The field of pharmacogenomics has seen some exciting advances in the recent past. The Human Genome Project and International HapMap projects have uncovered a wealth of information for researchers. The discovery of clinically predictive genotypes (e.g. UGT1A1*28, TYMS TSER), haplotypes (e.g. VKORC1 Haplotype A) and somatic mutations (e.g. epidermal growth factor receptor), along with the introduction of FDA approved pharmacogenetic tests (UGT1A1*28) and the initiation of a genotype-guided clinical trial for cancer therapy (TYMS TSER in rectal cancer) have provided the first steps towards the integration of pharmacogenomics into clinical practice. This review describes some of the recent advances in pharmacogenomics research.

INTRODUCTION

Variability in drug response is observed across all areas of medicine. In the UK, it has been estimated that ~7% of patients are affected by adverse drug reactions (ADRs). These ADRs result in the need for the equivalent of 15–20 400-bed hospitals and cost about £380 million a year (1). In 1994, an estimated 2 216 000 hospital patients in the US, suffered a serious ADR, leading to approximately 106 000 patient deaths, making ADR fatalities the fourth to sixth leading cause of death in the US in 1994 (2). Clearly, the current regime of ‘one dose fits all’ is not ideal for patients and is not cost-effective for the health service. With multiple drug strategies for many diseases now readily available, pharmacogenomics encompasses the search for answers to the hereditary basis for interindividual differences in drug response (3,4) with the ultimate goal of personalized therapy selection to reduce the incidence of ADRs and improve treatment efficacy (Fig. 1).

There is a great deal of variability at the DNA level between individuals. Single nucleotide polymorphisms (SNPs) account for over 90% of genetic variation in the human genome. The remainder of the variation is caused by insertions and deletions (indels), tandem repeats and microsatellites. With the completion of the human genome project and first phase of the HapMap, a wealth of polymorphism information is now readily accessible via publicly available databases (5). Initial estimates of approximately 1.42 million polymorphisms in the human genome (6) have been exceeded. Currently, the largest public SNP database, dbSNP has >27 000 000 submissions with >10 000 000 validated polymorphisms (Build 125, October 2005). In addition, affordable, high throughput-technologies are now available, making pre-treatment genotyping a realistic possibility (7,8).

With so much data a mere internet connection away, the problems are which polymorphisms of the many per gene to assess and whether haplotype provides more information than individual genotype for a given drug–gene interaction. In cancer chemotherapy, the added complications of the tumour genome—clinically predictive somatic mutations, gross chromosome rearrangements, gene/chromosome loss or duplication (9,10)—mean there are many issues still to be resolved before pharmacogenomics becomes commonplace in the clinic. However, advances towards bringing pharmacogenomics into focus have been made. Some of this recent progress is highlighted subsequently.

Pre-treatment polymorphism assessment

One of the most obvious applications of pharmacogenomics is the pre-treatment assessment of an individual patient’s probability of benefit and/or adverse events from a specific medication. In the past few years, the FDA has added thiopurine methyltransferase genetic information to the azathioprine and mercaptopurine package insert information, detailing a genetic risk for neutropenia.
Pharmacogenomics aims to identify patients at risk for toxicity or reduced response to therapy prior to medication selection.

(3) With at least one UGT1A1/C3 genotype information in the irinotecan package insert, with dosing guidelines based on UGT1A1 genotype (http://www.fda.gov/medwatch/safety/2005/jul_pi/camptosar_pi.pdf). The Roche Amplichip also received approval for detection of deficiencies in CYP2D6 and CYP2C19 metabolism (11). A more recent example of regulatory approval of pharmacogenetic information is with irinotecan (CPT-11; Camptosar), a camptothecin analogue approved for treatment of advanced colorectal cancer in combination with 5-fluorouracil/leucovorin. This drug is also active in the treatment of lung, brain and breast tumours. The active form of irinotecan, SN-38, can be inactivated through glucuronidation by a member of the UDP-glucuronosyltransferase family, UGT1A1, an enzyme responsible for hepatic bilirubin glucuronidation.

A polymorphic dinucleotide repeat has been identified in the UGT1A1 promoter TATA element and consists of 5, 6, 7 or 8 copies of a TA repeat [(TA)_nTAA], with the (TA)_nTAA allele considered wild-type and (TA)_7TAA (UGT1A1*28) the most frequently reported variant allele (12). The longer the repeat allele, the lower the corresponding UGT1A1 gene expression and individuals with seven or eight alleles have significantly lower UGT1A1 expression (13).

As UGT1A1 is responsible for the conversion of SN-38 to the inactive metabolite, SN-38G via glucuronidation, variability in UGT1A1 expression leads to interindividual variation in SN-38G formation (14,15). Consequently, the presence of greater than 6 TA repeats in the UGT1A1 promoter region leads to less SN-38G being formed and the potential for excess SN-38 to be retained in the cell, leading to severe toxicity, including potentially fatal neutropenia and diarrhoea.

In 2000, Ando et al. (16) published a study of 108 Asian patients treated with irinotecan-containing regimens. Patients with at least one UGT1A1*28 allele encountered severe toxicity compared with patients homozygous for the (TA)_nTAA allele (P < 0.001). In a recent prospective study of 66 cancer patients receiving irinotecan, UGT1A1*28 was corroborated as a predictive marker for severe neutropenia (P = 0.02) (17).

In 2005, the USA FDA approved the inclusion of UGT1A1 genotype information in the irinotecan package insert, with dosing guidelines based on UGT1A1 genotype (http://www.fda.gov/medwatch/safety/2005/jul_pi/Camptosar_PI.pdf) (18). One month later, the FDA approved a clinical test for the UGT1A1*28 allele (http://www.fda.gov/bbs/topics/NEWS/2005/NEW01220.html) (18), making the integration of pharmacogenomics into clinical practice for cancer treatment a reality.

Clinically relevant haplotypes

Where the functional polymorphism is unknown, haplotypes for specific genes may be more informative than individual genotypes. Haplotype tag SNPs (htSNPs) selected on the basis of linkage disequilibrium can substantially reduce the amount of genotyping needed per gene.

Warfarin is the most commonly prescribed anticoagulant with over 21 million prescriptions in the US alone in 2003 (19,20). Warfarin, a derivative of coumarin, is used to treat and prevent thromboembolic disorders including pulmonary embolism, stroke, atrial fibrillation and heart attacks (21). Warfarin has a narrow therapeutic index that varies widely between individuals and requires constant monitoring and adjustment (21,22). In a study of Taiwanese patients on warfarin, an average of 4.3 dose adjustments and 13.7 blood samples were needed for monitoring per patient per year (23). Ideally, the dose should be predicted in advance of warfarin therapy initiation to prevent undesirable side effects and/or delays in reaching the optimum therapeutic dose.

Warfarin targets Vitamin K, an essential cofactor for the modification of glutamic acid to γ-carboxyglutamate in coagulation factors VIII, IX and prothrombin by vitamin K-dependent γ-carboxylase. Vitamin K is recycled by vitamin K epoxide reductase (VKORC1) in the endoplasmic reticulum membrane (20,24). Warfarin is also metabolized via hydroxylation in the liver by cytochrome P450, subfamily 1IC, polypeptide 9 (CYP2C9). Two non-synonymous polymorphisms, CYP2C9*2 and CYP2C9*3, code for CYP2C9 enzymes with approximately 40 and 10% of the wild-type enzyme activity, respectively (25–27).

There is a strong association between genetic factors and warfarin dose. Patients with one or two of the functional CYP2C9 SNPs require reduced warfarin dose and experience a 2–3-fold increased risk of bleeding when beginning warfarin (28–30). However, SNPs in VKORC1 may be more important. Recent studies have identified haplotype-dependent predictions for warfarin dosing (22). VKORC1 haplotype A predicted 21–25% of the required warfarin dose, and the inclusion of CYP2C9 genotypes reduced the required warfarin dose up to 31% in Caucasians (22). Including non-genetic factors such as age, sex, body surface area (BSA) and drug interactions with genotype information predicts up to 60% of warfarin dose (Fig 2) (22,30,31). The remaining 40% of warfarin dosing variability remains unexplained.

Clinically relevant somatic mutations

The cancer genome undergoes many rearrangements and can contain clinically relevant genetic variation not found in the germline. Somatic mutations, acquired before or after chemotherapy can also affect drug efficacy and toxicity.

Gefitinib (Iressa) and erlotinib (Tarceva) are inhibitors of the epidermal growth factor receptor (EGFR), specifically inhibiting the EGFR tyrosine kinase domain. Approximately 10% of patients with non-small-cell lung cancer (NSCLC) who have failed standard therapies, experience a relative
response from these drugs. Consequently, efforts were undertaken to determine the mechanism of response (or lack of) in patients receiving EGFR inhibitors.

In 2004, Lynch et al. (32) resequenced the 28 exons of the EGFR gene in nine NSCLC patients who responded favourably to gefitinib, seven patients who did not respond to gefitinib and 25 patients with primary NSCLC who did not receive gefitinib therapy. Of the nine patients who responded to gefitinib therapy, eight had somatic EGFR mutations in the exons corresponding to the tyrosine kinase domain (exons 18–24). Two (of 25) patients who did not receive gefitinib also had EGFR mutations. No mutations were identified in the seven patients who did not respond to gefitinib, in addition, matched normal tissue for patients with mutations were wild-type at all tumour mutation loci (32). The most common mutations (4/9 patients and 2/25 patients who did not receive gefitinib) were deletions in exon 19 of 12–18 nucleotides, all including the deletion of at least amino acids 746–750. In a concurrent study, Paez et al. (33) screened tumour DNA from five NSCLC patients who responded to gefitinib and four patients who did not respond. No mutations were identified in the patients who did not respond, however, all five patients who responded had mutations in the EGFR tyrosine kinase domain exons (33). These mutations were confirmed as somatic as they were not present in matched normal tissue. These data were confirmed in a later study by Paez et al. (34), in which similar mutations were found in 7/10 patients who responded to EGFR inhibitors compared with 0/8 patients who did not respond. In addition, Pao et al. (34) identified that 7/15 non-smokers with adenocarcinoma carried EGFR mutations, whereas only 4/21 former or current smokers with NSCLC carried the mutations ($P$=0.0001) (34). There have been a multitude of publications depicting the presence of EGFR mutations in NSCLC from nearly every continent (35). However, there is also data emerging that the presence of an EGFR mutation may be a prognostic, rather than predictive, marker (36); patients with mutations have a better outcome regardless of therapy.

Interestingly, mutations in EGFR may also be related to resistance of NSCLC to gefitinib or erlotinib. In a study by Kobayashi et al. (37), a patient with beneficial EGFR mutations experienced response from gefitinib but subsequently relapsed. Further analysis of the EGFR gene in the tumour cells from this patient confirmed the remaining presence of the beneficial EGFR mutations and also the acquisition of a non-synonymous mutation (T790M) affecting the gefitinib/EGFR complex formation and leading to gefitinib resistance (37). A subsequent study by Pao et al. (38) described the same phenomena in three patients who relapsed after initial response to gefitinib or erlotinib. All three patients had acquired the T790M mutation in their tumour (38). Consequently, the presence of EGFR mutations in NSCLC patients who have failed previous therapies may provide useful prognostic information for eligibility for EGFR inhibitor therapy, but patients responding to therapy still need to be monitored for the acquisition of additional mutations that may lead to resistance to therapy.

Genotype-guided clinical studies

Thymidylate synthase catalyses the methylation of dUMP to dTMP. As the sole de novo source of thymidylate in the cell, it is an important target for drugs such as 5-fluorouracil, methotrexate, oral 5FU prodrugs (e.g. capecitabine) and other novel folate-based drugs (e.g. raltitrexed). Over-expression of TYMS has been linked to resistance to these drugs (39,40). The cause of the variability in TYMS expression is still unclear, however, polymorphisms in the 5$^{\prime}$ and 3$^{\prime}$ untranslated regions (5$^{\prime}$-UTR and 3$^{\prime}$-UTR) of the TYMS gene have been described (41) and these are suggested to have an impact on the efficacy of TYMS-targeted chemotherapy treatment.

A polymorphic tandem repeat in the 5$^{\prime}$-UTR of the TYMS enhancer region (TSER) resulting in 2–9 copies of a 28 bp repeated sequence has been identified (42) and initial data suggest that increased number of repeats increases TYMS RNA and protein expression (42,43). The function of the repeated sequence has recently been elucidated. Within the TYMS TSER an SNP was identified (44). This G>C SNP lies in the 12th nucleotide of the second repeat of the TSER$^{*}$3 allele and the presence of the cytosine allele abolishes a transcription factor (USF-1) binding site within the TSER resulting in a 3–4-fold reduction in TYMS expression (44).

The TSER polymorphism has been linked to tumour downstaging in patients with rectal cancer who were treated preoperatively with 5-fluorouracil-based chemoradiation (45). In a study of 65 patients with stage T2–T4 rectal cancer, patients with at least one TSER$^{*}$2 allele had an 38% increased frequency of tumour downstaging at the time of surgical resection compared with TSER$^{*}$3/TSER$^{*}$3 patients (60 versus 22%; $P$ = 0.036; Fig. 3) (45).

The first genotype-guided clinical cancer trial in North America is currently underway based on TYMS TSER genotype. Rectal cancer patients (stage T3 and T4) with the ‘good risk’ TSER$^{*}$2 allele are treated in a phase II study consisting of standard therapy (radiation and 5FU). The sample size was calculated to detect a downstaging rate of 60%, compared with the historical downstaging rate of 45%. Patients homozygous for TSER$^{*}$3 (‘bad risk’ genotype) are enrolled.
in a phase II study, in which they receive the standard radiation and 5FU therapy along with an additional chemotherapy agent (irinotecan). The sample size was calculated to detect an improvement from the previously reported TSER*3/*3 downstaging rate of 22–45%. Preliminary data implied an improved response rate in both treatment groups (46), suggesting an enrichment for positive response.

CONCLUSIONS

Although there is still a long way to go before pharmacogenomics becomes every day practice in the clinic, there has been significant progress towards achieving this goal. The identification and validation of functional, clinically relevant polymorphisms and haplotypes will provide the basis for similar studies for other drugs. The approval of genetic tests for pharmacogenomics becomes every day practice in the clinic, there has

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