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Prognostic Effectiveness of Carrying Genotypic Variants of Gene Polymorphisms VEGFA (Rs2010963) and HIF1A (Rs11549465) in the Development of Dopamine Agonist Resistant Prolactinomas

Z. Yu. Khalimova, M. S. Safarova

The Republican Specialized Scientific And Practical Medical Center Of Endocrinology Named After Academician Y.H. Turakulov, Tashkent, Uzbekistan

Abstract Research objective. To evaluate the prognostic effectiveness of carrying genotypic variants of VEGFA (rs2010963) and HIF1A (rs11549465) gene polymorphisms in the development of resistance of prolactinomas to dopamine agonist therapy. Methods. The study included cases of hormonally active prolactinomas (PRL) confirmed by clinical, laboratory, and instrumental investigations. Based on the selection results, a total sample of 128 PRL cases was formed. A prospective cross-sectional study included the randomization of patients with hormonally active PRL depending on PRL sensitivity to therapy. Gene polymorphisms VEGFA (rs2010963) and HIF1A (rs11549465) were examined using allele-specific polymerase chain reaction with SNP Express reagent kits in real-time mode. Results. The research results indicated that the GC genotype of the VEGFA gene polymorphism (rs2010963) exhibits moderate sensitivity (18.3%) and high specificity (76.1%), with the highest prognostic effectiveness. This is confirmed by a relatively high odds ratio with a confidence interval upper limit >1, highlighting its prognostic value. The CT genotype of the HIF1A gene polymorphism (rs11549465) demonstrates moderate sensitivity (16%) and high specificity (78.7%), with the highest prognostic effectiveness. Conclusion. Relatively high prognostic effectiveness has been established for carrying the GC genotype and C allele of the VEGFA gene polymorphism (rs2010963), as well as the CT genotype and T allele of the HIF1A gene polymorphism (rs11549465) in the development of prolactinoma resistance to dopamine agonist therapy.

Keywords Prolactinoma, Dopamine agonist resistance, VEGFA (rs2010963) polymorphism, HIF1A (rs11549465) polymorphism

1. Introduction

Genetic features inherent in hormonally active pituitary adenomas, including prolactinomas, have remained largely understudied [1,2]. In recent years, there has been a considerable amount of information regarding the genetic changes in prolactin-secreting pituitary adenomas. The search for genes associated with the formation of prolactinomas resistant to dopamine agonist therapy continues [3,4,5,6].

However, research on the genetic characteristics of prolactin-secreting pituitary adenomas with resistance to drug therapy remains limited at present. There are research results dedicated to mutations associated with prolactinoma resistance to dopamine agonist treatment [7,8,9].

Studying the molecular profile of prolactin-secreting pituitary adenomas is necessary to understand the key factors that form the basis of tumor development and its resistance to treatment. The application of molecular genetic methods in the early stages of treatment allows for determining a rational personalized management strategy for patients. In certain cases, the results of these studies enable the identification of indications for surgical treatment, prevention of negative changes in the topography and anatomy of the adenoma, and reduction of the risk of surgical intervention [5,10,11].

In connection with the above, the aim of this study was to evaluate the prognostic effectiveness of carrying genotypic variants of gene polymorphisms VEGFA (rs2010963) and HIF1A (rs11549465) in the development of prolactinoma resistance to dopamine agonist therapy.

2. Materials and Methods

The study was conducted from 2021 to 2024. The research

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took place at the Republican Specialized Scientific and Practical Medical Center of Endocrinology named after academician Y.X. Turakulov, Ministry of Health of the Republic of Uzbekistan. Clinical, imaging, and laboratory stages of the study were conductedwithin the framework of the research. The molecular-genetic stage of the study was carried out at the laboratory of the molecular-genetic department of the Specialized Scientific and Practical Medical Center of Hematology, Ministry of Health of the Republic of Uzbekistan.

Inclusion criteria:

Cases of hormonally active prolactinomas (PRL) confirmed by clinical, laboratory, and instrumental studies:

Microprolactinomas (less than 10 mm);

Macroprolactinomas (more than 10 mm);

Giant PRL (more than 4 cm).

They included PRL with aggressive behavior (APRL) (recurrent, resistant, rapid growth, and imaging characteristics of aggressive growth) and therapy-resistant PRL (TR-PRL) (resistance to therapy was defined as the absence of normalization of prolactin blood levels and/or no reduction in adenoma volume by 50% or more from the baseline on maximum tolerable doses of dopamine agonists, but not less than 15 mg/day bromocriptine or 3 mg/week cabergoline, for at least 6 months).

Exclusion criteria:

Oncological history;

Concurrent genetic diseases;

Hypothyroidism and other endocrine disorders;

Functional hyperprolactinemia;

Non-functioning adenoma, corticotropinoma, somatotropinoma, gonadotropinoma, thyrotropinoma;

Cirrhosis of the liver, chronic kidney disease, chronic heart failure.

As a result of the selection, a total sample of PRL cases was formed, consisting of 128 patients, with an average age of 44.2±8.7 years. The prospective cross-sectional study included the randomization of patients with hormonally active PRL depending on PRL sensitivity to therapy (sensitive to dopamine agonists and resistant to therapy).

Polymorphisms of the VEGFA (RS2010963) and HIF1A (RS11549465) genes were investigated using the allele-specific polymerase chain reaction (PCR) method with the SNP-Express reagent kits in real-time mode ("Syntol," Russia) PCR-RV (RT-PCR).

The prognostic effectiveness of each genetic indicator was calculated by determining sensitivity (SE), specificity (SP), and AUC (positive predictive value). The AUC of genes was determined as follows: if the AUC value <0.5, the marker was considered random; 0.5<AUC>0.6 – poor; 0.6<AUC>0.7 – fair; 0.7<AUC>0.8 – good; AUC>0.8 – excellent classifier.

The strength of the association between genetic indicators was assessed using the odds ratio (OR) and its 95% CI (confidence interval) according to the following formula:

$$OR=(a \times d)/(b \times c)$$

where a – frequency of the allele (or genotype) in the patient group, b – frequency of the allele (or genotype) in the control group, c – sum of the frequencies of the remaining alleles (or genotypes) in the patient group, d – sum of the frequencies of the remaining alleles (or genotypes) in the control group. An OR value of 1 indicated no association with the disease. An OR > 1 indicated an increased risk of pathology, while OR<1 indicated a reduced risk of pathology.

3. Results

Table 1 presents the results of assessing the prognostic effectiveness of carrying genotypic and allelic variants of the VEGFA gene polymorphism (RS2010963) in the context of the development of prolactin resistance to dopamine antagonist therapy. For this purpose, the following indicators characterizing prognostic effectiveness were calculated: SE – sensitivity; SP – specificity; AUC - positive predictive value; OR – odds ratio; 95% CI – confidence interval.

The calculation results revealed the following outcomes. In the assessment of the prognostic effectiveness of genotypes, it was determined that the GG genotype has a sensitivity of 73.3%, specificity of 20.5%, and positive predictive value of 0.333. The odds ratio for the development of prolactin resistance was 0.353 with a 95% confidence interval of 0.151-0.825. The GC genotype had a sensitivity of 18.3%, specificity of 76.1%, and AUC of 0.577. The odds ratio was 2.61 with a 95% CI of 1.084-6.286. The CC genotype had a sensitivity of 2.5%, specificity of 98%, and AUC of 0.667. The odds ratio was 3.208 with a 95% CI of 0.283-36.349.

In the assessment of the prognostic effectiveness of alleles, it was determined that allele C had a sensitivity of 18.1%, specificity of 80%, and AUC of 0.586. The odds ratio was 2.273 with a 95% CI of 0.999-5.171. Allele G had a sensitivity of 80%, specificity of 18.1%, and AUC of 0.384. The odds ratio was 0.44 with a 95% CI of 0.193-1.001.

The research results indicated that the GG genotype has average sensitivity (73.3%) and specificity (20.5%), and the AUC value suggests weak prognostic effectiveness in the development of prolactin resistance to dopamine antagonist therapy (AD). The GC genotype shows moderate sensitivity (18.3%) and high specificity (76.1%), with the highest prognostic effectiveness, as it has a relatively high odds ratio and the confidence interval's lower and upper bounds are >1, confirming its prognostic value. The CC genotype has very low sensitivity (2.5%) but high specificity (98.0%), indicating its potential use as a negative marker. Allele C also has high prognostic effectiveness, while allele G demonstrates weak prognostic ability.

Table 2 presents the results of assessing the prognostic effectiveness of carrying genotypic and allelic variants of the HIF1A gene polymorphism (rs11549465) in the context of developing prolactin resistance to dopamine antagonist therapy.

Table 1. Results of assessing the prognostic effectiveness of carrying genotypic and allelic variants of the VEGFA gene polymorphism (RS2010963) in the development of prolactin resistance to dopamine antagonist therapy (DA)

Genotypes	TP	TN	FP	FN	SE	SP	AUC	OR	95%CI
GG	33	17	66	12	73,3	20,5	0,333	0,353	0,151-0,825
GC	15	35	11	67	18,3	76,1	0,577	2,61	1,084-6,286
CC	2	48	1	77	2,5	98,0	0,667	3,208	0,283-36.349
Alleles									
C	17	48	12	77	18,1	80,0	0,586	2,273	0,999-5,171
G	48	17	77	12	80,0	18,1	0,384	0,44	0,193-1.001
TP – true positive; TN – true negative; FP – false positive; FN – false negative; SE – sensitivity; SP – specificity; AUC - predictive value of a positive result; OR – odds ratio; 95%CI – confidence interval.									

Table 2. Results of the Prognostic Efficiency Evaluation of Genotypic and Allelic Variants of the HIF1A Gene Polymorphism (rs11549465) in the Development of Prolactin Resistance to Dopamine Agonist Therapy (DA)

Генотипы	TP	TN	FP	FN	SE	SP	AUC	OR	95%CI
CC	34	16	67	13	72,3	19,3	0,337	0,412	0,178-0,955
CT	13	37	10	68	16,0	78,7	0,565	2,389	0,955-5,974
TT	3	47	1	77	3,8	97,9	0,750	4,915	0,497-48,637
аллели									
T	16	47	11	77	17,2	81,0	0,593	2,383	1,019-5,570
C	47	16	77	11	81,0	17,2	0,379	0,42	0,180-0.981
TP – true positive; TN – true negative; FP – false positive; FN – false negative; SE – sensitivity; SP – specificity; AUC - predictive value of a positive result; OR – odds ratio; 95%CI – confidence interval.									

The calculation results revealed the following outcomes. When evaluating the prognostic effectiveness of genotypes, it was determined that the CC genotype has sensitivity equal to 72.3%, specificity equal to 19.3%, and a positive predictive value of 0.337. The odds ratio for developing prolactin resistance was 0.412 with a 95% confidence interval of 0.178-0.955. The CT genotype had a sensitivity of 16%, specificity of 78.7%, and an AUC of 0.565. The odds ratio was 2.389 with a 95% CI of 0.955-5.974. The TT genotype had a sensitivity of 3.8%, specificity of 97.9%, and an AUC of 0.750. The odds ratio was 4.915 with a 95% CI of 0.497-48.637. When assessing the prognostic effectiveness of alleles, it was found that the T allele had a sensitivity of 17.2%, specificity of 81%, and an AUC of 0.593. The odds ratio was 2.383 with a 95% CI of 1.019-5.570. The C allele had a sensitivity of 81%, specificity of 17.2%, and an AUC of 0.379. The odds ratio was 0.42 with a 95% CI of 0.180-0.981.

The results of the research indicate that the CC genotype has moderate sensitivity (72.3%) and specificity (19.3%), with the AUC value indicating weak prognostic efficiency in the development of prolactin resistance to dopamine agonist (DA) therapy. The CT genotype demonstrates moderate sensitivity (16%) and high specificity (78.7%), showing the highest prognostic efficiency. Allele T also exhibits high prognostic efficiency, while allele C demonstrates weak prognostic capability.

4. Discussion

Some researchers have found that hypervascularization plays a key role in the invasiveness and spread of various tumor types, including invasive pituitary adenomas. Studies have also examined the role of vascular endothelial growth factor (VEGF) expression and its receptors VEGFR-2 in pituitary adenomas, revealing a significant correlation between adenoma volume and VEGFA expression levels. In conclusion, angiogenesis in tumorigenesis is a complex and dynamic process, and while the vascularity of pituitary adenomas, especially prolactinomas, remains unknown, for invasive or aggressive pituitary adenomas, angiogenesis is considered one of the important pathogenetic mechanisms [2,4,12,13].

Vascular endothelial growth factor (VEGF) is a signaling protein that promotes the formation of new blood vessels. VEGF plays a role in restoring oxygen supply to cells and tissues in cases of hypoxia caused by impaired blood circulation. Overexpression of VEGF contributes to tumor development by providing enhanced oxygenation to support continuous tumor growth. Tumors capable of producing VEGF can sustain their growth by oxygenating the expanding tissue, and this process is known as angiogenesis [1,7,14].

Hypoxia-inducible factor-1 alpha (HIF-1α) has the ability to regulate the level of vascular endothelial growth factor (VEGF) not only in pituitary adenomas but also in other

tumors. More than a decade ago, an experiment reducing HIF-1α levels demonstrated its protective function by reducing apoptosis in the human pituitary adenoma cell line (HP75) under hypoxic conditions. Subsequent studies confirmed that VEGF activation occurs under the influence of HIF-1α. It is reported that HIF-1α activates VEGF under hypoxic conditions, confirming the interaction between HIF-1α and VEGF. Studies have also identified excessive expression of HIF-1α and VEGF-A in postoperative surgical materials from invasive pituitary adenomas compared to non-invasive samples, confirming the role of HIF-1α as the top regulator of VEGF expression. All these findings contribute to the complex HIF-1α-VEGF pathway, activating invasive processes in pituitary adenomas [2,3,4,15].

In addition, von Hippel-Lindau tumor suppressor (pVHL) protein is known as a negative regulator of HIF-1α. Low expression of this protein with high VEGF expression is associated with an increased recurrence rate and more aggressive behavior of pituitary adenomas. All these facts complement each other and confirm the importance of pathways related to VEGF and HIF-1α in the invasiveness and aggressiveness of pituitary adenomas [1,3,4].

5. Conclusions

A relatively high prognostic efficiency of carrying the GC genotype and C allele of the VEGFA gene polymorphism (rs2010963), as well as the CT genotype and T allele of the HIF1A gene polymorphism (rs11549465), in the development of prolactin resistance to dopamine agonist therapy has been established. This allows for the use of these genetic markers for early prediction of prolactin resistance development to treatment.

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