New genetic causes of pheochromocytoma: current concepts and the clinical relevance

Hartmut P.H. Neumann,1 Markus Cybulla,1 Hirotaka Shibata,2 Mototsugu Oya,3 Mitsuhide Naruse,4 Eiji Higashihara,5 Toshiro Terachi,6 Hao Ling,1,7 Hiroshi Takami,8 Taro Shuin9 and Masaru Murai3

1Department of Medicine, Albert-Ludwigs-University, Freiburg, Germany, 2Department of Endocrinology, Health Center and Department of Internal Medicine, 3Department of Urology, School of Medicine, Keio University Tokyo, 4Department of Endocrinology, Kyoto Medical Center, Kyoto, 5Department of Urology, Kyorin University, Tokyo, 6Department of Urology, Tokai University, Kanagawa, Japan, 7Department of Medicine Tongji Hospital, Tongji University, Shanghai, China, 8Department of Surgery, Teikyo University, Tokyo 9Department of Urology, Kochi Medical School, Kochi, Japan

(Received for publication on September 1, 2004) (Revised for publication on December 7, 2004) (Accepted for publication on December 16, 2004)

Abstract. Pheochromocytoma and paraganglioma are tumors of the autonomous nervous system mainly occurring in the adrenal medulla, but also in the extraadrenal paraganglias of the abdomen, thorax, neck and skull basis. The etiology comprises germline mutations of now 6 genes. About 10 years known are the RET gene susceptible for multiple endocrine neoplasia type 2, the VHL gene for von Hippel-Lindau Disease, and the NF 1 gene for neurofibromatosis Recklinghausen (neurofibromatosis type 1). Since 2000 the genes for succinatedehydrogenase subunits SDHB, SDHC, and SDHD have been identified for paraganglioma syndromes type 4, type 3, and type 1 respectively. Investigations of series of pheochromocytoma patients identified germline mutations in one of the genes SDHB, SDHD, VHL and RET in 24% to 50% of the patients. Multifocal tumors, young age and positive family history, known features associated with inheritence, have not been present in all patients. Therefore, analyses of blood DNA for mutations in these genes are recommended. Positive tests provide the patients and their relatives with essential platforms for clinical care. Experiences in this field of medicine have shown that optimal management of patients with pheochromocytoma-associated syndromes is a high challenge. National registries may be instrumental in order to provide with adequate facilities. (Keio J Med 54 (1): 15–21, March 2005)

Key words: pheochromocytoma, paraganglioma, paraganglioma syndromes, genes of succinatedehydrogenase subunits B, genes of succinatedehydrogenase subunits D

Introduction

Pheochromocytoma and paragangliomas are the terms for tumors of the autonomous nervous system. We use the term pheochromocytoma for hormonally active tumors mainly located in the adrenals, but sometimes in paraganglias of the retroperitoneum or thorax. In contrast, paraganglioma is used for non secreting tumors, mostly located in paraganglias of the neck and skull basis.1,2

A Japanese study at 2000 revealed that high rates of hypertension (17%), mortality rate (15%) and recurrence (6%) are associated with malignancy.3 These features are worth to consider reevaluation of patients with pheochromocytoma under the aspect of risk factors, such as underlying inheritance, germline mutations of susceptibility genes, and associated disorders.

Syndromes Associated with Pheochromocytoma

First, thyroid C cell tumors and pheochromocytoma form multiple endocrine neoplasia type 2 (MEN 2), a
disorder based on germline mutations of the RET gene (Fig. 1). The mutations predisposing to pheochromocytoma are located in exons 10, 11 and 16. In Japan, a large series comprising 48 kindreds with MEN 2 revealed a sensitivity of 97% detection rate of the germline mutations.

Second, pheochromocytoma is a component of von Hippel-Lindau disease (VHL) together with hemangioblastomas of the eyes, the cerebellum, the brain stem and the spinal cord, as well as tumors and/or cysts of the kidneys and the pancreas (Fig. 2). In the kidneys these tumors are clear cell carcinomas and in the pancreas islet cell tumors. Some VHL patients have only pheochromocytoma. Pathogenetically relevant are mutations of the VHL gene. More than 300 different VHL mutations have been described, and those associated with pheochromocytoma are nearly all of missense type. Experiences from Japan including four VHL patients

Fig. 1  Multiple endocrine neoplasia type 2 in a 44 year old female patient. Multifocal (bilateral) medullary thyroid carcinoma shown by MIBG scintigraphy and operation specimen (arrows; arrow heads indicate the tissue bridge). Bilateral adrenal pheochromocytoma shown by MIBG, CT, and operation specimens (arrows indicate the tumors).

Bilateral adrenal pheochromocytoma and bilateral, multifocal, in part cystic renal cell carcinoma in a 36 year old male patient with von Hippel-Lindau disease.

Fig. 2  Bilateral adrenal pheochromocytoma and bilateral Renal Carcinomas and Cysts
with pheochromocytoma have been reported in 1995. In addition, in 2002 77 families, including 15 with pheochromocytomas have been moleculargenetically analyzed. The overall detection rate was 73%, but in families with pheochromocytoma even 93%. Third, pheochromocytoma is associated with von Recklinghausen’s neurofibromatosis in about 1–5% of such patients (Fig. 3). The susceptibility gene is NF1. Nowadays well recognized are patients with paraganglioma syndrome (PGL) (Fig. 4). The term was created on the observation of families with several patients developing tumors of paraganglias of the neck and skull basis. In 2000 the link of such families with a gene localized in the chromosomal area on chromosome 11q23, the SDHD gene, was elucidated. The gene encodes a subunit (subunit D) of succinate dehydrogenase (SDH). The enzyme plays a key role in two elementary biochemical pathways, the respiratory chain and the tricarboxylic acid – or Krebs cycle. SDH consist of subunits A–D, and SDHD was first identified to be altered in PGL. This is now named PGL1. Subsequently we showed that non MEN 2 – non VHL – non NF 1 pheochromocytoma patients could be identified as SDHD mutation carriers. Soon later mutations in the SDHB gene (SDH subunit B) have been described in patients with paragangliomas and in such with pheochromocytoma. Finally, in a small subset of paraganglioma patients mutations in the SDHC gene have been described. Now we distinguish 4 types of PGL, namely type 1 (associated with mutations of SDHD), type 4 (SDHB), type 3 (SDHC) and type 2 (gene unknown). In contrast to MEN 2 and VHL, families with SDHx (B, C and D) mutations are increasingly recognized but still rare. Therefore, the following data must be handled carefully.

Among all pheochromocytomas more than 25% can be attributed to one of the 6 syndromes (MEN 2, VHL, NF 1, PGL 1, 3, and 4) based on mutations of RET, VHL, SDHB, SDHC and SDHD or clinically to NF 1. We showed this for the genes RET, VHL, SDHB and SDHD in 271 non related index cases with pheochromocytomas. Not included have been the NF 1 cases. Further, not included have been the relatives of index cases, a considerable number depending by size of the

Fig. 3 Bilateral adrenal pheochromocytoma shown by MRI in coronal and axial projection in a patient with neurofibromatosis type 1.
families and the area where they live. We showed that most hereditary pheochromocytoma patients have VHL mutations followed by RET, SDHD and SDHB. Meanwhile a French series showed a frequency of 50% of germline mutations in an unselected series of pheochromocytoma patients which also included a priori familial cases.19

**Major Clinical Features of Presentation of Pheochromocytoma**

Age at presentation is much younger in hereditary than in sporadic pheochromocytoma, with an average of nearly 20 years difference. Mean age seems to be in VHL the lowest followed by PGL type 1 and 4, MEN 2 and NF 1. However, patients with germline mutations of one of the discussed genes may become symptomatic after the age of 60.21

**Location**

The adrenals are the preferent location for all hereditary pheochromocytomas. Nearly exclusively affected, however, are the adrenals in MEN 2.

Extraadrenal paragangliomas are found in about 50% of the PGL 4 patients followed by 45% in those with PGL 1 and 22% with VHL. Thus, extraadrenal location is statistically an attitude of heredity.21

The thorax is a unique area of location of pheochromocytoma. Only 1% of pheochromocytomas are located here. Most thoracic tumors (30%) are associated with mutations of SDHD followed by VHL (3%).

Paragangliomas of the neck and skull basis are characteristics of carriers of SDHx mutations. Only some very single cases have been reported in VHL and also in MEN 2.22–25 Clear differences for affected paraganglia in PGL 1 vs PGL 3 or PGL 4 are unknown.

Multifocal pheochromocytoma is a difficult term, since tumors may be detected simultaneously or arise as de novo lesions with various time intervals up to decades. Multifocal location, a striking feature for heredity is absent in many cases associated with any syndrome. Multifocal pheochromocytoma occur most often in MEN 2 followed by VHL, PGL 1 and PGL 4.21

Malignancy has been regarded as a typical biological behaviour of sporadic pheochromocytoma. About 5–10% of sporadic pheochromocytomas are malignant. Recently, it was recognized that a considerable percentage of carriers of SDHx mutations have malignant tumors26 whereas this is very seldom in VHL, exceptional in MEN 2 and not reported in PGL 1.

The knowledge about old and new cases of pheochromocytoma must be discussed in 4 major aspects.

1) How to do genetic analyses?
2) How to diagnose clinically pheochromocytoma/paraganglioma?
3) How to treat patients with pheochromocytomas and/or paragangliomas?
4) How to care for the patient and his family?

Informations regarding these complex features are given in Tables 1, 2, 3.

**Molecular genetic Analyses**

In a given patient, mutations occur only in one pheochromocytoma susceptibility gene. Therefore, the most likely mutated gene should be analysed first. Key informations can be drawn from family history regarding occurrence of pheochromocytoma and/or paraganglioma and other rare tumors or lesions typical for one of the syndromes. If these are unknown, it would be wise to do tests for germline mutations nevertheless for several reasons. 1st: New mutations are well documented in patients with pheochromocytoma for VHL or RET. 2nd: In VHL, mutation carriers may only develop pheochromocytomas. 3rd: The parental generation can carry the mutation, but may not express the disease. This is possible according to three phenomenons. One is that tumors may be asymptomatic lifelong.
The second feature is reduced penetrance, e.g. some carriers do not develop tumors at all. As far as we know, penetrance is high in VHL and MEN 2, but not 100%, whereas such figures are unknown for the PGLs. The third mechanism was found in carriers of SDHD mutations. These develop only tumors if the mutation is transmitted from the father. The term for this mechanism is maternal imprinting, and it explains why the disease can jump over generations.27

Based on the presentation of the tumors it can be recommended to start for adrenal tumors with RET and VHL followed by SDHD and SDHB. For extra-adrenal tumors RET can be postponed. For neck paraganglioma one should analyze SDHD and SDHB; SDHC may be added. In malignant tumors SDHB and VHL should have preference.

Table 1 Classification of Pheochromocytoma

<table>
<thead>
<tr>
<th>Gene</th>
<th>VHL</th>
<th>MEN 2 Multiple Endocrine Neoplasia Type 2</th>
<th>NF 1 Neurofibromatosis Recklinghausen (Type 1)</th>
<th>PGL 1</th>
<th>PGL 3</th>
<th>PGL 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal Locus</td>
<td>3p25–26</td>
<td>10q11.2</td>
<td>17q11</td>
<td>11q23</td>
<td>1q21–23</td>
<td>1p23–25</td>
</tr>
<tr>
<td>Exons</td>
<td>3</td>
<td>21</td>
<td>59</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Hot Spots</td>
<td>no</td>
<td>yes</td>
<td>unknown</td>
<td>no</td>
<td>no</td>
<td>unknown</td>
</tr>
<tr>
<td>Typical Tumor Location</td>
<td>Adrenal and extraadrenal, abdominal, seldom thoracic</td>
<td>Adrenal and extraadrenal abdominal</td>
<td>Adrenal, extraadrenal, abdominal, thoracic</td>
<td>Head and neck paranglioma</td>
<td>Adrenal, extraadrenal, abdominal, thoracic</td>
<td></td>
</tr>
<tr>
<td>Associated Lesions</td>
<td>CNS-Hemangioblastoma, Retinal Angioma, Renal Cell Cancer, Pancreatic Cysts, Islet Cell tumors, Endolymphatic Sac Tumors, Epididymal Cystadenoma</td>
<td>Medullary Thyroid Carcinoma, Hyperparathyroidism, Ganglieneuroma</td>
<td>Neurofibromas, Pigmented Spots, “Lisch-Nodes” of the Iris Nodules</td>
<td>no</td>
<td>no</td>
<td>Head and Neck Paraganglioma Renal Cell Carcinoma (seldom)</td>
</tr>
</tbody>
</table>

Table 2 When to consider Pheochromocytoma associated Syndromes?*

- Multifocal pheochromocytoma/paraganglioma
- Extraadrenal pheochromocytoma/paraganglioma
- Young age
- Family History for pheochromocytoma/paraganglioma
- Other tumors in the patient of brain, spinal cord, eyes, ears, thyroid gland, neck, parathyroid gland, kidney, pancreas, epididymis, skin
- Relatives with tumors of brain, spinal cord, eyes, ears, thyroid gland, neck, parathyroid gland, kidney, pancreas, epididymis, skin

* All patients should be investigated molecularly-genetically.

The second feature is reduced penetrance, e.g. some carriers do not develop tumors at all. As far as we know, penetrance is high in VHL and MEN 2, but not 100%, whereas such figures are unknown for the PGLs. The third mechanism was found in carriers of SDHD mutations. These develop only tumors if the mutation is transmitted from the father. The term for this mechanism is maternal imprinting, and it explains why the disease can jump over generations.27

Based on the presentation of the tumors it can be recommended to start for adrenal tumors with RET and VHL followed by SDHD and SDHB. For extra-adrenal tumors RET can be postponed. For neck paraganglioma one should analyze SDHD and SDHB; SDHC may be added. In malignant tumors SDHB and VHL should have preference.

Table 3 Clinical Investigation Programs for Pheochromocytoma associated Syndroms

When to consider Pheochromocytoma associated Syndromes?*

- Multifocal pheochromocytoma/paraganglioma
- Extraadrenal pheochromocytoma/paraganglioma
- Young age
- Family History for pheochromocytoma/paraganglioma
- Other tumors in the patient of brain, spinal cord, eyes, ears, thyroid gland, neck, parathyroid gland, kidney, pancreas, epididymis, skin
- Relatives with tumors of brain, spinal cord, eyes, ears, thyroid gland, neck, parathyroid gland, kidney, pancreas, epididymis, skin

* All patients should be investigated molecularly-genetically.

The second feature is reduced penetrance, e.g. some carriers do not develop tumors at all. As far as we know, penetrance is high in VHL and MEN 2, but not 100%, whereas such figures are unknown for the PGLs. The third mechanism was found in carriers of SDHD mutations. These develop only tumors if the mutation is transmitted from the father. The term for this mechanism is maternal imprinting, and it explains why the disease can jump over generations.27

Based on the presentation of the tumors it can be recommended to start for adrenal tumors with RET and VHL followed by SDHD and SDHB. For extra-adrenal tumors RET can be postponed. For neck paraganglioma one should analyze SDHD and SDHB; SDHC may be added. In malignant tumors SDHB and VHL should have preference.
Clinical Diagnosis

Diagnosis and documentation of the tumors should be optimized preoperatively. Biochemistry and radiology are equally important for the diagnosis of pheochromocytoma and paragangliomas. Based on our personal experience and recent studies, documentation of the tumors should be done using MRI as the first line method. We recommend that the lesion(s) should be confirmed by nuclear medicine imaging. In this regard, MIBG scintigraphy is well introduced, but with limited sensitivity of about 80–85 percent. Nowadays, dopa positron emission tomography (PET) or dopamin-PET seems to be the method of choice (Fig. 5). The use of two imaging methods helps to avoid an overlook of additional tumors. In addition, dopamin or dopa PET enables imaging of all areas, including abdomen, thorax and neck, and by this method such tumors can be visualized within only 2–3 hours investigation time.

Preoperatively, the blood pressure must be normalized. International recommendations include use of alpha blockers and beta blockers. Possibly any drug can be used which achieves normotension. A documentation of preoperative blood pressure for 24 h is advised.

Treatment

Treatment of pheochromocytoma is a high challenge in all these syndromes. The surgical considerations must include that these lesions are mostly benign, frequently multifocal, sometimes relapsing, often small and occur in young age. Endoscopic surgery is the modern access. Extensive experience has been gathered thoughout Japan for adrenalectomy using transperitoneal and retroperitoneal accesses, resulting in low complication rates. If located in the adrenals, organ sparing surgery should be attempted. Organ-sparing procedures can also be performed endoscopically and revealed excellent results in experienced hands. This is a striking argument to establish the diagnosis of pheochromocytoma/paraganglioma associated syndromes preoperatively.

After Care

After-care depends on the specific syndrome which is diagnosed molecularly. An adjusted clinical program has to be performed soon after treatment of the pheochromocytoma especially for MEN 2 and VHL. Still an open question is, if and how often and how extensively the paraganglial system needs controls. The major question is to anticipate the time when a pheochromocytoma will settle metastases. Carriers of an SDHB mutation surely need regular controls in this regard.

In Japan, the yearly detection rate for pheochromocytomas is about 1000 cases. Nation-wide projects need to be well supported in order to establish large registries, in order to perform molecular genetic investigations according to the current knowledge, to provide with clinical background informations based on extensive experience, and to perform the needed regular controls in patients identified with an inherited, pheochromocytoma associated condition.

Conclusion

In conclusion, pheochromocytomas and paragangliomas are a high challenge for diagnosis clinically and molecularly, for optimal treatment, and for adequate aftercare which includes the patient and in mutation positive cases also the family. Experiences in Germany, France and Poland have shown that dedicated, at best national registries should be established for such purposes.

References

15. Gimm O, Armanios M, Dziema H, Neumann HP, Eng C: Some- 
14. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Mys- 
13. Kennedy DW, Nager GT: Glomus tumor and multiple endocrine 
12. Walther MM, Herring J, Enquist E, Keiser HR, Linehan WM: 
10. Yoshida M, Ashida S, Kondo K, Kobayashi K, Kanno H, Shi- 
9. Bournhill RA, Dinsmore RE, Gillies S, Hargreaves CD, Rad- 
8. Neumann HP, Bender B, Zauner I, Berger DP, Eng C, Brauch 
7. Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, 
6. Neumann HP: Basic criteria for clinical diagnosis and genetic 
5. Egawa S, Futami H, Takasaki K, Iihara M, Okamoto T, Kanbe 
3. Terachi T, Yoshida O, Matsuda T, Orikasa S, Chiba Y, Takaha- 
2. Higashihara E, Tanaka Y, Horie S, Aruga S, Nutahara K, Min- 
1. Baysal BE, Willett-Brozick JE, Filho PA, Lawrence EC, Myers 