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Mini Review

Molecular Determinants of Isoniazid Drug Resistance *Mycobacterium tuberculosis*

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Abstract

Drug-resistant TB (TB) is a serious global problem, not only complicates the treatment of patients with resistant strains of TB, but also poses a threat to the global process to achieve the goals of the TB elimination strategy in the world by the World Health Organization (WHO) [1]. WHO estimates that 1.674 million people died of tuberculosis in 2016 alone, while between 2000 and 2016 they were healed 52.5 million lives thanks to improved diagnosis and treatment. At the same time, 13 of the 22 highest burden countries failed to meet the target for the decline in tuberculosis prevalence in 2014, highlighting the need to improve strategies to combat the disease [2]. Effective TB control is especially challenging among patients with multidrug-resistant TB (MDR-TB), which are resistant to at least isoniazid and rifampicin, the two most potent anti-TB drugs used in standard first-line treatment. For example, 2015 data show that by the end of 2014, 153 countries had reported the circulation of drug-resistant TB strains in the region, eighty of which have continuous surveillance systems, while others rely on epidemiological surveys. In the absence of an effective vaccine against tuberculosis, appropriate antibiotic therapy remains the most important tool in the fight against the spread of tuberculosis. N on at the same time, the factors of resistance, greatly exacerbating transmission from TB that are resistant to the first drug and the second line and show the importance of broader molecular genetic research in this area [1-5].

Keywords: *Tuberculosis; Drug Resistance; Molecular Mechanisms of Development of Mycobacterial Resistance; Gene Mutations*

With regard to TB drug resistance, there is a distinction between true genetic and acquired resistance. The true genetic stability, being a species attribute ohm pathogen related to the absence of the target or antibiotic action, poor cell wall permeability, in some cases, manifested as efflux. Thus, *M. tuberculosis* has true genetic resistance to many nonspecific antimicrobial drugs belonging to the families of penicillins, β-lactams, macrolides, carbapenems, cephalosporins, tetracyclines. Acquired drug resistance of TB is formed due to the development of point mutations in chromosomes and the formation of new genes that control the synthesis of new protein-enzymes that destroy or inactivate specific anti-TB drugs. Acquired drug resistance is subdivided into primary and secondary. Primary drug resistance is determined in patients infected with drug-resistant strains of *Mycobacterium tuberculosis*, despite the fact that these patients have not previously taken anti-tuberculosis drugs. Secondary drug resistance develops in the course of treatment of a patient with tuberculosis, the development of this phenomenon takes 3 - 6 months from the start of therapy [6].

As time shows, one of the important tasks that needs to be solved today, along with others, is the study of the genome of "problem" clinical isolates of *Mycobacterium tuberculosis*. The issues of drug resistance of TB strains, as well as the peculiarities of the mechanisms of its development, became especially relevant after the complex nature of genome changes was clarified. As for the complete decoding of

the genome of *Mycobacterium tuberculosis*, which contains about 4000 sequences encoding proteins and more than 70% of them are fully studied, in contrast to gene mutations associated with the development of resistance.

A special threat to patients is posed by multidrug resistant strains simultaneously resistant to isoniazid and rifampicin. It is known that diseases caused by such isolates of *M. tuberculosis* are acutely progressive and difficult to treat. Thus, scientific publications contain a large number of results of analysis of clinical isolates of MDR-TB with multiple gene mutations, some of which also concern isoniazid. This drug is included with the list of vital and essential medicines, would l first synthesized in 1912 a broad clinical application of which began only in 1952. Since then, isoniazid has become the most widely used drug in the treatment of tuberculosis caused by drug-susceptible strains of mycobacteria, along with rifampicin and pyrazinamide. In addition, prophylactic isoniazid monotherapy has been used in the treatment of latent tuberculosis. Inhibition of the synthesis of mycolic acids, a very important component of the cell wall of *M. tuberculosis*, by isoniazid leads to deprivation of acid resistance. The presence of mycolic acids in *M. tuberculosis* makes it resistant to many types of drug treatment, and their synthesis is absolutely essential for the survival of this pathogen. A number of studies have shown that isoniazid exerts its action against active mycobacteria in the presence of oxygen. The drug is not active in anaerobic conditions against bacteria in a latent state. In addition, considerable importance is the temperature, so at 37°C the activity is enhanced and reduced at 4°C, which shows the relationship isoniazid with enzymatic active NOSTA a bacterial cell. Isoniazid being a precursor of the active ingredient, in fact pro drug further manifestation of the antibacterial activity of which depends on activation within the bacterial cell enzyme and cat Alazeyas - peroxidases (KatG) [7].

Like other anti-TB drugs have of *Mycobacterium tuberculosis* resistance isoniazid appeared soon after its introduction into clinical practice and has achieved relatively high rates over the past two decades. Several intracellular targets of this drug are known - a complex of enzymes involved in the synthesis of mycolic acids. Mutations in the genes encoding these proteins (inhA, acpM, and kasA) can induce isoniazid resistance (INH).

Along with this, the resistance of mycobacteria to isoniazid occurs due to the overproduction of targets for the action of the active forms of the drug. For example, proteins involved in the transport of mycolic acid precursors and its biosynthesis: acylated carrier protein (acpM gene), synthetase (kasA gene) and reductase (inhA gene) of the carrier protein. Most of the mutations are detected in the promoter regions of the listed genes. The degree of resistance associated with overproduction of targets is usually lower than with mutations in the catalase-peroxidase genes. As noted in the scientific literature, the overall prevalence of isoniazid resistance, alone or in combination with other drugs Preview creases 13%, which means that every seventh case of tuberculosis may be caused by resistant strains to isoniazid. Consequently, this poses a serious threat to the governance and control of tuberculosis worldwide [8,9].

Today, there are quite a few scientific publications, where the main role is attributed to mutations at several loci of the genome of *Mycobacterium tuberculosis*, including katG, inhA (with its promoter region mabA-inhA), kasA, ahpC (with an upstream regulatory region oxyR-ahpC), ndh, nat and mshA. Most of the research in recent years has been based on mutation detection, DNA sequencing, hybridization on DNA chips, real-time PCR, etc [8].

According to scientific publications, the drug molecule is activated inside a microbial cell by the enzyme catalase- peroxidase (katG gene). Mutations in the katG gene (at position 315) lead to a decrease in the enzyme activity by about 50%, being the most common cause of resistance. So, Mr. eneticheskie studies conducted in the early 1990's, completely explain yayut a connected s between catalase activity of mycobacteria and their resistance to isoniazid. In the last century Y. The Zhang with colleagues [10] in his experiments proved that the transformation of INH-resistant strains of *Mycobacterium smegmatis* and *M. tuberculosis*, with is polz ment is functional the gene and katG answering it for encoding protein catalase- peroxidases (KatG), restores the sensitivity of these strains to INH. Therefore, these studies confirm the important role KatG in biological activity to isoniazid and involves camping, that and isoniazid is a prodrug [6,9]. Next, after the penetration INH after a number of metabolic processes of pharmacologically active derivative forming adducts with a molecule of the

coenzyme NAD + or NADP + acts as an inhibitor of the enzyme involved in the biosynthesis of nucleic acids, as well as mycolic acids, which are components of the cell wall of *Mycobacteria* [11]. In this case, at the end of the twentieth century have been published works, where they tried to prove that one of the main mechanisms of resistance to isoniazid is deletion of gene katG. This hypothesis has not been supported by numerous subsequent studies. So, Jagielski, T. With co-authors in his article confirms that complete deletion of the katG gene is rare and usually involves strains with a high level of resistance to INH (MIC > 5 μg/ml). As the authors point out, the main role in the development of INH resistance is played by spontaneous mutations in this gene, in the form of single-point mutations (missense mutations) or small (from 1 to 3 nucleotides) insertions or deletions. Proof of this is the results of research work obtained in 2004 by Polish scientists on the study of mutations in the katG gene in 46 museum strains of mycobacteria resistant to isoniazid. According to published data, the most common mutation was the replacement of codon 315, which was found in 34 (74%) strains out of 43 (93%) *M. tuberculosis* MDR strains tested. The researchers believe that the presence of mutations in this codon may serve as a predictive factor for INH resistance. As shown by the authors, among detected 16 different mutations at the nucleotide sequence of katG, 14 (87.5%) resulted in a change of amino acids in claim oligopeptide second circuit KatG (missense mutation) [9].

It should be noted that the issues of gene mutations associated with resistance of mycobacteria have been closely studied since the end of the last century. As indicated by sources, it was revealed more than 300 different mutations in the katG. At the same time, in most scientific publications, mutations in codon 315 of the katG gene are noted. In addition, one particular amino acid substitution (serine to threonine) accounts for 95% of all mutations in katG 315 [12]. Mutations in katG are associated with a wide range of moderate to high isoniazid resistance, above the commonly tested concentrations of 0.2 and 1 mg/l in solid, 0.1 and 0.4 mg/l in liquid nutrient medium. In terms of molecular mechanisms, many authors have argued that, in addition to katG mutations, isoniazid resistance results from mutations in the inhA promoter region. This phenomenon leads to overexpression of the isoniazid target (InhA), which requires higher doses of the drug to achieve complete inhibition. Results Most studies show that m. utatsii in the promoter region inhA tend to lead to low phenotypic stability, and also confer resistance to second line medications ethionamide and protionamide [13,14]. Also, there are a number of works, where n he most common mutation in the promoter region inhA believe mutation c-15t, which is present on average in 19% of isoniazid resistance of clinical isolates all over the world. According to many modern authors, in addition to the two most common causes of isoniazid resistance, mutations in genes that regulate katG expression (for example, the intergenic region furA-katG and sigI) and in the inhA coding region, there are also mutations involving inactivation of isoniazid, a change in redox potential, changing biosynthesis mikotiol well and excretion of the drug formulation via pump governmental pumps [12,14-16].

Raising the issues of resistance to known mycobacterial preparations, it should be noted that one of the new and little understood problems in the clinical and epidemiological assessment of the etiological agent in tuberculosis infection is the characteristic of the so-called Peking genotype. It should also be noted here that the use of genotyping methods in epidemiological studies (24-locus MIRU-VNTR typing, spoligotyping, etc.), based on the analysis of repetitive elements, made it possible to identify genetic differences between mycobacterial strains both within the same region and in different countries. In a comparative analysis of the total genomic sequences were identified differences between the strains at the level of single nucleotide (single nucleotide polymorphisms from Eng. Single-Nucleotide Polymorphisms; SNP) and over extended fragments (large-sequence polymorphisms; LSP). Thus, it was proposed to use SNP and LSP analysis for family-specific typing and study of mutations leading to the formation of drug resistance. As practice shows, the clinical manifestations of this infectious disease caused by this genotype are extremely unfavorable. At the same time, in many publications it was noted that extrapulmonary forms of this disease are significantly more common in patients with the Peking strain. The history of the discovery of representatives of the Beijing family (Beijing genotype), originally known as the W-strain, begins in the 90s of the last century. In earlier scientific publications, strains of the Peking genotype are divided into four groups: endemic, in which no reliable association of the genotype with drug resistance has been found; epidemic related to drug resistance; epidemic, not related to drug resistance; rare or undetected. There are many publications on the study of the distribution of this genotype. More recent works indicate distribution on a clearly in e data genotyping isolated ix 9 genetic families *Mycobacterium tuberculosis* complex, of which the most common in China, Japan,

Southeast Asia, the FSU are strains genetic family Beijing, propagation frequency of which in these regions varies from 30 to 70%. Thus, according to the SITVIT international database (http://www.pasteurguadeloupe.fr:8081/SITVIT_ONLINE/) [17,18], genotype strains are characterized by the SIT 1 spoligotype (from the English Spoligotype International Type, international spoligotype), which is determined by the presence in the DR locus of 9 out of 43 43 spacers (from 35 to 43). Today it is known that from 2 to more than a dozen groups are distinguished within the genotype, the differentiation of which is often very conditional and depends on the chosen typing method. So, in scientific practice, the division of representatives of this genotype into atypical/ancient and typical/modern strains is accepted, while the specificity of modern strains is the deletion of RD150 and/or RD142, as well as the presence of a repeating element IS6110 in the NTF region [19]. According to the international spoligotyping database SpolDB4, Beijing strains are present in the largest number of countries at the global level (13% of the world number of isolates), being a unique genotype for this indicator. The increased interest in the Beijing genotype is due to the fact that it has the ability to spread rapidly, can have a rapidly progressive course, cause disseminated forms of tuberculosis, leads to active bacterial excretion, has the property of high mutational variability; among strains of the Beijing genotype, broad drug resistance and multiple drug resistant options.

As for research work on assessing the population structure of the causative agent of tuberculosis, identifying the main genotypes and their subtypes responsible for the formation of multiple resistance in neighboring countries, for example, in Russia, there are a fairly large number of publications. In particular, Zhdanova SN., *et al*. [20] studying the distribution of these isolates in the Irkutsk region in their publications reports that in South America and in some of the nearest neighbors of the former Soviet Union/Russia, such as Bulgaria and Romania, non-indigenous isolates of the Beijing BL7 (MIT 642) and S (MIT 256) genotypes, which retained their epidemic significance in the vast territory of North Asia and adjacent regions of Asia and Europe, as well as the establishment of their role in the formation of high levels of multidrug resistance prevalence [21,22].

The situation is different on the European continent. The spread of the Peking genotype in this region is characterized by a significant diversity in the number of detected strains of this genetic family. So, for a number of countries: Russia, Ukraine, Kazakhstan, Uzbekistan, Turkmenistan, Kyrgyzstan, Azerbaijan, Latvia, Estonia, Armenia, Georgia, the share of the Pekin genotype was approximately 50%. At the same time, in neighboring countries such as Poland, Finland, Bulgaria, Turkey, its share did not exceed the conditional threshold of 10%. It is possible that the apparent "mosaicism" of the distribution of the genotype "Beijing" in a number of European countries is a manifestation of factors that were previously unknown and associated only with certain countries [23,24]. According to the publications of the Belarusian scientists, among strains circulating on the territory of Belarus, the most frequently observed mutations in the gene for the katG - at codon 315, simultaneously, MBT isolates isolated from patients, mutations leading to MDR MBT were also more often detected in the 315th codon of the katG gene, less often in the -15th and -8th codons of the inhA gene [25].

Proof of it is, holdings molecular genetic study by the VNTR-typing of isolates of *Mycobacterium tuberculosis* isolates from 46 patients with pulmonary tuberculosis, living in Astana (Kazakhstan). So, Kazakh e colleagues have shown the prevalence among isolates to 70% (32 isolates) representatives of the Beijing family. During the study, 23 different genetic profiles were identified, with the identification of mutations in the 315th codon of the KatG gene and in the 531st codon of the rpoB gene with amplification of the fragment containing the 315th codon of the katG gene and the fragment containing the 531st codon of the gene rpoB. As noted by the authors, the cluster of isolates belonging to the Beijing family had significant genetic homogeneity. The group of scientists emphasizes that both resistant and anti-TB drug-sensitive isolates of the Beijing family were encountered in this sample, and no association of this family with drug resistance was shown, which indicates the individual characteristics of these isolates circulating in a particular area [26]. More recent work of 2019, another group of scientists of Kazakhstan and also confirming etsya above presentation of the facts. Thus, evaluation of the spectrum of mutations in the gene katG, promoter regions fabGinhA, oxyR-ahpC and rpoB responsible for drug resistance of *M. tuberculosis* to isoniazid and rifampicin and genetic definition of families of 103 multiresistant clinical isolates of *M. tuberculosis* common in Kazakhstan was conducted by the method of Sanger and spoligotyping. The research results showed that among all studied isolates, mutations prevailed in codon 531 of the Ser → Leu rpoB gene, amounting to 87.4% and in codon 315 of Ser → Thr katG of the gene 97%, respectively, causing

resistance to rifampicin and isoniazid. Thus, more than 80% of multidrug-resistant *M. tuberculosis* strains were classified as the most virulent and widespread genotype in the world, Beijing [27].

However, many aspects of molecular mechanism and activity of INH is still unknown. Combinations of mutations in the katG and the promoter region inhA provide a high level of resistance (MIC > 10 mg/l). However, these variations do not explain all of the phenotypic heterogeneity observed for katG mutations. Typically, further research in this direction is investigating other (combinations of) genetic mutations that contribute to the wide spectrum of MICs observed among isoniazid-resistant *Mycobacterium tuberculosis* isolates [28,29].

Conclusion

Summarizing the above, it should be noted that modern molecular genetic methods for typing the causative agent of tuberculosis make it possible to see that, despite the genetic heterogeneity of the population of mycobacteria within each geographic territory, epidemically significant families of mycobacteria can be identified that pose the greatest danger. With regard to data the Republic of Uzbekistan, regional strains in this respect are poorly understood and needs of a deployed full genomic research. Existing scientific publications mostly cover clinical aspects and partly diagnostics of various forms of tuberculosis in the region.

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