PRECLINICAL DIAGNOSTICS OF VON HIPPEL-LINDAU SYNDROME IN A CHILD

© Oleg A. Malievskiy[1]*, Ramziya I. Malievskaya[1], Victor A. Malievskiy[1], Anatoliy N. Tulpakov[2,3]

[1]Bashkir State Medical University, Ufa, Russia
[2]Research Centre for Medical Genetics, Moscow, Russia
[3]Russian Children's Clinical Hospital, Moscow, Russia

The description of the child aged 5 months with the von Hippel-Lindau syndrome without any manifestations of this syndrome is presented. The reason for the molecular genetic examination was the presence of cases of this syndrome in the family (mother and sister). The heterozygous variant c.355T>C p.F119L was found in the *VHL* gene. An objective examination revealed no pathology. A comprehensive laboratory and instrumental examination aimed at searching for components of the von Hippel-Lindau syndrome, including a blood test for metanephrines and normetanephrines, ultrasound of the abdominal organs, examination of the fundus, also did not reveal any abnormalities. Given the results of molecular genetic diagnosis, the child remains under observation and will undergo regular examinations to identify components of the von Hippel-Lindau syndrome, including blood/urine tests for normetanephrines.

KEYWORDS: children; pheochromocytoma; von Hippel-Lindau syndrome.

ДОКЛИНИЧЕСКАЯ ДИАГНОСТИКА СИНДРОМА ФОН ХИППЕЛЯ-ЛИНДАУ У РЕБЕНКА ГРУДНОГО ВОЗРАСТА

© О.А. Малиевский[1]*, Р.И. Малиевская[1], В.А. Малиевский[1], А.Н. Тюльпаков[2,3]

[1]Башкирский государственный медицинский университет, Уфа, Россия
[2]Медико-генетический научный центр имени академика Н.П. Бочкова, Москва, Россия
[3]Российская детская клиническая больница, Москва, Россия

Представлено описание случая диагностики синдрома фон Хиппеля-Линдау у ребенка в возрасте пяти месяцев, не имеющего каких-либо проявлений данного заболевания. Поводом для молекулярно-генетического обследования стало наличие случаев данного синдрома в семье (мама и сестра). В гене *VHL* был выявлен гетерозиготный вариант c.355T>C p.F119L. При объективном обследовании со стороны внутренних органов патологии не выявлено. Комплексное лабораторно-инструментальное обследование, направленное на поиск компонентов синдрома фон Хиппеля-Линдау, в том числе анализ крови на метанефрины и норметанефрины, УЗИ органов брюшной полости, осмотр глазного дна, также не выявило каких-либо отклонений. Учитывая результаты молекулярно-генетической диагностики, ребенок остается под наблюдением и будет проходить регулярное обследование с целью выявления компонентов синдрома фон Хиппеля-Линдау, включая анализы крови/мочи на норметанефрины.

КЛЮЧЕВЫЕ СЛОВА: дети; феохромоцитома; синдром фон Хиппеля-Линдау.

RELEVANCE

Pheochromocytomas/paragangliomas (PCC/PGL) are neuroendocrine tumors that develop from the chromaffin cells of the adrenal medulla (80-90% of cases) and extra-adrenal localization, releasing catecholamines in excess amounts. In childhood, PCC/PGL is characterized by a predominance of extra-adrenal localization, bilateral damage to the adrenal glands in the case of adrenal localization of PCC, recurrent course and multifocal lesions [1, 2]. Also in childhood PCC/PGL is manifested by arterial hypertension in 64-93% of cases, headache in 39-95%, sweating in 90%, palpitations in 53%, signs and symptoms of a mass effect. In 30% of cases, the disease is detected in the form of accidental formation in the adrenal glands [3].

In contrast to the adult population, in childhood and young adulthood the proportion of monogenic diseases that cause the development of PCC/PGL is much higher (40 and 80%, respectively) [1, 2]. In children with PCC/PGL, mutations are most often observed in genes associated with the activation of hypoxia-induced signalling, in particular in the von Hippel-Lindau (VHL) gene, and in genes encoding one of the succinate dehydrogenase (SDH) subunits. Von Hippel-Lindau syndrome (VHL syndrome) in children is the most common genetic cause of PCC/PGL [4]. VHL syndrome develops due to an inactivating germinal mutation in the *VHL* suppressor gene on chromosome 3p25.3, has an autosomal dominant type of inheritance and high penetrance (more than 90%). This gene encodes two protein isoforms with molecular weights of 30 kDa (full form) and 19 kDa (shortened form), which play

Received: 13.03.2023. Accepted: 28.07.2023.

an important role in regulating the response to hypoxia and are necessary to maintain cellular homeostasis.

VHL mediates tumor invasion and metastasis by regulating HIFs protein expression [5, 6]. In approximately 20% of patients the disease is caused by do novo mutations [7]. Among germinal mutations, missense mutations are the most common (50-70%), while nonsense mutations and splice site mutations were less common [8, 9]. A specific feature of the course of VHL syndrome is the genotype-phenotype correlation. In particular, it has been shown that missense mutations are associated with greater risks of metastatic diseases and a more aggressive course with a rapid progression of clinical manifestations.

In addition to pheochromocytomas, in VHL syndrome, highly vascular tumors, neuroendocrine tumors of the pancreas, epididymis, central nervous system, retinal hemangioblastoma, carcinoid syndrome, cysts in the kidneys and pancreas can develop, which requires a multidisciplinary approach to diagnosis, follow-up of patients and their therapy. PCC/PGL develops in 10-25% of cases of VHL syndrome, pheochromocytomas are more common, and the risk of metastatic disease is 3-8% [10, 11].

CASE DESCRIPTION

Child K. was invited by a pediatric endocrinologist for examination due to family history of pheochromocytoma in several family members.

Mother has malignant pheochromocytoma (adrenalectomy on the right side – in 2001, on the left side – in 2015). Molecular genetic diagnosis was not available. A child's sibling was diagnosed with pheochromocytoma at the age of five years with no clinical manifestations, including arterial hypertension and tachycardia. Abdominal CT scan in the projection of both adrenal glands revealed soft tissue lesions having a rounded shape with clear and even contours with dimensions 24x13.5x24.5 mm on the right and 23x29.5x37 mm on the left, with heterogeneous density from +35 HU in the center to +55 +57 HU in the periphery. In spot urine the level of fractionated metanephrines (free + conjugated) was at 65 μg/g creatinine, fractionated normetanephrines at 5980 μg/g creatinine. Bilateral adrenalectomy was performed. Histological findings were consistent with pheochromocytoma with high metastatic potential (PASS score 6, GAPP score 6). Subsequently, there were two local relapses and lung metastases. With the opportunities of the AlphaEndo program, a molecular genetic study was conducted at the National Medical Research Center of Endocrinology of the Russian Ministry of Health, during which the heterozygous variant c.355T>C p.F119L was identified in the *VHL* gene during Sanger sequencing.

The maternal aunt was also previously diagnosed with bilateral pheochromocytoma, for which bilateral adrenalectomy was performed. Molecular genetic diagnosis was not carried out. Her daughter was diagnosed with pheochromocytoma accidentally at the age of 11 during a preventive medical examination at school. Ultrasound of the abdominal organs in the projection of the right adrenal gland revealed an oval mass with clear even contours of a liquid-solid structure measuring 58x30x40 mm, a solid component of a homogeneous structure with medium echogenicity, a liquid component in the form of several liquid inclusions measuring 7-22x13 mm. On CT in the area of the right adrenal gland, an oval mass was found with dimensions of 41x28x48 mm, density from +21 to +40 HU, with an area of irregular shape, reduced density to +10 HU. There were no clinical manifestations of the disease, including arterial hypertension. Plasma levels of adrenaline were 17 pg/ml (reference range 18-460), norepinephrine 20629 pg/ml (reference range 85-1250), dopamine 82.6 pg/ml (reference range 50-220), and serotonin 26 ng/ml (reference range 50-220 ng/ml). Urinary excretion of free metanephrines was 15.24 μg/day (reference range 7.69-33.33 μg/day), free normetanephrines – 2595.87 μg/day (reference range 7.91-35.18 μg/day), norepinephrine – 2517 μg/day (reference range 15-80 μg/day), and dopamine – 264 μg/day (reference range 65-400 μg/day). The heterozygous variant c.355T>C p.F119L was also identified in the *VHL* gene.

When examining child K., the parents did not make any complaints about his health. Physical examination of internal organs revealed no pathology. The blood pressure level was 88/54 mm Hg, heart rate 112 beats per minute. There were no symptoms of excess catecholamines in the form of sweating, arterial hypertension, tachycardia, and polyuria. The child is able to hold his head from a month and a half, he sits with support. There were no focal neurological symptoms, symptoms of cranial nerve damage, pathological reflexes. The child is able to fix gaze, recognizes parents and family members, which indicates the absence of visual impairment.

Taking into account the absence of hypertension and tachycardia in the sib with pheochromocytoma, the levels of metanephrines and normetanephrines in blood plasma were tested, although VHL Alliance experts recommend doing this after reaching the age of 5. The plasma level of free metanephrine was 0.25 nmol/L (reference range 0-0.49 nmol/L), free normetanephrine – 0.57 nmol/L (reference range 0-0.89 nmol/L), which made it possible to exclude pheochromocytoma. The comprehensive objective and laboratory-instrumental examination aimed at finding the components of von Hippel-Lindau syndrome, including examination of the fundus, ultrasound of the abdominal cavity and kidneys, did not reveal any abnormalities. The heterozygous variant c.355T>C p.F119L was identified in the *VHL* gene. Taking into account the results of molecular genetic diagnosis, the child remains under observation and will undergo regular examination in order to identify the components of von Hippel-Lindau syndrome, including blood/urine tests for normetanephrines.

DISCUSSION

This case report indicates the possibility of preclinical diagnosis of hereditary forms of pheochromocytomas by molecular genetic examination of adult patients and their families.

This will make it possible to detect both the development of pheochromocytoma and other components of VHL syndrome at an early stage, thereby avoiding their metastasis and improving the prognosis for the life of patients [2]. The need to actively identify children with familial forms of pheochromocytoma is justified by the fact that quite often they are asymptomatic. The pathogenic variant c.355T>C p.F119L identified in our patient belongs to the missense

mutations, which are the most common and which are associated with high risks of metastatic diseases and a more aggressive course with rapid progression of clinical manifestations [4, 9]. Thus, knowledge of the type of mutation enables not only to identify VHL syndrome before the development of pheochromocytoma and any other clinical manifestations, but also to predict the course of pheochromocytoma and, possibly, determine treatment tactics. The study by Michael Reich demonstrated that the probability of occurrence of various components of VHL syndrome increases with age [12]. This justifies the need for follow-up of patients by a multidisciplinary team.

Variant c.355T>C p.F119L has been previously described in patients with various manifestations of VHL syndrome. Kang et al. revealed this mutation in a patient with a CNS tumor and retinal hemangioblastoma and without pheochromocytoma [14], while in the series by Albattal et al. this mutation is described in a patient with bilateral pheochromocytoma without other components of the syndrome [15]. Individuals who have not been found to have changes in the *VHL* gene during DNA analysis do not need follow-up.

To predict the relative risk of certain manifestations in family members with this syndrome, several categories of VHL syndrome are distinguished. These categories are determined by the type of mutation and are characterized by a high or low risk of various manifestations of the syndrome depending on the mutation. Despite this, all patients with VHL syndrome are recommended to be monitored for all possible signs, regardless of subtype [13].

In accordance with the recommendations of experts on VHL syndrome (VHL Alliance, 2020), follow-up of children should be started at infancy [13]. Young people require ophthalmological examination, including fundus examination. The VHL Alliance recommends the "5-11-15 rule": annual 24-hour urine test or blood test for normetanephrines and metanephrines from 5 years of age, examination by audiologist and tomography of the brain and spinal cord every two years from the age of 11, and MRI of the abdominal cavity every two years from the age of 15 (Table 1).

In the VHL Patient and Caregiver Handbook the VHL Alliance recommends using MRI instead of CT in case of VHL syndrome in order to reduce the overall lifetime exposure

Table 1. Guidelines for surveillance of patients with VHL syndrome (VHL Alliance, 2020) [13]

Type of examination (tumor screening)	Age						Pregnancy
	Up to 5 years	Beginning at age 5 y	Beginning at age 11 y	Beginning at age 15 y	Beginning at age 30 y	Beginning at age 65 y	
Medical history and examination	Annually from age 1 year	Annually	Annually	Annually	Annually	Annually	Before conception
BP and pulse (pheochromocytoma, paraganglioma)	Annually from age 2 years	Annually	Annually	Annually	Annually	Annually	Before conception
Fundus (retinal hemangioblastoma)	Every 6-12 months from age 1 year	Every 6-12 months	Every 6-12 months	Every 6-12 months	Annually	Annually	Before conception and then every 6-12 months
Metanephrines (pheochromocytoma, paraganglioma)		Annually	Annually	Annually	Annually	Stop examinations	Before conception
CNS MRI with/without contrast (CNS hemangioblastoma)			Every 2 years	Every 2 years	Every 2 years	Stop examinations	Before conception
Audiogram (endolymphatic sac tumor)			Every 2 years	Every 2 years	Every 2 years	Stop examinations	
Abdominal MRI with/without contrast (renal cell carcinoma, pheochromocytoma/paragangliomas, neuroendocrine tumors/pancreatic cysts)				Every 2 years	Every 2 years	Stop examinations	Before conception
MRI of the internal auditory passages (endolymphatic sac tumor)				One time			

of people with VHL syndrome to radiation. To control particularly important areas of the brain and spinal cord, the most effective and cost-effective way is CNS MRI, including MRI of the brain, cervical, thoracic and lumbar spine. To exclude hemangioblastomas of the brain and spinal cord, it is necessary to perform MRI scans with a magnetic field strength of at least 1.5 T (Tesla) with or without contrast enhancement and necessarily with thin sections of the posterior cranial fossa [13]. Macrocyclic and gadolinium class II contrast agents are preferred for CNS MRI.

In addition to the generally accepted anamnesis collection and physical examination, patients undergo neurological examination, auditory and vestibuloneural tests, assessment of symptoms of excess catecholamines (headaches, palpitations, sweating, hyperactivity, anxiety, polyuria, abdominal pain).

An extended ophthalmic examination, including ophthalmoscopy, to exclude retinal hemangioblastoma should be performed every 6-12 months, depending on the result of the previous examination (especially in children) and the expected need for follow-up. If a detailed ophthalmic examination in the usual way is not possible, examining children under anesthesia shall be considered. It is also advisable to evaluate the possibility of performing ultra-wide-angle photography and ultra-wide-angle fluorescence angiography, which should not replace an examination by ophthalmologist.

To diagnose pheochromocytoma, it is advisable to determine the level of free plasma metanephrines due to their higher sensitivity compared to measuring the level of metanephrines in 24-hour urine test [13].

Abdominal MRI is aimed at early detection of cysts and carcinomas of the kidneys, serous cystadenomas and neuroendocrine tumors of the pancreas, PCC/PRG of the adrenal glands and extra-adrenal localization. It can be performed simultaneously with brain MRI under one long-term anesthesia, especially in children. However, CNS and abdominal MRI protocols should be performed sequentially. It is not recommended to assess changes in the spine using the abdominal MRI protocol or to assess the condition of the abdominal organs using the results of the CNS MRI protocol. If no CNS hemangioblastomas are detected, routine follow-up is continued every 2 years. In the presence of hemangioblastoma(s) and an increase in its (their) size and/or in the presence of concomitant symptoms, scans should be performed annually (or more often) depending on the situation (or a patient has to be referred to a neurosurgeon). If the initial scan shows no kidney lesions, routine follow-up is continued every 2 years.

If small kidney tumors (less than 3 cm) are detected, MRI must be repeated every 3-6 months until stability. After stability has been determined during three consecutive scans, repeated studies are considered once every 2 years. If the renal neoplasm is 3 cm or more, a consultation with urologist is recommended. High-resolution magnetic resonance imaging (section thickness 1 mm) for examination of the internal auditory meatus should be performed after 15 years old [13].

It is advisable to continue regular medical and ophthalmological examinations in persons over 65 years of age who have not shown any manifestations of VHL syndrome by this age [13].

CONCLUSION

Unfortunately, in the families described in our study, none of the adult patients were offered molecular genetic diagnostics, despite the fact that the clinical guidelines of the Russian Association of Endocrinologists for the diagnosis and treatment of pheochromocytoma/paraganglioma recommend genetic examination for all patients with PCC/PG [2]. The need for molecular genetic diagnosis is justified by the fact that more than a third of all patients with PCC/PG have hereditary mutations; the diagnosis of hereditary syndrome in the proband is the way to timely diagnosis and treatment of other family members, and the identification of a genetic defect creates the possibility of using preimplantation and prenatal diagnostics. The legal representatives of the children we examined were explained the goals and results of genetic testing and the importance of examining all lineal relatives. The long-term follow-up plan has been developed for the child with molecular genetically confirmed VHL syndrome, taking into account the recommendations of the VHL Alliance.

ADDITIONAL INFORMATION

Funding sources. This study was self-initiated by the authors and no funding was raised.

Conflict of interest. The authors hereby declare no actual or potential conflict of interest related to this publication.

Contribution of authors. All authors made substantial contributions to obtaining, analyzing, and interpreting results, searching for and evaluating literature data, writing the manuscript, or making significant (important) edits to it in order to enhance the scientific value of the paper. Every author approved the final version of the text prior to publication and agreed to accept responsibility for all aspects of this study, which implies due investigation and resolution of any issue related to the accuracy or integrity of any part thereof.

Patient consent. The legal representative of the patients voluntarily signed an informed consent to publish personal medical information in an impersonal form in the Problems of Endocrinology journal.

REFERENCES

1. Pamporaki C, Hamplova B, Peitzsch M, et al. Characteristics of Pediatric vs Adult Pheochromocytomas and Paragangliomas. *J Clin Endocrinol Metab* (2017) 102(4):1122–32. doi: https://doi.org/10.1210/jc.2016-3829
2. Мельниченко Г.А., Трошина Е.А., Бельцевич Д.Г., и соавт. Клинические рекомендации Российской ассоциации эндокринологов по диагностике и лечению феохромоцитомы/параганглиомы. — *Эндокринная хирургия.* — 2015; 9(3):15-33. doi: https://doi.org/10.14341/serg2015315-33
3. Park H, Kim MS, Lee J, et al. Clinical Presentation and Treatment Outcomes of Children and Adolescents With Pheochromocytoma and Paraganglioma in a Single Center in Korea. *Front Endocrinol* (Lausanne) (2020) 11:610746. doi: https://doi.org/10.3389/fendo.2020.610746
4. Petenuci J, Guimaraes AG, Fagundes GFC, et al. Genetic and Clinical Aspects of Paediatric Pheochromocytomas and Paragangliomas. *Clin Endocrinol* (Oxf) (2021) 95(1):117–24. doi: https://doi.org/10.1111/cen.14467
5. Peng S, Zhang J, Tan X, et al. The *VHL/HIF* axis in the development and treatment of pheochromocytoma/paraganglioma. Front Endocrinol (Lausanne). 2020; 11:586857
6. Doonachar A, Gallo MD, Doukas D, et al. Differential effects of HIF-alpha isoforms on apoptosis in renal carcinoma cell lines. *Cancer Cell Int.* 2015; 15:23
7. Реброва Д.В., Ворохобина Н.В., Имянитов Е.Н., и соавт. Клинико-лабораторные особенности наследственных феохромоцитом и параганглиом. — *Проблемы эндокринологии*. — 2022; 68(1):8-17. doi: https://doi.org/10.14341/probl12834
8. Fagundes G, Petenuci J, Lourenco DM, et al. New Insights Into Pheochromocytoma Surveillance of Young Patients With *VHL* Missense Mutations. *Journal of the Endocrine Society*, Volume 3, Issue 9, September 2019, Pages 1682–1692. doi: https://doi.org/10.1210/js.2019-00225
9. Hong B, Ma K, Zhou J, et al. Frequent Mutations of *VHL* Gene and the Clinical Phenotypes in the Largest Chinese Cohort With Von Hippel-Lindau Disease. *Front Genet.* 2019 Sep 18; 10:867. doi: https://doi.org/10.3389/fgene.2019.00867. PMID: 31620170; PMCID: PMC6759728
10. Crespigio J, Berbel LCL, Dias MA, et al. Von Hippel-Lindau disease: a single gene, several hereditary tumors. *Journal of Endocrinological Investigation*, 06 Jun 2017, 41(1):21-31. doi: https://doi.org/10.1007/s40618-017-0683-1
11. Nolting S, Bechmann N, Taieb D, et al. Personalized Management of Pheochromocytoma and Paraganglioma. *Endocr Rev* (2022) 43(2):199–239. doi: https://doi.org/10.1210/endrev/bnab019
12. Reich M, Jaegle S, Neumann-Haefelin E, et al. Genotype-phenotype correlation in von Hippel-Lindau disease. *Acta Ophthalmol.* 2021 Dec; 99(8):e1492-e1500. doi: https://doi.org/10.1111/aos.14843
13. VHLA Suggested Active Surveillance Guidelines: Пер. с англ. Руководство для людей с болезнью фон Хиппеля-Линдау, их семей и медицинских профессионалов, 148 с. — https://www.vhl.org/
14. Kang HC, Kim IJ, Park JH, et al. Three novel VHL germline mutations in Korean patients with von Hippel-Lindau disease and pheochromocytomas. *Oncol Rep.* 2005 Oct; 14(4):879-83. PMID: 16142346
15. Albattal S, Alswailem M, Moria Y, et al. Mutational profile and genotype/phenotype correlation of non-familial pheochromocytoma and paraganglioma. *Oncotarget.* 2019 Oct 15; 10(57):5919-5931. doi: https://doi.org/10.18632/oncotarget.27194. PMID: 31666924

Manuscript received: 13.03.2023. Approved for publication: 28.07.2023. Published online: 28.02.2024.

AUTHORS INFO

*__Oleg A. Malievskiy__, MD, PhD, Professor; address: 3 Lenina street, 450008 Ufa, Russia;
ORCID: https://orcid.org/0000-0003-2599-0867; SPIN-код: 6813-5061; e-mail: malievsky@list.ru

__Ramsiya I. Malievskaya__; ORCID: 0000-0001-9841-0611; e-mail: ramsiya1987@mail.ru
__Victor A. Malievskiy__, MD, PhD, Professor; ORCID: https://orcid.org/0000-0003-0522-7442; SPIN-код: 4429-2910;
e-mail: vmalievsky@mail.ru
__Anatoliy N. Tulpakov__; ORCID: https://orcid.org/0000-0001-8500-4841; SPIN-код: 8396-1798;
e-mail: anatolytiulpakov@gmail.com

TO CITE THIS ARTICLE:

Malievskiy OA, Malievskaya RI, Malievskiy VA, Tulpakov AN. Preclinical diagnostics of von Hippel-Lindau syndrome in a child. *Problems of Endocrinology*. 2024; 70(1): 100-104. doi: https://doi.org/10.14341/probl13280