Pharmacogenomic biomarkers: new tools in current and future drug therapy

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The rapid development of techniques in the area of genome analysis has facilitated identification of new pharmacogenomic biomarkers that can provide predictive tools for improved drug response and fewer adverse drug reactions. Such biomarkers mainly originate from genes encoding drug-metabolizing enzymes, drug transporters, drug targets and human leukocyte antigens. Some of these are now integrated by the USA Food and Drug Administration and the European Medicines Agency into drug label inserts. In this review, we examine the utility and mechanistic background of pharmacogenomic biomarkers in several areas of medicine, including cancer, infection and cardiovascular disease. We also discuss the use of these biomarkers in drug development and address the impact on personalized drug prescription, including opportunities and bottlenecks.

Introduction
Since the completion of the human genome sequence in 2003, the rapid development of next-generation sequencing methods and novel versatile gene-array techniques has provided fantastic opportunities for personalized medicine using pharmacogenomic biomarkers. These biomarkers, as defined by the European Medicines Agency (EMA), are measurable DNA and/or RNA characteristics that are indicators of normal biologic processes, pathogenic processes, and/or a response to therapeutic or other interventions, and they are currently being identified at an increasing rate. Of particular importance are genetic biomarkers for drug efficacy and adverse drug reactions (ADRs) (Box 1). ADRs account for ~7% of hospitalizations [1], ~20% of all readmissions to hospital [2], and ~4% of withdrawals of new chemical entities [3]. They are as costly as drug treatment itself, and are among the leading causes of death in the USA, with 100 000 deaths annually [4,5]. In this review, we focus on recent developments for pharmacogenomic biomarkers and their value in drug development and clinical practice.

Cancer and immunosuppression
Within the field of cancer therapy, pharmacogenomic biomarkers are highly important owing to the narrow therapeutic range for most drugs. In this section, we consider a range of cancer-related pharmacogenomic biomarkers (Table 1).

Pharmacokinetic biomarkers
Breast cancer Estrogen-dependent drug treatment [such as the estrogen receptor (ER) antagonist tamoxifen and aromatase inhibitors] relies on ER signaling, and thus only tumors expressing ERs are suitable for such therapy. The commonly used breast-cancer adjuvant tamoxifen is bioactivated by cytochrome P450 2D6 (CYP2D6) to its main active metabolite, endoxifen, which has a 100-fold higher affinity for ERα. Data from a large number of retrospective studies indicate that the CYP2D6 genotype indeed plays a role in tamoxifen treatment outcome [6-9]. Thus, carriers of defective CYP2D6 alleles such as CYP2D6*4 and CYP2D6*10 have an increased risk of breast cancer relapse and lower event-free survival rates compared to extensive metabolizers (EMs). Therefore, alternative adjuvant treatment with aromatase inhibitors might be a better choice for such patients. However, mandatory use of a CYP2D6 genetic test would require additional data from randomized prospective studies. Recent data also reveal a potential effect of CYP2C19 polymorphism on relapse risk during tamoxifen treatment, as evidenced by an unadjusted odds ratio of 0.15 in homozygous carriers for the rapid CYP2C19*17 allele compared to CYP2C19*1/*1 patients [10].

Colon cancer The topoisomerase 1 inhibitor irinotecan is used in combination regimens for treatment of metastatic colorectal cancer. It requires bioactivation by carboxylesterase to produce the chemotherapeutic metabolite SN-38. Excessive levels of SN-38 can cause neutropenia, and inactivation and excretion of SN-38 depend on UDP-glucuronosyltransferase 1A1 (UGT1A1) activity [11]. The wild-type allele UGT1A1*1 carries a six-repeat TATA box [A(TA)6TAA], whereas UGT1A1*28 has seven repeats. A higher number of TA repeats causes reduced UGT1A1 gene transcription, and the common UGT1A1*28 allele is associated with reduced clearance of SN-38 [12]. Owing to the increased risk of accumulated SN-38 levels and the resulting risk of neutropenia in UGT1A1*28 carriers, the US FDA decided in 2005 to include UGT1A1*28 as an indication for a lower initial irinotecan dose, although genetic testing is not required. Moreover, evaluation of the cost–benefit of UGT1A1 genotyping requires additional studies.

Leukemia Thiopurine methyltransferase (TPMT) is involved in the metabolism of the thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) that
Box 1. Pharmacogenomic biomarkers

Pharmacogenomic biomarkers could provide tools to:
- Avoid overdosing and subsequent ADRs
- Avoid underdosing and subsequent lack of efficacy
- Avoid drug use by hypersensitive individuals
- Improve clinical diagnosis
- Rescue drugs previously withdrawn because of ADRs

The biomarkers that can predict drug response or drug toxicity are mostly genetic variants of:
- Drug-metabolizing enzymes such as CYP2C9, CYP2C19, CYP2D6, UGT1A1 and TPMT
- Drug transporters such as SLCO1B1
- Drug targets such as EGF receptors and tyrosine kinases
- Human leukocyte antigens

The recommendations for pharmacogenomic implementation of absorption, distribution, metabolism and excretion (ADME) genes in drug development as defined by the EMA draft guidelines on drug development include:
- Identification of involvement of polymorphic enzymes early in development
- Biobanking of DNA samples in clinical trials for subsequent genetic analyses
- Evaluation of the effect of the polymorphism on the tolerated dose in Phase I trials
- Effect of the polymorphism on pharmacokinetics, safety and efficacy in Phase II and III trials
- Individualized doses based on genetics in Phase III trials when required

are used in treatment of leukemia, autoimmune diseases and inflammatory bowel diseases, as well as after organ transplantation to prevent rejection. AZA is non-enzymatically bioactivated to 6-MP, which is further metabolized to thioinosinic acid (TIMP) and 6-thioguanine. TPMT inactivates both 6-MP and TIMP, and TPMT polymorphism thus influences the pharmacokinetic profile of both AZA and 6-MP. Up to one in 20 patients treated with 6-MP is affected by serious myelosuppression [13] that can lead to life-threatening infections, anemia and severe bleeding events. Many studies have shown that the non-synonymous defective TPMT*2, *3A, *3B and *3C alleles, which together account for more than 80% of defective TPMT alleles [13], cause increased side effects. As many as 86% of TPMT-deficient patients on thiopurine treatment develop myelosuppression [14], and because of the risk of fatal toxicity, TPMT genotyping or phenotyping before AZA or 6-MP treatment is recommended, but not required, by the FDA. This test is commonly carried out at clinical centers in the USA and in Europe.

A recent genome-wide analysis (GWA) study of interindividual variability in methotrexate pharmacokinetics in 434 children with acute lymphoblastic leukemia suggests a strong effect of SLC01B1 polymorphism on methotrexate pharmacokinetics, as well as an association with the risk of gastrointestinal toxicity (odds ratio 15) [15]. However, the mechanisms behind these effects remain to be determined.

Pharmacodynamic biomarkers

Aggressive systemic mastocytosis Imatinib mesylate inhibits certain tyrosine kinases and is used in the treatment of several cancer types, such as gastrointestinal stromal tumor (GIST), chronic myelogenous leukemia and aggressive systemic mastocytosis (ASM). One of the targets of imatinib is the tyrosine kinase KIT (CD117, gene c-kit), which is genetically activated in up to 90–95% of all GIST and ASM cases. Some of these mutations cause increased imatinib response, whereas others confer resistance. Thus, the FDA has indicated treatment only for GIST cases with detectable KIT expression (as determined by immunohistochemistry, IHC). In ASM, the activating 2447A→T c-kit mutation (D816 V) is present in neoplastic cells of ~90% of patients and is associated with resistance to imatinib [16]. Consequentially, the FDA has indicated that imatinib treatment is only suitable in ASM patients lacking this mutation or with unknown c-kit status.

Breast cancer Gene amplification and thus overexpression of the human epidermal growth factor receptor 2 (HER2) develops in 20–25% of all breast cancers and is associated with an aggressive tumor phenotype. The monoclonal antibody trastuzumab targets HER2 to inhibit proliferation and induce tumor cell death in metastatic breast cancers. Owing to the necessity of HER2 overexpression for treatment response, trastuzumab is only administered to patients showing overexpression of HER2 protein (determined by IHC) or amplification of the HER2 gene (determined by fluorescent in situ hybridization, FISH) [17]. The EMA and FDA have recommended trastuzumab as an adjuvant first-line and single second-line treatment for metastatic breast cancer tumors that overexpress HER2. Trastuzumab as adjuvant treatment for HER2-positive metastatic gastric cancer (MGC) and neoadjuvant treatment for HER2-positive breast cancer is currently listed as pending an EU decision by the EMA. In this case, trastuzumab should only be used for MGC tumors with HER2 classification of IHC 3+, or IHC 2+ combined with confirmatory FISH+.

Colon cancer The antibodies panitumumab and cetuximab directed at the human epidermal growth factor (EGF) receptor (HER1, also known as EGFR) are used in the treatment of metastatic colorectal cancer because tumor growth often depends on signaling via EGF that involves downstream V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS). Owing to their HER1 dependence, panitumumab and cetuximab are used only for treatment of HER1-expressing tumors. Patients carrying activating KRAS mutations are non-responders to panitumumab and cetuximab and are highly predisposed to progressive disease [18–20]. The FDA and EMA have concluded that HER1-directed antibodies are useful for the treatment of HER1-expressing tumors lacking KRAS mutations. Thus, KRAS genotyping is a valuable tool for identifying responders, although antibody treatment only increases overall survival by 6–10 months.

Lung cancer Platinum-based combination (PBC) regimens are standard first-line treatments of non-small cell lung cancer (NSCLC), which is the most common cause of cancer mortality in the world. Due to common treatment resistance and the frequent over-expression of HER1 in lung carcinoma, HER1 inhibitors such as gefitinib and
<table>
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<td>Codeine</td>
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<td>CNS depression</td>
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<td>1-15% (carrier)</td>
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<td>NSAIDs</td>
<td>Pain</td>
<td>Inflammation</td>
<td>Gastrointestinal bleeding</td>
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<td>Cancer</td>
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<td>Response</td>
<td>c-kit mutation (D816V)</td>
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<td>Indicated for c-kit D816V-negative ASM (FDA)</td>
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<td>Panitumumab (Vectibix)</td>
<td>Metastatic colorectal carcinoma</td>
<td>Response</td>
<td>Presence of HER1 expression</td>
<td>HER1 expression 65-85% KRAS 70% (non-carrier)</td>
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<td>Cetuximab (Erbitux)</td>
<td>Metastatic colorectal carcinoma</td>
<td>Response</td>
<td>Presence of HER1 expression</td>
<td>HER1 expression 65-85% KRAS 70% (non-carrier)</td>
<td>Indicated or recommended for treatment of HER1-expressing tumors with wild-type KRAS (FDA, EMA)</td>
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<td>Trastuzumab (Herceptin)</td>
<td>Breast cancer Metastatic gastric cancer</td>
<td>Response</td>
<td>HER2 amplification or overexpression</td>
<td>20-25% (carrier)</td>
<td>Evaluation of HER2 overexpression is necessary for treatment (FDA, EMA)</td>
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<td></td>
<td>Azathioprine (Azasan, Imuran) 6-mercaptopurine (Purinethol)</td>
<td>Leukemia Autoimmune disease Transplantation</td>
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<td>Homozygosity for defective TPMT alleles (e.g. TPMT*2)</td>
<td>1% (hom)</td>
<td>TPMT geno- or phenotyping is recommended prior to treatment (FDA)</td>
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<td></td>
<td>Irinotecan (Camptosar, Campto)</td>
<td>Colon cancer Neutropenia</td>
<td>Response</td>
<td>UGT1A1*28 homozygosity</td>
<td>5-10% (hom)</td>
<td>Reduced initial dose should be considered for UGT1A1*28/*28 subjects (FDA)</td>
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<tr>
<td>Cardiovascular</td>
<td>Clopidogrel (Plavix)</td>
<td>Vascular disease</td>
<td>Response</td>
<td>Defective CYP2D6 alleles (e.g. CYP2D6*4 and *10)</td>
<td>1-7% (hom) Up to 40-50% (het)</td>
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<td>Response</td>
<td>CYP2C19*17 (increased)</td>
<td>1-8% (hom) Approx. 30% (het)</td>
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<td></td>
<td>Response, bleeding complications</td>
<td>CYP2C19*17 (increased)</td>
<td>1-8% (hom) Approx. 30% (het)</td>
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<td>Warfarin (Coumadin, Waran)</td>
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<td>Dose requirement</td>
<td>VKORC1 polymorphism</td>
<td>15-20% (hom) 50% (het)</td>
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<tr>
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<td>Acenocoumarol (Sintron, Sinthrome)</td>
<td></td>
<td>Dose requirement</td>
<td>Defective CYP2C9 alleles (e.g. CYP2C9*2 and *3)</td>
<td>3% (hom) 30% (het)</td>
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<td>Simvastatin (Zocor, Simlup, Simcard, Simvacor)</td>
<td>Hypercholesterolemia</td>
<td>Myopathy</td>
<td>OATP1B1*5</td>
<td>2% (hom)</td>
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erlotinib have been developed to target the tyrosine kinase domain (HER1-TKIs). Patients benefiting most from HER1-TKIs carry activating mutations in the HER1 gene or have a gene amplification. In NSCLC patients carrying HER1 mutations, gefitinib has a clear advantage compared to standard PBC treatment [21]. HER1 mutations also predict acquired resistance to gefitinib or erlotinib, usually within 1 year of HER1-TKI treatment [22]. The most common mutation causing resistance yields a T790M amino acid conversion, and this marker might be useful in predicting subgroups of patients with HER1 mutations who should not receive HER1-TKIs. In Europe, the EMA has approved gefitinib for advanced or metastatic NSCLC in patients with HER1 mutations in all lines of therapy. At present, gefitinib is only FDA-approved for patients who are or have been benefiting from gefitinib treatment.

Analgesia
In the context of analgesic drugs, the CYP2D6 polymorphism plays a major role in the activation of prodrugs such as codeine and tramadol (Table 1). Subjects who are ultrarapid metabolizers (UMs) have elevated enzyme levels (mainly due to CYP2D6 gene duplication) [23] and suffer from ADRs owing to excessive amounts of active metabolites. By contrast, subjects lacking CYP2D6 have reduced pain relief. Other predictive pharmacogenomic biomarkers in this field are relatively limited.

Pharmacokinetic biomarkers
Nonsteroidal anti-inflammatory drugs Subjects carrying defective CYP2C9*2 and *3 alleles show slower clearance of nonsteroidal anti-inflammatory drugs (NSAIDs) and a higher risk of gastrointestinal bleeding [24]. In a meta-analysis of three studies on gastrointestinal bleeding, CYP2C9*2 resulted in an odds ratio of 1.58 for all NSAIDs and 1.96 for NSAIDs that are CYP2C9 substrates [25]. Associations were also found for CYP2C9*3, but with lower statistical power [25]. Further studies are required in this area.

Opioids CYP2D6 converts codeine to its active metabolite, morphine. Whereas poor metabolizers (PMs) do not bioactivate enough codeine for a reasonable effect, UMs do so at risk of central nervous system (CNS) depression and other side effects due to elevated morphine production [24,26]. Koren et al. showed that the CYP2D6 UM phenotype in breastfeeding mothers is a potential risk for infant mortality owing to massive activation of codeine-to-morphine that can be passed on to the infant [27]. Furthermore, respiratory depression due to CYP2D6 UM has been observed in adults [26], although more studies in this area are needed. CYP2D6 is also active in the conversion of tramadol to the active metabolite 0-desmethyltramadol, and a lower response and higher drug consumption has been observed among CYP2D6 PMs [24,26].

Infection
Several different pharmacogenomic biomarkers can effectively predict response and ADRs in treatment of infectious diseases (Table 1).
Pharmacodynamic biomarkers

HIV  Maraviroc is a C-C chemokine receptor type 5 (CCR5) co-receptor antagonist indicated for combination treatment of patients infected with CCR5-tropic HIV-1. Maraviroc blocks human CCR5, which is used by CCR5-tropic HIV to enter human immune cells. CXCR4-tropic HIV does not use the CCR5 receptor and dual-tropic HIV can use both; thus, HIV tropism must be determined before starting maraviroc therapy. The sensitivity of the tropism test used is crucial to obtain the best possible response; the improved sensitivity of the Trofile™ test has enhanced the ability to detect low levels of CXCR4-using virus that could otherwise lead to misclassification of HIV tropism [28].

Immunological biomarkers

Bacterial infection  Flucloxacillin is a narrow-spectrum β-lactam penicillin used against bacteria such as Staphylococcus aureus. Approximately 8.5 of every 100 000 new flucloxacillin users experience a potentially serious drug-induced liver injury (DILI). A GWA study revealed that the human leukocyte antigen HLA-B*5701 allele was predictive for flucloxacillin-induced DILI, with an odds ratio of 80 [29]. Since only 1 of 1 000 carriers of the HLA-B*5701 allele will develop this specific ADR, a genetic test is not warranted. A similar but less specific association of HLA genotype with hepatitis has been described for antibacterial treatment with co-amoxiclav [30,31].

Hepatitis C virus  Hepatitis C virus (HCV) infection affects 170 million people worldwide and is the leading cause of cirrhosis in North America. Four recent GWA analyses revealed that single nucleotide polymorphisms (SNPs) in the IL28B gene (interferon-gamma-3 (IFN-γ3)) are associated with treatment outcome in patients on ribavirin plus peginterferon α treatment [32]. An approximately three-fold difference in treatment response was observed, depending on the IL28B genotype [33]. The mechanism behind the effect of IL28B genotype is not clear at present, although IL28B genotype also seems to affect virus load before treatment and the innate clearance of HCV [34].

HIV  Important work by Mallal and colleagues revealed that abacavir-induced hypersensitivity reactions (HSRs) during treatment of HIV infection and AIDS are strongly associated with the HLA-B*5701 allele (Table 1), which occurs in 2–6% of Caucasians [35]. In their prospective study, genotyping before therapy reduced the number of immunologically verified HSRs from 2.7% to 0% [35]. Because the specificity of the HLA-B*5701 biomarker is ~50% and the cost–benefit for predictive genotyping is high, the EMA has mandated a genetic test before starting abacavir therapy, and the FDA has also strongly recommended genotyping. Interestingly, implementation of genetic testing has reported to increase the prescription of abacavir [36], which is contrary to the general skepticism and the view of genetic tests as a burden in the drug industry. Furthermore, it has been shown that HLA-B*5701 testing is cost-effective in the clinical setting [37].

CNS disorders

Much research has focused on genetic variants for the prediction of treatment success for CNS-related diseases, particularly depression. Despite the efforts made, not many convincing results have been reported (Table 1).

Pharmacokinetic biomarkers

Depression  The highly polymorphic CYP2D6 enzyme metabolizes many tricyclic antidepressants (TCAs). A particular problem with TCA treatment is that UMs are five- to ten-fold overrepresented among non-responders [38]. Interestingly, Zackrisson et al. recently found a nine-fold higher frequency of UMs among suicide cases compared to natural death cases [39], an observation that can probably be explained by sub-therapeutic antidepressant levels. Genotyping for CYP2D6 UMs seems to be highly relevant before TCA therapy, particularly in Mediterranean areas, where this phenotype is common.

Immunological biomarkers

Epilepsy  Epileptic patients on carbamazepine therapy are at risk of developing the potentially fatal dermatological reactions Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), with a risk of 0.01–0.06% in Caucasians but an approximately ten-fold higher risk in Asians. The incidence of SJS/TEN is highly associated with HLA-B*1502 allele before starting carbamazepine therapy. The FDA has declared that patients of high-risk Asian ancestry should be genotyped for the HLA-B*1502 allele before starting carbamazepine therapy.

Cardiovascular disease

Pharmacokinetic biomarkers

Coagulation  Clopidogrel, an antiplatelet prodrug that inhibits the ADP-dependent P2Y12 receptor, is the second best-selling drug in the world (http://knol.google.com/k/top-ten-twenty-best-selling-drugs-2009#) and is for example used to prevent thrombotic events after myocardial infarction, ischemic stroke and coronary stent placement.
The drug requires activation by CYP2C19 [44,45] and thus CYP2C19 polymorphism affects clopidogrel response (Table 1, Figure 1). The effect of CYP2C19 genotype on clopidogrel treatment has recently attracted much attention. A recent meta-analysis of nine studies involving close to 10,000 patients revealed a significantly higher risk of adverse cardiovascular events in carriers of defective (reduced-function) alleles [46]. This effect was especially prominent in patients who had undergone percutaneous intervention, and especially for stent thrombosis as the major cardiovascular event [46]. Two recent large studies that could not be included in the meta-analysis because of the set up or data presentation showed differential effects with respect to CYP2C19 genotype, with a lack of effect in one [47] and a clear effect in the other [48]. However, two-thirds of the population in the latter study had undergone percutaneous intervention, whereas less than one-fifth of the population in the former study comprised such patients. Moreover, Paré et al. only presented data on the effect of CYP2C19 genotype on clopidogrel treatment in relation to placebo [47], which might lead to misinterpretation of the data. The increased risk of cardiovascular events in defective CYP2C19 carriers (typically two- to three-fold [46,48,49]) has prompted the FDA to issue a black box warning for clopidogrel regarding reduced effectiveness in patients carrying two defective CYP2C19 alleles (PM phenotype). Conversely, it has been reported that carriers of the rapid CYP2C19*17 allele exhibit a lower rate of cardiovascular events in a study of clopidogrel versus placebo [50] and in a study of clopidogrel only [50]. With regard to bleeding complications [48,51], the impact of CYP2C19 polymorphism on clopidogrel efficacy has been controversial. Depending on the reproducibility of the CYP2C19*17 findings, inclusion of CYP2C19*17 status in the FDA black box warning might be relevant in the future. At present, two novel drugs, ticagrelor and prasugrel, have been developed with kinetics independent of CYP2C19 genotype and thus have an
advantage over clopidogrel in this respect. For an overview of some representative effects of CYP2C19 genotypes on clopidogrel treatment, see Figure 1.

Coumarins such as warfarin and acenocoumarol are anticoagulants used to prevent thrombotic events. The effect of pharmacogenomic factors on warfarin treatment outcome has attracted much attention owing to the narrow therapeutic window, with bleeding complications on one hand and coagulation risk on the other. Polymorphisms of vitamin K epoxide reductase complex subunit 1 (VKORC1), CYP2C9 and CYP4F2 affect warfarin dosing, accounting for up to 25%, 15% and 3–5% of the variation in dosing, respectively (Table 1) [52]. Dosing algorithms reveal that the overall genetic contribution to the warfarin dose is 30–50%, and GWA studies indicate that no other genetic variations are important [53]. However, prospective studies have not yet provided strong evidence for biomarker-guided dosing [52,54]. CYP2C9 and VKORC1 polymorphisms have also been associated with acenocoumarol dosing in several studies, and a recent GWA study identified VKORC1 and CYP2C9 as the main genetic factors, with a lesser contribution by CYP4F2 [55]. For new anticoagulants, such as dabigatran, that act as direct thrombin inhibitors, dosing is expected not to be influenced by polymorphic genes.

Hypercholesterolemia Statin administration for treatment of hypercholesterolemia can cause myopathy. The incidence of myopathy is only ~1 per 10,000 patients for standard doses (20–40 mg simvastatin) but increases at higher doses (e.g. 80 mg simvastatin) [56]. A GWA study of subjects on 80 mg of simvastatin daily revealed strong association between a polymorphic site (rs4149056) in the organic anion transporter protein 1B1 (OATP1B1) gene (SLCO1B1) with myopathy [56]. This polymorphism is linked to the causative SNP of

Figure 2. Important pharmacogenomic biomarkers influencing treatment response and/or ADR incidence. For details and abbreviations, please see the main text.
OATP1B1*5 (c.521T→C, p.V174), which causes reduced simvastatin hepatic transport and increased plasma concentrations [57] with myopathy [56]. Indeed, OATP1B1*5 allele was highly indicative of the development of myopathy in the GWA study, with an odds ratio of 4.5 per copy of the C allele and 16.9 for C-allele homozygosity. Indeed, more than 60% of myopathy cases could be attributed to this variant, and the association between OATP1B1 polymorphism and myopathy was confirmed in a replicate study of 16,664 patients on a lower dose of 40 mg simvastatin per day [56]. Owing to the very rare incidence of statin-induced myopathy, routine genotyping for the OATP1B1 polymorphism would not be cost-effective but could be of value for retrospective analysis. It has also been shown that the OATP1B1*5 polymorphism affects the pharmacokinetics of many other statins (albeit to a lesser extent) and of the antidiabetic drug repaglinide, the antihistamine fexofenadine and the endothelin A receptor antagonist atrasentan [57].

**Other areas**

**Immunological biomarkers**

*Hyperuricemia* Allopurinol is used to treat inflammatory arthritis but can cause the life-threatening dermatological conditions SJS and TEN [58]. The occurrence of SJS or TEN seems to be highly associated with the HLA-B*5801 allele, with an odds ratio of 41–580, depending on the population [59–62]. Thus, HLA-B*5801 could be used as a biomarker for serious dermatological reactions, although the allele is also found in allopurinol-tolerant patients [59].

**Drug development**

A major application of pharmacogenomic biomarkers is their use as integrated tools during drug development, and both the FDA (www.FDA.gov) and the EMA (www.ema.europa.eu) provide guidelines in this context. It is important to consider inter-individual differences in drug pharmacokinetics and pharmacodynamics at an early stage in drug development (Box 1). Before drug candidates enter clinical trials, possible metabolism and transport pathways encoded by polymorphic genes must be considered and potential interactions identified through *in vitro* assays. It is also vital to identify the enzyme responsible for formation of the active drug metabolite before entering clinical trials.

Pharmacogenomic biomarkers might also aid market re-entry for drugs previously withdrawn because of ADRs [63]. Lumiracoxib, a prostaglandin endoperoxide synthase 2 inhibitor used to treat acute pain and osteoarthritis, was withdrawn in 2005 because of DILI cases. Retrospective genetic analyses revealed that HLA-DQ allelic variants could predict elevated transferase levels. Research revealed 100% sensitivity for the HLA-DQA1*0102 allele for patients with the highest transferase levels, with an additional negative predictive value of 99% [64]. Novartis has now submitted an application to the EMA for the use of lumiracoxib in genetically selected populations. It remains to be seen whether the drug efficacy in comparison to competitor drugs is high enough to overcome the costs and inconvenience of applying a genetic diagnostic test.

**Pharmacogenomic biomarkers for clinical use**

Given the promise of pharmacogenomic biomarkers in aiding treatment of a wide variety of disorders (Figure 2), translation of this potential to clinical practice is a major issue. Our experience is that pharmacogenomic biomarkers for drugs on the market are not emphasized by clinicians or by the drug industry. Box 2 summarizes the opportunities and the many bottlenecks that are evident in the translation of pharmacogenomic knowledge into clinical routine. Biases that should be considered in future research are also outlined in Box 2.

**Implementation and regulatory issues**

For clinical use of pharmacogenomic biomarkers, pharmacogenomic labeling of drugs are standardized so that labels contain information either on: (i) mandatory biomarker use; (ii) recommended use; or (iii) for informative purposes only. In the case of mandatory genotyping, there should be enough data available to establish the predictive value and clinical support. At present, this definition is given for abacavir, imatinib, maraviroc and certain cancer antibodies for which a clear basis for responsiveness has been demonstrated. Among biomarkers with recommended (but not mandated) use, none has undergone clinical trials and only a few are based on information from meta-analyses of several different studies. Pharmacogenomic information is contained on ~10% of drug labels approved by the FDA, the majority of which concerns the polymorphic cytochrome P450 genes CYP2C9, CYP2C19 and CYP2D6 [65]. However, implementation of pharmacogenomic biomarkers has been more rapid in drug development (Box 1). An initiative to implement pharmacogenomic information into clinical practice has been adopted by the Clinical Pharmacoge-
nomics Implementation Consortium to provide guidance on the use of such tests in the clinic [66].

Concluding remarks

Methods for genomic analyses are rapidly being developed. SNP arrays covering 5 million SNPs will soon become a reality, and the cost for whole-genome sequencing is rapidly decreasing [67], with companies now offering such services for as little as a few thousand US dollars. The first study to use information from whole-genome-sequencing for pharmacogenomic purposes was published last year [68]. The amount of data generated in a whole-genome sequencing approach is huge, but only genetic information specifically related to the drug treatment would be relevant and ethically approvable. In the future, there will probably be an arsenal of new pharmacogenomic biomarkers identified owing to the rapid development of techniques and the evolvement of relatively new fields such as epigenetic and small nuclear (sn)RNA-mediated mechanisms. However, the most important aspect will always be cost–benefit analysis of genetic testing. Overall, we can look forward to an exciting future in this area.

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