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ГЕНДЕРНІ ОСОБЛИВОСТІ РЕГУЛЯТОРНИХ ЗВ'ЯЗКІВ ГОМОЦИСТЕЇНУ, ГОРМОНІВ ГІПОФІЗУ І ЩИТОПОДІБНОЇ ЗАЛОЗИ У ДІТЕЙ ІЗ РАЙОНІВ, ЩО ПОСТРАЖДАЛИ У РЕЗУЛЬТАТІ АВАРІЇ НА ЧОРНОБИЛЬСЬКІЙ АТОМНІЙ ЕЛЕКТРОСТАНЦІЇ

Бандажевський Ю.І., Дубова Н.Ф.

GENDER CHARACTERISTICS OF REGULATORY CONNECTIONS BETWEEN HOMOCYSTEINE, PITUITARY AND THYROID HORMONES IN CHILDREN FROM AREAS AFFECTED BY THE ACCIDENT AT THE CHORNOBYL NUCLEAR POWER PLANT

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**BANDAZHEVSKIY Yu.I.,
DUBOVA N.F.**

Ecology and Health
Coordination and
Analytical Center,
Ivankyv, Ukraine

As a result of the accident at the Chornobyl Nuclear Power Plant (ChNPP), a huge number of adults and children were exposed to radioactive elements, with the greatest impact on the thyroid gland from short-lived ^{131}I [1] and long-lived ^{137}Cs [2].

The consequence of this process was an increase in

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Бандажевський Ю.І., Дубова Н.Ф.

Координаційний аналітичний центр «Екологія і здоров'я», Іванків, Україна

Метою роботи було визначення гендерних особливостей у формуванні регуляторних зв'язків гомоцистеїну (H_{cy}), гормонів гіпофізу та щитоподібної залози (ЩЗ) у дітей із районів України, що постраждали внаслідок аварії на Чорнобильській атомній електростанції.

Матеріали та методи: аналітичний, генетичний, лабораторний, математико-статистичний.

Результати. Було обстежено 379 підлітків (187 хлопчиків та 192 дівчинки) із Іванківського та Поліського районів Київської області. У крові дітей визначали кількість H_{cy} , тиреотропного гормону гіпофізу (TSH), вільного трийодтироніну (T_3), вільного тироксину (T_4). Проводили дослідження стану генетичної системи фолатного циклу (ФЦ). У групі хлопчиків із Поліського району вміст H_{cy} , TSH , T_3 у крові був достовірно більшим, ніж у групі дівчаток. Після лісових пожеж у Чорнобильській зоні відчуження (ЧЗВ) рівень у крові H_{cy} , T_3 , і T_4 у дівчаток із Іванківського району був достовірно вищим, ніж у дівчаток Поліського району. З концентраціями H_{cy} у крові, що не перевищують рівень 10,0 мкмоль/л, у під-

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the number of cases of malignant neoplasms of the thyroid gland among the adult and child population [3, 4].

In addition to oncological processes, in subsequent years, various types of structural and functional disorders of the thyroid gland were identified in residents of the affected areas [5].

To date, it has not been possible to fully determine their pathogenesis.

Social and medical projects of the European Commission and the Rhone-Alpes Region (France), carried out in regions of Ukraine bordering the Chernobyl Exclusion Zone (ChEZ) in 2013-2017, expanded scientific knowledge about the long-term humanitarian consequences of the Chernobyl accident [6].

During the implementation of these projects, a large number of adolescent children were found to have elevated levels in the blood of the sulfur-containing amino acid homocysteine (H_{cy}),

one of the main links in the internal metabolism of the essential amino acid methionine.

A large number of studies have revealed the association of hyperhomocysteinemia with a number of serious diseases in adults [6].

This issue has been very poorly studied in children.

In particular, this concerns the connection of H_{cy} with pathological processes in the thyroid gland.

Considering the existing knowledge about the participation of H_{cy} in metabolic processes, the study of its effect on regulatory connections in the hypothalamic-pituitary-thyroid system under radiation exposure is a relevant area of medical science.

The purpose of this study was to determine gender characteristics in the formation of regulatory connections between homocysteine, pituitary and thyroid hormones in children from regions of Ukraine affected by the accident at the

Chernobyl nuclear power plant.

Material and methods. The analytical study used information obtained during a genetic and laboratory examination of children aged 12-17 years old, permanently residing in the Ivankyivskiy and Polyskiy districts of the Kyiv region, whose soils are contaminated with radioactive elements due to the Chernobyl accident [7].

The survey of children, agreed upon with parents, was carried out in 2015, with the support of the European Commission and the Regional Council of Rhone-Alpes (France).

Group 1 included 201 children from the Polyskiy district, including 101 boys and 100 girls.

Group 2 included 178 children from the Ivankyivskiy district, including 86 boys and 92 girls.

The test material used was blood taken in the morning, on an empty stomach, from the ulnar vein, from children in the Polyskiy district on 04/02/2015, from children in the Ivankyivskiy district on 12/18/2015.

The obtained blood samples were examined in a laboratory certified in accordance with European Union quality standards.

The amount of H_{cy} , pituitary thyroid-stimulating hormone (TSH), free triiodothyronine (T_3), and free thyroxine (T_4) was determined in the blood of children. At the same time, a study was also carried out on the state of the genetic system of the folate cycle (FC).

Determination of H_{cy} in the blood was carried out using the immunochemical method with chemiluminescent detection (ECLIA). Analyzer and test system: Architect

групах обстежених дітей реєструвався прямий зв'язок між значеннями H_{cy} та T_4 , що свідчить про певний фізіологічний паритет між ФЦ та гіпофізарно-тиреоїдною віссю. З концентраціями H_{cy} , що перевищують 10,0 мкмоль/л у підгрупах дітей, TSH стимулював процес утворення T_3 за рахунок зменшення вмісту T_4 . Процес стимуляції активності ферментів ФЦ за допомогою T_3 , представлений зворотними зв'язками H_{cy} - T_3 та H_{cy} - T_3/T_4 , найбільш виражений у групі дівчаток порівняно з групою хлопчиків. Можливо, це є причиною того, що в їхній крові міститься менше H_{cy} .

Висновки. Підвищення концентрації H_{cy} у крові та посилення утворення T_3 під впливом TSH в організмі обстежених дітей пов'язане з генетичним фактором (алель T MTHFR:677 у геномі). Виражена зовнішньосередовищна радіаційна дія, пов'язана з лісовими пожежами у ЧЗВ, сприяла значному збільшенню вмісту H_{cy} у крові та посиленню впливу TSH на процеси тиреоїдного обміну у дітей із прилеглих до неї населених пунктів. T_3 стимулює вплив на ферменти ФЦ, що призводить до посилення метилювання H_{cy} та збільшення утворення внутрішнього метіоніну.

Ключові слова: фолатний цикл, гомоцистеїн, гормони гіпофіза та щитоподібної залози, діти, радіоактивно забруднені території.

1000 (ABBOT Diagnostics (USA). The level of Hcy in the blood of children over 10.0 $\mu\text{mol/l}$ was defined as a state of hyperhomocysteinemia.

Determination of TSH, T_3 and T_4 was carried out using an immunochemical method with electrochemiluminescent detection (ECLIA). Analyzer and test system: Cobas 6000, Roche Diagnostics (Switzerland).

The reference range of extreme values designated by the laboratory was: for TSH - 0.28-4.3 $\mu\text{MO/ml}$; for T_3 - 2.3-5.0 pg/ml ; for T_4 - 1.1-1.8 ng/dl .

During the genetic study of FC, the allelic variants C677T and A1298C of the methylenetetrahydrofolate reductase gene (MTHFR), A2756G of the gene associated with B_{12} -dependent methionine synthase (MTR), A66G of the gene associated with methionine synthase reductase (MTRR)



ПРОБЛЕМИ ЧОРНОБИЛЯ

centiles were calculated. The hypothesis about the type of distributions was tested (Kolmogorov-Smirnov test). All studied parameters did not correspond to the law of normal distribution, and therefore the non-parametric Mann-Whitney U test was used to compare values.

The statistical significance of the indicators was assessed by determining the significance level p using a statistical program.

The critical level of significance of the null statistical hypothesis (p) was taken as 0.05.

During the research, an

analysis of the correlations between H_{cy} , hormones TSH, T_3 , T_4 , T_3/T_4 index was carried out in subgroups of children in the Polyskyi and Ivankivskyi districts with different genotypes and different levels of H_{cy} in the blood ($>10.0 \mu\text{mol/l}$ and $10.0 \mu\text{mol/l}$).

The relationship between the indicators H_{cy} , TSH, T_3 , T_4 , T_3/T_4 was determined using the Spearman rank correlation coefficient (r). The strength of the correlation was assessed using a traditional scale: weak – from 0 to 0.299; average – from 0.3 to 0.699; strong – from 0.7 to 1.

Table 1

Statistical characteristics of indicators of children in the Polyskyi district

Groups of children	Analyzed indicators							
	H_{cy}		TSH		T_3		T_4	
	Me	IQR	Me	IQR	Me	IQR	Me	IQR
Boys	10.69	8.44-14.52	2.10	1.54-2.67	4.58	4.24-4.92	1.17	1.03-1.27
Girls	9.38	8.01-10.90	1.65	1.24-2.15	3.86	3.60-4.10	1.17	1.10-1.29

Note table 1-3: Me – median value; IQR – interquartile range.

were determined. The method used was Real-time PCR. Analyzer and test system Detection amplifier «DT-96», «DNA-Technolog» (Russia).

Statistical processing of the obtained results was carried out using the IBM SPSS Statistics 22 program (USA). For the analyzed indicators, the median (Me), interquartile range (IQR), minimum and maximum parameter values, and per-

Table 2

Results of statistically significant differences when comparing indicators in the analyzed groups of children in the Polyskyi district

Indicators	Groups comparisons	Number of comparison group	Average rank	U-criterion value, significance level p
H_{cy}	Boys	101	115.27	U = 3608.500; $p = 0.0001$
	Girls	100	86.59	
TSH	Boys	101	114.89	U = 3647.500; $p = 0.001$
	Girls	100	86.98	
T_3	Boys	101	133.83	U = 1734.500; $p = 0.0001$
	Girls	100	67.85	

Results and its discussion. In the group of boys from the Polyskyi district, the blood levels of H_{cy} , TSH, and

T_3 were significantly more than in the group of girls from this district (Tables 1, 2).

In the group of girls from the Ivankyvskyi district, the blood levels of H_{cy} , T_3 , T_4 were significantly more than

Table 3

Statistical characteristics of indicators of children in the Ivankyvskyi district

Groups of children	Analyzed indicators							
	H_{cy}		TSH		T_3		T_4	
	Me	IQR	Me	IQR	Me	IQR	Me	IQR
Boys	12.20	10.88-15.86	2.09	1.62-2.85	4.59	4.29-4.90	1.22	1.10-1.29
Girls	11.17	9.28-12.59	1.57	1.18-2.00	4.19	3.86-4.56	1.25	1.16-1.32

Note: Me – median value; IQR – interquartile range.

Table 4

Results of statistically significant differences when comparing indicators in the analyzed groups

Indicators	Groups comparisons	Number of cases	Average rank	U-criterion value, significance level p
H_{cy}	«1» - Girls	100	79.81	U = 2931.000; p = 0.0001
	«2» - Girls	92	114.64	
T_3	«1» - Girls	100	80.02	U = 2952.000; p = 0.0001
	«2» - Girls	92	114.41	
T_4	«1» - Girls	100	85.95	U = 3545.000; p = 0.006
	«2» - Girls	92	107.97	

Note: Group «1» – girls of the Polyskyi district; Group «2» – girls from Ivankyvskyi district.

Table 5

Variants of correlations in genetic subgroups of boys and girls in the Polyskyi district

Main genotype	Level H_{cy} , $\mu\text{mol/l}$	Correlations	
		Group 1	Group 2
A/AMTR:2756	10.0	$+H_{cy}-T_4$	$-H_{cy}-T_3/T_4, +T_3-T_4$
	>10.0	-TSH-T4	-
A/G, G/GMTR:2756	10.0	-	-
	>10.0	-	-
A/AMTHFR:1298	10.0	-	$-H_{cy}-T_3, -H_{cy}-T_3/T_4$
	>10.0	-	-
A/C, C/CMTHFR:1298	10.0	$+H_{cy}-T_4$	-
	>10.0	-TSH-T4	-
C/C MTHFR:677	10.0	$+H_{cy}-T_4, -H_{cy}-T_3/T_4$	-
	>10.0	-TSH-T4	-
C/T, T/T MTHFR:677	10.0	$+TSH-T_3/T_4$	$-H_{cy}-T_3/T_4$
	>10.0	-	-
A/AMTRR:66	10.0	$+H_{cy}-T_4$	$-H_{cy}-T_3, -H_{cy}-T_3/T_4$
	>10.0	-	-
A/G, G/G MTRR:66	10.0	-	$-H_{cy}-T_3/T_4$
	>10.0	-	-

Note: Group 1 – boys; Group 2 – girls. «+» – direct correlation; «-» – inverse correlation.

in the group of girls from the Polyskyi district (Tables 3, 4).

At the level of $H_{cy} \leq 10.0$ $\mu\text{mol/l}$ in the genetic subgroups of boys in the Polyskyi district (main genotypes A/AMTR:2756, A/C, C/CMTHFR:1298, C/C MTHFR:677 and A/AMTRR:66), a direct correlation was recorded $H_{cy}-T_4$ connection (Table 5).

At the level of $H_{cy} > 10.0$ $\mu\text{mol/l}$, in the genetic subgroups of boys in the Polyskyi district (main genotypes A/AMTR:2756, A/C, C/CMTHFR:1298 and C/C MTHFR:677), an inverse correlation TSH- T_4 (Table 5).

The proportion of cases with risk alleles of the MTHFR:C677T polymorphism in this case was greater than with the H_{cy} level ≤ 10.0 $\mu\text{mol/L}$ (Table 6).

In the genetic subgroups of girls from the Polyskyi district, with H_{cy} levels ≤ 10.0 $\mu\text{mol/l}$, inverse relationships between $H_{cy}-T_3$ and $H_{cy}-T_3/T_4$ were identified (Table 5).

In a group of children from the Ivankyvskyi district examined after forest fires in the ChEZ [8], in subgroups with H_{cy} levels > 10.0 $\mu\text{mol/l}$, direct correlations between TSH- T_3 and TSH- T_3/T_4 were revealed, more pronounced in the subgroups of girls, in comparison with subgroups of boys (Table 7).

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GENDER FEATURES OF REGULATORY RELATIONSHIPS OF HOMOCYSTEINE, PITUITARY AND THYROID HORMONES IN CHILDREN FROM AREAS AFFECTED BY THE ACCIDENT AT THE CHORNOBYL NUCLEAR POWER PLANT

Bandazhevskiy Y.I., Dubova N.F.
Coordination Analytical Center «Ecology and Health», Ivankyv, Ukraine

The purpose of the work was to determine gender characteristics in the formation of regulatory connections between homocysteine (H_{cy}), pituitary and thyroid hormones in children from regions of Ukraine affected by the accident at the Chornobyl nuclear power plant.

Materials and methods: analytical, genetic, laboratory, mathematical and statistical.

Results. 379 adolescents (187 boys and 192 girls) from the Ivankyvskiy and Polyskiy districts of the Kyiv region were examined. The amount of H_{cy} , pituitary thyroid-stimulating hormone (TSH), free triiodothyronine (T_3), and free thyroxine (T_4) was determined in the blood of children. A study was conducted on the state of the genetic system of the folate cycle (FC). In the group of boys from the Polyskiy district, the content of H_{cy} , TSH, T_3 in the blood was significantly more than in the group of girls. After forest fires in the Chornobyl Exclusion Zone (ChEZ), the blood levels of H_{cy} , T_3 , and T_4 in girls from the Ivankyvskiy district were significantly

more than in girls from the Polyskiy district. When H_{cy} concentrations in the blood did not exceed $10.0 \mu\text{mol/l}$, in subgroups of children examined, a direct relationship was recorded between the values of H_{cy} and T_4 , indicating a certain physiological parity between the FC and the pituitary-thyroid axis.

At H_{cy} concentrations exceeding $10.0 \mu\text{mol/l}$ in subgroups of children, the stimulating effect of TSH on the process of T_3 formation was revealed by reducing the T_4 content. The process of stimulating the activity of FC enzymes with the help of T_3 , represented by the $H_{cy}-T_3$ and $H_{cy}-T_3/T_4$ feedbacks, is most pronounced in the group of girls compared to the group of boys. This may be the reason that they have less H_{cy} in their blood.

Conclusions. An increase in the concentration of H_{cy} in the blood and an increase in the formation of T_3 under the influence of TSH in the body of the examined children is associated with a genetic factor (allele T MTHFR:677 in the genome). The pronounced external radiation impact associated with forest fires in the ChEZ contributed to a significant increase in the content of H_{cy} in the blood and an increase in the influence of TSH on the processes of thyroid metabolism in children from adjacent settlements. T_3 has a stimulating effect on FC enzymes, which leads to increased H_{cy} methylation and increased formation of internal methionine.

Keywords: folate cycle, homocysteine, pituitary and thyroid hormones, children, radioactively contaminated areas.

An example of hormonal connections of the pituitary-thyroid axis is a subgroup with the main genotype C/C MTHFR:677 (Table 7).

In this subgroup of girls, with H_{cy} levels $>10.0 \mu\text{mol/l}$, correlations were identified - direct TSH- T_3/T_4 ($r = 0.629$, $p = 0.002$, $n = 21$) and reverse TSH- T_4 ($r = -0.673$, $p = 0.001$, $n = 21$).

At H_{cy} levels $10 \mu\text{mol/L}$ in this subgroup of girls, an inverse correlation between $H_{cy}-T_3/T_4$ was recorded ($r = -0.632$, $p = 0.011$, $n = 15$), which was absent in the similar subgroup of boys (Table 7).

The absence in the genome of children of this subgroup of the risk allele T of the MTHFR:677 polymorphism, which significantly increases the level of H_{cy} in the blood [6], allows us to conclude that the identified effects of interaction between H_{cy} and hormones of the pituitary-thyroid axis are associated, to a greater extent, with external environmental radiation influence.

The results of a correlation analysis of genetic subgroups taking into account the level of H_{cy} in the blood indicate a certain relation-

ship between FC and the pituitary-thyroid axis.

When considering the cause-and-effect mechanisms of interaction between FC and the thyroid gland, it is possible to consider the hypothesis that H_{cy} has a stimulating effect on the processes of T_4 synthesis in the thyroid gland.

The direct correlation between $H_{cy}-T_4$ in subgroups of boys in the Polyskiy district illustrated the relationship between H_{cy} and T_4 under conditions when the level of H_{cy} in the blood did not exceed $10.0 \mu\text{mol/l}$.

The physiological parity between the blood concentrations of H_{cy} and T_4 is due to the close connection between the cycles of transsulfuration and deiodination reactions [9]. In this regard, H_{cy} utilization was accompanied by a decrease in T_4 content and an increase in T_3 .

TSH is involved in this process. Its effect on thyroid hormoneogenesis is re-

flected in the genetic subgroups of boys with blood H_{cy} levels $>10.0 \mu\text{mol/L}$, in the form of inverse TSH- T_4 correlations. At the same time, the influence of the C/T, T/TMTHFR:677 genotypes, which contribute to an increase in the H_{cy} content in the blood, is visible.

In subgroups of girls in the Polyskyi district, with blood H_{cy} levels $10.0 \mu\text{mol/l}$, inverse correlations between

H_{cy} - T_3 and H_{cy} - T_3/T_4 reflect the stimulating effect of T_3 on H_{cy} methylation processes. This is the reason for the lower concentrations of H_{cy} , TSH and T_3 in the blood.

Thus, the state of hyperhomocysteinemia in children of the Polyskyi district, formed to a large extent due to a genetic factor, is a condition for increased formation of T_3 , which can activate the processes of H_{cy} methylation through its effect on the FC enzyme systems [9].

In girls this mechanism is better expressed than in boys.

As a result, with the corresponding FC genotypes, the level of H_{cy} in their body is lower.

After forest fires in the ChEZ, the level of H_{cy} in the blood of children from adjacent settlements increased significantly, which led to an increased influence of TSH on the processes of thyroid hormonal metabolism.

The influence of the genetic factor in this case is not visible, since there were no differences between the groups of children in the Polyskyi and Ivankovsky districts in terms of the proportion of risk alleles for FC polymorphisms [10].

Direct associations between TSH- T_3 and TSH- T_3/T_4 , indicating increased T_3 formation under the influence of TSH, were present in most subgroups of girls and in some subgroups of boys with H_{cy} levels $>10.0 \mu\text{mol/L}$. At the same time, in the subgroups of boys there was no stimulating effect of T_3 on H_{cy} methylation processes.

Conclusions

In the group of boys from the Polyskyi district, the concentration in the blood of H_{cy} , TSH, T_3 was significantly more than in the group of

Variants of correlations and the proportion of genotypes C/T, T/T MTHFR:677 in genetic subgroups of boys

Table 6

Main genotype	Level H_{cy} , $\mu\text{mol/l}$	Correlations	Proportion of genotypes C/T, T/T MTHFR:677, %
A/A MTR:2756	10.0	+ H_{cy} - T_4	28.6
	> 10.0	-TSH- T_4	60.0
A/C, C/C MTHFR:1298	10.0	+ H_{cy} - T_4	7.1
	> 10.0	-TSH- T_4	39.3
A/A MTRR:66	10.0	+ H_{cy} - T_4	25.0
	> 10.0	-	37.5

Note: «+» – direct correlation; «-» – inverse correlation.

Variants of correlations in genetic subgroups of boys and girls in the Ivankovsky district

Table 7

Main genotype	Level H_{cy} , $\mu\text{mol/l}$	Correlations	
		Group 1	Group 2
A/A MTR:2756	10.0	-	-
	> 10.0	-	+TSH- T_3 , +TSH- T_3/T_4
A/G, G/G MTR:2756	10.0	-	-
	> 10.0	+TSH- T_3/T_4	- H_{cy} - T_3/T_4
A/A MTHFR:1298	10.0	-	-
	> 10.0	-	+TSH- T_3 , +TSH- T_3/T_4
A/C, C/C MTHFR:1298	10.0	+ H_{cy} - T_3/T_4	-
	> 10.0	-	+ H_{cy} - T_4 , - H_{cy} - T_3/T_4
C/C MTHFR:677	10.0	-	- H_{cy} - T_3/T_4
	> 10.0	-TSH- T_4 , +TSH- T_3/T_4	-TSH- T_4 , +TSH- T_3/T_4
C/T, T/T MTHFR:677	10.0	-	-TSH- T_4
	> 10.0	-	-
A/A MTRR:66	10.0	-	-
	> 10.0	-	+ H_{cy} - T_4
A/G, G/G MTRR:66	10.0	-TSH- T_3	-
	> 10.0	-	+ H_{cy} - T_4 , - H_{cy} - T_3/T_4

Note: Group 1 – boys; Group 2 – girls. «+» – direct correlation; «-» – inverse correlation.

girls from the same region.

After forest fires in the ChEZ, the concentrations of H_{cy} , T_3 , and T_4 in the blood of girls in the Ivankyivskiy district were significantly more than in the girls of the Polyskiy district.

When H_{cy} concentrations in the blood did not exceed $10.0 \mu\text{mol/l}$, in subgroups of children examined, a direct relationship was recorded between the values of H_{cy} and T_4 , indicating a certain physiological parity between the FC and the pituitary-thyroid axis.

At H_{cy} concentrations exceeding $10.0 \mu\text{mol/l}$ in subgroups of children, the stimulating effect of TSH on the process of T_3 formation was revealed by reducing the T_4 content.

An increase in the concentration of H_{cy} in the blood and an increase in the formation of T_3 under the influence of TSH in the body of the examined children is associated with a genetic factor (allele T MTHFR:677 in the genome).

The pronounced external radiation impact associated with forest fires in the ChEZ contributed to a significant increase in the content of H_{cy} in the blood and an increase in the influence of TSH on the processes of thyroid metabolism in children from adjacent settlements.

T_3 had a stimulating effect on FC enzymes, which led to increased H_{cy} methylation and increased formation of internal methionine. This process is most pronounced in the group of girls, in comparison with the group of boys.

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Конфлікт інтересів відсутній.

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