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Polymorphisms in Pharmacogenetics of Personalized Cancer Therapy

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Abstract

Therapy process of personalized cancer management covers surgery, chemotherapy, radiation therapy and targeted therapies. The choice of cancer chemotherapeutic agents and doses depends upon the location and stage of tumor, as well as the general state of the patient. On the chemotherapy, radiotherapy, and targeted therapy processes, pharmacogenetics offers customized solutions according to the personal genetic information. Especially for clinicians, genetic information obtained from polymorphism-based pharmacogenetic tests is highly crucial for the better prediction ability of drug response and life-threatening toxic reactions due to the narrow therapeutic index of cancer chemotherapeutic agents. Pharmacogenotyping utilizes different examination strategies, such as single nucleotide polymorphism analysis, somatic/germline mutation analysis and partial/full genome sequencing. The promising effect of pharmacogenetics on the solving of the individual variability in drug response and toxic reactions is being observed with the accumulation of the information that unravel the human genomic variations from large-scale population and multi-parameter-based pharmacogenetic studies of the post-genomic era. Polymorphisms contribute wide variations in human genome and may define how individuals respond to medications, either by changing the pharmacokinetics and pharmacodynamics of drugs or by altering the cellular response to therapeutic agents. To define the effect of polymorphisms on the targets of chemotherapeutics is necessary for the prediction of altered pharmacokinetics of therapeutic agents.

Keywords: personalized medicine, pharmacogenetics, polymorphisms

1. Introduction

1.1. Genetic polymorphisms

1.1.1. Mutation or polymorphism

Genetic and environmental factors are the two main reasons that cause human phenotype variations. If the genomic DNA sequences of two individuals are compared, substantial sequence variations can be detected at different points of the whole genome. There are many forms of these genetic variations [1]. Polymorphism term, arose from the combination of the Greek words 'poly' (meaning as multiple) and 'morph' (meaning as form), is used in genetics to describe multiple forms of a single gene that exist in a population. Polymorphisms are genetic variants and refer to the occurrence of various phenotypes in a certain population. A polymorphism is a DNA sequence variation and does not classify as mutation. In genetic polymorphisms, there are two or more equally acceptable sequence of a gene and the common allele must have a frequency of 1% or more in the population. If the frequency is lower than 1%, the allele is accepted as a mutation. On the other hand, a mutation is a change in DNA sequence away from normal allele and forms abnormal variant [2].

1.1.2. Nomenclature

The unique and universal nomenclature to refer specific single nucleotide polymorphisms (SNPs) is that using the rs number (reference sequence). It stands for Reference SNP cluster ID. The rs number allows the precise identification of a polymorphic variation in the numerous databases (NCBI, HapMap, SNP500 Cancer, etc.). For instance, a SNP causes a replacement of an amino acid by another amino acid; this can be defined by the name and the position of the replaced amino acid, followed by the name of the novel amino acid. As an example, a common SNP in the *DPYD* gene is identified as rs 1801160 [V732I or Val732Ile]. SNPs are also identified by the name and position of nucleotide in the reference DNA sequence. The same SNP in the *DPYD* gene, presented with rs 1801160, is identified as 2194G>A. The letters A, T, C, and G can be used for both nucleotides and amino acids, and this can cause confusion [3].

1.1.3. Types of polymorphisms

Developments in next generation sequencing technologies under the "Human Genome Project," simplify to investigate the allelic variants of a gene taken from different people of a population [4]. These various genetic polymorphisms include minor changes on DNA sequence, as substitutions, deletions, insertions, and repeats. These changes influence the three-dimensional structure, expression and activity of the proteins encoded by these genes. Alterations called as single nucleotide polymorphisms (SNPs, pronounced snip) are the most common form of polymorphisms on a gene sequence. They occur when alleles reveal only a single base pair (bp) change—A, T, C or G—in the genome sequence between individuals. For example, one variant may have an A nucleotide at a certain position and other has a G

nucleotide (**Figure 1**). This type of change generates single nucleotide variants (SNVs). SNPs can affect gene function due to the change of protein but can also occur in noncoding parts of the gene so they would not be seen in the protein product [5, 6].

1.1.4. Polymorphisms and ethnicity

Intrinsic factors and extrinsic factors cause variability in drug response. Extrinsic factors such as food and concomitant medications may be controllable, but intrinsic factors such as gender, ethnicity, age, renal or hepatic function, and genetic differences in the expression of enzymes need advance knowledge to control [7]. Ethnicity is one of the key factors that can explain the observed variability in both pharmacokinetics (PK) and pharmacodynamics (PD) of therapeutics, resulting in differences in response to drug therapy as well as chemotherapeutics. United States Food and Drug Administration (FDA) guidelines pay attention to "ethnically sensitive situations" for some drugs and suggests the types of solutions that may control such ethnic sensitivity. One of the most important factors that may contribute to this ethnic sensitivity of a drug, include genetic polymorphisms in metabolic pathways of drugs [7, 8]. To define the variability on the response or metabolism of specific therapeutics or drug targets, drug companies and prestigious research groups are biobanking DNA samples [9]. Although the clinical relevance of some variants is well characterized, the relevance of some variant alleles is as yet unknown.

1.1.5. Allele frequency and Hardy-Weinberg principle

There are some challenges on the use of disease-associated polymorphism knowledge. One of these challenges is the lack of the unique information regarding the frequency of specific polymorphism in the targeted population. Without a unique presenting style of the

Figure 1. Classes of DNA variation affecting a single nucleotide position. (A) Single nucleotide variant (SNV) in which two variants differ by having a G nucleotide or a C nucleotide (B) Insertion variation in which variant 1 has exactly same reference sequence, variant 2 has one more T nucleotide; Deletion variation in which variant 1 has a A nucleotide, variant 2 does not have; (C) Variable number of tandem repeats (VNTR) in which two variants differ by having repeats of nucleotides.

polymorphism-related data, prevention of the risk for disease and drugs remain unknown. In addition, determining the factors that may affect the association of the allele with disease or drugs, such as ethnicity, may not be possible without population-based allele frequencies [10–12]. SNPs can be assigned with an allele frequency—the ratio of chromosomes in the population carrying the less common variant to those with the more common variant. It is important to note that there are variations among different populations, so a common SNP allele may be much rare in one geographical or ethnic group than another [13, 14]. The Hardy-Weinberg principle can be used to calculate allele frequencies [15]. The Hardy-Weinberg principle (also known as the Hardy-Weinberg equilibrium, equation, theorem, or law) states that allele frequencies in a population will remain stable from generation to generation in the absence of other evolutionary factors, such as mutation, polymorphisms, genetic drift, gene flow, meiotic drive, and mate choice. Hardy-Weinberg principle describes that the ideal condition against the effects of these factors can be analyzed. The principle is named after Godfrey Harold Hardy and Wilhem Weinberg, who first demonstrated it mathematically. If Hardy-Weinberg principle is violated, the key interferences of a genetic polymorphisms-based study may be compromised. Thus, accumulating evidence suggests that Hardy-Weinberg principle-based reporting may be optimal in genetic and nongenetic journals, because variability in the analyzed data can cause errors or peculiarities [15].

2. Polymorphisms-based pharmacogenetics applications and personalized cancer therapy

Most of the chemotherapeutic agents for cancer treatment affect a minority of cancer patients and have a narrow therapeutic index that frequently causes life-threatening toxicities and even death. Even specific molecule-targeted therapies, which are safer than cytotoxic drugs, are associated with severe adverse events. Thus, novel treatment strategies that can increase the effectiveness of therapy and decrease the rate of adverse events will be developed. Under this approach, the aim of personalized medicine is to tailor the therapy options according to patient's molecular profile [16, 17]. Establishing the relation between molecular characteristics of patient and drug outcomes is crucial for the identification of predictive biomarkers and understands the base of personalized therapy. Personalized medicine can also be called as P4 medicine due to the various contents as predictive, personalized, preventive, and participatory medicine (P4); it separates patients into different groups with an individual's molecular profile. Thus, personalized medicine covers the determination of the safest and most effective chemotherapeutic agents [18]. The goal of discipline of pharmacogenetics, first used in the late 1950s, is to make 'personalized medicine' as applicable to various patient groups. It can be defined as the study of patients' genotype affecting drug response. In some patient groups, certain drugs work well but not as well in others. Pharmacogenetics-based studies (between genotype of patients and the response of therapeutics) allow designing more effective and population-specific therapeutic treatments (**Figure 2**). Polymorphism analysis, mutation analysis and genome sequencing are the backbones of discipline of pharmacogenetics.
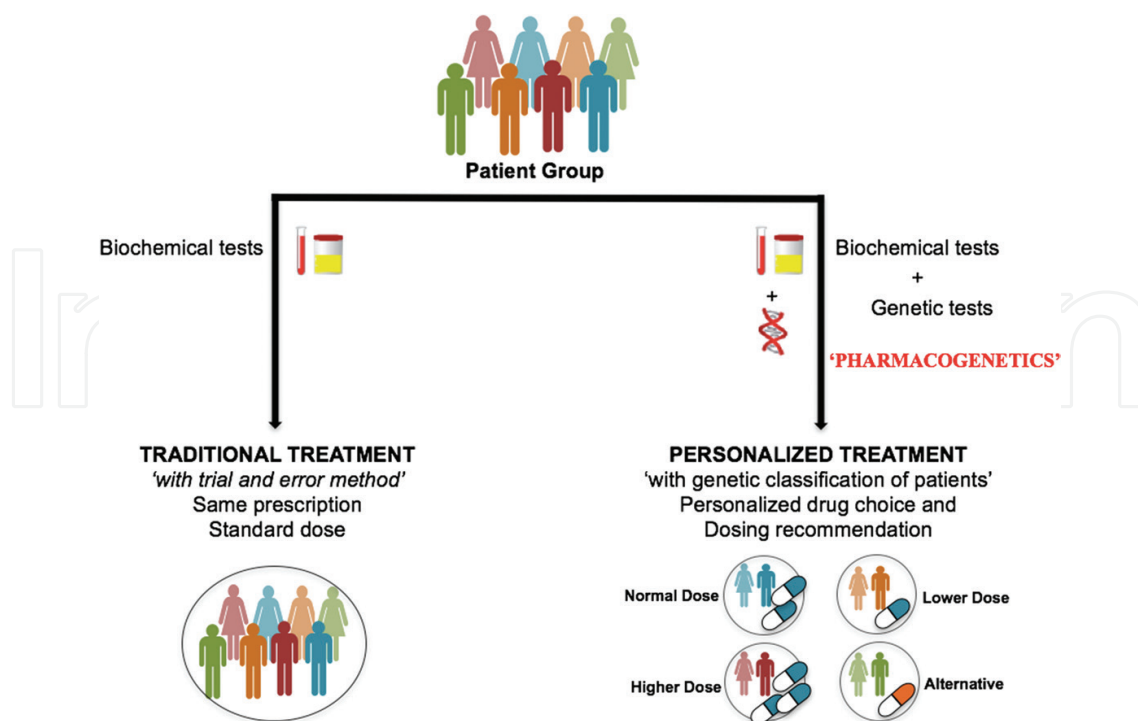
Figure 2. The traditional prescribing approaches in clinical therapies, such as 'trial and error', 'one drug fits all' and 'one dose fits all', have limits due to the drug safety. Pharmacogenetics combines standard biochemical tests with molecular genetics based tests to detect DNA variations in the human genome for the application of genomic-based prescribing.

Polymorphisms and their association with diseases should be handled based on gene as bio-markers, due to the relatively large frequency in the human genome. To learn, how to use and interpret the polymorphisms analysis and which test(s) should be chosen is essential.

2.1. Methods for polymorphism detection

After the deep sequencing of human genome with Human Genome Project, the detection of population-based DNA polymorphisms, especially that effect the development and the progress of diseases, is the second phase of human genomics. High-throughput polymorphism genotyping process includes fast and cost-effective identification of polymorphisms in different individuals and lead to the determination of associations between genotype and phenotype. Generally, genotyping steps start with the isolation of starting material as DNA from patient; it follows with amplification to increase the sample amount and then finalize with polymerase chain reaction (PCR), sequencing or array-based technologies. A number of good polymorphism genotyping technologies are currently in use to meet the needs of clinics and researches, but only one genotyping method is not ideal for all applications (**Table 1**). Slow speed of assays due to the time-consuming protocols, high instrument and consumable costs, and requirements on the performing multiple assays in parallel are the main challenges of polymorphism genotyping technologies. Studies on ideal polymorphism genotyping technologies are on development process [19–21].

Method	Advantages	Disadvantages
Allele-specific single-base primer extension	High success rate; High accuracy; Quantitative; Adaptation to multiplexing.	High instrument cost; Relatively long protocol; Tricky multiplex PCR.
Allele-specific enzymatic cleavage – restriction fragment length polymorphism (RFLP)	High accuracy; SNP at a restriction endonuclease recognition sequence.	High instrument cost; Long protocol; Difficult adaptation for high-throughput genotyping
Pyrosequencing	Provides sequence information surrounding the polymorphism; Real-time scoring; Quantitative.	High instrument and consumable cost; Long protocol; Limited throughput
Mass spectrometry	High accuracy (as long as the sample is very pure); Fast genotyping reactions in seconds; Allows thousands of reactions in a day.	High instrument and consumable cost; Long protocol; Limited throughput; Requires very pure sample for accuracy.
Invader assay	No PCR for amplification; Simple protocol; Isothermal reaction	Requires large amount of genomic DNA; Requires optimizations for each SNP; Key enzyme is not suitable for general research use.

Table 1. A summary of popular polymorphism detection methods with their advantages and disadvantages [19–21].

2.2. Polymorphisms as biomarkers for drug response and toxicity

Variations in the metabolism of a chemotherapeutic agent due to genetic alterations may cause significant differences in terms of efficacy and toxicity. Such pharmacological effects occur since oncologists schedule the dosing of chemotherapeutic agents according to patient's body surface area and other nongenetic factors. Genetic differences due to the polymorphisms are thought as one of the strongest reasons in adverse drug reactions (ADRs). Genetic polymorphisms are considered as molecular biomarkers in pharmacogenetic-based studies both in clinic and research to predict the ADRs and apply the medications as personal. The main objective of pharmacogenetics is to understand the nature of various responses including adverse drug reactions (ADRs) to drugs [22]. Pharmacogenetic associations are important in cancer chemotherapy due to the extremely narrow chemotherapeutic index of anticancer drugs given for cancer management. Polymorphisms in both patient's genome and tumor genome affect the regulation of drug transport, retention and efflux of anticancer drugs, determining the penetration into tumor tissue. Genetic information of tumors is not stabile as somatic tissues; new alterations on genetic material (as mutation or chromosomal loss) can occur continuously. Therefore, drug-related toxicities depend on the genotype of nontumor tissue. Thus, the tumor genome possesses most of the polymorphisms that influence the sensitivity or resistance of drugs (KRAS and EGFR, KIT, TS polymorphisms, etc.); hence, treatment efficacy and tumor genome will have a key role as a dose limiting factors in cancer management. Polymorphisms on the host

genome, which tumor genome does not present, are the main determinants of toxicity risk (e.g. polymorphisms on the genes of drug metabolism such as *dihydropyrimidine dehydrogenase gene (DPYD), thiopurine-S-methyltransferase (TPMT), UDP-glucuronosyltransferase (UGT)*, etc.) [23]. Genotyping studies have revealed that the gene encoding a specific protein can have a number of differences in sequence at the nucleotide level. These differences especially called as polymorphisms, sometimes do not cause significant alterations on the final product, but may have an effect on the substrate specificity and activity of the product (especially for enzymes) or other characteristics and functions. For example, polymorphisms in *cytochrome P450 2D6 (CYP2D6)* are one of the cytochrome P450 enzymes of the liver that can influence how humans metabolize cancer drugs, although the enzymes are basically the same sequence and structure. Polymorphisms in CYP2D6 have been seen in the general population about 10% and it has been associated with poor-metabolizer phenotype of enzyme. This is important for codeine-based pain medications due to the activation of codeine to morphine and includes CYP2D6-dependent step [24].

Pharmacogenetic tests based on the determination of genetic variants for drug efficacy or toxicity has begun to use in the 2000s, although genetic-based studies began in the 1950s. FDA has been working to improve pharmacogenetic technologies in the development, regulation and use of medications and revised drug labels in terms of pharmacogenetic biomarkers in oncology area (**Table 2**) [25, 26].

It is important to describe important gene polymorphisms and their clinical meaning in oncology field that may determine the optimum pharmacological treatment in terms of treatment outcomes, tolerability and the occurrence of serious, even life-threatening adverse reactions.

Pharmacogenetic biomarker	Drugs	Labeling	Outcome
UGT1A1	Irinotecan Belinostat Nilotinib Pazopanib	Dose determination and administration; Warnings and precautions, clinical pharmacology	Toxicity
DPYD	Capecitabine Fluorouracil	Warnings and precautions, patient counseling information	Toxicity
TPMT	Cisplatin	Adverse reactions	Toxicity
	Mercaptopurine Thioguanine	Dose determination and administration; Warnings and precautions; Adverse reactions; Clinical pharmacology	Toxicity
G6PD	Rasburicase Dabrafenib	Warnings and precautions; Contraindications; Adverse reactions; Patient counseling information	Toxicity
CYP2D6	Tamoxifen Rucaparib	Dose determination and administration; Clinical pharmacology	Toxicity

Table 2. FDA-approved pharmacogenetic biomarkers for anti-cancer drug labeling [26].

2.2.1. Uridinediphosphate glucuronosyl transferase 1A1 (UGT1A1)

The UGT super family includes four main UGT families, namely UGT1, UGT2, UGT3, and UGT8. The *UGT1* and *UGT2 genes*, encode 16 functional proteins, have been extensively studied and well characterized. A phase II metabolic enzyme, UGT1A1 is the most studied UGT enzyme due to its main role in glucuronidation of exogenous and endogenous substrates, including bilirubin. UGT1A1 also appears in the metabolism processes of most of the anti-cancer drugs, such as topoisomerase I inhibitor irinotecan, the topoisomerase II inhibitor etoposide [27]. Alterations on the glucuronidation activity of UGT1A1 caused by genetic or environmental factors may have significant physiological and pharmacological results on the metabolism of anticancer agents. Allelic variations have been identified in the promoter region and exon 5 of UGT1A1 region. The wild-type allele of *UGT1A1* gene (known as *UGT1A1*1*) has six thymine- adenine (TA) repeats in the promoter region of gene (TATA box). Allelic differences vary from five (*UGT1A1*36*, proficient allele) to eight (*UGT1A1*37*, deficient allele) TA repeats, and these differences affect the UGT1A1-mediated glucuronidation of SN-38 (7-ethyl-10-hydroxycamptothecin), active metabolite of anticancer drug irinotecan both *in vitro* and *in vivo*. Increasing number of TA repeats has been associated with decreased transcription of gene and overall UGT1A1 activity (**Table 3**) [28]. Patients (allele frequency in Caucasians 8–20% are homozygous, 40–50% are heterozygous) with seven TA repeat sequence (named UGT1A1*28) have severe toxicity risk after irinotecan treatment because of decreased gene expression and overall UGT1A1 activity (30% enzyme activity in *28 relative to *1 allele). These patients [with $(TA)_7$ repeats] have fourfold relative toxicity risk compared with patients with six repeat sequences [29]. *UGT1A1*60* (in linkage disequilibrium with TATA box variants) and *UGT1A1*93* are the other variants located in the promoter region. Both of them are found homozygous in around 10% of Caucasians [30]. *UGT1A1*6* and *UGT1A1*27* are located in coding region exon 1. *UGT1A1*6* is the most frequent variant in Asian populations (not found in Caucasians) and associated with ~30% decreased enzyme activity in homozygous patients. *UGT1A1*27* is almost completely eradicated enzyme activity. Nearly 3% of Asian people, are homozygous for both *6 and *27 variant [28, 31].

2.2.2. Dihydropyrimidine dehydrogenase gene (DPYD)

The pyrimidine antimetabolites 5-fluorouracil and its oral prodrug capecitabine are widely used chemotherapeutic agents in the management of variety of tumor types, including colorectal, breast, and head and neck cancers. They activate metabolically and inhibit thymidylate synthase enzyme, which takes role in cellular replication. However, 5-FU leads significant toxicities, such as myelosuppression, mucositis, hand-foot syndrome, and diarrhea. On the other hand, accumulation of knowledge on 5-FU mechanism has developed new strategies that increase the treatment efficacy and response [32, 33]. 5-FU is metabolized by dihydropyrimidine dehydrogenase (DPD) enzyme, and it converts the fluoropyrimidine to its inactive metabolite dihydrofluorouracil (**Figure 3**). DPD enzyme, which is encoded by *DPYD* gene, is a rate-limiting enzyme of 5-FU catabolism and is also used for evaluating the variability of 5-FU metabolism among patients [34]. SNPs in the *DPYD* gene are responsible for insufficient production of DPD enzyme; therefore, low levels of enzyme increase the half-life of the drug,

Genotype	Ref SNP	HGVS Region	Drug	Enzymatic activity Clinical phenotype
UGT1A1*1	Common allele-Wild type	(TA) 6TA	Irinotecan	Normal enzyme activity
UGT1A1*28	rs 8175347	(TA) 7 TA TATA box	Irinotecan	Reduced enzyme activity *1/*28 Irinotecan dosing based on clinical findings *28/*28 Dose reduction recommended.
UGT1A1*36	rs 8175347	(TA) 5 TA TATA box	Irinotecan	Normal enzyme activity Irinotecan dosing recommendations are less clear.
UGT1A1*37	rs 8175347	(TA) 8 TA TATA box	Irinotecan	Normal enzyme activity Irinotecan dosing recommendations are less clear.
UGT1A1*60	rs 4124874	c.-3279T>G Promoter region	Irinotecan	Normal enzyme activity Standard irinotecan dosing
UGT1A1*93	rs10929302	c.-3156G>A Promoter region	Irinotecan	Normal enzyme activity Standard irinotecan dosing
DPYD*9A	rs1801265	c.85T>C, p.Cys29Arg Exon 2	5-fluorouracil Capecitabine	Normal enzyme activity Standard irinotecan dosing
DPYD*2A	rs3918290	IVS14 + 1G>A Intron 14 and exon 14	5-fluorouracil Capecitabine	Reduced enzyme activity Increased toxicity risk
DPYD*13	rs55886062	c.1679T>G, p.Ile560Ser Exon 13	5-fluorouracil Capecitabine	Reduced enzyme activity Increased toxicity risk
TYMS 2R/2R, TYMS 2R/3RG, TYMS 3RG/3RG,	rs34743033	28bp VNTR (2R; 3R) Promoter enhancer region	5-fluorouracil Capecitabine	Enzyme activity based on the repeats 2R/2R: Decreased TYMS expression, increased 5-FU responsiveness, increased risk of toxicity 2R/3RG, 3RG/3RG: Increased TYMS expression, decreased 5-fluorouracil, capecitabine responsiveness, poor prognosis.

Genotype	Ref SNP	HGVS Region	Drug	Enzymatic activity Clinical phenotype
TYMS 3RG/3RC, *TYMS 2R/3RC,* *TYMS 3RC/3RC*	rs2853542	G>C SNP in 2nd repeat of 3R allele (3RC) Promoter enhancer region	5-fluorouracil Capecitabine	Enzyme activity based on the repeats *3RG/3RC: Increased TYMS expression, decreased 5-FU responsiveness, poor prognosis* *2R/3RC or 3RC/3RC: Decreased TYMS expression, increased 5-FU responsiveness, increased risk of toxicity*
MTHFR, 677C>T	rs1801133	c.677C>T, p.Ala222Val Exon 4	Methotrexate	Reduced enzyme activity *Homozygosity: Risk for toxicity from drugs and requirement for dosing adjustments/ discontinuation* *Heterozygosity: Associated with intermediate enzyme activity* *Lower dose requirements for methotrexate*
MTHFR, 1298A>C	rs1801131	c.1298A>C; p.Glu429Ala Exon 7	Methotrexate	Reduced enzyme activity *Homozygosity/Heterozygosity: Associated with intermediate enzyme activity. Lower dose requirements for methotrexate.*
*TPMT*1*	Common allele-Wild type	Common allele-Wild type	6-mercaptopurine; Azathioprine; Thioguanine	Normal enzyme activity
*TPMT*2*	rs1800462	c.238G>C, p.Ala80Pro Exon 5	6-mercaptopurine; Azathioprine; Thioguanine	Poor activity Homozygosity: very low/absent enzyme activity Heterozygosity: intermediate activity *Low/Absent Activity: Increased risk for developing life-threatening side effects at a standard dose of a thiopurine drug.* *Intermediate (Reduced) Activity: Increased risk for toxicity at a standard dose of a thiopurine drug.*

Genotype	Ref SNP	HGVS Region	Drug	Enzymatic activity Clinical phenotype
*TPMT*3A*	rs1800460 rs1142345	c.[460G>A;719A>G], p.[Ala154Thr;Tyr240Cys] Exon 7, 10	6-mercaptopurine; Azathioprine; Thioguanine	Poor activity *Homozygosity—Low/Absent Activity: Increased risk for developing life-threatening side effects from a standard dose of a thiopurine drug. Heterozygosity- Intermediate (Reduced) Activity: Increased risk for toxicity from a standard dose of a thiopurine drug.*
*TPMT*3B*	rs1800460	c.460G>A, p.Ala154Thr Exon 7	6-mercaptopurine, Azathioprine, Thioguanine	Poor activity Homozygosity—*Low/Absent Activity: Increased risk for developing life-threatening side effects at a standard dose of a thiopurine drug.* Heterozygosity—*Intermediate (Reduced) Activity: Increased risk for toxicity at a standard dose of a thiopurine drug.*
*TPMT*3C*	rs1142345	c.719A>G, p.Tyr240Cys Exon 10	6-mercaptopurine, Azathioprine, Thioguanine	Poor activity *Homozygosity—Low/Absent Activity: Increased risk for developing life-threatening side effects from a standard dose of a thiopurine drug. Heterozygosity—Intermediate (Reduced) Activity: Increased risk for toxicity from a standard dose of a thiopurine drug.*
*CYP2D6*1*	Common allele-Wild type	Common allele-Wild type	Tamoxifen	Normal activity *Extensive metabolizer*
*CYP2D6*2*	rs16947	c.584G>c, p.Arg296Cys Exon 2	Tamoxifen	Normal enzyme activity
*CYP2D6*4*	rs3892097	1846G>A Exon 3—Junction of intron 3 and exon 4 (Not applicable variant occurs in a noncoding region)	Tamoxifen	Inactive enzyme

Genotype	Ref SNP	HGVS Region	Drug	Enzymatic activity Clinical phenotype
CYP2D6*5		Whole gene deletion On allele	Tamoxifen	Inactive enzyme
CYP2D6*6	rs5030655	c.1707delT, p.Trp152Gly Exon 3	Tamoxifen	Inactive enzyme
CYP2D6*9		AGA deletion at 2613-2615, p.Lys281 del	Tamoxifen	Reduced enzyme activity (Partially functioning)
CYP2D6*10	rs1065852	c.188C>T, p.Pro34Ser Exon 1	Tamoxifen	Reduced enzyme activity
CYP2D6*17	rs28371706 rs16947	c.320C>T, p.Thr107Ile; c.886T>C, p.Cys296Arg Intron 1	Tamoxifen	Reduced enzyme activity
CYP2D6*41	rs28371725	c.2988G>A Intron 6 (Not applicable variant occurs in a noncoding region)	Tamoxifen	Reduced enzyme activity

Note: Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines.

Table 3. Biological impact of the important pharmacogenetic biomarker variants [28, 42–46].
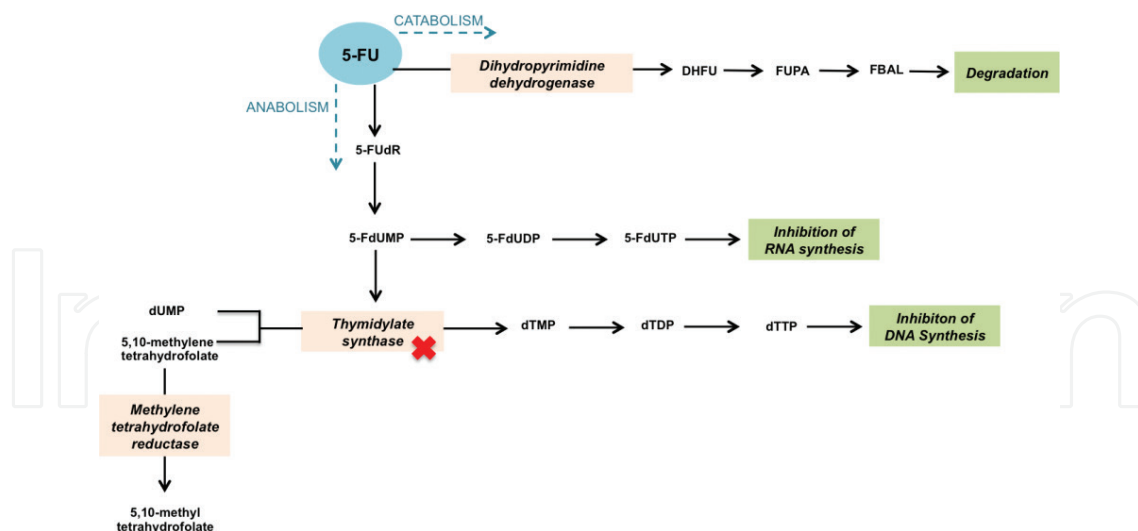
Figure 3. Metabolism of 5-fluorouracil (5-FU). The initial and rate-limiting enzyme of 5-FU is dihydropyrimidine dehydrogenase (DPD), catalysing the conversion of 5-FU into 5,6-dihydrofluorouracil (DHFU). DHFU is degraded into fluoro-beta-ureidopropionic acid (FUPA) and fluoro-beta-alanine (FBAL) through degradation cascade. 5-Fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP), is the cytotoxic product resulting from a multi-step activation of 5-FU, inhibits the enzyme thymidylate synthase (TS) enzyme. This inhibition leads to accumulation of deoxy-uridine-monophospate (dUMP) and depletion of deoxy-thymidine-monophosphate (dTMP), which leads to inhibition of DNA synthesis. 5-FU can also inhibit RNA synthesis in a pathway that involves 5-fluorouridine monophosphate (5-FUMP) and subsequent conversion to 5-fluorouridine triphosphate (5-FUTP) via 5-fluorouridine diphosphate (5-FUDP).

thus, resulting in excess drug accumulation and toxicity due to the inefficient catabolism of drug. Genetic testing of polymorphisms is being used for the classification of patients who would be at high risk for severe or fatal toxicity when receiving fluoropyrimidine-based che-motherapy (**Table 3**) [35]. Complete deficiency of DPD has been seen in approximately 5% of the overall population and also 3–5% of the population has a partial DPD deficiency due to sequence variations in DPYD gene [36].

The IVS14+ 1G>A change with the combination of a mutation in intron 14 and a deletion at 5′-splice consensus sequence of exon 14, the most known and frequent variant (known as DPYD*2A), is resulting the formation of a truncated enzyme product lacking activity. The estimated incidence of homozygous genotype of this allelic variant is 0.1% and heterozy-gous genotype is 0.5–2.0% in Caucasians [37, 38]. Other variants, which are associated with increased toxicity risk, include 496A>G in exon 6, T1679G (DPYD*13) in exon 13 and 2846A>T in exon 22 [39–41]. Genetic mutations in *DPYD* can be analyzed by highly sensitive methods even for heterozygous variants such as pyrosequencing. But determination of the 5-FU and dihydrofluorouracil concentration ratio in plasma by high-pressure liquid chromatography (HPLC) may be more reliable predictor test for toxicity [35]. Besides *DPYD*, there are some other pharmacogenetic biomarkers that are being used for the determination of the efficacy and toxicity of fluoropyrimidine-related therapies, such as *thymidylate synthase gene (TYMS)*.

2.2.3. Thymidylate synthase gene (TYMS)

The main intracellular target of fluoropyrimidine-related therapies is to inhibit thymidylate synthase (TS) enzyme (encoded by the *TYMS* gene) that catalyzes the transformation of dUMP,

which is essential for DNA replication (**Figure 3**) [47]. Fluorodeoxyuridylate (5-FdUMP), an activated metabolite from 5-FU, forms stable complexes with TS enzyme and folate to stop DNA synthesis over blocking the conversion of dUMP to dTMP. Therefore, low expression levels of TS enzyme in colorectal patients receiving 5-FU-based treatment were associated with desired drug response as well as to longer survival [47]. Polymorphisms, which lead *TYMS* variations (2R/2R, 2R/3R, or 3R/3R) by forming double-tandem repeat (2R) or a triple-tandem repeat (3R) on the *TYMS* promoter enhancer region (generally abbreviated as *TSER*), influence the translation of TYMS mRNA and toxicity (**Table 3**). It has been showed that homozygous 3R/3R cells overexpressed *TYMS* mRNA compared with homozygous 2R/2R cells [48]. Apart from these repeats, a G>C SNP has been showed on the 12th nucleotide of the second repeat at 3R allele, and it is causing a tri-allelic locus as 2R, 3RG, and 3RC. The 3RC allele has similar transcriptional activity of the 2R allele [49]. Another genetic polymorphism of the *TYMS* gene has been identified as a 6 basepair deletion at position 1494 in the 3′-untranslated region (3′-UTR). It was demonstrated that there is a strong association between the 6-bp deletion and low *TYMS* mRNA expression in colorectal tumor tissue [50, 51].

2.2.4. Methylene tetrahydrofolate reductase (MTHFR)

Methylene tetrahydrofolate reductase (MTHFR) a regulatory enzyme is involved in folate metabolism that redirects folate metabolites from pyrimidine synthesis towards methionine synthesis (**Figure 3**). Most of the *MTHFR* gene variants of enzyme contain polymorphisms that lead loss-of-function in the enzyme (**Table 3**). The level of loss-of-function depends on the type and number of polymorphisms in coding gene. Therefore, *MTHFR* polymorphisms affect drug metabolism, such as methotrexate, may be more likely to experience toxicity. c.677C>T (p.A222V) and c.1298A>C (p.G429A) are the most common polymorphisms of *MTHFR* gene that forms abnormal forms of enzyme. Both polymorphisms are associated with reduced MTHFR enzyme activity. Adjustment drug dosages and prediction of the toxicity risk, *MTHFR* polymorphism test may be performed for a patient who is treated with methotrexate [23].

2.2.5. Thiopurine-S-methyltransferase (TPMT)

Thiopurine-S-methyltransferase (TPMT) enzyme, encoding by *TPMT* gene, catalyzes the S-methylation of thiopurine drugs (such as 6-mercaptopurine, azathioprine, and thioguanine) for drug inactivation. The purine antimetabolites are converted to active thioguanine nucleotides that can be incorporated into DNA or RNA to block the DNA replication. During this activation process, purine antimetabolites cause cellular toxicity for both malign and benign cells. TPMT catalyzes inactivation of the formed thioguanine nucleotides [52]. It is known that some patients have low enzyme activity due to germline genetic variations. There are several *TPMT* polymorphisms that caused more than 20 variant alleles of *TPMT* gene (from *TPMT*2* to *TPMT*20*) and have different effects on TPMT function (**Table 3**). Among these variant alleles *TPMT*2, TPMT*3A, TPMT*3B*, and *TPMT*3C* have been determined as responsible for enzyme deficiency. Among these alleles TPMT*2, TPMT*3A and TPMT*3C are deficient alleles that present poor enzyme activity [45]. Accumulation of cytotoxic thiopurine

nucleotides leading to severe toxicity has been shown in people with deficient *TPMT* alleles, after receiving a standard dose thiopurine-based treatment. Patients with low TPMT activity expose more activated thioguanine nucleotides and more treatment toxicity as well as efficacy. Patient's genotype predicts thiopurine nucleotide levels and treatment outcomes in thiopurine drug metabolism that shows tri-modal distribution with 89–94% of patients having high enzyme activity, 6–11% of them having intermediate activity (as heterozygous), and 0.3% of them having low or no activity (as homozygous) [53].

2.2.6. Glucose-6-phosphate dehydrogenase (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) is a metabolic enzyme involved in pentose phosphate pathway that is important in red blood cell metabolism. The *G6PD* gene is highly polymorphic with more than 300 variants. *G6PD* gene deficiency is a common disease-causing enzymopathy and is associated with low levels of G6PD enzyme. The most remarkable symptom of *G6PD* gene deficiency is hemolytic anemia caused by the ingestion of drugs and food substances that result in oxidative stress. The excess peroxide due to deficiency has a risk for both hemolytic and methemoglobinemia. Rasburicase is a recombinant urate oxidase that has been used for the initial management of plasma uric acid in pediatric and adult patients with leukemia, lymphoma and solid tumors who are receiving chemotherapeutic agents and are expected for tumor lysis syndrome. Rasburicase is contraindicated in patients with *G6PD* gene deficiency; therefore, FDA recommends screening for G6PD deficiency before beginning rasburicase treatment. Dabrafenib is a kinase inhibitor that blocks the growth and spread of cancer cells in the body. Dabrafenib consists of a sulfonamide moiety and contains hemolytic anemia risk in patients with G6PD deficiency. Thus, FDA recommends monitoring for patients with G6PD deficiency started with dabrafenib [54–56].

2.2.7. Cytochrome P450 2D6 (CYP2D6)

Cytochrome P450 2D6 (CYP2D6) deficiency is the first discovered pharmacogenetic deficiency with the characterization of decreased enzyme expression-related polymorphisms. Decreased mRNA levels of *CY2D6* were determined as the reason of reduced metabolism and adverse reactions to an anti-hypertensive drug, debrisoquine. These findings based on molecular technologies display the importance of genotyping on the pharmacological phenotyping involved in drug metabolism [35]. CYP2D6 is a highly polymorphic enzyme (**Table 3**). According to increased or decreased enzyme activity due to SNPs, duplications or deletions, genetic variants of CYP2D6 were classified into four metabolic phenotypes: ultra-rapid metabolizer, extensive metabolizer, intermediate metabolizer and poor metabolizer (**Table 4**). This classification system has been used to control treatment recommendations for several drugs, including tamoxifen [57].

Tamoxifen is a selective estrogen receptor modulator, which is used in the treatment of estrogen receptor-positive breast cancer. Tamoxifen is a weak estrogen antagonist by itself, but it is converted into its main active metabolite, antiestrogenic endoxifen, by CYP2D6. CYP2D6 genotype is predictive of endoxifen exposure that is critical in determining treatment outcome [59]. Increased endoxifen exposure is associated with increased treatment efficacy and toxicity [60, 61].

Phenotype	Genotype
Poor metabolizer	Two inactive alleles
Intermediate metabolizer	Two active alleles
Extensive metabolizer	One active and one inactive alleles; Two decreased activity alleles; One decreased activity and one inactive allele
Ultra-rapid metabolizer	More than two copies of active allele

Notes: Active alleles: *1, *2, *33, *35; decreased activity alleles: *9, *10, *17, *29, *36, *41; inactive alleles: *3, *4, *5, *6, *7, *8, *11-*16, *19-*21, *38, *40, *42.

Table 4. CYP2D6 phenotypes according to activity status of *CYP2D6* alleles (adapted from Swen JJ., et al. 2011) [58].

2.3. Biomarker discovery studies for pharmacogenetic applications

Pharmacogenetics and its backbone studies in terms of polymorphisms present new developments and trends in the field of tailored medications and advancements in the modification of therapeutic choices utilizing genotypic information from polymorphism analysis. Pharmacogenetic biomarker studies have multiple processes from discovery to clinical implementation (**Figure 4**). The ultimate aim of the biomarker studies is to find a clinically accessible decision-maker biomarker to improve patient outcomes. However, many of the valid associations cannot be achieved for clinical implementation due to the lack of sufficient robustness or clinical importance for the question. Biomarker discovery studies should be performed as the screening of genotype-phenotype relations in large cohorts with statistical and bioinformatics tools. The most significant markers obtained from discovery studies are replicated for analytical validation in different cohorts with the evaluation of assay reproducibility and robustness. After successful analytical validity, the biomarker and assay must be evaluated to confirm its performance in diagnosing the clinical phenotype or predicting outcome of
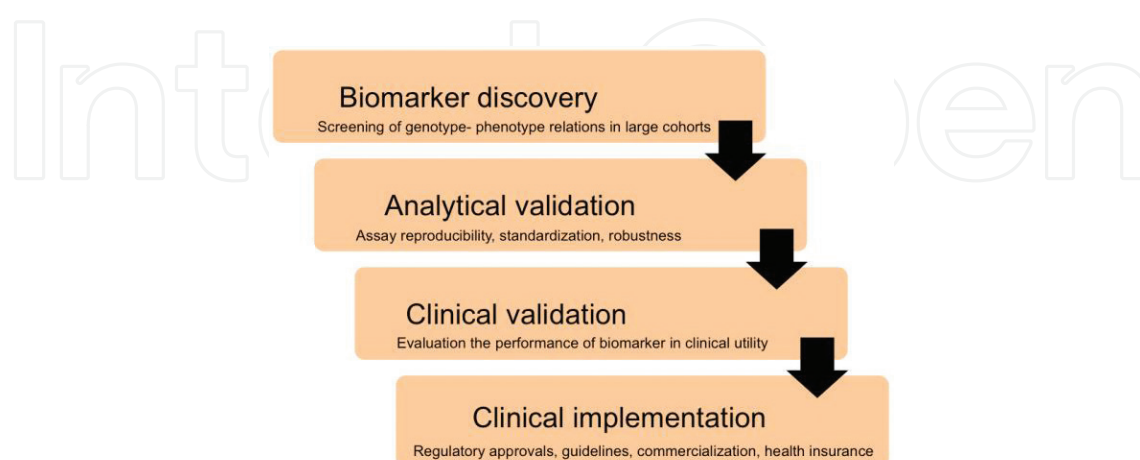

Figure 4. Schematic presentation of pharmacogenetic biomarker studies from development to clinical implementation.

interest. A clinically valid biomarker assay can undergo translation through prospective confirmation of clinical utility to improve patient outcomes. And finally, analytically and clinically validated biomarker assay is ready for implementation phase that includes regulatory approval and incorporation in clinical practice guidelines, commercialization and coverage by health insurance [52, 62].

2.4. Future directions and conclusion

After "Human Genome Project," science world began to use individual genotypic information to predict the risk of diseases, to prognose the disease, to guide therapeutic decision-making, and to develop targeted medications. Especially, the better prediction ability for drug response and life-threatening toxic reactions is highly crucial for clinicians due to the narrow therapeutic index of many cancer chemotherapeutic agents. The aim of personalized medicine is to prescribe a convenient chemotherapeutics to the right patients with right dose to achieve maximal therapeutic benefit with minimal toxicity. Deep observation of the human genome is still ongoing, and it will help to discover new targets and select the most efficacious drug for each patient's tumor, which is named as genomic prescribing system, the next evolution of systemic cancer management. Genetic polymorphisms, as significant biomarkers, have provided significant and crucial information in the management of toxicity and dosing of cancer treatments. So far the achievements have been limited due to the multifactorial challenges on the polymorphism-based personalized cancer management. Especially reliable clinical data for the effects of genetic alterations on the disease pathogenesis, drug metabolism and response are not always available; and performing the large-scale prospective clinical studies, to understand the associations of polymorphisms and the application of chemotherapeutics, is often laborious. However, these prospective studies are required for establishing possible relations for evaluating the utility and cost-effectiveness of polymorphism-based pharmacogenetic tests and personalized medicine. Ethical, social, and regulatory issues relevant with pharmacogenetic-based personalized medicine are the other complex and challenging factors to establish the relations between genetic polymorphisms and personalized drug responses.

The main application of genetic polymorphism knowledge is improving the futuristic health care through gene therapy, discovery of new drugs and drug targets and upgrade the discovery processes with advanced technologies. The use of single candidate genes can be useful as a part of initial treatment, but in near future, it will never be enough to provide fully tailored treatment decisions on the cancer management. Other omics technologies will complement the genotype-phenotype association-based pharmacological-pathway approach, such as transcriptomics, epigenomics, and miRNAomics.

2.4.1. Polymorphism and pharmacogenetics facts

- Humans share about 99.9% sequence identity. The other 0.1% are mostly SNPs.

- SNPs are the most common polymorphism type and occur about every 1000 bases.

- SNPs can be silent—99% of them are not in coding regions and not in genes and thus cause harmless, harmful, latent changes.

- Genetic polymorphisms are one of the most important factors that may contribute to ethnic sensitivity of a drug.

- FDA recommends for following guidelines relating to "ethnically sensitive situations" for some drugs and suggests solutions that may control such ethnic sensitivity in the context of therapeutic applications and study designs.

- The Hardy-Weinberg principle can be used to calculate allele frequencies.

- Hardy-Weinberg principle-based reporting may be optimal in genetic polymorphism-based studies.

- Personalized medicine separates patients into different groups with an individual's molecular profile and covers the determination of the safest and most effective chemotherapeutic agents.

- The aim of pharmacogenetics is to make 'personalized medicine' as applicable to various patient groups.

- Although a number of good polymorphism genotyping technologies are currently in use to meet the needs of clinics and researches, not only one genotyping method is ideal for all applications. Studies on ideal polymorphism genotyping technologies are still ongoing.

- FDA has been working to improve pharmacogenetics technologies in the development, regulation and use of medications and revise drug labels in terms of pharmacogenetic biomarkers in oncology area.

- Polymorphism in the *DPYD* gene is responsible for insufficient production of DPD enzyme; therefore, low levels of enzyme increase drug accumulation and toxicity due to the inefficient catabolism of drug.

- Allelic differences of *UGT1A1* affect the glucuronidation of SN-38, active metabolite of irinotecan both *in vitro* and *in vivo*.

- *TPMT* polymorphisms have different effects on function of TPMT that inactivates thioguanine nucleotides. Patients with low TPMT activity expose more activated thioguanine nucleotides and more treatment toxicity as well as efficacy.

- FDA recommends screening for G6PD deficiency before the beginning of rasburicase treatment.

- *CYP2D6* deficiency is the first discovered pharmacogenetic deficiency. Genetic variants of CYP2D6 are classified into ultra-rapid metabolizer, extensive metabolizer, intermediate metabolizer, and poor metabolizer.

- Biomarker development studies involve multiple steps, as biomarker assay development, analytical validation, clinical validation, and clinical implementation.

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