Endocr (2009) 36:10–15
DOI 10.1007/s12020-009-9204-0

CASE REPORT

R27X nonsense mutation of the *SDHB* gene in a patient with sporadic malignant paraganglioma

Masaki Naito · Takeshi Usui · Tamiko Tamanaha · Sachiko-Tsukamoto Kawashima · Hitomi Iogawa · Hanae Hagiwara · Takashi Kimura · Tetsuya Tagami · Manabu Kurosawa · Akira Shimatsu · Mitsuhide Naruse

Received: 22 September 2008 / Accepted: 10 April 2009 / Published online: 5 May 2009

Abstract It has been estimated that approximately 10% of pheochromocytomas and paragangliomas are part of a hereditary syndrome. Recent studies, however, suggest that the genetic involvement in pheochromocytoma/paraganglioma is actually far more common. Here, we report a case of malignant paraganglioma with no apparent family history. A 59-year-old man was referred to our services because of multiple abdominal masses. Plasma and urine adrenalin and noradrenalin levels were slightly elevated, and plasma dopamine and urine vanillylmandelic acid levels were remarkably elevated. Abdominal and chest computed tomography revealed multiple masses in the para-aortic region and in both lungs. Although ^{131}I-meta iodobenzylguanidine scintigraphy did not show significant uptake in these tumors, a 6-[^{18}F]fluorodeoxyglucose positron emission tomographic scanning study showed multiple areas of uptake corresponding to the tumors. Biopsy of the tumors revealed paraganglioma with chromogranin A-immunopositive cells. Genetic analysis indicated a nonsense mutation at codon 27 of the *SDHB* gene. As recently described, *SDHB* mutations may cause extra-adrenal and malignant paragangliomas, such as in the present case.

Keywords Paraganglioma · SDHB · Malignant · Sporadic

M. Naito · T. Usui · T. Tamanaha · S.-T. Kawashima · H. Iogawa · H. Hagiwara · T. Kimura · T. Tagami · A. Shimatsu · M. Naruse
Department of Endocrinology and Metabolism, National Hospital Organization, Kyoto Medical Center, Kyoto, Japan

T. Usui (✉) · T. Tagami · A. Shimatsu · M. Naruse
Clinical Research Institute Center for Endocrine and Metabolic Diseases, National Hospital Organization, Kyoto Medical Center, 1-1 Mukaihata-cho Fukakusa Fushimi-ku, Kyoto 612 8555, Japan
e-mail: tusui@kyotolan.hosp.go.jp

M. Kurosawa
Department of Diagnostic Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Introduction

Pheochromocytoma is a rare tumor of the adrenal gland, comprising less than 5% of all adrenal tumors [1]. Pheochromocytoma occurs sporadically or as part of an inherited multiple endocrine neoplasia type 2 (MEN2) syndrome or von Hippel–Lindau (VHL) disease [2–4]. *RET* proto-oncogene and *VHL* tumor suppressor genes are responsible for these two familial types of pheochromocytoma. In MEN2, most patients present with a phenotype of medullary thyroid carcinoma in addition to pheochromocytoma. In contrast, patients with VHL sometimes present with only the pheochromocytoma phenotype, which is recognized as type-2C VHL [5, 6]. *NF1* gene mutations, which cause neurofibromatosis type I, also contribute to the development of pheochromocytoma [7, 8]. The development of hereditary pheochromocytoma and paraganglioma was recently associated with genes encoding three mitochondrial complex II subunit proteins, *SDHB*, *SDHC*, and *SDHD* [9–11]. In a large number of patient studies, approximately 25% of the patients with apparent sporadic pheochromocytoma showed a germline mutation in one of the following four genes (*RET*, *VHL*, *SDHB*, and *SDHD*) [12]. More recently, the relation between the clinical phenotypes of pheochromocytoma/paraganglioma and the responsible genes has been investigated. *SDHB*-related paragangliomas often present as apparently sporadic paragangliomas with symptoms related to the tumor mass

rather than to excess catecholamine [13, 14]. In addition, 80% of *SDHB*-associated paragangliomas are located in extra-adrenal abdominal, intrapelvic, or thoracic locations. Patients with *SDHB* mutations are at increased risk for malignant paraganglioma and may be at risk for renal cell carcinoma [15]. To date, however, there has been no clear phenotype–genotype relation in *SDHB*-associated paraganglioma. Here, we report a case of malignant extra-adrenal paraganglioma with a nonsense mutation of the *SDHB* gene.

Case

A 59-year-old man was referred to our hospital for further examination and treatment for multiple masses in the abdominal and thoracic regions. During an examination for pollakiuria and anemia in the previous hospital, peri-aortic masses were detected on an abdominal computed tomography (CT) scan. On admission, his height was 175.9 cm, weight was 57.4 kg, blood pressure was 121/75 mmHg, and pulse rate was 117/min with a regular rhythm. The patient had episodic palpitations and lower back pain since presenting with pollakiuria. The patient had no familial history of hypertension or endocrinologic abnormality. The serum and urine catecholamines and their metabolite levels are shown in Table 1. Serum and urine noradrenalin and urine normetanephrine levels, as well as serum and urine dopamine levels, were elevated. Abdominal CT showed multiple peri-aortic masses (Fig. 1a) and multiple small nodules in both lungs (Fig. 1b). Although ^{131}I-meta iodobenzylguanidine scintigraphy (MIBG) revealed no significant uptake (Fig. 2), 6-[^{18}F]fluorodeoxyglucose positron emission tomographic scanning (FDG-PET) revealed intense uptake by masses consistent with those detected by CT scan (Fig. 3). A CT-guided biopsy was performed, and the histopathology of a specimen from a mass located left of the abdominal aorta was compatible with an extra-adrenal pheochromocytoma based on chromogranin A-immunopositive staining (Fig. 4). The patient was therefore diagnosed with malignant paraganglioma. Bone scintigraphy revealed no bone metastasis (data not shown). Combination cytotoxic chemotherapy with cyclophosphamide, vincristine, and dacarbazine, ^{131}I MIBG treatment, or both, were considered as treatment for the patient because of the tumor location and number. The patient declined these treatments, however, and opted to undergo only symptomatic treatment. A combination of doxazosin and atenolol was administered to prevent a pheochromocytoma crisis. Genetic counseling was provided for the patient.

Table 1 Biochemical test results

Biochemical test	Result	Reference range
Serum catecholamines		
Adrenalin (ng/ml)	<0.01	<0.17
Noradrenalin (ng/ml)	0.83	0.15–0.57
Dopamine (ng/ml)	12.0	<0.03
Urine catecholamines		
Adrenalin (mg/day)	10.3	1–23
Noradrenalin (mg/day)	341	29–120
Dopamine (mg/day)	59000	100–1000
Metanephrine (mg/day)	0.08	0.05–0.2
Normetanephrine (mg/day)	0.52	0.10–0.28

Methods

Genetic analysis

Informed consent for subsequent genetic analysis was obtained from the patient. Genomic DNA from the patient's peripheral blood was extracted using a DNA Blood Kit (Qiagen, Hilden, Germany). Genomic DNA from the removed tumor was extracted using a DNA Mini Kit (Qiagen). All eight exons of the *SDHB* gene were amplified by polymerase chain reaction (PCR), using the primers shown in Table 2. PCR was performed in a final volume of 25 µl containing 10 mM Tris–HCl(pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 nM of each dNTP, 1 mM of each primer, 100 ng genomic DNA, and 5 units of LA Taq polymerase (Takara, Shiga, Japan). Amplifications were performed for 30 cycles as follows: 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The PCR products were purified using Montage PCR Centrifugal Filter Devices (Millipore, Bellerica, MA) and subsequently sequenced. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The *RET*, *VHL*, and *SDHD* genes were also analyzed as described above.

Restriction fragment length polymorphism (RFLP) was performed by *Dde*I digestion of PCR products of exon 2 from blood DNA obtained from both the patients and a normal control as a template. The digested products were electrophoresed on a 1.5% agarose gel.

Results

Genomic DNA from the patient's peripheral blood showed a heterozygous nonsense mutation of CGA > TGA (R27X) in the *SDHB* gene (Fig. 5a). The mutation was confirmed by RFLP using the restriction enzyme *Dde*I (Fig. 5b). The

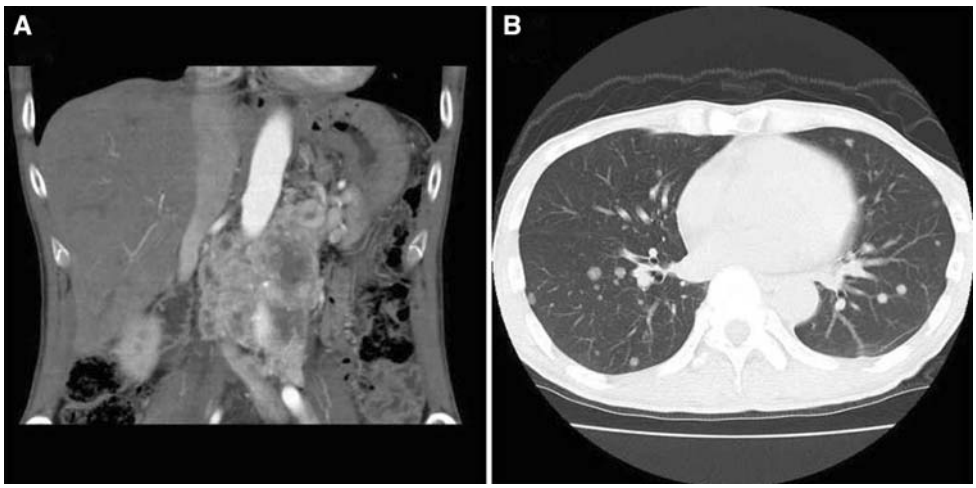

Fig. 1 Abdominal (**a**) and thoracic (**b**) CT. Abdominal CT showed irregularly-shaped large masses around the abdominal aorta. Chest CT shows multiple round masses in both lungs

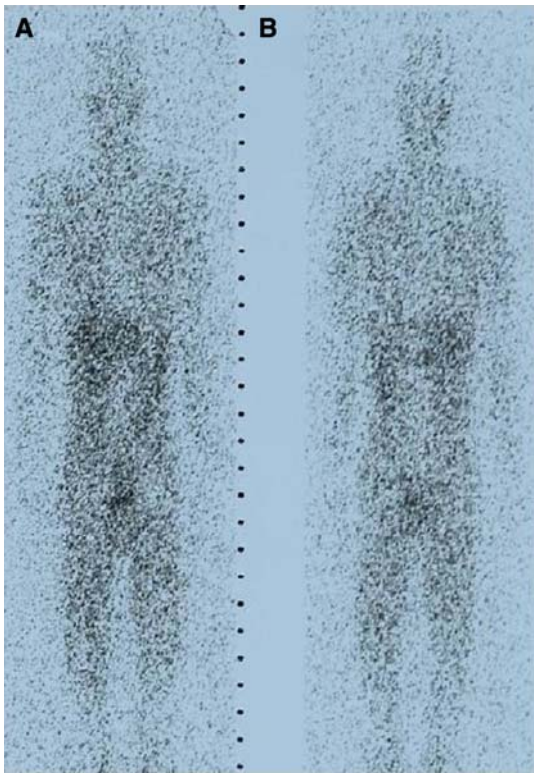

Fig. 2 ^{131}I-Meta iodobenzylguanidine (^{131}I-MIBG) scintigraphy. No remarkable uptake was observed in either the anterior (**a**) or posterior (**b**) view in MIBG scintigraphy

284-bp PCR fragment of exon 2 from the peripheral blood of the normal subject was not digested by the restriction enzyme *Dde*I, which recognizes the mutated sequence. On the other hand, the PCR fragment of exon 2 from the patient was partially digested by *Dde*I into 186-bp and 98-bp fragments, indicating a germline mutation in the patient in one allele of the *SDHB* gene. No mutation was observed in *RET*, *VHL*, and *SDHD* genes.

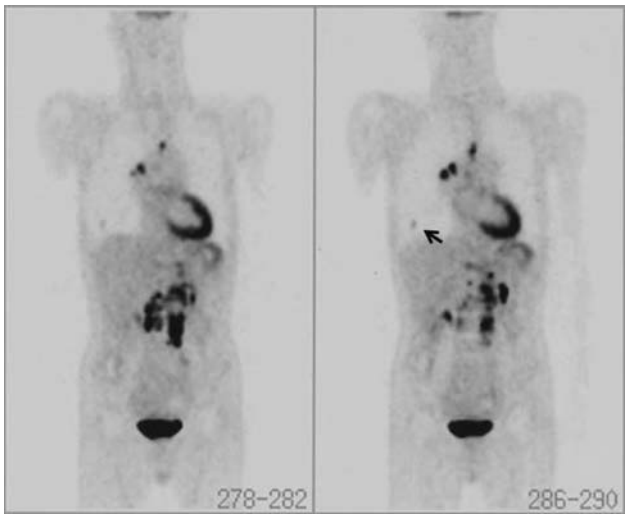

Fig. 3 6-[^{18}F]Fluorodopamine positron emission tomographic scanning (FDG-PET). Multiple areas of abnormal uptake were observed in the abdomen and thorax. *Arrow* indicates abnormal uptake in the lung

Discussion

A higher than expected frequency of genetic mutations in apparently sporadic pheochromocytomas was first reported by Neumann et al. [12]. Approximately 25% of patients with apparently sporadic pheochromocytomas carry a mutation of the *VHL*, *RET*, *SDHB*, or *SDHD* genes. Genetic testing in two other studies revealed mutations in 12.7% and 11.6% of cases with apparently sporadic presentation [16, 17]. Therefore, genetic involvement should be considered for all patients with pheochromocytomas and paragangliomas [18]. Because our patient had no known family history of pheochromocytomas or paragangliomas, we speculated that this was a case of sporadic paraganglioma. Six genes are reported to be involved in the development of pheochromocytoma: *NF1*, *RET*, *VHL*, *SDHB*, *SDHD*, and *SDHC*.

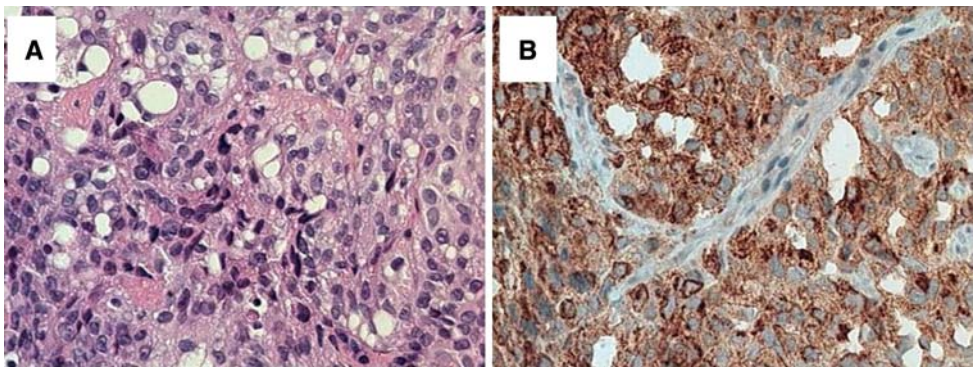

Fig. 4 Microscopic findings of the abdominal tumor biopsy. HE stain revealed a polygonal tumor with sharply defined cell borders. Tumor cells showed moderate nuclear enlargement and hyperchromasia (**a**). Most of the tumor cells were immunopositive for chromogranin A (**b**)

Table 2 Primers used for PCR and sequencing

	Forward primer (5′–3′)	Reverse primer (5′–3′)
Exon 1	GATGTTCGACGGGACACCGGCGGAG	CTCCGCAGCCCCATCAGCTCCAGGC
Exon 2	AATCCAGCGTTACATCTGTTGTGCC	AAGCATGTCCCTAAATCAAATCAAG
Exon 3	CTCCGATTATATTATGATAAAGTGT	CCAGCCCAAGCCTCTTTGGAAGACC
Exon 4	GAAGAAAGTATTTGGGGCAGGACTG	AAACTAATAGCGTAACACACATAGC
Exon 5	AAAGCTGAGGTGATGATGGAATCTG	CCACACTCCTGGCAATCATCTTTGC
Exon 6	AAAGGTAACATTTAATCAGCTGAAG	TTACAGCAATCTATTGTCCTCTTGG
Exon 7	TCTGCACTCCCAGAGCTTTGAGTTG	CTGCCAATCACCTCTTTGTGAGCAC
Exon 8	CCCAAGATTGTGGGTTTTCCCTTTC	CATTCCCTGCGGCAAGTAAAGGAAC

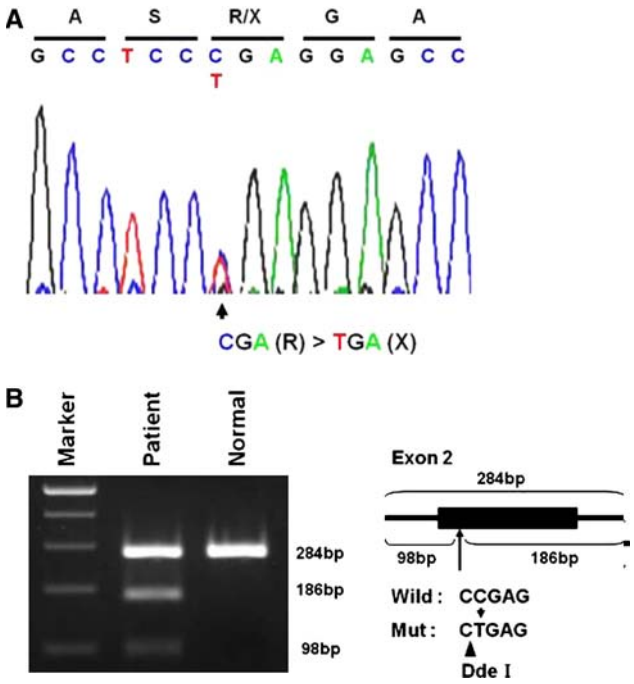

Fig. 5 Genetic analysis of the patient. Nucleotide sequence around codon 27 of the *SDHB* gene (**a**). RFLP of exon 2 of the *SDHB* gene (**b**)

Medical and family histories along with physical examination will help to pinpoint the features of the genetic syndrome. The typical *SDHB* mutation-associated paraganglioma originates from extra-adrenal abdominal or thoracic locations and carries a significant risk of malignant disease, as in the present case [19–22].

We identified an R27X nonsense mutation in the present case. The R27X mutation was previously reported in two families; one case from Finland that had cardiac paraganglioma and early-onset renal cell carcinoma [12, 15], and another case from Germany that carried the nonsense mutation [20]. Thus, both cases of the R27X mutation were reported in Europe. The reason for the emergence of this mutation in Japan, an apparently ethnically divergent population, is unclear, because our patient does not have any relatives in Europe.

Recently, IVS3 + 1G > A and R46Q mutations in *SDHB* were reported in Japan [23, 24]. The present case is the third case of paraganglioma with an *SDHB* mutation reported in Japan. All three of these cases presented with the malignant form of abdominal paraganglioma.

Genotype–phenotype correlations among patients with *SDHB* mutations in different exons did not exhibit obvious differences [14]. Neumann et al. reported that in a study of 32 patients with *SDHB* mutation, 11 (34%) patients had malignant pheochromocytomas or paragangliomas [12, 25]. Brouweres et al. reported that in a study of 44 patients with malignant paragangliomas, 13 (30%) patients had *SDHB* mutations; metastasis originated from an adrenal primary tumor in approximately one-third of the patients and from an extra-adrenal tumor in two-thirds of the patients [14, 26].

Some paragangliomas with *SDHB* mutations present with a high dopamine level [14]. The present case had a markedly elevated serum and urine dopamine levels.

Genetic testing should be offered to first-degree relatives of patients with *SDHB* mutations [16, 27]. Clinical evaluation and testing of family members revealed that 29% of *SDHB* carriers are diagnosed by 30 years of age and 45% of *SDHB* carriers are diagnosed by 40 years of age [27]. Penetrance data suggest that screening commencing at 10 years of age would lead to detection of the disease in approximately 96% patients with an *SDHB* mutation [27]. The present patient has two sons under the age of 20. Genetic testing for the sons was not performed because the patient refused to provide consent for the testing. The clinical significance of genetic testing of the family members, as well as the long-term follow-up of the asymptomatic carriers is not yet established.

Acknowledgments We are grateful to Ms. Matsuda for technical assistance in genetic testing. This work is a part of the Clinical Collaborating Research of National Hospital Organization.

References

1. W.F. Young Jr., Management approaches to adrenal incidentalomas. A view from Rochester, Minnesota. Endocrinol. Metab. Clin. North Am. **29**, 159–185 (2000)
2. C. Eng, Seminars in medicine of the Beth Israel Hospital, Boston. The RET proto-oncogene in multiple endocrine neoplasia type 2 and Hirschsprung's disease. N. Engl. J. Med. **335**, 943–951 (1996)
3. H.P. Neumann, D.P. Berger, G. Sigmund, U. Blum, D. Schmidt, R.J. Parmer, B. Volk, G. Kirste, Pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel–Lindau disease. N. Engl. J. Med. **329**, 1531–1538 (1993)
4. M.M. Walther, R. Reiter, H.R. Keiser, P.L. Choyke, D. Venzon, K. Hurley, J.R. Gnarra, J.C. Reynolds, G.M. Glenn, B. Zbar, W.M. Linehan, Clinical and genetic characterization of pheochromocytoma in von Hippel–Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. J. Urol. **162**, 659–664 (1999)
5. H.P. Neumann, C. Eng, L.M. Mulligan, D. Glavac, I. Zauner, B.A. Ponder, P.A. Crossey, E.R. Maher, H. Brauch, Consequences of direct genetic testing for germline mutations in the clinical management of families with multiple endocrine neoplasia, type II. JAMA **274**, 1149–1151 (1995)
6. M.M. Ritter, A. Frilling, P.A. Crossey, W. Hoppner, E.R. Maher, L. Mulligan, B.A. Ponder, D. Engelhardt, Isolated familial pheochromocytoma as a variant of von Hippel–Lindau disease. J. Clin. Endocrinol. Metab. **81**, 1035–1037 (1996)
7. M.M. Walther, J. Herring, E. Enquist, H.R. Keiser, W.M. Linehan, von Recklinghausen's disease and pheochromocytomas. J. Urol. **162**, 1582–1586 (1999)
8. M.E. Zoller, B. Rembeck, A. Oden, M. Samuelsson, L. Angervall, Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. Cancer **79**, 2125–2131 (1997)
9. D. Astuti, N. Hart-Holden, F. Latif, F. Lalloo, G.C. Black, C. Lim, A. Moran, A.B. Grossman, S.V. Hodgson, A. Freemont, R. Ramsden, C. Eng, D.G. Evans, E.R. Maher, Genetic analysis of mitochondrial complex II subunits SDHD, SDHB and SDHC in paraganglioma and phaeochromocytoma susceptibility. Clin. Endocrinol. (Oxf.) **59**, 728–733 (2003)
10. D. Astuti, F. Latif, A. Dallol, P.L. Dahia, F. Douglas, E. George, F. Skoldberg, E.S. Husebye, C. Eng, E.R. Maher, Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am. J. Hum. Genet. **69**, 49–54 (2001)
11. B.E. Baysal, R.E. Ferrell, J.E. Willett-Brozick, E.C. Lawrence, D. Myssiorek, A. Bosch, A. van der Mey, P.E. Taschner, W.S. Rubinstein, E.N. Myers, C.W. Richard 3rd, C.J. Cornelisse, P. Devilee, B. Devlin, Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science **287**, 848–851 (2000)
12. H.P. Neumann, B. Bausch, S.R. McWhinney, B.U. Bender, O. Gimm, G. Franke, J. Schipper, J. Klisch, C. Altehoefer, K. Zerres, A. Januszewicz, C. Eng, W.M. Smith, R. Munk, T. Manz, S. Glaesker, T.W. Apel, M. Treier, M. Reineke, M.K. Walz, C. Hoang-Vu, M. Brauckhoff, A. Klein-Franke, P. Klose, H. Schmidt, M. Maier-Woelfle, M. Peczkowska, C. Szmigielski, C. Eng, Germ-line mutations in nonsyndromic pheochromocytoma. N. Engl. J. Med. **346**, 1459–1466 (2002)
13. F.M. Brouwers, G. Eisenhofer, J.J. Tao, J.A. Kant, K.T. Adams, W.M. Linehan, K. Pacak, High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J. Clin. Endocrinol. Metab. **91**, 4505–4509 (2006)
14. H.J. Timmers, A. Kozupa, G. Eisenhofer, M. Raygada, K.T. Adams, D. Solis, J.W. Lenders, K. Pacak, Clinical presentations, biochemical phenotypes, and genotype–phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. J. Clin. Endocrinol. Metab. **92**, 779–786 (2007)
15. S. Vanharanta, M. Buchta, S.R. McWhinney, S.K. Virta, M. Peczkowska, C.D. Morrison, R. Lehtonen, A. Januszewicz, H. Jarvinen, M. Juhola, J.P. Mecklin, E. Pukkala, R. Herva, M. Kiuru, N.N. Nupponen, L.A. Aaltonen, H.P. Neumann, C. Eng, Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. Am. J. Hum. Genet. **74**, 153–159 (2004)
16. L. Amar, J. Bertherat, E. Baudin, C. Ajzenberg, B. Bressac-de Paillerets, O. Chabre, B. Chamontin, B. Delemer, S. Giraud, A. Murat, P. Niccoli-Sire, S. Richard, V. Rohmer, J.L. Sadoul, L. Strompf, M. Schlumberger, X. Bertagna, P.F. Plouin, X. Jeunemaitre, A.P. Gimenez-Roqueplo, Genetic testing in pheochromocytoma or functional paraganglioma. J. Clin. Oncol. **23**, 8812–8818 (2005)
17. A.P. Gimenez-Roqueplo, H. Lehnert, M. Mannelli, H. Neumann, G. Opocher, E.R. Maher, P.F. Plouin, Phaeochromocytoma, new genes and screening strategies. Clin. Endocrinol. (Oxf.) **65**, 699–705 (2006)
18. W.F. Young Jr., A.L. Abboud, Editorial: paraganglioma—all in the family. J. Clin. Endocrinol. Metab. **91**, 790–792 (2006)
19. D.E. Benn, A.L. Richardson, D.J. Marsh, B.G. Robinson, Genetic testing in pheochromocytoma- and paraganglioma-associated syndromes. Ann. N. Y. Acad. Sci. **1073**, 104–111 (2006)
20. H.P. Neumann, C. Pawlu, M. Peczkowska, B. Bausch, S.R. McWhinney, M. Muresan, M. Buchta, G. Franke, J. Klisch, T.A. Bley, S. Hoegerle, C.C. Boedeker, G. Opocher, J. Schipper, A. Januszewicz, C. Eng, Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA **292**, 943–951 (2004)
21. T.H. Pham, C. Moir, G.B. Thompson, A.E. Zarroug, C.E. Hamner, D. Farley, J. van Heerden, A.N. Lteif, W.F. Young Jr., Pheochromocytoma and paraganglioma in children: a review of medical and surgical management at a tertiary care center. Pediatrics **118**, 1109–1117 (2006)

22. A.L. Young, B.E. Baysal, A. Deb, W.F. Young Jr., Familial malignant catecholamine-secreting paraganglioma with prolonged survival associated with mutation in the succinate dehydrogenase B gene. J. Clin. Endocrinol. Metab. **87**, 4101–4105 (2002)
23. K. Isobe, S. Minowada, I. Tatsuno, K. Suzukawa, S. Nissato, T. Nanmoku, H. Hara, T. Yashiro, Y. Kawakami, K. Takekoshi, Novel germline mutations in the SDHB and SDHD genes in Japanese pheochromocytomas. Horm. Res. **68**, 68–71 (2007)
24. K. Takekoshi, K. Isobe, H. Suzuki, S. Nissato, Y. Kawakami, K. Kawai, N. Yamada, R46Q mutation in the succinate dehydrogenase B gene (SDHB) in a Japanese family with both abdominal and thoracic paraganglioma following metastasis. Endocr. J. **55**, 299–303 (2008)
25. A. Karagiannis, D.P. Mikhailidis, V.G. Athyros, F. Harsoulis, Pheochromocytoma: an update on genetics and management. Endocr. Relat. Cancer **14**, 935–956 (2007)
26. K. Gangopadhyay, V. Baskar, A. Toogood, A case of exclusive dopamine-secreting paraganglioma. Clin. Endocrinol. (Oxf.) **68**, 494–495 (2008)
27. D.E. Benn, A.P. Gimenez-Roqueplo, J.R. Reilly, J. Bertherat, J. Burgess, K. Byth, M. Croxson, P.L. Dahia, M. Elston, O. Gimm, D. Henley, P. Herman, V. Murday, P. Niccoli-Sire, J.L. Pasieka, V. Rohmer, K. Tucker, X. Jeunemaitre, D.J. Marsh, P.F. Plouin, B.G. Robinson, Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J. Clin. Endocrinol. Metab. **91**, 827–836 (2006)