PERSPECTIVES FOR OPTIMIZING THE MOLECULAR GENETIC LABORATORY IN EARLY DIAGNOSIS AND PREDICTION OF MULTIFACTORIAL DISEASES IN WOMEN AND CHILDREN

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Abstract
The widespread use of molecular genetic methods in multifactorial diseases makes it possible to improve the prediction of pathology long before the onset of clinical signs, which significantly helps in the prevention of the disease. Based on the study of molecular genetics and functional char-acteristics of children with acute obstructive pulmonary disease (AOB) and recurrent bronchial obstruction (RBO) with a predisposition to bronchial asthma (BA), the optimization of early diagnosis and medical rehabilitation measures was carried out. The distribution frequencies of alleles and genotypes of the Arg16Gly (rs1042713) and Gln27Glu (rs1042714) locus of the β2-adrenergic receptor (ADRB2) locus, children with RBO, OOB, AD, and healthy children were studied by real-time PCR using the SNP-express-SHOT reagent kit. on modern equipment "Rotor Gene 6000/Q". The need for specialized molecular genetic laboratories with certified equipment based on multidisciplinary clin-ics is a promising direction in optimizing the early diagnosis of diseases in children.

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At the present stage of development of medicine, the use of genetic examination meth-ods in clinical practice and understanding of the molecular genetic mechanisms of child-hood pathology is an urgent and necessary problem in pediatrics. Early diagnosis of child-hood pathologies based on the determination of hereditary genetic factors is not only an ur-gent medical, but also a socio-economic prob-lem. Medical genetics, aimed at early detection and prevention, has received particularly great development in the world in recent years due to the widespread introduction of new molecu-lar genetic technologies. The use of molecular genetic methods in multifactorial diseases makes it possible to improve the prediction of pathology long before the onset of clinical signs, which significantly helps in the imple-mentation of disease prevention [2,4,7].

The widespread use of genetic diagnostic methods makes it possible to improve the di-rection of pharmacogenetics and a personal-ized approach to patient therapy. Currently, clinical practice does not have a sufficiently wide range of laboratory diagnostic methods that allow not only diagnosing diseases, monitoring therapy, but also monitoring treatment. Until recently, laboratory diagnostic methods used in clinical practice had one common drawback - they did not take into account the patient's predisposition to various diseases ac-cording to genetic factors. Questions of the pa-tient's predisposition to various diseases under-lie a new direction of medicine - personalized medicine, which can be defined as a strategy, prevention and treatment of diseases based on the results of molecular genetic studies.

Due to to scientific research, it became known that genetic polymorphisms play an im-portant role in the development of various dis-eases - genome changes that occur in the hu-man population in at least 2 variants (alleles) with a frequency of at least 1%. The most com-mon type of genetic polymorphism is single nucleotide substitutions (SNPs), which are ge-netically unique to each individual [3,5,8]. Some polymorphic variants of genes (“suscep-tibility genes”), under certain unfavorable con-ditions, can contribute to the development of multifactorial diseases. Combinations of al-lelic variants of various genes that provide a normal metabolic process or are involved in the development of a specific pathology are called "gene networks". Elucidation of the components of the gene network of each mul-tifactorial disease, the development on this ba-sis of a set of preventive measures for a partic-ular patient form the basis of predictive medi-cine.

Abroad, at present, molecular diagnostic technologies are being developed, improved and introduced into clinical practice. So, even now clinical laboratory diagnostics has a wide range of methods based on the detection and diagnosis of nucleic acid analysis methods - polymerase chain reaction (PCR), genotyping, biochips, sequencing, etc. Currently, using the PCR method in Russia and abroad, methods for studying the human genome have been de-veloped - sequencing according to Sanger, Ed-man, pyrosequencing. The purpose of these studies is to determine the sequence of nucleo-tides. The pyrosequencing method based on the principle of “sequencing by synthesis” is already being introduced into clinical practice. When a nucleotide is included in the DNA chain under study, pyrophosphates are re-leased, then a chain of chemical reactions oc-curs with their participation,

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which leads to the formation of light quanta. The intensity of the glow is determined by a special device [6,9]. A series of reagents have been developed in sci-entific laboratories around the world to deter-mine predisposition to diseases of bronchial asthma, the cardiovascular system (arterial hy-pertension, myocardial infarction), diabetes mellitus, obesity, osteoporosis, etc. Upon re-ceipt of this information, the doctor can de-velop individual recommendations for prevention for the patient, and the patient, in turn, can take timely measures and thereby prevent the development of the disease.

PCR is one of the few methods of labor-atory diagnostics currently used in clinical practice, characterized by the highest specific-ity and sensitivity in the detection of many dis-eases. PCR has the following advantages over other methods of clinical laboratory diagnos-tics: universality, high specificity (up to 100%) of the method is due to the fact that due to the selection of specific primers, a unique DNA or RNA fragment is determined that is character-istic only for this pathogen; high sensitivity (at present, the sensitivity threshold of some am-plification test systems allows you to deter-mine single copies in the test sample; high manufacturability and automation of the method allow you to get the results of the study in the hands of the doctor and the patient on the day of the study; analysis is possible in a min-imum sample volume, which is extremely im-portant in neonatology, forensic medicine, clinical genetics, etc.; the possibility of simul-taneous diagnosis of several pathogens or ab-normal genes in one sample without compro-mising the sensitivity or specificity of the test result [2,4,7,8].

In the Central Asian region, the PCR method, in most cases, has private clinics that have the necessary equipment and high prices for genetic analysis. Given these circum-stances, most medical institutions that use PCR diagnostics and diagnostics of genetic predis-positions in their work tend to equip their own laboratories with appropriate equipment.

In the conditions of the 1st multidiscipli-nary clinic of the Tashkent Medical Academy, a research work (R&D) was carried out using molecular genetic research methods. Despite the fact that significant changes are taking place in the field of medicine in Uzbekistan, in-depth molecular genetic studies for early diag-nosis and prediction of recurrent bronchial ob-struction (RBO) and bronchial asthma in chil-dren of the Uzbek population have not been carried out.

The role of genetic factors in the devel-opment of bronchopulmonary diseases (BLD), in particular in children with recurrent bron-chial obstruction, is one of the least studied problems. The results of studies by many sci-entists are mainly devoted to AD and are con-tradictory: in some studies, the significance of the influence of polymorphic alleles of the ADRB2 gene in the pathogenesis of AD, as well as in the formation of the response of pa-tients to therapy with β2-agonists, has been de-termined, and in other studies it has been shown that these polymorphic variants of the ADRB2 gene do not associated with BA. In foreign and domestic literature, great im-portance is attached to the role of polymorphic variants rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu) of the β2-adrenergic receptor ADRB2 gene in the pathogenesis of BA, bron-chopulmonary dysplasia, and COPD. There are no studies studying the association of Arg16Gly and Gln27Glu polymorphic variants of the ADRB2 gene in recurrent bronchial ob-struction in children. Authors Ponomareva M.S., Furman E.G., Khuzina A.M. (2015) in children with BA from the city of Perm found that a mutation in the ADRB2 gene in children with BA occurs 2 times more often in Arg16Gly polymorphism and 3 times more of-ten in Gln27Glu, compared with practically healthy children [6,12].

Abroad, a large number of scientific works are devoted to the analysis of the rs1042713 A>G polymorphic variant of the ADRB2 gene and its effect on the development of AD and the effectiveness of β2-agonist ther-apy (Figueiredo RG, 2021 [11]. Bliker Y., Dir-cye C., et al. (2012) studied the polymorphism of the ADRB2 gene for long-term therapy with β2-agonists in combination with inhaled glucocorticosteroids [10]. of European origin, the absence of an association of the polymorphic variant rs1042713Gln27Glu A/G of the ADRB2 gene with the frequency of exacerba-tions and indicators of respiratory function was revealed (E. Israel et al., 2010). Scientists from the Asyut Medical University of Egypt found that carriers of the heterozygous Arg16Gly allele group were good responders to β2-agonist therapy, and carriers of the G/G mutation gen-otype were poor responders (Heba S. E. et al., (2018). Scientists Srinivas B., Jyoti A. et al. (2015) analyzed the ADRB2 gene variant (Arg16Gly) with pharmacogenetic response and disease severity in South Indian asthmatics. receptor insensitivity after exposure to a β2-agonist.

In our Republic, a number of works have been carried out to study the clinical, im-munological and some genetic aspects of acute obstructive bronchitis (AOB) and BA in chil-dren. Thus, immunological and genetic ap-proaches have been studied in optimizing

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the treatment of BA in adolescents (Ziyadullaev Sh.Kh., 2014); the role of the T-31c polymor-phism of the Il-1β gene in recurrent bronchitis in children was determined [1]; revealed path-ogenetic features of the treatment, rehabilita-tion and prevention of acute respiratory dis-eases occurring with SBO in children (Khai-darova M.M., 2018).

Zakirova U.I. (2021), based on the study of molecular genetics and functional character-istics of children with recurrent bronchitis, with a predisposition to bronchial asthma, op-timized early diagnosis and medical rehabilita-tion measures. The distribution frequencies of alleles and genotypes of the Arg16Gly (rs1042713) and Gln27Glu (rs1042714) locus of the β2-adrenergic receptor (ADRB2) locus, children with RBO, OOB, AD, and healthy children were studied by real-time PCR using the SNP-express-SHOT reagent kit. on modern equipment "Rotor Gene 6000/Q" (Real-time CFX96 C1000 Touch) Bio-Rad (Germany).

Differences in the frequency and nature of occurrence of ADRB2 gene genotypes de-pending on gender differences were estab-lished; for the first time, the predictor role of the polymorphic locus rs1042713 (Arg16Gly(46G>A)) was determined in carri-ers of the G/G genotype of the ADRB2 gene, which was a genetic marker of the incidence of RBO and AD in children of Uzbek ethnicity; for the first time, the predictor role of the pol-ymorphic locus rs1042714 (Gln27Glu (79C>G)) of the A/G and G/G gen-otypes of the ADRB2 gene was established, which was a genetic marker of the incidence of RBO in children and a factor in the formation of asthma in children in children of Uzbek eth-nicity; children with the homozygous G/G gen-otype of both ADRB2 gene loci, as well as those with the heterozygous C/G genotype of the Gln27Glu ADRB2 gene locus, are at risk for severe RBO in children. an allele with pol-ymorphism of the Gly16/Glu27 locus of the ADRB2 gene in carriers of the homozygous A/A genotype provided protection against the development of AD and was associated with a milder course of RBO and AD in children. In children with RBO, high efficacy of salbuta-mol was found in the greatest amount in carri-ers of the A/G and A/A genotypes, while low efficacy was found in carriers of the G/G gen-otype of both variants of the ADRB2.

The practical use of the obtained results of the study determined the effectiveness of the use of molecular genetic data as an indicator for determining the hereditary burden to RBO and AD in children; determination of alleles and genotypes of the ADRB2 gene in the blood showed effectiveness for the early diagnosis of RBO and the prediction of BA in children; assessed the clinical significance of the ADRB2 gene polymorphism with a therapeutic re-sponse to β2-agonists for children with RD and BA of the Uzbek population for the correction of anti-relapse treatment; in children with re-current obstructive bronchitis scientifically ef-fective use of kinesiohydrotherapy (KHT) at the stage of long-term rehabilitation; devel-oped and proven the effectiveness of using the electronic program "Model for predicting bronchial asthma in children with recurrent bronchial obstruction.

Today, molecular genetic studies are an integral part of the diagnosis and choice of treatment in modern oncology. At present, it has become possible to develop drugs that act directly on the molecular target in the tumor cell without causing serious damage to other organs and tissues of the patient. The use of such drugs is called "targeted therapy". Of great importance is also the identification of hereditarily caused forms of cancer. The use of new drugs focused on a "point" effect on mo-lecular mechanisms necessitates the manda-tory identification of genetic disorders [3,7].

Abroad and in Russia, the detection of genetic disorders by real-time allele-specific PCR (PCR-HRM) makes it possible to rela-tively quickly detect and quantify mutations in oncogenes, which is necessary for prescribing a treatment regimen and monitoring the effec-tiveness of therapy. Using the pyrosequencing method, it is also possible to determine the most common mutations in the BRCA1 and BRCA2 oncogenes, which are the cause of he-reditary cancer of the breast, ovaries, pancreas, and prostate. The use of modern diagnostic ap-proaches to detect somatic mutations in onco-genes and determine the genetic predisposition to cancer development allows targeted treat-ment depending on the individual genotype of some types of cancer.

Current health problems require manda-tory verification of modern methods of molec-ular genetic analysis and equipment. The clos-est to practical application is the FISH method, which is already widely used to diagnose not only frequent chromosomal diseases, but also multifactorial diseases. It should be noted once again that, despite its seeming simplicity, as our many years of experience show, the appli-cation of the method requires not only appro-priate equipment (an ABI 3600 type se-quencer), but, most importantly, a highly qual-ified specialist with extensive experience in molecular genetic diagnostics. Even higher requirements for the qualification of a specialist are made by the technology of comparative ge-nomic hybridization (GHG) on chips.

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In addition to the experience of molecu-lar genetic testing, the application of this method requires the ability to work with the appropriate computer programs necessary for the correct interpretation of the results of the analysis of microchromosomal rearrange-ments. When analyzing chromosomal abnor-malities by molecular methods, specialists should adhere to the recommendations adopted by the European Cyto-genetic Society, and to describe the results obtained by FISH, non-in-vasive prenatal diagnosis (NIPD), SGG, etc., use the rules of the International System for Cytogenetic Nomenclature. However, the high cost of the test, even by US standards, failures due to in-sufficient amounts of fetal DNA in a pregnant woman's blood, a hitherto unknown false-negative rate, and the lack of standards are a serious obstacle to the use of genetic anal-ysis methods.

The widespread use of molecular ge-netic meth-ods is relatively accessible to any medical institution through the use of public la-boratories. The creation of new molecular ge-netic laboratories is necessary and timely in the Central Asian region. When working with mo-lecular genetic laboratories, but also with clin-ical diagnostic laboratories, we face a large number of problems at all stages of analysis. At the preanalytical stage, the use of the ser-vices of third-party laborato-ries often entails the need to use additional consuma-bles for the collection of biological material and the neces-sary reagents. Today, we are forced to aban-don the universal method of sampling biological mate-rial, which can lead to errors both on the part of clini-cians and at the stage of sorting and transporting bio-logical material: at the analyti-cal stage, there is no possibility of quality con-trol of the work performed and operational in-formation on the use of one or an-other imple-mentation method. Taking into account the need to provide the most accurate results, as the basis of a PCR laboratory, there is a Rotor-Gene Q instrument widely used in scientific and practical medi-cal institutions (QIAGEN, Germany; Fig. 1)

Fig.1 Rotor-Gene Q instrument (“QI-AGEN”, Germany)

This equipment is character-ized by the highest quality of data obtained, high performance and flexibil-ity.

A possible way to solve these problems in the Cen-tral Asian region is to organize spe-cialized molecular genetic laboratories with certified equipment on the basis of existing molecular research centers, as well as ongoing cycles to improve the skills of pediatricians in medical genetics and prenatal diagnostics.

When planning the center, it is necessary to pro-ceed from the following tasks:

- organization of a high-performance PCR diagnos-tic laboratory;
- organization of a genetic laboratory;
- maximum automation of all stages of the labora-tory process;
- use of "open" type equipment, which allows to reduce the dependence of the labora-tory on suppliers of test systems;
- full compatibility of methods for sam-pling biologi-cal material with those already used;
- maximum adherence to official instruc-tions and recommendations on the organiza-tion of laboratories working with molecular bi-ological methods in order to improve the safety and quality of services.

Thus, the creation and optimization of a molecular genetic laboratory in the early diag-nosis and predic-tion of multifactorial diseases in women and children is an integral part of medical education. It is known that, in addition to the listed difficulties, the creation of molec-ular genetic laboratories is associated with ad-ditional difficulties, both financial and organi-zational.

New technologies that have signifi-cantly in-creased the possibilities of perinatal diagnosis and make it more efficient and safer can significantly re-duce the natural genetic burden of hereditary pathol-ogy in the popula-tion.

At the same time, the introduction of mo-lecular genetic diagnostic methods creates cer-tain organiza-tional and methodological diffi-culties, making it nec-essary to make adjust-ments to the traditional method of patient ther-apy that has been established for many years.

Today, the Tashkent Medical Academy has all the conditions for organizing a labora-tory for motherhood and childhood, there is an appropriate material and technical base, trained personnel, young people striv-ing for science, pregnant women and children who need timely diagnosis and effective treatment of vari-ous diseases.

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