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ENDOCRINE GENETICS/EPIGENETICS

One genotype, many phenotypes: *SDHB* p.R90X mutation-associated paragangliomas

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Abstract

Context *SDHB* p.R90X germline mutation is the most common genetic alteration in our patients with familial or apparently sporadic pheochromocytoma/paraganglioma (PPGL).

Objective To analyze the clinical and pathological characteristics, response to therapy, and outcome of patients with *SDHB* p.R90X-associated PPGL and describe the clinical phenotypic variability in the patients carrying this mutation.

Methods We reviewed the clinical and pathological characteristics and analyzed the phenotypic variability of all 13 patients that have *SDHB* p.R90X mutation-associated PPGL.

Results Thirteen patients (five females and eight males). The median age at diagnosis was 23 years (range 8–43). Although the mutation was the same, there was significant phenotypic variability between patients and even within the same family. Four patients (30.8%) had a family history of PPGL and six patients (46%) had distant metastasis. Surgery of the primary tumor was performed in 11 patients (84.6%). Two patients had inoperable PPGL. Patients with metastasis received different combinations of chemotherapy, Lu^{177} radiotherapy, multikinase inhibitors, and external irradiation. Only five patients (38.5%) were in remission at a follow-up duration of 4–9 years. The other patients either died due to their disease progression (four patients, 30.8%) or continue to have progressive disease (two patients, 15.4%) or recurrence (one patient, 7.7%). Patients with distant metastasis were older, had larger primary tumors, were more likely to have a family history of PPGL and had a worse outcome.

Conclusion *SDHB* p.*R90X* mutation-associated PPGL have significant phenotypic variability and are associated with a high risk of distant metastasis and mortality.

Keywords Paraganglioma · SDHx · SDHB · Mutation · Phenotype · Genotype

Introduction

Paragangliomas (PGL) and pheochromocytomas (PCC) are chromaffin cell-derived neuroendocrine tumors [1]. PCC and PGL, abbreviated collectively as PPGL are rare tumors arising from the same cell of origin but have distinct clinical and genetic differences [1]. PCC refers to intra-adrenal tumors while PGL refers to extra-adrenal tumors [2]. PPGL had been considered to be mostly sporadic but over the last 2 decades, it has become clear that about 40% of them are hereditary in origin due to several underlying predisposing genes [3]. In the 1980–1990s, the knowledge about the genetics of PPGL was limited to the fact that they sometimes occur as part of multiple endocrine neoplasia type 2, von Hippel Lindau syndrome or neurofibromatosis type 1 [4]. This represented <10% of cases of PPGL at that time [4]. In the year 2000, cases of head and neck PGL were found to carry germline mutations in the succinate dehydrogenase subunit D [5]. In 2002, Neumann et al., reported that about 24% of 271 apparently sporadic PPGL (mostly PCC) carried germline mutations in any of four genes, *SDHD*, *SDHB*, *VHL,* or *RET* [6]. Since that time, studies have added several novel genes to the list of underlying predisposing genes and currently, more than 30 genes have been identified to carry germline or somatic genetic alterations that are either the

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underlying or predisposing genetic alterations for familial or apparently sporadic cases of PPGL [1, 3]. These mutations occur in about 40% of cases of PPGL [2, 3]. There is a fairly good genotype/phenotype correlation [7–10]. For example, most cases of familial PGL have *SDHx* mutations while familial PCC usually carry *RET* or *VHL* mutations [9, 10]. In contrast to the usual types of PCC, *VHL*-associated PCC are usually characterized by norepinephrine secretion [11, 12]. *SDHB* is associated with a much higher risk of malignant PPGL than other types of mutations [12].

We have recently described the underlying genetics of PPGL in our highly consanguineous population [13]. Of 101 cases of apparently sporadic PPGL, 37 were found to have underlying germline mutations. *SDHB* mutations were the most common genetic alterations (21 cases) and the *SDHB* p.R90X nonsense mutation was the most common mutation occurring in 12/21 (57%) suggesting that it might be a founder mutation in our population. We were intrigued by the wide spectrum variability of the disease manifestations and course in patients carrying this germline mutation. We noticed significant intrafamilial and interindividual heterogeneity of the manifestations and the course of PPGL associated with this mutation. For this reason, we are focusing in this report on this aspect describing in detail the clinical and pathological phenotype, response to treatment, and outcome of patients carrying this unique mutation and highlighting the phenotypic variability associated with it.

Patients and methods

Of 199 patients with familial (98 patients) or apparently sporadic (101 cases) PPGL, 22 had germline *SDHB* mutations. *SDHB* p.R90X (c.268C>T) truncating mutation was the most common mutation occurring in 13 cases. Four out of the 13 patients (30.7%) are familial cases and the other nine patients are apparently sporadic cases. We studied the clinical, biochemical, radiological and pathological features, and outcome of these patients. We obtained an institutional Review Board Approval (RAC # 2150 015) from the Ethics Committee of the King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia. An informed consent was obtained from all cases in whom blood was collected prospectively while consent requirement was waived by the Ethics Committee for patients in whom the tissue was obtained from pathology archives. we collected data from the medical records of these patients. Genomic DNA was isolated from peripheral leukocytes or normal non-tumorous tissue as previously described [13]. The molecular testing was done using PCR and Sanger sequencing or next-generation sequencing confirmed subsequently by Sanger sequencing as previously described [13].

Statistical analysis

Numerical variables are expressed as median and range or mean ± SD and *T* test was used for analysis. Categorical variables are expressed as numbers and percentages and Fisher Exact test was used for analysis. The Statistical Package for the Social Sciences (SPSS) version 21 (IBM, Chicago) was used for the analysis. *P* value of ≤0.05 was considered significant.

Results

General characteristics of the patients

The patients were five females and eight males (Table 1). The median age at diagnosis was 23 years (range 8–43). The sites of the PGL were abdominal along the abdominal aorta in 11 cases (84.6%) and in the neck in one patient (carotid body tumor). One patient (7.7%) had synchronous abdominal and head PGL. Urine metanephrines were measured in ten patients. One had completely normal urine catecholamine metabolites including metanephrine, normetanephrine, and 3-methoxytyramine (3-MT) despite having a 10-cm PGL. All the other nine patients had elevated normetanephrine but normal metanephrines and 5 (55.6%) of them had elevated 3-MT levels. The median tumor size was 10.5 cm (range 3–18). Six tumors (46%) showed evidence of necrosis and 4 (30.8%) showed calcifications. Six patients (46%) had distant metastasis (Table 2). Two cases (15.4%) presented with widely metastatic PGL and inoperable primary tumors; one received chemotherapy and showed stability of the disease for about a year followed by progression and the other patient received at different stages chemotherapy, Lutetium 177 (Lu^{177}) peptide receptor radiotherapy, Sorafenib, and Pazopanib but continued to progress and died. Surgery for the primary tumor was done in the other 11 cases (84.6%). Only five patients (38.5%) were in remission without evidence of recurrence at a follow-up duration of 4–9 years. The other patients either died due to their disease progression (four patients, 30.8%) or continue to have progressive disease (two patients, 15.4%) or recurrence (one patient, 7.7%)

Phenotypic variability and *SDHB* p.R90X mutation

Of the 13 patients that harbor the *SDHB* p.R90X mutation, there were four patients with a family history of PGL; two were siblings and two had a family history of PGL (Table 1 and 2). The other nine patients had no family history of such tumors.

Table 1 Clinical and pathological characteristics, management, and outcome of 13 patients with *SDHB* p.R90X mutation-associated PPGL

No.	Age at Dx	Sex	Family Hx	Location	Size (cm)	Distant Mets.	Initial Tx	Additional Tx	Outcome	Duration (years)
1	23	F	Yes	Abdomen	5	Yes	Sx	Sx, MIBG, VCD	Death	15
2	43	M	Yes	Abdomen	10	Yes	VCD	Pazopanib	Progression	1.8
3	24	M	Yes	Abdomen	12	Yes	VCD	Lu^{177}, Sorafenib	Death	4
4	24	M	Yes	Abdomen	13	Yes	Sx	MIBG, XRT	Progression	14
5	36	M	No	Abdomen and head	18	Yes	Sx	MIBG, XRT	Death	6
6	21	M	No	Abdomen	10	Yes	Sx		Death	4
7	23	F	No	Abdomen	7	No	Sx		Recurrence	10
8	10	F	No	Abdomen	4.5	No	Sx		Remission	7
9	21	F	No	Adrenal	4	No	Sx		Remission	4
10	23	M	No	Neck (carotid)	4.5	No	Sx		Remission	10
11	8	F	No	Abdomen	3	No	Sx		Remission	3
12	17	M	No	Abdomen	14	No	Sx		Remission	6
13	13	M	No	Abdomen	13	No	Sx		Remission	9

Mets Metastasis, *Tx* Therapy, *Sx* Surgery, *MIBG* Meta-iodobenzylguanidine, *VCD* vincristine, cyclophosphamide, and doxorubicin, *Lu177*; Lutetium 177, *XRT* External radiotherapy

Patients with a family history of PPGL

Patients No. 1 and 2 (Table 1) are a sister and a brother. The sister was diagnosed at age 23 years with upper abdominal PGL. She underwent surgical resection and the pathology showed a PGL with a focal margin extension. She remained in a complete clinical, biochemical, and radiological remission for 7 years before she developed a recurrence at the same site of the original tumor. She underwent another surgery which could not resect the tumor completely. She received two doses of meta-iodobenzylguanidine-I-131 (MIBG) therapy but her tumor progressed locally and she developed metastases in the liver, lungs, and bone. She was subsequently treated with vincristine, cyclophosphamide, and doxorubicin (VCD) chemotherapy and showed a remarkable response to the first dose with almost 90% reduction in the size of the primary and metastatic tumors (Fig. 1). Unfortunately, she developed severe leucopenia and thrombocytopenia and could not continue receiving VCD. Her disease progressed and she died at age 37 years, 8 years after she developed recurrence and 15 years since her original diagnosis. Her brother presented at age 43 years with a large lower abdominal PGL and widely metastatic disease to the liver, lungs, and bone. His primary tumor was inoperable. He received six courses of the same chemotherapeutic regimen (VCD) without significant complications. He did not develop leucopenia or thrombocytopenia. Unlike the response in his sister, he showed a minimal response to VCD and his disease continued to slowly progress (Fig. 1). He was started recently on Pazopanib and the response is not yet clear.

Patient No. 3 (Table 1) presented at age 24 years with a 12-cm lower abdominal PGL and metastases to the liver and lungs. His primary tumor was inoperable and he was treated with six courses of VCD. The tumor showed minimal response initially but continued to progress after a few months. He received four doses of Lu^{177} but showed no response and finally he was treated with Sorafenib. Although he showed a significant subjective improvement with some radiological response in the first 3 months of Sorafenib therapy, his tumor subsequently continued to progress and he died from his disease 4 years after the diagnosis. His father had abdominal PGL and also died from progressive metastatic disease.

Patient No. 4 (Table 1) was diagnosed in 2006 at age 24 years with a left upper abdominal PGL metastatic to the left testis and spine. He underwent resection of the abdominal PGL along with resection left kidney and left adrenal gland. The tumor was 13.5 cm in size without invasion and Ki67 was <2%. He received two doses of MIBG, 155 and 225 mCi in 2006 and 2009 with no apparent response. He underwent resection of left testicular metastasis and left orchidectomy in 2012 and this showed a 6-cm metastasis from the PGL. He received external irradiation to the lumbar spine and he is still stable but with slow progression of his metastases. His father and an aunt died from metastatic abdominal PGL.

Patients without a family history of PPGL

Nine patients (Patients No. 5–13, Table 1) did not have a family history of PPGL but their relatives were not formally evaluated. Seven of them presented with abdominal PGL, one

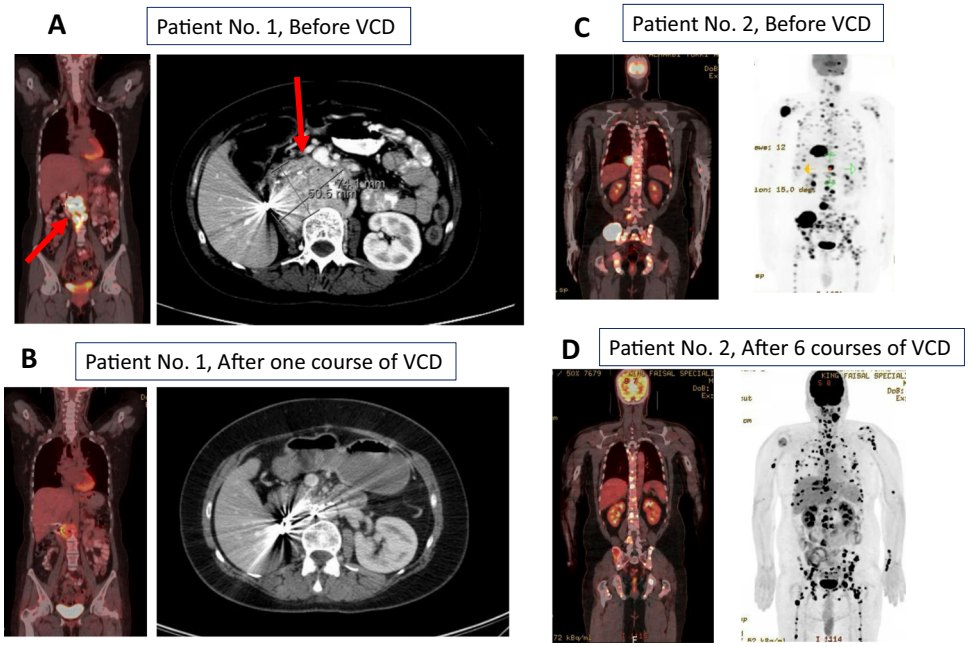

Fig. 1 The left panel shows a whole-body coronal section of a CT FDG PET and a cross-sectional section of a contrast-enhanced CT scan of the abdomen for patient No. 1 before chemotherapy (**a**) and after one course of VCD chemotherapy (**b**) with major resolution of the mid abdominal mass (arrow) and several foci on the PET scan. The right panel is for patient No. 2 (a brother of patient No. 1) showing a CT FDG PET before chemotherapy (**c**) and after six courses of VCD chemotherapy (**d**) showing the progression of extensive widespread metastasis

with a unilateral PCC, and one with synchronous abdominal and head PGL (Fig. 2). Two (Patients No. 5 and 6) had malignant metastatic PGL and succumbed to their disease while the other seven patients had local disease. One patient (Patient No. 7, Table 1) had a local recurrence 10 years after the initial resection. The patients who had metastatic disease include a 36-year-old man who was previously reported in detail [14]. He presented with headache and a change in voice. A computed tomography scan of the head revealed a midline nasopharyngeal tumor that was biopsied and turned out to be a PGL. A CT scan of the chest and abdomen revealed an 18-cm upper abdominal PGL that was resected (Fig. 2). The head PGL was inoperable. He was treated with MIBG twice and showed initially some reduction in tumor size but progressed later on with distant metastases and died 6 years after the initial diagnosis. The second patient (Patient No. 6) was a 21-year-old man who presented with a 10-cm abdominal PGL with extensive metastases to the retroperitoneal lymph nodes, lungs, and bone. He underwent resection of the primary tumor and adjacent malignant lymph nodes but refused additional therapies and his disease progressed and he died 4 years after the initial diagnosis. Patient No. 7 was a 23-year-old lady at the time of the initial presentation when she was found to have an abdominal PGL. She underwent resection of a 7-cm upper abdominal PGL which on histopathological examination showed no unusual features. She remained in clinical, biochemical, and radiological remission until 6 years later when she started to have a recurrent mass at the same location of the primary tumor. This was also associated with gradually increasing levels of urine normetanephrine and positive MIBG scan at the site of recurrence. So far, she has no evidence of distant metastases and is scheduled for another surgery. Other patients had "benign" PGL that were resected and they are in remission as summarized in Table 1.

Table 2 Comparison of patients with and without distant metastasis in 13 patients with *SDHB* p.R90X mutation-associated PPGL

Characteristic	PGL with metastasis	PGL without metastasis	*P* value
Median age ± SD (years)	28.5 ± 8.9	16.4 ± 6.2	0.02
Sex (M:F)	5:1	3:4	0.27
Family history	4/6 (66.6%)	0/7	0.021
U. Normetanephrine, mean ± SD (μmol/day)	81 ± 105	31 ± 25	0.33
Tumor size (cm), mean ± SD	12.6 ± 3.3	7.0 ± 4.6	0.043
Location			
Abdomen	4 (66.7%)	6 (85.7%)	0.34
Head and neck	0	1 (14.3%)	
Abdomen, head, and neck	2 (33.3%)	0	
Outcome			
Remission	0	6 (85.7%)	0.005
Recurrence	0	1 (14.3%)	
Progression	2 (33.3%)	0	
Death	4 (66.7%)	0	

Comparison of patients with and without metastatic PPGL

Table 2 shows a comparison between those with metastatic PPGL and those without distant metastases. Patients with distant metastasis were older, more likely to have a family history

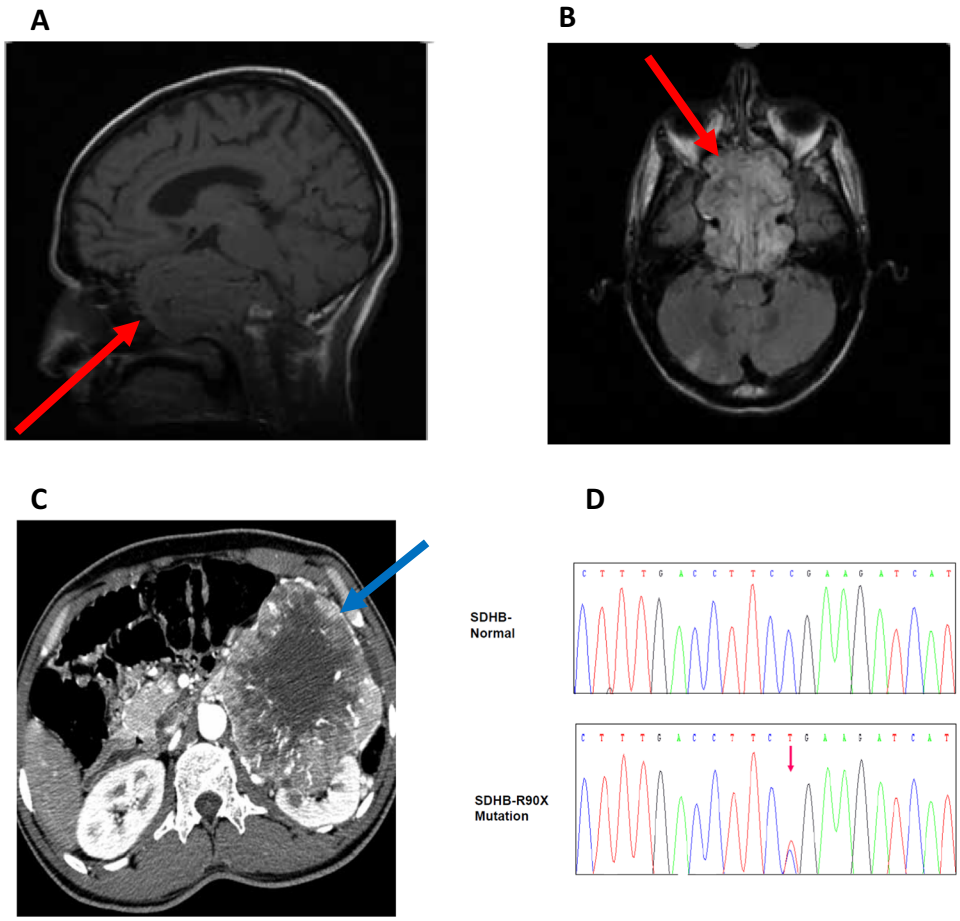

Fig. 2 A composite diagram for patient No. 5 with a non-contrast T1 weight sagittal MRI image (**a**) and a contrast-enhanced T1 axial MRI image showing a huge PGL at the nasopharyngeal area and base of the skull (red arrow). An enhanced CT axial abdominal image (**c**) shows a synchronous huge heterogeneously enhancing PGL with necrosis occupying the left side of the abdomen (blue arrow). A chromatogram (**d**) is showing the c.268C>T, p.R90X mutation

of PPGL, had larger size tumors and were more likely to progress or die from their disease. There was no difference in sex, location, and urine normetanephrine levels

Discussion

In this study, we have described a series of patients with a single *SDHB* mutation (p.R90X) causing PPGL with variable manifestations, course, response to therapy and outcome. This reflects significant tumor heterogeneity and strongly suggests that tumorogenesis is a highly complex process beyond germline genetics. Although all of these patients share the same initiating mutation, the age of onset, location of the tumors, secretory components, malignant potential, response to therapy, and outcome of the disease significantly differed as if they were caused by different mutations. This was most obvious in patients No. 1 and 2 (Table 1). Although they are siblings, they presented at different ages and with different manifestations. They also showed a different response to chemotherapy (Fig. 1). The first one presented at age 23 years with a localized PGL that was resected and she remained in remission until she had a recurrence 7 years later. Her recurrent disease progressed to widely metastatic disease in her late 30s close to her brother's age of initial presentation. She showed an impressive response to VCD therapy which was not the case in her brother (Fig. 1). However, she developed severe neutropenia and thrombocytopenia due to chemotherapy which were not seen in her brother who received six courses of the same chemotherapeutic agents (VCD). Lack of response to chemotherapy was seen in patient No. 3 who received six courses of VCD but continued to slowly progress (Fig. 1). In contrast to patient No. 1 who did not respond to sorafenib, this patient showed an excellent short-term clinical and radiological response to Sorafenib.

The risk of malignancy and development of metastatic disease was also variable. Six patients developed distant metastases while seven patients had localized "benign" PGL. Patients with distant metastases were older and had a larger tumor size. This suggests that the risk of metastasis might be just a matter of time in patients who did not have metastasis if they were not diagnosed early. Indeed, the youngest patient with distant metastasis was 21 years and only a few patients without distant metastasis are above this age (Table 1). One of those patients had a recurrence (Patient No. 7, Table 1) suggesting a propensity towards recurrence and metastatic spread.

This may have an implication on the surveillance and intervention. It is reasonable to recommend that active surveillance during the second decade of life should be strictly followed for subjects carrying this mutation and surgery should be performed as soon as the diagnosis of PPGL is made.

The reason for heterogeneity in the clinicopathological spectrum and outcome of PPGL in patients carrying the same underlying germline mutation is not fully understood. It is probably related to other factors including genetic and environmental factors [15]. It is well known that carcinogenesis is a multistep process including initiation, growth, progression, and metastasis [16]. At a molecular level, each of these stages is characterized by certain genomic and epigenetic changes [16]. A good example of this is colon cancer in patients with familial polyposis coli or medullary thyroid cancer in patients with multiple endocrine neoplasia type 2 [17, 18]. In these conditions, the process usually starts with cell hyperplasia, development of benign hyperplastic nodules/adenomas, progression to cancer, and finally further progression to more aggressive and metastatic cancer [17, 18]. Although the initiating event in these conditions is usually germline mutations in the *APC* tumor suppressor gene or *RET* proto-oncogene, respectively, additional genomic and epigenetic events take place and lead to further progression of the disease. In the case of *SDHB* p.R90X mutation, similar mechanisms likely occur with this mutation being essential for initiation of tumorigenesis but additional genetic and epigenetic alterations occurring after the initiation steps leading to variable course of the disease. Subclonal evolution where some cells formulate a subclone inside the tumor is an additional mechanism by which tumor progression varies [15]. In addition to the intrinsic tumor factors, extrinsic factors play important roles in tumor heterogeneity and clinical variability [15]. These include tumor microenvironment, immune system, blood, and nutrient supply and extracellular matrix composition [15, 19]. So, although the initiating event in our patients is the germline mutation p.R90X, additional genetic events including somatic mutations, copy number variation, epigenetic events, and metabolic changes probably contribute to further growth and progression of these tumors.

In the first report of the role of *the SDHB* gene in the pathogenesis of PPGL, Astuti et al., reported the p.R90X mutation in three unrelated families with PPGL [20]. The families were of different origin and haplotyping and loss of heterozygosity analysis showed that this was not a founder mutation in that study [20]. The mutation is due to a transition from C to T at nucleotide 268 of *SDHB* gene (Fig. 2) resulting in a truncated protein lacking the C-terminal 191 amino acids. This was predicted to result in a significant effect on the SDHB function [20]. This was confirmed in a recent study that used in Silico structural modeling and in vitro assessment of SDHB activity. p.R90X mutation was predicted to disrupt the assembly of the SDH complex and was shown in in vitro to lead to loss of expression of SDHB expression and loss of its enzymatic activity [21]. Interestingly, p.R90X was not reported in the recently completed TCGA study that included 173 patients from North America [3]. This may reflect ethnic differences in the occurrence of this mutation and may also suggest that it is a founder mutation in our population since it was the most common mutation.

Our study is the first to report on genotype/phenotype correlation in PPGL from an Arab population and the first to report specifically in details on this truncating mutation (p.R90X). Although the number of patients seems small, this is the largest series of patients with this single mutation. To our knowledge, no previous study has described the phenotype in patients with this mutation in the details we presented here. However, our study has some limitations including the fact that although the patient number is large for a single mutation, it remains small for statistical analysis. Second, we were unable to evaluate the relatives of those patients in details since they live in distant places and some of them were not interested in medical evaluation. Therefore, it is possible that some of the patients who were thought to have de novo mutations and are apparently sporadic are in fact part of families with the same mutation with other family members either affected but undiagnosed or are in the presymptomatic stage.

In summary, *SDHB* p.R90X mutation (Fig. 2) is the most common mutation in our population and PPGL associated with it shows significant variability in their manifestations, locations, malignant potential, response to therapy, and course of the disease. Overall, patients carrying this mutation present in their second decade of life and are at a significant risk of malignancy. Regular surveillance of subjects carrying this mutation and early intervention are recommended. After surgery, long-term follow-up and monitoring is also recommended even in patients who have been in a long-term remission as recurrence can occur after many years.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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