

Integration of Genomics and Epigenomics into Pharmaceutical Studies

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LILIANA BURLIBAȘA^{1,2*}, VICTOR TRĂISTARU³, ILEANA IONESCU³

¹Faculty of Biology, University of Bucharest, Romania

²Epigenetics Centre, Bucharest, Romania

³University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania

*Address correspondence to: Faculty of Biology, 1-3 Aleea Portocalilor, Sector 6, Bucharest, Romania, Email: liliana_burlibasa@yahoo.com.au

Abstract

A great challenge of personalized medicine is to recommend medication based on an individual genetic pattern. Pharmacogenomics is the study of how genetic and epigenetic profiles determine the response to a therapeutic intervention. Currently, numerous studies have demonstrated that both genetic variants (including Single Nucleotide Polymorphisms - SNPs and Copy Number Variants - CNVs) and epigenetic mechanisms (DNA methylation, histone modifications and microRNA) are involved in gene expression variation. This paper gives an overview of basic mechanisms involved in genes expression. Moreover it discusses the clinical and preclinical evidence for the contribution of genetic and epigenetic factors to pharmacogenomics, focusing on how this knowledge informs about the efficiency of medication.

Keywords: pharmacogenomics, SNPs, CNVs, epigenetics mechanisms, personalized medicine

1. From Genomics to Epigenomics

Genome sequence information has a great utility across a broad range of scientific areas. A new class of diagnostic test has been developed due to advances in human genomics. In medicine, genomics is applied to diagnose diseases, to develop optimized treatment for patients based on their hereditary and acquired genomic profile, to develop genetic therapies and to prevent disease initiation and progression. [1].

The Human Genome Project was an international, collaborative research program, whose goal was the complete mapping of entire genome of human beings [2]. It was the world's biggest biological project. The main goals of the Human Genome Project were to provide a complete and accurate sequence of the three billion DNA base pairs that constitute the human genome and to map all human genes.

In 2004, researchers from the International Human Genome Sequencing Consortium (IHGSC) announced a new estimate of 20,000 to 25,000 genes in the human genome [3]. Previously 30,000 ~ 40,000 genes had been predicted, while estimates at the start of the project reached up to as high as 2,000,000.

The sequencing phase of the Human Genome Project has been completed. However, studies of DNA variation continue in another international project, HapMap Project, whose goal is to identify patterns of single nucleotide polymorphism.

In September 2003 was launched another project, ENCODE (Encyclopedia of DNA Elements), intended as a follow-up to the Human Genome Project. ENCODE aims to identify functional elements in the entire human genome. To date, this project has facilitated the identification of a new DNA regulatory element, helping to get new insights into the

organization and regulation of our genome, as well as into the way the differences in DNA sequence could influence the progression of disease.

When the Human Genome Project was completed, most scientists were convinced that genetic diseases would become a thing of the past. However, scientists have not been able to provide answers to many problems relating to such disorders. Further study has resulted in the conclusion that the genetic code can be read in different ways, meaning that parts of the DNA can be blocked and others can be activated. Starting with these unsolved by the Human Genome Project problems, researchers have focused on the control of gene expression.

In the first half of the 20th century, genetics and developmental biology were separate disciplines. The term “epigenetics” was originally used by Waddington [4] to link the two fields. With the understanding that all cells of an organism carry the same DNA, and with increased knowledge of the mechanisms of gene expression, the definition was modified to focus on the ways in which heritable traits can be associated not with changes in nucleotide sequence, but with chemical modifications of DNA or of the structural and regulatory proteins that bind to it. Today, epigenetics refers to the study of heritable patterns of gene expression that are not caused by changes in DNA sequence. The heritability of gene expression model refers to both transgenerational inheritance and somatic cell division [5].

Epigenetics is the key to the current and future research that can elucidate genome functioning. Much of the control of gene expression is governed by epigenetic changes, such as differential DNA methylation, histone modifications and regulatory microRNAs (miRNAs). The involvement of epigenetic mechanisms in gene regulation, genetic imprinting, chromatin structure and some pathological conditions is now well established. Facilitated by the Human Genome Project, epigenetic phenomena can be studied on a genome-wide scale, giving rise to the new field of *Epigenomics*. There are many implications of epigenomic research in some areas of functional genomics. In this context, the two major projects: Roadmap Epigenomics Project and International Human Epigenome Consortium constitute the basis for human genomics, allowing an accurate interpretation of the organization of genes and thereby providing important insights into human health and disease. The Epigenome Network of Excellence (The Epigenome NoE) was a EC FP6 consortium (2004-2009) formed by 25 research groups and 16 associate partners to promote a coherent European Research Area and prioritize research into molecular mechanisms of genes expression. The research program of EpiNoE was focused on central questions, such as the existence of a epigenetic code in addition to the genetic code, the molecular mechanisms of epigenetic plasticity, and the how epigenetic dysfunction affects disease. Research efforts such as Human Epigenome Project which aims to identify, catalog and interpret genome-wide DNA methylation pattern of all human genes in all tissues, will provide new insights into the epigenetic components of the human genome. The integration of epigenomic profiling with the information provided by the HapMap Project will allow the identification of both genetic and non-genetic factors responsible for variation in organism’s response to medication therapies.

2. Pharmacogenomics in Medicine

The relationship between genome and drug response was established 55 years ago, with the discovery that the deficiency in glucose-6-phosphate dehydrogenase (G6PD) results in hemolytic anemia following the ingestion of primaquine, a drug used in treatment of malaria [6].

Numerous investigations have been focused on the single nucleotide polymorphisms (SNPs) of genes encoding metabolizing enzymes (e.g. cytochrome P450 enzyme superfamily [7-8]). The Human Genome Project has provided a “reference sequence” upon which

hereditary DNA sequence variation can be associated with different phenotypes. Approximately 11 million SNPs are estimated to exist in human population, with an average of one every 1,300 base pairs. An individual's response to a specific drug is often linked to these common DNA variations. In addition to SNPs, other genetic elements have been implicated in medication response [9-10]. Copy number variants (CNVs) have received considerable attention in recent years. CNVs are distinct from SNPs, being the subject of pharmacogenetic studies that show their importance as a genetic source of variability in the metabolic activity of some enzymes [11].

However, not only DNA sequence-based variation (SNPs and CNVs) and genetic mutations can affect gene expression, but also other non-genetic factors such as epigenetic pattern and habitat can regulate gene expression, suggesting the potential involvement of these non-genetic determinants in drug response variation [12] (Fig. 1).

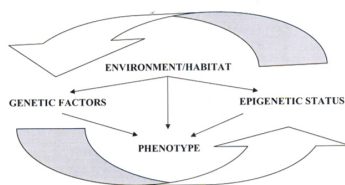


Fig. 1 *Phenotype can be affected by genetic variation, epigenetic factors and environment*

2.1. The use of SNPs in pharmacogenomics

Some of the SNPs are functionally silent, occurring in non-coding or non-regulatory regions of the genome. However, some of the SNPs are biologically functional, leading to altered protein structure and expression. The process of identifying these biologically relevant SNPs, in particular those that are associated with the risk of an individual's susceptibility to various diseases and medication responses is well underway.

In the 1980s, SNPs were detected using restriction enzymes to identify the presence or absence of cutting sites and scored by observing the resulting fragment length variation. Today, there are five commonly used methods for SNPs detection: Single strand conformation polymorphism (SSCPs), heteroduplex analysis, direct DNA sequencing, pyrosequencing, DNA microarray technology.

There are two main approaches for the use of SNPs maps in pharmacogenomics: **linkage disequilibrium mapping** and **candidate gene approach**.

Linkage disequilibrium mapping (LD) is a procedure for determining the non-random association of alleles at two or more loci on a chromosome. It is based on the premise that the regions adjacent to a gene of interest are transmitted through the generations along with that gene and can be identified by the specific pattern of markers (haplotypes) that they contain, so that the detection of haplotypes can be used to locate specific genes. Linkage-disequilibrium mapping is useful to identify genes involved in some complex diseases. In some genomic regions, LD expands over several thousands of Kilobases, whereas in other genomic regions surrounding single genes, LD can be small. The estimates of the average extent of LD in the human genome vary widely, ranging from 100 Kb to 3 Kb [13]. The strength of LD will

affect the magnitude of an association. A marker in LD with a SNP susceptibility will yield a relative risk that is smaller than if the SNP susceptibility were tested directly [13].

Candidate gene approach tests the effects of genetic variants of a potentially contributing gene in an association study. These studies may allow the identification of genes with small effects. This approach has already been extended to identifying candidate genes affecting drug response (e.g. gene variants in a drug-metabolizing enzyme - thiopurine methyltransferase, have been linked to adverse drug reaction [14].

An example of relevant polymorphism (SNP) that influences other drug metabolism is *cyp2d2* genotype. The following cytochrome P450 enzymes: CYP1(A1/A2), CYP2(A6/B6/C8/C9/C19/D6/E1 and CYP3(A4/A5), are the most abundant isoforms involved in drug pathways. Each form varies 100-fold or more within a given population due to genetic and non-genetic factors and numerous environmental factors, some of which are constant (sex and genotype), whereas others are dynamic (age, weight, drug exposure, diet, etc.). CYP3A4 is mainly influenced by sex and is inducible by a wide range of substances, whereas CYP2D6 is influenced by genetic polymorphisms. In this case, genetic polymorphisms can lead to the formation of less active or inactive enzymes. It is estimated that a considerable percent of the human population is homozygous for non-functional *cyp2d6* mutant alleles, leading to the ability to activate opioid analgesics. For example, codeine exerts its analgesic effects through morphine, its metabolite. "An individual that carry a *cyp2d6* genotype will have a rapid metabolism to morphine. He was taking drugs that inhibited CYP3A4 metabolism leading to more codeine being available for morphine metabolism. Renal impairment led to less morphine being removed from the circulation. As a consequence, morphine accumulation because of this combination of factors, led to toxicity" [15].

Tamoxifen has been used for the systemic treatment of patients with breast cancer for nearly three decades. Treatment success is primarily dependent on the presence of the estrogen receptor (ER +) in the breast carcinoma. While about half of patients with advanced ER-positive disease immediately fail to respond to tamoxifen, in the responding patients the disease ultimately progresses to a resistant phenotype. The possible causes for tamoxifen resistance have been attributed to the pharmacology of tamoxifen, alterations in the structure and function of the ER, the interactions with the tumour environment, genetic alterations in the tumour cells and some polymorphisms of cytochrome P450. It was discovered that women with certain mutation in their *cyp2d6* gene were not able to efficiently break down Tamoxifen, making it an ineffective treatment for their disease [16]. Since then, women have been genotyped for those specific mutations so that they can immediately benefit from the most effective therapy.

Drug response is a complex process, resulting not only from genotyping variation at many loci, but also from the level of gene expression, histones and regulatory proteins modifications, drug interactions and doses, diet and other non-genetic determinants.

2.2. Copy number variants and pharmacogenomics

CNVs are structural chromosomal rearrangements coming from non-allelic homologous recombination and unequal crossing-over events. If the recombination event occurs between genes, the result is a deletion respectively, a duplication without genes disruption (e.g. Charcot-Marie-Tooth disease type 1A). If the recombination event occurs within a gene, the structure and the function of that gene are disrupted. For example, a sequence of 9.5 Kb in intron 22 of the coagulation factor VIII gene is repeated twice at a region near the long arm end of the X chromosome. An intra-chromosomal recombination event involving this

repeated region causes the disruption of the Factor VIII gene and half of hemophilia A cases [17].

Each of these structural rearrangements can lead to a dosage imbalance in the genetic material, and therefore affect the expression levels and the activity of the protein.

Numerous studies have investigated the CNVs contribution to the susceptibility to disease such as Crohn's disease [18], vasculitis, microscopic polyangiitis and Wegener's granulomatosis [19-20], autism [21] and neuroblastoma [22]. CNVs can be detected with transcriptional analysis or copy number variation arrays. For example, the chromosomal region 12q13-q14 is amplified in many sarcomas. This chromosomal region encodes a protein named MDM2, which is known to bind to P53 (a tumor suppressor protein). When MDM2 is amplified, it prevents P53 from regulating cell growth, which can result in tumor development [23]. Additionally, certain breast cancers are associated with overexpression and increase in the *erbB2* gene copy number, which encodes for human epidermal growth factor receptor 2 [24]. The presence of a high number of *erbB2* copies has been associated with aggressive forms of breast cancer [25].

The rapid advances in the methods that facilitate the assay and analysis of copy number variation for genes encoding drug metabolizing enzymes have demonstrated the implication and their dramatic consequences on drug response. Such CNVs have been observed to alter gene dosage, being thus likely to play a role in drug efficacy and drug toxicity respectively [26].

cyp2d6 may occur in CNVs of 0 to 13 copies [27]. CNVs for this gene affect the plasma levels of the active metabolite of tamoxifen, called endoxifen, so that the ultra-rapid metabolizers that carry more copies of this gene can exhibit higher levels of endoxifen, than those that carry normal copy number for the gene [26]. Another drug metabolizing cytochrome P450 gene namely *cyp2a6*, also occurs in CNVs. This gene encodes an enzyme that metabolizes nicotine and its metabolite cotinine. An increased copy number of this gene leads to a higher level of activity responsible for increased risk of nicotine addiction and of tobacco-related cancers [28].

A polymorphic duplication of the voltage-gated potassium channel, shaker-related subfamily, member 5 (KCNA5), which is expressed in cardiac atrium, has been reported in CNV database, suggesting the potential genotypic effect of CNV associated with KCNA5 on the antiarrhythmic drug response phenotype [29]. Studying CNVs is not fundamentally different from studying SNPs. The techniques of detection and genotyping may differ, although the same principles apply.

3. Epigenetic Therapy – A Powerful Tool in Medicine

The study of genotype-phenotype relationship has challenged researchers and clinicians, because some observations cannot be explained. For example, monozygotic twins carrying the same pathological mutation can be clinically different. The study of such unusual cases has uncovered the role of epigenome (altered genetic information without changes in DNA sequence) in health, disease and drug response [30].

The epigenetic mechanisms consist of complex interactions between DNA and histones, which define the profile of gene expression. These DNA-histones interactions, as well as gene expression process, are also influenced by small non-coding RNA (miRNA).

A great surprise has been the discovery that epigenetic signals can be passed on from one generation to the next, even for several generations, without changing a single gene sequence. Unlike genetic mutations, epigenetic changes are potentially reversible, thus giving great hope to scientists in order to find new therapeutic strategies.

Numerous studies have demonstrated that gene expression signatures have the capability of predicting response to various cancer therapies [31].

Cytosine methylation from eukaryotic DNA is a modification in which a methyl group is enzymatically transferred from S-adenosylmethionine (SAM) to the 5-position of cytosine. The methyl group is situated in the major groove of the DNA and inhibits transcription by interfering with transcription factor binding proteins. The pattern of genome methylation is explained by two distinct processes: *de novo* methylation (involving DNA methyl-transferase 3a – DNMT3a, and DNA methyl-transferase 3b – DNMT3b) and maintenance methylation (involving DNA methyl-transferase 1 – DNMT1).

The recent progress in molecular biology techniques has provided the necessary tools to detect DNA methylation in the human genome, thus potentially allowing the study of the roles of DNA methylation in gene regulation. DNA methylation can be detected by bisulfite conversion, methylation-sensitive restriction enzyme digestion, chromatin immunoprecipitation assay, direct sequencing, pyrosequencing. Combining these techniques with DNA microarrays and high-throughput sequencing has made the mapping of DNA methylation feasible on a genome-wide scale.

Some studies demonstrated that O⁶-methylguanine DNA methyl-transferase (MGMT) promoter hypermethylation is a useful marker for predicting survival in patients with diffuse large B-cell lymphoma treated with multidrug regimens including cyclophosphamide [32]. Hypomethylation of MGMT promoter was shown to be involved in basic fibroblast growth factor induced resistance against temozolomide in human melanoma cells [33]. Shen and his coworkers correlated drug activity with DNA methylation, identifying a list of methylation markers that predicted sensitivity to chemotherapeutic drugs [34]. Many other studies suggest that the variation in DNA methylation status significantly contribute to the variation in gene expression, which in turn affects drug response [35].

Because of the dual effects carried out by the DNMTs, the development of drugs to counteract aberrant methylation is a difficult act. There are some drugs that are clinically effective, mostly in hematological diseases: cytosine analogue 5-aza-2'-deoxycytidine (5aza-dC), and non-nucleoside analogue DNMT1 (N-phthalyl-L-tryptophan). These agents are used in combination with histone-deacetylase (HDAC) inhibitors like trichostatin A (TSA) and the benzamide compound etinostat. For example, the combination of 5 aza-2dC with etinostat or decitabine with sodiumphenylbutyrate or valproic acid, showed a positive activity for the treatment of myelodysplastic syndrome [36].

There are ongoing clinical trials with decitabine in combination with more traditional anticancer agents like prednisone and doxorubicin, with the drug doses being reduced to avoid toxicities.

4. Pharmacogenomics and Personalized Medicine

Pharmacogenomics combines traditional pharmaceutical science (biochemistry) with the newly made advances in molecular genetics such as DNA variation and epigenetic profile in the human genome. Pharmacogenomics has the potential to provide personalized drug therapy based on genetically determined variation, which results in the following advantages: more effective and safer drugs; more accurate methods to determine optimal drug dosages (the current methods based only on weight and age will be replaced with dosages based on a personalized genetic and epigenetic profiles); more powerful medicines (developing therapies targeted on specific diseases, maximizing therapeutic effects while decreasing toxicity).

It is clear that medicine will be more oriented towards disease prevention rather than towards the efforts to cure people in the late stages of disease. Because individuals could have

different potential disease combinations, medicine will also become highly personalized, with the knowledge of individual genetic signature and lifestyle influences [37].

References

1. TRIPP S. and GRUEBER M, Economic impact of the Human Genome Project, *Battelle the Business of Innovation*, 2011
2. www.genome.gov
3. IHGSC, Finishing the euchromatic sequence of the human genome, *Nature*, 2004; **431** (7011): 931-945
4. WADDINGTON C.H., The epigenotype, *Endeavour*, 1942; **1**:18-20
5. NICULESCU M.D., HAGGARTY P., Nutrition in Epigenetics, Wiley-Blackwell, Oxford, 2011, p.2
6. BEUTLER E., The hemolytic effect of primaquine and related compounds: a review. *Blood*, 1959; **14**(2):103-139
7. BISHOP J.R., ELLINGROD V.L., Neuropsychiatric pharmacogenetics: moving toward a comprehensive understanding of predicting risks and response. *Pharmacogenomics*, 2004; **5**(5):643-655
8. MELLEN P.B., HERRINGTON D.M., Pharmacogenomics of blood pressure response to antihypertensive treatment. *J. Hypertens.*, 2005; **23**(7):1311-1325
9. CAPPUZZO F., VARELLA-GARCIA M., SHIGEMATSU H., et al., Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. *J. Clin. Oncol.*, 2005; **23**:5007-5018
10. GRESHOCK J., CHENG J., RUSNAK D., MARTIN AM, WOOSTER R, GILMER T, LEE K, WEBER BL, ZAKS T., Genome-wide DNA copy number predictors of lapatinib sensitivity in tumor-derived cell lines. *Mol. Cancer Ther.*, 2008; **7**:935-943
11. HEBBRING S.J., MOYER A.M., WEINSHILBOUM R.M., Sulfotransferase gene copy number variation: pharmacogenetics and function. *Cytogenet. Genome. Res.*, 2008; **123**:205-210
12. ZHANG W., HUANG S.R., DOLAN M.E., Integrating epigenomics in pharmacogenomics studies, *Pharm. and Personalized Med.*, 2008; **1**:7-14
13. ALWI Z.B., The use of SNPs in pharmacogenomics studies, *Malaysian J. of Med. Sci.*, 2005; **12**(9): 4-12
14. KRYNETSKI E.Y., EVANS W.E., Pharmacogenetics as a molecular basis for individualized drug therapy: the thiopurine S-methyltransferase paradigm. *Pharm. Res.* 1999 **16**:342-349
15. GASCHE Y.D.Y., FATHI M., CHIAPPE A., COTTINI S., DAYER P., DESMEULES J., Codeine intoxication associates with ultrarapid CYP2D6 metabolism. *New Engl. J. of Med*, 2004; **57**:2827-2831
16. ELLSWORTH R.E., DECEWICZ D.J., SHRIVER C.D., ELLSWORTH D.L., Breast cancer in personal genomics era. *Curr. Genomics*, 2010; **11**(3):146-161
17. OUAHCHIL K., LINDEMAN N., LEE C., Copy number variants and pharmacogenomics, *Pharmacogenomics*, 2006; **7**(1):25-29
18. FRANSEN K., MITROVIC M., VANDIEMEN C.C., WEERSMA R.K., The quest for genetic risk factors for Crohn's disease in the post-GWAS era, *Genome Med.*, 2011; **3**:13
19. FANCIULLI M., NORSWORTHY P.J., PETRETTO E., DONG R., HERPER L., KAMESH L., HEWARD J.M., GONGH S.C., DESMITH A., BLAKEMORE A.I. et al. FCGR38 copy number variation is associated with susceptibility to systemic, but not organ-specific autoimmunity. *Nat. Genet.*, 2007; **39**(6):721-723
20. WILLCOCKS LC, LYONS PA, CLATWORTHY MR, ROBINSON JI, YANG W, NEWLAND SA, PLAGNOL V, MCGOVERN NN, CONDLIFFE AM, CHILVERS ER, ADU D, JOLLY EC, WATTS R, LAU YL, MORGAN AW, NASH G, SMITH KG: Copy number of **FCGR3B**, which is associated with systematic lupus erythematosus, correlates with protein expression and immune complex uptake. *J. Exp. Med.* 2008; **205**:1573-1582
21. GLESSNER JT, WANG K, CAI G, KORVATSKA O, KIM CE, WOOD S, ZHANG H, ESTES A, BRUNE CW, BRADFIELD JP, IMIELINSKI M, FRACKELTON EC, REICHERT J, CRAWFORD EL, MUNSON J, SLEIMAN PM, CHIAVACCI R, ANNAIAH K, THOMAS K, HOU C, GLABERSON W, FLORY J, OTIENO F, GARRIS M, SOORYA L, KLEI L, PIVEN J, MEYER KJ, ANAGNOSTOU E, SAKURAI T, et al.: Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature*, 2009; **459**:569-573

22. DISKIN SJ, HOU C, GLESSNER JT, ATTIYEH EF, LAUDENSLAGER M, BOSSE K, COLE K, MOSSÉ YP, WOOD A, LYNCH JE, PECOR K, DIAMOND M, WINTER C, WANG K, KIM C, GEIGER EA, MCGRADY PW, BLAKEMORE AIF, LONDON WB, SHAIKH TH, BRADFIELD J, GRANT SFA, LI H, DEVOTO M, RAPPAPORT ER, HAKONARSON H, MARIS JM. Copy number variation at 1q21.1 associated with neuroblastoma, *Nature* 2009; **459**:987-991.
23. OLINER J.D. KINZLER K.W., MELTZER P.S., GEORGE D.L., VOGELSTEIN B., Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature*; 1992; **358**:80-83
24. POLLACK, J. R., PEROU CM, ALIZADEH AA, EISEN MB, PERGAMENSCHIKOV A, WILLIAMS CF, JEFFREY SS, BOTSTEIN D, BROWN PO Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat. Genet.*, 1999; **23**, 41–46
25. PEIRÓ, G., MAYR D, HILLEMANN S, LÖHRS U, DIEBOLD J., Analysis of HER-2/neu amplification in endometrial carcinoma by chromogenic *in situ* hybridization: Correlation with fluorescence *in situ* hybridization, HER-2/neu, p53, and Ki-67 protein expression, and outcome. *Modern Pathol.*, 2004; **17**:277-287
26. GAMAZON E.R., HUANG R.S., DOLAN M.E., COX N.J., Copy number polymorphisms and anticancer pharmacogenomics, *Genome Biol.* 2011; **12**(5) R46
27. JOHANSON I., LUNDQVIST E., BERTILSSON L., DAHL M.L., SJÖGVIST F., INGELMAN-SUNDBERG M., Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc. Natl. Acad. Sci. USA*, 1993; **90**:11825-11829
28. MALAIYANDI V., SELLERS E.M., TYNDALE R.F. , Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. *Clin Pharmacol. Ther.*, 2005; **77**:145-158
29. DROLET B., SIMARD C., MIZOUE L., RODEN D.M., Human cardiac potassium channel DNA polymorphism modulates access to drug-binding site and causes drug resistance. *J. Clin. Invest.* , 2005; **115**(8):2209-2213
30. ALLIS D., JENUWEIN T., REINBERG D., CAPARROS M.L., Epigenetics, Cold Spring Harbor Laboratory Press, New York, 2006, p.436
31. POTTI A., NEVINS J.R., Utilization of genomic signatures to direct use of primary chemotherapy. *Curr Opin. Genet. Dev.*, 2008; **18**:62-67
32. ESTELLER M., GAIDANO G., GOODMAN S.N., et al., Hypermethylation of the DNA repair gene O(6)-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma, *J. Natl. Cancer. Inst.*, 2002; **94**:26-32
33. FONTIJN D., ADEMA A.D., BHAKAT K.K., et al., O(6)-methylguanine-DNA-methyltransferase promoter demethylation is involved in basic fibroblast growth factor induced resistance against temozolomide in human melanoma cells, *Mol. Cancer Ther.* 2007; **6**:2807-2815
34. SHEN L., KONDO Y., AHMED S., BOMMBER Y., KONISHI K., GOU Y., CHEN X., VILAYTHONG J.N., ISSA Y.P., Drug sensitivity prediction by CpG island methylation profile in the Nci-60 cancer cell line panel, *Cancer Res*, 2007; **67**:11335-11343
35. MIHOCHA M., SHUKLA S., GOBBURN J., Integration of pharmacogenomics and pharmacometrics to support drug development, regulatory and therapeutic decisions, *Curr Pharmacogenomics and Personalized Med.*, 2014; **12**(1):32-42
36. LÜBBERT M., Targets of epigenetic therapy – gene reactivation as a novel approach in MDS treatment. *Cancer. Treat. Rev.* 2007; **33**:547-552
37. ABDOLMALEKY H.M., ZHOU J.R., THIAGALINGAM S., SMITH C.L., Epigenetic and pharmacoepigenomics studies of major psychoses and potentials for therapeutics. *Pharmacogenomics*, 2008; **9**(12):1809-1823