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The role of pharmacogenetics in cancer therapeutics

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The variability in treatment responses and narrow therapeutic index of anticancer drugs are some of the key challenges oncologists face. The knowledge of pharmacogenetics can potentially aid in the discovery, development and ultimately individualization of anticancer drugs. The identification of genetic variations that predict for drug response is the first step towards the translation of pharmacogenetics into clinical practice. This review provides an update on the results of studies assessing the effects of germline polymorphisms and somatic mutations on therapeutic outcomes and highlights the potential applications and future challenges in pharmacogenetic research pertaining to cancer therapeutics.

Introduction

Genetic constitution is an important cause for individual variations in the response and tolerance to drug treatment [1]. These variations are often due to germline mutations in genes that encode for drug-metabolizing enzymes, transporters, cellular targets and signalling pathways. An important distinction between pharmacogenetics in oncology and other therapeutic fields is that somatic mutations, frequently acquired in cancer tissues, also contribute to the variations in treatment outcome and could fortuitously be exploited in targeted therapy to maximize treatment efficacy. The application of pharmacogenetic testing in cancer therapy is particularly attractive because of the narrow therapeutic index of chemotherapeutic agents. This review aims to provide an update on the genetic basis for interindividual variations in therapeutic outcome relevant to key classes of anticancer agents and the potential application of pharmacogenetics in the treatment of cancer.

Drug metabolizing enzymes

Thiopurine methyltransferase and 6-mercaptopurine

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyses the methylation of aromatic and heterocyclic sulphydryl compounds. The substrates of TPMT include 6-mercaptopurine, 6-thioguanine and azathioprine. 6-mercaptopurine is used for the treatment of childhood acute lymphocytic leukaemia (ALL). Two other pathways compete with TPMT for the metabolism of 6-mercaptopurine: xanthine oxidase converts 6-

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mercaptopurine to an inactive thiouric acid, whereas hypoxanthine guanine phosphoribosyltransferase converts it to thioinosine monophosphate, the precursor of active thioguanine nucleotides [2]. The xanthine oxidase activity in haematopoietic tissues is negligible and TPMT is the major inactivating enzyme for 6-mercaptopurine in these tissues.

Genetic polymorphisms in *TPMT* have been associated with 6-mercaptopurine toxicity and therapeutic efficacy [3–6]. Patients with TPMT deficiency require dose reduction to prevent life-threatening toxicity. Even when treated at 10% of the standard dose of 6-mercaptopurine, patients homozygous for *TPMT* variants have similar or superior survival compared with patients with at least one wild-type allele [7]. In addition, a higher incidence of etoposide-induced myeloid leukaemia, and radiation-induced brain metastases were observed in patients with TPMT deficiency [8, 9]. It is postulated that increased exposure to thioguanine nucleotides may increase DNA damage and potentiate the leukaemogenic effect of etoposide and radiation. Patients homozygous for the wild-type allele are less likely to have severe treatment toxicity but may be at higher risk of disease relapse [10, 11].

Weinshilboum & Sladek demonstrated that red cell TPMT activity has a trimodal distribution and is inherited in an autosomal codominant fashion [12]. It is estimated that one in 300 caucasians carry two 'deficient' *TPMT* alleles (with reduced or no function) and have almost no detectable TPMT activity, while one in 10 has intermediate TPMT activity. At least 21 nonsynonymous mutations have been identified, of which 17 were shown to result in reduced TPMT activity [13, 14]. *TPMT*3A* is the most common variant in caucasians and, together with *TPMT*2* and *TPMT*3C*, accounts for over 95% of low activity phenotypes. In caucasians, the reported allelic frequencies were 4.4%, 0.4% and 0.2% for *TPMT*3A, TPMT*2* and *TPMT*3C*, respectively [14]. TPMT enzymes produced by *TPMT*2*, *TPMT*3A* and *TMPT*3C* variants were susceptible to proteosomal degradation resulting in lower catalytic activity [15, 16].

There are substantial differences in the frequency of TMPT variants across various population groups. In South-East Asian and African populations, *TPMT**3C is the most common *TPMT* variant. The estimated allele frequencies of *TPMT*3C* were 2.3–1% and 2.4% for South-East Asian and African populations, respectively [17–19]. Variable number tandem repeats (VNTR) have been found in the promoter region of TPMT. Although there is *in vitro* evidence to suggest that VNTR polymorphisms correlate negatively with TPMT activity, the importance of VNTR polymorphisms has not been clearly established in clinical studies [20–22].

The traditional way of assessing TPMT red blood cell activity has several limitations: (i) the test result is unreliable for up to 60 days following blood transfusion, (ii) it is time consuming, and (iii) thiopurine administration may increase enzyme activity by approximately 20%, especially in heterozygous individuals [10, 23]. Recently, a large-scale genotype–phenotype association study has demonstrated the feasibility of pharmacogenetic testing for *TPMT* polymorphisms (*TPMT*2*, *TPMT*3*, *TPMT**9, *TPMT*16, TMPT*17* and *TPMT*18*) [14]. A high overall concordance was observed between *TPMT* genotype and phenotype (98.4%). The sensitivity and specificity of the test were 90% and 99%, respectively, and the positive and negative predictive values were 94% and 99%, respectively. The Food and Drug Administration (FDA) has recommended patients with clinical evidence of severe toxicity, particularly myelosuppression, to be considered for TPMT testing.

UDP-glucuronosyltransferases and irinotecan

UDP-glucuronosyltransferases belong to a superfamily of enzymes that catalyse the glucuronidation of many lipophilic xenobiotics and endogenous substrates. The addition of a glycosyl group from a nucleotide sugar renders hydrophobic compounds more soluble for elimination via bile and urine. The *UGT1* gene, located on chromosome 2q37, expresses nine functional UGT1A proteins by alternative splicing of 13 different exons 1 with the common exons 2–5 [24]. UGT1A1 is the major isoform responsible for the glucuronidation of bilirubin and SN-38, the active metabolite of irinotecan [25, 26]. Iyer *et al.* had reported a wide interindividual variation in UGT1A1 activities, with a 17-fold difference in the rate of SN-38 glucuronidation observed *in vitro* [27].

Reduced glucuronidation of SN-38 has been associated with increased treatment-related diarrhoea and neutropenia [28, 29]. This observation led to several clinical studies that demonstrated the association between *UGT1A1*28*, hyperbilirubinaemia and irinotecan toxicity [30–33]. *UGT1A1*28* homozygosity is associated with Gilbert's syndrome, a benign form of familial hyperbilirubinaemia [25, 34]. It is defined as a dinucleotide (TA) insertion in the TATA box of the *UGT1A1* promoter $(TA)_7$ resulting in a reduction in the expression of UGT1A1 [25, 34, 35]. In one study, grade 4 neutropenia was observed in half of the patients homozygous for *UGT1A1*28*, whereas no grade 4 toxicity was reported in patients lacking this allele [32]. Based on this study, it is estimated that *UGT1A1*28*

genotyping could lead to a 50% relative reduction or 5% absolute reduction in grade 4 neutropenia. This translates into the prevention of one severe irinotecan toxicity for every 20 patients genotyped for *UGT1A1*28*. In the same study, variant −3156G→A also predicted for lower nadir neutrophil counts [32]. This latter polymorphism is common (frequency of 0.3) and in close proximity to the phenobarbital response enhancer module. It is also in linkage disequilibrum with *UGT1A1*28* but the functional significance of this polymorphism is still unknown [36].

There is a wide frequency variation in the *UGT1A1*28* genotype across different population groups. Homozygosity for *UGT1A1*28* occurs in 19–24% of the populations in the Indian subcontinent, 12–27% of African populations, 5–15% of caucasian populations but only 1.2–5% in South-east Asian and Pacific populations [35, 37–40]. In addition, several other polymorphisms that are more commonly associated with Gilbert's syndrome in East Asians were shown to have reduced SN-38 glucuronidation activities *in vitro*: *UGT1A1*60* (3279T→G), *UGT1A1*6* (211G→A, G71R), *UGT1A1*27* (686C→A, P229Q) and *UGT1A1*7* (1456T→G, Y486D) [36–41]. The reported allelic frequencies of these variants were 13–23% (*UGT1A1*6*), 13.6% (*UGT1A1*60*) and 0.5–2.8% (*UGT1A1*27*) [42–44]. Prospective studies should be performed in order better to ascertain the benefits and optimal genotyping strategy in different population groups.

Dihydropyrimidine dehydrogenase and 5-fluorouracil

5-fluorouracil (5-FU) has been a cornerstone in the treatment of colorectal cancer over the past few decades. 5-FU is converted to its cytotoxic nucleotides, which in turn inhibit thymidylate synthase or incorporates into RNA and DNA. It is metabolized to its inactive form, 5,6-dihydro-5-fluorouracil, by dihydropyrimidine dehydrogenase (DPYD) [45]. DPYD is the rate-limiting enzyme in the catabolism of pyrimidines such as uracil and thymidine, and the synthesis of β-alanine [46]. Decreased DPYD activity can lead to the accumulation of 5-FU and severe toxicities, including mucositis, neutropenia, neurological symptoms and death [47–49].

Over 40 different polymorphisms have been reported, of which 17 mutations are found in patients with severe 5-FU toxicity [50]. It is estimated that 3–5% of the population is heterozygous and 0.1% is homozygous for alleles with impaired DPYD function [51, 52]. *DPYD*2A* is the most common *DPYD* polymorphism associated with impaired DPYD activity. *DPYD*2A* is caused by a 5′ splice site mutation at intron 14 G1A resulting in the formation of a truncated protein [53]. It is estimated that about a quarter of patients suffering from severe 5-FU toxicity have *DPYD*2A* polymorphism [54, 55]. The allelic frequency of *DPYD*2A* is about 1.8% in European caucasians, while it has not been detectable in Egyptian and Japanese populations [56–58].

Although *DPYD* polymorphisms are associated with severe 5-FU toxicity, about one- to two-thirds of patients who experienced treatment toxicity do not have a molecular basis for DPYD deficiency [59–60]. In addition, *DPYD* genotyping correlates poorly with DPYD level [61]. The low frequency of *DPYD* polymorphisms as well as the low sensitivity and specificity of genotyping hampered the application of DPYD pharmacogenetics to clinical practice.

CYP2D6 and tamoxifen

Tamoxifen is widely used in the treatment for oestrogen receptor-positive breast cancer. It is metabolized by several CYP isoforms (CYP3A, CYP2D6, CYP2C9, CYP2C19, CYP2B6 and CYP1A2) to form several metabolites, including 4-hydroxy-tamoxifen, N-desmethyl-tamoxifen and 4-OH-N-desmethyl-tamoxifen (endoxifen) [62–63]. Both endoxifen and 4-OH-tamoxifen are 100 times more potent than tamoxifen, but endoxifen is present at a much higher plasma concentration than 4-OH-tamoxifen [64].

CYP2D6 appears to be the predominant CYP isoform that catalyses the formation of endoxifen [63]. There is a strong association between *CYP2D6* genotype and plasma levels of endoxifen [65]. A fourfold difference in endoxifen concentration was observed between subjects homozygous for wild type compared with those homozygous for the nonfunctional *CYP2D6* variants. Studies assessing the association between *CYP2D6* genotype with patient outcomes to tamoxifen have produced contradicting reports. Nowell *et al.* did not find a significant association between *CYP2D6* genotype and overall survival [66], whereas Wegman *et al.* suggested that tamoxifen treatment benefited patients with *CYP2D6**4 alleles but not *CYP2D6**1 homozygotes [67]. However, it is difficult to interpret the results of both retrospective studies as the number of patients homozygous for variant *CYP2D6* genotype was small and the comparison arms were not controlled for tumour stage and other treatment modalities.

At least 88 allelic variants have been described, many of which are nonfunctional or have reduced catalytic activity. It is estimated that 5–10% of caucasians have nonfunctional variants [68]. *CYP2D6*4* is the most common nonfunctioning variant in caucasians and, together with *CYP2D6*3*, *CYP2D6*5* and *CYP2D6*6*,

constitutes approximately 97% of all nonfunctioning phenotypes [69, 70]. In *CYP2D6*3* and *CYP2D6*6*, a single base deletion at 2637A and 1795T, respectively, results in a premature stop codon and the production of a nonfunctioning truncated protein, whereas *CYP2D6*5* is a gene deletion. *CYP2D6*4* allele has a 1934G→A transition at the junction of the intron 3 and exon 4, producing a splicing defect [71].

Gene duplication is responsible for ultrarapid CYP2D6 metabolism in only 1–3% of Europeans and up to 20% of some Middle-Eastern and North African populations [72]. The frequencies of nonfunctional alleles are relatively low in Asians, but there is a large proportion of the population with variant alleles that are associated with reduced CYP2D6 activity. *CYP2D6*10* (188C→T, P34S) occurs in about 50% of East Asians and largely accounts for lower CYP2D6 activity in the extensive metabolizer phenotype in Asians [73, 74], whereas *CYP2D6*2* and *CYP2D6*17* are more common in African-Americans [75]. Additional studies will be needed to address the impact of gene duplication and reduced function alleles.

Metabolizing enzymes of the folate pathway

Methylenetetrahydrofolate reductase and methotrexate

Methotrexate exerts its cytotoxic effects by inhibiting several folate-dependent enzymes, including dihydrofolate reductase, thymidylate synthase and aminoimidazole carboxamide transformylase. The treatment toxicity from high-dose methotrexate can be minimized with the administration of reduced folate, folinic acid [76]. Conversely, a reduction of intracellular folate pool can lead to an increase in methotrexate toxicity.

5,10-methylenetetrahydrofolate reductase (MTHFR) is an important enzyme that regulates folate and homocysteine homeostasis. It catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the carbon moiety required for the conversion of homocysteine to methionine [77].

Deficiency in MTHFR has been associated with a reduced folate pool, as well as neurological and vascular diseases [78–80]. 677C→T, a common functional polymorphism of *MTHFR*, produces an alanine to valine amino acid substitution within the predicted catalytic domain of the MTHFR enzyme. The resultant variant protein has reduced catalytic activity and is more thermolabile [81]. An A→C mutation at position 1298 of *MTHFR* gene abolishes an *Mbo*II recognition site. Although 1298A→C has been associated with reduced MTHFR activity, neither the homozygous nor heterozygous state is associated with a change in homocysteine or folate level. However, it appears that individuals heterozygous for both 677C→T and 1298A→C have a phenotype similar to that of 677TT homozygotes [82]. The allelic frequency of 677C→T variant ranges from 24 to 46% in Europeans, 26 to 44% in East Asians, 57% in Mexicans and 11% in African Americans [83, 84].

*MTHFR*677C→T variant is associated with a decreased folate level [85]. Patients with variant *MTHFR*677C→T allele are more likely to experience treatment-related toxicity after methotrexate, as part of the regimens for breast cancer, ovarian cancer and bone marrow transplant [86–89]. Testing for *MTHFR* polymorphisms may help to identify individuals at risk of severe treatment toxicity. It is likely that polymorphisms in other folate-dependent enzymes and transporters may also affect the response to methotrexate.

Drug targets

Thymidylate synthase and antimetabolites

Thymidylate synthase (TYMS) catalyses the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), the only source of intracellular thymidylate essential for DNA replication and repair [90]. It is the main target for 5-FU, capecitabine and raltitrexed. The active metabolite of 5-FU, fluorodeoxyuridine monophosphate (FdUMP) blocks the formation of dTMP by forming a stable complex with TYMS. The overexpression of TYMS is associated with resistance to 5-FU and other TYMS inhibitors such as raltitrexed [91–93] and reduced response to hepatic artery infusion of floxuridine [94].

TYMS gene contains seven exons and a 5′-flanking untranslated enhancer region containing a 28-bp tandem repeat sequence [95, 96]. The number of tandem repeats varies from two (2R) to nine (9R) copies [97]. The translational efficiency is correlated with the number of tandem repeats. *In vitro*, there is a 2.6–3.6-fold increase in TYMS expression with the 3R variant compared with the 2R variant [98, 99]. The distribution of tandem repeats in caucasians is 16% 2R/2R, 51–55% 2R/3R, 29–32% 3R/3R and <1% for other variants [100–101].

A G→C SNP located at the 12th nucleotide of the second tandem repeat of 3R has recently been identified (3RC) [102]. The polymorphism occurs within the USF consensus element and alters the transcriptional activity of TS gene. 3RC variant has a lower TS expression level and is associated with better clinical outcome with fluoropyrimidines when compared with the 3R variant [103–104]. This may explain some of the discrepancies seen when 5′ UTR tandem repeats were used alone in predicting 5-FU response. The 3RC allele (3RC) occurs

in 56%, 28% and 37% of all 3R alleles in caucasians, African-Americans and Chinese, respectively [102].

In patients with metastatic colorectal cancer, those homozygous for 3R had a poorer survival (12 months *vs.* 16 months) and a lower response rate (9% *vs.* 50%) to 5-FU-based chemotherapy [99, 105]. Similarly, patients homozygous for 3R benefited less from 5-FU adjuvant chemotherapy and neoadjuvant chemoradiation [106, 107]. In children with acute lymphoblastic leukaemia who were treated with methotrexate, those homozygous for 3R had poorer event-free survival [108].

Gene amplification can result in the overexpression of TYMS. Wang *et al.* has shown the feasibility of using fluorescence *in situ* hybridization to detect *TYMS* gene amplification in cancer tissues. In this study, patients with metastases containing *TYMS* amplification had poorer survival (329 *vs.* 1021 days), which suggests that *TYMS* amplification is a major mechanism of 5-FU resistance [109].

A 6-bp deletion located in the 3′ untranslated region (UTR), 447 bp downstream of the stop codon, has also been associated with decreased mRNA stability and intratumoral TYMS level [110]. This 3′-UTR polymorphism was found to be in linkage disequilibrium with 5′-flanking untranslated enhancer region polymorphism and the haplotypes 2R/ins 6-bp seemed to be associated with increased treatment toxicity [100]. In colorectal cancer tissues, the loss of heterozygosity (LOH) of the *TYMS* locus is a common phenomenon (62%) [111]. Patients with 3R/2R genotype can acquire 3R/loss or 2R/loss genotype in their cancer tissues. Patients with tumour 3R/loss genotype had a poorer treatment outcome compared with 2R/loss when treated with fluoropyrimidine-based therapy [112]. This highlights the importance of assessing somatic mutations in cancer tissues in order to predict for treatment outcome.

Epidermal growth factor receptor and tyrosine kinase inhibitors

The epidermal growth factor receptor (EGFR) is frequently dysregulated and overexpressed in a number of epithelial cancers including nonsmall cell lung cancer (NSCLC) and head and neck cancer. EGFR signalling is important for tumour cell proliferation and angiogenesis, and has become an attractive target for therapy [113].

Two oral EGFR tyrosine kinase inhibitors (TKI), gefitinib and erlotinib, have been approved for use as second- or third-line therapy in advanced non-small-cell lung cancer. These drugs have a favourable toxicity profile. The most frequent toxicities are diarrhoea and acneiform rash. Patients who develop skin toxicity are associated with a favourable outcome [114]. After evaluating the initial analysis of two large placebo-controlled phase III trials (BR21 and ISEL), FDA currently limits the use of gefitinib to patients who are already receiving and benefiting from gefitinib [115–116]. However, studies on gefitinib and EGFR have provided valuable insight into genetic variation and treatment outcomes.

Different population groups showed significant variability in the response to these drugs. The response rate of gefitinib is higher in Japanese patients compared with caucasians (27.5% *vs.* 10.5%) [117]. Furthermore, the intensity of immunohistochemical staining for tumour EGFR expression does not correlate well with response [118]. Recently, somatic mutations in the tyrosine kinase domain of the EGFR were found to be present in most patients who responded to gefitinib and erlotinib [119–121]. It is postulated that these mutations, which cluster around the ATP-binding site of the tyrosine kinase domain (exons 18, 19 and 21), stabilize the interaction between drug and the tyrosine kinase domain. The most common mutations are due to multinucleotide in-frame deletion in exon 19 and a point mutation L858RA in exon 20. Within the tyrosine kinase domain, a point mutation, T790M, seems to confer resistance to gefitinib [122]. Two Asian studies found that *EGFR* genotyping has a sensitivity of 52–92%, a specificity of 79–91% and a negative predictive value of 86–90%, which construes that nine out of 10 patients with a negative *EGFR* genotyping will not benefit from gefitinib treatment [123, 124].

In addition to EGFR tyrosine kinase domain mutations, several studies have linked EGFR TKI sensitivity to increased *EGFR* gene copy number in lung cancer [125, 126]. Cappuzzo *et al.* have demonstrated that a high *EGFR* gene copy number was associated with better response (36% *vs.* 3%) and survival (19 months *vs.* 7 months) when treated with gefitinib [126].

The potential importance of germline polymorphisms in determining clinical response to TKIs has not been clearly established. However, the presence of interethnic differences in the frequency of somatic mutations, and the positive correlation between rash and response and/or survival when treated with TKIs, may suggest a possible genetic basis for susceptibility to somatic mutation and skin toxicity [114, 117, 127]. Currently, research efforts have been focusing on identifying germline variants that may affect: (i) the cellular susceptibility to somatic mutations, (ii) the development of skin rash, and (iii) the transcriptional activity and expression of EGFR [128–130].

Application of pharmacogenetic testing to cancer therapeutics

Currently, there are strong data to support the use of pharmacogenetic testing for *UGT1A1* and *TPMT* polymorphisms. Pharmacogenetic information pertaining to irinotecan toxicity is now included in the revised drug labelling after the FDA advisory committee meeting in November 2004. Pharmacogenetic testing may enable clinicians to identify those patients who are less likely to benefit from expensive drugs, and those who are susceptible to severe treatment-related toxicities at standard treatment doses, thus making treatments safer and more cost-effective. The availability of high-throughput genotyping platforms has allowed a large set of SNP markers to be studied and may lower the cost of pharmacogenetic testing.

The utility of pharmacogenetics extends beyond cancer therapy. It has the potential to facilitate the identification of drug targets and accelerate drug discovery and development. Tumour tissues frequently acquire mutations in oncogenes, which themselves can confer sensitivity to drugs, as in the case of EGFR tyrosine kinase domain mutation and response to gefitinib. Incorporating pharmacogenetic testing in early clinical trials may provide vital information about pharmacogenetic profiles with treatment responses and tolerability. This information can help investigators identify patients with specific pharmacogenetic profiles, and may reduce the size and cost of phase III clinical trials needed to establish drug efficacy.

Future challenges

Early studies of pharmacogenetics were mostly monogenic candidate-gene association studies. These studies were often hypothesis driven, based on identifying phenotypic variability and correlating with genetic polymorphism. Although this approach has been extremely useful in advancing our knowledge in pharmacogenetics, monogenic association study has some limitations: (i) it is difficult to ascertain if the positive association observed could be due to the linkage with untyped functional variant allele or to intra-gene interaction; (ii) it is not often possible to evaluate each SNP directly because of cost constraints and incomplete knowledge of the polymorphisms; and (iii) drug disposition and drug response are usually determined by the interaction of multiple genes and pathways.

As there is approximately one SNP in every 1000–3000 base pairs throughout the human genome [131], it is possible that up to hundreds of different variable loci are present within a candidate gene. However, SNPs or alleles physically close together on the same chromosome are rarely separated by recombination, and hence they tend to occur more frequently together rather than by chance. This association between neighbouring SNPs or alleles is known as linkage equilibrium and it enables the selection of marker SNPs, known as haplotype tagging SNPs, to capture the genetic diversity across a region or haplotypes block, thus reducing the number of SNPs needed to represent all the common polymorphisms in a candidate gene to an average of five to seven SNPs. Recently, Sai *et al.* reported that the haplotype structure of *UGT1A1* in Japanese patients may be adequately represented by limited numbers of marker alleles, which illustrates the economy of this approach. There is emerging evidence that polymorphisms in *OATP1B1* and *ABCC2* may also affect the disposition of SN-38 [132, 133]. Haplotype association studies would allow the evaluation of the large set of candidate genes, for example, between UGT1A1 and various transporters in association studies. There is an ongoing global effort by the International HapMap Project to establish the haplotype structures of four different population groups. Once the population haplotype structure is available, empirical genome-wide screen can be performed to identify candidate genes with appropriate haplotype tagging SNPs. Expression microarrays and proteomic studies provide alternative strategies to identify candidate genes that will complement current haplotype–phenotype approaches.

Conclusions

Pharmacogenetic studies have provided strong evidence for the genetic basis of drug response and tolerability. The translation of pharmacogenetic research into clinical practice is time consuming, labour intensive and expensive. When possible, pharmacogenetic testing should be incorporated in early phase clinical trials. The biological functions of causal variants should be evaluated and the association validated across different population groups. All of these require a collaborative effort involving multiple disciplines and between academic centres with complementary areas of expertise. The Pharmacogenetics Research Network (PGRN), which comprises 12 institutes across the USA, is an example of one such collaboration (http://www.pharmgkb.org/). Although the extensive use of pretreatment pharmacogenetic testing is still limited, the prospects of using pharmacogenetic testings to tailor individual therapy regimens in the future are promising.

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