OPINION

# Pharmacogenetics in the evaluation of new drugs: a multiregional regulatory perspective

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Abstract | Pharmacogenetics, one of the cornerstones of personalized medicine, has the potential to change the way in which health care is offered by stratifying patients into various pretreatment categories, such as likely responders, likely non-responders or likely to experience adverse drug reactions. In order to advance drug development and regulatory science, regulatory agencies globally have promulgated guidelines on pharmacogenetics for nearly a decade. The aim of this article is to provide an overview of new guidelines for the implementation of pharmacogenetics in drug development from a multiregional regulatory perspective — encompassing Europe, the United States and Japan — with an emphasis on clinical pharmacokinetics.

Pharmacogenetics — the study of the associations between the genetics of individuals and their response to drugs, which is a subset of pharmacogenomics (BOX 1, note 1) — has become an important tool for drug development and in regulatory review[1–5]. So far, results from studies with a pharmacogenetics component have been used for several purposes including the following: elucidating the molecular or mechanistic basis for lack of drug efficacy or occurrence of adverse drug reactions (ADRs); clarifying variability in clinical response to drugs by ruling out the role of pathways involving the protein products of well-known polymorphic genes as clinically significant contributors to variable drug pharmacokinetics (PK) and/or pharmacodynamics (PD) parameters; estimating the magnitude of potential drug–drug interactions (DDIs); and designing clinical trials to test for greater treatment effect in genetic subpopulations[5].

Recently, the European Medicines Agency (EMA) published a guideline on the role of pharmacogenetics methodologies in the evaluation of drug PK properties and the US Food and Drug Administration (FDA) published a draft guidance on the use of clinical pharmacogenetics in early-phase clinical studies. These documents, along with similar guidelines from the equivalent agency in Japan (the Pharmaceuticals and Medical Devices Agency (PMDA)), are expected to affect drug development by providing a framework for using pharmacogenetics data throughout a drug's life cycle: from the preclinical phase to post-marketing pharmacovigilance. After providing brief background information on how genetic variations can affect drug response, the aim of this article is to describe the guidelines from the EMA, the FDA and the PMDA, focusing on critical issues for the use of pharmacogenetics during drug development related to drug PK parameters. These include the use of threshold values to guide decisions on the implementation of pharmacogenetics in different phases of drug development, and requirements for DNA sampling, genotyping and phenotyping (TABLE 1). This article also aims to compare the current guidelines from each agency and highlight future perspectives.

## Genetic variants and drug response

The responses to virtually all drugs can vary between individuals owing to intrinsic factors (such as age, health and genetics) and/or extrinsic factors (such as diet, the use of concomitant drugs and adherence) that may affect drug PK and/or PD parameters. In recent years, our understanding of the influence of genes on interindividual differences in drug response has developed rapidly with the availability of the human genome sequence and technologies that allow high-throughput genotyping[3,4,6]. Examples of genetic variants that influence drug response include single nucleotide polymorphisms (SNPs), insertions and deletions, and copy number variations.

An individual's response following administration of a drug depends on several factors. First, genes relevant to the drug's absorption, distribution, metabolism and excretion (ADME), which determine PK properties; second, genes that encode drug targets — either intended or unintended — and their associated pathways, which determine PD properties; and third, genes that influence disease susceptibility or progression (examples of each category are given in TABLE 2). Although genetic variants affect both the PK and PD parameters of drugs, thereby contributing to heterogeneous clinical outcomes (that is, toxicity and/or efficacy), this article focuses on how genetic variants affect PK parameters because the study of genetic variants related to PK properties, especially in terms of drug metabolism, is a relatively mature field in which sufficient data and experience within the regulatory agencies are available to provide detailed guidance. For certain drugs, for example, in oncology, genetic variants directly related to PD parameters may be more important than genetic variants related to PK properties in influencing variability in drug response, but we have fewer examples to form the basis for regulatory guidance, and the strategy for drug development is often more complex than linking a genetic factor to drug concentrations.

The ADME properties of a drug are determined by a complex interplay of systemic (such as cardiovascular) and molecular factors (such as drug transport proteins

Box 1 | **Regulatory documents mentioned in this article**

All links are active in the online PDF.

**International Conference on Harmonisation (ICH)**

*Note 1.* ICH guidelines (see also Further information) are developed to harmonize approaches to drug regulation in order to facilitate global drug development and approval processes. ICH Topic E15 is entitled *Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories*. Step 5 of ICH Topic E15 was approved by the Medicinal Products for Human Use, European Medicines Agency in November 2007 (EMEA/CHMP/ICH/437986/2006); published in the notification by the Japanese Ministry of Health, Labour and Welfare on 9 January 2008 (PFSB/ELD Notification No. 0109013 & PFSB/SD Notification No. 0109002); and published in the Federal Register by the US Food and Drug Administration on 8 April 2008 (Vol. 73, No. 68, 19074–19076).

**European Medicines Agency (EMA)**

*Note 2a.* Guideline on the use of Pharmacogenetic Methodologies in the Pharmacokinetic Evaluation of Medicinal Products (EMA/CHMP/37646/2009).

*Note 2b.* Note for Guidance on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 Corr.*).

*Note 2c.* Key Aspects on the Use of Pharmacogenomic Methodologies in the Pharmacovigilance Evaluation of Medicinal Products (EMA/CHMP/917570/2011).

*Note 2d.* Reflection Paper on Pharmacogenomics in Oncology (EMEA/CHMP/PGxWP/128435/2006).

*Note 2e.* Reflection Paper on Co-development of Pharmacogenomic Biomarkers and Assays in the Context of Drug Development (EMA/CHMP/641298/2008).

*Note 2f.* Reflection Paper on Pharmacogenomic Samples, Testing and Data Handling (EMEA/CHMP/PGxWP/201914/2006).

*Note 2g.* Reflection Paper for Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples (EMA/INS/GCP/532137/2010).

**Pharmaceuticals and Medical Devices Agency (PMDA), Japan**

*Note 3a.* Guideline on Clinical Pharmacokinetic Studies of Pharmaceuticals (Notification No. 796; 1 Jun 2001).

*Note 3b.* Guideline on Methods of Drug Interaction Study (Notification No. 813; 4 Jun 2001).

*Note 3c.* Guideline on General Principles for Clinical Trials Using Pharmacogenomics (Notification No. 0930007; 30 Sep 2008) (in Japanese).

*Note 3d.* Basic Principles on Global Clinical Trials (Notification No. 0928010; 28 Sep 2007).

*Note 3e.* Basic Principles on Global Clinical Trials (Reference Cases) (Administrative Notice; 5 Sep 2012).

*Note 3f.* Guideline on Clinical Evaluation of Anti-Cancer Drugs (Notification No. 1101001; 1 Nov 2005) (in Japanese).

*Note 3g.* Request to Cooperate in Research Regarding Severe Adverse Reactions (Skin Disorder and Rhabdomyolysis) (Notification No. 0926-2; 26 Sep 2011) (in Japanese).

*Note 3h.* Guideline on Evaluation of Diagnostic Device for Analyzing Genetic Profiles Based on DNA Chips (Notification No. 0404002; 4 Apr 2008) (in Japanese).

*Note 3i.* Draft Guideline on Evaluation of Diagnostic Device for Analyzing RNA Profiles (3 Jul 2012) (in Japanese).

*Note 3j.* Scientific Consultation on Pharmacogenomics/Biomarker: The Result of Biomarker Qualification for Drug-Induced Nephrotoxicity (31 May 2010).

**US Food and Drug Administration (FDA)**

*Note 4a.* Guidance on Pharmacogenomic Data Submissions (Mar 2005).

*Note 4b.* Guidance on Pharmacogenomic Data Submissions — Companion Guidance (Aug 2007).

*Note 4c.* Guidance on Clinical Pharmacogenomics: Premarketing Evaluation in Early Phase Clinical Studies (Feb 2011).

*Note 4d.* Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System (Mar 2005).

*Note 4e.* Guidance on Pharmacogenetic Tests and Genetic Tests for Heritable Markers (Aug 2007).

*Note 4f.* Guidance on In Vitro Companion Diagnostic Devices (Jul 2011).

and metabolizing enzymes), with pharmacogenetics focusing primarily on the latter (TABLE 3). Presently, genes encoding proteins involved in drug metabolism have been the most extensively studied and are most often (~80%) referenced in drug labelling[7] (see also the Table of Pharmacogenomic Biomarkers in Drug Labels on the FDA website; Further information).

Genetic variants in phase I or phase II metabolizing enzymes may lead to the following outcomes: increased or decreased clearance of the parent drug and/or its pharmacologically active or toxic metabolites; increased or decreased production of active metabolites from the respective prodrugs; or increased or decreased formation of toxic metabolites. In terms of metabolizing capacity, the normal (wild-type) phenotype is generally defined as the 'extensive metabolizer'. Relative to the extensive metabolizer phenotype, increased metabolism capacity occurs in the 'ultrarapid metabolizer' phenotype, and is usually the result of multiple active alleles (copy numbers) or gain-of-function mutations. Decreased metabolism occurs in the 'poor metabolizer' phenotype, and is often the result of genetic variants leading to reduced or abolished expression or function of the respective enzymes (in poor metabolizers the loss-of-function genetic variants are usually homozygous). Heterozygous loss-of-function genetic variants often result in the 'intermediate metabolizer' phenotype, with a metabolizing capacity that can range between that of extensive metabolizer and poor metabolizer phenotypes.

Of all clinically used drugs, 30–50% are metabolized by functionally polymorphic enzymes[8,9], including phase I cytochrome P450 enzymes (for example, CYP2C9, CYP2C19 and CYP2D6)[10] and phase II enzymes (for example, UDP-glucuronosyltransferases, *N*-acetyltransferase-2, sulphotransferases and some methyltransferases). Examples of drug classes for which genetic polymorphisms significantly affect PK parameters in genetic subpopulations of patients include antidepressants, antipsychotics and anticoagulants. Many antipsychotics and antidepressants are known CYP2D6 substrates and plasma levels of these drugs at the same dosage can vary 5-20-fold among individuals. Exposure to anticoagulants, such as warfarin and acenocoumarol, is dependent on the *CYP2C9* genotype of the patient[11,12], and multiple reports describe an increased frequency of ADRs among individuals with the poor metabolizer phenotype, which probably result from increased systemic exposure to the parent drug[13].

Table 1 | **Regulatory coverage of key issues in the application of PGx/PGt in drug development**

| Issue identified | Regulatory agency | | |
|---|---|---|---|
| | **European Medicines Agency (EMA)** | **Pharmaceuticals and Medical Devices Agency (PMDA), Japan** | **US Food and Drug Administration (FDA)** |
| DNA sampling during drug development | • Prospective DNA sampling and banking for PGx/PGt-related genotype analyses is highly recommended — even when there are no obvious indications of a relevant genetic influence on PK properties — to allow for retrospective analyses when clearer links between genetic influence and PK variability become evident | • Samples for genetic analysis in clinical trials are categorized into three types depending on the purpose and characteristics of the PGx/PGt analysis<br>• For each category, practical points to consider are described, such as necessary information to be included in the informed consent form and study protocol (BOX 1, note 3c) | • Baseline collection and storage of DNA samples from all participants in all arms of all clinical trials is strongly encouraged<br>• Obtain as high sample acquisition rate as possible if complete acquisition is not possible<br>• Reasons for incomplete sample acquisition should be described and potential for bias estimated |
| PGx/PGt sampling and genotyping (phenotyping) in PK studies | • DNA sampling and genotyping is required (see cut-off values below) in the following cases: when important PK variability is not explained by other intrinsic or extrinsic factors; when variability in exposure in genetic subpopulations exists that may require changes in the posology or treatment recommendation of the drug for the specific subpopulation; and when a different benefit–risk balance in certain genetic subpopulations prior to authorization needs to be identified<br>• Alternatively, phenotyping can be used if reproducible data can be generated at safe levels of the drug in the outlier population<br>• Genotyping during FIH and further Phase I studies is required in the following cases: when *in vitro* data indicate that >50% of the drug is predicted to be cleared via a single polymorphic enzyme; when results of *in silico* physiologically-based model simulations indicate importance of a polymorphic enzyme<br>• Genotyping in Phase II (dose-finding) studies is required in the following cases: when *in vivo* data indicate >25% of the parent drug is cleared by a functionally polymorphic enzyme; when >25% of the *in vivo* formation or elimination of an active metabolite — contributing to >50% of the PD effect or efficacy — is governed by a functionally polymorphic enzyme; when no genotype or phenotype, TDM or titration-based dosing is applied to normalize drug exposure, even though exposure is known to vary because of genetic variations<br>• Genotyping in Phase III for relevant genes in all patients is required to confirm the following: genotype or phenotype, TDM or titration-based dosing approach in genetic subpopulations, combined with sparse PK sampling; presumed lack of clinical significance of the different exposures between genetic subpopulations; and (lack of) clinical impact when exposure normalization is not feasible | • If a polymorphic enzyme is involved in the major metabolic pathway of a drug it is recommended that the extent to which PK parameters are influenced by genetic polymorphisms is assessed<br>• If large interindividual differences in PK parameters are expected owing to a genetic polymorphism it is recommended that the effects of the genetic factor are examined<br>• In the question and answer section of the guidance (BOX 1, note 3a) it is mentioned that non-Japanese clinical PK data may be used for regulatory submission if the frequency of a particular allele in individuals of Japanese ethnicity is rare and collection of Japanese PK data on such allele is difficult<br>• Need to assess the possibility of drug interaction in consideration of a phenotype and/or genotype of each patient if a polymorphic enzyme is significantly responsible for metabolism of a drug<br>• It is recommended that racial variability of genotypes among ethnicities is taken into consideration<br>• If genetic variation in metabolic enzymes or transporters is expected to affect the PK properties of the drug it is recommended that genetic tests in PK studies are conducted to examine the incidence of genetic variation in different ethnicities and the PK–genotype relationship (BOX 1, note 3e)<br>• Concrete criteria (numerical values) are not specified as to when PGx/PGt-related PK studies should be considered (such matters can be discussed on scientific consultation, if necessary) | • Recommended in single and multiple ascending dose PK studies if metabolism is a major route of elimination in humans and if the drug is primarily metabolized (or converted to an active metabolite) by well-established polymorphic genes<br>• Known differences in prevalence of ADME-related gene variants among racial or ethnically distinct groups should be considered and selected genetic markers should occur with acceptable prevalence in the population studied<br>• Screening of subjects in early clinical trials using high throughput methodologies (for example, ADME 'gene chips') may be considered instead of targeted candidate gene approach<br>• If genotypes are found to be of relevance in predicting exposure in early trials, subsequent patient studies should be designed accordingly (genotype based dose-adjustment, dose-stratification) |
| PGx/PGt sampling and genotyping in PD studies | • Although it is not the main focus of the guideline (BOX 1, note 2a) research is in progress<br>• However, it is partly covered by the reflection paper on PGx in oncology (BOX 1, note 2d) | • Partly described in the guideline of clinical evaluation for anti-oncology drugs (BOX 1, note 3f)<br>• Such matters can be discussed on scientific consultation if necessary | • If genotypes are found to be of relevance in predicting drug effect in early trials, subsequent patient studies should be designed accordingly (for example, enrichment or stratification designs) |

Table 1 (cont.) | **Regulatory coverage of key issues in the application of PGx/PGt in drug development**

| Issue identified | Regulatory agency | | |
|---|---|---|---|
| | **European Medicines Agency (EMA)** | **Pharmaceuticals and Medical Devices Agency (PMDA), Japan** | **US Food and Drug Administration (FDA)** |
| DNA sampling and genotyping (phenotyping) post-approval | • PGx in pharmacovigilance guideline in preparation | • It is requested that cooperation is needed in research regarding severe cutaneous adverse reactions and severe adverse reactions (skin disorder and rhabdomyolysis) (BOX 1, note 3g)<br>• Such matters may be discussed during a review for a marketing authorization and/or on scientific consultation, if necessary | • Not currently covered in FDA guidances |
| References for sample collection and handling | • Covered in the following reflection papers: co-development of PGx biomarkers and assays in the context of drug development (BOX 1, note 2e); PGx samples, testing and data handling (BOX 1, note 2f); laboratories that perform analyses or evaluation of clinical trial samples (BOX 1, note 2g) | • Partly covered in the following guideline: general principles for clinical trials using pharmacogenomics (BOX 1, note 3c)<br>• Such matters can be discussed on scientific consultation, if necessary | • Covered in the following guidances: pharmacogenomic data submission — companion guidance (BOX 1, notes 4a,b); genomic data and sample coding categories (BOX 1, note 1) |
| Authorized PGx/PGt test | • Covered in the reflection paper on co-development of PGx biomarkers and assays in the context of drug development (BOX 1, note 2e) | • Covered in the following guidelines: evaluation of diagnostic device for analyzing genetic profiles based on DNA chip (BOX 1, note 3h); evaluation of diagnostic device for analysing RNA profiles (BOX 1, note 3i)<br>• Consideration paper on co-development of a drug with biomarker assay is under preparation | • Covered in the following guidances: drug metabolizing enzyme genotyping system (BOX 1, note 4d); PGx tests and genetic tests for heritable markers (BOX 1, note 4e); *in vitro* companion diagnostic devices (BOX 1, note 4f) |
| Standardization for in-house PGx/PGt tests | • It is anticipated that the evolving legislation will address this issue<br>• Large public procurement networks are in place for developing reference standards | • Not currently covered in PMDA guidances | • Not currently covered in FDA guidances |
| Genotype testing performed by general providers | • EU legislation in preparation | • Not currently covered in PMDA guidances | • Not currently covered in FDA guidances |
| Validation of association studies | • Research in progress<br>• Issue is taken into account on a case-by-case basis in the 'qualification of novel methodologies for medicine development' process (see Further information) | • Issue is taken into account on a case-by-case basis in the special consultation of PGx and biomarkers or regular scientific consultation (BOX 1, note 3j) | • Independent replication of novel markers is recommended |

ADME, absorption, distribution, metabolism, excretion; EU, European Union; FIH, first-in-human; PD, pharmacodynamics; PGt, pharmacogenetics; PGx, pharmacogenomics; PK, pharmacokinetics; TDM, therapeutic drug monitoring.

By contrast, excessive prodrug activation may affect the safety of codeine, tramadol (both of which are CYP2D6 substrates) and clopidogrel (a CYP2C19 substrate) in individuals with the ultrarapid metabolizer phenotype[13]. In 20% of Asians bearing the *CYP2C19* poor metabolizer phenotype, the activation of clopidogrel is diminished, thereby resulting in lower anticoagulation effects and lower protection against cardiovascular events[14–16]. This example also illustrates that pharmacogenetically based variations in PK properties may affect the clinical PD properties of a drug and the associated benefit–risk considerations.

At present, most cases in which pharmacogenetic information has been included in drug labelling to help optimize the benefit–risk profile of the drug, such as those examples mentioned above, have been based on research conducted after the regulatory approval of the drug. However, as more knowledge has been gained on genes involved in drug response (particularly drug metabolism), and technological advances have aided the timely and less costly characterization of relevant genetic variants in patients involved in clinical trials, new opportunities to investigate pharmacogenetics during the development of novel drugs have emerged. In recognition of this, regulatory agencies globally have been developing guidance for drug developers for several years, with the goal of ensuring satisfactory efficacy and lowering the incidence of ADRs associated with novel drugs[13,14].

Recently published guidance documents from the EMA, the FDA and the PMDA provide recommendations on the conduct of pharmacogenetics studies at different phases of drug development to optimize drug PK parameters. The issues discussed below include the following: situations in which pharmacogenetics studies are required or recommended; the banking of DNA from trial participants, which can help ensure that unknown genetic variants or important metabolic pathways can be retrospectively identified and their clinical effects tested with sufficient power; and the translation of knowledge into drug labelling (for example, in recommendations for dosing adjustments). First, we summarize key aspects of the guidances from each regulatory agency and then discuss differences between the guidances and future perspectives.

Table 2 | **Examples of genes affecting PK, PD or disease susceptibility or progression**

| Area involved | Treatment or disease | Gene involved |
|---|---|---|
| ADME (PK) | Clopidogrel | *CYP2C19* variants[14–16] |
| | Simvastatin | *SLCO1B1* variants[13,32] |
| PD | Vemurafenib | *BRAF* variants (for example, BRAF-V600E)[47] |
| | Cetuximab and panitumumab | *KRAS* variants (for example, wild-type KRAS)[48] |
| Disease susceptibility or progression | HIV | *CCR5* variants (that is, CCR5-Δ32)[49] |
| | Rheumatoid arthritis | *HLA-DRB1* and *HLA-DRB4* variants[50] |

ADME, absorption, distribution, metabolism and excretion; *CCR5*, chemokine (C-C motif) receptor 5; *CYP2C19*, cytochrome P450, family 2, subfamily C, polypeptide 19; *HLA-DRB*, major histocompatibility complex, class II, DR beta; PD, pharmacodynamics, PK, pharmacokinetics; *SLCO1B1*, solute carrier organic anion transporter family, member 1B1.

## The new EMA guideline

The new EMA guideline (BOX 1, note 2a) provides information on several critical issues for the implementation of pharmacogenetics into the PK-related evaluation of novel drugs (TABLE 1). The Pharmacogenomics Working Party (PGWP; see Further information) at the EMA outlined the first reflection paper on the use of pharmacogenetic methodologies in the PK evaluation of medicinal products in 2007. Since the beginning of 2009, this initial paper was subsequently updated and upgraded into a guideline. In doing so, the PGWP built an intimate collaboration with the Pharmacokinetics Working Party (PKWP; see Further information) of the Committee for Medicinal Products for Human Use (CHMP; see Further information) at the EMA. In 2010, the draft version of the guideline was opened to the public for consultation, and subsequent comments from external academic and industrial parties were implemented. The CHMP approved the new guideline in February 2012 and it entered into force in the European Union in August 2012.

The EMA guideline describes the situations and stage(s) throughout the clinical development programme for which pharmacogenetics-related PK studies should be performed. It states the regulatory considerations and/or requirements (for example, related to study design, selection of subjects and sampling) for pharmacogenetics-related PK studies that investigate the effects of polymorphisms at the ADME level (such as enzymes, drug transporters, binding proteins and other relevant proteins). It also provides information on when the clinical impact of genetic differences on PK parameters should be evaluated, as well as advice on the type of supporting studies that may be needed for posology and treatment recommendations for genetic subpopulations. Moreover, it discusses the possible consequences of genetically determined differences in PK parameters for treatment recommendations and labelling. Finally, special considerations on the integration of DDIs, as well as the effect of impaired or immature organ function in conjunction with pharmacogenetics-related PK studies are given.

Below, we summarize and directly compare key principles of the EMA guideline with those from the FDA and PMDA guidelines that are relevant for the following: situations and stages of drug development for which the effect of pharmacogenetics on PK properties should be considered; DNA sampling during drug development; the evaluation of the clinical consequences due to genetic variants; and the translation of those consequences into treatment recommendations.

***When pharmacogenetic studies for PK properties are required or recommended.*** The EMA guideline distinguishes between required and recommended procedures throughout the drug development process. The discrimination between these terms is based on cut-off values that define an "important pathway" for decision-making purposes (FIGS 1,2). Studies evaluating the effect of pharmacogenetics on the PK parameters of an active substance (parent drug and/or its active metabolites), as well as the implications for efficacy and safety, are generally required when the magnitude of interindividual variation in exposure is likely to negatively influence the efficacy and/or safety in genetically defined subpopulations. Factors that identify such situations are listed in BOX 2.

If important interindividual variability in PK properties is observed and no apparent genetic polymorphism that can predict the PK outliers has been identified, dose adjustment in further phases of the development programme can be based on phenotyped individuals. Factors identifying situations in which studies of the effects of pharmacogenetics on the PK parameters of an active substance and their implications for efficacy and safety are generally recommended are listed in BOX 2. Genotyping or phenotyping is not required during the clinical development programme when the results of PK studies obtained before the first-in-human investigations clearly demonstrate that the impact of pharmacogenetics is irrelevant to the clinical outcome of the medicinal product.

***Requirements for DNA banking.*** In all clinical phases of development, prospective banking of DNA for genotyping is highly recommended, even when there are no obvious indications of a relevant genetic influence on PK properties. These strong recommendations ensure that unknown genetic variants or biochemical pathways of importance can be retrospectively identified and their clinical effects tested with adequate statistical power. It is important to keep in mind that during the different phases of drug development, only a limited number of patients can be included, and rare ADRs may only become apparent after marketing (that is, at Phase IV) when a larger number of patients have received the drug. Such a situation is exemplified by clopidogrel, for

Table 3 | **Systemic and molecular factors affecting ADME properties**

| Property | Examples of systemic factor | Examples of molecular factors |
|---|---|---|
| Absorption | Gastrointestinal function, lung function | Variants in genes encoding drug transporters and channel proteins |
| Distribution | Cardiovascular function | Variants in genes encoding drug transporters and channel proteins |
| Metabolism | Liver function, kidney function | Variants in genes encoding enzymes involved in phase I and phase II of metabolism |
| Excretion | Gastrointestinal function, liver function, kidney function | Variants in genes encoding drug transporters and channel proteins |

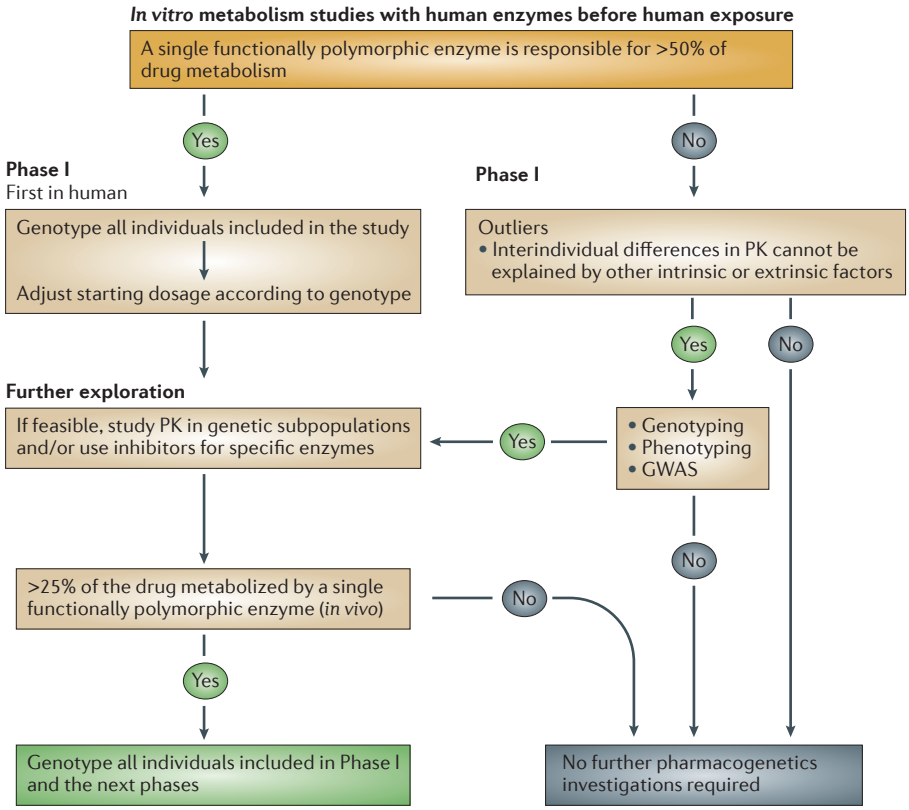


Figure 1 | **The European Medicine Agency's decision-making tree for *in vitro* studies prior to human exposure and Phase I studies.** For polymorphic enzyme systems for which well-validated *in silico* physiologically based pharmacokinetic (PK) models have been developed, pharmacogenetics differences in humans may be predicted and used as a guide for clinical study design with respect to pharmacogenetics investigation. GWAS, genome-wide association study.

which activation by polymorphic enzymes was identified only after the drug was in wide clinical use[14]; if stored DNA samples were available it would have probably facilitated clarification of the consequences.

***Integrating pharmacogenetics effects on PK properties into drug development.*** In the following subsections, recommendations are made on how to implement pharmacogenetics during the different phases of drug development, starting with the *in vitro* studies that are conducted before clinical investigation in Phase I–IV studies (FIGS 1,2). The final goal of the clinical development programme should be to obtain a clear dosing or treatment recommendation at the time of marketing authorization that yields effective and safe treatment not only in the main population, but also in genetically or phenotypically defined subpopulations.

**In vitro *studies before human exposure.*** *In vitro* metabolism studies using human enzymes should be conducted before Phase I (BOX 1, note 2b). Such studies preferably include identification of *in vitro* metabolizing enzymes, and the identification and characterization of metabolites formed through candidate metabolic pathways (both pharmacologically active and/or toxic metabolites of the drug). In the EMA guideline, a pathway can be considered "important" when *in vitro* data indicate that >50% of the drug is cleared by a single polymorphic metabolizing enzyme. Although arbitrary, it is assumed that the increased drug exposure under these conditions is likely to be relevant for efficacy and/or safety, as such a reduction in drug clearance could double the intended exposure *in vivo*, which is typically equivalent to increasing the dose to the next level in an early PK study. The aim of this cut-off level is to avoid individuals with a poor metabolizer phenotype from being exposed to unsafe doses.

Involvement of drug transporters may also be indicated by *in vitro* data obtained before Phase I trials. The *in vivo* importance of a drug transporter may be implied through use of animal models and *in vitro* systems or from information on similar substances. It may be difficult to make quantitative predictions of the *in vivo* contribution of drug transport proteins and no cut-off values for these entities have been provided in the guideline thus far.

***Phase I (exploratory, first-in-human).*** When the *in vitro* data indicate that >50% of the drug is cleared by a single functionally polymorphic enzyme, it is advised to genotype the relevant gene in the first-in-human study population to avoid safety issues related to genetically determined differences in active substance exposure (FIG. 1). Subjects with a genotype predicted to result in markedly increased exposures of active drug or its metabolites should preferably be allowed to participate in the first-in-human study only at doses lower than those expected to be safe in individuals that have an extensive metabolizer phenotype.

***Phase I (further exploration).*** In this phase, the relative contribution of an important polymorphic enzyme on the *in vivo* PK properties of a drug or active metabolite is investigated. It is recommended to investigate the relevance of the genotype of an important metabolizing enzyme on the PK parameters of a drug in a conventional PK study within genetically defined subpopulations whenever feasible (FIG. 1).

When this is impractical, and when there is ample supporting scientific literature or validated data available, the effect of a genotype may be confidently mirrored by treatment with an inhibitor of the respective metabolizing enzyme, and the effect of the polymorphism could be estimated from the results. In such cases, it is important to consider off-target effects of the respective inhibitors. If a marked effect of a polymorphism is confirmed *in vivo* (arbitrarily defined in the EMA guideline when >25% of the parent drug is cleared by the polymorphic enzyme *in vivo*), it is recommended to genotype the indicated genes in as many of the Phase I studies and the next phases as possible. This is to maximize the amount of supporting data for recommendations utilized for studies in genetically defined subpopulations. Moreover, where relevant, it is necessary to expand the Phase I study and evaluate relevant drug interactions and consequences of impaired or immature organ function in defined subpopulations. The >25% cut-off is consistent with that applied to DDIs (BOX 1, note 2b).

***Phase II (dose finding, exploratory).*** The ultimate aim of Phase II investigations is to optimize the posology and design of the

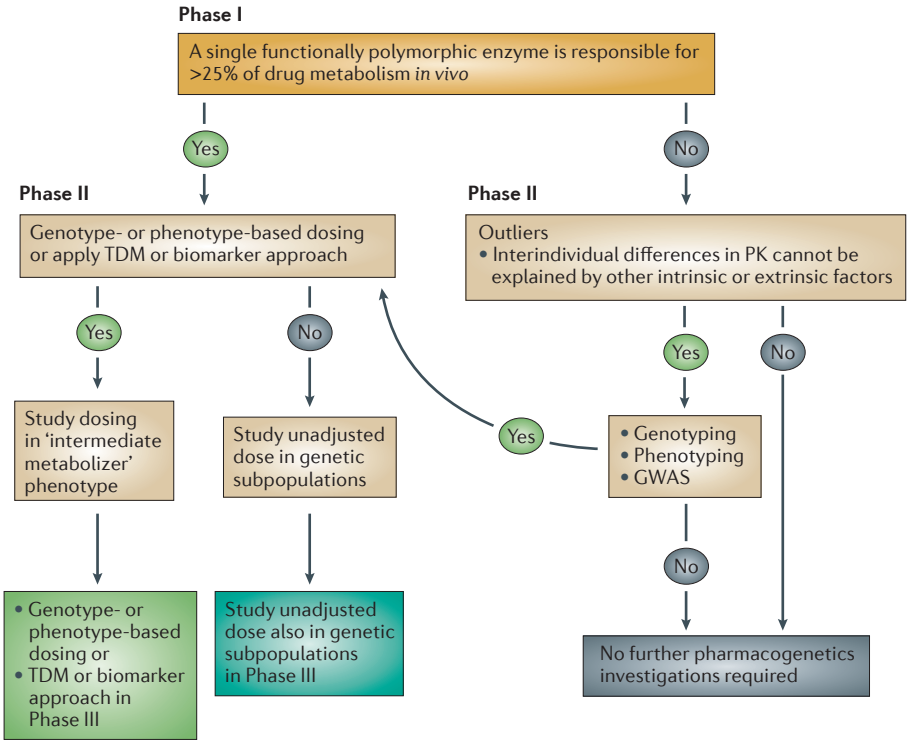


Figure 2 | **The European Medicine Agency's decision-making tree for Phase I and Phase II studies.** For polymorphic enzyme systems for which well-validated *in silico* physiologically based pharmacokinetic (PK) models have been developed, pharmacogenetics differences in humans may be predicted and used as a guide for clinical study design with respect to pharmacogenetics investigation. GWAS, genome-wide association study; TDM, therapeutic drug monitoring.

Phase III studies, including the decision of whether genotype-based dosing should be applied or not. Potential clinically relevant pharmacogenetic influences on drug PK properties from Phase I studies must be considered in the design of Phase II studies, either by investigating genotype-based or phenotype-based dosing or exploring therapeutic drug monitoring (TDM) or biomarker-guided dosing (FIG. 2).

When Phase II data indicate that the difference in exposure observed between genetically defined subpopulations is indeed likely to be of clinical importance, individuals with an intermediate metabolizer phenotype should be investigated in a further PK study. If the sponsor decides that dose adjustment is not needed based on genotype, TDM or biomarker data, the exposure level obtained with an unadjusted dose in the genotypically defined subpopulation should also be used in Phase II studies.

***Phase III (confirmatory).*** When data from Phase II studies indicate a significant difference in exposure or distribution of the drug or metabolite within the genetically or phenotypically defined subpopulation, genotyping and/or phenotyping of all relevant genes in all patients in Phase III trials is required and doses need to be adjusted accordingly. There are several ways to apply knowledge regarding the likely consequences of a polymorphism on the efficacy and/or safety to designing a Phase III trial (BOX 2).

For polymorphic drug transport proteins, plasma levels may not vary between different genotypes. Instead, altered intracellular or inter-organ distribution may occur (affecting target exposure at the cellular level). The consequences here depend on the relationships between the local exposure and PD properties of the drug. If indicated, genotyping for the relevant drug transporter genes is encouraged.

***Phase IV (post-marketing).*** At the time of marketing authorization, information on the safety of a drug is relatively limited owing to factors including the low numbers of subjects (together with genetic subpopulations) in clinical trials, restricted inclusion criteria and restricted conditions for drug treatment. Furthermore, rare but serious ADRs may be identified late in development, or may only be discovered and characterized after marketing authorization and increased population exposure. Therefore, systematic consideration of pharmacogenetics in the risk management plan (RMP) is warranted.

The EMA is currently formulating a guideline on the use of pharmacogenomic methodologies in pharmacovigilance (BOX 1, note 2c). Retrospective analyses on the collection of stored, high-quality genomic material during clinical trials or after obtaining marketing authorization may prove indispensable for clarifying the contribution of pharmacogenetics to observed ADRs or lack of efficacy. For retrospective analyses, it is essential that the genomic information can still be linked to sufficient clinical information from the respective patients. Furthermore, these types of analyses are more effective with a larger amount of data (DNA and respective clinical information). Therefore, as stated in the EMA guideline, DNA samples from all individuals enrolled in clinical studies (Phases I–III) should be stored.

***Involvement of relevant polymorphic proteins identified during Phases I to IV.*** FIGURE 1 and FIGURE 2 outline an ideal situation for the decision-making process (that is, the potential effect of pharmacogenetics on PK parameters is detected early in drug development and further investigated throughout the remaining phases of clinical development) as conceived by the EMA. However, in reality, it is likely that knowledge regarding the potential effect of pharmacogenetics on drug PK properties will be limited at the initial stages of clinical development. Under such conditions, acquired PK data, as well as clinical efficacy and safety information obtained at later developmental stages, may trigger the need for pharmacogenetics-guided studies for evaluating the impact of pharmacogenetics on the clinical outcome. Such situations may arise on the following occasions: first, a previously unknown or sparsely studied functionally polymorphic enzyme or drug transporter is found to be involved in the metabolism or transport of the drug. Second, the enzyme or drug transporter involved in the metabolism or transport is known but there is no prior knowledge regarding functional polymorphisms of the gene. Third, PK outliers are observed throughout Phases I to IV.

For the first and second scenarios, appropriate standard pharmacogenetic considerations need to be implemented in the subsequent evaluation of the drug. Special attention must be paid to outliers (as in the third scenario) whereby an important

Box 2 | **Pharmacogenetics studies in the European Medicines Agency guidance**

Here, the factors influencing the need for pharmacogenetics investigations are outlined.

**Factors describing when pharmacogenetics studies are required**

- If *in vitro* and/or clinical (*in vivo*) studies indicate that a known functionally polymorphic enzyme or drug transporter is likely to be important in the disposition of the drug.
- Or if *in vitro* and/or clinical studies indicate that a known functionally polymorphic enzyme or drug transporter is likely to represent an important factor in the formation, elimination or distribution of a pharmacologically active or toxic metabolite.
- Or if clinical studies indicate that substantial interindividual differences in the pharmacokinetic (PK) properties of a drug that cannot be explained by other intrinsic or extrinsic factors are likely to influence the efficacy or safety of the drug in a genetically variable subpopulation.

**Factors describing when pharmacogenetics studies are recommended**

- If the available *in vitro* data indicate that a polymorphic enzyme or drug transporter contributes to the PK properties of the active substance, but that the quantitative role is relatively low based on the *in vitro* data.
- Or if there is high interindividual PK variability, or there are PK outliers with higher or lower exposure to the active substance that cannot be attributed to other known intrinsic or extrinsic factors, but which could possibly give rise to clinical efficacy and/or safety concerns based on the existing knowledge.
- Or if major PK differences between ethnic groups cannot be attributed to other known intrinsic or extrinsic factors.

**Factors describing when pharmacogenetics studies are considered in Phase III studies**

- If all previously acquired data suggest (but are insufficient to prove) that a marked difference in drug exposure lacks clinical relevance, and no genotype- or phenotype-specific treatment is under consideration. In this situation, a goal of the Phase III study should be to confirm this presumed lack of clinical significance. The conclusion on comparable efficacy and/or safety obtained from subjects exposed to high or low levels of the parent drug due to their genotype must be supported by conclusive clinical data obtained from those exposure levels. Therefore, a sufficient number of genetically defined patients should be included in Phase III (enrichment studies). For cases in which the prevalence of the poor metabolizer phenotype is low, an additional treatment group that consists of subjects that have the extensive metabolizer phenotype and implementation of larger doses may be needed. PK and pharmacodynamics (PD) data related to efficacy and/or safety may be supportive of a lack of clinical significance in this respect.
- If all previously acquired data suggest that the difference in dosage is likely to be clinically relevant, and a genotype- or phenotype-based dosing regimen yielding comparable dosages was developed in Phase I and Phase II studies. The posology of active substances used in Phase III studies is to be adjusted on a genotype or phenotype basis, and sparse sampling with population PK analyses may be applied in the Phase III studies to confirm the dose normalization.
- If all previously acquired data indicate that the difference in dosage is likely to be clinically relevant, and dose titration is pursued regardless of genotype (if suitable markers exist). The Phase III study should then aim to confirm that there are no efficacy and/or safety concerns for the genetically defined subpopulation when the proposed general dose titration is applied. PK and PD data related to efficacy and safety may be supportive of clinical significance in this respect.
- If all previously acquired data indicate that the difference in exposure is likely to be clinically relevant, but owing to the available marketed formulations it is not possible to adjust the doses. Patients tested positive for a specific genotype or phenotype (patients at risk) should then be excluded from trials.

PK alteration may be caused by a rare but functionally relevant genetic variant. For outliers occurring during Phase I and II trials, additional investigations including detailed genotyping (beyond known candidate genes), phenotyping and/or genome-wide association studies are recommended (FIGS 1,2). Proactive analyses of all possible pertinent genes are recommended in such cases. To further guide drug development, meta-analyses on pooled data from different PK or clinical studies can be considered. If possible, the included studies should be similar with respect to non-genetic factors that may affect the PK parameters of a drug. For all three scenarios, population PK analyses may be used as a hypothesis-generating tool.

Conclusions from retrospective analyses carried out in response to emerging data may be acceptable for genetic issues related to PK parameters if they are mechanistically supported by available *in vitro* or PK information. In this case, it is preferable that DNA from a representative proportion of patients enrolled in the Phase I, II and III studies should be available. If new genetic associations are discovered from retrospective analyses, complementary *in vitro* or PK examinations aimed at investigating the mechanism of action and confirming the PK consequences are expected to be carried out.

***Clinical consequences of genetic variants and translation into treatment recommendations.*** The clinical consequences of observed variability in drug exposure due to genetic variants in genetically defined subpopulations depend on the following: first, the magnitude of drug exposure caused by the polymorphism; second, the relationship between PK and PD properties of the drug; third, the relationship between drug dose and clinical effect/ADRs; and fourth, the severity of possible ADRs and/or clinical consequences of reduced efficacy.

Dosing recommendations should ensure that patients receive effective and safe drugs. In principle, unless it is reliably shown that a difference in active substance and metabolite exposure has little consequence for efficacy and safety, the EMA expects genetic variants to be compensated with dose adjustments. For this, either genotype-based or phenotype-based dosing can be applied or individual dose titration based on TDM can be used to improve efficacy or reduce ADRs. If dose titration based on clinical markers is applied, data showing satisfactory efficacy and/or safety of the drug within the genetically defined subpopulation must be provided.

## The PMDA, Japan, guideline

The PMDA has been promoting the use of pharmacogenetics in drug development through multiple efforts, such as formation of guidelines (in collaboration with the Ministry of Health, Labour and Welfare) and conducting scientific consultation with the pharmaceutical and medical device industries and biomarker qualification meetings[17,18]. The following section provides an overview of how the current PMDA guidelines relate to pharmacogenetics and the current perspective of the PMDA on the use of pharmacogenetics in drug development (TABLE 1).

***Evaluation of pharmacogenetics in PK studies.*** Two guidelines relating to PK studies published in 2001 (BOX 1, notes 3a,b) recommend that a sponsor should examine how

genetic variants of metabolizing enzymes affect PK parameters and DDIs of drugs. It is recommended that genetic tests should be incorporated in PK studies in order to select a stratified population when marked interindividual differences in PK parameters of the drug are expected and/or when a drug is metabolized mainly by polymorphic enzymes. The phenotype and/or genotype of an individual subject should be taken into consideration when examining possible DDIs. In the question and answers document attached to the guideline on clinical PK studies of pharmaceuticals (BOX 1, note 3a), it is also mentioned that if it is not feasible to conduct PK studies that include Japanese individuals with a poor metabolizer phenotype, extrapolation of non-Japanese clinical PK data may be considered for review by the PMDA.

Compared with the EMA guideline, concrete criteria (numerical values) are not specified as to when pharmacogenetics-related PK studies should be considered. Although such discussions are currently being carried out between the PMDA and respective sponsors on a case-by-case basis, the general principles are similar to that described in the EMA guideline. In addition, a significant amount of scientific knowledge, such as the influence of drug transporters on PK parameters and experiences of pharmacogenetics-related PK studies, has rapidly accumulated since these guidelines were published.

***Requirements for DNA banking.*** The PMDA encourages a sponsor to collect DNA samples in clinical trials for the prospective and/or retrospective examination of the involvement of genetic variants in the efficacy or safety of a drug. In the guideline published in 2008 (BOX 1, note 3c), sample collection in clinical trials is categorized into three types depending on the purpose and characteristics of the pharmacogenetics analyses.

In the first and second categories (category A and B, respectively), genes for analysis are specified before the initiation of clinical trials and limited to the scope of the investigational drug. One difference between category A and B is the definition of the timing for pharmacogenetics analyses. In category A, pharmacogenetics analyses are conducted throughout clinical trials, whereas the timing of genomic or genetic analyses in category B is undefined. In the third category (category C), exploratory pharmacogenetics analyses that are not directly related to the investigational drug may be included. Banking of DNA samples is allowed only in categories B and C. Because all genetic testing is prespecified and conducted during clinical trials in category A, banking of DNA samples for future use is not thought to be necessary.

The PMDA guidelines describe the points to consider in planning a pharmacogenetics study for each category, such as the necessary information to include in the informed consent form and study protocol. Thus, these documents clearly support the collection of DNA samples in clinical trials by a sponsor. Although the EMA guideline provides more details, both agencies are working towards a common goal.

***Pharmacogenetics in the globalization of drug development.*** Consideration of pharmacogenetics is also important in global drug development. Pharmacogenetic analysis in multi-regional clinical trials (MRCTs) will provide useful scientific data for understanding similarities and differences in drug responses (efficacy and/or safety) among various ethnicities. Specifically, when large differences in PK parameters among different populations is observed, pharmacogenetic analyses are useful for the examination of the reason or reasons for the differences and to set an appropriate dose for each population in later clinical trials (such as an exploratory dose-finding study).

When genetic variants in metabolic enzymes or transporters are expected to affect the PK properties of the investigational drug, the PMDA encourages the conduct of pharmacogenetic analyses in MRCTs to characterize the incidence of genetic variants in different ethnicities, as well as the relationship between PK properties and genotype. The PMDA has worked on a new document describing the points to consider in planning MRCTs and evaluating the data. This is an accompanying document to the former 2007 notification entitled *Basic Principles On Global Clinical Trials* (BOX 1, note 3d), and was published in September 2012 (BOX 1, note 3e).

## The US FDA guidance

Approximately a decade ago, the FDA realized that the completion of the Human Genome Project would bring with it significant advances in personalized medicine and that there was a need for pharmacogenetically informed drug development[19]. Consequently, several pharmacogenetics-related programmes (for example, the Voluntary Genomic Data Submissions Program[20,21], which is held regularly with EMA) and guidances were developed to facilitate communication and translate pharmacogenetics into drug development and regulation (BOX 1, notes 1, 4a and 4b). There has been a subsequent growth in the proactive incorporation of pharmacogenetic principles into various aspects of clinical development (TABLE 1), most notably in clinical pharmacology studies and in patient selection for later confirmatory studies[5].

The FDA draft guidance released for public comment in February 2011 (BOX 1, note 4c) is among the latest in a series of guidances related to individualized therapy and stratified medicine. It was formulated to aid drug developers and regulatory staff in considering how to leverage early-phase clinical studies to better characterize the genetic sources of variability in PK and PD responses and in safety predisposition. Improved understanding in these areas could aid in the design of later phase clinical studies or serve as evidence supportive of drug benefit relative to risks. The FDA received feedback from external stakeholders and is currently updating the original draft version.

The following sections provide an overview of the FDA guidance and current FDA thinking on the potential for genomics to help answer questions that arise during early drug development. Of note, the guidance does not address trial design or statistical analyses considerations for later phase, randomized controlled confirmatory clinical trials or co-development of a drug and *in vitro* diagnostic. Rather, it focuses on exploratory and observational studies that are intended to determine the effect of genetic variants on PK and PD parameters, safety or response that may generate genomic hypotheses that could be applied in late-phase confirmatory trials. As with the FDA's other guidance documents, this guidance (BOX 1, note 4c) does not establish legally enforceable responsibilities for the pharmaceutical industry. Rather, it describes the FDA's current perspective and should be viewed only as advice, except where specific regulatory or statutory requirements are cited.

***General pharmacogenetic considerations in drug development.*** To date, the FDA experience suggests that pharmacogenetic studies may contribute to a better understanding of interindividual differences in the exposure to or efficacy and safety of investigational drugs. Additionally, dose–response or concentration–response relationships can be affected by genetic variants in PD-related genes. When considering non-genetic covariates (intrinsic and extrinsic factors), genetic differences between individuals can affect

patients' disease trajectory (that is, as prognostic factors) or likelihood for treatment response (that is, as predictive factors).

The FDA recognizes the potential utility of genetic variants in PK-related or PD-related genes in prognostic or predictive enrichment of clinical trials, which in turn could enhance clinical phases of drug development by increasing the efficiency of clinical trials (for example, by decreasing the sample size or shortening the treatment duration needed to demonstrate a treatment effect — if one exists). Preliminary data from early-phase trials can serve to inform enrichment strategies in later phases of development. Furthermore, results from studies with a pharmacogenetics component can be used in various ways to optimize knowledge about a given compound and inform regulatory review as previously described[5].

***Requirements for DNA banking.*** A major issue in the meaningful conduct of pharmacogenetic analyses is the availability of DNA from a sufficient number of patients. Similar to the EMA guidance, the FDA guidance strongly encourages baseline collection and storage of DNA samples from all participants in all arms of all clinical trials. This includes exploratory, adequate and well-controlled clinical trials that are intended to support the effectiveness and safety of a novel drug. Complete DNA collection at baseline is recommended in order to minimize the potential for biases that may occur from delayed sampling. This is likely to be most critical in situations in which fatal or morbid conditions are being studied, a high drop-out rate occurs or tolerability of treatment is an issue. The guidance also recommends storage of DNA samples, as questions of drug response variability or safety amenable to pharmacogenetics investigation can emerge any time during drug development and putative candidate genes may not have been considered in advance of initiating the clinical trial.

The FDA received various comments from the public regarding its position on the acquisition of DNA samples. Many applauded the recommendation of complete sample acquisition in the context of early exploratory trials as essential for advancing the field. Other comments from the public posited that complete sample acquisition is not practical given the global nature of drug development and regional heterogeneity of institutional review boards and ethics committees in reviewing protocols with a pharmacogenetics component. The FDA acknowledges the challenges of conducting clinical trials in the global context. Nevertheless, DNA collection is particularly important to characterize the role of genetic variants for drugs for many reasons. These include characterizing high interindividual variability in PK or PD properties; multimodal distributions for measured PK or PD parameters; observed PK or PD differences between racial or ethnic groups; narrow therapeutic ranges; and potential safety issues that have been previously linked to genetic predisposition (for example, liver injury, severe cutaneous reactions, QT prolongation). To the extent that these drug characteristics may not be predictable before initiating clinical development, the FDA considers it good practice to ensure high rates of DNA sample acquisition for most drugs entering clinical testing. If complete acquisition is not possible, efforts should be made to obtain as high a sample acquisition rate as possible. Additionally, it is important to describe specific reasons for incomplete sample acquisition, report where the "missingness" occurs (for example, domestic versus non-domestic) and to estimate the potential for bias where possible.

***Evaluation of pharmacogenetics in clinical pharmacology studies.*** The FDA guidance emphasizes the importance of considering data from preclinical assessment in determining the best approach for integrating pharmacogenetics in early-phase clinical studies. Subsequent to *in vitro* studies, clinical pharmacology studies may be used to identify a potential association between the drug's PK and PD properties, as well as genetic variants in metabolizing enzymes, drug transporters and drug targets. In cases for which metabolism is a major route of elimination in humans and the drug is primarily metabolized by well-established polymorphic genes (for example, CYP2D6 and CYP2C19), as identified during preclinical development, the contribution of genetic variants to PK variability and subsequently to dose or dosing regimen selection in human studies should be evaluated. However, whereas the EMA guideline recommends that the contribution of a specific polymorphic metabolizing enzyme to PK variability should be assessed when *in vitro* data show >50% of the drug is predicted to be cleared by the enzyme, the FDA guidance does not propose such a threshold as lesser degrees of metabolism could be relevant for drugs with a steep exposure–response relationship.

It is generally recommended that pharmacogenetics should be considered in single dose and in multiple ascending dose PK studies. These studies may be able to provide information on the influence of common ADME gene variants with large effects on PK parameters. DNA samples from all enrolled subjects in early-phase clinical studies should be collected for potential analyses of the genetic cause of PK outliers, and to determine the influence of genetic variation on PK parameter distribution. Prospective genotyping may be needed when there is a high likelihood that a subset of patients will experience excessive exposure due to their genetically mediated altered drug metabolism (which is particularly relevant if drug toxicity is suspected to be dose-related). These subjects may either receive an adjusted dose in early-phase dose-ranging studies, or be excluded from the initial dosing cohorts until a better understanding of the *in vivo* relevance of the PK variation has been developed.

For cases in which *in vitro* studies suggest a drug is metabolized extensively by a polymorphic enzyme the FDA guidance recommends that pharmacogenetic analyses should be conducted in single and/or multiple-dose PK studies to evaluate the impact of common genetic variants on *in vivo* drug concentrations (and relevant PD measurements if available). Consideration of known differences in the prevalence of ADME-related gene variants among racial or ethnically distinct groups should be incorporated into the planning for such analyses. Selected genetic variants (for example, SNPs in genes encoding metabolizing enzymes) occurring with acceptable prevalence in the population should be studied. A dedicated clinical pharmacology study with targeted, genotype-based enrolment may be desirable in certain situations and should allow for a meaningful retrospective analysis.

Attention should also be given to drugs for which conversion to an active metabolite occurs through a polymorphic metabolism pathway, as differences in metabolite exposure among individuals may have implications for dosing, efficacy and safety. Notably, strategies other than a targeted candidate gene approach may be useful for assessing PK variability in early drug development. For example, screening of subjects in early-phase clinical trials using high-throughput methodologies (for example, ADME 'gene chips') may generate valuable information on the sources of PK and/or PD variability.

The FDA guidance recognizes that important PK variability of an active parent drug (or metabolites), if observed in Phase I trials, should shape the design of subsequent clinical studies (for example, dose–response studies in genetically defined

Table 4 | **Summary of differences between the three regulatory guidelines on pharmacogenetics**

| Issue | Regulatory agency | | |
|---|---|---|---|
| | **European Medicines Agency** | **Pharmaceutical and Medical Devices Agency, Japan** | **US Food and Drug Administration** |
| Development phases covered in guideline or guidance | Preclinical and clinical development (Phases I–IV; focusing on PK) | Clinical development (Phases I–IV) | Early clinical development (Phases I and II) |
| Banking of DNA samples | Highly recommended | Encouraged* | Strongly encouraged |
| Genomic testing | Required‡ | Recommended | Recommended |
| *In vitro* cut-off values§ | >50% | None | None |
| *In vivo* cut-off values§ | >25% | None | None |

*Does not apply to category A (see main text for more details). ‡Is a firm requirement only when *in vitro* (>50%) or *in vivo* (>25%) cut-off values are met. §For when pharmacogenetics-related testing is required in pharmacokinetics (PK) studies.

subpopulations). For cases in which genotypes are found to be of relevance in predicting exposure and drug effect, this knowledge should be used in the design of subsequent clinical trials. For example, genotypes can be used to select patients for trials (for example, enrichment with responders or exclusion of patients likely to experience toxicity); stratify groups within trials; and adjust doses for trials. This strategy may increase average effect, decrease toxicity and improve the chances for overall success of the study.

Another important aspect for the design of clinical pharmacology studies is the pharmacogenetic evaluation of dose–response relationships. If genotype is shown to be associated with systemic exposure–response (activity and/or safety) in early PK or PD studies, subsequent dose–response studies with methodological features including, but not limited to, genotype-based dose stratification should be considered.

## Discussion and future perspectives

The three guidelines described in this article represent current regulatory thinking on the application of pharmacogenetics in PK studies and are intended to complement early and continuous scientific discussions between drug developers and regulatory agencies during drug development. The guidelines are primarily relevant for drugs that are still under development, and applying them should help ensure that benefit–risk assessments of genetic variants that affect drug PK properties are made before drug registration, as opposed to post-registration, which was the case for older drugs such as warfarin, acenocoumarol, codeine, tramadol and clopidogrel. With respect to drugs that are already registered (original or generic), pharmacogenetics-related investigations triggered by observations in Phase IV of the drug's life cycle may be necessary, and are at the moment underserved by the guidelines of the three agencies (TABLE 1). It is an upcoming challenge to address these critical issues in future guidelines — an exercise under way at the different agencies.

***Differences between approaches to key issues.*** There are several differences in how the three agencies deal with critical issues in the application of pharmacogenetics to PK parameters and these are highlighted in TABLE 4. First, whereas the EMA guideline covers preclinical and clinical Phases I–IV, with a main focus on PK parameters, the PMDA guideline only covers clinical Phases I–IV and the FDA guidance focuses only on early clinical Phases (I–II). One potential factor underlying these differences is that the EMA, in contrast to the FDA and PMDA, is not systematically involved in early clinical trial design, when many facets of pharmacogenetics are relevant to drug development. This introduces variation in the levels of exposure and data to rely upon. Therefore, the EMA adopted a pragmatic and focused approach that facilitates clinically relevant discussions in scientific advice meetings and allows for gathering data sets in a defined context from a regulatory point of view.

Second, there are differences with respect to the stringency of banking of DNA samples recommended for pharmacogenetics analyses. Whereas banking of DNA samples is highly recommended by the EMA and strongly encouraged by the FDA, it is only encouraged by the PMDA and only for those cases in which pharmacogenetic analyses are not a priori necessary. One factor underlying these differences is that DNA banking in clinical studies requires approval by regional ethics committees, and may also be subject to national laws and regulations. Therefore, general DNA banking for pharmacogenetic studies can only be mandated within the guidelines if permitted under legislation and the ethics committees agree that this procedure is in the interest of the subject and/or public; for example, by allowing for retrospective analyses in cases in which clearer links between genetic influence and PK properties become evident. Thorough science-based regulatory guidance might provide useful information to ethics committees about the need for pharmacogenetic sampling, and encourage the development of consistent and robust approaches to the oversight of sample collection, data collection and data protection.

A third area in which there are differences among the three agencies is when pharmacogenetics-related PK studies are required or recommended. The clear *in-vitro*/*in vivo* cut-off values used to guide developers' decision-making during early-phase drug development put forward by the EMA are not shared by the respective agencies in the United States or Japan. The experience of the EMA with the respective cut-off values will undoubtedly help to stimulate discussion regarding a harmonization process among the three agencies. Such future harmonization of the respective guidelines is and will continue to be a major challenge, but could substantially aid global drug development programmes[22] by providing pragmatic, unified and transparent guidance for drug developers. Such an enterprise will, however, also need to involve national legislations, as regional ethics committees are intimately involved in governing clinical drug development.

***Other future issues relevant to regulatory guidance on pharmacogenetics.*** A major issue in translating well-validated pharmacogenetics-related data into everyday patient care is the difficulty in making it useful for clinicians. Conversely, the rapid proliferation of non-validated pharmacogenetics associations and tools could be prematurely or inappropriately introduced into clinical decision-making. For example, research with high-content genome-wide panels may lead to spurious associations (dubbed the 'incidentalome'[23–25]) that could undermine hard-won achievements

## Glossary

**Adverse drug reactions**
(ADRs). Noxious, undesired or unintended responses to pharmacological treatments that occur at dosages used for prophylaxis, diagnosis or therapy of diseases.

**Enrichment studies**
Clinical studies in which patient subsets are enrolled or analysed in order to increase the likelihood for demonstrating a specific treatment effect (if one exists).

**Genetic subpopulations**
Groups of individuals sharing the same genetic variants. Ethnicity is not included in the context here.

**Interactome**
The entire set of protein–protein interactions that occur in a cell.

**Pharmacodynamics**
(PD). The desired or adverse biological (for example, biochemical or physiological) effect of a drug on the body.

**Pharmacogenetics**
According to the definitions set by the International Conference on Harmonisation Topic E15 guideline, pharmacogenetics is a subset of pharmacogenomics that studies variations in DNA sequence as related to drug response.

**Pharmacogenomics**
According to the definitions set by the International Conference on Harmonisation Topic E15 guideline, pharmacogenomics is the study of variations in DNA and RNA characteristics as related to drug response.

**Pharmacokinetics**
(PK). How the body affects a drug over a period of time as a function of absorption, distribution, metabolism and excretion.

**Pharmacovigilance**
A pharmacological science related to the detection, assessment and prevention of adverse drug reactions in the post-marketing period of a drug's life cycle.

**Phenotyping**
Grouping of individuals based on measurement of an observable characteristic (for example, the extent to which they are able to metabolize a drug or other substrate).

in translating functionally important mutations into adjusted drug dosing. The use of "informed cohorts"[26] (genetically defined and phenotypically characterized subpopulations) throughout drug development programmes might aid the delivery of expert-vetted recommendations for drug dose adjustments, as well as health-care considerations[27].

The EMA is implementing a policy of transparency and involvement of stakeholders in collaborations with, for example, the PGWP, in open conferences such as the recent workshop on *Pharmacogenomics: from science to clinical care* (see Further information). This approach will provide dissemination of state-of-the-art information to ensure balanced understanding of the contribution of validated pharmacogenetics tests to public health. The ongoing revisions of legislation on *in vitro* diagnostics in Europe will further reinforce the oversight of genomic testing and limit inappropriate claims. The FDA has also conducted a series of open workshops in collaboration with the Drug Information Association in order to enhance implementation of pharmacogenetics in drug development, to inform policy development for pharmacogenetics and to increase transparency among stakeholders.

Ongoing and future challenges in the area of pharmacogenetics include the successful and streamlined implementation of drug and device co-development in order to bring the clinical utility and implementation of a personalized medicine approach to the next level. As mentioned in the guidances, apart from metabolizing enzymes other molecular determinants of ADME include drug transporters and/or channels, which allow movement of drugs across the plasma membrane[28]. In recent years, a multitude of articles describing the possible effects of genetic variants of drug transporters on the efficacy and/or safety of medicinal products have been published[29]. One example is certain polymorphisms in *SLCO1B1* — which encodes the organic anion transporter protein 1B1 (OATP1B1) — which significantly alter the PK properties and associated ADRs of drugs such as statins[30–32]. Importantly, genetic variants affecting drug transport may also alter target exposure at the cellular level, which is more complex to measure and to monitor compared to systemic exposure. In general, the effects of these genetic variants on PK parameters has not been extensively evaluated relative to those of metabolizing enzymes, and it is anticipated that additional functionally important genetic variants affecting drug transport will be defined as research continues.

Until now, translation of knowledge regarding the effect of genetic variants (both metabolizing enzymes and proteins responsible for drug transport) into specific regulatory recommendations has been challenging. With the expected expansion of our existing knowledge on such genetic variants and the functional interactome[33–40] they are involved in — as well as emerging research regarding the role of microRNAs, small regulatory RNAs and epigenetic alterations of genes involved in ADME — it is anticipated that the resolution for understanding the basis for interindividual differences in ADME gene expression and function will exponentially increase in coming years, and new tools in the field will thus evolve. Regulatory agencies are attentive to the evolution of such science as it has an impact on various aspects of the drug's life cycle. Therefore, mechanisms such as Innovation Task Force meetings, the 'qualification of novel methods methodologies for medicine' process (see Further Information) and scientific advice (provided through the regulatory agencies), are available to the scientific community to ensure platforms for dialogue and the evolution of regulatory standards in line with scientific progress.

Finally, as mentioned earlier, even a complete understanding of the influence of genetic variants on drug PK properties would only be one component of what has been described as "precision medicine"[41]. For some drugs, genetic variants directly related to PD properties — for example, a genetic variant in the target protein — may be more important than genetic variants related to PK parameters in influencing variability in drug response. This is particularly true for targeted medicines for which the genetic variant of the pathway is key for the clinical effect, such as vemurafenib, cetuximab and panitumumab. The study of such genetic variants is most advanced in the field of oncology, which has been the focus of particular regulatory attention. For example, the experience of the EMA with pharmacogenetics in the oncology field has been summarized in a reflection paper (BOX 1, note 2d), and the need for further regulatory guidances will be considered by the regulatory agencies in upcoming years. Moreover, the effects of genetic variants in drug targets and off-targets on interindividual variability in drug response could be further exacerbated if these proteins are part of large protein interaction networks (and particularly if they are key nodes), rather than only being single components of a targeted pathway. Ultimately, as highlighted in a recent commentary[24], precision medicine[41,42] "… informed by molecular phenotypes, environmental modulators of physiology and a systems-oriented view of multiple pharmacological interactions…", together with the integration of pharmacogenetics[13,43–46] into PK data, will build the foundation for individualized or personalized medicine.

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