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Review article
Pregledni članak

MOLECULAR GENETIC MARKERS AS A BASIS FOR PERSONALIZED MEDICINE

MOLEKULARNO-GENETIČKI MARKERI KAO OSNOV ZA PERSONALIZOVANU MEDICINU

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Summary

Nowadays, genetics and genomics are fully integrated into medical practice. Personalized medicine, also called genome-based medicine, uses the knowledge of the genetic basis of disease to individualize treatment for each patient. A number of genetic variants, molecular genetic markers, are already in use in medical practice for the diagnosis, prognosis and follow-up of diseases (monogenic hereditary disorders, fusion genes and rearrangements in pediatric and adult leukemia) and presymptomatic risk assessment (*BRCA* 1/2 for breast cancer). Additionally, the application of pharmacogenomics in clinical practice has significantly contributed to the individualization of therapy in accordance with the patient's genotype and gene expression profile. Genetic testing for several pharmacogenomic markers (*TPMT, UGT1A1, CYP2C9, VKORC1*) is mandatory or recommended prior to the initiation of therapy. The most important achievement of genome-based medicine is molecular-targeted therapy, tailored to the genetic profile of a disease. Testing for gene variants in cancer (*BCR-ABL, PML/RARα, RAS, BCL-2*) is part of the recommended evaluation for different cancers, in order to achieve better management of the disease. The ultimate goal of medical science is to develop gene therapy which will fight or prevent a disease by targeting the disease-causing genetic defect. Gene therapy technology is rapidly developing, and has already been used with success. Although medicine has always been essentially »personal« to each patient, personalized medicine today uses modern technology and knowledge in the field of molecular genetics and genomics, enabling a level of personalization which leads to significant improvement in health care.

Keywords: gene therapy, molecular diagnosis, molecular genetic markers, molecular-targeted therapy, personalized medicine, pharmacogenomics

Kratak sadržaj

Genetika i genomika su danas potpuno integrisane u medicinsku praksu. Personalizovana medicina, poznata i kao medicina zasnovana na genomu, koristi znanja o genetičkoj osnovi bolesti da bi se individualizovalo lečenje svakog pacijenta. Veliki broj genetičkih varijanti, molekularno-genetičkih markera, već se koristi u kliničkoj praksi za dijagnozu, prognozu i praćenje bolesti (monogenska nasledna oboljenja, fuzioni geni i rearanžmani u pedijatrijskim i adultnim leukemijama) i presimptomatsku procenu rizika od obolevanja (*BRCA1/2* za kancer dojke). Osim toga, primena farmakogenomike u kliničkoj praksi značajno je doprinela individualizaciji terapije u skladu sa genotipom i profilom ekspresije gena pacijenta. Genetičko testiranje za nekoliko farmakogenomičkih markera (*TPMT*, *UGT1A1*, *CYP2C9*, *VKORC1*) obavezno je ili se preporučuje pre započinjanja terapije. Najvažniji doprinos medicine zasnovane na genomu je ciljana molekularna terapija, prilagođena genetskom profilu bolesti. Testiranje genetičkih varijanti u malignim oboljenjima (*BCR-ABL*, *PML/RARα*, *RAS*, *BCL-2*, *KIT*, *PDGFR*, *EGF*) doprinosi tačnijoj stratifikaciji različitih kancera i adekvatnom izboru terapije. Krajnji cilj medicinske nauke je da primeni gensku terapiju koja bi eliminisala uzrok bolesti ili prevenirala bolest, ciljajući genetički defekt koji leži u osnovi bolesti. Tehnologija koja prati gensku terapiju veoma se brzo razvija i već se uspešno primenjuje. Iako je medicina oduvek suštinski bila »personalizovana«, prilagođena svakom pacijentu, personalizovana medicina danas koristi modernu tehnologiju i znanja iz oblasti molekularne genetike i genomike, omogućujući stepen personalizacije koji vodi ka značajnom napretku medicinske prakse.

Ključne reči: genska terapija, molekularna dijagnostika, molekularno-genetički markeri, ciljana molekularna terapija, personalizovana medicina, farmakogenomika

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Personalized medicine

Nowadays, it is thought that virtually all human diseases, except perhaps trauma, have a genetic component. Genetic information is stored in the DNA molecule. Certain portions of DNA are unique to each individual. Any two unrelated people are 99.9 percent identical at the genetic level, with 0.1% being different and making us all individuals (genetic variation). Genetic variation influences every aspect of human physiology, development, and adaptation. Consequently, understanding human genetic variation could play an important role in promoting health and combating disease.

Fascinating recent developments in molecular genetics, especially the improvement in modern technology for human genetic profiling, as well as growing knowledge regarding the genetic base of diseases, have led to the introduction of the principles of personalized medicine in clinical practice.

Personalized medicine principles aim to reach an individualized treatment for each patient. These principles, shared by medical genetics and genomics, include the use of genetic variants as markers for diagnosis, prognosis and prevention, as well as targets for treatment (1) (*Table I*).

Personalized medicine is frequently called genome-based medicine. It is »a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease« (2). It is defined as »any clinical practice model that emphasizes the systematic use of preventive, diagnostic and therapeutic interventions that use genome and family history information to improve health outcome« (3).

There has long been interest in personalizing medicine. Hippocrates individualized diagnosis and treatment, for example, by giving cold food to a »phlegmatic« person (4). Personalized genomics follows several decades of scientific discovery and clinical translation in human genetics. Genetic analyses have been used in medicine for years. Genetics examines individual genes and their effects as they relate to diseases. Single gene diseases include thalassemia, phenylketonuria and cystic fibrosis. However, even these monogenic hereditary disorders can be influenced by other, modifier genes.

Genomic and personalized medicine aim to tackle more complex diseases, such as cancer, heart disease and diabetes. It is now well-known that these diseases have a strong polygenic background. Therefore, they can be better understood using a whole-genome approach.

High-throughput analysis of the whole genome (the complete set of DNA within a single cell of an organism), comprising the DNA sequencing analysis and functional genomic analysis (mainly concerned with the patterns of gene expression during various conditions), opened the door wide for personalized medicine. The application of genomics in clinical practice is the best example of successful translational research, the research that aims to move »from bench to bedside« or from laboratory experiments through clinical trials to point-of-care patient applications.

Molecular genetic markers

Molecular genetic markers represent one of the most powerful tools for the analysis of genomes and enable the association of heritable traits with underlying genomic variation. Availability of a wide array of molecular genetic markers offers tools for quick detection and characterization of genetic variation. Two forms of DNA sequence-based markers, single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs), predominate in modern genetic analysis (5). The most studied molecular genetic markers, SNPs, are distributed over the whole genome. The number of SNPs is estimated to range from 0.5 to 1 SNP per 100 base pairs (bp). Besides SNPs, there are other important classes of genetic variants frequently used as molecular genetic markers, such as VNTRs (variable number of tandem repeats, a polymorphic sequence containing 20–50 copies of 6–100 bp repeats), STRs (short tandem repeats, also known as SSRs or microsatellites, a subclass of VNTR in which a repeat unit consists of only 2–7 nucleotides) and CNP (copy number polymorphisms, variation in the number of copies (CNV) of a DNA sequence in the >1 kb size range, which are common and widely distributed in the human genome) (6).

DNA sequence-based markers may affect levels and patterns of gene expression. The amount of transcript of each gene is treated as a phenotypic trait, since it reflects changes in protein function more reliably than DNA markers. Gene expression profiling represents a potent tool for exploring functional genetic variation using RNA molecular genetic markers (7).

The systematic study of protein structures, post-translational modifications, protein profiles, protein–protein, protein–nucleic acid, and protein–small molecule interactions, and the spatial and temporal expression of proteins in eukaryotic cells, are crucial to understanding complex biological phenomena. Proteins are essential to the structure of living cells and their functions. However, the technology for protein profiling is still very expensive and time consuming. Therefore, protein-based molecular markers are not widely used yet (8).

High-throughput methodology for genome-wide genetic and gene expression profiling

There are several approaches for the comprehensive analysis of the genetic profiles of a large

Table I Major genes and associated molecular genetic markers applied in personalized medicine.

Gene/molecular genetic marker	Disease	Application	Reference
HBB	thalassemia	molecular diagnosis	23, 26, 27
PAH	phenylketonuria	molecular diagnosis	24, 28
CFTR	cystic fibrosis	molecular diagnosis	25, 29
A1AT	alpha-1 antitrypsin deficiency	molecular diagnosis	70
HLA	celiac disease	molecular diagnosis preventive medicine	74
t(9;22)(q34;q11) – BCR/ABL	ALL CML	risk stratification molecular-targeted therapy	37 83
t(4;11)(q21;q23) – MLL/AF4	ALL	risk stratification	37
t(12;21)(p13;q22) – TEL/AML1	ALL	risk stratification	37
t(1;19)(q23;p13) – E2A/PBX1	ALL	risk stratification	37
BRCA 1/2	breast, ovarian, prostate and pancreatic cancers	preventive medicine molecular-targeted therapy	57, 58, 107
TPMT	ALL, IBD, transplantation medicine	pharmacogenomics	47, 48
UGT1A1	Gilbert syndrome	molecular diagnosis pharmacogenomics	46
VCORC1	thrombosis and thromboembolism	pharmacogenomics	43, 44
CYP2C9	thrombosis and thromboembolism	pharmacogenomics	45
PML/RARα	APL	molecular-targeted therapy	89–95
RAS	various human cancers	molecular-targeted therapy	103
BCL-2	AML	molecular-targeted therapy	104–106
KIT	sarcoma, glioma, melanoma, liver and renal cancer	molecular-targeted therapy	107
PDGFR	sarcoma, glioma, melanoma, liver and renal cancer	molecular-targeted therapy	107
EGFR	lung cancer, glioblastoma	molecular-targeted therapy	107
BRAF	melanoma	molecular-targeted therapy	107
HER2	breast cancer	molecular-targeted therapy	107
ADA	SCID (ADA deficiency)	gene therapy	113–118
LPL	LPL deficiency	gene therapy	119–122

number of people which have provided sufficient data on molecular genetic markers that may be used in the diagnosis, prognosis and treatment of certain diseases (9). The best known are platforms for DNA analyses (DNA microarrays, genotyping arrays, SNP arrays, Next-generation sequencing) and hybridization platforms for the analyses of gene expression, or the amount of transcribed messenger RNA (hybridization microarrays, expression profiling) (10). A special kind of studies, GWAS (genome-wide association study) analyses, have contributed to the implementation of personalized medicine in clinical practice, analyzing a large number of genetic markers in different individuals suffering from the same disease. GWAS analyses establish the relationship of molecular genetic markers with the pathological phenotype (11). Biomedical professionals are keen to understand the personal genetic profile of every person. Sequencing the complete genome is therefore imposing as the ultimate genetic test. It can be performed once in a lifetime, as early as possible, and the data can be used throughout life, with the aim to achieve better health and longer life using the principles of preventive and personalized medicine (12). It is important to note that for all these methods, bioinformatics data processing has a significant role.

Several international projects have contributed to the development and permanent improvement of methodology for a comprehensive analysis of genetic profiles. Biobanks, the repository of human genetic material, as the major outcome of these projects, provided a sufficient number of samples for comprehensive studies. The Human Genome Project was completed in 2003. Its main achievement is the information on the first sequence of the entire human genome. Results of this research allowed a better understanding of the structure, organization and variability of the human genome, and also became the basis for the study of normal and abnormal gene functioning (13). International HapMap Project has identified the most common genetic variants in the human populations, which are later used in designing genotyping platforms (14).

DNA microarrays, known also as DNA chips, are used for detection of a large number of SNPs in populations and differences between patients and healthy controls. Gene expression profiling platforms use the same technology, except the starting material that is analyzed is total RNA of an individual (10).

Comparative Genomic Hybridization (CGH), as a step ahead of cytogenetic analysis and standard FISH analysis, is a molecular-cytogenetic method that detects copy number variants, very common and very heterogeneous in human DNA (15).

The most accurate method, the so-called gold standard, for determining nucleotide changes in DNA is the sequencing analysis. The sequencing method »reads« the DNA nucleotide by nucleotide. Automatic sequencing, based on the Sanger method, was absolutely dominating in genetics (16), but the need for accurate genetic information to be obtained quickly and cheaply was a catalyst for a fundamental shift in the sequencing technology. Nowadays, there are various platforms for new »next-generation sequencing« technologies that are based on different strategies and are able to produce very large amounts of data. Today, using this methodology, up to 500 000 different DNA samples can be sequenced in one step (17–19).

Sequencing of the complete genome provides 3000 times more information than the platforms for the analysis of DNA variants that exist today. It is a method that allows the analysis of all the genes and regulatory sequences in an individual (20, 21). Comparison of genomes analyzed in two groups (patients and healthy controls) would contribute to a true understanding of the genetic basis of certain diseases.

Sequencing of the complete genome does provide information on complete DNA in the genome of an individual. However, our knowledge is not sufficient to understand how to use this information in clinical practice and preventive medicine. Nowadays, many studies are devoted to the bioinformatics analysis of data obtained from genomic sequences and their possible applications in medicine. Sequencing of the entire genome of each newborn baby and monitoring its health until old age could give valuable information on the genotype–phenotype association for the future of medicine, personalized medicine.

Genome-wide association studies (GWAS) use modern methodology (next generation sequencing technologies, as well as expression profiling platforms) to examine the presence or absence of thousands or millions of genetic variants in the genomes of different individuals that have the same disease and compare it with genetic variants in the genomes of healthy individuals, with the aim to determine the associations of certain genetic variants with normal or pathologic conditions. DNA profiles from a group of healthy individuals are compared with DNA profiles from a group of patients carrying a certain disease. If a genetic variant is more frequent in the group of patients, then it could be an attribute of the disease and should be considered as a diagnostic, prognostic or targeted therapy marker (11).

In January 2008, the National Institute of Health (NIH), USA, decided to combine all the available GWAS studies and to put up their results for public health care usage. Thousands of people were tested for over 200 diseases in 1200 GWAS studies till the end of 2011, and over 4000 genetic variants associated with different diseases were discovered (22).

Molecular genetic markers and health care strategies

Study of the genetic basis of different diseases and the analysis of a number of human genome-wide profiles have led to the implementation of the principles of personalized medicine in clinical practice.

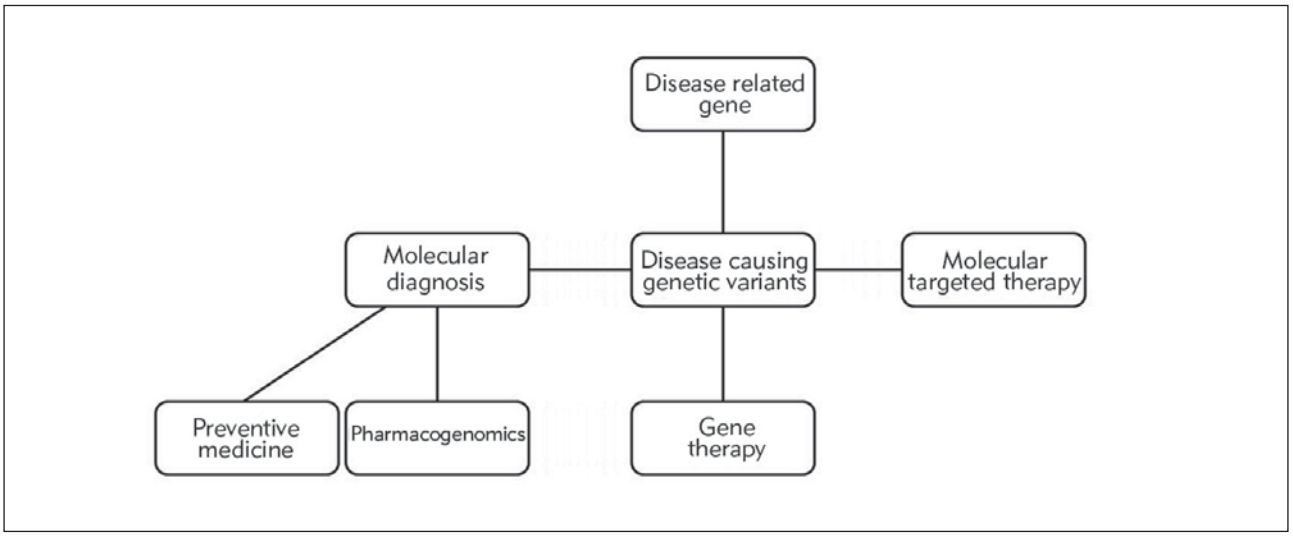

Figure 1 Molecular genetic markers and health care strategies.

Identification of disease related genes and disease causing genetic variants (molecular genetic markers) enables accurate diagnosis, prognosis and follow-up of the disease. It is also a basis for design of the strategies that minimize risk for developing the disease (preventive medicine) and establishment of the guidelines for using therapeutics according to a person's genotype (pharmacogenomics). The final achievement of the study of molecular genetic markers is the implementation of therapeutic approaches that »repair« the affected genes (gene therapy) and design of molecular therapeutics which target the biological mechanism that causes the disease (molecular targeted therapy).

There are several health care strategies based on the application of molecular genetic markers, such as: molecular diagnosis, prognosis and follow-up of the disease, predictive genetics, pharmacogenomics, molecular-targeted and gene therapy (*Figure 1*).

Molecular genetic markers in diagnosis, prognosis and follow-up of disease

The association of molecular markers with human diseases has led to the identification of genes and genetic mutations responsible for many heritable diseases such as thalassemia, phenylketonuria, cystic fibrosis, etc. Thalassemias, the most frequent hereditary disorders in the world, are characterized by genetic defects in one or more globin genes which impair the synthesis of hemoglobin's polypeptide chains (23). Phenylketonuria is a metabolic disease inherited in an autosomal recessive fashion. PKU is caused by mutations in the human phenylalanine hydroxylase gene which affect the structure and/or function of the phenylalanine hydroxylase enzyme, thus decreasing catabolism of L-phenylalanine (24). Cystic fibrosis is an autosomal recessive genetic disorder caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). It represents the most common genetic disorder among Caucasians (25).

The characterization of the most common mutations causing hereditary disorders has created the basis for screening, counseling and first trimester prenatal diagnosis.

Also, better definition of the genetic basis of the most common genetic disorders in the Serbian at risk population has improved the strategy for screening prospective parents and making prenatal diagnosis. Data on molecular genetic markers of the most common genetic disorders are the result of an over 20-year systematic survey in Serbia (26–29).

Molecular genetic markers have been proven to be crucial in the diagnosis of single gene disorders. However, genetic profiles nowadays have diagnostic, prognostic and therapeutic applications in several fields, especially cancer care. One of the most prominent examples for the role of genetic profiling in oncology is the detection of fusion genes and rearrangements in pediatric leukemia (30).

Acute lymphoblastic leukemia (ALL) is one of the most common malignancies in childhood and adolescence, with a successful treatment rate of 80 percent (31, 32). The treatment options were continuously improving during the past few decades, but still 10–15% of the patients develop relapse of the disease (33). Emerging new therapy concepts are focused on individualization of the therapy, that can be achieved through precise risk stratification based on the patients' specific genetic aberrations (34), detection of early treatment response and detection of minimal residual disease (MRD) (35, 36).

Genetic alterations that are the most important in ALL risk stratification, and the MRD follow-up, are translocations t(9;22)(q34;q11) – *BCR/ABL*, t(4;11)(q21;q23) – *MLL/AF4*, t(12;21)(p13;q22) – *TEL/AML1* and t(1;19)(q23;p13) – *E2A/PBX1* (37).

Minimal residual disease (MRD) studies of these translocations allow sensitive detection of leukemic cells undetectable by normal cytomorphologic examination, thereby providing accurate information about the *in vivo* efficacy of cytotoxic treatment (38).

Pharmacogenetics and Pharmacogenomics

Pharmacogenomics is referred to as the study of variation in the DNA sequence and gene expression as related to drug efficacy and toxicity. It is a base for the implementation of personalized medicine, a young but rapidly advancing field of health care. The goal of pharmacogenomics is to identify genomic and clinical information in order to predict the response to treatment of a person. Pharmacogenomic research is being developed in two main directions: identification of specific genes and gene products correlated with different diseases, which could represent the target for new therapeutics, and identification of genes and gene allelic variants that might influence the response to a drug that has already been used in therapy (39).

Pharmacogenomics completely changes the old-fashioned therapeutic paradigm of »one dose fits all patients« and »trial-and-error« prescription, to a novel, personalized concept of »matching the right therapeutic and the right dose to the specific genetic signature of the patient«.

Due to the rapid development of technology, pharmacogenetics became more and more applied to the whole genome and grew into pharmacogenomics. Pharmacogenomic testing is provided through medical and research institutions that developed it in order to make the treatment of patients more efficient, and also by direct-to-consumer companies, mostly accessible through the Internet (40). Many pharmacogenomic tests are routinely used in clinical practice worldwide. Before administering certain medications to a patient, it is mandatory to perform some pharmacogenomic analyses. For some medications pharmacogenomic testing is just recommended, but for the majority of drugs, the testing used today is only informative (41). Introducing routine pharmacogenomic testing into clinical practice enables patients to get an adequate therapy (correct medications and correct drug dose) in accordance with their genotype (42). This approach reduces duration of treatment, saves the health care system a lot of money for unnecessary medications and provides minimal complications and adverse reactions to the drug. Detection of polymorphisms in certain genes involved in the metabolism of a particular drug defines the metabolic status of a person. This is a criterion for the adequacy of a particular drug and also for a drug dose. However, some other factors, including copy number of the gene, presence or absence of secondary or tertiary modifiers, interactions of different drugs and some environmental factors, can also influence the metabolic category of a person. Basic research gives us ever more information that makes the pharmacogenomic testing more accurate. The most clinically relevant pharmacogenomic markers are found in genes for *VKORC* (vitamin K epoxide reductase) (43, 44) and *CYP2C9* (member of the cytochrome P450 family) (45) and they have to be tested before administering anticoagulant therapy (coumarin derivatives and warfarin). Additionally, prior to application of irinotecan therapy, used for colorectal and pulmonary cancer treatment, pharmacogenomic markers in the *UGT1A1* (uridine diphosphate glucuronosyltransferase 1 family, polypeptide A1) gene need to be analyzed (46). Variants in the *TPMT* gene (thiopurine S-methyltransferase) are tested in order to adjust immunosuppressive therapy (6-mercaptopurine, azathioprine and thioguanine), used in the treatment of acute leukemia, inflammatory and autoimmune diseases and in transplantation medicine (47, 48).

Recently, the research in the field of population pharmacogenomics has shown that the study of pharmacogenomic markers in a population and in a certain ethnic community is of great importance (49). The international PGENI project (Pharmacogenetic for Every Nation Initiative) coordinated by the University of North Carolina, USA, has a goal to help the incorporation of genomic risk data into medication decision-making in every country (50). PGENI's model is to look at the genetic incidences of causative risk or drug-efficacy markers in a given population and then to try to individualize the health policy, rather than to introduce treatment individualization for each person. PGENI's bioinformatics tool compares the SNPs (pharmacogenomic markers) found in a particular population with the World Health Organization's »clinical decision trees«, to come up with a prioritized list of medications that should be chosen for the treatment of each disease or trait. Classification of population-specific pharmacogenomic marker frequency profiles could lead to country-specific recommendations for drug efficacy and safety. Serbia is an active member of the PGENI project. Our preliminary data showed that, due to high frequency of the *UGT1A1* and *CYP2C9* pharmacogenomic markers in the Serbian population, routine testing of these markers for every patient should be performed before administering irinotecan and warfarin drugs.

Easily accessible Internet databases on pharmacogenomics, designed by authoritative agencies, have an important role in building up awareness of the significance of pharmacogenomic testing in both the scientific community and general population. Pharmacogenomics Knowledge Base (PharmGKB) is a freely accessible web database that collects, curates and disseminates knowledge about the impact of human

genetic variation on drug responses (51). American Food and Drug Administration (FDA) (52), European Medicines Agency (EMA) (53) and Pharmaceutical and Medical Devices Agency (PMDA) (54) from Japan are the most relevant world agencies that work on the improvement of public health and safety by reviewing and evaluating clinical information on medications and medical devices, including dosing guidelines and drug labels, potentially clinically actionable gene–drug associations and genotype–phenotype correlations. Pharmacogenomic testing before administering drugs became validated and approved by those agencies, based on clinical studies. The international HapMap Project is focused on the identification and catalogization of genetic similarities and differences in the human population, thus enabling biomedical researchers from all over the world to find the genes involved in diseases and responses to therapeutic drugs (14).

The rapid development and application of »next-generation sequencing« technology have opened the possibility of successful application of pharmacogenomic testing in order to individualize therapy. The ultimate genetic test at a reasonable price, complete human genome sequencing, could change the future of pharmacogenomic testing. Before routine application of this modern technology, it is necessary to intensify basic research and find answers about the influence of genetic variants on phenotype in order to develop appropriate bioinformatics tools. At that point, pharmacogenomic testing will get true clinical significance and personalized medicine will really find the path to each patient.

From predictive genetics to preventive medicine

Predictive genetic testing represents the genetic analysis of a healthy individual in order to predict risk for developing a certain disease before the appearance of early symptoms (presymptomatic risk assessment). The aim of predictive genetics is to define predictive genetic risks factors and determinants of health and disease, based on comprehensive epidemiological studies. Predictive genetic risk markers can be used separately or in combination with other markers in algorithms.

Predictive genetic testing can be very important for people that have any cancer history in the family (5–10% of familial adenomatous polyposis, hereditary nonpolipose colon cancer, breast cancer and ovarian cancer) (55–57). If family cancer history suggests an increased risk of developing a certain disease, performing genetic testing could be particularly important for the denial of risk. As an illustration, for particular variants of the *BRCA1* and *BRCA2* genes, it has been demonstrated that they are associated with increased risk of breast and ovarian cancers. Variants of *BRCA1* gene account for 5 percent of all breast cancers and about 50% of all inherited breast cancers. Variants of *BRCA2* gene account for about 30–40% of all inherited breast cancers. Furthermore, these genetic variants contribute to the risk of developing breast or prostate cancer in men. If a woman has a history of breast cancer in her family, preventive genetic testing could reveal her possible carrier status and risk could be assessed. In the case of positive testing results for risk contributing genetic variants, preventive measures could be carried out with the aim to »catch« the disease in the very beginning and to achieve better quality of life (57, 58).

A large number of predictive tests reveal the risk, but do not provide information that the disease will really develop, when it will happen and how severe the symptoms will be. Such tests are used for Crohn's disease (59, 60), cardiovascular disease (61), hypertension (62, 63), rheumatoid arthritis (64), ulcerative colitis (65, 66), venous thromboembolism (67). The positive side of knowing the genetic risks of various diseases and conditions is the awareness of the patient and the physician that preventive measures and diagnostic examinations should be performed on time. The most useful are predictive genetic tests accompanied by efficient diagnostic methods to determine the symptoms and the effectiveness of therapy.

Genetic tests for the diagnosis of hereditary diseases, such as alpha and beta thalassemia (23, 26), cystic fibrosis (25, 68), phenylketonuria (24), Gaucher's disease (69), alpha-1 antitrypsin deficiency (70), hemochromatosis (71), tyrosinemia (72), mucopolysaccharidosis (73) etc. should be performed after the first symptoms of the disease. Genetic tests have a predictive value in the patient's family members. The advantage of using such tests is that, if a person knows that they carry genetic risk at the time of planning the offspring, he/she may turn to genetic counseling for help. Because the sequencing of the human genome in the future will become a financially feasible option, it is possible that the complete genome sequence would be determined for the child at birth, instead of neonatal screening tests. Then, genetic tests that identify genetic disease will become truly predictive genetic tests. This approach would also be of great importance for the diagnosis of rare diseases, which are nowadays characterized by time-consuming diagnostic analyses.

Predictive tests are not only performed when searching for the risk of developing a serious disease. Predictive genetic tests can indicate that a person needs to modify the diet, to avoid the harmful effects of nutrients, for example gluten (for celiac disease) (74), lactose (for adult hypolactasia) (75), caffeine (for hypersensitivity) (76, 77) or fat (for obesity) (78–82). Nutrigenomics, based on the individual's genetic background, provides the ability to correct a congenital metabolic imbalance with proper diet or certain food supplements.

Molecular genetic markers as therapeutic targets

Knowledge of the molecular structure of disease related genes is also changing the way researchers approach developing new drugs.

The best known molecular-targeted therapy is imatinib mesylate, a tyrosine-kinase inhibitor used in the treatment of Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia (CML) (83). The exact chromosomal defect in Philadelphia chromosome is a reciprocal translocation between chromosomes 9 and 22, designated as t(9;22). As a result of the translocation, the oncogenic *BCR-ABL* gene fusion is formed, producing a constitutive active tyrosine kinase enzyme, which phosphorylates subsequent proteins and initiates the signaling cascade necessary for cancer development. Imatinib mesylate works by preventing BCR-ABL enzyme from permanent activation of the »downstream« proteins, thus inhibiting the growth of cancer cells and leading to their death by apoptosis (83). The BCR-ABL tyrosine kinase enzyme exists only in cancer cells. Therefore, only cancer cells are killed through the drug's action (84, 85). Imatinib mesylate, an authentic molecular-targeted therapy, was not as efficient as it was expected. Analysis of the BCR-ABL enzyme active site of imatinib mesylate resistant CML patients revealed genetic-based changes which prevent binding of the drug. Consequently, new tyrosine-kinase inhibitors were designed to target these molecular defects (86). A brand new approach in the treatment of CML is based on RNA interference. Small interfering RNAs have been designed to inhibit *BCR-ABL* gene expression (87).

During the last decade, research in the field of molecular genetics has made substantial advances in understanding the molecular basis of acute myeloid leukemia (AML). A great number of specific genetic alterations in AML have been identified and characterized. These molecular genetic markers represent a target for the development of new therapeutic agents specifically directed toward leukemic cells (88).

Acute promyelocytic leukemia (APL) is the first AML subtype which is treated with an agent targeted to a molecular genetic aberration. More than 98% of APL cases are characterized by the presence of PML/RARα fusion protein which blocks the differentiation of leukemia cells in the promyelocytic stage. *PML/RARα* fusion gene was the target for the design of a specific therapeutic agent – all-trans retinoic acid (ATRA).

ATRA leads to a conformational change of the multifunctional complex which includes PML-RARα, leading to normal regulation of RARα-responsive genes and the induction of the terminal differentiation of APL cells (89). ATRA is commonly used in the treatment of newly diagnosed APL patients. Introduction of ATRA in therapeutic protocols for APL resulted in high clinical remission rates of APL patients (90–92). However, some patients in time become resistant to ATRA. A new therapeutic agent, arsenic trioxide (ATO), emerged as an option for overcoming ATRA resistance (93, 94). ATO induces differentiation of APL cells, as well as their apoptosis, thus eliminating the effects of *PML-RARα* genetic defect (95). Therapeutic approach based on ATRA and ATO used for the treatment of APL is the most successful example of differentiation therapy. It represents a prototype for the development of similar therapeutic agents for treatment of other hematological malignancies and cancers.

Another approach of molecular-targeted therapy in AML is based on the principle that the block in the differentiation process of cells can be reversed by abrogation of the epigenetic silencing (96, 97). This is a universal approach used in development of potential drugs for treatment of many diseases, including cancer. The two most common mechanisms of epigenetic silencing, altering the regulation of transcription, have led to the development of clinically applicable drugs. The first mechanism of epigenetic silencing is aberrant DNA methylation. Cytidine analogs such as 5-aza-cytidine or 5-aza-2-deoxycytidine integrate into DNA as alternative nucleotides and trap DNA methyltransferases, causing the formation of demethylated DNA (98). Due to this mechanism, hypermethylation of DNA in malignant cells is reversed (99), generally leading to the induction of differentiation and the inhibition of proliferation of the malignant cells (100). These drugs could replace cytotoxic chemotherapy in the near future (101). The second mechanism of epigenetic silencing, used as a target for molecular therapeutics, is the modification of histones. Deacetylation of histones results in their stronger binding to DNA and eventually to transcriptional repression. Newly developed histonedeacetylase (HDAC) inhibitors work as modulators of transcriptional repression of tumor suppressors or factors responsible for normal differentiation and cell growth (102).

Farnesyltransferase inhibitors (FTIs) are small-molecule inhibitors that selectively inhibit farnesylation of a number of intracellular substrate proteins such as RAS. *RAS* is the most common oncogene in human cancer. Mutations that permanently activate the RAS protein are found in 20–25% of all human tumors and up to 90% in certain types of cancer (e.g. pancreatic cancer) (103). For this reason, RAS inhibitors are studied as a potential therapy for the treatment of malignancies and other diseases with *RAS* overexpression.

Another molecular target, successfully used in the design of molecular therapy, is apoptosis. Overexpression of the Bcl-2, an antiapoptotic protein, was observed in hematological malignancies. Cells that have less Bcl-2 are not only more susceptible to apoptosis, but also more sensitive to chemotherapy (104).

Antisense oligonucleotides block target mRNA specifically. An antisense oligonucleotide-based therapy inhibits Bcl-2 overexpression, promotes apoptosis and diminishes drug resistance in patients with AML (105, 106).

A number of therapies based on targeting gene variants responsible for malignant transformation have been used: genetic variants of *EGFR* gene in lung cancer and glioblastoma are treated with cetuximab, gefitinib, etc., *KIT* and *PDGFR* gene variants in sarcoma, glioma, melanoma, liver and renal cancer are treated with imatinib, nilotinib etc., *BRAF* gene variants in melanoma are treated with RAF inhibitors, *BRCA* gene variants in breast, ovarian, prostate and pancreatic cancers are treated with PARP inhibitors, *HER2*-positive breast cancer is treated with Herceptin (107).

Up until recently, revolutionary discoveries in the field of molecular genetics had to wait for years to be applied in medical practice. Today, each novel molecular mechanism is applied immediately after its identification. Therefore, new emerging molecular-targeted therapy is constantly being introduced into clinical practice.

Gene therapy

Disease related genes and disease causing genetic variants can be treated by introducing genetic material into a cell to fight or prevent disease. The idea of gene therapy, the way to repair defective genes, was born thirty years ago and is still considered controversial in some scientific communities (108, 109). However, research on gene therapy has been conducted for a number of diseases, especially monogenic diseases (thalassemia, cystic fibrosis, hemophilia) and cancer, through various approaches (110).

Genetic material can be delivered to a cell using a »vector«. The most commonly used vectors in gene therapy are viruses, since they are natural deliverers of genetic material (their own) into a human cell. Viral genome is altered in a manner to make a virus safe and non-infective, and to carry a therapeutic gene (111, 112). A therapeutic gene is not only a »healthy« copy of the gene which replaces a mutated gene, but also a genetic material which inactivates a mutated gene that functions improperly or any other genetic material that can fight a disease, when introduced in a cell or incorporated in a human genome. Virtually all cells and tissues are potential targets for gene therapy. However, all gene therapy protocols in humans are directed to somatic cells which are non-reproductive. Somatic cell therapy affects only the targeted cells in the patient, and is not passed on to future generations. Germline gene therapy remains controversial and prohibited in most of the countries.

Somatic gene therapy is divided in three categories: *ex vivo*, *in vivo* and *in situ*. In *ex vivo* gene therapy, patient's cells are removed from the body and then grown and genetically modified outside the body. After insertion of the therapeutic gene into the patient's cells, they are returned to the patient. Interior, *in vivo*, gene therapy means that genetic manipulation and the transfer of the therapeutic gene to cell is performed inside the patient's body, while *in situ* gene therapy means that the therapeutic gene is delivered directly to the tissue that has to be treated in order to restore the missing function (113).

In the early 1990s, gene therapy was successful in combating SCID (Severe Combined Immunodeficiency, also called ADA deficiency or »bubble baby disease«) for the first time. *Ex vivo* approach was applied. Retroviral vectors were used to introduce the normal allele of the adenosine deaminase (*ADA*) gene into the cells of a 4-year-old girl, born with ADA deficiency. In this disease, an abnormal variant of the *ADA* gene fails to make ADA, a protein indispensable for the correct function of T-lymphocytes. The girl, and many more after her suffering from SCID, was cured and had a normal life, although she had to repeat the gene therapy protocol every few months. From 1993, SCID immunodeficiency was considered 100% cured by gene therapy (113, 114). However, in 2002, two cases of T-cell ALL were newly diagnosed after retrovirus-mediated gene therapy of SCID immunodeficiency. It was confirmed that the therapeutic gene was integrated in the regulatory region of *LMO2* oncogene, most probably causing the malignant phenotype (115, 116). Moreover, an 18-year-old high-school graduate died after adenovirus-mediated gene therapy of ornithine transcarbamylase. Both incidents were the result of well-known weaknesses of the gene therapy of today, the use of viral vectors for the delivery of the therapeutic gene in the patient's cell (the position of viral integration in the human genome is hard to control and production of noninfective viral particles is not yet efficient enough) (117, 118).

Gene therapy had its best and worst times. However, researchers continue to improve gene therapy and develop new approaches. Today, there are more than a thousand on-going clinical trials for gene therapy. Finally, in November 2012, the first gene therapy received marketing authorization from the European Commission, for patients with lipoprotein lipase (LPL) deficiency (119). The gene therapy product, alipogene tiparvovec, is based on an adeno-associated virus vector and the replacement of the gene responsible for LPL expression, which is defective in patients with LPL deficiency (120). These patients have an extremely high level of serum triglycerides causing recurrent and life threatening pancreatitis (121). Definitely, the first commercially-approved gene therapy product in the West represents an outstanding medical achievement.

»Gene therapy, like every other major new technology, takes time to develop. It will succeed with

time. And it is important that it does succeed, because no other area of medicine holds as much promise for providing cures for the many devastating diseases that now ravage humankind« (122).

Conclusion

The future of medicine, without a doubt, lies in the realization of the idea of personalized medicine. The final achievement of the Human Genome Project was the creation of a catalogue of human genes. Molecular biologists of today are facing an ambitious goal of understanding the function of all genes, associating DNA content with individual phenotype as well as medically relevant features. Gene expression profiling will be able to reveal all genes relevant for certain pathologies, guiding medical doctors toward specific molecular therapy. As soon as gene manipulation begins to cure, a large number of people will have a long and better life, despite the predispositions. In this way, an old proverb will finally become true: »Fato prudentia maior est« (Wisdom is stronger than destiny).

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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