Tatjana Isailović, Jovana Vignjević,
Milan Petakov, Bojana Popović,
Sanja Ognjanović, Đuro Macut,
Ivana Bozić, Svetozar Damjanović

GENETIC ANALYSIS OF PATIENTS WITH PHEOCHROMOCYTOMA

Case report: A 45-year old woman was admitted to the hospital because of a large adrenal tumour. The history of illness began in April 2004, with paroxysmal hypertension up to 200/110 mmHg, along with redness and warmth in the face, profuse sweating with occasional nausea and urge to vomit. These symptoms would appear spontaneously and would stop after couple of minutes, and after these episodes the patient felt exhausted. These attacks became more often up to several times a week. The patient said that she was suffering from sustained hypertension for 10 years, with average value of 150/90mmHg. She was periodically treated with antihypertensive (metoprolol, fosinopril, amlodipine). Ultrasound of the abdomen revealed an enlargement of the right adrenal gland (about 41.4mm) with two smaller cystic changes within it. CT scan confirmed the presence of heterogeneous tumour mass (56 x 64 cm) in the adrenal area with necrotic central zone. Due to the specific family situation, although the attacks were more often, the patient did not undergo any medical exams until January 2005. At that time control ultrasound showed further progression of tumour changes and increase in diameter of tumour to 75 x 65 mm. Then she was sent to the Institute of Endocrinology KCS. She didn't loose any weight. In the history of previous illness the patient was operated on follicular cyst of the right ovary in 2000. Her mother suffered from hypertension as well.

On physical examination: the patient was obese with a regular distribution of fat tissue. The weight was 89 kg, height 170 cm and body mass index was 31.0 kg/m¸. The skin was pale. Diffuse goitre was present, there were no palpable lymph nodes. The lungs and heart appeared normal. The blood pressure was 150/90 mm Hg, heart rate 88 per minute, there was no orthostatic hypotension. The abdomen was soft, no mass was palpable.

The laboratory exams revealed microcytic anaemia (normal level of reticulocytes, MCV 71.6/l), elevated fasting glucose (the mean blood glucose was 8.7mmol/l), diabetic curve was observed during OGTT (9.2, 12.9, 16.2, 10.9, 14.6 mmol/l. Normal results were obtained for the rest of the measurements. ***Tumour markers:*** Chromogranin A was elevated (359.4 ěg/ml n.v. do 98 ěg/ml); calcitonine, thyroglobulin, CEA, AFP and 5-HIAA were normal. ***Immune analysis*****:** anti Tg antibody and anti TPO antibody were negative. ***Hormonal analysis:*** highly elevated free urinary 24h catecholamines (norepinephrine and epinephrine) (NOR: 1503.56, 1884.44, 1704.97, 389.85, 98.64, 107.98ěg/24h; ADR: 12.8, 7.10, 11.62, 8.23, 6.20, 13.94 ěg/24h; DM: 221.45, 242.34, 231.67, 251.22, 152.17, 142.63 ěg/24h). Cortisol, 17 OH progesterone, testosterone, SHBG, sTSH, FT4 were normal. ***Chest X-ray*** appeared normal. ***Abdominal ultrasound:*** In the area of the right adrenal gland the tumour mass of 78 x 58 mm was observed. ***Abdominal CT scan:*** a round, heterogeneous tumour mass with a central necrotic zone was seen in the area of the right adrenal gland. The measure of the change were 5x6x10cm. Based on the elevated urinary 24h catecholamines the diagnosis of pheochromocytoma was made. The premedication was started with 40 mg/day of phenoxybenzamine and the patient was transferred to the surgical department where right adrenalectomy was preformed. ***Hystopatological finding*** confirmed the diagnosis: pheochromocytoma of adrenal gland. The tumour was well rounded, diameter was 75x65x30mm and weight 78g, encapsulated by a thick fibrous capsule with signs of local invasion.

Due to the hystopatological results new hospitalization of the patient was planned, when ąłąl-labeled meta-iodobenzylguanidine (MIBG) scintigraphy of the whole body would be done as well as measuring of free urinary 24h catecholamines. **Genetic analysis** of perifereal blood showed the presense of germline mutations in codon 46 (GTG® GTA, V46V) of the SDHD gene (succinate dehydrogenase D). (Picture 1.)

Discussion

Pheochromocytoma is a neuroendocrine, catecholamine-producing tumour of chromaffin cells. In 90% of the cases they are arising from adrenomedullary tissue and in about 10% of cases from extra-adrenal chromaffin tissue (paragangliomas). They are benign in 90% of the cases, although there is no reliable hystopatological examination that can distinguish malignant pheochromocytoma from benign. The features that can correlate with malignancy include degree of necrosis, nuclear pleomorphism, mitotic rate, capsular invasion, and vascular invasion. Only the presence of metastatic disease is an absolute indicator of malignancy. High concentration of dopamine in blood and urine can also be distinctive. (1)

There are two distinct subpopulations of chromaffin cells: adrenergic cells that contain phenylthanolamine N-methyltransferase (PNMT) and store predominantly epinephrine, and noradrenergic cells, that do not express PNMT and store mainly norepinephrine. PNMT converts norepinephrine to epinephrine. Most pheochromocytomas produce predominantly norepinephrine, many produce both norepinephrine and epinephrine, but these tumors rarely produce predominantly epinephrine. Epinephrine producing tumors arise mostly from adrenal gland, while paragangliomas produce norepinephrine only. Pheocromocytomas can also produce a variety of peptides: chromogranin A, endogenous opoids, erythropoetin, PTH-related peptide, neuropeptide Y etc. and they can be used as markers of the treatment efficiency.

Clinical signs and symptoms depend on the metabolic and hemodynamic actions of circulating catecholamines, as well as associated peptides. Hypertension, especially paroxysmal hypertension (50%), is the most common clinical sign, although a smaller number of patients can be normotensive (10%). Orthostatic hypotension is also frequent and is followed by tachycardia. Pallor is a typical sign of catecholamine excess in approximately 30% of patients, whereas redness and flushing is much less common. More than half of patients can be glucose intolerant, but this disorder does not need to be treated because the symptoms retire after the surgery. However, the classical triad of signs that should arouse suspicion for a pheochromocytoma includes headache, palpitations and sweating in hypertensive patients.

Pheochromocytoma can occur as a sporadic form or as a part of four familial cancer syndromes: 1) MEN 2a and MEN 2b, 2) von Hippel – Lindau syndrome, 3) neurofibromatosis type 1 and 4) familial pheochromocytoma. (4, 5) Patients with familial pheochromocytoma are usually younger at the time pheochromocytoma diagnosis is made, often presenting as a bilateral or multicentric tumour and they have lower risk of developing malignant tumor. (2, 3)

Multiple endocrine neoplasia 2a (MEN 2a) is an autosomal dominant cancer syndrome consisting of medullary thyroid carcinoma (MTC), hyperparathyroidism and pheochromocytoma. MEN 2b is a combination of MTC, pheochromocytoma, marfanoid habitus and multiple mucosal neuromas. Pheochromocytomas occur mostly between 30 and 40 years of age. About 50% of patients with MEN 2a or MEN 2b harbour pheochromocytoma, and it is bilateral in about 50% of the affected. In 25.1% of patients with MEN 2a, pheochromocytoma is a first manifestation of the disease. (7). All of the patients with MEN 2 syndrome have germ-line mutation of the RET-protooncogene, localised in chromosome 10q11.2. RET-protooncogene is expressed in cells derived from neural crest such as chromaffin and parafolicular C cells. Mutations in RET-protooncogene lead to ligand independent constitutive activity of the tyrosine kinase receptor. Most frequently mutations are found in exone 10 (codons 609, 611, 618, 620) and in exone 11 (codons 631 and 634). Codon 634 mutations occur in 80% of MEN 2a kindreds and are most commonly associated with pheochromocytoma. A single methionine to threonine substitution at codon 918 in exon 16 of the RET protooncogene is associated with the development of pheochromocytoma in MEN 2b syndrome.

Von Hippel – Lindau syndrome (vHL) is an autosomal dominant cancer syndrome. VHL consists of haemangioma of cranial axis, renal cysts, renal cell carcinoma, pancreatic neuroendocrine tumours and cysts, epidydimal cysts and pheochromocytoma. The mutations that cause the development of vHL syndrome are located in VHL gene at chromosome 3p26-25. VHL gene encodes vHL tumour-supressor protein, involved in tumorigenesis in many different ways (indirectly, by hypoxia inducible factor HIF 1 or directly, by disabling G0 phase of cell cycle, disrupting of angiogenesis or creating some irregularity in extracelular cell matrix). There are several types of vHL syndrome, but pheochromocytoma is a part of three types: Type 2a (pheochromocytoma, haemangioblastoma of retina and

CNS), 2b (pheochormocytoma, haemangioblastoma of retina and CNS, renal cell carcinoma, pancreatic neuroendocrine tumours and cysts) and 2c (pheochormocytoma). (8) About 10 – 20% of patients with vHL syndrome harbour pheochromcytoma, but the incidence depend on the type of mutation. Pheochromcytomas associated with vHL syndrome are bilateral rather than unilateral; the diagnosis in vHL patients is made at earlier age than in MEN 2 patients but they are more often presented as extra-adrenal tumours than in MEN 2 patients (12%). (3, 4) Pheochromocytomas associated with MEN 2 are adrenergic, whereas those in vHL syndrome are noradrenergic. Because of the lower expression of phenylethanolamine-*N*-methyltransferase (PNMT), these patients usually have elevated norepinephrine only.

Neurofibromatosis type 1 (NF1, Recklinghausen's disease) is an autosomal dominant disease, characterized by café-au-lait macules, neurofibromas, iris hamartomas (Lisch nodules). Tumor-supressor gene for NF1 is located at 17q11.2 chromosome and encodes protein called neurofibromin. The role of this protein is not elucidated yet. NF1 gene contains of 51 exons and because of many different types of mutations troughout this large gene, the identification of specific mutations is very difficult. Patients with NF1 have a greater risk of developing additional tumours such as pheochromocytomas and juvenile myeloid leukaemia. Pheochromocytoma is present at 0.1 – 5.7% of patients with NF1 (9)

Familial pheochormocytoma is characterized by mutations in subunit D of succinate dehydrogenase. Succinate dehydrogenase (SDH) is a mitochondrial enzyme that contains 4 subunit (SDHA, SDHB, SDHD, SDHD), forming a mitochondrial complex II, known as a succinate: ubiquinone oxidoreductase complex.

SDH partially contacts mitochondrial matrix and is insert in the internal part of mitochondrial membrane through SDHD and SDHC subunits. In that way it takes part in Crebs cicle and oxidative phophorylation. SDHD is a tumor-supressor gene consisting of 4 exons. Although the exact mechanism of tumorigenesis is not completely understood, it appears that loss of function of mitochondrial enzymes with consecutive elevation of free radicals is a crucial moment in this process. This situation is recognised by mitochondria as a state of hypoxia and it activates hypoxia inducible factor HIF, wich is followed by activation of antiapoptotic and proliferative genes (TGF, EGF, VEGF, PDGF). Patients with pheochromocytoma that carry germline mutation in SDHD gene in 75% of the cases have mutations at 5' end of gene in first two exons. Patients with paragangliomas have mutations at 3' end of gene in 3. and 4. exons. Disorders in these regions change the signaling peptide (presequence) and insertion of enzyme in mitochondrial membrane becomes impossible. As the consequence the mitochondrial complex is disassembled with the loss of its cathalitic activity. The types of mutations in SDHD gene are missense, nonsence, frameshift and silent mutations. (10)

Diagnosis od pheochromocytoma is made by characteristic clinical presentation, biochemical parameters (urinary 24h cathecholamines, chromogranin A), precise visualisation (abdominal CT scan or NMR) and ąłąI-labeled meta-iodobenzylguanidine (MIBG) scintigraphy of adrenal gland. MIBG scintigrafy of whole body is used for detection of metastatic disease.

The therapeutic treatment of pheochromocytoma is surgical excision of the tumour. But prior to surgery, patients with pheochromocytoma should always undergo pharmacological blockade of catecholamine effects. Phenoxybenzamine is an alpha adrenergic blocker that opposes catecholamine-induced vasoconstriction and it is used for premedicaton in dose of 40 –80 mg per day. Alpha adrenergic blockers are also used to stop attacks, along with small doses of beta –blockers to prevent reflex tachycardia. Beta blockade alone can be dangerous for patients with pheochromocytoma because it can augment effects of catecholamines at alpha adrenoreceptors.

Conclusion

Genetic screening for mutations of patients with pheochromocytoma should be mandatory and done routinely. It includes analyses of RET, VHL and SDH genes, considering association of pheochromocytoma with other malignancies as well as more frequent bilateral presence. These mutations can be detected in genetic laboratory of Institute for Endocrinology KCS. The type of mutation correlates with clinical manifestations especially in cases of MEN 2a syndrome. It can enable prophylactic therapy, as well as adequate following of patient and timely treatment of the illness.

We found germline heterozygotic mutation in codone 46 (GTG?GTA, V46V) at our patient. This substitute does not lead to aminoacid change because both codons encode the same one amino-acid, valin. Since there are no changes at the protein level we can not conclude that this is the mutation that led to pheochromocytoma. Further following of the patient and her family is neceassry in order to prevent development of the familial pheochromocytoma. Genetic screening of SDHD gene should be done routinely at patients with apparently sporadic pheochromocytoma.

Picture 1. DNA sequence od SDHD gene: germline heterozygotic mutation in codone 46 (GTG?GTA, V46V)

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Institute of Endocrinology, Belgrade, Serbia and Montenegro; Department for endocrine related tumours