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Spectrum and functional properties of *ERG11* gene mutations in fluconazole-resistant *Candida albicans* strains isolated from HIV-infected patients

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Abstract

Rationale. The low efficacy of azole antimycotics in treatment of *Candida* infections, especially in HIV-infected patients, is often associated with overexpression of the *ERG11* gene in *Candida* spp., which results in increased production of ergosterol – the target of the above antimycotic drugs. Researchers have found *ERG11* gene mutations that can modify its overexpression effects by increasing or decreasing it. However, the findings reported by different laboratories and countries are highly contradictory.

The **purpose** of the study is to explore the spectrum and functional properties of *ERG11* gene mutations in fluconazole-resistant *Candida albicans* strains isolated from HIV-infected patients.

Materials and methods. The study was performed using 10 *C. albicans* strains inherently resistant to fluconazole and voriconazole and isolated from the oropharynx of HIV-infected patients; the strains were provided from the collection of the Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology. The strains were assessed by their sensitivity to antimycotic agents: anidulafungin, micafungin, caspofungin, posaconazole, voriconazole, itraconazole, fluconazole, amphotericin B, 5-flucytosine. Expression levels of the *ERG11* gene were measured by quantitative PCR. *ERG11* gene mutations were identified by Sanger sequencing.

Results. Five mutations (*E266D*, *G464S*, *I471L*, *D116E*, and *V488I*) were detected in the *ERG11* gene in seven *C. albicans* strains; six strains carried non-associated co-occurring mutations. Increased expression of the *ERG11* gene was found in six *C. albicans* strains. The *V488I* mutation demonstrated a strong negative association with the increased expression of the *ERG11* gene ($r = –0.845$; $p < 0.05$). The minimum inhibitory concentration (MIC) in strains carrying mutations was a hundred times as low ($p < 0.05$) as MIC in strains without mutations. In mutation carriers, posaconazole and itraconazole MICs were on average 16.5 times as low as MICs of voriconazole and fluconazole ($p < 0.001$). The presence of mutations in the *ERG11* gene had almost no effect on MICs of the tested antimycotics of the echinocandin, polyene, and pyrimidine groups.

Conclusion. Multiple mutations were detected in the *ERG11* gene in most of the *C. albicans* strains isolated from HIV-infected patients and resistant to fluconazole and voriconazole. Except for the *V488I* mutation, the detected mutations were not associated with the overexpression of the *ERG11* gene and decreased the effects of overexpression of the *ERG11* gene by up to 100 times, though they did not eliminate the inherent resistance to triazole antimycotics.

Keywords: *Candida, ERG11 gene, mutations, resistance to antimycotic agents, HIV infection*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the South Ural State Medical University (protocol No. 4, April 25, 2014).

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Оригинальное исследование
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Спектр и функциональные свойства мутаций гена *ERG11* флуконазол-резистентных грибов *Candida albicans*, выделенных от ВИЧ-инфицированных пациентов

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Аннотация

Актуальность. Низкая эффективность терапии кандидозной инфекции азоловыми препаратами, особенно у ВИЧ-инфицированных пациентов, зачастую связана с гиперэкспрессией в грибах *Candida* spp. гена *ERG11*, которая обусловливает повышение объёма синтеза эргостерола — мишени данных препаратов. Обнаружены мутации гена *ERG11*, способные модифицировать эффекты его гиперэкспрессии путём как усиления, так и снижения. Однако сведения, полученные в различных лабораториях и странах, весьма противоречивы.

Цель работы — исследовать спектр и функциональные свойства мутаций гена *ERG11* в устойчивых к флуконазолу штаммах *Candida albicans*, выделенных от ВИЧ-инфицированных пациентов.

Материалы и методы. *И*сследование выполнено на 10 штаммах грибов *C. albicans*, выделенных из ротоглотки ВИЧ-инфицированных пациентов и изначально устойчивых к действию флуконазола и вориконазола, из коллекции Московского научно-исследовательского института эпидемиологии и микробиологии им. Г.Н. Габричевского. Штаммы были охарактеризованы по чувствительности к антимикотическим препаратам: анидулафунгину, микафунгину, каспофунгину, позаконазолу, вориконазолу, итраконазолу, флуконазолу, амфотерицину В, 5-флуцитозину. Уровень экспрессии гена *ERG11* измеряли с помощью количественной ПЦР. Мутации гена *ERG11* выявляли путём его секвенирования по Сэнгеру.

Результаты. В 7 штаммах *C. albicans* в структуре гена *ERG11* были обнаружены 5 вариантов мутаций (*E266D*, *G464S*, *I471L*, *D116E* и *V488I*), 6 штаммов оказались носителями сочетанных мутаций, которые не имели сопряжения. В 6 исследованных штаммах *C. albicans* была установлена повышенная экспрессия гена *ERG11*. Для мутации *V488I* была характерна сильная отрицательная связь с повышенной экспрессией гена *ERG11* ($r = –0,845$; $p < 0,05$). Минимальная ингибирующая концентрация (МИК) штаммов — носителей мутации была на 2 порядка ниже ($p < 0,05$), чем штаммов без мутаций. У носителей мутаций МИК позаконазола и итраконазола были в среднем в 16,5 раза ниже, чем МИК вориконазола и флуконазола ($p < 0,001$). Наличие мутаций в гене *ERG11* практически не отражалось на уровне МИК тестированных антимикотиков группы эхинокандинов, полиенов и пиримидина.

Заключение. В большинстве штаммов *C. albicans*, выделенных от ВИЧ-инфицированных пациентов и устойчивых к флуканазолу и вориконазолу, выявлен ряд мутаций в гене *ERG11*. За исключением *V488I* обнаруженные мутации не имели сопряжения с повышенной экспрессией гена *ERG11* и снижали эффекты гиперэкспрессии гена *ERG11* до 100 раз, хотя полностью не отменяли исходной резистентности к триазоловым препаратам.

Ключевые слова: *Candida, ген ERG11, мутации, резистентность к антимикотическим препаратам, ВИЧ-инфекция*

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The widespread use of fluconazole in the prevention and treatment of *Candida* infection, especially among immunocompromised individuals, promoted the resistance to azole antimycotics among fungi of the genus *Candida* [1, 2]. *Candida* spp. frequently display overexpression of genes encoding the synthesis of the antimycotic drug target. An important role belongs to the *ERG11* gene encoding lanosterol 14-α-demethylase. This enzyme participates in the final step of the synthesis of ergosterol – an integral part of the fungal cell membrane and the target of azole antimycotics. Overexpression of the *ERG11* gene results in elevated ergosterol synthesis, thus decreasing the *Candida* species sensitivity to therapeutic dosages of antimycotics [3]. At the same time, fluconazole can promote the development of the mechanism of microbial drug resistance [4], which, in its turn, is especially high against short-chain azoles like fluconazole [5].

A number of mutations detected in the *ERG11* gene can modify the effects of its overexpression to a certain extent. They were associated both with an increase and with a decrease in resistance to azoles [6–10]. However, the findings reported by different laboratories and countries are highly contradictory.

The **purpose** of the study is to explore the spectrum and functional properties of *ERG11* gene mutations in fluconazole-resistant *C. albicans* strains isolated from HIV-infected patients.

Materials and methods

The study was performed using 10 *C. albicans* strains inherently resistant to fluconazole and voriconazole from the collection of the Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology of Rospotrebnadzor.

C. albicans strains were assessed by:

- the level of expression and the presence of mutations in the ERG11 gene encoding lanosterol 14-α-demethylase;
- sensitivity to a number of antimycotic drugs belonging to triazole, echinocandin, polyene, and pyrimidine groups.

The *C. albicans* strains were isolated from the oropharynx of HIV-infected patients aged 20–69 years, having clinical manifestations of oropharyngeal candidiasis and undergoing treatment in Infectious Disease Clinical Hospital No. 2 in Moscow. HIV infection in all the patients was diagnosed using clinical and epidemiological data and confirmed by the detection of specific antibodies/antigens using enzyme immunoassay and lysate-based immunoblot assay for antibodies against human immunodeficiency virus proteins (Profiblot 48 TECAN, AutoBlot 3000) in accordance with the clinical classification of HIV infection[1]. All the participating patients signed their informed consent allowing the use of the laboratory test data for scientific purposes. All studies were carried out with the approval of the Ethics Committee of the South Ural State Medical University (minutes No. 4, 25/4/2014) in accordance with the requirements of the Declaration of Helsinki adopted by the World Medical Association in 1964 and outlining the ethical principles for medical research involving human subjects.

Identification of *C. albicans* species was performed using different methods:

1. Approximate differentiation of fungi by colony color after incubation on specific chromogenic media (Oxoid, HiMedia) at 37ºC for 24–48 hours in accordance with the manufacturer's instruction;

2. Assessment of biochemical activity following the incubation of standardized cell suspensions in the wells of plates of the Remel RapID YEAST PLUS and ErbaLachema commercial biochemical test systems at 37ºC in accordance with the manufacturer's instruction. The results were measured visually or semi-automatically in each well and interpreted in accordance with the manufacturer's instruction or using the respective software.

3. Real-time multiplex polymerase chain reaction (real-time PCR) using the AmpliSens *C.albicans*/*C. glabrata*/*C. krusei* — MULTIPRIME-FL reagent kit for simultaneous hybridization-fluorescence detection of *C. albicans*, *C. glabrata,* and *C. krusei* DNA. DNA was extracted from *Candida* spp. pure cultures using DNA-sorb-AM reagent kits (Central Research Institute of Epidemiology of Rospotrebnadzor) in accordance with the manufacturer's instruction. The Applied Biosystems 7500 Real Time PCR System was used for amplification.

The sensitivity to echinocandins (anidulafungin, micafungin, caspofungin), azoles (posaconazole, voriconazole, itraconazole, fluconazole), amphotericin B, and 5-flucytosine was evaluated. The analysis was performed in accordance with the recommendations issued by the Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy for testing sensitivity of microorganisms to antimicrobial agents with reference to CLSI M44 and M60 standards for *Candida* spp. as well as standards and criteria of the European Committee on Antimicrobial Susceptibility Testing for the microdilution method and bacterial cultures[2].

The minimum inhibitory concentration of the agent (MIC; mg/ml) was measured by serial microdilutions using Sensititre YeastOne10 plates (Trek Diagnostic System) in accordance with the manufacturer's instruction. The inoculum was prepared in the similar way as for the

[1] Russian clinical classification of HIV infection. URL: https://base.garant.ru/12145892 (In Russ.)

[2] Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy. Determination of the sensitivity of microorganisms to antimicrobial drugs: Recommendations. 2021. URL: https://www.antibiotic.ru/minzdrav/category/clinical-recommendations/

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disk diffusion method, then it was placed into the modified RPMI-1640 medium and distributed into 96-well plates containing serial microdilutions of antimycotic substances [11]. The results were measured visually by comparing with the growth in the positive control well in accordance with the criteria of the European Committee on Antimicrobial Susceptibility Testing [12].

Levels of *ERG11* gene expression were measured by quantitative PCR and the $2^{-\Delta\Delta CT}$ method [13]. RNA was extracted from a pure daily culture of the studied strain using the ExtractRNA reagent (Evrogen) in accordance with the manufacturer's instruction. The reverse transcription was carried out using the Reverta-L kit (Central Research Institute of Epidemiology of Rospotrebnadzor) in accordance with the manufacturer's instruction: 30 min at 37ºC. The following primers were used for PCR:

ERG11:
- F — aactacttttgtttataatttaagatggactattga;
- R — aatgatttctgctggttcagtaggt;

PMA1:
- F — ttgaagatgaccacccaatcc;
- R — gaaacctctggaagcaaattgg;

ACT1:
- F — ttggtgatgaagcccaatcc;
- R — catatcgtcccagttggaaaca.

The amplification was performed using the reagent kit for real-time PCR in the presence of Sybr-Green I intercalating dyes (Syntol) and the Applied Biosystems 7500 Real Time PCR System in accordance with the following parameters: 95ºC, 3 min; 40 cycles at 95ºC, 10 sec, 55ºC, 20 sec.

The *ACT* and *PMA* housekeeping genes were used as control genes. The reference $2^{-\Delta\Delta CT}$ values for the *ERG11* gene were obtained by analyzing sensitive isolates (n = 7). The expression level of the studied strain was considered significantly increased, if it was higher than the reference mean values for sensitive isolates (m) by more than 3 standard deviations (3σ).

For Sanger sequencing of the ERG11 gene [14], the following primers were used:

ERG11-1:
- F — atggctattgttgaaactgtcatt;
- R — ggatcaatatcaccacgttctc;

ERG11-2:
- F — attggagacgtgatgctgctcaa;
- R — ccaaatgatttctgctggttcagt.

The *ERG11* gene was amplified for sequencing using the Qiagen PCR Master Mix, 2x reagent kit and the Applied Biosystems Veriti thermal cycler in accordance with the protocol: 95ºC for 15 min; 35 cycles at 95ºC for 40 sec, 60ºC for 40 sec, 72ºC for 1.5 min; then at 72ºC for 10 min. The PCR products were purified using the ExoSAP-IT kit (Thermo Fisher Scientific Inc.) in accordance with the manufacturer's instruction. The sequencing reaction was performed with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and the following parameters: 95ºC for 15 min, 35 cycles at 95ºC for 15 sec, 55ºC for 15 sec, 72ºC for 30 sec; 72ºC for 7 min. The products were further purified using the BigDye Xterminator Purification Kit (Applied Biosystems); the Applied Biosystems 3500 genetic analyzer (Applied Biosystems) was used for sequencing.

Microsoft Excel, SciPy [15], Matplotlib [16] software was used for the statistical analysis and data visualization. The significance of differences between groups was assessed using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The significance level for the statistical hypothesis test was set at $p < 0.05$, the value universally practiced in medical research. The strength of relationships between variables was measured using the Pearson correlation coefficient.

Table 1. Mutations identified in the *ERG11* gene

Mutation	Abs.	%
E266D	4	40
G464S	2	20
I471L	1	10
D116E	3	30
V488I	3	30

Table 2. Associations of mutations identified in the *ERG11* gene

Mutation	Abs.	%	Association coefficient
E266D + G464S	1	10	0,100
E266D + D116E	2	20	0,356
E266D + V488I	1	10	0,089
V488I + I471L	1	10	0,409
V488I + D116E	1	10	0,045

Results

The study showed that 7 (70%) *C. albicans* strains had 5 mutations in the *ERG11* gene, which were identified as *E266D*, *G464S*, *I471L*, *D116E,* and *V488I*. The highest frequency of occurrence was demonstrated by the *E266D* mutation, while the *I471L* mutation was detected most rarely (**Table 1**). The total number of mutations was 13.

Compound, two-component mutations were carried by 6 (92.3%) strains (**Table 2**). The highest tendency toward forming compound mutations was demonstrated by *E266D* and *V488I* – 3 (30%) mutations in each. No noticeable association between mutations was observed — the correlation coefficient was 0.410 or lower.

Overexpression of the *ERG11* gene was detected in 60% of the tested *C. albicans* strains. The detected mutations occurred much more frequently in strains with overexpression of the above gene (**Table 3**). In the meantime, the statistical analysis did not reveal

any significant associations between them. At the same time, the *V488I* mutation demonstrated a strong negative relationship with the overexpression of the *ERG11* gene ($r = –0.845$; $p < 0.05$).

The results of the analysis of the association between the mutations and the sensitivity to antimycotic agents are presented in **Table 4**, showing that MIC in strains carrying some mutation was approximately equal to or significantly lower than MIC in strains without mutations. The noticeably significant difference was demonstrated by the sensitivity to azole antimycotics, MIC of which was 100 times lower in mutation carriers ($p < 0.05$) compared to the strains without mutations.

Among triazoles, significant differences were demonstrated by posaconazole and itraconazole, MIC of which in mutation carriers was 100 times as low ($p < 0.05$) as MIC in strains without mutations in the *ERG11* gene. Furthermore, MIC of these agents was on average 16.5 times as low as MIC of voriconazole and fluconazole ($p < 0.001$). Among the detected mutations, the *G464S* mutation deserves close attention: In its carriers, MIC of triazoles decreased less significantly than with other mutations ($p < 0.05$). The correlation analysis did not reveal any relationship between the chemical structure and molecular weight of the triazole agent and the presence of mutations.

The presence of mutations in the *ERG11* gene did not have any significant effect on MICs of the tested echinocandins, amphotericin B, and 5-flucytosine. However, in carriers of the *G464S* mutation, MICs of anidulafungin, caspofungin, and amphotericin B tended to shift insignificantly towards resistance ($p > 0.05$).

Discussion

During our molecular and genetic study of *C. albicans* strains that were inherently resistant to fluconazole and voriconazole, we detected high occurrence of overexpression of the *ERG11* gene as well as a number of mutations in the above gene: *D116E*, *E266D*, *G464S*, *I471L,* and *V488I*. The *E266D* mutation was most frequently detected in our subset of *C. albicans*. The above mutations were described previously; however, they are not ubiquitous [17–26]. Since all strains were viable, we concluded that the location of these mutations did not affect critical regions of the genome, and they were not lethal.

Hypothetically, the gene overexpression must create favorable conditions for mutation or recombination process. However, as our findings show, the mutations in the *ERG11* gene are not associated with its overexpression. Moreover, in most cases, the overexpression of the gene and its *V488I* mutation occurred discordantly. We can assume that the occurrence of the *V488I* mutation disables the ability of the gene to multiply.

One of the characteristics of the detected mutations was their co-occurrence. The co-occurrence of *E266D* and *G464S* mutations was previously described by researchers from China, the Unites States, and some other countries [7, 20, 25, 27–30]. In the meantime, based on the low likelihood of the linkage between individual mutations, the above co-occurrence should be seen as a random event. It means that, most likely, mutations are not linked with each other, i.e. they emerge independently in various regions of the gene, and their location does not depend on anything.

The continuous use of azole agents in treatment of HIV-infected patients with oropharyngeal candidiasis puts strong pressure on the *C. albicans* population, which starts accumulating resistant strains, including strains with overexpression of the *ERG11* gene. Functionally, this mechanism promotes the synthesis of the azole target. At the same time, nonsynonymous mutations in the *ERG11* gene lead to modification of the target molecule and, consequently, to altered affinity of antifungal agents to their target [21]. As a result, the effects of gene overexpression are reduced. This phenomenon was pointed out when strains with mutations *D116E*, *G464S,* and *E266D* were studied [5–7, 9, 10, 17, 22, 24, 31–36]; these mutations were found to be associated with a manifold increase in MIC of azole agents. At the same time, the *V488I* mutation as well as *E266D* and *D116E*, as demonstrated by some studied, remained neutral and had no effect on MIC [4–6, 9, 32, 37]. It is believed that this mechanism may remain idle if overexpression of the *ERG11* gene is absent [20].

Compared to studies of other researchers cited above, all the mutations detected in our study were associated with increased sensitivity to triazole agents

Table 3. Association of mutations in the *ERG11* gene with its hyperexpression

Mutation	Strains with overexpression of the gene		Strains without overexpression of the gene		Association coefficient
	abs.	%	abs.	%	
E266D	3	75,0	1	25,0	0,251
G464S	1	50,0	1	50,0	0,457
I471L	1	100,0	0	0,0	–
D116E	2	66,7	1	33,3	0,094
V488I	1	33,3	2	66,7	–0,845
The sum	8	61,5	5	38,5	0,089
Combined	4	66,7	2	33,3	0,251

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Table 4. Relationship of mutations in the *ERG11* gene with the sensitivity of *C. albicans* to antimycotic drugs

Mutation		*n*	Anidulafungin	Micafungin	Caspofungin	Posaconasole	Voriconasole	Itraconazole	Fluconazole	Amphotericin B	5-Flucitosine
The sum	+	12	0,03 ± 0,003	0,012 ± 0,001	0,08 ± 0,009	0,043 ± 0,019	1,083 ± 0,393	0,082 ± 0,038	33,333 ± 10,130	0,708 ± 0,074	0,065 ± 0,005
	–	28	0,041 ± 0,003	0,013 ± 0,001	0,086 ± 0,006	3,471 ± 1,117	4,036 ± 1,067	6,941 ± 2,234	98,857 ± 20,285	0,768 ± 0,048	0,066 ± 0,004
E266D	+	4	0,026 ± 0,004	0,012 ± 0,002	0,075 ± 0,015	0,023 ± 0,004	1,375 ± 0,875	0,038 ± 0,008	28,00 ± 12,000	0,75 ± 0,144	0,06 ± 0,000
	–	6	0,045 ± 0,007	0,013 ± 0,001	0,09 ± 0,013	4,057 ± 2,716	4,333 ± 2,635	8,113 ± 5,432	113,333 ± 48,637	0,75 ± 0,112	0,07 ± 0,010
G464S	+	2	0,045 ± 0,015	0,012 ± 0,004	0,12 ± 0,000	0,133 ± 0,118	2,25 ± 1,750	0,265 ± 0,235	96,00 ± 32,000	1,00 ± 0,000	0,06 ± 0,000
	–	8	0,036 ± 0,006	0,012 ± 0,001	0,075 ± 0,010	3,021 ± 2,100	3,375 ± 2,028	6,038 ± 4,201	75,00 ± 39,509	0,688 ± 0,091	0,068 ± 0,007
D116E	+	3	0,025 ± 0,005	0,01 ± 0,002	0,06 ± 0,000	0,03 ± 0,000	0,50 ± 0,000	0,05 ± 0,010	16,00 ± 0,000	0,667 ± 0,167	0,06 ± 0,000
	–	7	0,043 ± 0,006	0,013 ± 0,001	0,094 ± 0,012	3,477 ± 2,368	4,286 ± 2,228	6,954 ± 4,735	106,286 ± 41,705	0,786 ± 0,101	0,069 ± 0,009
V488I	+	3	0,03 ± 0,000	0,013 ± 0,002	0,08 ± 0,020	0,025 ± 0,005	0,50 ± 0,000	0,05 ± 0,010	16,00 ± 0,000	0,50 ± 0,000	0,08 ± 0,020
	–	7	0,041 ± 0,007	0,012 ± 0,001	0,086 ± 0,012	3,479 ± 2,367	4,286 ± 2,228	6,954 ± 4,735	106,286 ± 41,705	0,857 ± 0,092	0,06 ± 0,000

Note. "+" — mutation is present, "–" — mutation is absent.

unlike the strains without mutations, though the tested *C. albicans* strains were resistant. Only the *G464S* mutation was slightly slow in manifestation of these properties. It can be assumed that the detected mutations significantly affected the structure of the site of interaction between the target molecule and triazoles, which reduced their affinity. At the same time, we did not find any association between MIC in mutant strains and the chemical structure of the therapeutic agent, though overexpression of the *ERG11* gene was more efficient against short-chain azoles [3]. The detected mutations had no effect on the sensitivity of the tested strains to echinocandins, amphotericin B, and 5-flucytosine.

In our study, mutant *C. albicans* strains showed higher sensitivity to itraconazole and posaconazole than to voriconazole and fluconazole. This difference may be associated with the rare administration of the first two drugs for treatment of HIV-infected patients and with the targeted selection of strains by their resistance to the last two drugs.

Thus, most of the studied *C. albicans* strains, which are resistant to fluconazole and voriconazole, had mutations in the *ERG11* gene: *D116E*, *E266D*, *G464S*, *I471L,* and *V488I*, which, except for the *V488I* mutation, are not associated with the overexpression of the above gene. The detected mutations decreased the effects of *ERG11* gene overexpression up to 100 times, though they did not eliminate the inherent resistance to triazole antimycotics and did not affect the sensitivity to echinocandins, amphotericin B, and 5-flucytosine.

It should be remembered that the studied strains were isolated from HIV-infected patients – permanent residents of Moscow. Therefore, the obtained results should be interpreted taking into account the specific features of the Moscow Region. The absence of any firm conclusion about the effects of *ERG11* gene mutations necessitates further research, including clinical studies.

Conclusions

1. *D116E*, *E266D*, *G464S*, *I471L,* and *V488I* mutations are detected in the *ERG11* gene in *C. albicans* strains isolated from HIV-infected patients – residents of Moscow.

2. Except for *V488I*, the detected mutations do not have any association with the *ERG11* gene overexpression.

3. *C. albicans* strains – mutation carriers – were up to 100 times more sensitive to triazole antimycotics. The presence of mutations had no effect on the sensitivity to echinocandins, polyene, and pyrimidine.

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