupting cell division and new vessel formation in hypoxic areas (pseudohypoxic signature) [4]. Studies showed that SDH inactivation induces angiogenesis and tumorigenesis through the inhibition of hypoxia-inducible factors (HIF)-prolyl hydroxylase [13]. Loss of SDHB expression indicates biallelic inactivation of one of the SDHx component proteins [14].

Germline mutations in the fumarate hydratase (FH) gene, which encodes the enzyme fumarate hydratase in the Krebs cycle, cause hereditary leiomyomatosis and renal cell carcinoma syndrome [6,15,16]. Hypoxia can also lead to mutations in the *FH* gene, and it is believed that this condition triggers tumorigenesis by conducting an immunosupressive tumor microenvironment [17]. The SDHx complex and FH protein are integral components of the Krebs cycle, and FH – and SDHx-deficient renal cell carcinoma may show similar morphology [18]. Fumarate hydratase mutation and FH-IHC deficiency was also defined in PPGLs [19], and found to be associated with poor prognosis as well [4].

At least thirty percent of PPGLs are hereditary and perhaps as many as half of these familial cases are caused by germline mutations of the *SDH* subunits [20]. Clinicians frequently overlook patients with inherited PPGLs. This oversight stems from multiple factors, such as limited family history data, similarities in age distribution between inherited and non-inherited cases, spontaneous mutations, incomplete expression of genetic traits (particularly with *SDHB*), obscured familial inheritance, the diverse clinical presentation of the disease, and inadequate awareness among medical professionals [1]. There is ongoing debate among experts about whether genetic testing should be universally conducted for all patients with PPGL, and if so, the appropriate timing for such testing, particularly in children and young adults [21]. Clinical indicators for the presence of *SDHB, SDHC*, and *SDHD* germline mutations in these patients are often lacking. Identifying inherited PPGLs is crucial for both patients and their relatives, as they face an elevated risk of developing multiple malignant tumors. Some advocate for mutation analysis of *SDHB, SDHC*, and *SDHD* in all PPGL cases lacking clear clinical or familial indications [1,22]. Although, a recent study did not confirm the prognostic value of *SDHB* mutation [23], SDHB-IHC is known for its' high sensitivity, specifity and predictive values for a long time [9,19], and advised to be an important part of the pathology reports by World Health Organization (WHO) [2,5,24].

In this retrospective study, our aim was to assess the utility of SDHB and FH immunohistochemistry (IHC) and SDHB fluorescence insitu hybridization (FISH) in identifying SDH-related PPGL cases in our institution.

## 2. MATERIALS and METHODS

### *Patient selection and demographic data (n=54)*

This study was reviewed and approved by the Clinical Research Ethics Committee of Marmara University, School of Medicine (issue date 13/07/2018, approval number 09.2018.528) and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Our study included 40 pheochromocytomas and 14 paragangliomas. All cases were sampled and diagnosed at the same pathology department by the same endocrine pathologists (PB, HK). Consultation cases were excluded. Data on diagnosis, age, tumor localization, gender, clinical history, symptoms, and catecholamine levels were obtained from the hospital automation system. Tumor localization (adrenal vs. extra-adrenal: cervical, intra-abdominal, other), diameter, number, metastasis, and recurrence status were recorded. Survival status was obtained from the death notification system.

This cohort had no gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC) and NF1 case.

### *Pathological analysis*

The slides of all cases diagnosed in our department were retrieved from the archive and re-evaluated.

**PASS score assessment:** Diffuse growth, central necrosis/confluent necrosis, high cellularity, cell monotony, tumor cell spindling, mitosis, atypical mitotic figures, extension into adipose tissue, capsular invasion, vascular invasion, pleomorphism, and hyperchromasia were noted. The score was grouped as <4 and ≥4.

**GAPP score assessment:** Histologic pattern, cellularity, Ki-67 proliferation index, necrosis, vascular/capsular invasion, and catecholamine type were noted. A score of 0-2 indicated well-differentiated tumors, 3-6 indicated moderately differentiated tumors, and a score above 6 indicated poorly differentiated tumors.

For multiple pheochromocytomas, the tumors with the highest scores were considered. –

### *-Immunohistochemistry (IHC) (n=43):*

Eleven cases were excluded due to unavailability for SDHB and/or FH IHC analyses. Consequently, 32 pheochromocytomas and 11 paragangliomas were included in the immunhistochemical study.

**Case selection for succinate dehydrogenase (SDHB), and fumarate hydratase (FH):** These antibodies were applied to all possible cases, totally blind to the primary diagnosis and clinical information indicating familial predisposition.

**Technical Data:** Three-micrometer-thick sections were taken from formalin-fixed, paraffin-embedded tissue blocks and placed on positively charged slides. The slides were kept in an oven at 70°C. The immunohistochemical staining process was performed using a fully automated Ventana Benchmark Ultra instrument (Ventana Medical Systems, Tucson, AZ). Deparaffinization and antigen retrieval were carried out automatically. A ready-to-use

kit (ultraView™ Universal DAB Detection Kit, Catalog Number 760-500, Ventana Medical Systems, Tucson, AZ), which includes a biotin-free, HRP multimer-based, hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochloride (DAB), was used in the device. Counter stain was performed using Bluing Reagent for 4 minutes for background staining. Finally, the slides were air-dried, and a coverslip with a mounting medium was placed on each slide.

The primary antibodies used were; SDHB (Clone BSB-131, BioSB, 1:200, USA), and FH (Clone J-13, Santa Cruz, 1:100, Germany).

**Evaluation:** The slides were evaluated and scored by two pathologists (BKO, PB). The methods used for scoring each antibody were as follows:

- **SDHB**: Scored as negative (complete loss), weak and patchy staining (partial loss), or positive (preserved) as defined in prior studies. Mitochondrial type true granularity without background blush was considered as positive [5,19,25].
- **FH**: Scored similar to SDHB antibody as defined in prior studies [19,26].

### *-Fluorescence in situ hybridisation (FISH) for SDHB (Table I) (n=30)*

**Case selection:** FISH was applied to 26 cases that a pathologist suspected might be hereditary/familial at the time of diagnosis. These cases included:

- 12 cases with complete or partial loss of SDHB immunohistochemical staining
- 7 cases with a complete loss of FH immunohistochemical staining
- 1 case known for *RET* mutation with a medullary thyroid carcinoma [27] diagnosed in our department
- 1 case known for *VHL* mutation with a synchronous pancreatic neuroendocrine tumor excision
- 1 case with an indefinite history of MEN2A syndrome
- 1 abdominal paraganglioma [4] with a papillary thyroid carcinoma diagnosed in our department
- 1 abdominal paraganglioma [4]
- 2 cases with patients aged ≤18 years [21]
- Additionally 4 cases with no spesific features of IHC and/or clinical information were used as a control group.

Among these 30 cases selected for FISH analysis, two cases from 2015 and 2018 underwent the analysis twice but did not exhibit any signaling (n=28).

**Technical Data:** Formalin-fixed paraffin-embedded (FFPE) tissue sections were used to perform FISH. A commercial FISH Pretreatment kit (ZytoVision GmbH, Bremerhaven, Germany) was utilized to prepare the histological specimens according to a standardized protocol. This kit is specifically designed for manual use to ensure a consistent procedure. For the hybridization step, we employed the SDHB FISH Probe Kit by Empire Genomics ® (NY, USA, https://empiregenomics.com/fish-probes/gene/SDHB). This break apart probe is designed to flank the human *SDHB* gene located on chromosome band 1p36.13, and is typically used for detecting *SDHB* rearrangements such as translocation, inversion, or fusion with other genes, and marks them with 5-Florescein and 5-TAMRA fluorochromes.

After 19 hours of hybridization with the FISH probe, the sections were cleaned with a post-hybridization buffer to remove nonspecific labeling. Subsequently, a DAPI-antifade mounting medium, included in the kit, was used to observe and preserve the fluorescence.

The evaluation was performed using an Olympus BX63F imager fluorescence microscope at 60X magnification. At least 100 nuclei were counted with the assistance of two independent observers (BA, SH). *SDHB* rearrangements were interpreted based on the presence of a predominant atypical signal pattern with extra signals of *SDHB* in tumor cell nuclei and an isolated break-apart pattern.

### Statistical Analysis

Statistical evaluations were performed utilizing version 27.0 of the Statistical Package for the Social Sciences (SPSS). Descriptive statistics for numerical variables were reported as mean and median (range). Categorical variables were analyzed with the Pearson chi-square test, Yates's chi-square test, and Fisher's exact test. Overall survival (OS) rates were determined using the Cox proportional hazards model. A p-value of less than 0.05 was regarded as statistically significant.

## 3. RESULTS

### *Demographic data (n=54)*

The cohort included 40 pheochromocytomas, 14 paragangliomas.

Patients were 29 female and 25 male, with an mean age of 45 years (range: 8-74). The mean tumor diameter was 6 cm (range: 1.7-18 cm). Catecholamine levels were measured in 37 cases before surgery. Elevated levels of metanephrine were found in 15 cases, and elevated normetanephrine levels were found in 27 cases. In 13 cases, both metanephrine and normetanephrine were elevated. Vanillylmandelic acid (VMA) was elevated in 22 cases, 5-Hydroxyindoleacetic acid (5HIAA) was elevated in 6 cases, and homovanillic acid (HVA) was elevated in 8 cases. Plasma renin levels were available in 8 cases of pheochromocytoma (0.19-146.4 ng/ml/hour), and high in 3 cases. Aldosterone levels were available in 9 cases of pheochromocytoma (24-316ng/dl), and high in 1 case. ACTH was measured in 21 cases, with elevated levels in 19 cases (5-49.9pg/ml). Dexamethasone suppression (1 mg) was measured in 21 cases of pheochromocytoma (0.1-2.53 nmol/L), and was not high in any of the cases. Cortisol levels were measured in 31 cases (1.5-63 mcg/dl), and was high in only 1 case.

Six of the 40 pheochromocytoma cases were multiple (2-4 tumors). The locations of the paragangliomas (n=14) were intra-abdominal (8 cases), and head-neck (6 cases). The paragangliomas of the 2 cases of synchrone pheochromocytoma+paraganglioma were also located in the head-neck area.

Clinical information regarding familial disease or hereditary setting was present in 11 cases; a family history of pheochromocytoma


http://doi.org/10.5472/marumj.1629043
Marmara Med J 2025;38(1): 76-83

was present in 2 cases but no genetic analyses were performed, VHL syndrome was present in 4 cases, MEN2A syndrome found in 2 cases, and *RET* mutation was present in 1 case. One case was found negative for *RET* germline mutation, and another one was negative for *MEN1* germline mutation.

### *PASS and GAPP scores (n=54)*

For multiple cases, the tumors with the highest scores were considered.

PASS scores; Fifteen cases had scores <4, while 39 cases had scores ≥4**.** The PASS score was higher in patients over 45 years of age (p = 0.034).

GAPP scores; Eighteen cases were well differentiated, 34 were moderately differentiated, and 2 were poorly differentiated.

### *Other microscopic features*

The adrenal cortex showed pressure atrophy in 3 cases, it was infiltrated in 1 case, and was hyperplastic (both diffuse and nodular) in 1 case. The 2 cases with elevated aldosteron and cortisol levels had normal cortexes. The adjacent medulla was thickened in only 1 case with germline *RET* mutation.

Degenerative changes, including edema, cystic changes, fibrosis, calcification, hemorrhage, hyalinization, sinusoidal dilatation, and intravascular synthetic material due to embolization, were present in 44 cases.

Nuclear pseudoinclusions were found in 5 cases, hyaline globules in 13 cases, and lipofuscin and/or melanin pigment in 4 cases. Ganglion cells were present in 3 cases, and small cell change was seen in 7 cases. Amphophilic cells were present in 22 cases. Anomalies associated with the vascular pattern were found in 15 cases (including 1 case with cavernous change and 1 case with thick septation in the tumor center). Intraseptal chronic inflammation was observed in 9 cases, and the surgical margin was positive in 2 cases, and ruptured in 2 cases.

The statistical analyses did not reveal any significant associations between these microscopic features and other clinicopathological parameters or survival outcomes.

### *Results of IHC evaluation (n=46) (Table I)*

Succinate dehydrogenase-B staining was completely lost in 8 cases (Figure 1), partially lost in 4 cases, and preserved in 31 cases. Fumarate hydratase was completely lost in 10 cases, and preserved in 33 cases. In 3 cases both SDHB and FH was completely lost.

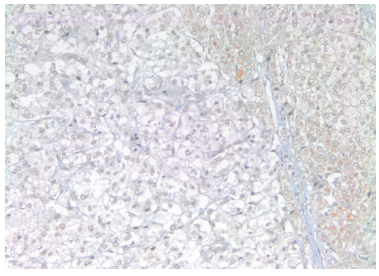

***Figure 1:*** *SDHB immunohistochemistry. On the right side there is normal adrenal cortex with preserved staining and on the left the tumor shows loss of staining, SDHB antibody, X20.*

The statistical analysis of the IHC results in comparison with other clinicopathological variables revealed that a diffuse growth pattern was significantly more common in both the complete SDHB and FH loss group and the complete SDHB loss group (p = 0.038). Furthermore, capsular invasion was significantly more prevalent in the partial SDHB loss group (p = 0.007).

The PASS scores were significantly correlated with the IHC results (Table I). Nine cases with PASS score ≥ 4 showed partial or complete loss of SDHB-IHC, while the rest preserved IHC staining. Conversely, almost half of the cases with PASS score <4 exhibited FH IHC loss (n=6/14) (p = 0.007).

***Table I.*** *PASS scores and IHC results*

| PASS score | Complete SDHB and complete FH loss | Complete SDHB loss | Partial SDHB loss | Complete FH loss | SDHB and FH preserved |
|---|---|---|---|---|---|
| <4 (n) | 1 | 1 | 1 | 6 | 5 |
| ≥4 (n) | 2 | 4 | 3 | 1 | 22 |

*SDHB: Succinate dehydrogenase-B, FH: Fumarate hydratase*

Ki-67 did not show any significant associations when compared with the other clinicopathological parameters.

### *Results of FISH evaluation (n=28) (Tables II, III)*

Among the 30 cases selected for FISH analysis, 2 cases with complete SDHB loss from 2015 and 2018 underwent the analysis twice but did not exhibit any signaling (n=28). The total cell count ranged from 100 to 149, with a mean of 116. Break-apart pattern (split) was found in 20 cases (71.4%) (Figure 2), with the number of splits ranging from 1 to 8, and a mean of 3.4. The percentage of splits ranged from 0.7% to 6%, with a mean of 2.96% (Table II).

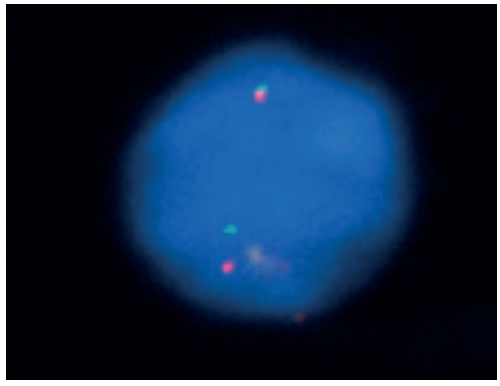

***Figure 2:*** *Break apart (split) pattern detected in FISH.*

Splits were observed in 2 cases with complete SDHB and FH loss, 3 cases with complete SDHB loss, 1 case with partial SDHB loss, 5 cases with total loss of FH staining, 6 cases with positive clinicopathological information, and in 3 control cases (Table III). The highest split percentage (6%) was found in a control case with no particular IHC or clinical information, as well as in a case with MEN2A syndrome, which involved metastatic medullary thyroid carcinoma, total loss of SDHB, and total loss of FH staining.

***Table II:*** *Case selection steps for FISH; 1) IHC results 2) Additional clinicopathological clues gathered by the pathologist , and the case based FISH results along with the survival information*

| IHC RESULTS | ADDITIONAL CLINICAL INFORMATION | FISH RESULTS | | | | | PASS / GAPP SCORES | SURVIVAL (months) |
|---|---|---|---|---|---|---|---|---|
| | | TOTAL CELL COUNT (n) | FUSION (n) | SPLIT (n) | SPLIT (%) | RED SIGNAL ONLY COMPONENT | | |
| Complete SDHB and complete FH loss | VHL GERMLINE MUTATION (+) | 120 | 120 | 0 | 0 | | 4/ 4 | 90 |
| | FAMILIAL | 124 | 121 | 3 | 2,4 | Present | 2/ 2 | 57 |
| | MEN2A GERMLINE MUTATION (+) & METASTATIC MTC | 100 | 94 | 6 | 6 | | 10/ 4 | 47 |
| Complete SDHB loss | VHL GERMLINE MUTATION (+) & PANNET | 100 | 100 | 0 | 0 | | 2/ 1 | 57 |
| | AGE 18 | 149 | 141 | 8 | 5,4 | | 5/ 3 | 36 |
| | None | 100 | 99 | 1 | 1 | Present | 9/ 4 | 112 |
| | None | 100 | 97 | 3 | 3 | Present | 6/ 4 | 73 |
| Partial SDHB loss | PHEO+PGL | 102 | 101 | 1 | 0,9 | | 8/ 6 | 60 |
| | None | 122 | 122 | 0 | 0 | | 7/ 5 | 121 |
| | None | 77 | 77 | 0 | 0 | | 3/ 2 | 102 |
| Complete FH loss | MULTIPLE TUMORS | 124 | 117 | 7 | 5,9 | Present | 0/ 0 | 99 |
| | PHEO+PGL | 122 | 122 | 0 | 0 | | 0/ 2 | 98 |
| | VHL GERMLINE MUTATION (+) | 110 | 105 | 5 | 4,5 | Present | 4/ 5 | 92 |
| | FAMILIAL | 133 | 132 | 1 | 0,8 | Present | 2/ 4 | 91 |
| | MULTIPLE TUMORS & AGE 12 | 105 | 99 | 6 | 5,7 | Present | 4/ 4 | 43 |
| | None | 106 | 104 | 2 | 1,9 | Present | 2/ 1 | 85 |
| | None | 114 | 114 | 0 | 0 | | 3/ 4 | 74 |
| SDHB and FH PRESERVED | RET GERMLINE MUTATION (+) & MTC | 135 | 134 | 1 | 0,7 | | 6/ 4 | 113 |
| | VHL GERMLINE MUTATION (+) & PANNET | 128 | 124 | 4 | 3,1 | | 1/ 2 | 85 |
| | MEN2A GERMLINE MUTATION (+) & AGE 16 | 165 | 163 | 2 | 1 | | 6/ 3 | 39 |
| | ABDOMINAL PGL + PTC | 101 | 101 | 0 | 0 | | 6/ 3 | 137 |
| | ABDOMINAL PGL | 143 | 139 | 4 | 2,8 | Present | 5/ 4 | 60 |
| | AGE 18 | 145 | 142 | 3 | 2,1 | Present | 12/ 7 | 61 |
| | AGE 8 | 120 | 119 | 1 | 0,8 | | 8/ 4 | 35 |
| | CONTROL CASE-1 (2014) | 106 | 106 | 0 | 0 | | 6/ 4 | 117 |
| | CONTROL CASE-2 (2020) | 100 | 94 | 6 | 6 | | 1/ 1 | 43 |
| | CONTROL CASE-3 (2021) | 100 | 99 | 1 | 1 | | 14/ 5 | 33 |
| | CONTROL CASE-4 (2021) | 133 | 131 | 2 | 2,3 | | 3/ 2 | 33 |

*SDHB: Succinate dehydrogenase-B, FH: Fumarate hydratase, IHC: Immunhistochemistry, FISH:Fluorescence in situ hybridization*

***Table III.*** *Distribution of FISH anomalies by the indication types*

| n=28 | COMPLETE SDHB and FH LOSS | COMPLETE LOSS OF SDHB | PARTIAL LOSS OF SDHB | COMPLETE LOSS OF FH | ADDITIONAL CLINICAL INFORMATION | CONTROL GROUP |
|---|---|---|---|---|---|---|
| FISH NEGATIVE | 1 | 1 | 2 | 2 | 1 | 1 |
| SPLIT POSITIVE | 2 | 3 | 1 | 5 | 6 | 3 |
| SPLIT (%)<br>Range<br>Mean | <br>2.4 and 6<br>4.2 | <br>1, 3 and 5.4<br>3.1 | <br>0,9 | <br>0.8-5.9<br>3.76 | <br>0.7-3.1<br>1.75 | <br>1,2 and 6<br>3 |
| RED SIGNAL ONLY COMPONENT | 1 | 2 | 0 | 5 | 2 | 0 |


http://doi.org/10.5472/marumj.1629043
Marmara Med J 2025;38(1): 76-83

Ten cases (35.7%) showed at least 1 cell with a red-signal only pattern (Figure 3). These cases were reviewed twice to account for the possibility that they may represent a different 3D version of the break-apart pattern. Five of them had complete FH-IHC loss, 2 of them had complete SDHB-IHC loss, 1 of the cases had both SDHB and FH-IHC loss, 1 case was 18 years of age, and 1 case had abdominal paraganglioma (Table II). Four of the cases (40%) with a red-signal only component had PASS scores <4, while 6 had (60%) PASS scores of ≥4 (p = 0.442). Additionally, the red-signal only pattern did not correlate with the Ki-67 scores (p = 0.275) or other clinicopathological parameters.

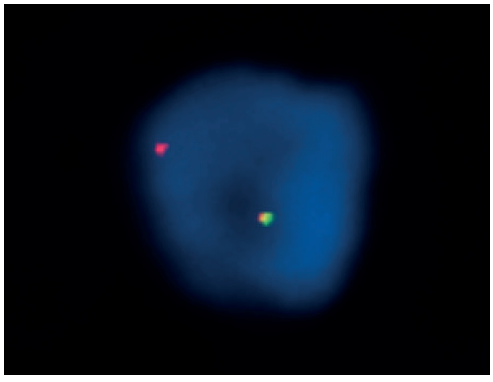

***Figure 3:*** *"Red signal only" component detected in FISH.*

### *Survival Data*

All 54 cases were followed up at our institution. The follow-up period ranged from 11 to 144 months, with a median duration of 85 months. Only 2 patients were lost during the follow-up, with causes of death being malignant pancreatic tumor (no tissue diagnosis) and unknown neurological events. There were no recurrences and/or metastasis in the cohort.

The group with red-signal only component did not have any deaths, so statistical analysis could not be performed.

## 4. DISCUSSION

The role of the pathologist in diagnosing pheochromocytomas and paragangliomas (PPGLs) is essential. Over the years, five pathological scoring systems have been developed to predict the metastatic or malignant potential of these tumors: the Pheochromocytoma of the Adrenal Gland Scaled Score (PASS), the Grading System for Adrenal Pheochromocytoma and Paraganglioma (GAPP), the Composite Pheochromocytoma/Paraganglioma Prognostic Score (COPPS), the Age, Size, Extra-adrenal location, Secretion type (ASES) score, and the Size, Genetic, Age, and PASS (SGAP) model. Unfortunately, none of these pathology-based scoring systems can accurately predict the metastatic risk of PPGLs [5,28].

It has been demonstrated that patients with PPGL syndrome, even those with an apparently sporadic presentation, can be identified through SDHB immunohistochemistry on PPGLs. This testing is both technically and financially feasible to perform routinely on all PPGLs, particularly when there are no familial or clinical indications of a specific inherited form of pheochromocytoma or paraganglioma. Surgical pathologists are now expected not only to provide a definitive diagnosis based on tissue samples but also to conduct additional tests that can help indicate prognosis, guide treatment, or facilitate genetic testing. By routinely performing SDHB immunohistochemistry, hereditary syndromes caused by germline mutations in SDHB, SDHC, or SDHD can be identified with a high degree of reliability [1,5].

Since, it is a mitochondrial staining pattern, the consantration of the SDHB antibody is a significant issue to evaluate the IHC staining properly [25,29] . Here in this study we used a dilution (1:200) not very low [1]or not very high , and used the same 3-tiered system similar to Gill AJ et al. [25]and ENSET [29]. The varying sensitivities and specificities associated with different antibody dilutions underscore the technical challenges of performing SDHB immunohistochemistry (IHC) [29]. This emphasizes the importance of conducting SDHB IHC in experienced centers that maintain rigorous quality assurance protocols [25]. The interobserver variation of IHC interpretation is pretty high even among the expert endocrine pathologists, especially in cases with SDHD mutation . Even in tumors with weak diffuse SDHB IHC staining, SDHD [30] and *VHL* mutations are still possible [19,25,29].

In this study, we demonstrated that SDHB and FH IHC loss are associated with diffuse growth patterns and capsular invasion. Additionally, the PASS scores were higher in the SDHB IHC weak/loss groups.

As there were only two deaths in the cohort, the survival analysis did not provide enough data to draw conclusions. Notably, there was only one disease-related death, which occurred in the 11th month due to a malignant pancreatic tumor without a tissue diagnosis.

For PPGL, due to the identification of multiple driver genes, including succinate dehydrogenase (SDH)-related genes, *RET*, *NF1*, and *VHL*, universal multi-gene germline panel testing is recommended as a comprehensive approach [31] despite not being cost-effective at all [14]. Recently, Rana HQ et al., published the most comprehensive genetic data on PPGLs, revealing that 37 out of 109 PPGL cases were positive for pathogenic germline variations in SDHx genes. Among these, 35% (13/37) exhibited a somatic inactivating allele consistent with loss of heterozygosity in the assessed SDHx gene. Specifically, 11 of these 37 cases showed a one-copy loss of the somatic allele: 8 had SDHB loss, 2 had SDHA loss, and 1 had SDHD loss. However, only eight cases demonstrated an IHC pattern consistent with biallelic inactivation of the relevant SDH subunit gene. Overall, 23 out of the 37 cases did not show a somatic inactivating single nucleotide variant (SNV), copy number, or structural alteration [14]. As more susceptibility genes are discovered there is an urgent need to create additional screening methods for each gene.

In this study, we explored a novel approach to the pathologist's role in diagnosing PPGL and introduced a cost-effective diagnostic tool to be used prior to expensive genetic testing.

Fluorescence in situ hybridization (FISH) has been a widely used method in pathology laboratories, particularly for lung and breast carcinomas. Given the variability in interobserver interpretations of immunohistochemistry (IHC) for the SDHB antibody, an additional confirmatory study, similar to the HER2 IHC-FISH sequence used for gastric carcinomas, may be beneficial.

The interpretation and calculation of SDHB FISH analysis are not as clearly defined in the literature or in the guidelines as they are for NTRK-ROS [32]. Given that any anomaly might be significant, we aimed to count as many cells as possible. Our study utilizing a break-apart SDHB FISH probe revealed variable results. Initially, we observed two atypical patterns: a break-apart pattern and a red-signal-only pattern. Considering that these might represent different 3D versions of the same signaling pattern, we reviewed them twice to confirm the absence of the green signal. Although, the red-signal-only pattern did not show any significant clinicopathological correlation, we chose to report these patterns separately, as they may hold significance for future studies. Among the four cases (control group) with no specific clinical information or IHC status, three exhibited splits, with one showing the highest split score. Conversely, on the other end of the spectrum, 4 out of 10 cases with partial or complete SDHB IHC loss did not exhibit any splitting. However, the break-apart pattern was predominantly observed in groups with SDHB-IHC loss and positive clinical information. Therefore, triaging FISH analyses could be effectively guided by the loss of SDHB-IHC staining and positive clinical history.

The most significant result of this study was observed in the group with complete FH-IHC loss. This group exhibited break-aparts in 71.4% of the cases, one of the highest upper limits of the break-apart pattern (5.9%), and one of the highest mean numbers of splits (3.76). Additionally, all cases had a red-signal-only component. We believe the red-signal-only component might indicate copy number variation, as suggested by Rana HQ et al. [14], or it could be a variation of the break-apart pattern due to the 2D structure of the cells on the slide.

Another important finding was the high incidences of the break-apart pattern in the control group (3/4) and IHC preserved group (6/7). This suggests that all cases might benefit from SDHB FISH analyses, especially in the context of ambiguous SDHB-IHC results or no clinical information.

In this study, we aimed to assess the utility of SDHB FISH in a small cohort of PPGLs. Although the control group was also limited in size, which restricts the ability to draw definitive conclusions, our findings provide a foundation for future research. Larger studies, particularly those including metastatic cases, could further explore the potential of SDHB FISH as a complementary test to SDHB-IHC.

In conclusion, the loss of SDHB-IHC and FH-IHC, combined with positive SDHB FISH results such as the red-signal-only pattern and break-apart pattern, could provide a more cost-effective and simpler method for triaging patients for genetic testing. SDHB FISH might also be useful in all PPGLs, as it could detect anomalies even in cases with preserved IHC staining.

**Acknowledgement**

The authors would like to extend their gratitude to FISH technicians Basak Atmaca and Selin Halil for their invaluable assistance with the FISH procedures and interpretation.

**Compliance with Ethical Standards**

**Ethical approval:** This study was reviewed and approved by the Clinical Research Ethics Committee of Marmara University, School of Medicine (issue date 13/07/2018, approval number 09.2018.528) and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

**Financial support:** The financial support was received from the Marmara University Committee of Scientific Research Project (Project No: SAG-A-250.919.0299)

**Conflict of interest:** The authors declare that they have no potential conflict of interest regarding the investigation, authorship, and/or publication of this article.

**Authors contributions:** BKO, KEA and PB: Conception, design, data collection, analysis and interpretation, PB: literature review, writing, BKO and MHT: Materials, data processing, LC and HK: Suoervision, Critical review. All of the authors declare that they have all participated in the design, execution and the analysis of the paper and they have approved the final version.

## REFERENCES

[1] van Nederveen FH, Gaal J, Favier J, et al. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol 2009;10:764-71. doi:10.1016/s1470-2045(09)70164-0

[2] Mete O, Tischler AS, de Krijger R, et al. Protocol for the examination of specimens from patients with pheochromocytomas and extra-adrenal paragangliomas. Arch Pathol Lab Med 2014;138:182-8. doi:10.5858/arpa.2012-0551-OA

[3] Zhikrivetskaya SO, Snezhkina AV, Zaretsky AR, et al. Molecular markers of paragangliomas/pheochromocytomas. Oncotarget 2017;8:25756-25782. doi:10.18632/oncotarget.15201

[4] Cascón A, Calsina B, Monteagudo M, et al. Genetic bases of pheochromocytoma and paraganglioma. J Mol Endocrinol 2023;70:e220167. doi:10.1530/jme-22-0167

[5] Mete O, Asa SL, Gill AJ, Kimura N, de Krijger RR, Tischler A. Overview of the 2022 WHO Classification of Paragangliomas and Pheochromocytomas. Endocr Pathol 2022;33:90-114. doi:10.1007/s12022.022.09704-6

[6] Ricketts C, Woodward ER, Killick P, et al. Germline SDHB mutations and familial renal cell carcinoma. J Natl Cancer 2008;100:1260-2. doi:10.1093/jnci/djn254

[7] Eisenhofer G, Tischler AS, de Krijger RR. Diagnostic tests and biomarkers for pheochromocytoma and extra-adrenal paraganglioma: from routine laboratory methods to disease stratification. Endocr Pathol 2012;23:4-14. doi:10.1007/s12022.011.9188-1

[8] Brouwers FM, Eisenhofer G, Tao JJ, et al. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab 2006;91:4505-9. doi:10.1210/jc.2006-0423

[9] Su T, Yang Y, Jiang L, et al. SDHB immunohistochemistry for prognosis of pheochromocytoma and paraganglioma: A retrospective and prospective analysis. Front Endocrinol (Lausanne) 2023;14:1121397. doi:10.3389/fendo.2023.112.1397

[10] Koh JM, Ahn SH, Kim H, et al. Validation of pathological grading systems for predicting metastatic potential in pheochromocytoma and paraganglioma. PLoS One 2017;12:e0187398. doi:10.1371/journal.pone.0187398

[11] Pierre C, Agopiantz M, Brunaud L, et al. COPPS, a composite score integrating pathological features, PS100 and SDHB losses, predicts the risk of metastasis and progression-free survival in pheochromocytomas/paragangliomas. Virchows Arch 2019;474:721-34. doi:10.1007/s00428.019.02553-5

[12] Chatzikyriakou P, Brempou D, Quinn M, et al. A comprehensive characterisation of phaeochromocytoma and paraganglioma tumours through histone protein profiling, DNA methylation and transcriptomic analysis genome wide. Clin Epigenetics 2023;15:196. doi:10.1186/s13148.023.01598-3

[13] Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. Nat Rev Cancer 2005;5:857-66. doi:10.1038/nrc1737

[14] Rana HQ, Koeller DR, Walker M, et al. Advancing precision oncology in hereditary paraganglioma-pheochromocytoma syndromes: Integrated interpretation and data sharing of the germline and tumor genomes. Cancers (Basel) 2024;16.947. doi:10.3390/cancers16050947

[15] Clark GR, Sciacovelli M, Gaude E, et al. Germline FH mutations presenting with pheochromocytoma. J Clin Endocrinol Metab 2014;99:E2046-50. doi:10.1210/jc.2014-1659

[16] Castro-Vega LJ, Buffet A, De Cubas AA, et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. Hum Mol Genet 2014;23:2440-6. doi:10.1093/hmg/ddt639

[17] Wang Y, Liu B, Li F, et al. The connection between tricarboxylic acid cycle enzyme mutations and pseudohypoxic signaling in pheochromocytoma and paraganglioma. Front Endocrinol (Lausanne) 2023;14:1274239. doi:10.3389/fendo.2023.127.4239

[18] Smith SC, Sirohi D, Ohe C, et al. A distinctive, low-grade oncocytic fumarate hydratase-deficient renal cell carcinoma, morphologically reminiscent of succinate dehydrogenase-deficient renal cell carcinoma. Histopathology 2017;71:42-52. doi:10.1111/his.13183

[19] Udager AM, Magers MJ, Goerke DM, et al. The utility of SDHB and FH immunohistochemistry in patients evaluated for hereditary paraganglioma-pheochromocytoma syndromes. Hum Pathol 2018;71:47-54. doi:10.1016/j.humpath.2017.10.013

[20] Gill AJ. Succinate dehydrogenase (SDH) and mitochondrial driven neoplasia. Pathology 2012;44:285-92. doi:10.1097/PAT.0b013e328.353.9932

[21] Wong MY, Andrews KA, Challis BG, et al. Clinical Practice Guidance: Surveillance for phaeochromocytoma and paraganglioma in paediatric succinate dehydrogenase gene mutation carriers. Clin Endocrinol (Oxf) 2019;90:499-505. doi:10.1111/cen.13926

[22] Bholah R, Bunchman TE. Review of pediatric pheochromocytoma and paraganglioma. Front Pediatr 2017;5:155. doi:10.3389/fped.2017.00155

[23] Hescot S, Curras-Freixes M, Deutschbein T, et al. Prognosis of malignant pheochromocytoma and paraganglioma (MAPP-Prono Study): A European network for the study of adrenal tumors retrospective study. J Clin Endocrinol Metab 2019;104:2367-74. doi:10.1210/jc.2018-01968

[24] Broudin C, Favier J, Verkarre V, Méatchi T. [Pathologist contribution in the diagnosis of hereditary predisposition to paranganglioma and pheochromocytoma]. Ann Pathol 2020;40:134-41. Apport du pathologiste dans les prédispositions héréditaires aux paragangliomes et phéochromocytomes. doi:10.1016/j.annpat.2020.01.010

[25] Gill AJ, Benn DE, Chou A, et al. Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. Hum Pathol 2010;41:805-14. doi:10.1016/j.humpath.2009.12.005

[26] Cheung VKY, Gill AJ, Chou A. Old, new, and emerging immunohistochemical markers in pheochromocytoma and paraganglioma. Endocr Pathol 2018;29:169-75. doi:10.1007/s12022.018.9534-7

[27] Thosani S, Ayala-Ramirez M, Palmer L, et al. The characterization of pheochromocytoma and its impact on overall survival in multiple endocrine neoplasia type 2. J Clin Endocrinol Metab 2013;98:E1813-9. doi:10.1210/jc.2013-1653

[28] Li Q, Lan Z, Jiang Y, Wang R, Li Z, Jiang X. Validation and evaluation of 5 scoring systems for predicting metastatic risk in pheochromocytoma and paraganglioma. Am J Surg Pathol 2024;48:855-65. doi:10.1097/pas.000.000.0000002238

[29] Papathomas TG, Oudijk L, Persu A, et al. SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a multinational study of the european network for the study of adrenal tumors (ENS@T). Mod Pathol 2015;28:807-21. doi:10.1038/modpathol.2015.41

[30] Santi R, Rapizzi E, Canu L, et al. Potential pitfalls of SDH immunohistochemical detection in paragangliomas and phaeochromocytomas harbouring germline SDHx gene mutation. Anticancer Res 2017;37:805-12. doi:10.21873/anticanres.11381

[31] Ohmoto A, Hayashi N, Takahashi S, Ueki A. Current prospects of hereditary adrenal tumors: towards better clinical management. Hered Cancer Clin Pract 2024;22:4. doi:10.1186/s13053.024.00276-6

[32] National Comprehensive Cancer Network N. Neuroendocrine and Adrenal Tumors. 2023.