BASIC PHARMACOGENETICS

Pharmacogenetics (modern term – pharmacogenomics) is the study of genetic factors that underlie variation in drug response. Pharmacogenomics implies a recognition that more than one genetic variant may contribute to variation in drug response.

Historically, the field began with observations of severe adverse drug reactions in certain individuals, who were found to harbor genetic variants in drug-metabolizing enzymes. As a scientific field, pharmacogenomics has advanced rapidly since the sequencing of the human genome.

- I stage – discription of pharmacogenetic phenomenon (1930-1960-yy);
- II stage – development of pharmacogenetics as a discipline (1960-1990-yy);
- III stage – develompent of the clinical approach of the pharmacogenetics (pharmacogenomics) (since 2000 and to present).

In the last decade, powerful genomewide association (GWA) studies, in which hundreds of thousands of genetic variants across the genome are tested for association with drug response, led to the discovery of many other important polymorphisms that underlie variation in both therapeutic and adverse drug response. In addition to polymorphisms in genes that encode *drug-metabolizing enzymes*, it is now known that polymorphisms in genes that encode *transporters, human leukocyte antigen (HLA) loci, cytokines,* and various other proteins are also predictive of variation in therapeutic and adverse drug responses. In addition to the new discoveries that have been made, the past decade has ushered in **"precision medicine,"** also known as "**stratified or personalized medicine**," in which genetic information is used to guide drug and dosing selection for subgroups of patients or individual patients in medical practice.

Single nucleotide polymorphism (SNP) is a base-pair substitution that occur in the genome that could result in an amino acid change of the correspondent protein. In turn, it can alter pharmacokinetics or pharmacodynamic processesses .

SNPs could have impact on:

- pharmacokinetics that include biotransformation enzymes (CYP-450, N-acetlytransferase, etc) and drug transporters (P-glycoproteins etc);

- pharmacodynamics that include target molecules for the drugs (receptors, enzymes etc) and proteins, which are predictive of variation in therapeutic and adverse drug responses (NOS, HLA).

Pharmacogenetic tests

Study of the patients samples blood or of buccal epithelium with the help of polymerase chain reaction (PCR) allow us reveal presence or absence of the SNPs of the certaine genes. It is a so-called procedure of patients genotyping. For test's implementation into practice it should match a few criteria:

- It should be proved an association between SNP and alteration of drug responses (adverse effect development, alteration of therapeutic effect);
- It should show significant specificity, sensitivity, and reproducibility;
- It should prove its advantage over traditional therapy (higher efficacy or safety, lower cost of the treatment);
- The special algorithm of the therapy modification according to testing results is needed (dose or drug change).

During last decades the number of pharmacogenetic tests is extremely growing. Внесением фармакогенетических данных в инструкции для использования лекарственных средств (их маркировка) занимается организация Food Drug Administration (FDA), USA is responsible for inclusion of pharmacogenetic labelling. In 2016 there were 165 drugs with pharmacogenetic labelling. Basically it concerns:

— cautions of the drugs application (80 drugs);
— indications for application (53 drugs);
— development of the adverse effects (50 drugs);
— change of dose or way of administration (42 drugs);
— drugs interactions (21 drugs).

Currently on FDA web-site (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations#section1) the information is represented in 3 tables:

- Section 1: Pharmacogenetic Associations for which the Data Support Therapeutic Management Recommendations;
- Section 2: Pharmacogenetic Associations for which the Data Indicate a Potential Impact on Safety or Response
- Section 3: Pharmacogenetic Associations for which the Data Demonstrate a Potential Impact on Pharmacokinetic Properties Only.

Analysis of the genetic variation in response to drugs and ADRs has been enabled somewhat by the **Pharmacogenomics Knowledge Base (PharmGKB)**, Pharmacogenomics Research Network (PGRN), and the Clinical Pharmacogenetics Implementation Consortium (CPIC). The **Dutch Pharmacogenetics Working Group (DPWG)** was established in 2005 by the Royal Dutch Pharmacist's Association

(KNMP). The DPWG is multidisciplinary and includes clinical pharmacists, physicians, clinical pharmacologists, clinical chemists, epidemiologists, and toxicologists. The objectives of the DPWG are:

- To develop pharmacogenetics-based therapeutic (dose) recommendations.
- To assist drug prescribers and pharmacists by integrating the recommendations into computerized systems for drug prescription and automated medication surveillance

Clinical Pharmacogenetics Implementation Consortium (CPIC) published a series of guidelines for using genetic information in selecting medications and in dosing. These highly informative guidelines are being used by practitioners in prescribing drugs to more effectively treat patients. Where appropriate, CPIC recommendations are included to provide information on how to use genetic variant data appropriately in therapeutic medicine.

On website https://www.pharmgkb.org one could see the results of DPWG and other research groups concerning 68 drugs (2016).

Durgs with pharmacogenetic labelling belong to the next groups:

— antidepressants — tricylclic antidepressants (6 drugs), selective serotonine reuptake inhibitors (6 drugs), other groups (2 drugs);

— antipsychotics (7 drugs);

— anticonvulsants (3);

— narcotic analgesics (3);

— anticancer/immunosupressants — fluoropyrimidines (3), antracyclines (2), other (7);

— proton pump inhibitors (5);

— anticoagulants (3), antiaggregant (1);

— antiviral (4);

— oral antidiabetics — sulfonurea derivatives (4)

The vast majority of the pharmacogenetic tests related to the genes that determine the activity of hepatic enzymes:

CYP2C9 — 8 (out of them 4 don’t need dose change of the drugs);

CYP2C19 — 16 (out of them 1 doesn’t need dose change of the drug);

CYP2D6 — 28;

UGT (uridine diphosphate glucuronosyltransferase) — 4;

DPYD (dihydropyrimidine dehydrogenase) — 3;

TPMT (thiopurine-S-methyltransferase) — 3;

CYP3A5 — 1.

Также несколько тестов определяют вероятность развития побочного или терапевтического эффектов:

HLA-A; HLA-B – 5;

IFNL3 (ген, модулирующий активность интерферона) – 2.

Також кілька тестів визначають імовірність розвитку побічного або терапевтичного ефектів:

HLA-A; HLA-B — 5;

IFNL3 (ген, що модулює активність інтерферону) — 2

Currently on website https://www.pharmgkb.org/ there are 868 notice concerning drugs' labelling; 189 clinical recommendations etc

Allele is any one of two or more genes that may occur alternatively at a given site (locus) on a chromosome. If polymorphism (mutation) is absent in both alleles then the carrier has *extensive (rapid) metabolizer* genotype. For such persons common doses are recommended.

If one allele out of two alleles is mutated then the carrier has *intermediate metabolizer* genotype; if both alleles are mutated then the carrier has *slow metabolizer* genotype. поліморфізм, то йдеться про генотип повільного метаболізатора. Such patients are characterized by lower enzymatic activity that can lead to:

1) Drugs' cummulation, enhancement of the pharmacologic effect and even overdosing. That is why slow metabolizer need reduction of standart dose or substitution by another drug, which is metabolized by another enzyme;
2) Decreasing of response could appear if the drug is a prodrug and decreasing of enzymatic activity result in dropping of active metabolite level (clopidogrel, codein, tramadol). In this case it is better to substitute by another drug.

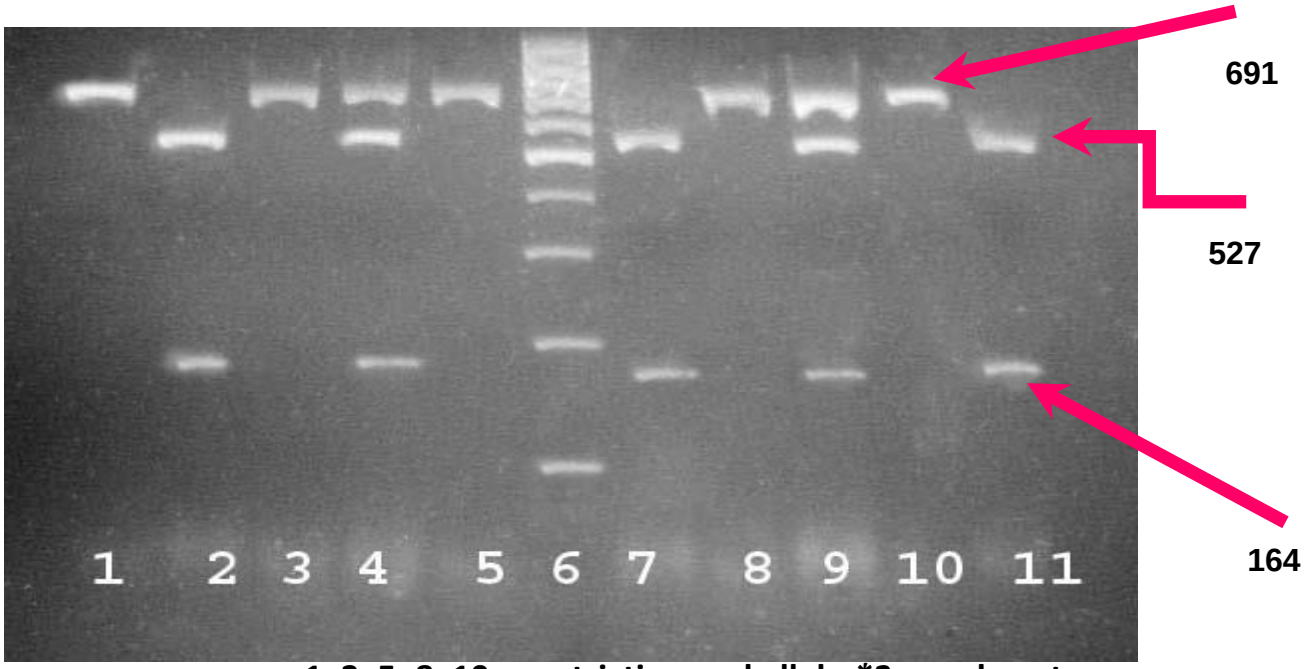
Biotransformation reactions mediated by P450 phase I enzymes typically modify functional groups (−OH, −SH, −NH2, −OCH3) of endogenous and xenobiotic compounds, resulting in an alteration of the biological activity of the compound. Phase I enzymes are involved in the biotransformation of over 75% of prescription drugs; therefore, polymorphisms in these enzymes may significantly affect blood levels, which in turn may alter response to many drugs. Of these, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 appear to be the most important forms, accounting for approximately 85% of the total human liver P450 content. Together, they are responsible for catalyzing the bulk of the hepatic drug and xenobiotic metabolism.

It is noteworthy that CYP3A4 alone is responsible for the metabolism of over 50% of the prescription drugs metabolized by the liver. The involvement of individual P450s in the metabolism of a given drug may be screened in vitro by means of selective functional markers, selective chemical P450 inhibitors, and P450 antibodies. In vivo, such screening may be accomplished by means of relatively selective noninvasive markers, which include breath tests or urinary analyses of specific metabolites after administration of a P450-selective substrate probe.

Polymorphisms in drug-metabolizing enzymes dominated the field of pharmacogenomics for many years, and for some years, metabolic phenotypes such as *extensive metabolizer (EM)*, reflecting an individual's metabolic rate of a particular drug that is a known substrate of a specific enzyme, were used to describe genetic effects on drug metabolism. After genotypic information became available, a new nomenclature was used to characterize an individual's metabolic rate. In particular, diplotypes, consisting of one maternal and one paternal allele, using star (∗) allele nomenclature, have been used. Each star (∗) allele is defined by specific sequence variation(s) within the gene locus, eg, single nucleotide polymorphisms (SNPs), and may be assigned a functional activity score when the functional characterization is known, eg, 0 for nonfunctional, 0.5 for reduced function, and 1.0 for fully functional. Some genes, such as CYP2D6, are subject to whole gene deletions, eg, CYP2D6∗5, and whole gene duplications or multiplications, eg, ∗1xN, ∗2xN, where N is the number of copies. If more than one copy of the gene is detected, the activity score is then multiplied by the number of copies observed (*ultra-rapid metabolizer*). Enzyme activity is generally a co-dominant or additive trait. For example, if an individual carries one normal function allele and one nonfunctional allele, he will have an intermediate metabolic activity or be considered an intermediate metabolizer (IM). The sum of allelic activity scores typically ranges between 0 and ≥ 3.0 and is most often used to define phenotypes as follows: 0 = PM (poor metabolizer), 0.5 = IM, 1.0–2.0 = EM, and ≥ 2.0 = UM (*ultra-rapid metabolizer*)

Detection of *CYP2C9* genotype

For detection of the mutated (variant) alleles **2* & **3* there were used two pairs of correspondant primers. After that PCR-products were exposed under restrictase enzymes. Since the site of restriction is absent in variant (mutated) alleles, the PCR-products avoided the restriction by correspondent enzyme.



1, 3, 5, 8, 10 – restriction and allele **2* are absent
2, 7, 11 – restriction and allele **2* are absent *(*2/*2)*
4, 9 – heterozygous genotype *(*1/*2* or **2/*3)*
PRODUCTS OF *Ava*II RESTRICTION

The enzyme *Ava*II is responsible for cleavage of DNA fragment 691 b.p. into two parts – 527 & 164 b.p., that reveal presence of **1* or **3* allele, while absence of cleavage (remaining 691 b.p.) reveal the presence mutated **2* allele.

Restriction products (b.p.)

	Endonuclease	
Allele	***AvaII***	***NsiI***
****1***	**527+164**	**112+29**
****2***	**691**	**112+29**
****3***	**527+164**	**141**

It is known that there are interethnic differences concerning frequency of ultrarapid/extensive/intermediate/slow metabolizers that results in differences of effectiveness and toxicity of certain drugs. That is why the national screening programs for study of genes polymorphism, which control drugs metabolism, is wise. For example, in 2014 we have studied polymorphism of CYP2C19 & 2C9

genes in Odesa region. It was established that according to CYP2C19 genotype, 79 % individuals were *extensive metabolizers*, 20 % — *intermediate metabolizers* і 1 % — *slow metabolizers*. In European countries around 68,2–76,6% are *extensive metabolizers*, while in Asia (South Korea) — around 40 %.

According to CYP2C9 genotype, in Odesa region 76 % individuals are *extensive metabolizers*, 22 % — *intermediate mebatolizers*, 2 % — *slow metabolizers*. In European countries 62–86%, 29–34% and 2–5 % correspondently; in Asia (Iran) — 41, 47 and 11 % correspondently. Thus, one can see certain interethnic differences in genotype spreading.

Some of the chemically dissimilar P450 substrate drugs, on repeated administration, **induce P450** expression by enhancing the rate of its synthesis or reducing its rate of degradation (phenobarbital, rifampicin etc). Induction results in accelerated substrate metabolism and usually in a decrease in the pharmacologic action of the inducer and also of co-administered drugs. However, in the case of drugs metabolically transformed to reactive metabolites, enzyme induction may exacerbate metabolite-mediated toxicity.

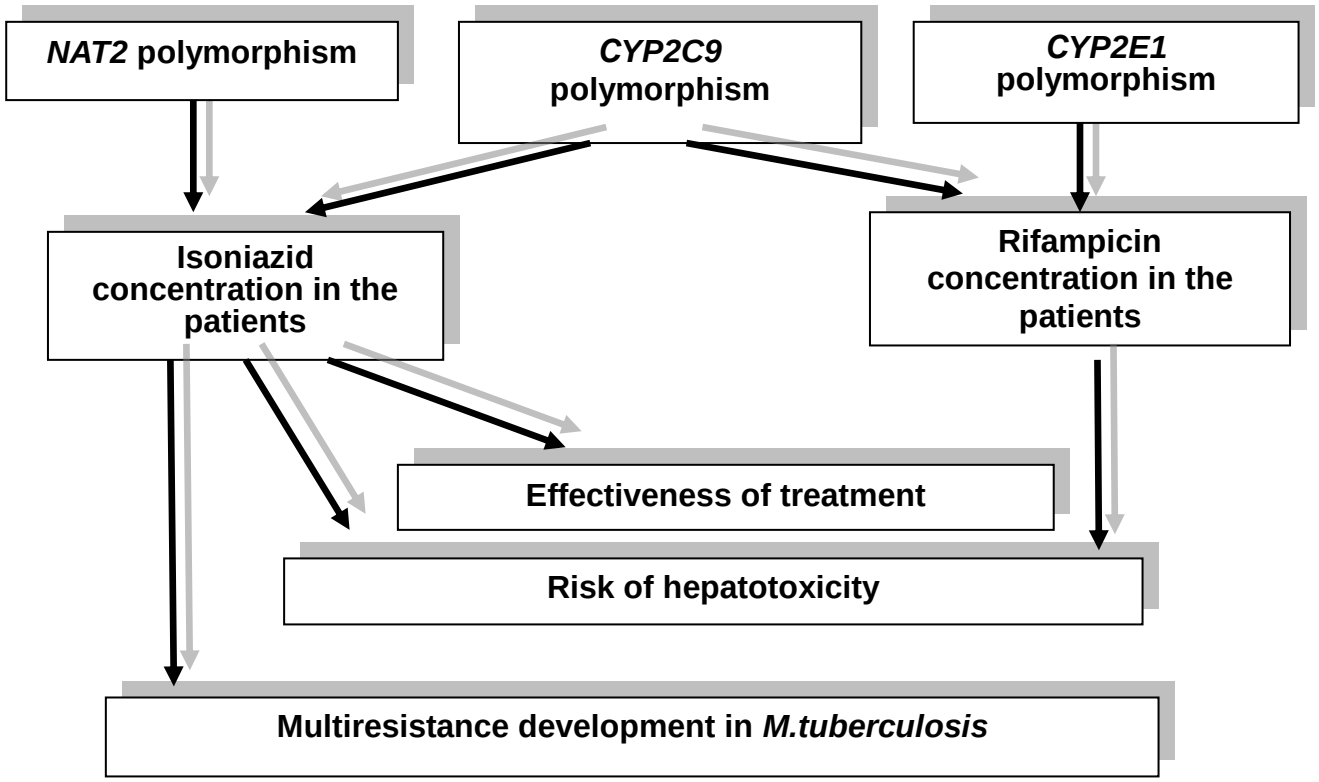
Certain drug substrates **inhibit cytochrome P450** enzyme activity. Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the P450 heme iron and effectively reduce the metabolism of endogenous substrates (eg, testosterone) or other co-administered drugs through competitive inhibition.

Phase II enzyme biotransformation reactions typically conjugate endogenous molecules, eg, sulfuric acid, glucuronic acid (glucuronidation), and acetic acid (acetylation), onto a wide variety of substrates in order to enhance their elimination from the body.

Consequently, polymorphic phase II enzymes may diminish drug elimination and increase risks for toxicities.

According to the results of the scientific work of Odesa National Medical University employees, it was established that the determination of polymorphisms of *NAT2, CYP2C9* genes in patients with pulmonary tuberculosis helps in predicting the effectiveness and safety of pharmacotherapy, as well as the appearance of multiresistant strains of *M. tuberculosis*. Determining the CYP2E1 genotype in patients with pulmonary tuberculosis and viral hepatitis C makes it possible to single out a group of patients with a high risk of toxic liver damage and low effectiveness of antiviral therapy, thus increasing the safety of

treatment. The significance of glutathione-S-transferase gene polymorphism in the development of side effects of antituberculosis therapy was also proven.



RELATION of TB-PATIENTS PHARMACOGENETICS WITH TREATMENT EFFECTIVENESS & SAFETY

Plasma membrane transporters, located on epithelial cells of many tissues, eg, intestinal, renal, and hepatic membranes, mediate selective uptake (OATP1B1) transporter and efflux (P-glycoprotein) of endogenous compounds and xenobiotics including many drug products. Transporters, which often work in concert with drug-metabolizing enzymes, play important roles in determining plasma and tissue concentrations of drugs and their metabolites. Genetic differences in transporter genes can dramatically alter drug disposition and response and thus may increase risk for toxicities.

Gene polymorphism can determine another enzymes activity (G-6-PDG, butyrylChE etc) that can promote certain adverse effects; determine immune reactions (severe intolerance); target molecules sensitivity for the drugs.

Despite our improved understanding of the molecular basis ofpharmacogenetic defects in drug-metabolizing enzymes, their impact on drug therapy and ADRs, and the availability of validated pharmacogenetic biomarkers to identify patients at risk, this clinically relevant information has not been effectively translated to patient care. Thus, the much-heralded potential for personalized medicine, except in a few instances of drugs with a relatively low therapeutic index (eg, warfarin), has remained largely unrealized. This is so even though 98% of US physicians are apparently aware that such genetic information may significantly influence therapy. This is partly due to the lack of adequate training in translating this knowledge to medical practice, and partly due to the logistics of genetic testing and the issue of cost-effectiveness. Severe ADRs are known to contribute to 100,000 annual US deaths, about 7% of all hospital admissions, and an increased average length of hospital stay. Genotype information could greatly enhance safe and efficacious clinical therapy through dose adjustment or alternative drug therapy, thereby curbing much of the rising ADR incidence and its associated costs.

Despite modern achievements, there are many difficulties in clinical pharmacogenetics. There is still a lack of data supporting the feasibility of routine pharmacogenetic screening. Thus, in many published studies, the issues of pharmacogenetics were secondary, so a significant number of works are based on an insufficient number of samples.

Secondly, the end point in the assessment of genetic polymorphism was pharmacokinetic data obtained under the conditions of administration of one drug in healthy people, which is not representative for everyday clinical practice. At the same time, the number of studies where pharmacogenetic issues are of primary importance is gradually increasing.

Dose adjustment recommendations mainly apply to patients for whom the genotype is known. Today, there are few such patients, they are mainly patients who underwent genotyping after unexplained unwanted effects or lack of therapeutic effect.

However, along with the decrease in the cost of pharmacogenetic testing and the increase in the number of laboratories with the possibility of genotyping, the number of such patients will increase.

The interaction gene–drug and drug–drug is more challenge. So far, research has only concerned the interaction between the drugs themselves.However, taking into account the data of pharmacogenetics, this approach may become incorrect. For example, the interaction between a CYP2C9

inhibitor and a drug that is a CYP2C9 substrate in *intermediate metabolizers*, according to CYP2C9, may require a differentiated approach compared to *slow metabolizers*. That is why the interaction gene–drug and drug–drug could have certain significance for drugs usage. Works in this direction have already begun to appear in the literature.

Nevertheless, pharmacogenetic research is the foundation for the further implementation of individual pharmacotherapy of patients taking into account their genetic passport, which, in turn, will contribute to increasing the effectiveness and reducing the toxicity of medicinal products.