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Germinal mutations of *RET*, *SDHB*, *SDHD*, and *VHL* genes in patients with apparently sporadic pheochromocytomas and paragangliomas

Mutacje germinalne genów *RET*, *SDHB*, *SDHD* i *VHL* u chorych z pozornie sporadycznymi guzami chromochłonnymi i nerwiakami przyzwojowymi

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Abstract

Introduction: Pheochromocytomas and paragangliomas are derived from neural crest cells and are localized mainly in adrenal medulla and sympathetic or parasympathetic ganglia. They can be inherited (25%) and be part of multi-endocrine syndromes such as MEN2 syndrome, von Hippel-Lindau syndrome, pheochromocytoma/paraganglioma syndrome, neurofibromatosis type 1, and Sturge-Weber syndrome. Clinical presentation can sometimes be atypical and does not always allow proper diagnosis. In such situations, DNA analysis can be helpful, especially when the pheochromocytoma is the first and only symptom.

Material and methods: We analyzed DNA from 60 patients diagnosed and treated in the Centre of Oncology with a diagnosis of pheochromocytoma or paraganglioma. DNA analysis was carried out for *RET* (exons 10, 11, 13, and 16), *SDHB*, *SDHD*, and *VHL* genes. Techniques used for the analysis were direct sequence analysis, MSSCP, and RFLP.

Results: Germinal mutations were found in 16 patients (26,7%). Most frequent were mutations in *RET* proto-oncogene, followed by *VHL* gene, one mutation in *SDHB*, and one in *SDHD* genes. A comparison of some of the clinical features of both groups (with and without mutation) showed statistically significant differences.

Conclusions: The results of our study show that genetic predisposition is frequent in chromaffin tissue tumours, which indicates that DNA analysis is necessary in every case, also because of possible atypical clinical presentation.

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Key words: *pheochromocytoma, paraganglioma,* SDHB, SDHD, VHL, RET, *DNA analysis*

Streszczenie

Wstęp: Guzy chromochłonne i nerwiaki przyzwojowe wywodzą się z komórek grzebienia nerwowego i zlokalizowane są głównie w rdzeniu nadnerczy oraz w zwojach autonomicznych współczulnych i przywspółczulnych. Mogą one (25%) stanowić składnik zespołów wielogruczołowych, takich jak zespół MEN2, zespół von Hippela-Lindaua, zespół mnogich guzów chromochłonnych i nerwiaków przyzwojowych (PPS, *pheochromocytoma/paraganglinoma syndrome*), a także nerwiakowłókniakowatości typu 1 czy zespołu Sturge-Webera. Nie zawsze obraz kliniczny pozwala jednoznacznie określić rodzaj zespołu, dlatego badanie DNA może być pomocne w ustaleniu rozpoznania. Celem pracy była ocena częstości występowania dziedzicznie uwarunkowanych guzów chromochłonnych i przyzwojaków u zgłaszanych jako pierwszy objaw.

Materiał i metody: Przeanalizowano DNA pochodzące od 60 chorych leczonych i diagnozowanych w Centrum Onkologii z powodu guza chromochłonnego i nerwiaków przyzwojowych. Przeprowadzono analizę DNA w zakresie następujących genów: *RET* (eksony 10, 11, 13 i 16), *SDHB*, *SDHD* i *VHL*. Wykorzystywano sekwencjonowanie DNA, analizę MSSCP oraz analizę restrykcyjną.

Wyniki: Mutacje germinalne znaleziono u 16 chorych (26,7%). Najczęstsze były mutacje w genie *RET*, następnie w genie *VHL* oraz po jednej mutacji w genach *SDHB* i *SDHD*. Analiza danych klinicznych chorych będących nosicielami mutacji wykazała znamienne statystycznie różnice w porównaniu z grupą chorych z guzami sporadycznymi.

Wnioski: Przeprowadzone badania wskazują na częsty udział predyspozycji dziedzicznej w wystąpieniu guzów chromochłonnych, co wskazuje na konieczność wykonywania badania DNA w każdym przypadku, także ze względu na możliwość nietypowego przebiegu klinicznego, zwłaszcza w grupie chorych w wieku 20–40 lat. **(Endokrynol Pol 2010; 61 (1): 43–48)**

Słowa kluczowe: *guz chromochłonny, nerwiak przyzwojowy,* SDHB, SDHD, VHL, RET, *analiza DNA*

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Introduction

Pheochromocytomas and other paragangliomas are derived from neural crest cells, which eventually differentiate into adrenal medulla and autonomic nervous system paraganglia. Depending on the localization and the ability of secretion and metabolisation of catecholamines, they are called pheochromocytomas or paragangliomas, and can be sympathetic or parasympathetic [1, 2].

Until recently, all secreting tumours, regardless of localization, were called pheochromocytomas, i.e. tumours derived from adrenal medulla, as well as sympathetic paragangliomas, usually located in the retroperitoneal space. The name "paraganglioma" was used only for non-secreting parasympathetic paragangliomas usually located in mediastinum or in the region of bifurcation of the jugular artery (tumours of glomus jugulare — chemodectomas) and the skull base. Currently the name "pheochromocytoma" is used only for tumours of the adrenal medulla; other tumours are referred to as paragangliomas.

The majority of these tumours are benign. The only criterion for diagnosing malignancy is the presence of metastases in the organs which normally lack the chromaffin cells [3, 4].

Pheochromocytomas often develop because of inherited syndromes like MEN2 (multiple endocrine neoplasia type 2), VHL (von Hippel-Lindau), or NF1 (neurofibromatosis 1). In the past it was believed that these tumours were inherited only in 10% of cases; at present it is known that they have familial origin in about 25% of apparently sporadic tumours [5, 6]. These syndromes are caused by germinal mutations of the predisposing genes: *RET* proto-oncogene, *VHL*, *NF-1* genes, and three genes encoding subunits of succinyl dehydrogenase: *SDHB*, *SDHD*, and *SDHC*.

The role of these last genes in the development of chromaffin tissue tumours was discovered only in 2002 [7]. Succinyl dehydrogenase is an enzyme playing a role in cellular respiration as a component of both the tricarboxylic acid cycle and the respiratory chain. Mutations in these genes are the cause of pheochromocytoma/paraganglioma syndrome (PPS), traditionally called PGL syndrome. In this syndrome, multiple tumours are common: *SDHD* mutations connected mainly with benign tumours of the head and neck region [8, 9], *SDHB* mutations more often causing hormonally active and malignant lesions. *SDHC* mutations are very rare and mainly cause paragangliomas.

MEN2 syndromes are caused by mutations in *RET* proto-oncogene activating the transmembrane protein tyrosine kinase. In these syndromes, the main component is medullary thyroid cancer. Pheochromocytomas are present in about 50% of cases, in most cases benign, often bilateral. In less than 20% of patients, hyperparathyroidism can be present. In MEN2B syndrome, apart from medullary thyroid cancer patients, the typical habitus presents thin posture, long, thin limbs, big lips, and mucosal neuromas. Due to transmural neural plexus hypertrophy, symptoms of megacolon may occur [8, 9, 11].

Von Hippel-Lindau (VHL) syndrome symptoms can vary and include haemangioblastomas of the central nervous system (CNS) and retina, clear cell renal carcinoma and pheochromocytoma, and other symptoms with less specificity, such as cysts of the pancreas, kidneys, epididymis, and broad ligament of the uterus. Pheochromocytomas appear in 10–26% of cases, mainly located in the adrenal medulla, in most cases as benign tumours. VHL syndrome is caused by inactivating mutations of the *VHL* gene involved in angiogenesis and regulating hypoxia [12, 13].

NF1 syndrome is quite frequent and is characterized by neurofibromas, *café au lait* spots, iris lesions (Lisch nodules), and adrenal pheochromocytomas. The latter are a rare component of the syndrome (0.1––5.7%) and are more frequent in patients with hypertension [14–16].

The presented study is part of a multicenter project concerned with the genetic predisposition to chromaffin tissue tumours.

The aim of the study is to evaluate the frequency of hereditary chromaffin tissue tumours in patients with apparently sporadic pheochromocytomas from the material of the Institute of our department and cooperating centres.

Material and methods

The subjects of the study were 60 patients with diagnosis of pheochromocytoma or paraganglioma referred for genetic evaluation. The diagnosis was made based on histopathology in 55 patients. The remaining five patients were not operated on, and the diagnosis was based on typical clinical manifestation, biochemical abnormalities, and radiological or scintigraphic imaging. Pheochromocytoma was diagnosed in 52 (77.4%) and paraganglioma in 9 (15%) patients; among them was one case of chemodectoma. One patient had both pheochromocytoma and paraganglioma (Table I).

Among the patients with pheochromocytoma, 41 had benign tumours (78.8%) and malignant lesion was present in 11 (20.7%). Malignancy was diagnosed when distant metastases were discovered. Sites of metastases were bones (4 patients), liver (5 patients), lungs (5 patients), and lymph nodes of the thoracic or abdominal cavity (5 patients). One person had multiple metastases in the left kidney.

Table I. *Description of the studied group*

Tabela I. *Opis badanej grupy*

Diagnosis	Number of cases	Percentage
Pheochromocytoma*	53	88.4
Paraganglioma	8	13.3
Chemodectoma	1	1.7

*One case of pheochromocytoma and paraganglioma

Mean age of diagnosis was 35.6 years (11–65 years).

Genetic diagnosis was carried out for MEN2, VHL, and PPS syndromes. This was based on the clinical examination and DNA analysis.

For the analysis, a blood sample was taken and DNA of the leukocytes was isolated with the use of the proteinase K and SDS. Then was extracted with NaCl and ethyl alcohol and dissolved in sterile deionised water. Isolated DNA was then amplified with the use of polymerase chain reaction (PCR). The amplification was carried out for all exons of *SDHB*, *SDHD*, and *VHL* genes, and exons 10, 11, 14, and 16 of *RET* proto-oncogene under conditions specific for each exon. The reaction was held in a mixture containing dNTPS, F, and R primers, $MgCl_2$, buffer, water, and DNA polymerase HotStartTaq. The reaction consisted of 35 cycles of different temperature conditions. The result of the reaction was then visualized on agarose gel with the use of ethidine bromide.

For the *SDHB* and *SDHD* genes, multi-temperature, single-strand conformation (MSSCP) analysis on the polyacrylamide gel was then performed with the use of a DNA Pointer System (Kucharczyk TE) and the results visualized with the use of the silver stain method. In the case of detection of DNA conformation change, sequence analysis was performed. For the exons of *VHL* and *RET* genes, sequence analysis was carried out as well (Applied Biosystem/Hitachi).

Results

Patients with germline mutations found in analyzed genes are characterized in Table II and the frequency of the mutations in Table III.

Germinal mutations were found in 16 out of 60 patients, which is 26.7%. Most common were mutations in the proto-oncogene *RET* (n = 11; 18.3%). Next were the *VHL* mutations (n = 3; 7.7%), and the mutations in *SDHB* and *SDHD* genes were found in two patients (3.4%).

In the group of patients with pheochromocytoma, mutations were found in 14 cases (26.9%), in benign tumours in 12 cases (29.3%), and malignant in 2 cases (18.2%). These were mutations in *RET* proto-oncogene (codons 634 and 791) and the *VHL* gene.

In the group of patients with paragangliomas, mutations were found in two cases (25%): in one patient with benign chemodectoma (mutation in *SDHD* gene) and one patient with malignant paraganglioma of the bladder with metastases in the iliac artery lymph nodes (*SDHB* mutation).

Among the *RET* mutations, most common was the mutation in exon 11 codon 634, present in six cases. All of these patients underwent full diagnostic procedures, and medullary thyroid cancer was eventually diagnosed in all cases. Two of them also had primary hyperparathyreoidismus. The pheochromocytomas in these patients were all secreting and localized in the adrenal medulla. Three patients had unilateral lesion, two developed second tumours and required a second adrenalectomy in the course of follow-up, and one patient had two tumours at presentation, which were then removed during one operation.

Among five patients with codon 791 mutation, thyroid nodules were diagnosed only in two of them but repeated FNAB revealed benign lesions. In all of these patients, repeated pentagastrin stimulation tests were within the normal range, thus no indications for prophylactic thyroidectomy were stated. Three of these patients had single adrenal tumours which were removed. In one patient, diagnosed because of unstable hypertension at the age of 14 years, a small paraganglioma of the retroperitoneal space was found. Another patient was operated on twice because of the recurrence and also underwent ^{131}I-MIBG treatment because of metastases in the abdominal lymph nodes.

In the *VHL* gene we found three different mutations: in codon 64 associated with bilateral pheochromocytomas; in codon 112 in a patient with malignant pheochromocytoma with abdominal lymph node and bone metastases; in codon 125 in a patient with bilateral pheochromocytomas, clear cell renal cancer, and blindness of the left eye because of retinal haemangioblastoma history — in the course of later diagnostic procedures, cerebellar haemangioblastoma was also found.

A mutation was found in exon 6 of *SDHB* gene in a patient with malignant paraganglioma of the bladder with concomitant regional lymph node metastases. In *SDHD* gene, a mutation was found in exon 1 in a patient with benign chemodectoma, who completely recovered after operation.

The average age of diagnosis was 30 years ± 16 years (11–68 years). Nine of those patients (50%) were under 20 years old at diagnosis, 5 (27.8%) were less than 40 years old, and only 22.2% of this group (n = 4) were older than 40 years. When compared to the group of patients in whom familial predisposition was excluded

Table II. *Clinical characteristic of the studied group*

Tabela II. *Charakterystyka kliniczna badanej grupy*

Lp	Initials	AOD	Dgn	Localization	Single/multiple	Malignant	Syndrome	Mutation
1	MB	19	Pgl	Bladder	Multiple	Yes	PGL	**SDHB**
2	PG	39	Pheo	Right adrenal gland	Single	No	MEN2A	RET 791
3	MJ	28	Pheo	Both adrenal glands	Multiple	No	MEN2A	RET 634
4	BJ	16	Pheo	Both adrenal glands	Multiple	No	–	VHL 64
5	JJ	28	Pheo	Both adrenal glands	Multiple	No	MEN2A	RET 634
6	BK	20	Pheo	Left adrenal gland	Single	Yes	MEN2A	RET 791
7	MK	14	Pgl	Both adrenal glands	Multiple	No	–	SDHD 196
8	MK	20	Pheo	Both adrenal glands	Multiple	No	MEN2A	RET 634
9	JL	14	Pgl	Retroperitoneum	Single	No	MEN2A	RET 791
10	AL	18	Pheo	Both adrenal glands	Multiple	No	–	VHL 125
11	JM	53	Pheo	Both adrenal glands	Multiple	No	MEN2A	RET 634
12	WP	31	Pheo	Both adrenal glands	Multiple	No	MEN2A	RET 634
13	MS	11	Pheo	Right adrenal gland	Single	Yes	–	VHL 112
14	RT	68	Pheo	Left adrenal gland	Single	No	MEN2A	RET 791
15	IW	23	Pheo	Left adrenal gland	Single	No	MEN2A	RET 791
16	HŻ	50	Pheo	Right adrenal gland	Single	No	MEN2A	RET 634
17	BL	49	Pheo	Left adrenal gland	Single	No	NF1	Not analyzed
18	GJ	11	Pgl	Retroperitoneum	Multiple	Yes	Sturge-Weber	Not analyzed

Table III. *Frequency of mutations/inherited syndromes*

Tabela III. *Częstość mutacji/zespołów dziedzicznych*

Gene	Localization	Number	Frequency
RET	codon 634	6	10%
	codon 791	5	8.3%
	Total	**11**	**18.3%**
vHL	codon 64	1	2.6%
	codon 112	1	2.6%
	codon 125	1	2.6%
	Total	**3**	**7.7%**
SDHB-D	N 721 G-A	1	2.6%
	codon 11	1	2.6%
	Total	**2**	**5.2%**
NF1		1	2.6%
Sturge-Weber		1	2.6%
Total		18	30%

because of DNA analysis, the age of diagnosis was higher — 41 years ± 13.7 years (12–72 years). In the age range less than 20 years old only three out of 42 sporadic patients (7%) had undergone operation. This difference was statistically significant (p = 0.003). Thirteen (31%) patients were diagnosed in the age of 20–40 years, and the majority were operated on during the age range over 40 years (n = 26; 62%). This difference is not statistically significant (p = 0.12). Only 1/3 of these patients (n = 6) had single tumours: adrenal (n = 4) or extra-adrenal (n = 2). In eight out of the 18 inherited cases, additional lesions appeared in the course of the disease, whereas in the group of sporadic tumours, multiple lesions were present only in 12%. This difference was also statistically significant (p = 0.03).

The majority (n = 12; 66%) had multiple or malignant lesions. In comparison with the group without familial predisposition, the age of diagnosis was higher — 41 years ± 13.7 years (12–72 years). In the age range less than 20, only three out of 42 patients (7%) had undergone the operation, 13 (31%) in the age of 20––40 years, and the majority in the age over 40 (n = 26; 62%). In this group, complete remission was achieved in 30 (72%) patients. The difference between the number of multiple and malignant lesions in these two groups is also statistically significant (p = 0.03).

Discussion

We analyzed the genetic cause of pheochromocytomas and paragangliomas in patients with apparently sporadic tumours. The DNA analysis was carried out for four genes: *RET*, *VHL*, *SDHB*, and *D* [17]. The third *SDH* gene — *SDHC* — was not analyzed at this point because its mutations are extremely rare — 4% in paragangliomas

only. NF1 diagnosis was based on clinical symptoms only [22].

Based on the DNA analysis, hereditary origin of pheochromocytomas was found in 16 cases (26.7%). Additionally, in one patient we found clinical features pointing to NF 1 syndrome and in another, family history pointed to Sturge-Weber syndrome, so these patients could also be treated as hereditary cases. With the addition of these two cases, the prevalence of familial tumours rises to 30%. Our findings are similar to these of a larger multicentre international study in which pheochromocytomas were found out to be inherited in 25% [6] of cases, higher than in other surveys. The presented material consists not only of the more difficult cases transferred for further diagnosis, but also of patients with unequivocal diagnosis of hereditary tumours transferred for further follow-up. This explains the higher contribution of hereditary pheochromocytomas.

In our material, *RET* proto-oncogene mutations were most frequent. They were observed in 11 patients, 18.3% of all cases and 68% of all found mutations. Mutations in codon 634 were found in six and in codon 791 in five cases. In the patients with codon 634 mutations [21], further procedures led to the diagnosis of medullary thyroid cancer, thus they could be regarded as missed clinically apparent MEN2 cases. Nevertheless, in the situation of high frequency of thyroid goiter in Poland and limited access to calcitonin estimation, referral to DNA diagnosis was the deciding step in the proper diagnosis and speeded up the detection of medullary thyroid cancer. The pheochromocytomas in these cases were all located in the adrenal glands, were benign, and in many cases bilateral, which is a typical feature of MEN2A syndrome according to the literature [15].

The clinical course of the disease in patients with codon 791 mutation was different. None of these patients developed thyroid cancer, one of the pheochromocytomas was malignant, and there was one case of paraganglioma.

Management of the carriers of these two mutations is also different. Most recommendations also advise prophylactic thyroidectomy in patients with codon 791 mutation. However, they concern children. In most of our patients, DNA analysis had already been performed in adulthood and further diagnosis showed no cause for suspicion of MTC. It can thus be presumed that the transforming potential of the mutation is weak, especially when no C cell hyperplasia is present, which was proven by negative pentagastrin stimulation test in all of our patients. In addition, the family history was negative in all cases. In this situation, we decided not to perform prophylactic thyroidectomy [19].

Mutations in *VHL* gene were associated with adrenal tumours and relatively young age of diagnosis. The risk of the malignancy is known to be very low [6, 15], although in our studied group we describe one patient with an obviously malignant, metastatic tumour.

Mutations in *SDHB* and *SDHD* genes were associated with the young age of onset. The only carrier of *SDHD* mutation found in our group had benign chemodectoma with complete remission after surgery. This mutation is the most common mutation ("hot spot") in the central European population (Poland/Germany) [20]. A mutation in the *SDHB* gene was observed in one patient with extra-adrenal paraganglioma with metastases of the lymph nodes. These findings are also in accordance with the literature data [10].

It is still not fully understood why the course of disease is different in the carriers of *SDHB* mutations from in those with *SDHD* mutations. Baysal suggests that there is a different pattern of expression of the tumours located above and below the diaphragm [7], whereas Jimenez points out the fact that *SDHB* encodes the subunit with full catalytic ability whereas *SDHD* and *SDHC* encode subunits harbouring catalytic subunits to the mitochondrial membrane [15].

It is believed that tumours appearing at a younger age are caused by hereditary syndrome [1, 2, 21]. When the criterion of a minimum of 20 years of age was taken into consideration, the difference was significant. We think that in the age group 20–40 years of age, DNA analysis, especially when a single tumour is present, can be most helpful in diagnosing inherited syndromes.

Inherited tumours were strongly associated with multiple disease foci and were statistically significant. Malignant tumours were present in four mutation carriers (28%) and in nine patients with sporadic tumours (21%). Thus, in contrast to some other publications [23, 24] we did not observe a strong correlation between malignancy and predisposing gene mutation related tumours, which is perhaps due to the low number of cases or to the bias related to the reference of more malignant cases in the truly sporadic group as well.

Conclusions

Our results point out that DNA analysis is necessary in every case of apparently sporadic pheochromocytoma and paraganglioma in order to diagnose hereditary syndromes that may have an untypical course.

Reference

1. Lenders J, Eisenhofer G, Manelli M et al. Phaechromocytoma. The Lancet 2005; 366: 665–675.
2. Kaltsas G, Besser M, Grossman A. The Diagnosis and Medical Management of Advanced Neuroendocrine Tumors. Endocr Rev 2004; 25: 458–511.
3. Thompson L. Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) to Separate Benign from Malignant Neoplasms. Am J Surg Pathol 2002; 26: 551–566.
4. Eisenhofer G, Bornstein S, Brouwers F et al. Malignant pheochromocytoma: current status and initiatives for future progress. Endocrine Related Cancer 2004;11: 423–436.
5. Dluhy R. Pheochromocytoma — Death of an axiom. N Engl J Med 2002; 346: 1486–1488.
6. Neumann H, Bausch B, McWhinney S et al. Germ line mutations in nonsyndromic pheochromocytoma. N Engl J Med 2002; 346: 1459–1465.
7. Baysal BE. Hereditary paraganglioma targets diverse paraganglia. J Med Genet 2002; 39: 617–22.
8. Bryant J, Farmer J, Kessler L. Pheochromocytoma: The expanding genetic differential diagnosis. J Natl Cancer Inst 2003; 95: 1196–1204.
9. Maher E, Eng C. The pressure rises: update on the genetics of pheochromocytoma. Human Molecular Genetics 2002; 20: 2347–2354.
10. Neumann H, Pawlu C, Pęczkowska P. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 2004; 292: 943–951.
11. Gagel R, Tashijan Jr A, Cummings T. The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a; an 18 year experience N Engl J Med 1992; 318: 478–484.
12. Maher E, Iselius L, Yates J. Von Hippel Lindau disease: a genetic study J Med Genet 1991; 28: 443–447.
13. Hes F, Hoppenher J, Lips C. Pheochromocytoma in von Hippel-Lindau disease: J Clin Endocrinol Metab 2003; 88: 969–974.
14. Zoller M, Rembeck B, Oden A. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population Cancer 1997; 79: 2125–2131.
15. Jimenez C, Cote G, Arnold A. Should patients with apparently sporadic pheochromocytomas or paragangliomas be screened for hereditary syndromes? J Clin Endocrinol Metab 2006; 91: 2851–2858.
16. Koch C, Vortmeyer A, Zhuang Z. New insights into the genetics of familial chromaffin cell tumors. Ann NY Acad Sci 2002; 970: 11–28.
17. Gimm O. Pheochromocytoma associated syndromes: genes, proteins and functions of RET, VHL and SDHx. Familial Cancer 2005; 4: 17–23.
18. Vieira A, Mello M, Elias L. Molecular and biochemical screening for the diagnosis and management of medullary thyroid carcinoma In multiple endocrine neoplasia type 2A. Horm Metab Res 2002; 34: 202–206.
19. Brauer V, Scholz G, Neumann S. RET germline mutation in codon 791 in a family representing 3 generations from age 5 to age 70 years: should thyroidectomy be performed? Endocr Pract 2004; 10: 5–9.
20. Pawlu C, Bausch B, Neumann H. Mutations of the SDHB and SDHD genes. Familial Cancer 2005; 4: 49–54.
21. Bauters C, Vantyghem M, Leteurtse E. Hereditary pheochromocytomas and paragangliomas: a study of five susceptibility genes. J Med Genet 2003; 40: e75.
22. Schiavi F, Boedeker CC, Bausch B et al.; European-American Paraganglioma Study Group. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. JAMA 2005; 294: 2057–2063.
23. Ercolino T, Taurino C, Sestini R et al. Malignant extra-adrenal pheochromocytoma caused by an SDHB intronic variation leading to a 54-bp deletion in exon 4. J Endocrinol Invest 2009; 32: 111–114.
24. Havekes B, Corssmit EP, Jansen JC et al. Malignant paragangliomas associated with mutations in the succinate dehydrogenase D gene. J Clin Endocrinol Metab 2007; 92: 1245–1248.