
The Role of Genotyping in Pharmacological Therapy

K. Lindpaintner

VP Research, Roche Genetics, F. Hoffmann-La Roche, Bldg 93/532,
4070 Basel, Switzerland
e-mail: klaus.lindpaintner@roche.com

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Abstract This communication is intended to provide a view of what the disciplines of genetics and genomics stand to contribute (and how they have actually contributed for many years) to drug discovery and development and, more broadly, to the practice of health care. Particular emphasis will be placed on examining the role of genetics, that is, acquired or inherited variations at the level of DNA-encoded information, with regard to common complex diseases. A realistic understanding of this role is essential for a balanced assessment of the impact of genetics on health care in the future. Definitions for some of the terms that are in wide and often unreflected use today will be provided. A more systematic classification of pharmacogenetics will be attempted. It is important to

be aware that what will be discussed is to a large extent still uncharted territory. So by necessity, many of the positions taken on today's understanding and knowledge must be viewed as somewhat speculative in nature. Where appropriate and possible, select examples will be provided, although it should be pointed out that much of the literature in the area of genetic epidemiology and pharmacogenetics lacks the stringent standards normally applied to peer-reviewed research, and replicate data are generally absent.

Keywords Pharmacogenetics · Pharmacogenomics · Toxicogenetics · Drug discovery · Drug regulation

1

Introduction

The advances made over the last 30 years in molecular biology, molecular genetics and genomics and the development and refinement of associated methods and technologies have had a major impact on our understanding of biology, including the action of drugs and other biologically active xenobiotics. The tools that have been developed to allow these advances, and the knowledge of fundamental principles underlying cellular function thus derived, have become indispensable to almost any field of biological research, including future progress in biomedicine and health care.

It is important to realize that with regard to pharmacology and drug discovery, these accomplishments, starting sometime in the last third or quarter of the 20th century, have led gradually to a fundamental shift from the chemical paradigm to a biological paradigm. Whereas previously medicinal chemistry drove new developments in drug discovery, with biology almost an ancillary service that examined new molecules for biological function, biology has now taken the lead, based on a new-found understanding of physiological effects of biomolecules and pathways, requesting from the chemist compounds that modulate the function of these biomolecules or pathways, with—at least theoretically—a predictable functional impact in the setting of integrated physiology.

One particular aspect has uniquely captured the imagination of both scientists and the public, namely our understanding of genetics, especially our cataloguing of genome sequences. While understandable—given the austere beauty of Mendel's laws, the compelling esthetics of the double helix structure, and the awe-inspiring accomplishment (coupled with an unprecedented public relations campaign) of the human genome project—the public excitement about genetics and genomics and the high expectations regarding the impact they will have on the practice of health care are almost certainly unrealistic. Thus, at the interface between genetics/genomics and pharmacology, pharmacogenetics and pharmacogenomics (usually in the most loosely defined terms) are commonly touted as heralding a revolution in medicine. Yet, as soon as one begins to probe more carefully, little substance is yet to be found to support these enthusiastic claims.

Table 1 Terminology

Pharmacogenetics
Differential effects of a drug, in vivo, in different patients, dependent on the presence of inherited gene variants
Assessed primarily genetic (SNP) and genomic (expression) approaches
A concept to provide more patient/disease-specific health care
One drug, many genomes (i.e., different patients)
Focus: patient variability
Pharmacogenomics
Differential effects of compounds, in vivo or in vitro, on gene expression, among the entirety of expressed genes
Assessed by expression profiling
A tool for compound selection/drug discovery
Many drugs (i.e., early-stage compounds), one genome [i.e., normative genome (database, technology platform)]
Focus: compound variability

Indeed, as pointed out above, the major change in how we discover drugs, from the chemical to the biological paradigm, already occurred some time ago; what the current advances promise to allow us to do in due time is to move from a physiology-based to a (molecular) pathology-based approach towards drug discovery, promising the advancement from a largely palliative to a more cause/contribution-targeting pharmacopoeia.

2 Definition of Terms

There is widespread indiscriminate use of the terms “pharmacogenetics” and “pharmacogenomics”, causing some confusion. While no universally accepted definition exists, there is an emerging consensus on their differential meaning and use (Table 1).

2.1 Pharmacogenetics

The term “genetics” relates etymologically to the presence of individual properties, and inter-individual differences in these properties, due to inheritance. The term “pharmacogenetics” describes the interactions between a drug and an individual’s (or perhaps more accurately, groups of individuals) response to it as it relates to differences in DNA-based information. It is concerned with the assessment of clinical efficacy and/or the safety and tolerability profile; in other words, the pharmacological response phenotype of a drug in groups of individuals that differ with regard to certain DNA-encoded characteristics. It tests the hypothesis that these differences may allow prediction of individual drug response. Assessment of DNA-encoded characteristics is based most commonly on the presence or absence of polymorphisms at the level of nuclear DNA. How-

ever, this assessment may occur also at different levels where such DNA variation translates into different characteristics, such as differential mRNA expression or splicing, protein levels or functional characteristics, or even physiological phenotypes, all of which may be seen as surrogate or more highly integrated markers of the underlying genetic variant. It should be noted, however, that some authors continue to subsume all applications of expression profiling under the term “pharmacogenomics”, in a definition of the terms that is more driven by the technology used rather than by functional context.

2.2

Pharmacogenomics

In contrast, the terms “pharmacogenomics”, and its close relative, “toxicogenomics”, are etymologically linked to “genomics”, the study of the genome and of the entirety of expressed and non-expressed genes in any given physiological state. These two fields of study are concerned with a comprehensive, genome-wide assessment of the effects of pharmacological agents, including toxins/toxicants, on gene expression patterns. Pharmacogenomic studies are thus used to evaluate the differential effects of a number of chemical compounds (in the process of drug discovery commonly applied to lead selection) with regard to inducing or suppressing gene transcription in an experimental setting. Except for situations in which pharmacogenetic considerations are front-loaded into the discovery process, inter-individual variations in gene sequence are not usually taken into account in this process. Therefore, unlike pharmacogenetics, pharmacogenomics does not focus on differences among individuals with regard to the drug’s effects, but rather examines differences among several (prospective) drugs or compounds with regard to their biological effects across the entire genome or some significant part thereof. The basis of comparison is quantitative measures of expression, using a number of more or less comprehensive gene-expression-profiling methods, commonly based on microarray formats. By extrapolation from the experimental results to theoretically desirable patterns of activation or inactivation of gene expression in the setting of integrative pathophysiology, this approach is expected to provide a faster, more comprehensive, and perhaps even more reliable way to assess the likelihood of finding an ultimately successful drug than previously available schemes, involving mostly in vivo animal experimentation.

Thus, although both pharmacogenetics and pharmacogenomics refer to the evaluation of drug effects using (primarily) nucleic acid markers and technology, the directionalities of their approaches are distinctly different: pharmacogenetics represents the study of differences among a number of individuals with regard to clinical response to a particular drug (“one drug, many genomes”), whereas pharmacogenomics represents the study of differences among a number of compounds with regard to gene expression response in a single (normative) genome/expressome (“many drugs, one genome”). Accordingly, the fields of intended use are distinct: the former will help, in the clinical setting, to find

the medicine most likely to be optimal for a patient (or to find the patients most likely to respond to a drug), the latter will aid in the setting of pharmaceutical research to find the most suitable drug candidate from a given series of compounds under evaluation.

3

Pharmacogenomics: Finding New Medicines Quicker and More Efficiently

Once a screen (assay) has been set up in a drug discovery project and lead compounds are identified, the major task becomes the identification of an optimized clinical candidate molecule among the many compounds synthesized by medicinal chemists. Conventionally, such compounds are screened in a number of animal or cell models for efficacy and toxicity, experiments that, while having the advantage of being conducted in the *in vivo* setting, commonly take significant amounts of time and depend entirely on the similarity between the experimental animal condition/setting and its human counterpart, i.e., the validity of the model.

Although such experiments will never be entirely replaced by expression profiling at either the nucleic acid (genomics) or the protein (proteomics) level, the latter technique offers powerful advantages and complimentary information. First, the efficacy and profile of induced changes can be assessed in a comprehensive fashion (within the limitations, primarily sensitivity and completeness of transcript representation, of the technology platform used). Second, these assessments of differential efficacy can be carried out much more expeditiously than in conventionally used, (patho)physiology-based animal models. Third, the complex pattern of expression changes revealed by such experiments may provide new insights into possible biological interactions between the actual drug target and other biomolecules, and thus reveal new elements or branch-points of a biological pathway that may be useful as surrogate markers, novel diagnostic analytes, or as additional drug targets. Fourth, and increasingly important, these tools serve to determine specificity of action among members of gene families that may be highly important for both the efficacy and safety of a new drug. It must be borne in mind that any and all such experiments are limited by the coefficient of correlation with which the expression patterns determined are linked to the desired *in vivo* physiological action of the compound.

A word of caution regarding micro-array-based expression profiling would appear to be in order: It is important to remain aware of the fact that all micro-array expression data are of only associative character, i.e., they do not infer causation, and must be interpreted mindful of this limitation.

As a subcategory of this approach, toxicogenomics is evolving as a powerful adjuvant to classic toxicological testing. As pertinent databases are being created from experiments with known toxicants, revealing expression patterns that may be predictive of the longer-term toxic liabilities of compounds, future drug discovery efforts should benefit from insights allowing earlier rejection of compounds likely to cause such complications.

When using these approaches in drug discovery, even if implemented with proper biostatistics and analytical rigor, it is imperative to understand the probabilistic nature of such experiments: a promising profile on pharmacogenomic and toxicogenomic screens will enhance the likelihood of having selected an ultimately successful compound, and will achieve this goal quicker than conventional animal experimentation, but will do so only with a certain likelihood of success. The less reductionist approach of the animal experiment will still be needed to evaluate the chosen compound. It is to be anticipated, however, that such approaches will constitute an important time- and resource-saving first evaluation or screening step that will help to focus and reduce the number of animal experiments that will ultimately need to be conducted.

4

Pharmacogenetics: More Targeted, More Effective Medicines for Our Patients

4.1

Genes and Environment

It is common knowledge that today's pharmacopoeia, although representing enormous progress compared with what our physicians had only 15 or 20 years ago, is far from perfect. Many patients respond only partially, or fail to respond altogether to the drugs they are given, and others suffer adverse events that range from unpleasant to serious and life-threatening.

There is an emerging consensus that all common complex diseases are multifactorial in nature, i.e., that they are brought upon by the coincidence of certain intrinsic (inborn or acquired) predispositions and susceptibilities on the one hand, and extrinsic, environment-derived influences on the other. The relative importance of these two influences varies across a broad spectrum. In some diseases external factors appear to be more important, while in others intrinsic predispositions prevail. In almost all cases, a number of both intrinsic (genetic) as well as extrinsic factors appear to contribute, although it is not clear from the currently available literature how much this reflects the requirement of several intrinsic and extrinsic factors to coincide in any one individual, or how much this reflects the causative heterogeneity of each of today's conventional clinical diagnoses. In either case, the disease-causing (or better, -contributing) role that intrinsic, genetically encoded properties play with regard to the occurrence of the disease is fundamentally different in these common, complex diseases as compared to the classic monogenic mendelian diseases. While in the latter the impact of the genetic variant is typically categorical in nature, i.e., deterministic, in the former case, the presence of a disease-associated genetic variant is merely of probabilistic influence, raising (or lowering) the likelihood of disease occurrence to some extent but never predicting it in a black-and-white fashion.

If we regard a pharmacological agent as an extrinsic, environmental factor with a potential to affect the health-status of the individual to whom it is admin-

istered, then individual differences in response to such an agent would be expected, under the paradigm just elaborated upon, to be based on differences regarding the intrinsic characteristics of these patients, as long as we can exclude variation in the exposure to the drug (this is important, as in clinical practice non-adherence to prescribed regimens of administration, or drug-drug interactions interfering with bioavailability of the drug, are perhaps the most likely culprits when such differences in response phenotype are observed). The influence of such intrinsic variation on drug response may be more easily recognizable and more relevant in drugs with a steep dose-response curve. The argument for the greater likelihood of observing environmental factor/gene interactions with drugs as compared to, say, food-stuffs, goes along the same lines.

Clearly a better fundamental and mechanistic understanding of the molecular pathology of disease and of the role of intrinsic, biological properties predisposing to such diseases, as well as of drug action at the molecular level, will be essential for future progress in health care. Current progress in molecular biology and genetics has provided us with some of the prerequisite tools that should help us reach the goal of a more refined understanding.

4.2

An Attempt at a Systematic Classification of Pharmacogenetics

Two conceptually quite different categories of inter-individually differential drug response may be distinguished on the basis of the underlying biological variance (Table 2):

1. In the first case, the underlying biological variation is *in itself not disease-causing* or -contributing, and becomes clinically relevant *only* in response to the exposure to the drug in question (classical pharmacogenetics).

Table 2 Pharmacogenetics systematic classification

Classic pharmacogenetics
Pharmacokinetics
Absorption
Metabolism
Activation of prodrugs
De-activation
Generation of biologically active metabolites
Distribution
Elimination
Pharmacodynamics
Palliative drug action (modulation of disease-symptoms or disease signs by targeting physiologically relevant systems, without addressing those mechanisms that cause or causally contribute to the disease)
Molecular differential-diagnosis-related pharmacogenetics
Causative drug action (modulation of actual causative or contributory mechanisms)

2. In the second case, the biological variation is *directly disease-related*, is of pathological importance per se, and represents a subgroup of the overall clinical disease/diagnostic entity. The differential response to a drug is thus related to how well this drug addresses or is matched to the presence or relative importance of the pathological mechanism it targets in different patients, i.e., the molecular differential diagnosis of the patient (disease-mechanism-related pharmacogenetics).

Although these two scenarios are conceptually rather different, they result in similar practical consequences with regard to the administration of a drug, namely stratification of patients based on a particular, DNA-encoded marker. It seems therefore legitimate to subsume both under the umbrella of “pharmacogenetics”.

4.2.1

Classical Pharmacogenetics

This category includes differential pharmacokinetics and pharmacodynamics.

Pharmacokinetics. Drug response may vary due to inter-individual differences in absorption, distribution, metabolism (with regard to both activation of pro-drugs, inactivation of the active molecule, and generation of derivative molecules with biological activity) or excretion of the drug. In any of these cases, the differential effects observed are due to the presence—at the intended site of action—either of inappropriate concentrations of the pharmaceutical agent, or of inappropriate metabolites, or of both, resulting either in lack of efficacy or in toxic effects. Pharmacogenetics, as it relates to pharmacokinetics, has been recognized as an entity for more than 100 years, going back to the observation, commonly credited to Archibald Garrod, that a subset of psychiatric patients treated with the hypnotic, sulphonal, developed porphyria. We have since then come to understand the underlying genetic causes for many of the previously known differences in enzymatic activity, most prominently with regard to the P450 enzyme family (Tables 3 and 4), and these have been the subject of recent reviews (Dickins and Tucker 2001; Evans and Relling 1999). However, such pharmacokinetic effects are also seen with membrane transporters, such as in the case of differential activity of genetic variants of MDR-1 that affects the effective intracellular concentration of anti-retrovirals (Fellay et al. 2002), or of the purine-analogue-metabolizing enzyme, thiomethyl-purine-transferase (Dubinsky et al. 2000).

Despite the widespread recognition of isoenzymes with differential metabolizing potential since the middle of the 20th century, the practical application and implementation of this knowledge has been minimal so far. This may be the consequence, on one hand, of the irrelevance of such differences in the presence of relatively flat dose-effect-curves (i.e., a sufficiently wide therapeutic window), as well as, on the other hand, the fact that many drugs are subject to complex, parallel metabolizing pathways, where in the case of underperformance of one

Table 3 Pharmacogenetics: chronology

Pharmacogenetic phenotype	Described	Underlying gene/mutation	Identified
Sulphonal porphyria	ca. 1890	Porphobilinogen deaminase?	1985
Suxamethonium hypersensitivity	1957–1960	Pseudocholinesterase	1990–1992
Primaquine hypersensitivity; favism	1958	G-6-PD	1988
long QT syndrome	1957–1960	<i>Herg</i> , etc.	1991–1997
Isoniazid slow/fast acetylation	1959–1960	<i>N</i> -acetyltransferase	1989–1993
Malignant hyperthermia	1960–1962	Ryanodine receptor	1991–1997
Fructose intolerance	1963	Aldolase B	1988–1995
Vasopressin insensitivity	1969	Vasopressin receptor2	1992
Alcohol susceptibility	1969	Aldehyde dehydrogenase	1988
Debrisoquine hypersensitivity	1977	CYP2D6	1988–1993
Retinoic acid resistance	1970	PML-RARA fusion-gene	1991–1993
6-Mercaptopurin-toxicity	1980	Thiopurine methyltransferase	1995
Mephenytoin resistance	1984	CYP2C19	1993–1994
Insulin insensitivity	1988	Insulin receptor	1988–1993

Table 4 Pharmacogenetics: pharmacological phenotyping

Phase I enzyme	Testing substance
Aldehyde dehydrogenase	Acetaldehyde
Alcohol dehydrogenase	Ethanol
CYP1A2	Caffeine
CYP2A6	Nicotine, coumarin
CYP2C9	Warfarin
CYP2C19	Mephenytoin, omeprazole
CYP2D6	Dextromethorphan, debrisoquine, sparteine
CYP2E1	Chlorzoxazone, caffeine
CYP3A4	Erythromycin
CYP3A5	Midazolam
Serum cholinesterase	Benzoylcholine, butyrylcholine
Paraoxonase/arylesterase	Paraaxon
Phase II enzyme	Testing substance
Acetyltransferase (NAT1)	<i>Para</i> -aminosalizylsäure
Acetyltransferase (NAT2)	Isoniazid, sulfamethazine, caffeine
Dihydropyrimidine dehydrogenase	5-fluorouracil
Glutathione transferase (GST-M1)	<i>Trans</i> -stilbene-Oxid
Thiomethyltransferase	2-mercaptoethanol, d-penicillamine, captopril
Thiopurine methyltransferase	6-mercaptopurine, 6-thioguanine, 8-azathioprine
UDP-glucuronosyl transferase (UGT1A)	Bilirubin
UDP-glucuronosyl transferase (UGT2B7)	Oxazepam, ketoprofen, oestradiol, morphine

enzyme, another one may compensate. Such compensatory pathways may well have somewhat different substrate affinities, but allow plasma levels to remain within therapeutic concentrations. Thus, the number of such polymorphisms that have found practical applicability is rather limited and, by and large, so far restricted to determinations of the presence of functionally deficient variants of the enzyme, thiopurine-methyl-transferase, in patients prior to treatment with purine-analogue chemotherapeutics.

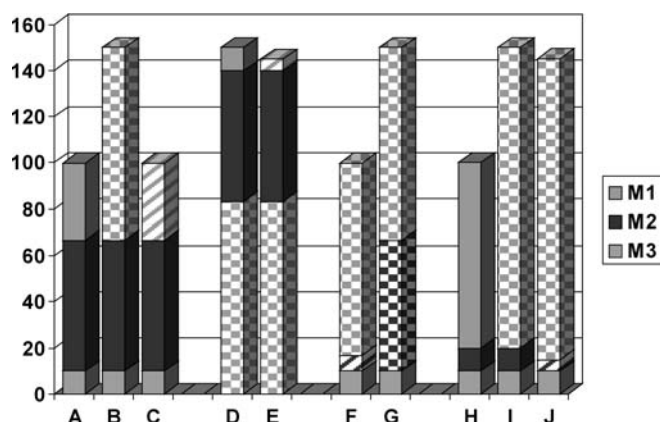


Fig. 1 A, Normal physiology: three molecular mechanisms ($M1$, $M2$, $M3$) contribute to a trait. B, Diseased physiology D1: derailment (cause/contribution) of molecular mechanism 1 ($M1$). C, Diseased physiology D1: causal treatment T1 (aimed at $M1$). D, Diseased physiology D3: derailment (cause/contribution) of molecular mechanism 3 ($M3$). E, Diseased physiology D3, treatment T1: treatment does not address cause. F, Diseased physiology D1, palliative treatment T2 (aimed at $M2$). G, Diseased physiology D1, palliative treatment T2; T2-refractory gene variant in $M2$. H, Normal physiology variant: differential contribution of $M1$ and $M2$ to normal trait. I, Diseased physiology D1-variant: derailment of mechanism $M1$. J, Diseased physiology D1-variant: treatment with T2. Solid colors indicate normal function, stippling indicates pathologic dysfunction, hatching indicates therapeutic modulation

Pharmacodynamics. Pharmacodynamic effects, in contrast, may lead to inter-individual differences in a drug's effects despite the presence of appropriate concentrations of the intended active (or activated) drug compound at the intended site of action. Here, DNA-based variation in how the target molecule, or another (downstream) member of the target molecule's mechanistic pathway, can respond to the medicine modulates the effects of the drug. This will apply primarily to palliatively working medicines that improve a condition symptomatically by modulating disease-phenotype-relevant (but not disease-cause-relevant) pathways that are not dysfunctional but can be used to counterbalance the effect of a dysfunctional, disease-causing pathway and therefore allow mitigation of symptoms. A classic example of such an approach is the acute treatment of thyrotoxicity with beta-adrenergic blocking agents: even though the sympathetic nervous system does not in this case contribute causally to tachycardia and hypertension, dampening even its baseline tonus through this class of drugs relieves the cardiovascular symptoms and signs of this condition, before the causal treatment (in this case available through partial chemical ablation of the hyperactive thyroid gland) can take effect. Notably, the majority of today's pharmacopoeia actually belongs to this class of palliatively acting medicines.

A schematic (Fig. 1) is provided to help clarify these somewhat complex concepts. A hypothetical case of a complex trait/disease is depicted where excessive, dysregulated function of one of the trait-controlling/-contributing pathways (Fig. 1, A, B) causes symptomatic disease; the example used refers to blood

pressure as the trait, and hypertension as the disease in question, respectively (for the case of a defective or diminished function of a pathway, an analogous schematic could be constructed and again for a deviant function). A palliative treatment would be one that addresses one of the pathways that, while not dys-regulated, contributes to the overall deviant physiology (Fig. 1, F), while the respective pharmacogenetic-pharmacodynamic scenario would occur if this particular pathway was, due to a genetic variant, not responsive to the drug chosen (Fig. 1, G). A palliative treatment may also be ineffective if the particular mechanism targeted by the palliative drug (due to the presence of a molecular variant) provides less than the physiologically expected baseline contribution to the relevant phenotype (Fig. 1, H). In such a case, modulating an *a-priori* unimportant pathway in the disease scenario will not yield successful palliative treatment results (Fig. 1, I, J).

Some of the most persuasive examples we have to date of such a palliative drug-related pharmacogenetic effect are in the field of asthma. The treatment of asthma relies on an array of drugs aimed at modulating different generic pathways, thus mediating bronchodilation or anti-inflammatory effects, often without regard to the possible causative contribution of the targeted mechanism to the disease. One of the mainstays of the treatment of asthma is activation of the beta-2-adrenoceptor by specific agonists, which leads to relaxation of bronchial smooth muscles and, consequently, bronchodilation. Recently, several molecular variants of the beta-2-adrenoceptor have been shown to be associated with differential treatment response to beta-2-agonists (Martinez et al. 1997; Tan et al. 1997). Individuals carrying one or two copies of a variant allele that contains a glycine in place of arginine in position 16 were found to have a three- and five-fold reduced response to the agonist, respectively. This was shown in both *in vitro* (Green et al. 1994, 1995) and *in vivo* (Green et al. 1995) studies to correlate with an enhanced rate of agonist-induced receptor down-regulation, but not with any difference in transcriptional or translational activity of the gene, or with agonist binding. In contrast, a second polymorphism affecting position 19 of the beta upstream peptide was shown to affect translation (but not transcription) of the receptor itself, with a 50% decrease in receptor numbers associated with the variant allele, which happens to be in strong linkage disequilibrium with the variant allele at position 16 in the receptor. The simultaneous presence of both mutations would be predicted to result in low expression and enhanced down-regulation of an otherwise functionally normal receptor, depriving patients carrying such alleles of the benefits of effective bronchodilation as a palliative (*i.e.*, non-causal) counter-measure to their pathological airway hyper-reactivity. Importantly, there is no evidence that any of the allelic variants encountered are associated with the prevalence or incidence, and thus potentially the etiology of the underlying disease (Reihnsaus et al. 1993; Dewar et al. 1998). This would reflect the scenario depicted in Fig. 1, H.

Inhibition of leukotriene synthesis, another palliative approach towards the treatment of asthma, proved clinically ineffective in a small fraction of patients who carried only non-wild-type alleles of the 5-lipoxygenase promoter region

(Drazen et al. 1999). These allelic variants had previously been shown to be associated with decreased transcriptional activity of the gene (In et al. 1997). It stands to reason, and consistent with clinical observations, that in the presence of already reduced 5-lipoxygenase activity, pharmacological inhibition may be less effective (Fig. 1, H–J). Of note again, there is no evidence for a primary, disease-causing or -contributing role of any 5-lipoxygenase variants; all of them were observed at equal frequencies in disease-affected and non-affected individuals (In et al. 1997).

Pharmacogenetic effects may not only account for differential efficacy, but also contribute to the differential occurrence of adverse effects. An example of this scenario is provided by the well-documented pharmacogenetic association between molecular sequence variants of the 12S rRNA, a mitochondrion-encoded gene, and aminoglycoside-induced ototoxicity (Fischel-Ghodsian et al. 1999). Intriguingly, the mutation that is associated with susceptibility to ototoxicity renders the sequence of the human 12S rRNA similar to that of the bacterial 12S rRNA gene, and thus effectively turns the human 12S rRNA into the (bacterial) target for aminoglycoside drug action, presumably mimicking the structure of the bacterial binding site of the drug (Hutchin and Cortopassi et al. 1994). As in the other examples, presence of the 12S rRNA mutation *per se* has no primary, drug-treatment-independent pathological effect *per se*.

By analogy, one may speculate that such molecular mimicry may occur within one species: adverse events may arise if the selectivity of a drug is lost because a gene that belongs to the same gene family as the primary target, loses its identity *vis-à-vis* the drug and attains, based on its structural similarity with the principal target, similar or at least increased affinity for the drug. Depending on the biological role of the imposter molecule, adverse events may occur, even though the variant molecule may be quite silent with regard to any contribution to disease causation. Although we currently have no obvious examples for this scenario, it is certainly plausible for various classes of receptors and enzymes.

4.2.2

Pharmacogenetics as a Consequence of Molecular Differential Diagnosis

As alluded to earlier, there is general agreement today that any of the major clinical diagnoses in the field of common complex disease, such as diabetes, hypertension or cancer, are comprised of a number of etiologically (i.e., at the molecular level) more or less distinct subcategories. In the case of a causally acting drug, this may imply that the agent will only be appropriate, or will work best, in that fraction of all the patients who carry the (all-inclusive and imprecise) clinical diagnosis in whom the dominant molecular etiology, or at least one of the contributing etiological factors, matches the mechanism of action of the drug in question (Fig. 1, C). If the mechanism of action of the drug addresses a pathway that is not disease relevant, perhaps because it is already down-regulated as an appropriate physiological response to the disease, then logically, the

drug would be expected not to show efficacy (Fig. 1, D, E). Thus, unrecognized and undiagnosed disease heterogeneity, disclosed indirectly by the presence or absence of response to a drug targeting a mechanism that contributes to only one of several molecular subgroups of the disease, provides an important explanation for differential drug response and likely represents a substantial fraction of what we today somewhat indiscriminately subsume under the term "pharmacogenetics".

Currently, the most frequently cited example for this category of pharmacogenetics is trastuzumab (Herceptin), a humanized monoclonal antibody directed against the *her-2* oncogene. This breast cancer treatment is prescribed based on the level of *her-2*-oncogene expression in the patient's tumor tissue. Differential diagnosis at the molecular level not only provides an added level of diagnostic sophistication, but also actually represents the prerequisite for choosing the appropriate therapy. Because trastuzumab specifically inhibits a gain-of-function variant of the oncogene, it is ineffective in the two-thirds of patients who do not over-express the drug's target, whereas it significantly improves survival in the one-third of patients who constitute the subentity of the broader diagnosis of breast cancer in whom the gene is expressed (Baselga et al. 1996). Some have argued against this being an example of pharmacogenetics, because the parameter for patient stratification (i.e., for differential diagnosis) is the somatic gene expression level rather than particular genotype data (Haseltine 1998). This is a difficult argument to follow, since in the case of a treatment-effect-modifying germ-line mutation it would obviously not be the nuclear gene variant per se, but also its specific impact on either structure/function or on expression of the respective gene/gene product that would represent the actual physiological corollary underlying the differential drug action. Conversely, an a-priori observed expression difference is highly likely to reflect a potentially, as yet undiscovered, sequence variant. Indeed, as pointed out earlier, there are a number of examples in the field of pharmacogenomics where the connection between genotypic variant and altered expression has already been demonstrated (In et al. 1997; McGraw et al. 1998).

Another example, although still hypothetical, of how proper molecular diagnosis of relevant pathological mechanisms will significantly influence drug efficacy is in the evolving class of anti-AIDS/HIV drugs that target the CCR5 cell-surface receptor (Huang et al. 1996; Dean et al. 1996; Samson et al. 1996). These drugs would be predicted to be ineffective in those rare patients who carry the delta-32 variant, but who nevertheless have contracted AIDS or test HIV-positive (most likely due to infection with an SI-virus phenotype that utilizes CXCR4) (O'Brien et al. 1997; Theodorou et al. 1997).

It should be noted that the pharmacogenetically relevant molecular variant need not affect the primary drug target, but may equally well be located in another molecule belonging to the system or pathway in question, both upstream and downstream in the biological cascade with respect to the primary drug target.

4.2.3

Different Classes of Markers

Pharmacogenetic phenomena, as pointed out previously, need not be restricted to the observation of a direct association between allelic sequence variation and phenotype, but may extend to a broad variety of indirect manifestations of underlying, but often (as yet) unrecognized sequence variation. Thus, differential methylation of the promoter region of O6-methylguanine-DNA-methylase has recently been reported to be associated with differential efficacy of chemotherapy with alkylating agents. If methylation is present, expression of the enzyme that rapidly reverses alkylation and induces drug-resistance is inhibited, and therapeutic efficacy is greatly enhanced (Esteller et al. 2000).

4.2.4

Complexity Is to Be Expected

In the real world, it is likely that a combination of the scenarios depicted affect how well a patient responds to a given treatment, or how likely it is that he or she will suffer an adverse event. Thus, a fast-metabolizing patient with poor-responder pharmacodynamics may be particularly unlikely to gain any benefit from taking the drug in question, while a slow-metabolizing status may counterbalance in another patient the same inopportune pharmacodynamics, and a third patient, who is a slow metabolizer and displaying normal pharmacodynamics, may be more likely to suffer adverse events. In all of them, both the pharmacokinetic and pharmacodynamic properties may result from the interaction of several of the mechanisms described above. In addition, we know of course that co-administration of other drugs, or even the consumption of certain foods, may affect and further complicate the picture for any given treatment.

5

Incorporating Pharmacogenetics into Drug Development Strategy

It is important to note that despite the public hyperbole and the high expectations surrounding the use of pharmacogenetics to provide personalized care, these approaches are likely to be applicable only to a fraction of medicines that are being developed. Further, if and when such approaches are used, they will represent no radical new direction or concept in drug development but simply a stratification strategy akin to others which we have been using it all along.

The opportunity to subdivide today's clinical diagnosis into molecular subtypes, based on a deeper, more differentiated understanding of pathology at the molecular level, will permit a more sophisticated and precise diagnosis of disease and foster medical advances which will appear as pharmacogenetic phenomena. However, the sequence of events that is today often presented as characteristic for a pharmacogenetic scenario—namely, exposing patients to a drug,

recognizing a differential [i.e. (quasi-)bimodal-] response pattern, discovering a marker that predicts this response, and creating a diagnostic product to be co-marketed with the drug henceforth—is likely to be reversed. Rather, the search for new drugs will be based specifically, and a priori, on a new mechanistic understanding of disease causation or contribution (i.e., a newly found ability to diagnose a molecular subentity of a previously more encompassing, broader, and less precise clinical disease definition). Thus, pharmacogenetics will not be so much about finding the “right medicine for the right patient”, but about finding the correct medicine for a given disease (subtype), as we have aspired to do all along throughout the history of medical progress. This is, in fact, good news: the conventional pharmacogenetic scenario would invariably present major challenges from both a regulatory and a business development and marketing standpoint, as it will confront development teams with a critical change in the drug’s profile at a very late point during the development process. In addition, the timely development of an approvable diagnostic in this situation is difficult at best, and its marketing as an add-on to the drug is a less than attractive proposition to diagnostics business. Thus, the practice of pharmacogenetics will, in many instances, be marked by progress along the very same path that has been one of the main avenues of medical progress all along: differential diagnosis first, followed by the development of appropriate, more specific treatment modalities.

Thus, the first step in the sequence of events in this case is likely to involve the development of an *in vitro* diagnostic test as a stand-alone product that may be marketed on its own merits, allowing the physician to establish an accurate, state-of-the-art diagnosis of the molecular subtype of the patient’s disease. Sometimes such a diagnostic may prove helpful, even in the absence of specific therapy, by guiding the choice of existing medicines and/or of non-drug treatment modalities such as specific changes in diet or lifestyle. The availability of such a diagnostic, as part of the more sophisticated understanding of disease, will undoubtedly foster and stimulate the search for new, more specific drugs; and once such drugs are found, the availability of the specific diagnostic test will be important for carrying out the appropriate clinical trials. This will allow a prospectively planned, much more systematic approach towards clinical and business development, with a commensurate greater chance of actual realization and success.

In practice, some degree of guesswork will remain, due to the nature of common complex disease. First, all diagnostic approaches, including those based on DNA analysis in common complex disease, as stressed above, will provide only a measure of probability. Although the variances of drug response among patients who do (or do not) carry the drug-specific subdiagnosis will be smaller, there will still be a distribution of differential responses: although by and large the drug will work better in the responder group, there will be some patients in this subgroup who will respond less or not at all, and conversely, not everyone belonging to the non-responder group will fail completely to respond, depending perhaps on the relative magnitude with which the particular mechanism

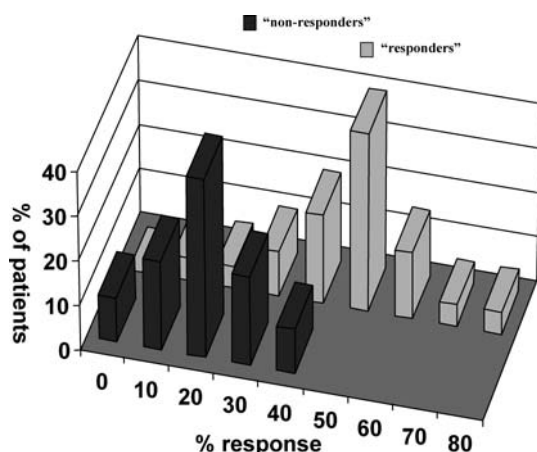


Fig. 2 Hypothetical example of bimodal distribution according to marker that indicates non-responder or responder status. Note that in both cases a distribution is present, with overlaps; thus the categorization into responders or non-responders based on the marker must be understood to convey only the probability of belonging to one or the other group

contributes to the disease. It is important to bear in mind, therefore, that even in the case of fairly obvious bi-modality, patient responses will still show distribution patterns and that all predictions as to responder or non-responder status will only have a certain probability of being accurate (Fig. 2). The terms “responder” and “non-responder” as applied to groups of patients stratified based on a DNA marker represent mendelian-thinking-inspired misnomers that should be replaced by more appropriate terms that reflect the probabilistic nature of any such classification, e.g., likely (non-) responder.

In addition, based on our current understanding of the polygenic and heterogeneous nature of complex disorders, we will only be able to exclude in any one patient those genetic variants that do not appear to contribute to the disease, and therefore deselect certain treatments, even in an ideal world where we would know about all possible susceptibility gene variants for a given disease and have treatments for them. We will, however, most likely find ourselves left with a small number, perhaps two to four, potential disease-contributing gene variants whose relative contribution to the disease will be very difficult, if not impossible, to rank in an individual patient. It is likely then that trial and error, and this great intangible quantity, physician experience, will still play an important role, albeit on a more limited and subselective basis.

Where differential drug response and/or safety occurs as a consequence of a pathologically irrelevant, purely drug-response-related pharmacogenetics scenario, there will be greater difficulty in planning and executing a clinical development program because it will be more difficult to anticipate or predict differential responses a priori. In this situation, it may also be more difficult to find the relevant marker(s), unless it happens to be among the obvious candidate

genes implicated in the disease physiopathology or the treatment's mode of action. Although screening for molecular variants of these genes, and testing for their possible associations with differential drug response, is a logical first step, if this is unsuccessful, it may be necessary to embark on an unbiased genome-wide screen for such a marker or markers. Despite recent progress in high-throughput genotyping, the obstacles that will have to be overcome on the technical, data-analysis and cost levels are formidable. They will limit the deployment of such programs, at least for the foreseeable future, to select cases in which there are very solid indications for doing so, based on clinical data showing a near-categorical (e.g., bi-modal) distribution of treatment outcomes. Even then we may expect to encounter for every success, due a favorably strong linkage disequilibrium across considerable genomic distance in the relevant chromosomal region, as many or more failures, where the culpable gene variant cannot be found due to the higher recombination rate or other characteristics of the stretch of genome on which it is located.

6 Regulatory Aspects

At the time of writing, regulatory agencies in both Europe and the United States are beginning to show keen interest in the potential role that pharmacogenetic approaches may play in the development and clinical use of new drugs and in the potential challenges that such approaches may present to the regulatory approval process. While no formal guidelines have been issued, the pharmaceutical industry has already been reproached, albeit in a rather non-specific manner, for not being more proactive in the use of pharmacogenetic markers. It will be of key importance for all concerned to engage in an intensive dialogue at the end of which, it is hoped, will emerge a joint understanding that stratification according to DNA-based markers is fundamentally nothing new, and not different from stratification according to any other clinical or demographic parameter, as has been used all along.

Still, based on the perception that DNA-based markers represent a different class of stratification parameters, a number of important questions will need to be addressed and answered, hopefully always in analogy to conventional stratification parameters, including those referring to ethical aspects. Among the most important ones are questions concerning:

- The need and/or ethical justification (or lack thereof) to include likely non-responders in a trial for the sake of meeting safety criteria, which, given the restricted indication of the drug, may indeed be excessively broad
- The need to use active controls if the patient/disease stratum is different from that in which the active control was originally tested
- The strategies to develop and gain approval for the applicable first-generation diagnostic, as well as for the regulatory approval of subsequent generations of tests to be used to determine eligibility for prescription of the drug,

as well as a number of ethical and legal questions relating to the unique requirements regarding privacy and confidentiality for genetic testing that may raise novel problems with regard to regulatory audits of patient data (see below).

A concerted effort to avoid what has been termed genetic exceptionalism—the differential treatment of DNA-based markers as compared with other personal medical data—should be made so as not to further complicate the already very difficult process of obtaining regulatory approval. This seems justified based on the recognized fact that in the field of common complex disease, DNA-based markers are not at all different from conventional medical data in all relevant aspects, namely specificity, sensitivity, and predictive value.

7

Pharmacogenetic Testing for Drug Efficacy Versus Safety

In principle, pharmacogenetic approaches may be useful both to raise efficacy and to avoid adverse events, by stratifying patient eligibility for a drug according to appropriate markers. In both cases, clinical decisions and recommendations must be supported by data that have undergone rigorous biostatistical scrutiny. Based on the substantially different prerequisites and opportunities for acquiring such data, and applying them to clinical decision-making, we expect the use of pharmacogenetics for enhanced efficacy to be considerably more common than for the avoidance of adverse events.

The chances of generating adequate data on efficacy in a subgroup is reasonably high, given the fact that unless the drug is viable in a reasonably sizeable number of patients, it will probably not be developed for lack of a viable business case, or at least only under the protected environment of orphan drug guidelines. Implementation of pharmacogenetic testing to stratify for efficacy, provided that safety in the non-responder group is not an issue, will primarily be a matter of physician preference and sophistication, and potentially of third-party payer directives, but would appear less likely to become a matter of regulatory mandate, unless a drug has been developed selectively in a particular stratum of the overall indication (in which case the indication label will be restricted to this stratum). Indeed, an argument can be made against depriving those who carry the likely non-responder genotype regarding eligibility for the drug, but who individually, of course, may respond to the drug with a certain, albeit lower probability. From a regulatory aspect, the use of pharmacogenetics for efficacy, if adequate safety data exist, appears largely unproblematic; the worst-case scenario (a genotypically inappropriate patient receiving the drug) would result in treatment without expected beneficial effect, but with no increased odds to suffer adverse consequences, i.e., much of what one would expect under conventional paradigms.

The usefulness and clinical application of pharmacogenetic strategies for improving safety, particularly with regard to serious adverse events, will meet with

considerably greater hurdles and is less likely to become practical. A number of reasons are cited for this. First, in the event of serious adverse events associated with the use of a widely-prescribed medicine, withdrawal of the drug from the market is usually based largely on anecdotal evidence from a rather small number of cases, in accordance with the Hippocratic mandate *primum non nocere*. If the sample size is insufficient to demonstrate a statistically significant association between drug exposure and event, as is typically the case, it will most certainly be insufficient to allow meaningful testing for genotype–phenotype correlations; the biostatistical hurdles become progressively more difficult as many markers are tested and the number of degrees of freedom applicable to the analysis for association continues to rise. Therefore, the fraction of attributable risk shown to be associated with a given at-risk (combination of) genotype(s) would have to be very substantial for regulators to accept such data. Indeed, the low prior probability of the adverse event, by definition, can be expected to yield an equally low positive (or negative) predictive value.

Second, the very nature of safety issues raises the hurdles substantially because in this situation the worst-case scenario, administration of the drug to the wrong patient, will result in a higher probability of harm to the patient. Therefore, it is likely that the practical application of pharmacogenetics for the purpose of limiting adverse events will be restricted to diseases with a dire prognosis, where a high medical need exists, where the drug in question offers unique potential advantages (usually bearing the characteristics of a life-saving drug), and where, therefore, the tolerance even for relatively severe side effects is much greater than for other drugs. This applies primarily to areas such as oncology or HIV/AIDS. In most other indications, the sobering biostatistical and regulatory considerations discussed represent barriers that are unlikely to be overcome easily; and the proposed, conceptually highly attractive, routine deployment of pharmacogenetics as a generalized drug surveillance or pharmaco-vigilance practice following the introduction of a new pharmaceutical agent (Roses 2000) faces these scientific as well as formidable economic hurdles.

8

Ethical and Societal Aspects of Pharmacogenetics

No discussion about the use of genetic/genomic approaches to health care can be complete without considering their impact on ethics, society and the law.

Much of the discussion about ethical and legal issues relating to pharmacogenetics is centered on the issue of genetic testing, a topic that has recently been the focus of a number of guidelines, advisories, white papers, etc., issued by a number of committees in both Europe and the United States. It is interesting to note that the one characteristic that almost all these documents share is a studious avoidance of defining exactly what a genetic test is. Where definitions are given, they tend to be very broad, including not only the analysis of DNA but also of transcription and translation products affected by inherited variation. In as much as the most sensible solution to this dilemma would be a consensus to

treat all personal medical data in a similar fashion regardless of the degree to which DNA-encoded information affects it (noting that there really is not any medical data that are not to some extent affected by intrinsic patient properties), it may, for the time being, be helpful to let the definition of what constitutes genetic data be guided by the public perception of genetic data, in as much as the whole discussion of this topic is prompted by these public perceptions.

In the public eye, a genetic test is usually understood either (1) as any kind of test that establishes the diagnosis (or predisposition) of a classic monogenic, heritable disease, or (2) as any kind of test based on nucleic acid analysis. This includes the (non-DNA-based) Guthrie test for phenylketonuria as well as forensic and paternity testing and the DNA-based test for Lp(a), but not the plasma-protein-based test for the same marker (even though the information derived is identical). Since monogenic disease is, in effect, excluded from this discussion, it stands to reason to restrict the definition of genetic testing to the analysis of (human) DNA sequence.

Based on the perceived particular sensitivity of genetic data, institutional review boards commonly apply a specific set of rules for granting permission to test for DNA-based markers in the course of drug trials or other clinical research, including (variably) separate informed consent forms, the anonymization of samples and data, specific stipulations about availability of genetic counseling, provision to be able to withdraw samples at any time in the future, etc.

Arguments have been advanced (Roses 2000) that genotype determinations for pharmacogenetic characterization, in contrast to genetic testing for primary disease risk assessment, are less likely to raise potentially sensitive issues with regard to patient confidentiality, the misuse of genotyping data or other nucleic-acid-derived information, and the possibility of stigmatization. While this is certainly true when pharmacogenetic testing is compared to predictive genotyping for highly penetrant mendelian disorders, it is not apparent why in common complex disorders, issues surrounding predictors of primary disease risk would be any more or less sensitive than those pertaining to predictors of likely treatment success or failure. Indeed, two lines of reasoning may actually indicate an increased potential for ethical issues and complex confrontations among the various stakeholders to arise from pharmacogenetic data.

First, while access to genotyping and other nucleic acid-derived data related to disease susceptibility can be strictly limited, the very nature of pharmacogenetic data calls for a rather more liberal position regarding use: if this information is to serve its intended purpose, i.e., improving the patient's chance for successful treatment, then it is essential that it is shared among at least a somewhat wider circle of participants in the health care process. Thus, the prescription for a drug that is limited to a group of patients with a particular genotype will inevitably disclose those patients' genotype to anyone of a large number of individuals involved in the care of those patients at the medical and administrative level. The only way to limit this quasi-public disclosure of this type of patient genotype data would be if he or she were to sacrifice the benefits of the indicated treatment for the sake of data confidentiality.

Second, patients profiled to carry a high disease probability along with a high likelihood for treatment response may be viewed, from the standpoint of insurance risk, for example, as comparable to patients displaying the opposite profile, i.e., a low risk to develop the disease, but having a high likelihood not to respond to medical treatment, if the disease indeed occurs. For any given disease risk, then patients less likely to respond to treatment would be seen as a more unfavorable insurance risk, particularly if non-responder status is associated with chronic, costly illness rather than with early mortality, the first case having much more far-reaching economic consequences. The pharmacogenetic profile may thus, under certain circumstances, become a more important (financial) risk-assessment parameter than primary disease susceptibility, and would be expected, in as much as it represents but one stone in the complex disease mosaic, to be treated with similar weight, or lack thereof, as other genetic and environmental risk factors.

Practically speaking, the critical issue is not only, and perhaps not even predominantly, the sensitive nature of the information and how it is disseminated and disclosed, but how and to what end it is used. Obviously, the generation and acquisition of personal medical information must always be contingent on the individual's free choice and consent, as must be all the application of such data for specific purposes. Beyond this, however, there is today an urgent need for the requisite dialogue and discourse among all stakeholders within society to develop and endorse a set of criteria by which the use of genetic, and indeed of all personal medical information, should occur. It will be critically important that society as a whole endorses, in an act of solidarity with those destined to develop a certain disease, guidelines that support the beneficial and legitimate use of the data in the patient's interest while at the same time prohibiting their use in ways that may harm the individual, personally, financially, or otherwise. As long as we trust our political decision processes to reflect the consensus of society, and as long as such consensus reflects the principles of justice and equality, the resulting set of principles should assert such proper use of medical information. Indeed, both aspects, data protection and patient/subject protection, are seminal components of the mandates included in the WHO's "Proposed International Guidelines on Ethical Issues in Medical Genetics and Genetic Services" (<http://www.who.int/ncd/hgn/hgnethic.htm>) which mandate autonomy, beneficence, no maleficence, and justice.

9 Conclusion

Pharmacogenetics, in the different scenarios included in this term, will represent an important new avenue towards understanding disease pathology and drug action, and will offer new opportunities of stratifying patients to achieve optimal treatment success. As such, it represents a logical, consequent step in the history of medicine—but an evolutionary, rather than a revolutionary one. Its implementation will take time and will not apply to all diseases and all treat-

ments equally. If society finds ways to sanction the proper use of this information, thus allowing and protecting its unencumbered use for the patient's benefit, important progress in health care will be made.

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