Pharmacogenetics: implementing personalized medicine

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Summary

Pharmacogenetics and pharmacogenomics have been widely recognized as fundamental steps toward personalized medicine. They deal with genetically determined variants in how individuals respond to drugs, and hold the promise to revolutionize drug therapy by tailoring it according to individual genotypes.
The clinical need for novel approaches to improve drug therapy derives from the high rate of adverse reactions to drugs and their lack of efficacy in many individuals that may be predicted by pharmacogenetic testing.
Significant advances in pharmacogenetic research have been made since inherited differences in response to drugs such as isoniazid and succinylcholine were explored in the 1950s. The clinical utility and applications of pharmacogenetics and pharmacogenomics are at present particularly evident in some therapeutic areas (anticancer, psycotrophic, and anticoagulant drugs).
Recent evidence derived from several studies includes screening for thiopurine methyl transferase or uridine 5'-diphosphoglucuronosyl-transferase 1A1 gene polymorphisms to prevent mercaptopurine and azathioprine or irinotecan induced myelosuppression, respectively. Also there is a large body of information concerning cytochrome P450 gene polymorphisms and their relationship to drug toxicity and response. Further examples include screening the presence of the HLA-B*5701 allele to prevent the hypersensitivity reactions to abacavir and the assessment of the human epidermal growth factor receptor (HER-2) expression for trastuzumab therapy of breast cancer or that of *KRAS* mutation status for cetuximab or panitumumab therapy in colorectal cancer.
Moreover, the application of pharmacogenetics and pharmacogenomics to therapies used in the treatment of osteoarticular diseases (e.g. rheumatoid arthritis, osteoporosis) holds great promise for tailoring therapy with clinically relevant drugs (e.g. disease-modifying antirheumatic drugs, vitamin D, and estrogens).
Although the classical candidate gene approach has helped unravel genetic variants that influence clinical drug responsiveness, gene-wide association studies have recently gained attention as they enable to associate specific genetic variants or quantitative differences in gene expression with drug response.
Although research findings are accumulating, most of the potential of pharmacogenetics and pharmacogenomics remains to be explored and must be validated in prospective randomized clinical trials.
The genetic and molecular foundations of personalized medicine appear solid and evidence indicates its growing importance in healthcare.

KEY WORDS: pharmacogenetics, drug effects, drug metabolism, drug therapy, antineoplastic agents.

Introduction

The vision of personalized medicine is a compelling one for the future of medical care. It foresees the use of molecular data to better classify disease, to facilitate the development and validation of new targeted therapies, to treat patients with more specificity and efficacy but fewer adverse events, and to more accurately determine disease predisposition. This vision is driven by the results of the Human Genome Project (Lander et al., 2001; Venter et al., 2001) and of the HapMap Project (The International HapMap Consortium, 2005; The International HapMap Consortium, 2007). Research findings in biomedical research based on this knowledge have unfolded a series of new predictive sciences among which are pharmacogenetics and pharmacogenomics. These sciences have been widely recognized as fundamental steps toward personalized medicine. They hold the promise to revolutionize how medicine is practiced. No longer will medicine focus, as it has historically, on symptomatic disease management and empirical drug-prescribing regimens but will move forward to a new era of individualized medicine. The clinical need for novel approaches to improve drug therapy derives from the high rate of adverse reactions to drugs and their lack of efficacy in many individuals that may be predicted by knowledge of their specific genetic make-up (Wilke et al., 2007). These clinical occurrences are due to interindividual variability in drug response. All patients do not respond to the same medicine in the same way. Some patients may experience adverse drug reactions that do not occur in other patients taking the same drug at the same dose. A drug may also display varied efficacy in different patients. In the past, the differences in the risk-benefit ratio between patients taking the same drug was attributed to non genetic factors such as age, sex, nutritional state, general medical condition (e.g. hepatic and renal function), lifestyle (diet, alcohol abuse, smoking), concomitant therapy or the presence of comorbidity. Today, in addition to these factors the differences in patient genetic make-up have been recognized to play an important role in the individual response to drugs. Results from studies on monozygotic and dizygotic twins have shown that genetic factors account for most of the variation in pharmacokinetics and pharmacodynamics of highly metabolized drugs (Relling and Giacomini, 2006).

History and terminology

Friederich Vogel coined the term “pharmacogenetics” in 1959 (Vogel, 1959) to define a new science aimed to study the influence of inherited factors on drug response variability through genetic and pharmacological knowledge and methods.

Evidence for a genetic basis of clinical syndromes associated with the administration of drugs emerged in the early '50s when antimalarial drugs (e.g. primaquine) were shown to induce haemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency (Carson et al., 1956). In the same period, further observations revealed that adverse reactions to drugs (isoniazide-induced peripheral neuropathy and a succinylcholine-induced apnea) were associated to inherited deficit of enzymatic activities (N-acetyl transferase and succinylcholine esterase), respectively (Kalow and Staron, 1957; Evans et al., 1960; Goedde et al., 1968).

The discovery of the first pharmacogenetic deficiency at the molecular level occurred in the 1980s when Gonzales et al. cloned the *CYP2D6* gene and characterized the genetic polymorphism responsible for the decreased expression of the CYP2D6 enzyme (Gonzales et al., 1988). Decreased expression levels of CYP2D6 mRNA were previously identified as responsible for reduced metabolism and adverse response to debrisoquine, an anti-hypertensive drug (Mahgoub et al., 1977; Bertilsson et al., 1980). This research gave rise to several studies based on the use of efficient molecular technologies linked to classical pharmacological phenotypization and genetic population studies that permitted the identification of several polymorphisms in genes involved in drug metabolism and mechanism of action.

At the end of the 1990s, the term "pharmacogenomics" was introduced in the medical literature. At present there is no consensus regarding the working definition of "pharmacogenomics" and this term is often used interchangeably with that of "pharmacogenetics".

The European Agency for the Evaluation of Medicinal Products (EMEA) defines "pharmacogenetics" as "the study of interindividual variations in DNA sequence related to drug response" and "pharmacogenomics" as "the study of the variability of the expression of individual genes relevant to disease susceptibility as well as drug response at cellular, tissue, individual or population level" (The European Agency for the Evaluation of Medicinal Products (EMEA), 2002). This definition agrees with the most common meaning of the term that considers pharmacogenomics as the evolution of pharmacogenetics on a genomic scale. Pharmacogenetics utilizes in fact available genetic technologies to study a limited number of genes to characterize the molecular basis of the individual response to drugs, while pharmacogenomics involves the study of the whole genome (DNA) or its products (RNAs) as they relate to drug response utilizing high-throughput technologies characteristic of this science.

Through these approaches, it has already been possible to identify several variations in the structure of genes codifying enzymes of drug metabolism, transporter proteins or target proteins (receptors, ion channels, enzymes) of drugs and to correlate these gene variations to interindividual variations in the response to xenobiotics. Many genetic factors, predictive of toxicity and response to pharmacological treatments, have thus been identified. In the future, pharmacogenetic and pharmacogenomic tests may help clinicians to choose the best treatment and safest dose for each patient. The identification of individuals that very presumably may manifest an adverse reaction to a specific pharmacological treatment will avoid the long process of dose adjustment and the risk of toxicity. In the meantime, these tests are potentially useful for selection of patients who may benefit from a specific pharmacological treatment.

Genetic polymorphisms influencing drug response

The disposition and fate of drugs (*pharmacokinetics*) and their therapeutic and toxicological effects (*pharmacodynamics*) depend on complex processes involving proteins codified by different genes influencing drug transport, metabolism, and mechanism of action.

It is thought that most genes contain casual variations in their nucleotide sequence developed during evolution. Variations located in a codifying region may lead to substitution of an amino acid in a specific position of a protein and consequently may affect protein function. When variations occur in a regulatory region, they may influence transcriptional and translational mechanisms with consequent modulation of gene product (mRNA and proteins) expression levels (Relling and Giacomini, 2006; Court, 2007).

Variation in the DNA sequence with a 1% allelic frequency or greater in a population is defined as *polymorphism*, while variation characterized by less frequency is defined as *mutation*. Gene mutations and polymorphisms codify for enzymes characterized by different metabolic activity or receptors with different affinity for the drug. They modify the pharmacological response in individuals or, in case of variations particularly frequent in some ethnic groups, even in a population (Relling and Giacomini, 2006; Court, 2007).

Genetic variations concerning single base pair substitutions, the simplest genetic variants, are defined as *single nucleotide polymorphisms* (SNPs). Genetic variations may also involve several nucleotides or long DNA traits. In this case they are considered large mutations and defined *substitutions, insertions, deletions, amplifications and translocations* (Relling and Giacomini, 2006; Court, 2007).

Prototypes in pharmacogenetics refer to monogenic traits. They consist of polymorphisms of a single gene codifying for a protein involved in the metabolism or in the effects of a drug that cause variable individual responses to this drug. Some examples are reported in Table I.

Allelic variants of CYP proteins are responsible for an increased response and toxicity from drugs belonging to very different classes (e.g. anticoagulant, psycothropic and immunosuppressive drugs) or for the diminished response to prodrugs such as codein that requires metabolism to morphine to be active (Ingelman-Sundberg et al., 2007).

This concept is shown in Figure 1: homozygous individuals for the *polymorphic* or *variant* allele (V/V), with a reduced drug metabolism have higher plasma drug concentrations than those obtained in wild-type homozygous individuals (Wt/Wt) even after a single dose (panel A). This condition may lead to achieve and exceed drug plasma concentrations causing toxic effects (dotted gray line).

The multidrug resistance gene (*MDR1* or *ABCB1*) codifies for P-glycoprotein (PgP), a member of the ABC superfamily of transporters (ATP-Binding Cassette family). It is a potent efflux pump for xenobiotics and physiological substrates whose overexpression plays an important role in the development of resistance to several anticancer drugs by their extrusion from the neoplastic cell (Nobili et al., 2006). Pgp is also responsible for the biliary and renal excretion of several drugs and may modify their intestinal absorption or their distribution into the central nervous system (Ho and Kim, 2005). The 3435C→T SNP on exon 26 of the *MDR1* gene is associated with pharmacokinetic alterations of various drugs including digoxin. This allelic variant leads to a significant increase in digoxin plasma concentrations (following the same oral dose) in homozygous 3435TT patients, in relation to the increased bioavailability of the drug due to the diminished expression of PgP at the duodenal mucosal level (Chowbay et al., 2005).

Asmatic patients with SNPs leading to amino acid substitutions (e.g. Arg→Gly at codon 16) in the β_2-adrenergic receptor whose stimulation produces bronchodilation, develop a diminished response to β_2-agonistic drugs such as terbutaline compared to wild-type patients (Arg16) (Green et al., 1995; Snyder et al., 2006). The modification in the dose-effect curve due to a

Table I - Examples of genetic polymorphisms that influence drug effects in humans.

Drug	Variable clinical effect	Genes with associated variants	Possible mechanism
Azathioprine and mercaptopurine	Increased haematopoietic toxicity	TPMT	Hypofunctional alleles
	Reduced therapeutic effect at standard doses		Wild-types alleles
Irinotecan	Increased hematopoietic toxicity	UGT1A1	Decreased expression due to regulatory polymorphism
Fluorouracil	Increased toxicity	DPD	Abrogation of enzymatic activity due to exonic mutation
Antidepressants, β-blockers	Increased toxicity	CYP2D6	Hypofunctional alleles
	Decreased activity		Gene duplication
Codeine	Decreased analgesia		Hypofunctional alleles
Omeprazole	Peptic ulcer response	CYP2C19	Hypofunctional alleles
Warfarin	Increased anticoagulant effects	CYP2C9	Coding region variants causing reduced S-warfarin clearance
	Reduced anticoagulant effects	VKORC1	Variant haplotypes in regulatory regions leading to variable expression
HIV protease inhibitors, digoxin	Decreased CD4 response in HIV-infected patients, decreased digoxin bioavailability	ABCB1 (MDR-1)	Altered P-glycoprotein function
Abacavir	Immunologic reactions	HLA variants	Altered immunologic responses
β1-antagonists	Decreased cardiovascular response	β1-adrenergic receptor	Altered receptor function or number
β2-agonists	Decreased bronchodilation	β2-adrenergic receptor	Altered receptor function or number
Diuretics	Blood pressure lowering	Adducin	Altered cytoskeletal function by adducin variants
QT prolonging drugs	Drug-induced arrythmia	Ion channels (HERG, KvLQT1, Mink, MiRP1)	Exposure of subclinical reduction in repolarizing currents by drugs
HMG-CoA reductase inhibitors (statins)	Low density lipoprotein cholesterol lowering	HMGCR	Altered HMG-CoA reductase activity

From Roden et al., Ann Intern Med 2006; 145: 749-57 (modified)

polymorphism of the pharmacological target (e.g. a receptor) is reported in Figure 1B. Reduced efficacy is generally not accompanied by reduced toxicity to the drug, since therapeutic and toxic effects often depend on different pathways. In the case of bronchodilators, the same allelic variant of the β_2-adrenergic receptor does not impede the tachycardic and proarrythmic effects of β_2-agonistic drugs on the heart (Kell, 2005). Consequently, the therapeutic index of the drug may be reduced.

Drugs must interact with specific targets localized in the plasma, on the cellular membrane or in cytoplasm to be efficacious. Qualitative (e.g. in the amino acid sequence) or quantitative (in the levels of gene expression) modifications of these effectors lead to the commonly observed biological variability, but they may also cause genetically determined pathologies (see Table I). In both cases, the administration of a drug which is safe and efficacious in the general population, may cause severe adverse effects in individuals carrying the disease-gene and make manifest a subclinical alteration as it occurs in a relatively rare but clinically important syndrome such as the long QT syndrome (LQTS). LQTS, in the congenital form, is determined by genetic alterations in ion channels that control the ventricular repolarization phase. The LQTS predisposes to the development of potentially fatal cardiac arrythmia, such as *torsade de point* and to sudden death.

A large number of cardiovascular and non cardiovascular drugs may provoke *torsade de point* in LQTS patients (Kannankeril, 2008). Most of these drugs produce this effect by interaction with an ion K^+ channel codified by the *HERG* gene. Some drugs such as terfenadine and cisapride are no longer commercially available because of their high probability for inducing *torsade de point.*

Pharmacogenetics in clinical practice

Significant advances in pharmacogenetic research have been made since inherited differences in response to drugs such as isoniazid and succinylcholine were explored in the 1950s. Table I shows some of the various clinical conditions in which genetic variability may lead to reduced therapeutic efficacy or increased risk of adverse reactions. The implications are partic-

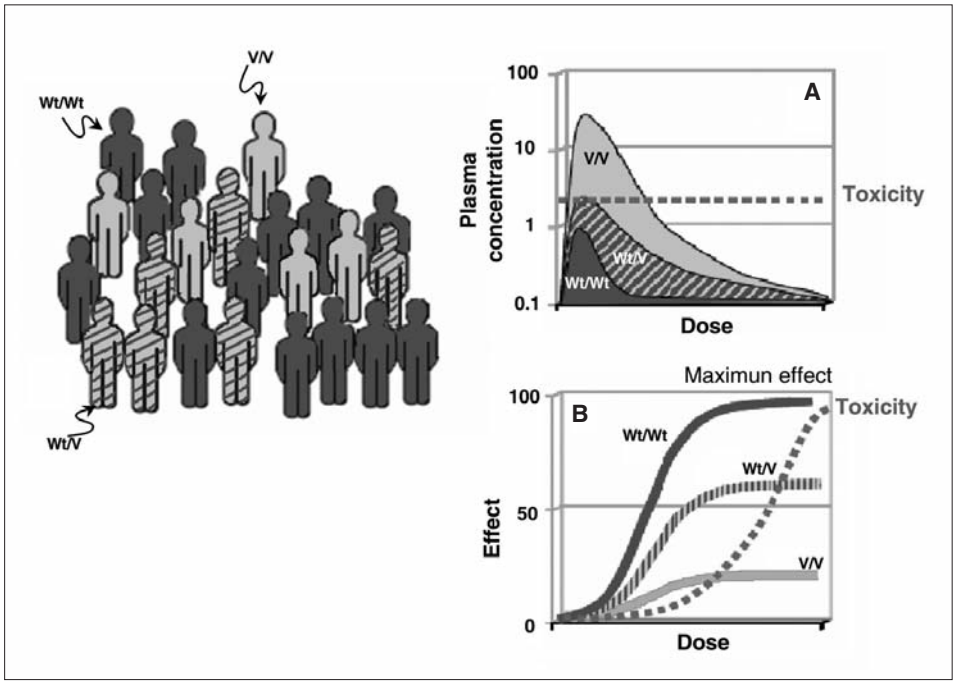

Figure 1 - Consequences of polymorphisms on plasma concentrations (A) and on the effect (B) of drugs. From Evans and McLeod, New Engl J Med 2003;348:538-549 (modified).

ularly relevant in case of chronic therapies or therapies with drugs characterized by a narrow therapeutic index, or in patients at risk for age and presence of concomitant pathologies requiring the coadministration of several drugs. Several convincing studies in the areas of cardiovascular (Lowes and Buttrick, 2008), respiratory (Rubin and Fink, 2007), gastrointestinal (Camilleri and Saito 2008), autoimmune (Ross et al., 2007), and neuropsychiatric (Hopkins and Martin, 2006) therapies have recently contributed to the recognition of potential clinical utility and applications of pharmacogenetics and pharmacogenomics in these therapeutic areas. Also, the application of pharmacogenetics and pharmacogenomics to therapies used in the treatment of osteoarticular diseases (e.g. rheumatoid arthritis, osteoporosis) holds great promise for tailoring therapy with clinically relevant drugs (e.g. disease-modifying antirheumatic drugs, vitamin D, and estrogens) (Ranganathan, 2008; Carbonell Sala and Brandi, 2007).

Cancer pharmacogenetics and pharmacogenomics

The problem of interindividual variability in drug response acquires particular relevance in the treatment of cancer due to the extremely narrow therapeutic index of anticancer drugs. Even limited alterations in the metabolism of an anticancer drug due to genetic variations may cause important changes in its pharmacological effect both in terms of toxicity and efficacy. This occurs unfortunately often since medical oncologists establish anticancer drug dosing on a standardized fashion, i.e. based on the patient body surface area and other non genetic factors.

As for other diseases, variation in the DNA sequence may regard the structure of genes coding for enzymes of metabolism, drug transport or activity, thus affecting drug disposition and fate, toxicity and efficacy.

Not only polymorphisms of the host genome but also those of the tumor genome may influence the response to anticancer drugs. Both host and tumor genomic variations regulate transport, retention and efflux of anticancer drugs, determining the degree of penetration into tumor tissue.

The tumor genome possesses most of the polymorphisms that influence tumor aggressiveness and its drug sensitivity or resistance (e.g. *p53*, *KIT*, *EGFR* and *KRAS* mutations, *TS* polymorphisms, etc.), hence treatment efficacy. Host genome polymorphisms are the main determinants of toxicity risk (e.g. polymorphisms of metabolism genes such as thiopurine methyltransferase, dihydropyrimidine dehydrogenase, UDP glucuronosyltransferase, etc.) which tumor genome polymorphisms do not appear to affect.

It is particularly important to briefly describe some examples of gene polymorphisms and their clinical meaning in a field in which knowledge is more developed and choice of the optimum pharmacological treatment for patients may determine the difference between cure, or at least a prolonged survival, and an unfavorable short-term outcome as well as that between good treatment tolerability and the occurrence of serious, even life-threatening adverse reactions.

Thiopurine S-methyltransferase (TPMT) is the enzyme responsible for the inactivation, by S-methylation, of mercaptopurine, which is mainly used in the treatment of acute lymphoblastic leukemia in children. Polymorphisms of the encoding gene of this enzyme are responsible for a marked decrease in enzymatic activity, and thus make difficult the administration of a safe dose of this drug. Leukemic children with an inherited deficiency of TPMT, if treated with mercaptopurine, develop severe bone marrow toxicity. Thus, although relatively rare *TPMT* polymorphisms are very important from a clinical point of view. Polymorphic homozygous individuals at high risk of toxicity represent only 0.3% of Caucasians (Weinshilboum et al., 1980) but those heterozygous with an intermediate risk are about 10% of the population) (McLeod et al., 2002).

Diagnosis of this phenotype (functional TPMT status) and the relative genotypes may thus be very useful for a more rational clinical use of mercaptopurine. Tests for genotypization of TPMT are currently commercially available in the USA. These tests are performed in certified clinical laboratories and their use is proposed for selecting optimal dosing in different categories of patients.

Dihydropyrimidine dehydrogenase (DPD), rate-limiting enzyme of 5-fluorouracil (5-FU) catabolism, converts this fluoropyrimi-

dine to its inactive metabolite dihydrofluorouracil (Heggie et al., 1987). Low levels of DPD have been associated with 5-FU-induced toxicity (Diasio et al., 1988; Johnson, 1999). Several SNPs in the complex structure of the gene, responsible for inefficient metabolism of 5-FU and the consequent increased risk of severe or fatal toxicity, have been identified. Of these, the G→A point mutation at 5'-splice consensus sequence of exon 14 (IVS14+ 1G>A) that causes the loss of the exon itself and the formation of a truncated product lacking enzymatic activity, is the most frequent (Johnson, 1999; Van Kuilenburg et al., 2004). It is estimated that the incidence of homozygous genotype individuals for this allelic variant (*DPYD*2A*) is 0.1% and that of heterozygous is 0.5-2.0% in Caucasians (Etienne et al., 1994; Lu et al., 1993).

Although the correlation between DPD enzymatic activity and genotype is adequate, its analysis is still laborious since it requires the determination of several known allelic variants; thus, it is believed that standard testing of DPD phenotype by determining the 5-FU and dihydrofluorouracil concentration ratio in plasma by high pressure liquid chromatograpy (HPLC) may be a more reliable predictor of toxicity (Di Paolo et al., 2001; Di Paolo et al., 2002).

Uridine diphosphoglucuronosyltransferases (UGTs) belong to a superfamily of enzymes that catalyse the glucuronidation of several endobiotics and xenobiotics, a process leading to the formation of metabolites with greater polarity and water solubility (Nagar and Remmel, 2006).

Several anticancer drugs such as irinotecan, etoposide and epirubicin are substrates of the UGT1A subfamily (Bosch et al., 2006). The UGT1A1*28 variant, the most extensively investigated, is a microsatellite mutation characterized by a TA insertion in the regulatory TATA box of the UGT1A1 promoter region (seven TA inserts $(TA)_7$ instead of six (TA)6 that lead to reduced expression and activity of the enzyme) (Bosma et al., 1995; Monaghan et al., 1996).

UGT1A1 inactivates SN-38, the active metabolite of irinotecan, into the more polar SN-38 glucuronide (Gupta et al., 1994). The presence of $(TA)_7$, in the UGT1A1 promoter reduces enzyme expression and levels of SN-38 glucuronidation (Iyer et al., 1998). Thus, patients who are homozygous or heterozygous for UGT1A1*28 have higher levels of SN-38 and experience severe neutropenia (Innocenti et al., 2004; Marcuello et al., 2004).

The frequency of the $(TA)_7$ allele ranges from 32 to 39% in Caucasians (Lampe et al., 1999; Beutler et al., 1998).

Based on the available data for UGT1A1*28 and irinotecan toxicity, the FDA has requested the inclusion of *UGT1A1* genotype information in the drug package insert, with recommended dose reduction based on genotype (No Authors listed, 2005). It has been observed that, at standard doses of the common regimen FOLFIRI, *UGT1A1* wild type and heterozygous patients could be underdosed with regard to irinotecan since homozygous variant patients showed a higher response and survival (Toffoli et al., 2006). Thus, a UGT1A1 dose-escalation study of irinotecan in colorectal cancer patients treated with the FOLFIRI regimen has been performed and it was observed that irinotecan is tolerated up to 310 mg/m^2 for heterozygous and to 370 mg/m^2 for wild-type patients. Preliminary data suggest that an increase in irinotecan dose in these UGT1A1 genotypes could increase tumor response (Toffoli et al., 2008).

The tumor response to chemotherapy depends on a wide series of genes including those that control the availability and action of drugs at tumor level, those codifying for oncogenes, those involved in signal transduction, in cell proliferation and in DNA repair, and in the apoptotic process.

There is a large body of information concerning single molecular determinants and their relationship to anticancer drug response.

Some examples include the 9:22 chromosome translocation in chronic myeloid leukemia (CML) and *KIT* mutations in gastrointestinal stromal tumors (GISTs) in relation to the response to imatinib, the overexpression of the HER-2 receptor in breast cancer in relation to the response to the monoclonal antibody trastuzumab and the promoter thymidylate synthase (TS) polymorphisms in the regulation of enzyme expression and in response to 5-FU, the activating mutations of the epidermal growth factor receptor (EGFR) and its gene amplification in non-small cell lung cancer (NSCLC) in relation to the response to tyrosine kinase inhibitors associated with this receptor, the *KRAS* mutation status in colorectal cancer in relation to the response to the anti-EGFR monoclonal antibodies cetuximab and panitumumab. In several cases (CML, breast cancer, colorectal cancer) this has brought to approval by regulatory agencies (FDA and EMEA) in these selected sensitive patient populations.

It has been shown that mutations of the kinase domain of the fusion product bcr-abl, that arises from a reciprocal chromosome translocation between chromosome 9 and 22 known as Philadelphia (Ph) chromosome and present in CML, confer resistance to the selective inhibitor of this tyrosine kinase, imatinib mesylate (Apperley, 2007).

Primary mutations have been observed in the KIT receptor tyrosin kinase gene of patients with GISTs. The most common mutations occur in the sequence of the *KIT* gene encoding the extracellular domain (exon 9) or the juxtamembrane domain (exon 11) (Hornick et al., 2007). Their occurrence has been associated with a higher response and more prolonged disease-free survival after treatment with imatinib (Hornick et al., 2007).

TS, the enzyme responsible for *de novo* synthesis of DNA, is the main target of 5-fluorodeoxyuridine monophosphate (5-FdUMP), the active metabolite of 5-FU (Mini et al., 1990). TS expression is the most important determinant of clinical efficacy of this drug since it correlates inversely with the sensitivity of tumors to 5-FU (Rose et al., 2002). Several polymorphisms located in the TS promoter affect the efficiency of gene transcription and consequently the TS mRNA and protein expression levels. It has been shown that the TS genotype is predictive for the tumor expression level of its product and consequently for the response to the fluoropyrimidine-based treatment.

One of these polymorphisms is represented by the variation in the number of repeats in the 28-bp sequence in the 5' promoter enhance region of TS (TSER) (Horie et al., 1995) whereas TSER*2 and TSER*3 genotypes are predominant. It has been shown that patients homozygous for the 3 tandem repeats TSER*3 (TSER*3/*3) have a lower probability of response to 5-FU-based chemotherapy and a worse prognosis compared to patients homozygous for the variant with 2 tandem repeats TSER*2 (TSER*2/*2) or heterozygous (TSER*2/*3) (Iacopetta et al., 2001, Pullarkat 2001; Villafranca et al., 2001; Morganti et al., 2005).

Another polymorphism is a SNP within the second repeat of TSER*3 (G→C at nucleotide 12). The two alleles are defined as 3RG and 3RC, respectively. The 3RC allele can abolish the increased transcriptional activity of the 3R variant in vitro, by altering a transcription factor binding site (Mandola et al. 2003).

A third polymorphism has also been discovered (Ulrich et al., 2002). It is represented by a deletion of 6 bp starting at position 1494 of the 3' untranslated region (3'UTR) (447 bp upstream of the stop codon), which has been shown to be associated with mRNA instability and decreased intratumoral TS mRNA levels (Mandola et al., 2004). However, its clinical relevance has still to be confirmed (McLeod et al., 2005).

Lynch et al. (2004) and Paez et al. (2004) first reported that somatic mutations in the *EGFR* gene were associated with clinical response to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib in non-small cell lung cancer (NSCLC). About

70% of NSCLCs with EGFR mutations respond to EGFR-TKIs, whereas 10% of tumors without EGFR mutations do so (Mitsudomi and Yatabe, 2007). Short *in-frame* deletions or amino acid substitutions in the region encoding the ATP-binding pocket of the EGFR tyrosine kinase domain (e.g. Gly719Cys in exon 18, Leu858Arg in exon 21 and deletions in exon 19) have been described. These mutations have been almost exclusively identified in patients responding to therapy with gefitinib and erlotinib and seem to be an ideal marker for the identification of patients who might obtain a satisfactory clinical response to these treatments (Lynch et al., 2004; Paez et al., 2004; Mitsudomi and Yatabe, 2007). These mutations are also strongly correlated with some clinical-pathological characteristics: they are more frequent in adenocarcinoma compared with other NSCLCs, in women compared with men and in non-smoking versus smoking patients and in Asian compared with non-Asian ethnicity (Mitsudomi and Yatabe, 2007).

The presence of an *EGFR* point mutation in exon 20 (Thr790Met mutation) in patients who have relapsed following initial successful treatment with erlotinib or gefitinib has also been identified (Kobayashi et al., 2005).

The KRAS mutation status is today considered a robust marker for predicting treatment outcome in patients with metastatic colorectal cancer treated with cetuximab or panitumumab. In particular the presence of *KRAS* mutations located within codons 12 and 13 is associated with resistance to these drugs (De Roock et al., 2008; Lièvre et al., 2008; Amado et al., 2008, Van Cutsem et al., 2008).

On the basis of these findings EMEA has recently given positive opinion for the use of panitumumab and cetuximab in EGFR expressing, *KRAS* wild-type only, metastatic colorectal cancer patients.

Although the classical candidate gene approach has helped to unravel relevant genetic variants that influence clinical drug response, gene-wide association studies have recently gained attention as they enable the association of specific genetic variants or the quantification of differences in gene expression with drug response.

In the future, genomic strategies based on the determination of multiple gene markers, in particular on the analysis of the expression of the tumor genomic profile or the patient haplotype, could be useful for the choice of anticancer treatment even more than those based on a monogenic approach. One example of the possible success of this strategy has been provided by the Netherlands cancer Institute. By the global analysis of tumor RNA, 70 differentially expressed genes have been identified. They represent a molecular profile able to predict prognosis in breast cancer patients with age < 55 years, tumor size < 5 cm, lymph node negative, who have not undergone adjuvant therapy, with a higher accuracy compared to the currently used clinical and pathological criteria (van der Vijver et al., 2002). A similar gene expression assay has been developed by an American group. This assay determines the 10-year risk for disease recurrence in tamoxifen-treated, node-negative breast cancer patients (Paik et al., 2004).

Prospective studies whose treatment is based on these gene profiles are ongoing (MINDACT Trial; TAILORX Trial).

Conclusions

The genetic and molecular foundations of personalized medicine appear solid and evidence indicates its growing importance in healthcare. However, most of the potential of pharmacogenetics and pharmacogenomics still remains to be explored. Many pharmacogenetic and pharmacogenomic controlled clinical studies, designed both to identify and to validate predictive factors for individual drug tolerability and efficacy, are ongoing. The use of molecular tests and markers in clinical practice is rapidly increasing but it is still at an initial stage. The most relevant example of such an approach is testing for the presence of the HLA-B*5701 allele to prevent hypersensitivity reactions to abacavir. Its use has been validated by a prospective clinical trial (Mallal et al., 2008) and is now recommended by drug regulatory agencies. Further results on the involvement of specific polymorphisms with a specific phenotype will be needed before these tools can be applied on a large scale.

Another objective of pharmacogenomics is to identify new pharmacological targets and new therapies by genomic high-throughput biotechnologies; these applications seem feasible and pharmacological research has been focussed on them for some time with interesting results (Remmers et al., 2007).

Advancement in knowledge and current results mean that pharmacogenetics and pharmacogenomics will have increased impact on drug research and development, clinical trials and clinical practice. In the latter case, the definition of the genetic make-up of each individual will add a further relevant factor to the non-genomic ones to assist the clinician in tailoring treatment options.

Acknowledgements

Supported by grants from Ente Cassa di Risparmio di Firenze and Associazione Giacomo Onlus.

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